BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED 11 (2025) 735–750

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

A comprehensive analysis of coffee silverskin bioconversion by *Hermetia illucens* larvae

F. De Filippis[1,2], *G. Sequino*[1], *D. Bruno*[3], *M. Bonelli*[4], *A. Nolasco*[1], *F. Esposito*[1], *T. Cirillo*[1], *G. Tettamanti*[3,5], *D. Ercolini*[1,2], *M. Casartelli*[4,5] *and S. Caccia*[2,4]*

[1]Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055, Portici (NA), Italy; [2]Task Force on Microbiome Studies, University of Naples Federico II, 80100, Naples, Italy; [3]Department of Biotechnology and Life Sciences, University of Insubria, via Dunant 3, 21100, Varese, Italy; [4]Department of Biosciences, University of Milan, via Celoria 26, 20133, Milan, Italy; [5]Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology (BAT Center), University of Naples Federico II, via Università 100, 80055, Portici (NA), Italy; *silvia.caccia@unimi.it

Received 12 April 2024 | Accepted 4 September 2024 | Published online 30 September 2024 | Published in issue 27 February 2025

Abstract

Coffee silverskin, the outer layer of the green coffee bean, represents a major by-product of the coffee industry derived from the roasting process. In recent years the development of sustainable and circular strategies to manage and valorise organic wastes and by-products has become increasingly relevant and the potential of coffee silverskin in food industry, cosmetics, and bioconversion applications is gaining attention. In the present work we addressed the valorisation of coffee silverskin through insect bioconversion using the larvae of the black soldier fly *Hermetia illucens*, one of the most promising bioconversion agents among insects. These larvae grow on a huge variety of organic substrates due to their outstanding adaptability, that is conferred by the plasticity of the midgut physiology and associated microbiota. Our results demonstrate that black soldier fly larvae were able to grow and develop on coffee silverskin. The larvae reduced this by-product by 25% and a high protein insect biomass (i.e. 56 g per 100 g of dry matter) was obtained. Interestingly, 25% of the hemicellulose fraction of coffee silverskin was degraded by the larvae. In addition, the larval gut microbiota, which plays a key role in larvae digestion adaptability and bioconversion, was shaped by growing the larvae on coffee silverskin and bacterial taxa involved in complex polysaccharide degradation were selected. In conclusion, black soldier fly larvae may represent (1) a valuable tool for the development of new and sustainable strategies for coffee silverskin bioconversion and valorisation, and (2) an effective bioincubator for the selection and isolation of microbial strains with peculiar degrading capacity.

Keywords

black soldier fly larvae – coffee by-products – circular economy – insect-mediated bioconversion – insect gut microbiota

1 Introduction

Circular economy is based on the recycling and valorisation of by-products to obtain new resources and reducing the amount of waste. Tonnes of organic by-products are discarded every year without considering the huge potential of their reuse, thus resulting in high disposal costs for companies and in negative impacts on the environment (Gavahian *et al.*, 2021).

As the global demand for food is rising, in particular of meat (OECD/FAO, 2022), the reuse of agri-food by-products could be an interesting solution for feed production. Actually, about 15% (i.e. 940 million tonnes of dry matter) of raw materials used to produce feed for livestock and aquaculture could be rather used as food for human nutrition (Sandström *et al.*, 2022). Such reuse could thus reduce competition for food resources and minimize environmental pressure on arable land and freshwater ecosystems, also contributing to the reduction of greenhouse gas emissions (Schader *et al.*, 2015; Van Hal *et al.*, 2019; Van Selm *et al.*, 2022).

The in-depth characterisation of the quality of these by-products (e.g. chemical composition, nutritional value, presence of biotic and abiotic contaminants) is a key aspect to identify those ones most suitable for substituting specific ingredients for feed production (Čolović *et al.*, 2019). Some by-products of crop processing are rich in low-digestible fibres, proteins of variable quality, and micronutrients, but are poorly nutritional for livestock or humans since they have a low metabolizable and net energy content (Van Hal *et al.*, 2019; Bindelle *et al.*, 2008; Ertl *et al.*, 2015). The combination of various by-products and/or physical, chemical, and microbiological treatments could improve their nutritional value (Yang *et al.*, 2021), but an alternative and successful approach for their valorisation could be the use of insects. Indeed, insects are gaining attention as efficient bioconversion agents of organic waste and by-products which can represent a suitable substrate for the growth of some species (Fowles and Nansen, 2019; Ojha *et al.*, 2020; Girotto and Piazza, 2022). In this context, the larvae of the black soldier fly *Hermetia illucens* (BSFL) have been largely used in the bioconversion of organic residues thanks to advantageous features, such as saprophagy, high consumption rate, and rapid development (Surendra *et al.*, 2020; Siddiqui *et al.*, 2022). Multiple valuable compounds can be obtained from the insect biomass (i.e. proteins, lipids, chitin, and antimicrobial peptides) which, according to current legislation and depending on the rearing substrate, can be used as raw materials or additives to produce animal feed, bioplastics, cosmetics, and medical products (Surendra *et al.*, 2020; Siddiqui *et al.*, 2022). In addition, the rearing residue can be valorised as organic fertilizer or for biogas production (Surendra *et al.*, 2020).

Among the by-products from agri-food supply chains, coffee silverskin (CS), which is generated during the roasting phase of the green coffee, has a valid perspective of reuse due to its chemical and physical profile (Nolasco *et al.*, 2022a,b; Lee *et al.*, 2023). CS is a thin integument (1-2% of the coffee bean's weight) that covers the two hemispheres of unroasted coffee beans (i.e. green coffee beans) and is detached during the roasting phase due to the high temperature. The recycling of this by-product is relevant for the development of a more sustainable coffee chain, due to the tons of coffee consumed every year at global level (Lee *et al.*, 2023). Moreover, the development of strategies for the valorisation of pre-consumer organic by-products of the food supply chain represents a challenging economic opportunity since they are, as CS, often rich in nutrients and active compounds (Fowles and Nansen, 2019; Gottstein *et al.*, 2021; Lee *et al.*, 2023).

Herein we investigated the capacity of BSFL and their associated gut microbiota to bioconvert CS. In particular, since in previous studies information on these aspects is fragmented, we aimed to provide a comprehensive view about the bioconversion process in a single experimental set up including: (1) the evaluation of larval performance and bioconversion indexes, (2) chemical compositional analyses of rearing substrate, insect biomass, rearing residue, and (3) the characterisation of microbiota composition. Importantly, the latter took into consideration, for the first time, the different tracts of the BSFL gut (i.e. the 3 districts of the midgut and the hindgut), demonstrating that taxonomic composition of the bacterial community was significantly tract-dependent and showing that bacteria likely involved in CS bioconversion were present in specific regions of BSFL gut.

This work sets the stage for developing green strategies for the management of this by-product which, although rich proteins and lipids, still represents a valuable but underexploited non-edible lignocellulosic biomass.

2 Materials and methods

Insect rearing

Experimental black soldier fly (BSF) strain was established in 2015 at University of Insubria (Varese, Italy).

Eggs were collected and maintained in a humid chamber with standard diet (STD) (Hogsette, 1992) at 27 °C until hatching (Bruno *et al.*, 2019). Larvae were reared for 4 days on STD with ethyl 4-hydroxybenzoate (nipagin) (Merck KGaA, Darmstadt, Germany), as previously described (Pimentel *et al.*, 2017) to prevent mould growth, then moved to STD without additives for 4 days. After a weaning period on STD, at day 9 after hatching (T_{zero}) they were moved to CS diet (CSD, obtained moistening CS with distilled water at 20% w/v) and fed *ad libitum*. Larvae were maintained in plastic boxes at 27.0 ± 0.5 °C, 70 ± 5% relative humidity, in the dark. Four independent rearing experiments on CSD were performed. Each batch consisted of 150 larvae except for those used for chemical analyses which consisted of 300 larvae. To monitor larval growth pools (5 or 10 pools for batches of 150 or 300 larvae, respectively) of 5 randomly selected larvae for each rearing were weighted every 2-3 days from T_{zero}. This procedure was repeated until insects started pupating, i.e. day 22 from T_{zero} (mean percentage of pupation at day 22 from T_{zero} with standard error: 11.5 ± 3.4%, n = 4). As control, larvae were reared on STD and growth was monitored until day 18 from hatching (mean percentage of pupation with standard error: 25 ± 2.1%, n = 3). To standardize rearing conditions, batches of 300 larvae were reared in 16 × 16 × 9 cm plastic boxes while batches of 150 in 14 × 14 × 7.5 cm plastic boxes. The ratio between the number of larvae/gr of rearing substrate was the same regardless of the rearing substrate.

Two coffee companies from Campania region (Italy) provided the CS from the roasting process of *Coffea arabica* and *Coffea canephora* (in unspecified ratio). Both coffee varieties are usually mixed by companies during the roasting process, and their thin integuments were recovered by suction cyclones and collected (Lachenmeier *et al.*, 2022). The samples were stored in the dark at 20 °C.

Determination of chemical composition of coffee silverskin, rearing residues, and larval biomass

Actively feeding larvae, most of which in last instar, were separated from CSD 19 days after T_{zero}, accurately washed and wiped dry to remove diet debris, and put in plastic boxes for 24 h without rearing substrate at the same rearing conditions described above ("*Insect rearing*") to allow emptying of the gut lumen. This procedure ensured the determination of the chemical composition of the larvae avoiding contaminations by ingested food. Samples of CS powder were analysed as they were, while rearing residues and larvae were lyophilized with a freeze-dryer (Alpha 2-4 LD plus, Martin Christ GmbH, Osterode, Germany) at 12-15 mbar at –80 °C and then analysed to determine the chemical composition. The analyses were conducted at the Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua (Agripolis, Legnaro, Italy) by the certified laboratory LACHI as reported in Bonelli *et al.* (2020). The crude protein content was calculated by considering the nitrogen-to-protein conversion factor (K_p) of 6.25 for substrate samples and of 5.6 for insect samples (Janssen *et al.*, 2017). Chitin was calculated by using Van Soest acid detergent fibre method (Stelmock *et al.*, 1985).

Calculation of bioconversion indexes and BSFL growth performance

Bioconversion indexes of CSD by BSFL were calculated considering T_{zero} and day 19 after T_{zero} (just before pupation started) the beginning and the end of the bioconversion process, respectively.

The efficiency of the bioconversion process and the growth performance of the larvae were evaluated through the following indexes:

substrate reduction (D): $((W - R) / W) \times 100$;

waste reduction index (WRI): (D / t);

efficiency of conversion of ingested food (ECI): $[(B_{fin} - B_{ini}) / (W - R)] \times 100$;

relative growth rate (RGR): $(B_{fin} - B_{ini}) / (t \times B_{fin})$;

survival rate (SR): $(I_{fin} / I_{ini}) \times 100$;

nitrogen conversion efficiency (NCE): $[(N_{ins} \times B_{fin}) / (N_w \times W)] \times 100$;

where W is the total amount of substrate provided to the larvae; R is the rearing residue (frass) at the end of bioconversion process; t indicates the time (days) spent by the larvae on the feeding substrate; B_{fin} and B_{ini} are the total amount of the insect biomass at the end and at the beginning of the bioconversion process, respectively; I_{fin} and I_{ini} are the number of insects at the end and at the beginning of the bioconversion process, respectively; and N_{ins} and N_w are the nitrogen content of insects at the end of the bioconversion process and of substrate provided to the larvae, respectively. Protein content in the samples was calculated as reported above ("*Determination of chemical composition of coffee silverskin, rearing residues, and larval biomass*"). All the indexes were calculated on a dry matter basis. To estimate the total amount of dry matter, samples (substrate provided to the larvae, rearing residues at the end of bioconversion process, insect biomass) were lyophilized with a freeze-dryer (Alpha 2-4 LD plus) under 12-15 mbar at –80 °C. Before lyophili-

sation, insects were removed from the rearing substrate, washed, wiped dry, and then frozen. Indexes were calculated using data obtained from 4 independent rearing experiments.

Collection of samples from insect gut

Larvae were dissected as reported in Bruno *et al.* (2019) and the gut was isolated in sterile phosphate-buffered saline (in mM: 137 NaCl, 2.7 KCl, 4.3 Na_2HPO_4, 1.4 KH_2PO_4, pH 7.4) in a sterile Petri dish (5.5 × 1.3 cm). Hindgut was separated from the midgut and immediately frozen in dry ice. Midgut was then divided into 3 districts, i.e. anterior, middle, and posterior region (Bruno *et al.*, 2019), and the peritrophic matrix with midgut content was separated from the epithelium of each tract and immediately frozen in a 1.5 ml tube in dry ice. Samples were kept at –80 °C until processing. For each gut tract, 5 samples (each consisting of 5-7 hindguts or midgut tract contents) were analysed. Samples were obtained from 3 independent rearing experiments, for a total of 15 samples from each gut tract to be processed for DNA extraction and metagenomics.

DNA extraction and purification from fresh rearing substrate, rearing residues, and insect samples

After thawing, samples were homogenized in 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8, with Eppendorf fitting pestles and vigorously vortexed. To favour microbial cell lysis, homogenates were frozen again in dry ice, thawed after 1 hour and vigorously vortexed. DNA was then extracted following standard procedures (SOP_07; www.microbiome-standards.org). Due to the presence of brownish pigments and/or other unknown contaminants inhibiting *16S rRNA* gene amplification (see "*Metataxonomics by* 16S rRNA *gene sequencing, bioinformatic analysis and statistics*"), samples were purified developing an *ad hoc* DNA purification protocol. In brief, samples were treated with solutions C2 and C3 of the DNeasy® PowerSoil® kit (Qiagen, Milan, Italy) according to manufacturer's instructions. Total DNA was then purified using the NucleoSpin® gDNA Clean up was then purified using the NucleoSpin® gDNA Clean up kit (Macherey-Nagel, Dueren, Germany) and quantified by the Qubit™ dsDNA High-Sensitivity Assay kit (ThermoFisher Scientific, MA, USA). The same protocol was adopted for DNA extraction from the fresh rearing substrate (i.e. moistened CS) and rearing residues. Five samples of both fresh and rearing residues were collected and processed for DNA extraction and metataxonomics for each of 3 independent rearing experiments.

Metataxonomics by 16S rRNA *gene sequencing, bioinformatic analysis and statistics*

The V3-V4 region of the *16S rRNA* gene was amplified using primers and PCR conditions previously described (Berni Canani *et al.*, 2017) with minor modifications. In brief, since PCR inhibitors persisted after DNA purification step, 4.5% (w/v) of polyethylene glycol and 0.072% (w/v) of bovine serum albumin were added to the PCR reaction mixture. These additives allow amplification of small amounts of DNA in the presence of contaminants (Wilson, 1997; Roux, 2009; Farell and Alexandre, 2012; Lorenz, 2012).

Amplicon libraries were prepared according to the Illumina standard protocol for amplicon sequencing using a MicroLab Starlet platform (Hamilton) and sequenced by Illumina MiSeq Platform by Novogene (Cambridge, UK), leading to 2 × 250 bp reads. Data analysis was carried out in QIIME 2 (q2cli version 2020.11.1; Bolyen *et al.*, 2019), using the 'dada2' plugin with default parameters. Filtering and Amplicon Sequence Variant (ASV) table generation were carried out as described by Sequino *et al.* (2022). Statistical analyses and plotting were carried out in R environment (http://www.r-project.org). Alpha-diversity indices (Shannon and Simpson) were calculated using the function 'diversity', while Bray-Curtis distance matrices were computed using 'vegdist' ('vegan' package). Pairwise Wilcoxon's tests were used to evaluate statistical significance.

3 Results

BSFL growth on coffee silverskin

The analysis of the composition of CS used in the present study showed that, along with an expected high fibre content (Gottstein *et al.*, 2021; Nolasco *et al.*, 2022b; Narita and Inouye, 2014), this organic substrate was rich in valuable nutrients as proteins and lipids (Table 1). In particular, it contained significantly much more proteins and lipids than other substrates used in previous BSFL rearing trials by this research group (Table 1). CS might thus represent an optimal candidate to challenge BSFL bioconversion potential. Larvae well tolerated and effectively consumed the CSD, indeed survival was higher than 95% (Table 2).

Nevertheless, they showed a lower rate of weight gain and a significant developmental delay compared to larvae grown on STD, an optimal diet to rear this insect species (Figure 1).

Larval weight gain on CSD was also markedly low compared to what observed on other organic wastes,

TABLE 1 Chemical composition of coffee silverskin (CS) (fresh) compared to other rearing substrates and of the rearing residues represented by CS processed by the larvae and insect remains (e.g. exuviae, frass, and dead insects). Values are expressed as g per 100 g of dry matter. n.d. = not detectable

Component	CS (fresh)	Rearing residue	Standard diet[a]	Vegetable mix diet[a]
Dry matter	93.1	94.0	91.0	89.1
Crude protein	20.7	23.1	14.1	10.3
Crude lipids	4.1	2.7	2.7	0.7
Crude fibre[b]	29.5	34.6	10.8	4.4
Nitrogen-free extract[c]	34.5	26.2	67.3	80.0
Ash	11.2	13.4	5.1	4.6
Hemicellulose[d]	12.8	9.6	21.3	3.6
Cellulose[d]	22.1	26.6	9.7	4.6
Lignin[d]	18.6	21.2	3.7	1.3
Starch	0.02	0.02	18.8	11.6
Glucose and fructose	n.d.	n.d.	3.3	12.8

a Values from Bonelli *et al.* (2020) obtained with the same experimental protocols and analytical procedures as in this study
b includes most of cellulose and insoluble lignin
c includes sugars, carbohydrates, organic acids, pectins, soluble lignin, hemicellulose and a small percentage of cellulose
d values calculated from neutral and acid detergent fibre analyses

TABLE 2 Black soldier fly larvae growth parameters, coffee silverskin diet reduction, and bioconversion indexes. Data are reported as mean ± standard error obtained in 4 independent rearing experiments. Indexes were calculated on dry weight basis and considering a duration of the bioconversion process of 19 days

Parameter	Value
Survival rate (SR) (%)	96.3 ± 0.6
Relative growth rate (RGR)	0.028 ± 0.004
Maximum weight (mg)	96.6 ± 3.1
Substrate reduction (D) (%)	24.5 ± 0.5
Waste reduction index (WRI) (%)	1.288 ± 0.024
Efficiency of conversion of ingested food (ECI) (%)	28.7 ± 4.1
Nitrogen conversion efficiency (NCE) (%)	36.3 ± 3.4

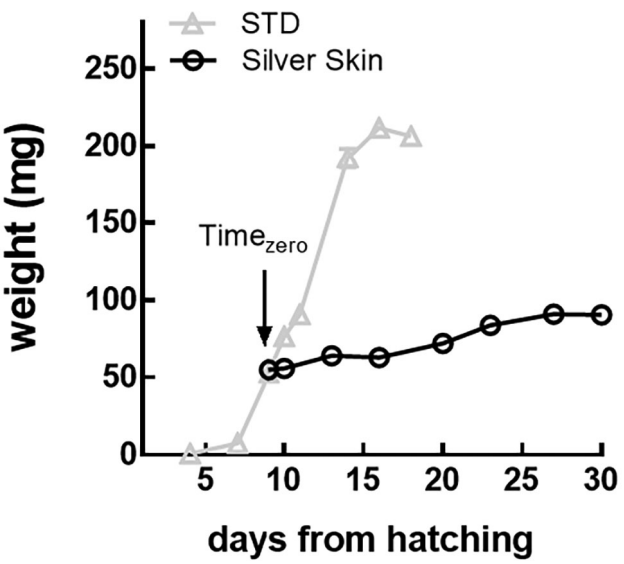

FIGURE 1 Growth of black soldier fly larvae on coffee silverskin diet and standard diet. The larval weight was recorded until pupation. The day in which the larvae reached the maximum weight was considered the end of the larval stage, then insects entered the prepupal stage and stopped feeding. The data represent the mean ± error standard of at least 3 replicates.

with only a few exceptions (e.g. manure, brewer's spent grain, and shrimp waste) (Diener *et al.*, 2009; Oonincx *et al.*, 2015; ur Rehman *et al.*, 2017; Meneguz *et al.*, 2018; Jucker *et al.*, 2020; Pliantiangtam *et al.*, 2021; Veldkamp *et al.*, 2021; Eggink *et al.*, 2022).

Rearing substrate reduction and bioconversion

BSFL were able to reduce the substrate of about 25% in dry weight (Table 2, Figure 2).

The modest substrate reduction compared to other organic wastes (Nyakeri *et al.*, 2017; Meneguz *et al.*, 2018; Jucker *et al.*, 2020; Lu *et al.*, 2021; Pliantiangtam *et al.*, 2021) was likely due to the high content of cellulose, lignin, and ash, which were not relevantly degraded and metabolized by the larvae as demonstrated by their percentage increase in the rearing residue (Table 1). Interestingly, unlike the aforementioned fibres, hemicellulose appeared to be consumed and degraded by the larvae (Table 1).

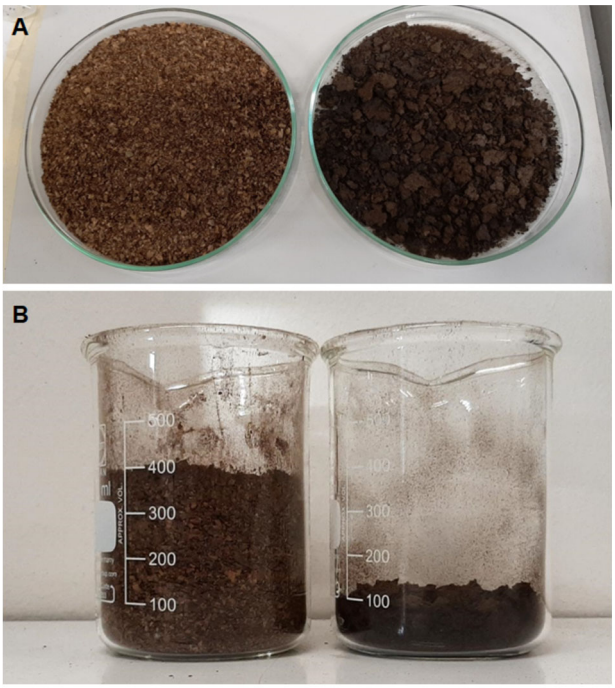

FIGURE 2 Coffee silverskin (CS) used to prepare the rearing substrate (on the left in A and B panels) and dry rearing residue obtained after black soldier fly larvae rearing for 19 days (on the right in A and B panels). The different appearance of the substrate and the strong reduction in volume after insect rearing are clearly appreciable. The substrate was reduced by 25% in dry weight.

WRI (Table 2), that along with substrate reduction takes into account the time spent by the larvae on the substrate, was in line, or even higher, compared to other organic wastes, as manure and silage grass (ur Rehman *et al.*, 2017; Veldkamp *et al.*, 2021). Importantly, ECI and NCE indexes (Table 2) demonstrated an effective CS bioconversion, in line with the high crude protein content of the biomass (Table 3), which was higher than, or comparable to, the values obtained for BSFL reared on many different organic wastes and by-products, or even on optimal substrates (Meneguz *et al.*, 2018; Cappellozza *et al.*, 2019; Jucker *et al.*, 2020; Surendra *et al.*, 2020; Eggink *et al.*, 2022).

It is worth noting that also chitin content was fulfilling, as it was higher than in most published studies (Cappellozza *et al.*, 2019; Surendra *et al.*, 2020; Eggink *et al.*, 2022). On the contrary, crude lipids content was relatively low compared to larvae grown on optimal substrates or wastes (from 5 to 12 times less) (Meneguz *et al.*, 2018; Cappellozza *et al.*, 2019; Jucker *et al.*, 2020; Parodi *et al.*, 2020; Surendra *et al.*, 2020; Lu *et al.*, 2021; Eggink *et al.*, 2022).

TABLE 3 Chemical composition of black soldier fly larvae reared on coffee silverskin diet (CSD). As a comparison, the composition of larvae reared on a fruit and vegetable mix (Vegetable Mix Diet, VMD), determined with the same analytical procedure (Cappellozza *et al.*, 2019), is reported. Values are expressed as g per 100 g of dry matter. n.d., not detectable

Component	CSD	VMD
Dry matter	92.7	97.0
Crude protein	55.9	39.4
Crude lipids	3.7	35.6
Ash	22.4	7.1
Chitin	7.2	4.0
Nitrogen-free extract[a]	10.8	13.9
Starch	1.0	1.8
Glucose and fructose	n.d.	0.3

a Includes sugars, carbohydrates, and organic acids.

Microbiota composition in the different regions of BSFL midgut, hindgut, rearing substrate, and rearing residues

As in other flies, the intermediate region of BSFL gut, the midgut, is characterized by striking regionalization (Terra, 1990; Buchon *et al.*, 2013; Buchon and Osman, 2015; Bonelli *et al.*, 2019, 2020; Caccia *et al.*, 2019). In particular, the midgut can be divided into 3 regions (i.e. anterior, middle, and posterior) each characterized by unique structural and functional features, luminal pH (mildly acidic, highly acidic, and alkaline in the anterior, middle, and posterior midgut, respectively) and microbiota composition (Bonelli *et al.*, 2019, 2020; Bruno *et al.*, 2019; Bruno *et al.*, 2024). Therefore, in the present study the composition of the bacterial community was analysed in the 3 midgut regions separately. In addition, as in some insects the posterior part of the gut (i.e. the hindgut) is the major site where digestion of dietary fibres occurs with the support of hydrolytic degradation by resident microorganisms (Engel and Moran, 2013; Brune, 2014; Jang and Kikuchi, 2020), the microbial community of the hindgut was investigated, too.

Microbial diversity decreased from the anterior to the posterior part of the midgut (Figure 3), as previously reported for BSFL grown on other substrates (Bruno et al., 2019). Consistently, we observed a clear clustering of the samples according to the intestinal region (Figure 4). Interestingly, microbiota composition of rearing residues was similar to that of the anterior midgut (Figures 4, 5 and 6) corroborating previous results on BSFL reared on STD (Bruno *et al.*, 2019).

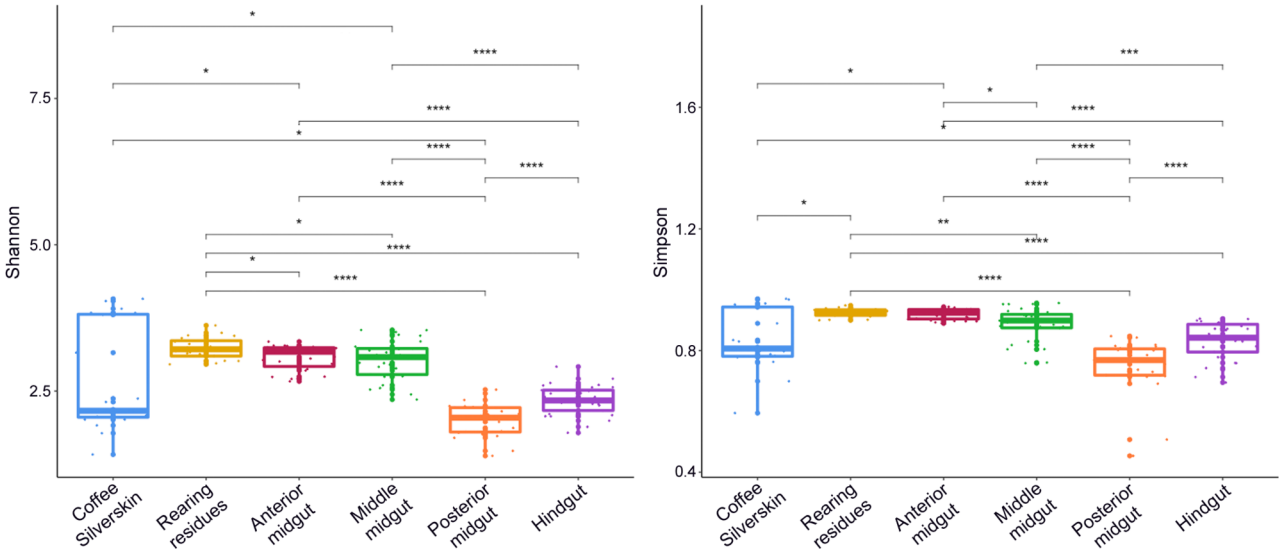

FIGURE 3 Box plot showing Shannon and Simpson biodiversity indices in the different samples. Significance was evaluated by pairwise Wilcoxon's tests (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; **** $P \leq 0.0001$).

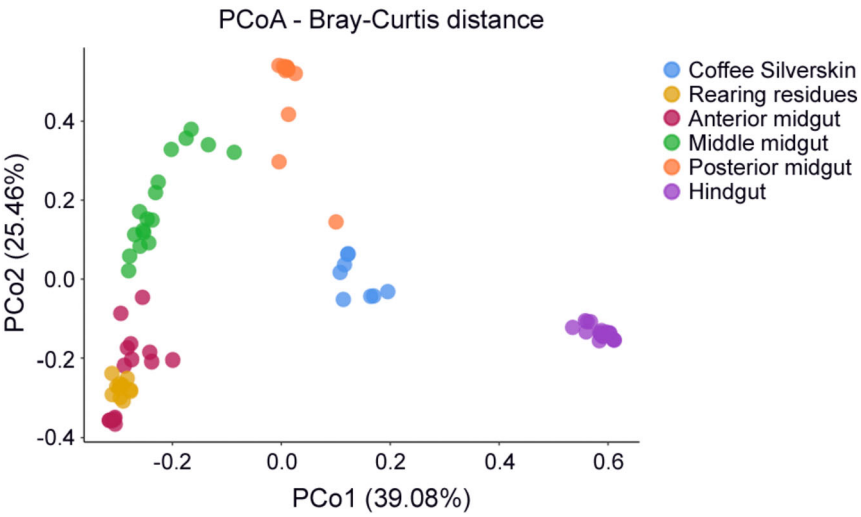

FIGURE 4 PCoA based on Bray-Curtis distance matrices obtained from microbiota composition at genus level.

At phylum level, Verrucomicrobia and Planctomyces were present, albeit at low percentages, in the microbial community of the anterior midgut, but were not represented in posterior midgut and hindgut, nor in the rearing residue (Figure 5). In addition, it is worth noting that Proteobacteria, dominant in the anterior and middle midgut, almost disappeared in the posterior midgut (Figure 5). Similarly to Proteobacteria, Bacteroidetes well tolerated anterior and middle midgut environment, but disappeared in posterior midgut. Actinobacteria and Firmicutes were dominant in the posterior midgut, while Bacteroidetes and Firmicutes characterised the microbial community in the hindgut, together with Proteobacteria.

More specifically, *Actinomyces* and *Paenibacillus* increased from the anterior to the posterior midgut, while *Brevundimonas*, *Devosia*, Sphyngomonadaceae, and Phyllobacteriaceae, that were present in the anterior and middle midgut, were not found in the posterior tract (Figure 6). In addition, *Asticcacaulis*, Flavobacteriaceae, and Microbacteriaceae decreased from the anterior to the posterior part. Finally, several taxa, absent or present at very low abundance in the midgut, appeared in the hindgut, such as *Dysgonomonas* and unidentified Lachnospiraceae and Ruminococcaceae (Figure 6). The hindgut was indeed characterized by a peculiar microbiota composition that did not mirror any midgut tract, nor the substrate, and had lost the Actinobacteria com-

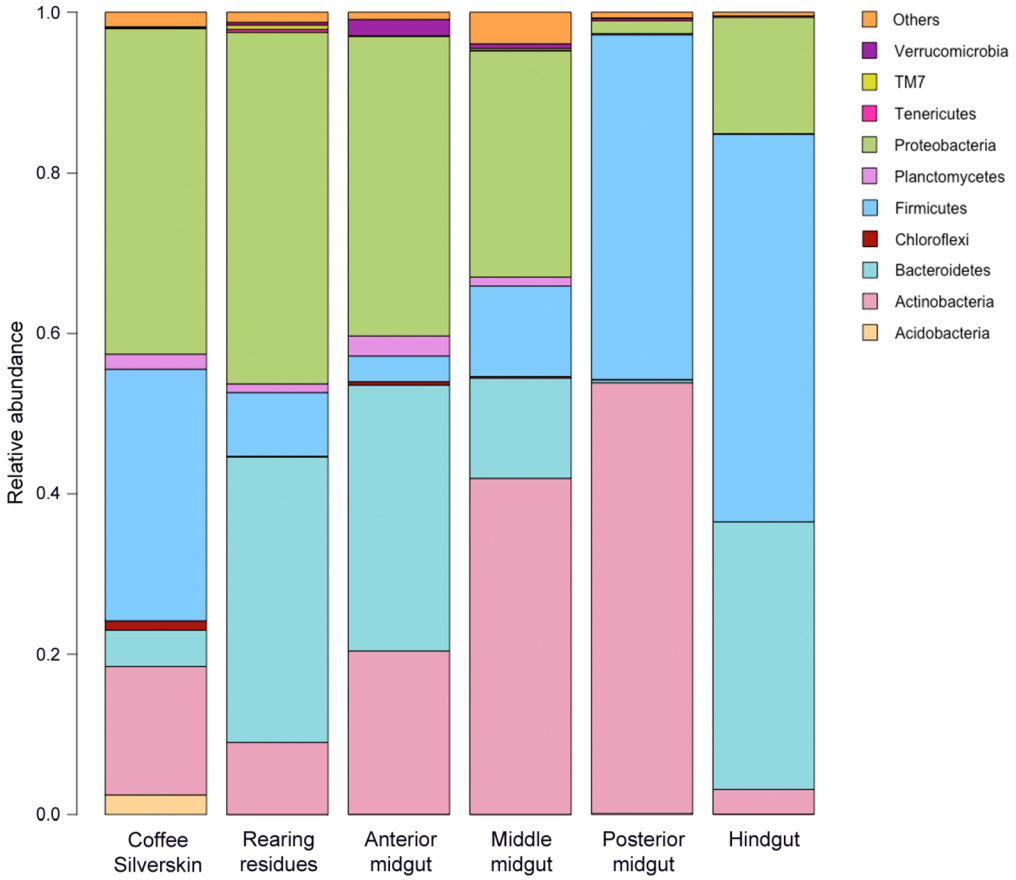

FIGURE 5 Stacked bar chart that shows the relative abundances of bacterial phyla identified in the midgut (anterior, middle, and posterior), hindgut and substrate samples. Values are the average of 3 replicates. Phyla with an abundance of <2% are summed up and shown as "others."

ponent which was in contrast highly represented in the middle and posterior midgut (Figures 5 and 6).

4 Discussion

The present study aimed to provide a comprehensive evaluation of BSFL ability to grow and biotransform CS, one of the major by-products of the coffee industry, and of the nutritional quality of the insect biomass. The different aspects herein explored provide a complete picture which is essential to exploit BSFL as bioconversion agents of CS and to set the stage for the development of strategies to increase their efficacy at industrial level. Moreover, the microbiota composition was evaluated in the different gut regions to shed light on microbes that may contribute to CS bioconversion thanks to their ability to digest complex plant polymers (e.g. hemicellulose and lignocellulose) and on the gut district where these activities may take place.

Despite a modest weight gain of BSFL, the obtained biomass has valuable characteristics in terms of chemical composition. Indeed, crude protein content is remarkably high compared to BSFL reared on other substrates. Interestingly, the crude protein yield (i.e. 56 g per 100 gr of dry biomass) is higher not only than in larvae reared on organic wastes or by-products, but also than those grown on optimal substrates in terms of nutrient content and availability (Diener *et al.*, 2009; Oonincx *et al.*, 2015; Meneguz *et al.*, 2018; Jucker *et al.*, 2020; Surendra *et al.*, 2020; Eggink *et al.*, 2022), such as a vegetable mix diet (Cappellozza *et al.*, 2019). This evidence highlights that the insect biomass obtained from CS bioconversion could be used as a sustainable, cost-effective alternative to traditional protein sources like soybean and fishmeal in animal nutrition, minimising the environmental impact of the substrates used for feed production (Smetana *et al.*, 2016, 2021; Ferronato *et al.*, 2024). In addition, chitin, also present in excellent amount in larvae reared on CS (see, for comparison, Cappellozza *et al.*, 2019; Surendra *et al.*, 2020; Galassi *et al.*, 2021; Eggink *et al.*, 2022), can positively modulate gut microbiota community in fish (Rimoldi *et al.*, 2023) and broilers (Sedgh-Gooya *et al.*, 2021), as well

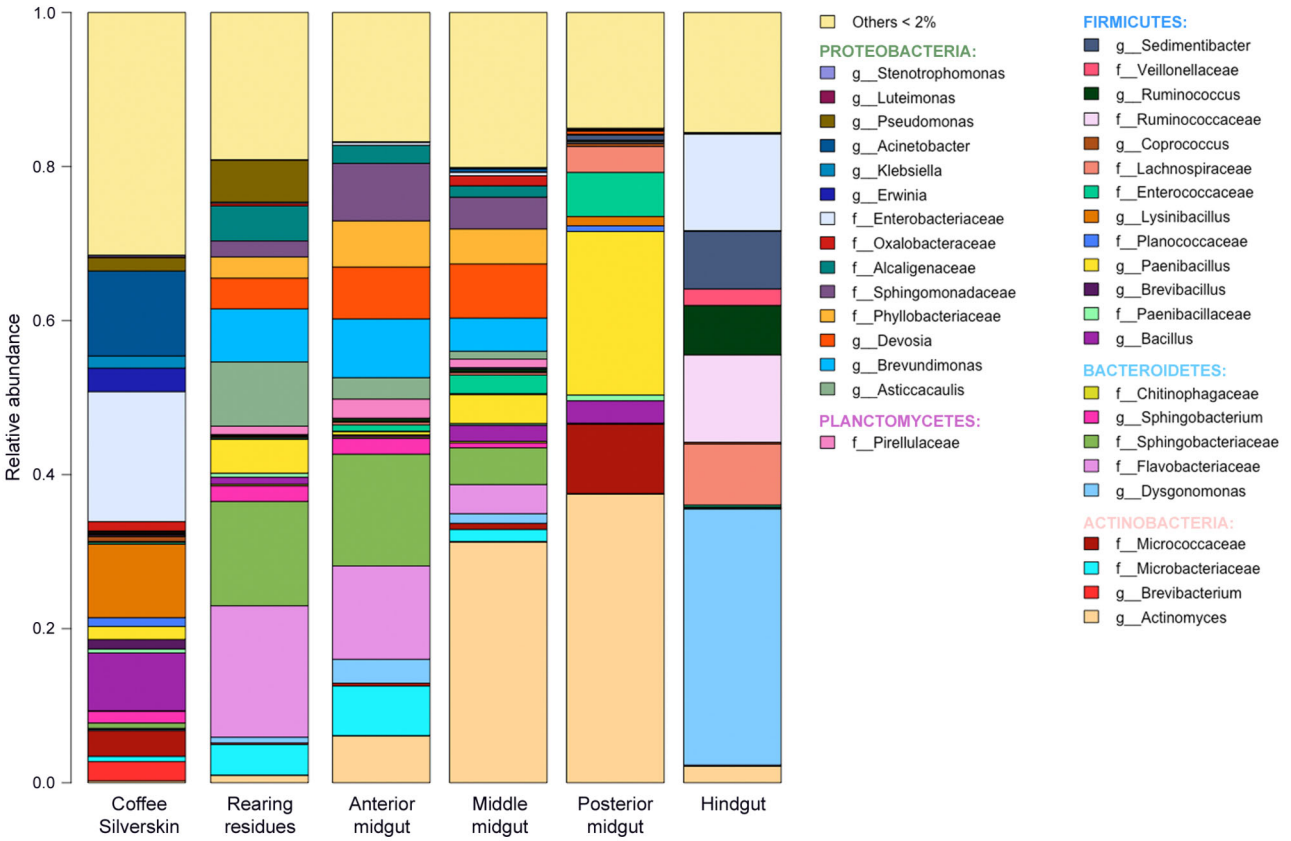

FIGURE 6 Stacked bar chart that shows the relative abundances of bacterial genera identified in the midgut (anterior, middle, and posterior), hindgut and substrate samples analysed. Values are the average of 3 replicates. Genera with an abundance of <2% are summed up and shown as "others."

as their growth rate (Lokman *et al.*, 2019). Due to the astonishing variety of applications in medicine, cosmetics, plant protection, feedstock, the demand of chitin and its derivative chitosan is rapidly increasing (Crini, 2019; Bakshia *et al.*, 2020; Huang *et al.*, 2023; Yanat and Schröen, 2023), and insects represent a more sustainable and reliable alternative source of these molecules compared to the current one, i.e. seafood waste (Hahn *et al.*, 2020; Bulak *et al.*, 2023).

Taking into account that proper supplementation of CS-based rearing substrate can result in an improved weight gain of the larvae and in their lipid content (crude lipids account for less than 5% of dry biomass in our experimental conditions), the use of CS for BSFL rearing can have practical applications in feed formulation (Olivotto *et al.*, 2017). The addition of various amounts of microalgae to CS led to an interesting increase of unsaturated fatty acids in the larvae, particularly of omega-3 (Truzzi *et al.*, 2020; Zarantoniello *et al.*, 2020). However, in the framework of circular economy, it is important to address all those issues related to the use of environmental resources, finding new alternative ingredients for marine aquafeeds without exploiting marine bioresources (e.g. microalgae; Rodrigues *et al.*, 2022). Indeed, several studies have demonstrated that blends of organic wastes and agri-food by-products with different chemical composition can improve BSFL growth and the quality of the obtained biomass (Oonincx *et al.*, 2015; ur Rehman *et al.*, 2017; Pliantiangtam *et al.*, 2021; Veldkamp *et al.*, 2021; Ceccotti *et al.*, 2022). Similarly, in line with the current demand of sustainable and circular product chains, blends of CS and other organic by-products and wastes could be designed to improve CS exploitation.

Summarising, CS is a by-product that can be bioconverted and valorised by BSFL; moreover, the obtained insect biomass is particularly rich in protein and chitin and therefore can be used for feed production. On the contrary, the use of CS for colony maintenance is not recommended as the larval growth performance on this by-product is not satisfactory and cheap nutrient-balanced substrates are available to this purpose.

Further efforts will be devoted to finely characterize the ash content and explore sustainable methods to reduce its levels in a potential BSFL-based feed, as high mineral content may exert detrimental effects, primarily

on monogastric species (Bikker *et al.*, 2020). In addition, composition of crude lipids and amino acid profiles of crude proteins will also deserve further investigations to better define the proper type of biomass exploitation (Surendra *et al.*, 2020).

This study describes, for the first time, the bacterial gut microbiota of BSFL reared on CS in the different regions of the gut (i.e. the 3 districts of the midgut and the hindgut). We showed that the morphofunctional regionalization of the BSFL gut (Bonelli *et al.*, 2019, 2020; Bruno *et al.*, 2024) affected microbiota composition in BSFL reared on CS as previously demonstrated for other substrates (Bruno *et al.*, 2019; Auger *et al.*, 2023; Vandeweyer *et al.*, 2023). Moreover, since the microbiota associated to fresh rearing substrate and rearing residues were herein analysed, possible correlations among them and those present in gut regions can be drawn. In particular, microbiota associated to the rearing residue and to the anterior midgut were very similar; the middle midgut, characterized by luminal highly acidic pH (Bonelli *et al.*, 2019), significantly shaped the composition of the bacterial community as in the posterior midgut the associated microbiota was less diversified and dominated by Firmicutes and Actinobacteria (i.e. 97% of posterior midgut species belong to these phyla). Importantly, hindgut was characterized by a unique microbiota with a few, but highly represented, taxa.

Microbiota regionalization may imply changes in the genetic pool of bacterial communities in the different tracts and thus to functions, including those related to specific degradation pathways of potential biotechnological interest (e.g. degradation of complex plant polymers as lignocellulose) as recently demonstrated for plastic polymers (De Filippis *et al.*, 2023). The results here reported supported this hypothesis. *Paenibacillus* and *Actinomyces*, which increased in abundance in the middle and, most of all, in the posterior midgut, have been reported as hemicellulose degraders (Schäfer *et al.*, 1996; Saini *et al.*, 2015; López-Mondéjar *et al.*, 2016). This may suggest a specific selection occurring in the different tracts of the BSFL midgut, that confers the posterior midgut the ability to degrade complex carbohydrates thanks to the resident microbiota. In accordance, the hemicellulose content in the rearing residues was lower than in CS, indicating that this fibre was effectively degraded by BSFL larvae. A very intriguing aspect that emerged from microbiota analysis was the bacterial community of the hindgut, dominated by strictly anaerobic Firmicutes and the Bacteroidetes *Dysgonomonas*. The latter was reported as dominant in the posterior midgut of BSFL reared on STD and a vegetable-based diet (Bruno *et al.*, 2019). In contrast, *Dysgonomonas* spp. were absent in the midgut of BSFL reared on a high protein diet (Bruno *et al.*, 2019) and both in the midgut and hindgut of BSFL reared on 3 different industrial residual streams (Vandeweyer *et al.*, 2023). In the present work, *Dysgonomonas* represents the most abundant taxon in the hindgut (about 30%). This genus is common in insect gut (Bridges and Gage, 2021) and has been reported as common inhabitant of xylophagous insects such as termites and wood-feeding cockroaches and it is attracting interest for its lignocellulosic activity (Luo *et al.*, 2019; Bridges and Gage, 2021). In addition, it was identified as a major component of the bamboo snout beetle *Cyrtotrachelus buqueti*, that showed a high lignocellulosic degradation activity (Luo *et al.*, 2019). Moreover, the genus *Dysgonomonas* was abundant in the gut of BSFL reared on lignocellulosic diets and metatranscriptomes were enriched of transcripts related to enzymes with hemicellulolytic activity (Kariuki *et al.*, 2023). This evidence and the absence of *Dysgonomonas* in the hindgut of BSFL reared on 3 different substrates (Vandeweyer *et al.*, 2023) suggests a correlation between these bacteria and the high lignocellulose content of CS and, possibly, their involvement in lignocellulose degradation.

As concerns Firmicutes, members of the Ruminococcaceae and Lachnospiraceae families, they represented about 25% of taxa found in the hindgut. These are the most abundant families of the order Clostridiales in the mammalian gut environment and share a common role as active degraders of complex plant material (Biddle *et al.*, 2013). Indeed, the abundance of Lachnospiraceae and Ruminococcaceae has been correlated to a high fibre diet (Liu *et al.*, 2022; Pu *et al.*, 2022) and *Ruminococcus* species are universally present and predominant in rumen bacterial community where they are responsible for cellulose and hemicellulose digestion) (Weimer, 2022). Although a decrease in cellulose and lignin content was not detected, a strong selection of cellulolytic and/or ligninolytic bacteria and functions cannot be excluded.

The picture of the gut microbiota of BSFL reared on CS that emerged from the present study is significantly different from that previously obtained by Osimani *et al.* (2021). This is likely due to the difference in the starting material (i.e. DNA from separated midgut tracts content or hindgut in the present study *versus* DNA from whole larvae). Biodiversity is higher and bacterial communities are more complex in the present study compared to the previous report and this is evident both at

phylum and lower taxa levels (Figures 5 and 6). Most of the taxa detected in that study were also identified in the present work, except for those associated to the hindgut that were not detected in the microbiota from whole larvae. The overall comparison suggests that using DNA from whole larvae may cause an underestimation of bacterial community complexity, likely due to the PCR amplification step (Huys *et al.*, 2008; Walker *et al.*, 2020). Indeed, bacterial amplicons to be sequenced were obtained using a DNA template in which bacterial DNA represented a very minor fraction compared to eukaryotic DNA (Huys *et al.*, 2008; Walker *et al.*, 2020).

The present work highlights the possibility of using BSFL-mediated biotransformation for developing sustainable value chains for CS waste valorisation. In addition, as CS significantly shaped the gut microbiota of BSFL and some bacterial spp. may be related to fibre degradation, the microbiota of BSFL reared on CS may represent a valuable source of bacterial strains and genes with biotechnological potential for the degradation of lignocellulosic biomass. Since sets of diverse enzymes are required for the complete depolymerization of such plant material (Cann *et al.*, 2020; Weimer, 2022; dos Santos *et al.*, 2023; Grgas *et al.*, 2023), it is important to search for consortia with the comprehensive metabolic machinery for depolymerizing polysaccharides to their carbon/energy-rich building blocks.

Conflict of interest

The authors have no conflict of interest to declare.

Data availability

The raw sequence reads generated in this study are available on the Sequence Read Archive (SRA) of the NCBI with the accession number PRJNA1113432. Other data will be made available on request.

Funding

This work was supported by Fondazione Cariplo (grant number 2020-0900).

References

Auger, L., Deschamps, M.-H., Vandenberg, G. and Derome, N., 2023. Microbiota is structured by gut regions, life stage, and diet in the Black Soldier Fly (*Hermetia illucens*). Frontiers in Microbiology 14: 1221728. https://doi.org/10.3389/fmicb.2023.1221728

Bakshi, P.S., Selvakumar, D., Kadirvelu, K. and Kumar, N.S., 2020. Chitosan as an environment friendly biomaterial – a review on recent modifications and applications. International Journal of Biological Macromolecules 150: 1072-1083. https://doi.org/10.1016/j.ijbiomac.2019.10.113

Berni Canani, R., De Filippis, F., Nocerino, R., Laiola, M., Paparo, L., Calignano, A., De Caro, C., Coretti, L., Chiariotti, L., Gilbert, J.A. and Ercolini, D., 2017. Specific signatures of the gut microbiota and increased levels of butyrate in children treated with fermented cow's milk containing heat-killed *Lactobacillus paracasei* CBA L74. Applied and Environmental Microbiology 83: e01206-17. https://doi.org/10.1128/AEM.01206-17

Biddle, A., Stewart, L., Blanchard, J. and Leschine, S., 2013. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. Diversity 5: 627-640. https://doi.org/10.3390/d5030627

Bikker, P., Stokvis, L., Van Krimpen, M.M., Van Wikselaar, P.G. and Cone, J.W., 2020. Evaluation of seaweeds from marine waters in Northwestern Europe for application in animal nutrition. Animal Feed Science and Technology 263: 114460. https://doi.org/10.1016/j.anifeedsci.2020.114460

Bindelle, J., Leterme, P. and Buldgen, A., 2008. Nutritional and environmental consequences of dietary fibre in pig nutrition: a review. Biotechnology, Agronomy, Society and Environment 12: 69-80.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K. and Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852-857. https://doi.org/10.1038/s41587-019-0209-9

Bonelli, M., Bruno, D., Caccia, S., Sgambetterra, G., Cappellozza, S., Jucker, C., Tettamanti, G. and Casartelli, M., 2019. Structural and functional characterization of *Hermetia illucens* larval midgut. Frontiers in Physiology 10: 204. https://doi.org/10.3389/fphys.2019.00204

Bonelli, M., Bruno, D., Brilli, M., Gianfranceschi, N., Tian, L., Tettamanti, G., Caccia, S. and Casartelli, M., 2020. Black soldier fly larvae adapt to different food substrates

through morphological and functional responses of the midgut. International Journal of Molecular Science 21: 4955. https://doi.org/10.3390/ijms21144955

Bridges, C.M. and Gage, D.J., 2021. Development and application of aerobic, chemically defined media for *Dysgonomonas*. Anaerobe 67: 102302. https://doi.org/10.1016/j.anaerobe.2020.102302

Brune, A., 2014. Symbiotic digestion of lignocellulose in termite guts. Nature Reviews Microbiology 12: 168-180. https://doi.org/10.1038/nrmicro3182

Bruno, D., Bonelli, M., De Filippis, F., Di Lelio, I., Tettamanti, G., Casartelli, M., Ercolini, D. and Caccia, S., 2019. The intestinal microbiota of *Hermetia illucens* larvae is affected by diet and shows a diverse composition in the different midgut regions. Applied and Environmental Microbiology 85: e01864-18. https://doi.org/10.1128/AEM.01864-18

Bruno, D., Bonelli, M., Valoroso, M.C., Roma, D., Montali, A., Pellegrino, M.G., Marzari, M., Caccia, S., Tettamanti, G. and Casartelli, M., 2024. Black soldier fly larvae efficiently bioconvert the organic fraction of municipal solid waste thanks to the functional plasticity of their midgut. Journal of Insects as Food and Feed. https://doi.org/10.1163/23524588-00001193

Buchon, N., Osman, D., David, F.P., Fang, H.Y., Boquete, J.P., Deplancke, B. and Lemaitre, B., 2013. Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. Cell Reports 3: 1725-1738. https://doi.org/10.1016/j.celrep.2013.04.001

Buchon, N. and Osman, D., 2015. All for one and one for all: regionalization of the *Drosophila* intestine. Insect Biochemistry and Molecular Biology 67: 2-8. https://doi.org/10.1016/j.ibmb.2015.05.015

Bulak, P., Proc-Pietrycha, K., Kaczor, M., Złotko, K., Polakowski, C., Wiącek, D., Waniak-Nowicka, H., Zięba, E., Waśko, A., Oleszczuk, P. and Bieganowski, A., 2023. A novel type of biochar from chitinous *Hermetia illucens* waste with a built-in stimulating effect on plants and soil arthropods. Scientific Reports 13: 8306. https://doi.org/10.1038/s41598-023-35460-6

Caccia, S., Casartelli, M. and Tettamanti, G., 2019. The amazing complexity of insect midgut cells: types, peculiarities, and functions. Cell Tissue and Research 377: 505-525. https://doi.org/10.1007/s00441-019-03076-w

Cann, I., Pereira, G.V., Abdel-Hamid, A.M., Kim, H., Wefers, D., Kayang, B.B., Kanai, T., Sato, T., Bernardi, R.C., Atomi, H. and Mackie, R.I., 2020. Thermophilic degradation of hemicellulose, a critical feedstock in the production of bioenergy and other value-added products. Applied and Environmental Microbiology 86: e02296-19. https://doi.org/10.1128/AEM.02296-19

Cappellozza, S., Leonardi, M.G., Savoldelli, S., Carminati, D., Rizzolo, A., Cortellino, G., Terova, G., Moretto, E., Badaile, A., Concheri, G., Saviane, A., Bruno, D., Bonelli, M., Caccia, S., Casartelli, M. and Tettamanti, G., 2019. A first attempt to produce proteins from insects by means of a circular economy. Animals 9: 278. https://doi.org/10.3390/ani9050278

Ceccotti, C., Bruno, D., Tettamanti, G., Branduardi, P., Bertacchi, S., Labra, M., Rimoldi, S. and Terova, G., 2022. New value from food and industrial wastes – bioaccumulation of omega-3 fatty acids from an oleaginous microbial biomass paired with a brewery by-product using black soldier fly (*Hermetia illucens*) larvae. Waste Management 143: 95-104. https://doi.org/10.1016/j.wasman.2022.02.029

Čolović, D., Rakita, S., Banjac, V., Đuragić, O. and Čabarkapa, I., 2019. Plant food by-products as feed: characteristics, possibilities, environmental benefits, and negative sides. Food Reviews International 35: 363-389. https://doi.org/10.1080/87559129.2019.1573431

Crini, G., 2019. Historical review on chitin and chitosan biopolymers. Environmental Chemistry Letters 17: 1623-1643. https://doi.org/10.1007/s10311-019-00901-0

De Filippis, F., Bonelli, M., Bruno, D., Sequino, G., Montali, A., Reguzzoni, M., Pasolli, E., Savy, D., Cangemi, S., Cozzolino, V., Tettamanti, G., Ercolini, D., Casartelli, M. and Caccia, S., 2023. Plastics shape the black soldier fly larvae gut microbiome and select for biodegrading functions. Microbiome 11: 1-18. https://doi.org/10.1186/s40168-023-01649-0

Diener, S., Zurbrügg, C. and Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management Resources 27: 603-610. https://doi.org/10.1177/0734242X09103838

dos Santos, J.R., de Souza Moreira, L.R. and Filho, E.X.F., 2023. Cellulose-degrading enzymes: key players in biorefinery development. Biologia 78: 1759-1772. https://doi.org/10.1007/s11756-022-01274-6

Eggink, K.M., Lund, I., Pedersen, P.B., Hansen, B.W. and Dalsgaard, J., 2022. Biowaste and by-products as rearing substrates for black soldier fly (*Hermetia illucens*) larvae: effects on larval body composition and performance. PLOS ONE 17: e0275213. https://doi.org/10.1371/journal.pone.0275213

Engel, P. and Moran, N.A., 2013. The gut microbiota of insects-diversity in structure and function. FEMS Microbiology Reviews 37: 699-735. https://doi.org/10.1111/1574-6976.12025

Ertl, P., Zebeli, Q., Zollitsch, W. and Knaus, W., 2015. Feeding of by-products completely replaced cereals and pulses in dairy cows and enhanced edible feed conversion ratio. Journal of Dairy Science 98: 1225-1233. https://doi.org/10.3168/jds.2014-8810

Farell, E.M. and Alexandre, G., 2012. Bovine serum albumin further enhances the effects of organic solvents on increased yield of polymerase chain reaction of GC-rich templates. BMC Research Notes 5: 257. https://doi.org/10.1186/1756-0500-5-257

Ferronato, N., Paoli, R., Romagnoli, F., Tettamanti, G., Bruno, D. and Torretta, V., 2024. Environmental impact scenarios of organic fraction municipal solid waste treatment with Black Soldier Fly larvae based on a life cycle assessment. Environmental Science and Pollution Research 31: 1-19. https://doi.org/10.1007/s11356-023-27140-9

Fowles, T.M. and Nansen, C., 2019. Artificial selection of insects to bioconvert pre-consumer organic wastes. A review. Agronomy for Sustainable Development 39: 31. https://doi.org/10.1007/s13593-019-0577-z

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S. and Colombini, S., 2021. Impact of agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the Black Soldier Fly (Diptera: Stratiomyidae) larvae. Journal of Insect Science 21: 8. https://10.1093/jisesa/ieaa148

Gavahian, M., Mathad, G.N., Pandiselvam, R., Lin, J. and Sun, D.W., 2021. Emerging technologies to obtain pectin from food processing by-products: a strategy for enhancing resource efficiency. Trends in Food Science and Technology 115: 42-54. https://doi.org/10.1016/j.tifs.2021.06.018

Girotto, F. and Piazza, L., 2022. Food waste bioconversion into new food: A mini-review on nutrients circularity in the production of mushrooms, microalgae and insects. Waste Management Resources 40: 47-53. https://doi.org/10.1177/0734242X211038189

Gottstein, V., Bernhardt, M., Dilger, E., Keller, J., Breitling-Utzmann, C.M., Schwarz, S., Kuballa, T., Lachenmeier, D.W. and Bunzel, M., 2021. Coffee silver skin: chemical characterization with special consideration of dietary fiber and heat-induced contaminants. Foods 10: 1705. https://doi.org/10.3390/foods10081705

Grgas, D., Rukavina, M., Bešlo, D., Štefanac, T., Crnek, V., Šikić, T., Habuda-Stanić, M. and Landeka Dragicevic, T., 2023. The bacterial degradation of lignin – a review. Water 15: 1272. https://doi.org/10.3390/w15071272

Hahn, T., Tafi, E., Paul, A., Salvia, R., Falabella, P. and Zibek, S., 2020. Current state of chitin purification and chitosan production from insects. Journal of Chemical Technology & Biotechnology 95: 2775-2795. https://doi.org/10.1002/jctb.6533

Hogsette, J.A., 1992. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294. https://doi.org/10.1093/jee/85.6.2291

Huang, K.X., Zhou, L.Y., Chen, J.Q., Peng, N., Chen, H.X., Gu, H.Z. and Zou, T., 2023. Applications and perspectives of quaternized cellulose, chitin and chitosan: a review. International Journal of Biological Macromolecules 242: 124990. https://doi.org/10.1016/j.ijbiomac.2023.124990

Huys, G., Vanhoutte, T., Joossens, M., Mahious, A.S., De Brandt, E., Vermeire, S. and Swings, J., 2008. Coamplification of eukaryotic DNA with *16S rRNA* gene-based PCR primers: possible consequences for population fingerprinting of complex microbial communities. Current Microbiology 5: 553-557. https://doi.org/10.1007/s00284-008-9122-z

Jang, S. and Kikuchi, Y., 2020. Impact of the insect gut microbiota on ecology, evolution, and industry. Current Opinion in Insect Science 41: 33-39. https://doi.org/10.1016/j.cois.2020.06.004

Janssen, R.H., Vincken, J.-P., van den Broek, L.A.M., Fogliano, V. and Lakemond, C.M.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. Journal of Agricultural and Food Chemistry. 65: 2275-2278. https://doi.org/10.1021/acs.jafc.7b00471

Jucker, C., Lupi, D., Moore, C.D., Leonardi, M.G. and Savoldelli, S., 2020. Nutrient recapture from insect farm waste: bioconversion with *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Sustainability 12: 362. https://doi.org/10.3390/su12010362

Kariuki, E.G., Kibet, C., Paredes, J.C., Mboowa, G., Mwaura, O., Njogu, J., Masiga, D., Bugg, T.D.H. and Tanga, C.M., 2023. Metatranscriptomic analysis of the gut microbiome of black soldier fly larvae reared on lignocellulose-rich fiber diets unveils key lignocellulolytic enzymes. Frontiers in Microbiology 14: 1120224. https://doi.org/10.3389/fmicb.2023.1120224

Lachenmeier, D.W., Schwarz, S., Rieke-Zapp, J., Cantergiani, E., Rawel, H., Martín-Cabrejas, M.A., Martuscelli, M., Gottstein, V. and Angeloni, S., 2022. Coffee by-products as sustainable novel foods: report of the 2nd International electronic conference on foods "Future foods and food technologies for a sustainable world". Foods 11: 3. https://doi.org/10.3390/foods11010003

Lee, Y.G., Cho, E.J., Maskey, S., Nguyen, D.T. and Bae, H.J., 2023. Value-added products from coffee waste: a review. Molecules 28: 3562. https://doi.org/10.3390/molecules28083562

Liu, Z., Li, N., Zhou, X., Zheng, Z., Zhang, C., Liang, S., Li, Y., Yan, J., Li, Q. and Mu, S., 2022. Effects of fermented bamboo powder supplementation on serum biochemical parameters, immune indices, and fecal microbial composition in growing-finishing pigs. Animals 12: 3127. https://doi.org/10.3390/ani12223127

Lokman, I.H., Ibitoye, E.B., Hezmee, M.N.M., Goh, Y.M., Zuki, A.B.Z. and Jimoh, A.A., 2019. Effects of chitin and chitosan from cricket and shrimp on growth and carcass performance of broiler chickens. Tropical Animal Health and Production 51: 2219-2225. https://doi.org/10.1007/s11250-019-01936-9

López-Mondéjar, R., Zühlke, D., Větrovský, T., Becher, D., Riedel, K. and Baldrian, P., 2016. Decoding the complete arsenal for cellulose and hemicellulose deconstruction in the highly efficient cellulose decomposer *Paenibacillus* O199. Biotechnology for Biofuels 9: 104. https://doi.org/10.1186/s13068-016-0518-x

Lorenz, T.C., 2012. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. Journal of Visualized Experiments 22: e3998. https://doi.org/10.3791/3998

Lu, Y., Zhang, S., Sun, S., Wu, M., Bao, Y., Tong, H., Ren, M., Jin, N., Xu, J., Zhou, H. and Xu, W., 2021. Effects of different nitrogen sources and ratios to carbon on larval development and bioconversion efficiency in food waste treatment by Black Soldier Fly larvae (*Hermetia illucens*). Insects 12: 507. https://doi.org/10.3390/insects12060507

Luo, C., Li, Y., Chen, Y., Fu, C., Long, W., Xiao, X., Liao, H. and Yang, Y., 2019. Bamboo lignocellulose degradation by gut symbiotic microbiota of the bamboo snout beetle *Cyrtotrachelus buqueti*. Biotechnology for Biofuels 12: 70. https://doi.org/10.1186/s13068-019-1411-1

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. and Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. Journal of the Science of Food and Agriculture 98: 5776-5784. https://doi.org/10.1002/jsfa.9127

Narita, Y. and Inouye, K., 2014. Review on utilization and composition of coffee silverskin. Food Research International 61: 16-22. https://doi.org/10.1016/j.foodres.2014.01.023

Nolasco, A., Squillante, J., Esposito, F., Velotto, S., Romano, R., Aponte, M., Giarra, A., Toscanesi, M., Montella, E. and Cirillo, T., 2022a. Coffee silverskin: chemical and biological risk assessment and health profile for its potential use in functional foods. Foods 11: 2834. https://doi.org/10.3390/foods11182834

Nolasco, A., Squillante, J., Velotto, S., D'Auria, G., Ferranti, P., Mamone, G., Errico, M.E., Avolio, R., Castaldo, R., Cirillo, T. and Esposito, F., 2022b. Valorization of coffee industry wastes: comprehensive physicochemical characterization of coffee silverskin and multipurpose recycling applications. Journal of Cleaner Production 370: 133520. https://doi.org/10.1016/j.jclepro.2022.133520

Nyakeri, E.M., Ogola, H.J.O., Ayieko, M.A. and Amimo, F.A., 2017. Valorisation of organic waste material: growth performance of wild black soldier fly larvae (*Hermetia illucens*) reared on different organic wastes. Journal of Insects as Food and Feed 3: 193-202. https://doi.org/10.3920/JIFF2017.0004

OECD/FAO, 2022. OECD-FAO Agricultural Outlook 2022-2031. OECD Publishing, Paris, France. https://doi.org/10.1787/f1b0b29c-en

Ojha, S., Bußler, S. and Schlüter, O.K., 2020. Food waste valorisation and circular economy concepts in insect production and processing. Waste Management 118: 600-609. https://doi.org/10.1016/j.wasman.2020.09.010

Olivotto, I., Oliver, M.P. and Turchi, C., 2017. Larval diets and nutrition. In: Calado, R., Olivotto, I., Oliver, M.P. and Holt, G.J. (eds.) Marine ornamental species aquaculture. John Wiley & Sons, Inc., Hoboken, NJ, USA. https://doi.org/10.1002/9781119169147.ch9

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. and van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLOS ONE 10: e0144601. https://doi.org/10.1371/journal.pone.0144601

Osimani, A., Ferrocino, I., Corvaglia, M.R., Roncolini, A., Milanović, V., Garofalo, C., Aquilanti, L., Riolo, P., Ruschioni, S., Jamshidi, E., Isidoro, N., Zarantoniello, M., Cocolin, L., Olivotto, I. and Clementi, F., 2021. Microbial dynamics in rearing trials of *Hermetia illucens* larvae fed coffee silverskin and microalgae. Food Research International 140: 110028. https://doi.org/10.1016/j.foodres.2020.110028

Parodi, A., De Boer, I.J., Gerrits, W.J., Van Loon, J.J., Heetkamp, M.J., Van Schelt, J., Bolhuis, J.E. and Van Zanten, H.H., 2020. Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing – a mass balance approach. Journal of Cleaner Production 271: 122488. https://doi.org/10.1016/j.jclepro.2020.122488

Pimentel, A.C., Montali, A., Bruno, D. and Tettamanti, G., 2017. Metabolic adjustment of the larval fat body in *Hermetia illucens* to dietary conditions. Journal of Asia-Pacific Entomology 20: 1307-1313. https://doi.org/10.1016/j.aspen.2017.09.017

Pliantiangtam, N., Chundang, P. and Kovitvadhi, A., 2021. Growth performance, waste reduction efficiency and nutritional composition of Black Soldier Fly (*Hermetia illucens*) larvae and prepupae reared on coconut endosperm and soybean curd residue with or without supplementation. Insects 12: 682. https://doi.org/10.3390/insects12080682

Pu, G., Hou, L., Du, T., Wang, B., Liu, H., Li, K., Niu, P., Zhou, W., Huang, R. and Li, P., 2022. Effects of short-term feeding with high fiber diets on growth, utilization of dietary fiber, and microbiota in pigs. Frontiers in Microbiology 13: 963917. https://doi.org/10.3389/fmicb.2022.963917

Rimoldi, S., Ceccotti, C., Brambilla, F., Faccenda, F., Antonini, M. and Terova, G., 2023. Potential of shrimp waste meal and insect exuviae as sustainable sources of chitin for fish feeds. Aquaculture 567: 739256. https://doi.org/10.1016/j.aquaculture.2023.739256

Rodrigues, D.P., Ameixa, O.M., Vázquez, J.A. and Calado, R., 2022. Improving the lipid profile of black soldier fly (*Hermetia illucens*) larvae for marine aquafeeds: current state of knowledge. Sustainability 14: 6472. https://doi.org/10.3390/su14116472

Roux, K.H., 2009. Optimization and troubleshooting in PCR. Cold Spring Harbor Protocols 2009: pdb.ip66. https://doi.org/10.1101/pdb.ip66

Saini, A., Aggarwal, N.K., Sharma, A. and Yadav, A., 2015. Actinomycetes: a source of lignocellulolytic enzymes. Enzyme Research 2015: 279381. https://doi.org/10.1155/2015/279381

Sandström, V., Chrysafi, A., Lamminen, M., Troell, M., Jalava, M., Piipponen, J., Siebert, S., van Hal, O., Virkki, V. and Kummu, M., 2022. Food system by-products upcycled in livestock and aquaculture feeds can increase global food supply. Nature Food 3: 729-740. https://doi.org/10.1038/s43016-022-00589-6

Schader, C., Muller, A., Scialabba, N.E.H., Hecht, J., Isensee, A., Erb, K.H., Smith, P., Makkar, H.P., Klocke, P., Leiber, F., Schwegler, P., Stolze, M. and Niggli, U., 2015. Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. Journal of the Royal Society, Interface 12: 20150891. https://doi.org/10.1098/rsif.2015.0891

Schäfer, A., Konrad, R., Kuhnigk, T., Kämpfer, P., Hertel, H. and König, H., 1996. Hemicellulose-degrading bacteria and yeasts from the termite gut. Journal of Applied Bacteriology 80: 471-478. https://doi.org/10.1111/j.1365-2672.1996.tb03245.x

Sedgh-Gooya, S., Torki, M., Darbemamieh, M., Khamisabadi, H., Karimi Torshizi, M.A. and Abdolmohamadi, A., 2021. Yellow mealworm, *Tenebrio molitor* (Col: Tenebrionidae), larvae powder as dietary protein sources for broiler chickens: effects on growth performance, carcass traits, selected intestinal microbiota and blood parameters. Journal of Animal Physiology and Animal Nutrition 105: 119-128. https://doi.org/10.1111/jpn.13434

Sequino, G., Valentino, V., Torrieri, E. and De Filippis, F., 2022. Specific microbial communities are selected in minimally-processed fruit and vegetables according to the type of product. Foods 11: 2164. https://doi.org/10.3390/foods11142164

Siddiqui, S.A., Ristow, B., Rahayu, T., Putra, N.S., Yuwono, N.W., Mategeko, B., Smetana, S., Saki, M., Nawaz, A. and Nagdalian, A., 2022. Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Management 140: 1-13. https://doi.org/10.1016/j.wasman.2021.12.044

Smetana, S., Palanisamy, M., Mathys, A. and Heinz, V., 2016. Sustainability of insect use for feed and food: life cycle assessment perspective. Journal of Cleaner Production 137: 741-751. https://doi.org/10.1016/j.jclepro.2016.07.148

Smetana, S., Spykman, R. and Heinz, V., 2021. Environmental aspects of insect mass production. Journal of Insects as Food and Feed 7: 553-571. https://doi.org/10.3920/JIFF2020.0116

Stelmock, R.L., Husby, F.M. and Brundage, A.L., 1985. Application of Van Soest acid detergent fiber method for analysis of shellfish chitin. Journal of Dairy Science 68: 1502-1506. https://doi.org/10.3168/jds.S0022-0302(85)80989-9

Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.L. and Khanal, S.K., 2020. Rethinking organic wastes bioconversion: evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). Waste Management 117: 58-80. https://doi.org/10.1016/j.wasman.2020.07.050

Terra, W., 1990. Evolution of digestive systems in insects. Annual Reviews of Entomology 35: 181-200. https://doi.org/10.1146/annurev.en.35.010190.001145

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. and Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Animal Feed Science and Technology 259: 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

ur Rehman, K., Rehman, A., Cai, M., Zheng, L., Xiao, X., Somroo, A.A., Wang, H., Li, W., Yu, Z. and Zhang, J., 2017. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). Journal of Cleaner Production 154: 366-373. https://doi.org/10.1016/j.jclepro.2017.04.019

van Hal, O., De Boer, I.J.M., Muller, A., de Vries, S., Erb, K.H., Schader, C., Gerrits, W.J.J. and van Zanten, H.H.E., 2019. Upcycling food leftovers and grass resources through livestock: impact of livestock system and productivity. Journal of Cleaner Production 219: 485-496. https://doi.org/10.1016/j.jclepro.2019.01.329

van Selm, B., Frehner, A., de Boer, I.J., van Hal, O., Hijbeek, R., van Ittersum, M.K., Talsma, E.F., Lesschen, J.P., Hendriks, C.M.J., Herrero, M. and van Zanten, H.H., 2022. Circularity in animal production requires a change in the EAT-Lancet diet in Europe. Nature Food 3: 66-73. https://doi.org/10.1038/s43016-021-00425-3

Vandeweyer, D., Bruno, D., Bonelli, M., Ijdema, F., Lievens, B., Crauwels, S., Casartelli, M., Tettamanti, G. and De Smet, J.,

2023. Bacterial biota composition in gut regions of black soldier fly larvae reared on industrial residual streams: revealing community dynamics along its intestinal tract. Frontiers in Microbiology 14: 1276187. https://doi.org/10.3389/fmicb.2023.1276187

Veldkamp, T., van Rozen, K., Elissen, H., van Wikselaar, P. and van der Weide, R., 2021. Bioconversion of digestate, pig manure and vegetal residue-based waste operated by Black Soldier Fly Larvae, *Hermetia illucens* L. (Diptera: Stratiomyidae). Animals 11: 3082. https://doi.org/10.3390/ani11113082

Walker, S.P., Barrett, M., Hogan, G., Flores Bueso, Y., Claesson, M.J. and Tangney, M., 2020. Non-specific amplification of human DNA is a major challenge for *16S rRNA* gene sequence analysis. Scientific Reports 10: 16356. https://doi.org/10.1038/s41598-020-73403-7

Weimer, P.J., 2022. Degradation of cellulose and hemicellulose by ruminal microorganisms. Microorganisms 10: 2345. https://doi.org/10.3390/microorganisms10122345.

Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Applied and Environmental Microbiology 63: 3741-3751. https://doi.org/10.1128/aem.63.10.3741-3751.1997

Yanat, M. and Schroën, K., 2023. Advances in chitin-based nanoparticle use in biodegradable polymers: A review. Carbohydrate Polymers 312: 120789. https://doi.org/10.1016/j.carbpol.2023.120789

Yang, K., Qing, Y., Yu, Q., Tang, X., Chen, G., Fang, R. and Liu, H., 2021. By-product feeds: current understanding and future perspectives. Agriculture 11: 207. https://doi.org/10.3390/agriculture11030207

Zarantoniello, M., Zimbelli, A., Randazzo, B., Compagni, M.D., Truzzi, C., Antonucci, M., Riolo, P., Loreto, N., Osimani, A., Milanović, V., Giorgini, E., Cardinaletti, G., Tulli, F., Cipriani, R., Gioacchini, G. and Olivotto, I., 2020. Black Soldier Fly (*Hermetia illucens*) reared on roasted coffee by-product and *Schizochytrium* sp. as a sustainable terrestrial ingredient for aquafeeds production. Aquaculture 518: 734659. https://doi.org/10.1016/j.aquaculture.2019.734659