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Effects of brewery by-products on growth performance, bioconversion efficiency, nutritional profile, and microbiota and mycobiota of black soldier fly larvae

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ABSTRACT

Brewery by-products are recognised as suitable rearing substrates for *Hermetia illucens*, better known as black soldier fly (**BSF**) but information about the impact of different ratios of brewer's spent grains (**BSG**) and brewer's spent yeast (**BSY**) are still scarce. This study evaluated the effects of BSG-BSY-based diets on BSF larval growth, survival, bioconversion efficiency, nutritional profile, and microbiota and mycobiota. A total of 3 000 6-day-old BSF larvae were allotted to five dietary treatments (six replicate boxes/diet, 100 larvae/box): (i) BSY2.5 (25 g/kg of BSY+975 g/kg of BSG), (ii) BSY5 (50 g/kg of BSY+950 g/kg of BSG), (iii) BSY7.5 (75 g/kg of BSY+925 g/kg of BSG), (iv) BSY10 (100 g/kg of BSY+900 g/kg of BSG), and (v) control (Gainesville diet). Larval weight and substrate pH were recorded every 4 days. At the end of the trial (5% of prepupae), bioconversion efficiency corrected for residue (**BER**), reduction rate (**RR**), and waste reduction index (**WRI**) were calculated, and the larval proximate composition, microbiota and mycobiota characterised. At 10 and 14 days of age, BSY7.5 and BSY10 larvae displayed higher weight than BSY2.5 and BSY5 ($P < 0.05$), with BSY10 larvae showing the highest weight among the BSG-BSY-based diets at the end of the trial ($P < 0.05$). The BSY7.5 and BSY10 larvae also displayed a better BER than BSY2.5 and BSY5 ($P < 0.01$), whereas similar RR, WRI, survival and development time, as well as pH, were, however, observed among the BSG-BSY-based diets ($P > 0.05$). The BSY10 larvae displayed lower ether extract content than the other BSG-BSY-based diets ($P > 0.001$). The use of BSG-BSY-based diets did not influence the alpha diversity of larval microbiota and mycobiota ($P > 0.05$), but a specific microbial signature was identified per each dietary treatment (*Porphyromonadaceae* [BSY5], *Sphingomonas* [BSY7.5], *Bacillus* [BSY10] and *Ruminococcus* and *Myroides* [BSG-BSY-based diets]; $P < 0.05$). Co-occurrence and co-exclusion analysis also showed that *Saccharomyces cerevisiae* and *Pichia* excluded and favoured, respectively, the presence of *Streptomyces* and *Fluviicola*, while *Clavispora lusitaniae* was associated with *Myroides* ($P < 0.05$). In conclusion, BSG-BSY-based diets are suitable for rearing HI in terms of larval performance, nutritional profile, and microbiota and mycobiota, with 7.5 and 10% of BSY inclusion levels being able to improve larval growth and bioconversion efficiency.

Implications

The black soldier fly is capable of efficiently converting organic matter into protein-rich biomass. Using food by-products as substrate for the insect larvae reduces the environmental footprint of waste and also contributes to the development of a circular economy, where organic materials are repurposed, emphasising the potential for eco-friendly and economically viable solutions. This study focuses on insect bioconversion of food by-products. Brewer's spent grains and brewer's spent yeast have proven to be valuable substrates for black soldier fly larvae. Higher inclusions tested have resulted in better larval growth. Inclusion at 10% has improved bioconversion efficiency compared to the control.

Introduction

Most agro-industrial processes create waste and by-products as side-streams of the main manufactured commodities. Beer, one of the most produced beverages around the globe, is not an exception. The main by-products from the process of brewing are brewer's spent grains (**BSG**) and brewer's spent yeast (**BSY**). The BSG accounts for roughly 85% of the total by-products generated

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from the beer-making process (Mussatto, 2014), and BSY is an underutilised by-product, rich in nutrients, particularly proteins, vitamins and minerals (Jaeger et al., 2020). Despite their high nutritional value, the large quantities available, and the low cost, these by-products are often discarded and not used to their full potential. Regarding BSG, the reason is that their composition can change in a very short time. Processes of endogenous metabolism occur if not kept at low temperatures, thus making hard to stock them in a cost-efficient manner (Aliyu and Bala, 2011; Feyissa et al., 2015; Acin-Albiac et al., 2022). Therefore, conservation of BSG can be a problem, especially in developing countries (Aranguiz et al., 2019). Several methods to stabilise BSG have been tested, such as filtration, freezing, or different types of drying (Jackowski et al., 2020), but each method may need machinery that is not always available or may be too expensive. The BSY, although amounting to a much smaller fraction of the total process by-products, has an interesting nutrient composition. Using BSY in animal feed has proven to be beneficial for other species such as poultry, ruminants, fish, and crustaceans (Line et al., 1998; Parmar et al., 2012; Harlow et al., 2016; San Martin et al., 2020; Ciurescu et al., 2021), not only because of its nutritive qualities but also for the ability of improving digestion by acting on the gut microbiota of the animals (Jaeger et al., 2020). Although, the positive features of BSY in animal nutrition, using it can pose a problem since is prone to fast degradation (Podpora et al., 2016). For this reason, it is often discarded instead of being used as a valuable feed ingredient (Boateng et al., 2015; Puligundla et al., 2020). Moreover, the reason behind its limited use in the food industry is the high quantity of purines, which may cause problems regarding human health (Jaeger et al., 2020). Feeding brewery residues to farmed animals is a valuable solution to reduce waste and limit land competition between food and feed crops (Dou et al., 2018). Since BSG are rich in readily available sugars and amino acids, as well as cellulose and hemicellulose, they may represent a good feed ingredient for ruminants and monogastrics (Aranguiz et al., 2019; Negash, 2021). However, another interesting alternative could be using brewery residues to feed larvae of black soldier fly (**BSF**, *Hermetia illucens*), without drying or further transforming them. The BSF larvae are voracious consumers of a wide range of organic material, and their capacity of digesting BSG has already been tested (Meneguz et al., 2018a; Bava et al., 2019; Permana et al., 2021; Broeckx et al., 2021). The high humidity of BSG (20–27% average DM content, Aranguiz et al., 2019), which makes their use for other purposes problematic, would be beneficial for BSF larvae, since it is close to the ideal humidity content of the feed for this species. Besides the benefit of being able to consume these by-products as they are, the BSF larvae can be used as feed ingredient rich in protein for fish, poultry and pigs (Biasato et al., 2019; El-Hack et al., 2020; Agbohessou et al., 2021; Bellezza Oddon et al., 2021; Biasato et al., 2022a), thus decreasing the pressure on feed crops by freeing more land for food use by utilising waste and by-products that would have been composted or sent to landfills. Considering the large volumes of BSG and BSY produced globally, and the need to dispose them, this study aims at testing the feasibility of rearing BSF larvae on a substrate entirely composed of brewery by-products, such as BSG and BSY. While several studies have been conducted on the effects that a BSG diet may have on BSF larvae growth in comparison with other sub-streams (Liu et al., 2018; Meneguz et al., 2018a; Bava et al., 2019; Broeckx et al., 2021), less has been done on the synergistic effects that a diet composed of both the BSG and BSY may have (Chia et al., 2018). Furthermore, it has ben proven that the rearing substrate can influence the microbiota (Bruno et al., 2019; Wynants et al., 2019; Tanga et al., 2021; Greenwood et al., 2021) and the mycobiota (Varotto Boccazzi et al., 2017, Tanga et al., 2021) of the larvae. Previous research has also showed that the use of BSG has the potential to modulate both the microbiota (Galassi et al., 2021; Greenwood et al., 2021) and the mycobiota (Tanga et al., 2021) of the BSF larvae gut, in terms of decreased alpha diversity (Galassi et al., 2021; Greenwood et al., 2021; Tanga et al., 2021), as well as increased abundance of genera belonging to Proteobacteria (Galassi et al., 2021; Greenwood et al., 2021) and Bacteroidetes (Galassi et al., 2021) phyla, and *Pichia* (Tanga et al., 2021), when compared to other by-products or a reference diet. Preliminary results explaining the effects of diets containing brewery by-products on larval growth, capacity of bioconversion and substrate reduction, have already been published in abstract form (Biasato et al., 2022b, Biasato et al., 2022c). However, data are still very limited and inconsistent, and no information are currently available about the influence of diets containing different rations of BSG and BSY on larval gut microbiota and mycobiota. Therefore, the present study aimed to test increasing inclusion levels of BSY in diets fully based on BSG by assessing their effects on larval growth, survival, substrate reduction, bioconversion efficiency, microbiota and mycobiota.

Material and methods

Study design

The BSF larvae used for the trial came from the colony maintained at the experimental facility of the Department of Agricultural, Forest and Food Sciences of the University of Turin, where the larvae are fed Gainesville diet (**GA**; Hogsette, 1992) and reared in a climatic chamber set at 29 °C, 65% RH, 0:24 L:D conditions. The adult flies are kept in another climatic chamber (MONTI & C. – Tecnologie del Freddo S.r.L.; Potenza, Italy) with artificial lighting, set at 29 °C, 70% Relative humidity (**RH**), 12:12 L:D conditions.

Four BSG-BSY-based diets with increasing levels of BSY were tested against the GA, used as a control diet, which also served as environmental control.

The ingredients (BSG and BSY) used for the BSG-BSY-based diets were analysed for the DM content (AOAC #934.01), the CP (AOAC #984.13; conversion factor N × 6.25) and the ash (AOAC #942.05), according to the International AOAC (2000). The ether extract (**EE**; AOAC #2003.05) was, instead, analysed following the International AOAC (2003). The chemical composition of the ingredients is shown in Table 1. The four BSG-BSY-based diets contained increasing levels of BSY at the expense of BSG:

- BSY2.5, experimental diet composed by 25 g/kg of BSY and 975 g/kg of BSG
- BSY5, experimental diet composed by 50 g/kg of BSY and 950 g/kg of BSG
- BSY7.5, experimental diet composed by 75 g/kg of BSY and 925 g/kg of BSG
- BSY10, experimental diet composed by 100 g/kg of BSY and 900 g/kg of BSG

The BSG-BSY-based diets and the GA were analysed for the DM, CP, ash and EE with the same method as the ingredients for the diets. Furthermore, the amylase neutral detergent fibre was also characterised (Mertens, 2002).

The non-structural carbohydrates (**NSC**) were calculated as follows:

$$NSC = 100 - (CP + EE + Ash + NDF)$$

The gross energy (**GE**) was analysed using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany).

The chemical composition of the BSG-BSY-based diets and of the GA is shown in Table 2.

Table 1
Chemical composition (g/100 g on DM basis) of the ingredients used in the BSG-BSY-based diets for rearing black soldier flies.

Ingredients	BSY	BSG
DM	17.82	21.89
CP	40.33	19.23
EE	0.43	7.04
Ash	6.35	4.37

Abbreviations: BSY=brewer's spent yeast; BSG=brewer's spent grains; EE=ether extract.
Values are reported as mean of duplicate analyses.

Growth trial

At the start of the experiment, the eggs that were used for the experiment were left to hatch on an empty container for 24 h, and then weighed. One kilogram of GA was given for each gram of newly hatched larvae, and the container was stored in a climatic chamber set at 29 °C, 60% RH and 0:24 L:D conditions. The larvae were left feeding for 5 days; then, the 6 days-old-larvae were sieved manually with a 0.5 mm and a 0.2 mm diameter sieve, and the fraction between these two diameters was used. A total of 100 g of 6 days-old-larvae were isolated to characterise their initial chemical composition. Larvae were washed and gently dried; then, the sample was frozen at −80 °C to kill them and then stored at −20 °C. Afterwards, a total of 3 000 6 days-old-larvae were taken individually with forceps and pooled in groups of five larvae. The groups were then weighed (Kern & Sohn GmbH; Balingen, Germany; d = 0.001) and pooled to form six replicates of 100 larvae per each dietary treatment (SD among groups was < 0.003); statistical analysis was performed to exclude the differences in weight. Per dietary treatment, six replicates were prepared (referred to as replicates for growth trial), each of them consisting of a plastic box (19 cm long, 13 wide, 6 cm tall) filled with 100 6 days-old-larvae and the corresponding dietary treatment. The plastic boxes had a plastic lid with silicon sealing to prevent larvae from escaping, of which the centre was cut out and replaced by a fine nylon mesh glued to the borders of the lid to allow air circulation, but still prevent larvae from escaping. All measurements described in the "Growth trial" paragraph were taken on these replicates.

Fig. 1 shows the grams of each macronutrient (on DM basis) at disposal for each individual larva. The quantity of macronutrient per larva is normalised with the DM of the substrate with the following formula:

$$g\ of\ macronutrient\ per\ larva = 0.9 * \left(\frac{DM}{100}\right) * macronutrient\ in\ \%/100)$$

where 0.9 are the grams of fresh substrate at disposal for each larva. Additionally, Fig. 1 shows the ratio of crude fat/CP in the dietary treatments.

All the 30 replicate boxes were weighed (Kern & Sohn GmbH; Balingen, Germany; d = 0.1) and filled with 90 g of the corresponding BSG-BSY-based diet or GA and 100 6 days-old-larvae. To avoid a temperature shock during the larvae inoculation, the young larvae were placed in the centre of the substrate and the boxes were left in the climatic chamber for 1 h. The replicates were placed in a shelf in a climatic chamber with the previously stated conditions and were randomly moved every day to avoid temperature or humidity biases.

At the beginning of the trial (T0) and every 4 days (T1 and T2), the weight (Kern & Sohn GmbH; Balingen, Germany; d = 0.1) of each replicate and its pH, (taken in two standardised points, HI99163, Hanna Instruments; Romania) were recorded, as well the weight of at least 30 larvae (Kern & Sohn GmbH; Balingen, Germany; d = 0.001). In detail, larvae were sampled by taking both the larvae and the substrate in three standardised points (two corners and centre) and putting them inside an empty container which had previously been weighed (Kern & Sohn GmbH; Balingen, Germany; d = 0.1). Subsequently, larvae were isolated from the substrate, counted and gently dried before weighing them (if the number of sampled larvae was less than 30, another, larger sample, was taken, until the amount of 30 larvae has been reached). The larvae and the substrate were then placed back in the replicate box, and the container was weighed again, in order to assess the potential substrate loss.

Daily, all replicates exposed to BSG-BSY diets were monitored. When a replicate reached 5% prepupae (Bosch et al., 2020), it was marked as "END." The weight of the residue substrate and the larval biomass, as well as the final pH of the substrate and the individual weight of at least 30 larvae, were registered following the same procedures previously described. All the larvae and the prepupae were also counted to calculate the survival rate (%). The following indices were calculated to assess the waste conversion efficiency of the larvae (Bosch et al., 2020):

$$\text{Bioconversion efficiency corrected for residue (}\mathbf{BER}\text{)} = [(larval\ biomass\ end - larval\ biomass\ start)/(D - R)] * 100$$

$$\text{Reduction rate (}\mathbf{RR}\text{)} = [(D - R)/D] * 100\%$$

$$\text{Waste reduction index (}\mathbf{WRI}\text{)} = RR/days$$

where:

Larval biomass end = Larval biomass at the end of the experiment (g on DM).

Larval biomass start = Larval biomass at the start of the experiment (g on DM).

Table 2
Chemical composition (g/100 g on DM basis) and gross energy (MJ/kg on DM basis) of the BSG-BSY-based and control diets for rearing black soldier flies.

Diet	BSY2.5	BSY5	BSY7.5	BSY10	GA
DM	20.86	20.45	20.51	20.6	26.3
CP	20.71	21.57	23.29	24.2	15.51
EE	6.45	6.38	6.97	6.01	2.1
Ash	4.19	4.34	4.58	4.41	6.03
NDF	49.04	48.16	49.99	48.3	37.44
NSC[1]	19.62	19.56	15.17	17.1	38.92
GE	18.97	18.86	19.08	19.1	17.02

Abbreviations: BSY2.5 = diet composed by 25 g/kg of BSY and 975 g/kg of BSG; BSY5 = diet composed by 50 g/kg of BSY and 950 g/kg of BSG; BSY7.5 = diet composed by 75 g/kg of BSY and 925 g/kg of BSG; BSY10 = diet composed by 100 g/kg of BSY and 900 g/kg of BSG; GA=Gainesville diet; EE=ether extract; GE=gross energy; NSC=non-structural carbohydrates; BSY=brewer's spent yeast; BSG=brewer's spent grains.
Values are reported as mean of duplicate analyses.
[1] Calculated as 100 – (CP+EE+Ash + NDF).

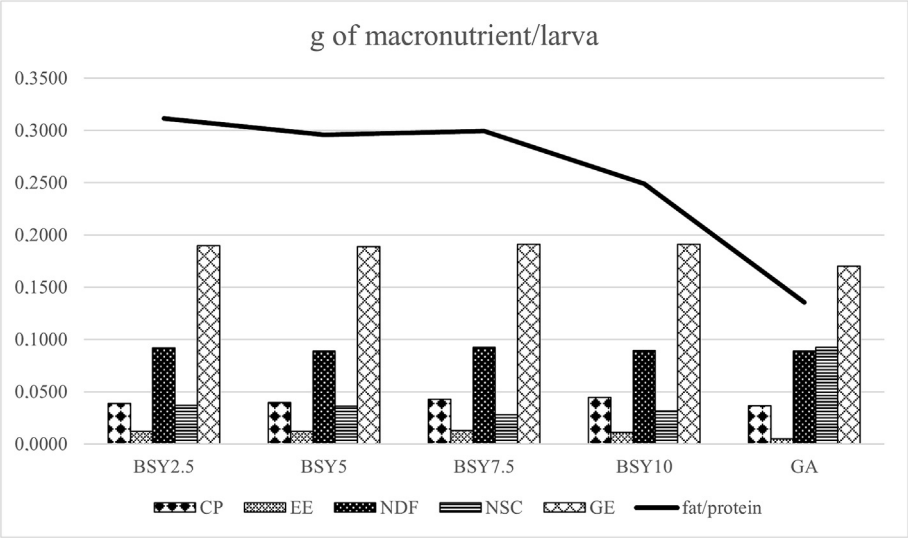

Fig. 1. Analysis of the g of each macronutrient available per black soldier fly larva on a DM basis. Abbreviations: See Table 2.

D=diet provided (g on DM).
R=residue obtained (g on DM).
Days = number of days from the start to end of the experiment.
To assess the suitability of each experimental diet, survival (%) and development time (days) were also calculated.

Chemical analyses

In parallel to the growth trial, three replicates per each dietary treatment, on which no measurements were performed, were also prepared to obtain the needed quantity of larvae for the chemical analysis and the microbiota and mycobiota characterisation. Each replicate consisted of a plastic box (23 cm long, 30 cm wide and 9 cm tall) with a lid with silicon sealing and a nylon mesh on the lid to allow air circulation, filled with 2 925 g of substrate and 3 250 6 days-old-larvae, to maintain the 0.9 g/larva feeding rate. The larvae used for the replicates were estimated as follows: after a careful homogenisation of the substrate containing the larvae, at least six samples (amounting at least to 5% of the weight of the larvae and remaining substrate) were randomly sampled and the total weight recorded (Kern & Sohn GmbH; Balingen, Germany; d = 0.001). Thereafter, larvae were separated from the substrate and counted. Subsequent samples were taken until the coefficient of variance among them was <10% (Deruytter et al., 2023). This method allowed to estimate the quantity of substrate + larvae to sample to have 3 250 larvae per replicate. The replicate boxes were left on a shelf in the same climatic chamber as the growth trial, and their position on the shelf was randomised every day to avoid temperature and humidity biases. Larvae were sampled for analysis when at least half of the replicates from the growth trial terminated the larval phase. At that moment, a total of 100 g of larvae was taken from each of the three replicates. Larvae were then washed, gently dried and frozen at −80 °C for an hour to kill them and stored at −20 °C until lyophilisation and chemical analyses were performed. Larvae were analysed for their DM (AOAC #934.01), CP (AOAC #984.13; conversion factor N × 4.67) and ash (AOAC #942.05) according to the International AOAC, 2000, while the EE (AOAC #2003.05) was analysed following the International AOAC, 2003. The GE was analysed using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany).

Microbiota and mycobiota characterisation

A total of 3 g of BSF larvae were also sampled per each replicate, washed in a solution of water and ethanol, stored in sterile plastic tubs, cooled at 4 °C (for a period not longer than 2 h), and frozen at −80 °C for the DNA extraction. Total DNAs from BSF larvae samples (200 mg) were extracted using the RNeasy Power Microbiome KIT (Qiagen, Milan, Italy) following the instructions from the manufacturer. The RNase (5 ng/mL) was added to digest RNA in the DNA samples, after an incubation of 1 h at 37 °C. The DNA was quantified using the QUBIT dsDNA Assay kit (Life Technologies, Milan, Italy) and standardised at 5 ng/µl. One µl of each DNA suspension was used as template for PCR amplification by using primers 16SR and 16SF spanning the V3-V4 region of the 16S rRNA gene following the procedure described by Klindworth et al. (2013). Fungi were detected by amplifying the D1 domain of the 26S rRNA gene (Mota-Gutierrez et al., 2019). Library preparation and sequencing by MiSeq instrument (Illumina, San Diego, CA) were carried out according to the manufacturing instructions.

Bioinformatics and statistical analysis of data

The dataset collected from the growth trial was analysed using IBM SPSS Statistics software (V20.0.0). The normality or non-normality distribution of data and the residuals was determined by Shapiro-Wilk test. Regarding RR, BER, WRI, and pH, the experimental unit considered was the replicate box, while for larval growth the experimental unit was the individual larva. The bioconversion indices and the chemical composition of the larvae were analysed by means of One-way ANOVA (posthoc test: Tukey), while pH and larval weights were analysed by fitting a generalised linear mixed model in order to assess the effects of two fixed effects (diet and time) and their interaction. The replicate was included as a random effect to account for repeated measurements on the same box. The results were expressed as least square mean (larval weights and pH) or mean (bioconversion indices and chemical composition) and pooled SE of the mean. The level of significance considered was ≤ 0.05. Amplicon sequencing data were analysed by using Quantitative Insights into Microbial Ecology 2 (Bolyen et al., 2019). Low quality and short reads were filtered

by DADA2 algorithm of Quantitative Insights into Microbial Ecology 2 (Callahan et al., 2016). Quantitative Insights into Microbial Ecology feature-classifier was used against the Greengenes 16S rRNA gene database or against the manual database (Mota-Gutierrez et al., 2019) for the microbiota and mycobiota, respectively. Alpha and beta diversity calculations were performed by the Quantitative Insights into Microbial Ecology 2 diversity script. Amplicon Sequence Variants were compared by the pairwise Kruskal-Wallis test. Venn diagrams were constructed by Venn Diagram Maker (https://goodcalculators.com/venn-diagram-maker/). Pairwise Spearman's non-parametric correlations were performed by using the *psyc* package of R-Studio in order to find co-occurrence and co-exclusion between bacteria and fungi. The level of significance considered was ≤ 0.05.

Results

Growth trial

Table 3 describes the effects of the diet and the time on the growth of the larvae. The larvae from GA were heavier than the BSG-BSY-based diets ($P < 0.05$). The BSY7.5 and BSY10 groups displayed the greatest results when compared to the other BSG-BSY-based diets ($P < 0.05$), while BSY2.5 showed the worst ($P < 0.001$). The BSY5 weight was intermediate, but different from all the dietary treatments ($P < 0.001$). Larvae were heavier at END than at T2 and T1 ($P < 0.05$). Fig. 2 shows the interaction between diet and time. At T0, the weight of the larvae was the same among all the dietary treatments. At T1, GA (0.089 g) showed a higher value ($P < 0.05$) than BSY7.5 (0.082 g), BSY5 (0.080 g) and BSY2.5 (0.067 g). The BSY10 larval weight (0.086 g) was comparable to GA and BSY7.5 ($P > 0.05$), but it was higher than BSY5 and BSY2.5 ($P < 0.05$). The BSY2.5 experimental diet produced the smallest larvae among all the groups at T1 ($P < 0.001$). The value of BSY5 and BSY7.5 were, instead, similar ($P > 0.05$). At T2, GA (0.132 g) maintained the heaviest larvae among all the groups ($P < 0.001$). Similarly to T1, BSY10 (0.119 g) showed a higher weight ($P < 0.05$) than BSY5 (0.105 g) and BSY2.5 (0.090 g). The weight of BSY7.5 (0.112 g) was analogous to BSY5 and BSY10 ($P > 0.05$). The BSY2.5 larvae showed the lowest weight ($P < 0.001$). At END, GA (0.135 g) and BSY10 (0.125 g) were comparable ($P > 0.05$) and had higher weights than the other BSG-BSY-based diets ($P < 0.05$). The larval weight of BSY7.5 (0.117 g) was higher when compared to BSY5 (0.107 g) and BSY2.5 (0.094 g, $P < 0.01$) and BSY5 weight was higher than BSY2.5 ($P < 0.001$). Table 4 depicts the effects that the diets had on the larval bioconversion efficiency. Regarding the BER, BSY10 and BSY7.5 displayed higher values than the other BSG-BSY-based diets ($P < 0.01$), with BSY5 and BSY2.5 also showing higher BER than GA ($P < 0.001$). The RR of the substrate and WRI were higher in GA when compared to the BSG-BSY-based diets ($P < 0.001$ and $P < 0.05$. respectively). The BSG-BSY-based diets did not differ between each other in terms of RR and WRI ($P > 0.05$). The development time showed no differences among the BSG-BSY-based diets nor the control ($P > 0.05$). Survival was not altered by the dietary treatment ($P > 0.05$).

pH

Table 5 shows the effects of diet and time on the pH of the substrate during the whole trial. The pH of GA differed from the BSG-BSY-based diets ($P < 0.001$), while it did not differ among the BSG-BSY-based diets ($P > 0.05$). Considering the time of experiment, the pH of T0 was the lowest ($P < 0.001$). The pH of T1 was higher than the other sampling times ($P < 0.001$), and the pH of END and T2 had an intermediate value between T1 and T0 and were statistically different from both ($P < 0.001$).

Fig. 3 shows the interaction between diet and time on the pH of the substrate. At T0, GA (5.93) presented a higher pH when compared to the BSG-BSY-based diets ($P < 0.001$). The BSY2.5 (4.43) had a value different from all other BSG-BSY-based diets ($P < 0.05$), while BSY5 and BSY10 had the same value (4.30) but differed statistically from all the others ($P < 0.001$). The BSY7.5 diet had also a pH value different from all the other groups at the start (4.39, $P < 0.05$). At T1, the pH of GA (7.53) was different from the BSG-BSY-based diets ($P < 0.001$), with them being statistically equal ($P > 0.05$). At T2, BSY5 differed from BSY2.5 ($P < 0.05$), with the other diets showing no significant differences among them ($P > 0.05$). At END, BSY2.5 showed a lower pH value than GA (8.17 and 8.49, respectively, $P < 0.01$), while the other BSG-BSY-based diets were equal ($P > 0.05$).

Chemical analyses

Table 6 reports the chemical composition of the larvae fed the BSG-BSY-based diets and the control. The larval DM content differed only between BSY7.5 and GA ($P < 0.05$). Regarding CP, no significant difference was observed among the BSG-BSY-based diets ($P > 0.05$), but BSY5 and BSY7.5 had a lower CP content than GA (36.98 g/100 g, 37.51 g/100 g and 40.05 g/100 g on DM, respectively, $P < 0.05$). For the EE, BSY10 (17.80 g/100 g) showed a lower value when compared to the other BSG-BSY-based diets ($P < 0.01$), which did not differ among each other ($P > 0.05$). Ash percentage was the highest in GA (10.43 g/100 g, $P < 0.001$), followed by BSY10, which had the highest numerical value (7.39%) among the BSG-BSY-based diets, but it was not statistically different ($P > 0.05$) from BSY7.5 (7.04 g/100 g) and BSY2.5 (6.86 g/100 g). The GE of the larvae fed the BSG-BSY-based diets was equal ($P > 0.05$), but higher than GA ($P < 0.01$).

Microbiota and mycobiota characterisation

After sequencing and quality filtering, 145 534 and 146 946 reads for 16S and 26S, respectively, were used for the downstream analysis (with a median value of 9 702 ± 4 661 and 9 766 ± 5 152 reads/sample for 16S and 26S, respectively). The rarefaction analysis and the estimated sample coverage indicated that there was a satisfactory coverage of all the samples (median value of 100% for both the target genes). The use of BSG-BSY-based diets did not influence the larval bacterial alpha diversity ($P > 0.05$, Supplementary Table S1). Similarly, by plotting the principal component analysis, no clear separation between the larvae fed the BSG-BSY-based diets and the GA was observed ($P > 0.05$, Fig. 4A). Independently of the administered diets, *Dysgonomonas*, *Sphingomonas*, unclassified members of *Sphingobacteriaceae* and *Sphingomonadaceae*, and *Morganella* resulted to be the most abundant genera in the BSF larvae microbiota (Fig. 4B). Comparing the presence of the main amplicon sequence variants among the dietary treatments by Venn diagram analysis (Fig. 4C), a specific microbiota signature was observed for the BSG-BSY-based diets. In particular, the larvae fed the BSY5 and the BSY10 diets were characterised by the presence of U.m. of *Porphyromonadaceae* and *Bacillus*, respectively, while *Ruminoccocus* and *Myroides* were shared among the BSG-BSY-based diets only. The BSY7.5 diet also revealed an increased abundance of *Sphingomonas* when compared to the other diets (false discovery rate < 0.05). The use of BSG-BSY-based diets did not influence the larval fungal alpha diversity ($P > 0.05$, Supplementary Table S1). Analogously, by plotting the principal component analysis, no clear separation between the BSF larvae fed the BSG-BSY-based diets and the GA was observed ($P > 0.05$, Fig. 5A). Independently of the administered diets, *Pichia*, *Saccharomyces*,

Table 3
Effects of the diet on the black soldier fly larval growth, weight measured as is (g).

Diet (D)					Time (T)			SEM		*P*-value		
BSY2.5	BSY5	BSY7.5	BSY10	GA	T1	T2	END	D	T	D	T	DxT
0.083^{d}	0.097^{c}	0.103^{b}	0.109^{b}	0.116^{a}	0.080^{c}	0.111^{b}	0.115^{a}	0.042	0.033	<0.001	<0.001	<0.001

Abbreviations: BSY2.5 = diet composed by 25 g/kg of BSY and 975 g/kg of BSG; BSY5 = diet composed by 50 g/kg of BSY and 950 g/kg of BSG; BSY7.5 = diet composed by 75 g/kg of BSY and 925 g/kg of BSG; BSY10 = diet composed by 100 g/kg of BSY and 900 g/kg of BSG; GA=Gainesville diet; T1 = 10 days after larvae hatching; T2 = 14 days after larvae hatching; END=end of the trial; BSY=brewer's spent yeast; BSG=brewer's spent grains.
Means with different superscript letters within the same row differ significantly ($P < 0.05$).

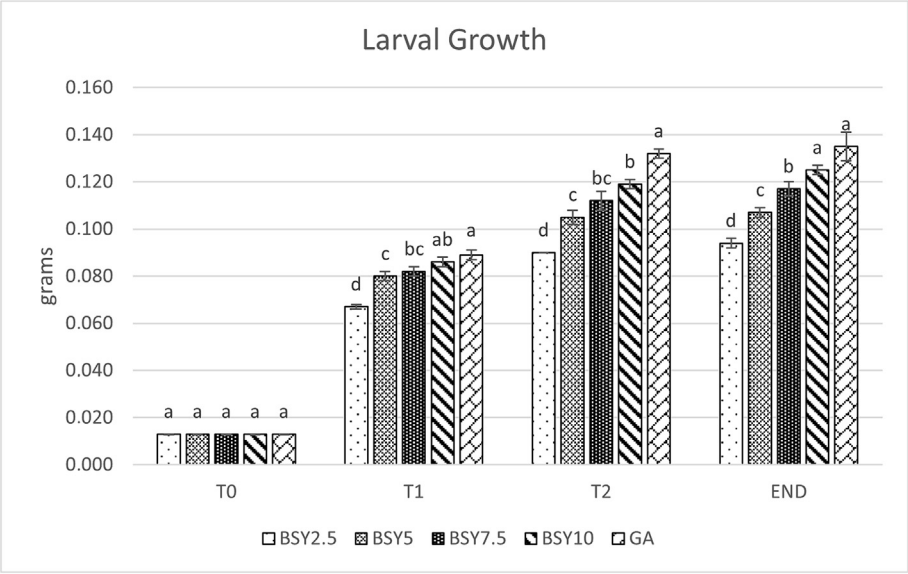

Fig. 2. Effects of the interaction between diet and time on black soldier fly larval growth. Abbreviations: T0 = 6 days after larvae hatching; T1 = 10 days after larvae hatching; T2 = 14 days after larvae hatching; END=end of the trial; see Table 2 for other abbreviations. Means with different letters (a, b, c) within the same time interval differ significantly ($P < 0.05$).

Table 4
Effects of the diet on the BER, RR, WRI, development time and survival of the black soldier fly larvae.

Parameters	BSY2.5	BSY5	BSY7.5	BSY10	GA	SEM	*P*-value
BER (%)	21.37^{b}	21.98^{b}	25.75^{a}	26.92^{a}	15.82^{c}	0.588	<0.001
RR (%)	44.88^{b}	48.83^{b}	45.92^{b}	48.37^{b}	66.85^{a}	0.982	<0.001
WRI	4.89^{b}	5.25^{b}	5.10^{b}	5.27^{b}	7.21^{a}	0.192	0.002
Development time (days)	15.17	15.33	15.00	15.17	15.33	0.182	0.223
Survival (%)	91.83	88.17	91.00	92.83	90.17	0.835	0.486

Abbreviations: BER=Bioconversion efficiency corrected for residue (%); RR=Reduction rate of the substrate (%); WRI=Waste reduction index; BSY2.5 = diet composed by 25 g/kg of BSY and 975 g/kg of BSG; BSY5 = diet composed by 50 g/kg of BSY and 950 g/kg of BSG; BSY7.5 = diet composed by 75 g/kg of BSY and 925 g/kg of BSG; BSY10 = diet composed by 100 g/kg of BSY and 900 g/kg of BSG; GA=Gainesville diet; BSY=brewer's spent yeast; BSG=brewer's spent grains.
Means with different superscript letters within the same row differ significantly ($P < 0.05$).

Table 5
Effects of the diet and time on the pH of the substrate used for rearing black soldier flies.

	Diet (D)					Time (T)				SEM		*P*-value		
	BSY2.5	BY5	BSY7.5	BSY10	GA	T0	T1	T2	END	D	T	D	T	DxT
pH	7.27^{b}	7.22^{b}	7.27^{b}	7.25^{b}	7.54^{a}	4.63^{c}	8.77^{a}	8.43^{b}	8.33^{b}	0.201	0.046	<0.001	<0.001	<0.001

Abbreviations: BSY2.5 = diet composed by 25 g/kg of BSY and 975 g/kg of BSG; BSY5 = diet composed by 50 g/kg of BSY and 950 g/kg of BSG; BSY7.5 = diet composed by 75 g/kg of BSY and 925 g/kg of BSG; BSY10 = diet composed by 100 g/kg of BSY and 900 g/kg of BSG; GA=Gainesville diet; T0 = 6 days after larvae hatching; T1 = 10 days after larvae hatching; T2 = 14 days after larvae hatching; END=end of the trial; BSY=brewer's spent yeast; BSG=brewer's spent grains.
Means with different superscript letters within the same row differ significantly ($P < 0.05$).

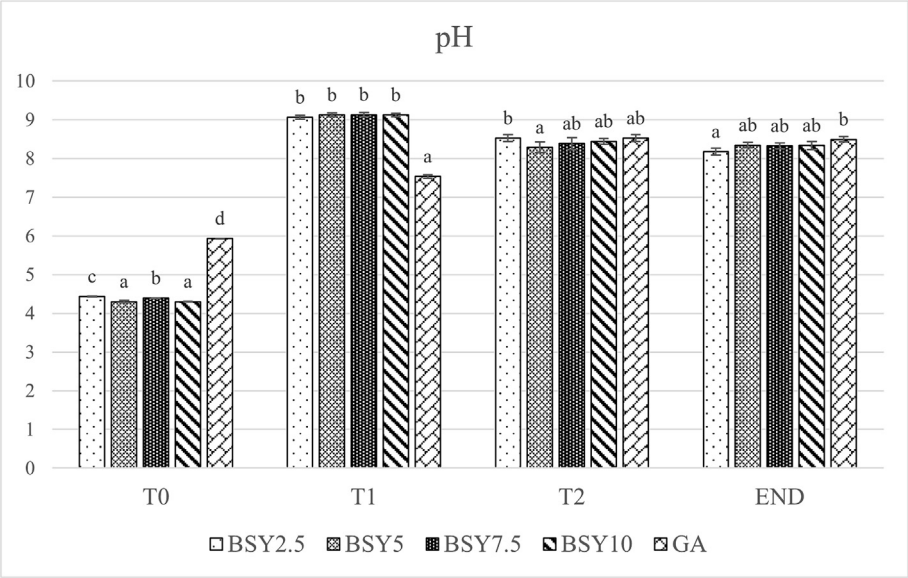

Fig. 3. Effects of the interaction between diet and time on the pH of substrates used for rearing black soldier flies. Abbreviations: T0 = 6 days after larvae hatching; T1 = 10 days after larvae hatching; T2 = 14 days after larvae hatching; END=end of the trial; see Table 2 for other abbreviations. Means with different letters (a, b, c) within the same time interval differ significantly ($P < 0.05$).

Table 6
Chemical composition (g/100 g on DM basis) and gross energy (MJ/kg on DM basis) of the black soldier fly larvae.

	Diet						
Item	BSY2.5	BSY5	BSY7.5	BSY10	GA	SEM	*P*-value
DM	24.47^{ab}	24.33^{ab}	24.68^{a}	23.91^{ab}	23.56^{b}	0.135	0.027
CP	38.69^{ab}	36.98^{b}	37.51^{b}	38.58^{ab}	40.05^{a}	0.327	0.004
EE	23.99^{a}	22.96^{a}	22.05^{a}	17.80^{b}	12.87^{c}	1.119	<0.001
Ash	6.86^{bc}	6.49^{c}	7.04^{bc}	7.39^{b}	10.43^{a}	0.384	<0.001
GE	24.36^{a}	24.16^{a}	24.24^{a}	24.07^{a}	22.91^{b}	0.151	<0.001

Abbreviations: BSY2.5 = diet composed by 25 g/kg of BSY and 975 g/kg of BSG; BSY5 = diet composed by 50 g/kg of BSY and 950 g/kg of BSG; BSY7.5 = diet composed by 75 g/kg of BSY and 925 g/kg of BSG; BSY10 = diet composed by 100 g/kg of BSY and 900 g/kg of BSG; GA=Gainesville diet; EE=ether extract; GE=gross energy; BSY=brewer's spent yeast; BSG=brewer's spent grains.
Means with different superscript letters within the same time interval differ significantly ($P < 0.05$).

and *Aureobasidium* resulted to be the most abundant genera in the BSF larvae mycobiota (Fig. 5B). Differently from microbiota, Venn diagram analysis did not reveal any specific mycobiota signature depending on the administered diets (Fig. 5C). *Saccharomyces cerevisiae*, *Aureobasidium proteae* and *Pichia* were the three ASVs shared among the datasets.

Co-occurrence and co-exclusion analysis showed that *S. cerevisiae* was negatively correlated with *Streptomyces*, while *Fluviicola* and *Myroides* were positively correlated with *Pichia* and *Clavispora lusitaniae*, respectively ($P < 0.05$, Fig. 6).

Discussion

The present study aimed to investigate the feasibility of rearing BSF larvae on a diet entirely composed by brewery by-products and, since these organic products are used fresh and have a high prebiotic/probiotic potential (BSY in particular), to test if an increasing inclusion level of BSY in the diet would have any effects on the growth and microbiota and mycobiota of the larvae. The reason behind the low inclusion of BSY in the diets is because BSY represents a much smaller fraction of the total beer production by-products when compared to BSG, and realistically, if the diets tested would be replicated at industrial scale, it would be difficult to have high percentages of BSY in the diet for availability issues. Results from the control diet are in line with what is normally observed at the research facility, thus allowing us to exclude those factors linked to the environment that altered the results. The feeding ratio chosen for the trial was decided based on literature findings. A study conducted in the same rearing centre as our trial (testing different larval substrates, among which a diet entirely composed by BSG), used a ratio of 0.8 g/larva (Meneguz et al., 2018a), while another study performed by Meneguz et al. (2018b) at the Texas A&M University Facility was performed with a ratio of 0.96 g/larva. Moreover, the larvae in our experimental facility generally take, starting from 6 days-old-larvae, 9 days to reach the prepupal stage when fed on GA, and Diener et al. (2009) reported that the adequate amount of feed for the larval stage is around 0.1 g/larva/day. Based on these studies and on our experience in breeding BSF larvae, a feeding ratio of 0.9 g/larva was deemed correct.

Regarding larval growth, the present study agrees with what was found by Broeckx et al. (2021), who tested beer draff (side-stream from beer production) as larval substrate. The highest average weight found in our study was 0.125 g (BSY10) while Broeckx et al. (2021) had a result of 0.131 g. The maximum larval weight was higher in BSY10 when compared to the other BSG-BSY-based diets, and BSY10 differed from the other treatments for a numerically lower fat/protein ratio and a slightly higher protein content (Fig. 1). This outcome agrees with the model developed by

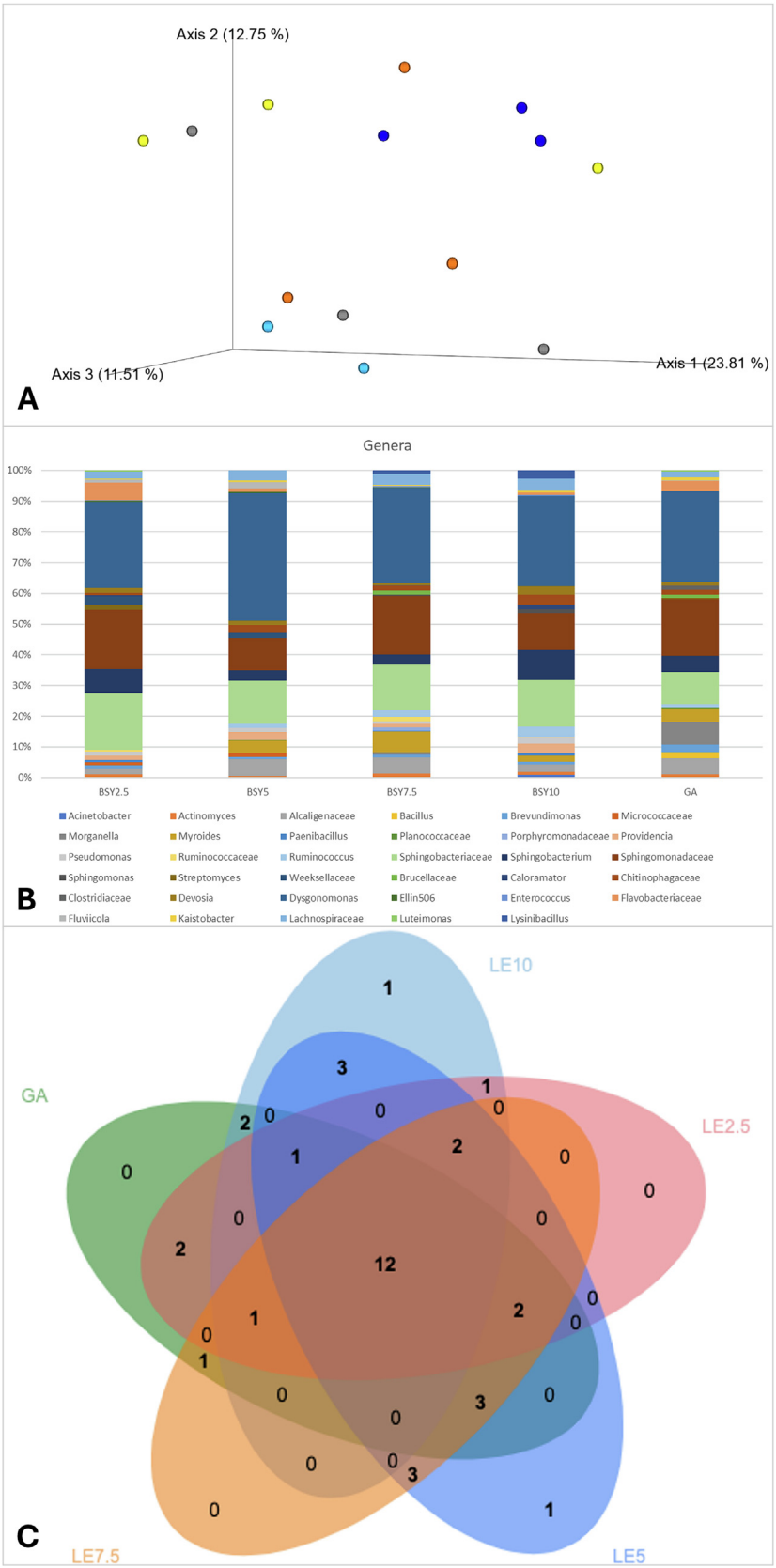

Fig. 4. Effects of diet on black soldier fly (BSF) larvae microbiota. (A) Principal coordinate analysis based on Bray-Curtis distance matrix as a function of the dietary treatment. (B) Metataxonomic composition of BSF larvae microbiota at the lowest taxonomic resolution (genus or family). (C) Venn diagram analysis. Abbreviations: see Table 2.

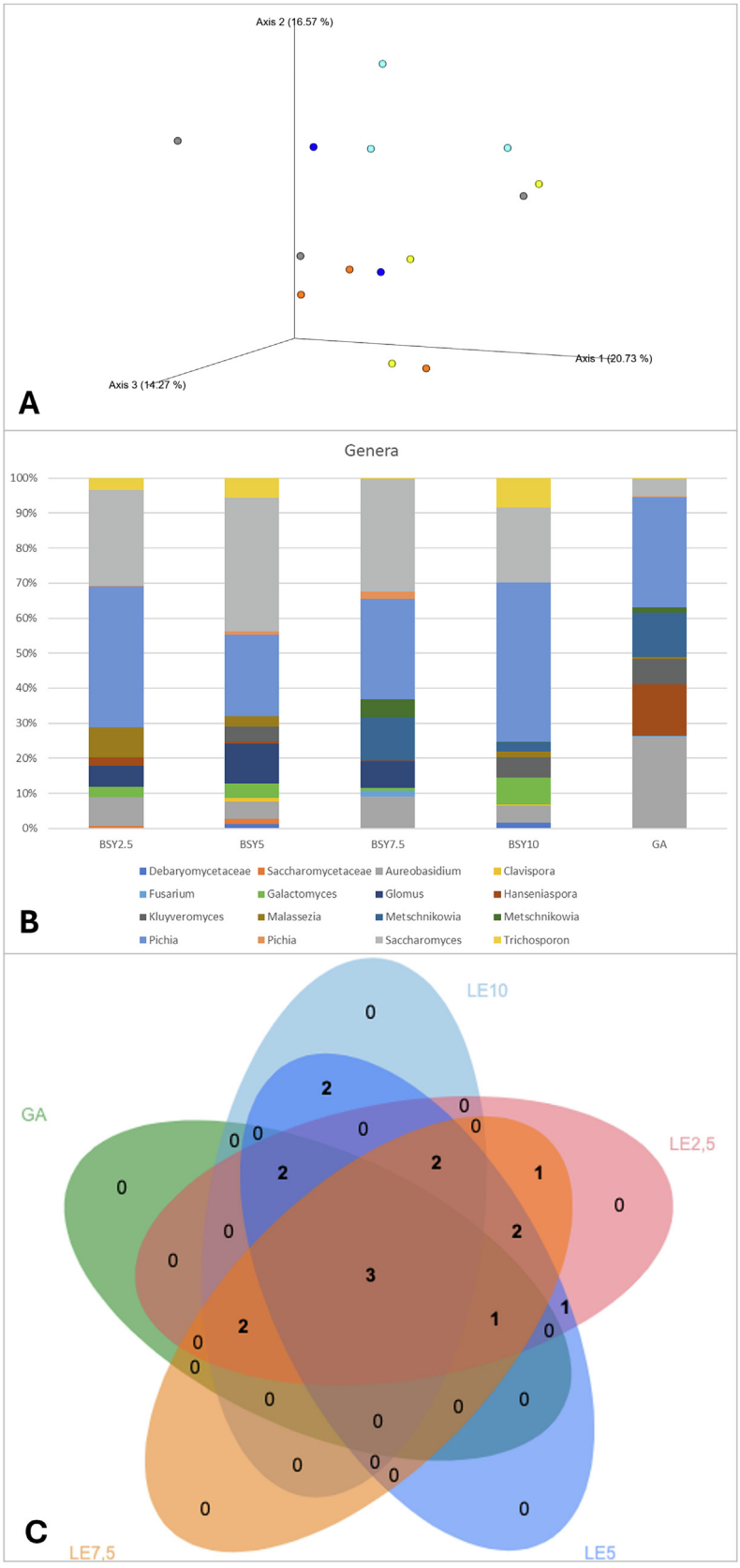

Fig. 5. Effects of diet on black soldier fly (BSF) larvae mycobiota. (A) Principal coordinate analysis based on Bray-Curtis distance matrix as a function of the dietary treatment. (B) Metataxonomic composition of BSF larvae mycobiota at the lowest taxonomic resolution (genus or family). (C) Venn diagram analysis. Abbreviations: see Table 2.

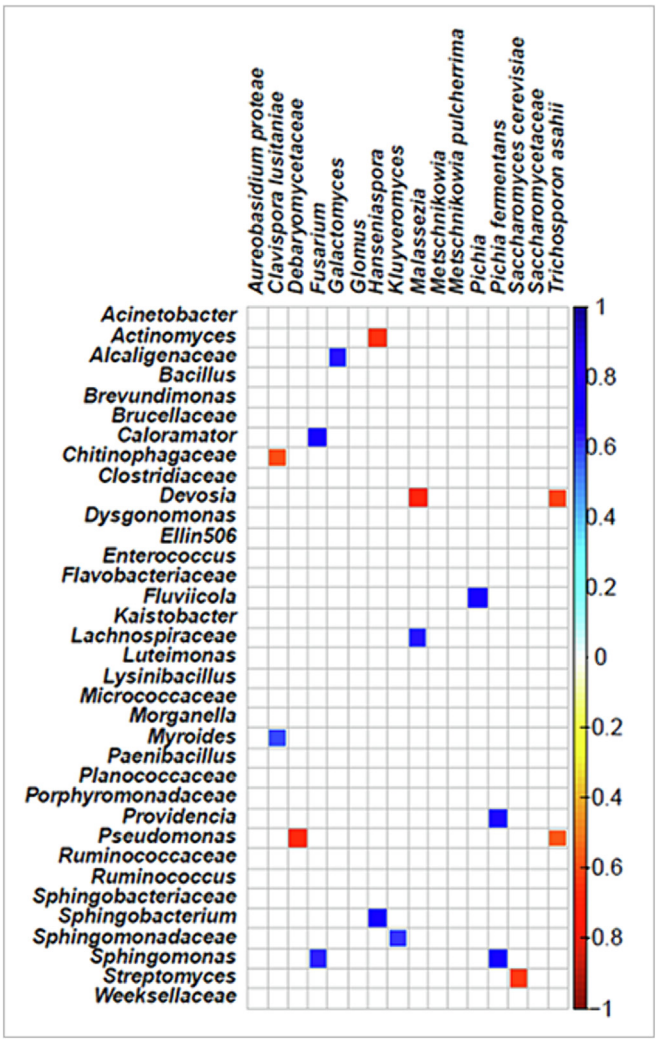

Fig. 6. Spearman's correlation between amplicon sequence variants and black soldier flies. Only significant associations are shown ($P < 0.01$). The intensity of the colors represents the degree of correlation between the bacteria and fungi, as measured by Spearman's correlation, where the blue color represents a positive degree of correlation and the red a negative correlation.

Broeckx et al. (2021), in which the protein and fat content of a substrate is a key element in prediction of its suitability for growth, which is maximal with a low fat/protein ratio (fat content below 0.9% and protein content above 15% on DM basis). Moreover, a recent study by Barragan-Fonseca et al. (2021) shows that protein concentration is important in the larval stage for performance, survival and development time.

The BER results for the BSG-BSY-based diets (21.37–26.92%) are higher than the value found by Broeckx et al. (2021) (13.91 ± 0.4%) and Meneguz et al. (2018a) (14 ± 3.4%). However, it is difficult to hypothesise that the higher result is due to the BSY inclusion of our study. In the study by Broeckx et al. (2021), the authors did not consider the residue (frass) in the formula, and that could be part of the reason for the higher BER of our study. Moreover, the parameter calculated by Meneguz et al. (2018a) is the efficiency of conversion of digested food and was calculated using values referring to the fresh substrate. We found higher BER values in BSY7.5 and BSY10, which can be related to the higher larval weights recorded in these treatments. Larval survival in this trial (88.17% the lowest recorded value, BSY5) was lower than what was found in other studies that tested brewery by-products by themselves (Broeckx et al., 2021, Bava et al., 2019, Liu et al., 2018), but in line with what observed by Chia et al. (2018), which tested different combinations of BSG and BSY. In particular, Bava et al. (2019) observed a survival of 95.87%, Broeckx et al. (2021) a value of 95.2%, and Liu et al. (2018) of 98.0%. Since methodologies used to rear the larvae, as well as experimental protocol and the larvae genetic origin, differ, it is difficult to point out if the differences in survival can be ascribed to the use of BSY or other factors. Nonetheless, given that the survival for the control diet is similar to what was found in the other treatments, it is possible to state that the different inclusions of BSY are not the cause of the slightly lower survival in this experiment. The RR did not differ among the BSG-BSY-based diets, but it was significantly lower when compared to the control diet. This outcome could be explained by the fact that the control diet was composed of smaller particles than the other treatments, and BSF larvae have a mouthpart that is typical of scavenger insects (Kim et al., 2010), benefitting from a smaller granulometry of the substrate. Indeed, at the end of the experiment, it was clearly visible that the frass from the BSG-containing diets was composed of bigger lumps of materials that may have dried before being totally consumed by the larvae. The coarser structure of BSG may have hampered the substrate reduction and could also have prevented larvae from fully exploiting the dietary nutrients. Furthermore, the residue from the BSG-BSY-based diets was also more humid than GA. However, the RR values found for the BSG-BSY-based diets are in line with what has been observed by Broeckx et al. (2021), so it appears that the BSY inclusions in our diets did not affect the larval capacity to consume the diet, since the paper cited, the diet was composed only by BSG. Moreover, Bruno et al. (2020), in a study that investigates the morphology of the head and mouthparts of the BSF larvae mouthparts, hypothesise that BSF larvae are more suited to ingest semi-liquid substrates. The WRI values observed during the experiment are higher than what was found by Bava et al. (2019), who tested the development of BSF larvae on different by-products (among which BSG). However, it has to be noted that our study considered the end of the larval cycle when a replicate reached 5% of prepupae vs the 40% considered by Bava et al. (2019). Therefore, the higher WRI is probably due to a shorter duration of the experiment rather than better digestion of substrate by the larvae.

The development time of the larvae (15 ± 0.93 days until 5% prepupae) was slightly shorter than what was found by Chia et al. (2018) when growing BSF larvae on a mixture of BSG of sorghum and barley with BSY (16.6 ± 0.4 days). On the contrary, it was 1 day longer than what was found by Broeckx et al. (2021), who tested BSF larvae development on different substrates (among which BSG). In the latter case, the authors mentioned the time needed to reach maximum larval weight rather than the end of the larval phase, so it is reasonable that they found a slightly shorter period.

The initial pH of the BSG-BSY-based diets was similar and acidic (in a range between 4.30 and 4.43), and significantly different ($P < 0.001$) from the pH of GA (5.93). However, during the consumption of the substrate by the BSF larvae (T1), the pH of the BSG-BSY-based diets spiked to more basic values (all above 9, $P > 0.05$), while the pH of GA was 7.53 and differed from the pH of the BSG-BSY-based diets ($P < 0.001$). At T2 and the end of the larval stage, all the diets stabilised in a pH between 8 and 9, thus allowing us to better ascribe differences in growth parameters to the composition of the diet rather than its pH. The fact that a substrate evolves from acid to alkaline during the stages of BSF larvae colonisation and digestion has already been reported (Kim et al., 2011, Meneguz et al., 2018a). Similar trends have also been observed by Wynants et al. (2019), who studied the microbiota of BSF larvae reared on different substrates both at industrial and laboratory scales. Alkalinisation of the substrate by BSF larvae is caused by the release of ammonium ions and ammonia (Ma

et al., 2018), and it is a process which takes place as well during the treatment of waste by vermicomposting (Alidadi et al., 2016). The fact that the BSG-BSY-based diets' pH showed a steep rise at T1 could be caused by the higher amount of CP, which results in a higher release of ammonium ions.

Regarding the chemical composition of the larvae, the different inclusions of BSY in the diet did not affect the final CP content of larvae among the BSG-BSY-based diets, but, curiously, larvae fed on GA had a higher CP content when compared to BSY5 and BSY7.5. This may seem counterintuitive, since the CP content of GA at the start of the trial was lower when compared to the BSG-BSY-based diets. However, Fuso et al. (2021) stated that the protein quantity present in the diet strongly influences the protein content in the larvae that feed on it up to a certain threshold. Therefore, even if the protein content in the diet is high, the protein content of the resulting larvae is not much altered. In particular, the capacity of BSF LARVAE to convert substrate protein into larval protein reached a plateau starting from values close to 20% (of protein in the absolute amount of nutrients of the substrate, calculated on DM). A recent study by Bellezza Oddon et al. (2022) assumes that the optimal CP for larval growth in the diet is around 16 g/100 g on DM, and the BSG-BSY-based diets tested had higher contents of protein (from 20.71 g/100 g to 24.16 g/100 g on DM). Since the protein content of the BSG-BSY-based diets was already high with an inclusion of 25 g/kg of BSY, the treatments which included increasing quantities of BSY could have no effects on the final CP content of the larvae because the nutrient requirements were already met, and larvae were not able to metabolise the excess protein in the diets. It has been observed that the larval protein content seems to have a positive correlation with the fibre content of the substrate (Fuso et al., 2021). However, in our study, the higher CP content of the larvae reared on GA could simply be the reason of the availability of the protein and the fact that the larvae are normally reared on this diet, thus operating a passive selection of individuals which thrive on it. Nevertheless, Gold et al. (2018) and Lee et al. (2014) found cellulase enzymes in the gut microbiota of BSF larvae, proving that they are able to digest fibre and transform it into protein. It may be speculated that, since the specimens from our colony have been reared for several generations on a GA diet, a passive selection of a strain that develops better on GA than any other diet may have occurred. This particularity has to be taken into consideration when comparing the treatments to the control, if the control is also used as the basal diet for the colony maintenance. In relation to the fat content of the larvae, the present study displays that the BSG-BSY-based diets with lower amount of BSY tended to produce larvae with a higher amount of fat. In particular, BSY2.5, BSY5 and BSY7.5 had higher EE content when compared to BSY10, and BSY2.5 and BSY5 larvae also contained more fat than BSY7.5 larvae. If we observe the quantity of fat in the substrate, it appears hard to find a correlation, since the BSY7.5 substrate had a higher amount of fat than BSY2.5 and BSY5. The NSC content of the substrate could explain such a trend, previous studies demonstrated that BSF is able to convert excess NSC into lipids (Gold et al., 2018; Barragan-Fonseca et al., 2021). Indeed, the BSY2.5 and BSY5 diets had higher amounts of NSC than BSY7.5, and the resulting larvae had higher lipid content, even though the BSY7.5 substrate contained more fat.

Nonetheless, when we observe the results from GA, we notice a lower amount of lipid content in the larvae, although the substrate has a much higher NSC content than the other diets. Possibly, the lipid content of the diet was so low (2.10 g/100 g vs the range 6.01–6.97 g/100 g on DM of the BSG-BSY-based diets) that the excess carbohydrates in the substrate could not compensate. Spranghers et al. (2018) reported that the larval fat content is strongly linked to the fat content of the substrate, and energy-rich diets result in larvae with a higher fat content. Similar results have been found by Georgescu et al. (2021), who reported that diets with an increasing energy content yielded larvae with an increasing fat content. In our study, where the BSG-BSY-based diets were isoenergetic but had a higher energy content when compared to GA, the larvae resulting from the BSG-BSY-based diets had more fat. The lipids accumulated during the last instars of the life of BSF larvae1 are needed to sustain the adult phase (Tomberlin et al., 2002; Hoc et al., 2020). Adult flies are capable of feeding, and therefore able to accumulate energy even after the larval phase (Bruno et al., 2020). However, Georgescu et al. (2021) pointed out that the egg clutches of the flies that fed on energy-rich diets had higher amounts of body fat than energy-poor diets and produced the heaviest egg clutches. For these reasons, although a lower fat content in the larvae may not be desirable if the production is aimed at producing fertile adults, it is more desirable if the reared larvae are destined to the production of insect meal. Considering this assumption, although BSY10 had a beneficial impact on larval growth, it may be possible that the resulting adults would have a lower reproductive capacity compared to the other BSG-BSY-based diets. The administration of BSG-BSY-based diets did not affect the microbiota nor the mycobiota alpha diversity of the BSF larvae of the present study. This is in contrast with the previous studies about the characterisation of gut microbiota and mycobiota of BSLF–fed diets containing BSG, which all highlighted a significant decrease in alpha diversity (Galassi et al., 2021; Greenwood et al., 2021; Tanga et al., 2021). On the one hand, such an outcome can potentially be attributable to the fact that the main ingredients of both the BSG-BSY-based diets and the GA are cereals or cereal processing by-products, thus representing a less diversified rearing substrate when compared to a complete feed such as the hen diet (Galassi et al., 2021). On the other hand, as differences between the whole BSF larval microbiota and the BSF larval gut microbiota have recently been underlined, the choice of the insect sample type – gut (Galassi et al., 2021; Greenwood et al., 2021; Tanga et al., 2021) vs whole larva (current research) – could have exerted a significant influence on the obtained results. Indeed, considering that the BSF larvae gut seems to be characterised by a lower alpha diversity than its whole body (IJdema et al., 2022), additional microbes from the BSF larvae cuticle or body may have contributed to the unaffected microbial diversity observed in the present study. However, Wynants et al. (2019) analogously analysed the microbiome of the whole larvae rather than the gut microbiome, identifying *Morganella*, *Enterococcus*, *Pseudomonas* and/or *Providencia* as recurring genera regardless of the substrate. Other studies that have investigated the BSF larvae gut microbiota similarly found that the main genera were *Dysgonomonas* and *Morganella* – along with *Enterococcus* and *Providencia* (Galassi et al., 2021; Gorrens et al., 2021; Greenwood et al., 2021; Shumo et al., 2021; Tanga et al., 2021). These findings reflect what we found in our study, where BSF larvae microbiota was mainly colonised by *Dysgonomonas*, *Sphingomonas*, U.m. of Sphingobacteriaceae and Sphingomonadaceae, and *Morganella*. Furthermore, high relative abundances of *Sphingobacterium* have previously been reported in BSF larvae fed BSG- or grain-based feeds (Galassi et al., 2021; Greenwood et al., 2021), thus suggesting its relationship with high-NSC rearing substrates. Investigating the differences in the 16S rRNA gene sequences between larvae fed the BSG-BSY-based diets and the GA, a specific genus signature was identified in the former group only. The identification of *Bacillus* and *Myroides* agrees with the available literature, as they can be observed in BSF larvae brewery waste–rearing residues (Gold et al., 2020). Furthermore, as Proteobacteria members are commonly highlighted in the gut microbiota of BSF larvae reared on BSG (Galassi et al., 2021; Greenwood et al., 2021), the identification of *Sphingomonas* (belonging to Alphaproteobacteria) represents a logical finding. As

far as Porphyromonadaceae are concerned, they have recently been defined as a biomarker of BSF larvae gut (Jiang et al., 2019), thus suggesting that the use of BSG-BSY-based diets allows preserving the physiological BSF larvae microbiota. Lastly, since bacteria belonging to *Ruminococcus* genus are able to digest cellulose (Liu et al., 2018), their predominant observation in the BSG-BSY-based diets (which are characterised by greater NDF when compared to the GA) appears reasonable.

The mycobiota of the BSF larvae fed either the BSG-BSY-based diets or the GA in the present study was mainly colonised by *Pichia*, *Saccharomyces*, and *Aureobasidium*. The identification of high relative abundances of *Pichia* and *Saccharomyces* agrees with the findings of Tanga et al. (2021) and Varotto Boccazzi et al. (2017), which observed them as dominant genera in the BSF larvae gut along with *Cyberlindnera*, *Saccharomycodes*, *Yamadazyma*, and *Scopulariopsis*. Furthermore, as *Aureobasidium* is one of the few yeasts capable of degrading cellulose (Fleet, 2011), its high prevalence in both the BSG-BSY-based diets and the GA (rich in NDF and non-starch polysaccharides) seems logical. Independently of the administered diets, significant correlations were also observed between bacterial and fungal genera. The negative correlation between *Streptomyces* and *Saccharomyces cerevisiae* finds its explanation in the research of Jones et al. (2017), which underlined that the physical association with *Saccharomyces* yeasts stimulates *Streptomyces* exploratory behaviour and growth at the expense of the formers. Furthermore, as the growth of *Pichia* is associated with an increase in the environmental pH (Wang et al., 2022), *Fluviicola,* which can grow well till a pH of 9.0–10.0 (Dahal and Kim, 2018), can find the ideal conditions to proliferate, thus explaining the positive correlation between them observed in the current research. Differently, the relationship between *Clavispora lusitaniae* and *Myroides* appears difficult to be explained. However, considering that both are opportunistic pathogens and are able to develop resistant towards antibiotics and antifungal agents (Choudhary et al., 2019; Mendoza-Reyes et al., 2022), a potential synergism between them cannot be excluded.

Conclusions

In conclusion, the BSG-BSY-based diets with increasing inclusions of BSY performed well and the development time and survival were comparable to the control diet. An inclusion of BSY in a BSG−based diet from 25 g/kg to 100 g/kg has no effect on the pH of the substrate during larval consumption, and no effect on the larval ability to consume it. There are positive changes in the BER with the inclusions of 75 g/kg and 100 g/kg, and the larval growth also showed positive significant changes with higher inclusion levels of BSY, thus being reasonably attributable to the increased, BSY-related CP content of the rearing substrate. In future trials, it would be interesting to test higher inclusions of BSY in the diet. Different inclusion levels of BSY did not affect the CP nor the energy content of the larvae, but the BSY10 diet yielded larvae with a lower fat content when compared to the other BSG-BSY-based diets. Even though the experiment was performed at a laboratory scale, the results give insight into the use and effects of the by-products tested as BSF feed and could be used as benchmark for industrial applications. The use of BSG and BSY as rearing substrates can partially modulate the microbiota of the BSF larvae, without influencing its mycobiota. The complex interactions between bacteria and fungi herein highlighted may potentially explain the absence of differences among the fungal compositions, but an "-omic" approach (consisting of metagenomics and metabolomics) is strongly recommended to explore the functional potential of BSG- and BSY-related microbiota and mycobiota changes. Furthermore, as the insect sample type may affect the observed results, future research should preferably focus on the BSF larvae gut rather than the whole larvae. Future studies keeping the CP content similar among the BSG-BSY-based diets could be useful to discern if potential, positive effects could be observed with increasing BSY in the diet as a consequence of its prebiotic/probiotic effect.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101288.

Ethics approval

None.

Data and model availability statement

The dataset analysed in this study is available on request to the corresponding author. None of the data were deposited in an official repository. The data that support the findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

There are no competing financial, professional, or personal interests that might have influenced the presentation of the work described in this manuscript.

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