Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Sustainable Food Technol.*, 2025, **3**, 811

Insect-mediated valorisation of anaerobically digested aquaculture waste: bioconversion performances, nutritional composition and microbial safety of black soldier fly larvae†

Giacomo Rossi, [a] Shikha Ojha, [ab] Julia Hankel [c] and Oliver K. Schlüter *[ad]

Recent studies have suggested that fresh aquaculture waste (ASW) could be satisfactorily treated with black soldier fly larvae (BSFL). However, pre-treatments such as drying or dewatering, which significantly modified the chemical, physical and microbiological properties of the waste, were always applied. On the other hand, industrially generated aquaculture waste might be bulk-accumulated for a long time and may not always be suitable for pre-treatments. Therefore, the present study aimed to evaluate the ability of BSFL in converting bulk-accumulated ASW from an aquaculture industrial facility, while generating high quality and safe insects. Five substrates, consisting of different mixtures of ASW (0, 25, 50, 75, and 100%) and chicken feed, were prepared and offered to the larvae. Increasing amounts of ASW in diet resulted in progressively lower larval growth, with a final larval weight reducing from 162.65 ± 4.81 g (treatment 0ASW) to 91.48 ± 3.38 g (treatment 75ASW), while no growth was observed in the substrate 100ASW. Larvae raised on substrates containing high amounts of ASW showed decreased concentration of lipids (from 35.52 ± 1.21 (treatment 0ASW) to 17.27 ± 0.52 (treatment 75ASW) % dm) and protein (from 40.62 ± 0.61 (treatment 0ASW) to 35.87 ± 0.34 (treatment 75ASW) % dm), while the amount of ash increased from 11.03 ± 0.34 (treatment 0ASW) to 31.74 ± 0.08 (treatment 75ASW) % dm). The amino acid composition and fatty acid profile of BSFL appeared to be stable. High microbial contamination (total viable count ranging between 8.39 and 9.28 log CFU g^{-1}) was always detected in the reared larvae, although no pathogens were found in any sample. It was concluded that, although the current EU legislation does not allow the use of animal manure for rearing insects, BSFL could be satisfactorily used for managing anaerobically digested ASW, reducing waste while recovering nutrients. However, the presence of an amending material capable of improving the quality of the initial waste was needed. Obtained insects might be valorised as feed or utilised to extract nutritional components for incorporation into food, veterinary, pharmaceutical or agricultural products, boosting the transition to the circular economy.

Received 31st December 2024
Accepted 18th March 2025

DOI: 10.1039/d4fb00392f

rsc.li/susfoodtech

Sustainability spotlight

Food production generates a huge amount of waste, including animal manure. While livestock manure management techniques are widely studied, management of waste generated by fish production has been less investigated so far. Insects have been described as an interesting tool for converting waste while recovering important nutrients to be valorised in the food and feed sectors. Therefore, this work aims to promote a sustainable production of food, starting from otherwise lost resources. The work perfectly aligns with the SDGs 2 – zero hunger, 12 – responsible production and consumption, 14 – life below water, and 15 – life on land.

[a]*Department of Systems Process Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Max-Eyth-Allee 100, 14469, Potsdam, Germany. E-mail: oschlueter@atb-potsdam.de*

[b]*Department of Land Sciences, School of Science and Computing, South East Technological University, Cork Road, X91 K0EK, Waterford, Ireland*

[c]*University of Veterinary Medicine Hannover (TiHo), Institute for Animal Nutrition, Bischofsholer Damm 15, 30173 Hannover, Germany*

[d]*Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521, Cesena, Italy*

† Electronic supplementary information (ESI) available: ESI S1 A: chemical composition (% dry matter, average ± standard error, $n = 3$) of the raw material used in the experiment. ESI S1 B: fatty acid profile (% total fatty acid, average ± standard error, $n = 3$) of the raw material used in the experiment. ESI S2: regression analyses between ASW weight in initial diet (g) and final larval weight (mg). ESI S3 A: correlation analyses between larval growth and bioconversion performances and chemical quality of the substrates. ESI S3 B: correlation analyses between larval proximate composition and chemical quality of the substrates. ESI S3 C: correlation analyses between larval fatty acid and fatty acid profile of the substrates. ESI S3 D: correlation analyses between larval amino acids and amino acid profile of the substrates. ESI S4: microbial confirmation results from MALDI-ToF analyses. See DOI: https://doi.org/10.1039/d4fb00392f

Introduction

As the importance of aquaculture production is rising,[1] an increase in the production of aquaculture solid waste (a.k.a. aquaculture sludge; ASW) is inevitable. Such waste consists of uneaten feed and fish faeces[2] and is produced after concentration processes are applied to the aquaculture wastewater.[3] Being rich in nitrogen, phosphorus and organic matter, ASW may represent a threat for aquatic organisms and the environment.[4] Traditional systems of aquaculture waste management include landfill discharge, incineration, wetland and phytoremediation, aerobic and anaerobic composting, fermentation, chemical or enzymatic hydrolysis and fertilization.[5,6] Although characterized by high efficiency, these methods are associated with high capital or operating costs and/or generation of undesirable byproducts.[5] Modern technologies combining aquaculture with other organisms including plants and microorganisms have also been developed. Such methods, referred to as integrated multi-trophic aquaculture systems, aim to associate nutrients sequestration and waste management with biomass production.[5] Based on such a concept, the idea of using insects and heartworms for ASW management has been proposed.[7,8]

Several insect species have been described as capable of growing on decaying materials such as waste and manure.[9] Use of waste for rearing insects has been shown to be responsible for significantly reducing the global warming potential of traditional waste disposal technologies.[10] Among the different insect species, black soldier fly (*Hermetia illucens*) larvae (BSFL) were identified as the most promising one.[11] Biodegradation of waste performed by BSFL is faster than that of traditional waste management systems,[12] while the greenhouse gas emissions calculated as CO_2-equivalent are up to 47% lower than the emissions associated with aerobic composting.[13] Further advantages should then lie in the lower land needed for insect rearing in comparison to the other technologies[14] as well as in the possibility of recovering nutrients, which would otherwise be lost.

Recent studies have shown that ASW may be utilized by these larvae, although pure aquaculture waste did not result in satisfactory growth performances.[7,15] The physical state and the nutritional composition of the waste have been suggested as crucial variables,[16] with fresh ASW showing better insect growth than oven dried ASW.[17]

ASW originating from a pilot-scale RAS (recirculating aquaculture system) unit was used in a recent study by Rossi *et al.* (2023).[17] Since ASW was collected daily, it did not accumulate in sedimentation ponds, suggesting that its physio-chemical properties were not comparable to those of the ASW produced at the industrial scale, where bulk accumulation is expected.[18,19] Additionally, this study did not consider the quality of the generated insect biomass. Insects are indeed the main output of any insect-mediated bioconversion process; therefore, an investigation into nutrient recovery, microbial safety and chemical quality of the insects is essential for a transition to a circular economy.[11] Generated insect biomass might indeed be used for feeding animals as well for extracting single components to be used as raw materials for energy or drug production.[20–22]

Therefore, the present study first aimed to investigate the ability of BSFL to utilize anaerobically digested ASW as a feeding source. Furthermore, the chemical quality and the microbiological safety of the obtained insects were evaluated with the idea of using them as a novel feed source, reintroducing nutrients within the food supply chain.

Materials and methods

Raw material

ASW produced by an industrial inland aquaculture facility located at the Blankensee lake (Trebbin, Germany), consisting of a mixture of European catfish (*Silurus glanis*) and pikeperch (*Sander lucioperca*) manure, was used in this experiment. According to the farm layout, aquaculture waste, generated from 10 breeding tanks (overall volume 84 m^3) hosting approximately 500 animals (average age 1 year, average weight 300 g for pikeperch and 3 kg for catfish), was collected in conical compartments and mechanically drained to a settling tank. Excess water was removed and dewatered waste was collected in an anaerobic sedimentation pond (overall volume 4 m^3). Approximately 50 kg of six month old ASW were collected and submitted to a concentration process consisting of sedimentation (1 hour, room temperature) and filtration (Φ 0.2 mm). The final material was frozen at −18 °C until further use.

Black soldier fly eggs laid on corrugated cardboard were purchased from Hermetia Baruth GmbH (Baruth/Mark, Germany) and placed in a climate chamber at 30 ± 1 °C and 70 ± 5% RH for hatching. Neonate larvae were transferred into a 2 L polypropylene transparent box (Ikea Deutschland GmbH & Co. KG, Munich, Germany) half filled with commercial chicken feed (Deuka Companion GmbH & Co. KG, Dusseldorf, Germany) and moistened with distilled water at 50% of final moisture. Boxes were housed in a climate chamber set at 28 ± 1 °C, 65 ± 5% RH and 0 : 24 (L : D). Larvae were let develop for 6 days before the beginning of the experiment.

Experimental setup

Frozen ASW was thawed overnight at room temperature and hand-mixed. Following the experimental setup reported in Rossi *et al.* (2023),[17] five substrates consisting of different percentages (0, 25, 50, 75, and 100%) of ASW (dry matter = 25.01 ± 1.75%) and chicken feed (dry matter = 89.98 ± 0.19%) were prepared (Table 1). Distilled water was added when needed with the aim of reaching a final substrate dry matter of approximately 30%. For every mixture, the correct amount of each ingredient (ASW, chicken feed, distilled water) was calculated by considering a final substrate mass of 300 g. The computed masses were therefore directly added into a 2 L polypropylene rearing box and thoroughly hand mixed. As pure ASW (substrate 100ASW) showed a final moisture content higher than 70%, a surplus of material was added. It allowed ensuring the same feeding ratio in all the treatments (0.45 g-dry substrate per larvae), avoiding that the different total solid content could hinder the results.[17]

For each treatment, six boxes were prepared. Three boxes were used for evaluating growth, bioconversion performances

Table 1 Composition, dry matter (% of fresh matter) and gross energy content (kJ per g dry matter) of the experimental substrates

Treatment	ASW[a] (%)	CF[b] (%)	ASW (g)	CF (g)	Water (g)	Total weight (g)	Dry matter[c] (%)	Gross energy (kJ g^{-1})
0ASW	0	100	0.00	100.09	199.91	300.00	32.91 ± 1.36*a*	18.56 ± 0.93*a*
25ASW	25	75	30.79	92.38	176.83	300.00	33.20 ± 0.86*a*	18.14 ± 0.04*a*
50ASW	50	50	80.05	80.05	139.90	300.00	29.49 ± 0.77*ab*	15.05 ± 1.78*b*
75ASW	75	25	171.48	57.16	71.36	300.00	29.97 ± 0.18*ab*	14.03 ± 0.57*b*
100ASW	100	0	360.00	0.00	0.00	360.00[d]	25.56 ± 1.89*b*	8.17 ± 0.14*c*

[a] ASW, aquaculture solid waste. [b] CF, chicken feed. [c] Dry matter is expressed as mean ± standard error of 3 independent repetitions. [d] The higher amount of substrate provided in the 100ASW treatment aimed to correct for the lower feeding rate (calculated on dry matter basis) observed in the treatment.

and chemical quality of insects (3 biological replicates), while the other three were intended for microbial investigation (3 biological replicates). After preparation, each substrate was left to stabilize for 24 hours at 25 °C prior to adding the larvae. Such operation was needed to allow the free water to be absorbed by the dry components of the medium, resulting in a homogeneous substrate. After this period, two-hundred 6-day old larvae were added on top of each substrate and the boxes were closed with a polypropylene transparent lid (Ikea Deutschland GmbH & Co. KG, Munich, Germany) equipped with a rectangular 15 × 10.5 cm hole screened by polyester black mosquito 1 × 1 mm net (HaGa-Welt GmbH & Co. KG, Nordstemmen, Germany). All the boxes were transferred into a HGC 1514 V Simpac Fitotron (Vötsch Industrietechnik, Balingen-Frommern, Germany) chamber set at 27 ± 1 °C, 70 ± 5% RH, 0 : 24 (L : D) and let develop for 12 days, *i.e.* up to the appearance of the first prepupae in all the substrates.

Evolution of growth and bioconversion performances

In order to understand the suitability of anaerobically digested ASW to be used as a rearing substrate for BSFL, exact weights of the initial substrate, starting larvae, frass and mature larvae were recorded. Additionally, numbers of seeded and harvested larvae were also documented. Larvae performances were evaluated through the following equations (Rossi *et al.*, 2023):[17]

$$\text{Larval survival (\%)} = (n_{Lf}/n_{Li}) \times 100$$

$$\text{Assimilated feed (g)} = dW_s - dW_f$$

$$\text{Feed conversion ratio (FCR)} = \text{Assimilated feed}/(dW_{Lf} - dW_{Li})$$

$$\text{Bioconversion rate (\%, BCR)} = ((W_{Lf} - W_{Li})/W_s) \times 100$$

$$\text{Substrate reduction (\%)} = ((dW_s - dW_f)/dW_s) \times 100$$

where n_{Lf} and n_{Li} were the final and the initial larval number respectively, W_{Lf}, W_{Li} and W_s indicated the final larvae fresh weight, initial larvae fresh weight and substrate fresh weight, while dW_{Lf}, dW_{Li}, dW_s and dW_f stood for final larval dry weight, initial larvae dry weight, substrate dry weight and frass dry weight.

Larval growth on the different substrates was evaluated during the rearing experiment by sampling 10-larvae every second experimental day (day 2, 4, 6, 8, 10, 12). Such larvae were randomly picked up with forceps, cleaned with towel paper and weighed on an analytical scale (Sartorius AG, Goettingen, Germany), prior to be returned to the respective substrate.

Chemical analysis

Proximate analyses, including dry matter (DM), ash, total lipids, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), total carbon (C), total nitrogen (N) and total sulphur (S) of substrates and insects, were performed according to Rossi *et al.* (2023).[17] DM was determined by oven drying at 105 °C overnight, ash was quantified through sample incineration at 550 °C for 4H, while total lipids were extracted with petroleum ether by using the Soxhlet apparatus and gravimetrically measured. N was quantified with the Kjeldahl method and crude protein was computed by applying the nitrogen-to-protein conversion factors 5.70 for CF, 3.11 for ASW and 4.76 for BSF larvae. C and S were measured after catalytic combustion using the elemental analyser VARIO EL (Elementar Analysensysteme GmbH, Langenselbold, Germany), while NDF and ADF were gravimetrically measured after sample digestion with neutral (NDF) or acid (ADF) detergent solution. ADL was instead measured after further digestion in H_2SO_4. Gross energy was quantified through the bomb calorimetry method as reported in Hopper *et al.* (2023).[23] Hemicellulose, cellulose and lignin contents were calculated according to Peguero *et al.* (2023).[24] The amount of non-fibrous carbohydrates (NFC) was calculated as the difference between 100 and the sum of crude protein, total lipids, NDF and ash.

The fatty acid profile of substrates and larvae was determined according to Lepage and Roy (1986)[25] with modifications. Specifically, a defined amount of analytical material was placed in a glass tube and a methanol–hexane mixture was added. Subsequently, acetyl chloride was added and the sample was heated, followed by the addition of potassium carbonate solution. After centrifugation, the measurement was carried out by gas chromatography (GC TRACE 1300, Thermo Scientific, Dreieich, Germany).

The amino acid profile was determined by ion-exchange chromatography (biochrom 30+ Amino Acid Analyzer, Laborservice Onken GmbH, Gründau, Germany) after hydrolysation. In the case of methionine and cysteine, sample hydrolysis was preceded by oxidation.

All the chemical analyses were carried out in duplicate, and the results were mediated. As treatment 100ASW did not result

in satisfactory insect growth, BSFL originating from this treatment were not analysed.

Evaluation of microbial safety

The three boxes for each treatment intended for microbial analyses were prepared by following the same procedure as in Section 2.2, but ensuring the highest hygienic standards. Briefly, prior to adding the substrate and larvae, experimental boxes and utensils were carefully cleaned and disinfected with 70% ethanol. After disinfection, approximately 310 g (300 g for rearing + 10 g for analyses) of each substrate were prepared and left to stabilize for 24 hours. Before adding insects, approximately 10 g of substrate from each box were sampled with sterile spoons, placed in a Nasco Whirl-Pak sterile bag and frozen at −80 °C for further microbiological analysis. The remaining 300 g were seeded with 200 BSFL and incubated for 12 days.

After this period, approximately 10 g of mature larvae or prepupae were randomly picked up with sterilized forceps, washed in autoclaved distilled water, dried with autoclaved tissue and transferred into a Nasco Whirl-Pak sterile bag.

For microbial analyses, 3 g of material and 27 g of autoclaved buffered peptone water were weighed in a sterile stomacher bag and homogenised at speed 4 for 2 min. The homogenate was serially diluted with sterile buffered peptone water and plated on selective media, according to the ISO standards. Aerobic mesophilic bacteria (plate count agar incubated for 72 h at 30 °C, DIN EN ISO 4833-2:2013), yeasts and moulds (Bengal red chloramphenicol agar, 7 days at 25 °C, DIN EN ISO 21527-1:2008), Enterobacteriaceae (crystal violet neutral red bile glucose agar, 24 h at 37 °C, DIN EN ISO 21528-2:2009-1), *Escherichia coli* (Tryptone Bilex X-glucuronide agar, 4 h at 37 °C followed by 20 h at 44 °C, DIN EN ISO 16649-2:2001), *Clostridium perfringens* (tryptose-sulfite-cycloserine agar, 20 h at 37 °C and anaerobiosis, DIN EN ISO 7937) and coagulase positive Staphylococci (Baird-Parker agar, 48 h at 37 °C, DIN EN ISO 6888-1:1999) were evaluated through the traditional plate count method. Prevalence of *Salmonella* spp. (DIN EN ISO 6579), *Listeria monocytogenes* (DIN EN ISO 11290-1&2) and *Campylobacter* spp. (DIN EN ISO 10272-1) was also considered. MALDI-ToF MS (matrix assisted laser desorption/ionisation time of flight mass spectroscopy) equipped with the AnagnosTes SARAMIS database (bioMerieux Deutschland GmbH, Nürtingen, Germany) was used for confirming the microbial identity.[26] All the analyses were carried out in duplicate and the results were mediated.

Statistical analyses

One-way ANOVA followed by the Tukey HSD post hoc test was applied for computing statistical differences between treatments in terms of chemical quality, larvae performances and microbial composition. The general linear model (GLM) with "quasipoisson" distribution was applied when the absence of normality on model residuals was detected. GLM with binomial distribution was instead used for detecting differences on larval survival, while the general linear mixed model (GLMM) with substrate replicates as the random effect and observations nested within the same box was implemented for over-time larval growth evaluation. For all the analyses, a significance level of 0.05 was used. Spearman's rank correlation test was applied for evaluating the effect of substrate chemical composition on larvae performance as well as of substrate fatty acid and amino-acid profiles on larval fatty acid and amino–acid profiles. All the statistical analyses were implemented in R statistical environment version 4.1.0.[27]

Results and discussion

The aim of the present study was to evaluate the ability of BSFL to convert anaerobically digested ASW, as well as to investigate the quality and safety of the produced insects.

BSFL growth and waste bioconversion

Growth curves of BSFL reared on the different substrates are displayed in Fig. 1. While no statistically relevant differences attributable to the different treatments were computed between samples 0ASW, 25ASW, 50ASW and 75ASW (GLMM, Chi-Square = 4.9634, Df = 3, P = 0.174), no growth was detected in substrate 100ASW over 12 days of composting. GLMM analyses carried out on the growth curves highlighted no direct effect of the treatments, while interaction between treatment and time appeared highly significant (GLMM, Chi-Square = 131.6039, Df = 15, $P < 2 \times 10^{-16}$), suggesting that differences between treatments could be detected in some analytical days, but not in others. Specifically, as clearly displayed in Fig. 1, excluding the 100ASW treatment, all the other substrates performed similarly during the first 6 days of rearing, while a worsening of the larval growth in the substrate 75ASW was detected in the following days (P = 0.002). The main reason for these differences in growth, which resulted in significantly lower final larval weight in substrate 75ASW (P = 0.001, Table 2), can be attributed to the chemical composition of the ASW used in the present experiment. High amounts of ash and lignin and low levels of lipids and carbohydrates were indeed measured on this material, while the protein level and hemicellulose and cellulose contents were not different from chicken feed (ESI S1-A†). This

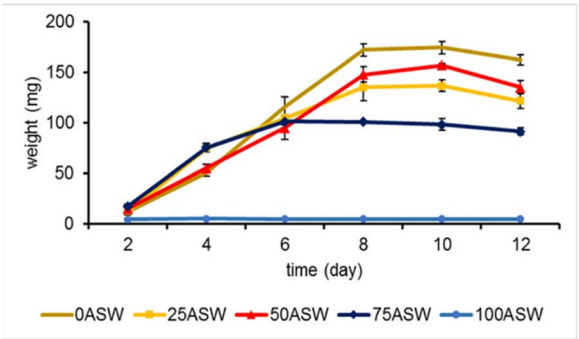

Fig. 1 Averaged weight increase trend of 10 BSF larvae reared on the experimental substrates. Error bars correspond to the standard error computed on 3 replicates per treatment.

Table 2 Growth and bioconversion performances (average ± standard error, $n = 3$) of BSFL reared on the experimental diets. Values within the same column followed by different letters are significantly different ($P < 0.05$)

Treatment	Larval survival (%)	Final weight (mg)	Assimilated feed (g)	FCR	BCR (%)	Substrate reduction (%)
0ASW	83.17 ± 1.92*b*	162.65 ± 4.81*a*	42.35 ± 2.17*a*	5.74 ± 0.75*b*	6.89 ± 0.39*a*	42.97 ± 1.26*a*
25ASW	78.50 ± 1.00*b*	121.82 ± 7.59*a*	48.92 ± 13.91*a*	8.82 ± 1.94*ab*	5.07 ± 0.33*bc*	43.95 ± 7.39*a*
50ASW	82.67 ± 1.96*b*	135.38 ± 6.53*a*	31.23 ± 1.32*b*	5.66 ± 0.25*b*	5.78 ± 0.01*ab*	35.27 ± 1.17*b*
75ASW	91.83 ± 1.01*a*	91.48 ± 3.38*b*	37.46 ± 0.55*ab*	13.11 ± 0.38*a*	4.34 ± 0.20*c*	42.08 ± 0.51*a*

composition might be due to the origin of the ASW as well as to the anaerobic digestion occurring during the stabilisation period in anaerobic settling ponds. ASW used in the present experiment indeed originated from two carnivorous fish species, which traditionally are fed with diets rich in protein and poor in carbohydrates and lipids.[28] It is therefore conceivable that manure from these species contained relatively low amounts of lipids and carbohydrates. The following anaerobic digestion might have further worsened this condition with anaerobic microorganisms responsible for consuming significant amounts of organic matter,[29–31] generating an ash-rich material.

As BSFL growth is negatively correlated with the ash level in the diet,[32,33] and proper equilibrium of proteins, lipids and carbohydrates is important for supporting larval development,[34] it is conceivable that substrates rich in ASW resulted in poor larval growth. Ash is a non-energetic component of the diet, whose presence is responsible for limiting the amount of energy contained in the substrate and available for larval growth and metabolic activities (Table 1). The strong positive correlation ($P < 0.001$, adjusted $R^2 = 0.79$) computed between final larval weight and energy content of the substrate further supports this observation (ESI S3†).

Addition of increasing percentages of chicken feed to ASW led to a progressive reduction of ash ($P < 0.001$), while the levels of carbohydrates ($P = 0.003$) and lipids ($P < 0.001$) increased (Fig. 2). Such conditions directly affected the larval growth (Table 2) as suggested by the significant linear correlation (adjusted $R^2 = 0.930$) computed between the weight of ASW in diet and the final larval weight (ESI S2†). Increasing lipid and carbohydrate levels in diet also resulted in a better substrate consumption by the BSFL, as suggested by the higher assimilated feed ($P = 0.012$) computed for substrates 0ASW and 25ASW. However, even though an evident direct relationship was observed between ASW in diet and assimilated feed (Table 2), no correlation was computed between this parameter and lipids or carbohydrates in diet (ESI S3-A†). This suggests that other variables such as pH (Bohm *et al.*, 2022)[35] and the carbon to nitrogen (C/N) ratio[36] should be considered. While pH was not evaluated in the present study, the C/N ratio was stable between the different treatments, with values ranging between 10.32 and 13.20 (except for substrate 100ASW, ESI S1-A†), which indicates high nutrient balance and therefore optimum conditions for BSFL growth and substrate utilization.[37,38]

No direct correlation was computed between substrate reduction and chemical properties of the substrates, albeit a progressive worsening of performances was observed when higher levels of ASW were included in the diet. Noteworthy, the highest level of ASW inclusion (treatment 75ASW) resulted in a substrate reduction not significantly different from the substrate 0ASW, indicating that while high ASW in diet is not suitable for insect biomass production, it might be a viable possibility for ASW management. It might be explained by the higher larval survival and microbial activity recorded in this substrate. Several microorganisms are indeed known for degrading indigestible components of the diet, allowing a more exhaustive utilisation of the rearing substrate by the larvae,[11] while higher larval survival may indicate higher larval activity.[39] However, while yeast and moulds recorded on substrate 75ASW were significantly lower than in the other substrates (as discussed in the Microbial evaluation of BSFL section), suggesting low microbial contribution on the substrate reduction, larval survival on the same treatment was significantly higher (Table 2). Such a condition, together with the low BCR and high FCR computed in substrate 75ASW, led to conclude that ASW was mainly indigested by the BSFL, which however were the main contributors to the substrate utilisation.

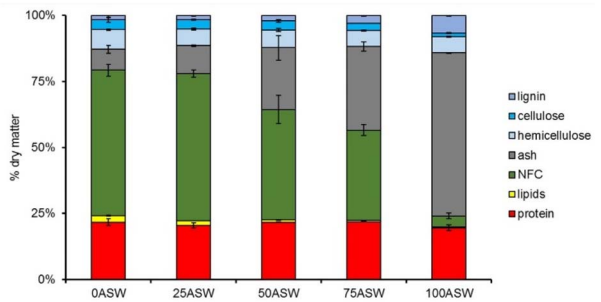

Fig. 2 Chemical composition (% DM) of diets used for BSFL rearing. Error bars correspond to the standard error computed on 3 replicates per treatment.

Nutritional quality of BSFL

Rearing substrates greatly affected the insects' proximate composition (Table 3). Progressive decreases in dry matter ($P < 0.001$), protein ($P < 0.001$) and lipid ($P < 0.001$) contents were noted when higher amounts of ASW were included in the diet. On the other hand, ash content increased ($P < 0.001$), following a similar trend to the diet. Such observations are consistent with already published data, which shows high dependence of the insect chemical composition on the substrate chemical composition.[40–42]

Table 3 Proximate composition (% dry matter, average ± standard error, $n = 3$) of BSFL reared on different experimental diets. Values within the same row followed by different letters are significantly different ($P < 0.05$)

Parameter	0ASW	25ASW	50ASW	75ASW
Dry matter (% fresh matter)	36.43 ± 1.02*a*	33.28 ± 0.29*b*	31.84 ± 0.15*b*	22.24 ± 0.32*c*
Lipids	35.52 ± 1.21*a*	27.26 ± 1.63*b*	26.07 ± 0.88*b*	17.27 ± 0.52*c*
Protein	40.62 ± 0.61*a*	39.86 ± 0.55*a*	37.54 ± 0.38*b*	35.87 ± 0.34*b*
Ash	11.03 ± 0.34*d*	17.19 ± 0.61*c*	22.58 ± 0.41*b*	31.74 ± 0.08*a*
NDF	12.84 ± 0.87	15.69 ± 1.40	13.81 ± 0.78	15.11 ± 0.42
ADF	8.54 ± 0.25	9.58 ± 0.52	8.14 ± 0.31	8.16 ± 0.18
ADL	1.60 ± 0.13	2.05 ± 0.14	1.74 ± 0.14	1.59 ± 0.14

While larval dry matter appeared for being strongly affected by several substrate parameters (ESI S3-A†), with lower dry matter measured in insects growth on the substrate with lower dry matter and energy content, larval fat was significantly correlated with volatile solids ($P = 0.001$), lipids ($P < 0.001$) and gross energy ($P = 0.009$) contained in the substrate. A direct relationship was also detected between insect lipids and amount of carbohydrates in the diet ($P = 0.024$), although the correlation coefficient was lower than 0.7 (ESI S3-B†). No correlation was observed between insect lipids and dietary protein ($P = 0.308$). These observations are in line with previous studies[31,43–45] and confirm that BSFL are able to assimilate dietary lipids[46] as well as to convert carbohydrates in lipids.[47] However, as the lowest lipid content was measured for the BSFL showing the lowest BCR, specific conditions, such as adequate feed availability and dietary digestibility, should be addressed. As a low BCR indicates ineffective consumption of the substrate,[48] an apparent food limitation might be hypothesized in treatment 75ASW. Such a condition, which might be due to the high ash and indigestible fibres in the diet, has already been described as responsible for the production of larvae with low fat reserves[49] and seems to be satisfactory for explaining the lower lipids in BSFL growth on substrates containing higher amounts of ASW.

Crude protein in BSFL produced in the present study was in line with previous research studies, where values ranged between 35 and 49% were often recorded.[50,51] Differences in the protein content might be explained by the different larval growth efficiency observed in different substrates. A lower crude protein level was indeed computed for BSFL reared on substrates containing high ASW, *i.e.* the substrates that resulted in smaller and lighter larvae, and several studies have shown that the protein level in BSFL increased fast in the first few days of life, and less during the fattening stage.[17,52]

The ash level in larvae was observed to mimic the ash level in diet ($P < 0.001$). It confirms the observation of Ewald *et al.* (2020)[53] and Liland *et al.* (2017)[54] for BSFL reared on mussel waste and algae. Similar results have also been obtained by Spranghers *et al.* (2017)[31] for BSFL reared on anaerobically digested vegetable waste and Rossi *et al.* (2023)[17] when growing percentages of fresh ASW were included in the diet. The most suitable explanation for such differences should be the presence of some undigested substrate in the larval gut.[41]

Concerning the fatty acid profile, addition of ASW to the diet led to a reduction in SFA ($P < 0.001$) and increase in MUFA ($P < 0.001$) and PUFA ($P < 0.001$) (Table 4). Despite this, no critical effects were observed in insects, with only significant difference detected in BSFL raised on substrate 75ASW (Table 4). Similar results were also obtained by Liland *et al.* (2023)[15] for BSFL reared on substrates containing increasing amounts of oven dried ASW, although raising amounts of ASW in diet led to an increase in MUFA but not PUFA in insects. On the other hand, in our study, BSFL produced on the substrate richer in ASW did show approximately 20% more PUFA than BSFL from treatment 0ASW. This is in line with previous findings and confirm that the fatty acid profile of BSFL can be modified by the diet,[55] although correlation was only found for few fatty acids (ESI S3-C†). However, although ASW appeared to be an important source of PUFA n-3 (ESI S1-B†), and higher percentages of these fatty acids were measured in substrates richer in ASW (Table 4), BSFL raised on substrates containing ASW did not show any increase of these fatty acids. Significantly higher concentrations of PUFA n-6 were instead detected for larvae harvested from substrates with higher amounts of ASW ($P < 0.001$). These data, on the one hand, confirm the inability of BSFL to synthesize PUFA n-3,[54] while on the other hand show a strong preference of BSFL to accumulate PUFA n-6.

Considering individual fatty acids, lauric acid was the most abundant in all the larvae, even though it was quite low in the substrates. This observation is in line with Fitriana *et al.* (2022),[51] confirming that lauric acid can be actively synthesized by BSFL. However, the amount of lauric acid measured in BSFL from treatment 75ASW was approximately half of the other treatments (Table 4). Such differences might be explained by the lower level of carbohydrates measured in substrate 75ASW (Fig. 2). Carbohydrates are the precursors of acetyl-CoA, a molecule crucial for the synthesis of lauric acid.[48] Despite this, since the amount of lauric acid measured in 75ASW insects was very high in comparison with the rearing substrate, simple bioaccumulation may not be a satisfactory explanation. An alternative hypothesis might therefore be connected to a possible oxidation of UFA present in the initial substrates.[54,56] The lower amount of lauric acid in BSFL from the treatment 75ASW was offset by the higher concentrations of palmitic and oleic acids, which were almost double that from insects harvested from the other substrates (Table 4). An inverse correlation between lauric and palmitic acid in BSFL has already been

Table 4 Fatty acid profile (% total fatty acids, average ± standard error, $n = 3$) and amino acid profile (% crude protein, average ± standard error, $n = 3$) of experimental substrates and BSFL reared on the correspondent experimental diet. Values within the same row followed by different letters are significantly different ($P < 0.05$)

		0ASW	25ASW	50ASW	75ASW
Fatty acid[a]					
Butyric (4:0)	Substrate	<0.005	0.22 ± 0.01*b*	1.12 ± 0.11*b*	8.11 ± 0.76*a*
	BSFL	<0.005	<0.005	0.02 ± 0.02*a*	0.45 ± 0.40*a*
Capric (10:0)	Substrate	<0.005	<0.005	<0.005	<0.005
	BSFL	1.03 ± 0.01*a*	0.98 ± 0.01*ab*	0.92 ± 0.01*b*	0.71 ± 0.02*c*
Lauric (12:0)	Substrate	0.25 ± 0.00*b*	0.24 ± 0.01*b*	0.27 ± 0.01*b*	0.36 ± 0.01*a*
	BSFL	56.13 ± 0.86*a*	57.43 ± 0.62*a*	56.93 ± 0.43*a*	29.00 ± 0.84*b*
Myristic (14:0)	Substrate	0.24 ± 0.01*c*	0.30 ± 0.00*bc*	0.43 ± 0.01*b*	0.82 ± 0.06*a*
	BSFL	7.28 ± 0.11*b*	8.27 ± 0.11*a*	8.53 ± 0.06*a*	5.83 ± 0.14*c*
Myristoleic (14:1)	Substrate	<0.005	<0.005	<0.005	<0.005
	BSFL	0.09 ± 0.00*c*	0.08 ± 0.00*d*	0.10 ± 0.00*b*	0.15 ± 0.00*a*
Pentadecanoic (15:0)	Substrate	<0.005	<0.005	<0.005	<0.005
	BSFL	0.08 ± 0.00*c*	0.10 ± 0.00*c*	0.13 ± 0.00*b*	0.45 ± 0.01*a*
Palmitic (16:0)	Substrate	16.88 ± 0.11*d*	17.76 ± 0.17*c*	19.11 ± 0.05*b*	19.99 ± 0.17*a*
	BSFL	9.46 ± 0.14*b*	9.66 ± 0.11*b*	10.28 ± 0.12*b*	16.86 ± 0.29*a*
Palmitoleic (16:1)	Substrate	0.19 ± 0.00*b*	0.25 ± 0.01*b*	0.39 ± 0.01*b*	0.79 ± 0.10*a*
	BSFL	1.52 ± 0.03*c*	1.58 ± 0.01*c*	2.06 ± 0.03*b*	6.63 ± 0.08*a*
Margaric (17:0)	Substrate	<0.005	<0.005	<0.005	0.14 ± 0.07*a*
	BSFL	0.12 ± 0.00*c*	0.14 ± 0.00*bc*	0.16 ± 0.00*b*	0.36 ± 0.01*a*
Stearic (18:0)	Substrate	2.73 ± 0.03*c*	2.96 ± 0.11*bc*	3.19 ± 0.07*b*	4.09 ± 0.10*a*
	BSFL	1.40 ± 0.02*b*	1.39 ± 0.02*b*	1.50 ± 0.01*b*	3.52 ± 0.04*a*
Elaidic (18:1 n-9 *trans*)	Substrate	0.17 ± 0.02*b*	0.19 ± 0.01*bc*	0.24 ± 0.01*b*	0.45 ± 0.03*a*
	BSFL	0.06 ± 0.01*b*	0.06 ± 0.01*b*	0.12 ± 0.01*b*	0.18 ± 0.02*a*
Oleic (18:1 n-9 *cis*)	Substrate	25.58 ± 0.30*a*	25.59 ± 0.15*a*	24.69 ± 0.02*a*	21.81 ± 0.28*b*
	BSFL	8.79 ± 0.25*b*	7.78 ± 0.19*c*	7.86 ± 0.12*bc*	17.10 ± 0.29*a*
Linoleic (18:2 n-6 *cis*)	Substrate	49.85 ± 0.22*a*	48.17 ± 0.12*b*	45.39 ± 0.27*c*	37.03 ± 1.10*d*
	BSFL	12.88 ± 0.55*b*	11.51 ± 0.41*bc*	10.47 ± 0.18*c*	16.65 ± 0.79*a*
Arachidic (20:0)	Substrate	0.31 ± 0.00*c*	0.35 ± 0.00*bc*	0.42 ± 0.01*b*	0.63 ± 0.04*a*
	BSFL	0.08 ± 0.00*c*	0.09 ± 0.00*bc*	0.09 ± 0.00*b*	0.41 ± 0.01*a*
Gondoic (20:1)	Substrate	0.27 ± 0.01*c*	0.29 ± 0.00*c*	0.33 ± 0.00*b*	0.44 ± 0.02*a*
	BSFL	0.03 ± 0.00*a*	0.02 ± 0.00*a*	0.02 ± 0.00*a*	<0.005*b*
α-Linolenic (18:3 n-3)	Substrate	2.87 ± 0.05*a*	2.89 ± 0.02*a*	2.83 ± 0.01*a*	2.38 ± 0.12*b*
	BSFL	0.92 ± 0.03*a*	0.76 ± 0.02*b*	0.58 ± 0.01*c*	0.69 ± 0.04*bc*
Heicosanoic (21:0)	Substrate	<0.005	<0.005	<0.005	<0.005
	BSFL	0.03 ± 0.00*b*	0.07 ± 0.01*b*	0.10 ± 0.00*b*	0.42 ± 0.05*a*
Eicosadienoic (20:2)	Substrate	0.14 ± 0.01*c*	0.15 ± 0.00*c*	0.18 ± 0.00*b*	0.24 ± 0.00*a*
	BSFL	0.03 ± 0.00*a*	0.02 ± 0.00*b*	<0.005	<0.005
Behenic (22:0)	Substrate	0.32 ± 0.02*c*	0.38 ± 0.00*c*	0.56 ± 0.02*b*	1.08 ± 0.02*a*
	BSFL	0.07 ± 0.00*b*	0.06 ± 0.00*b*	0.07 ± 0.00*b*	0.38 ± 0.01*a*
Di-omo-γ-linolenic (20:3 n-6)	Substrate	<0.005*c*	<0.005*c*	0.22 ± 0.01*b*	0.29 ± 0.02*a*
	BSFL	<0.005	<0.005	<0.005	<0.005
Lignoceric (24:0)	Substrate	0.20 ± 0.02*b*	0.20 ± 0.00*b*	0.25 ± 0.01*ab*	0.50 ± 0.08*a*
	BSFL	<0.005	<0.005	0.02 ± 0.00*b*	0.11 ± 0.00*a*
EPA (20:5 n-3)	Substrate	<0.005	<0.005	<0.005	0.07 ± 0.07*a*
	BSFL	<0.005	<0.005	0.02 ± 0.00*b*	0.10 ± 0.01*a*
Nervonic (24:1)	Substrate	<0.005	0.08 ± 0.04*b*	0.17 ± 0.01*b*	0.29 ± 0.03*a*
	BSFL	<0.005	<0.005	<0.005	<0.005
DHA (22:6 n-3)	Substrate	<0.005	<0.005	0.21 ± 0.02*b*	0.58 ± 0.02*a*
	BSFL	<0.005	<0.005	<0.005	<0.005
SFA	Substrate	20.93 ± 0.11*d*	22.41 ± 0.20*c*	25.36 ± 0.22*b*	35.73 ± 1.23*a*
	BSFL	75.69 ± 0.82*a*	78.19 ± 0.60*a*	78.76 ± 0.34*a*	58.51 ± 1.10*b*
UFA	Substrate	79.07 ± 0.11*a*	77.59 ± 0.20*b*	74.64 ± 0.22*c*	64.27 ± 1.23*d*
	BSFL	24.31 ± 0.82*b*	21.81 ± 0.60*b*	21.24 ± 0.34*b*	41.49 ± 1.10*a*
MUFA	Substrate	26.20 ± 0.28*a*	26.39 ± 0.20*a*	25.82 ± 0.04*a*	23.77 ± 0.12*b*
	BSFL	10.49 ± 0.26*b*	9.52 ± 0.19*b*	10.17 ± 0.15*b*	24.05 ± 0.34*a*
PUFA	Substrate	52.87 ± 0.18*a*	51.21 ± 0.14*b*	48.82 ± 0.27*c*	40.50 ± 1.11*d*
	BSFL	13.83 ± 0.58*b*	12.29 ± 0.42*bc*	11.07 ± 0.19*c*	17.44 ± 0.84*a*
PUFA n-6	Substrate	50.00 ± 0.23*a*	48.32 ± 0.12*ab*	45.79 ± 0.26*b*	37.47 ± 1.08*c*
	BSFL	12.91 ± 0.55*b*	11.53 ± 0.41*bc*	10.47 ± 0.18*c*	16.65 ± 0.79*a*
PUFA n-3	Substrate	2.87 ± 0.05*b*	2.89 ± 0.02*ab*	3.04 ± 0.03*a*	3.03 ± 0.03*ab*
	BSFL	0.92 ± 0.03*a*	0.76 ± 0.02*b*	0.60 ± 0.01*c*	0.78 ± 0.06*ab*

Table 4 (*Contd.*)

		0ASW	25ASW	50ASW	75ASW
Amino acid[b]					
Aspartic acid	Substrate	9.06 ± 0.19*b*	9.41 ± 0.24*b*	9.75 ± 0.09*ab*	10.16 ± 0.08*a*
	BSFL	9.99 ± 0.20	9.39 ± 0.29	9.50 ± 0.14	10.20 ± 0.08
Threonine	Substrate	3.50 ± 0.04*c*	3.53 ± 0.07*bc*	5.35 ± 1.47*b*	4.10 ± 0.03*a*
	BSFL	4.87 ± 0.11*a*	3.67 ± 0.15*b*	3.98 ± 0.03*b*	3.91 ± 0.08*b*
Serine	Substrate	4.84 ± 0.09*b*	4.91 ± 0.10*b*	5.17 ± 0.08*ab*	5.36 ± 0.04*a*
	BSFL	4.29 ± 0.09*a*	3.30 ± 0.13*b*	3.54 ± 0.15*b*	4.34 ± 0.13*a*
Glutamic acid	Substrate	21.70 ± 0.43*a*	20.18 ± 0.06*b*	18.62 ± 0.18*c*	15.59 ± 0.22*d*
	BSFL	10.85 ± 0.56*b*	9.84 ± 0.68*b*	10.65 ± 0.43*b*	14.32 ± 0.24*a*
Glycine	Substrate	4.44 ± 0.06*c*	4.76 ± 0.06*c*	5.35 ± 0.12*b*	6.14 ± 0.13*a*
	BSFL	6.37 ± 0.16*ab*	6.07 ± 0.10*b*	6.10 ± 0.04*b*	6.90 ± 0.22*a*
Alanine	Substrate	4.95 ± 0.06*c*	5.29 ± 0.04*c*	5.89 ± 0.05*b*	6.80 ± 0.21*a*
	BSFL	7.51 ± 0.22*b*	7.41 ± 0.32*b*	7.43 ± 0.06*b*	9.64 ± 0.62*a*
Valine	Substrate	4.79 ± 0.03*c*	5.02 ± 0.01*b*	5.40 ± 0.06*a*	5.63 ± 0.07*a*
	BSFL	7.27 ± 0.22	7.22 ± 0.14	7.10 ± 0.07	7.57 ± 0.11
Cysteine	Substrate	1.72 ± 0.02	1.66 ± 0.14	1.60 ± 0.02	1.60 ± 0.04
	BSFL	0.64 ± 0.05*b*	0.63 ± 0.00*b*	0.74 ± 0.02*b*	1.18 ± 0.09*a*
Methionine	Substrate	1.39 ± 0.02	1.33 ± 0.10	1.24 ± 0.07	1.13 ± 0.07
	BSFL	1.90 ± 0.12	1.84 ± 0.04	1.93 ± 0.05	1.75 ± 0.07
Iso-leucie	Substrate	4.12 ± 0.08*b*	4.23 ± 0.06*ab*	4.48 ± 0.05*ab*	4.35 ± 0.04*a*
	BSFL	4.99 ± 0.13*b*	5.02 ± 0.05*ab*	5.05 ± 0.04*ab*	5.40 ± 0.08*a*
Leucine	Substrate	7.97 ± 0.10	8.10 ± 0.07	8.23 ± 0.08	8.03 ± 0.06
	BSFL	7.71 ± 0.20	7.42 ± 0.16	7.39 ± 0.07	8.02 ± 0.12
Tyrosine	Substrate	3.23 ± 0.05	3.12 ± 0.02	3.28 ± 0.05	3.37 ± 0.07
	BSFL	5.72 ± 0.05*a*	4.29 ± 0.28*b*	4.94 ± 0.33*ab*	5.55 ± 0.37*ab*
Phenylalanine	Substrate	4.97 ± 0.04	5.13 ± 0.04	5.17 ± 0.04	5.05 ± 0.07
	BSFL	4.79 ± 0.11	4.51 ± 0.06	4.54 ± 0.03	4.70 ± 0.07
Histidine	Substrate	2.58 ± 0.03	2.56 ± 0.03	2.51 ± 0.08	2.36 ± 0.03
	BSFL	3.57 ± 0.08	3.07 ± 0.14	3.09 ± 0.12	3.10 ± 0.22
Lysine	Substrate	4.73 ± 0.13	4.66 ± 0.14	4.81 ± 0.08	4.89 ± 0.04
	BSFL	6.19 ± 0.07*b*	6.14 ± 0.21*b*	6.43 ± 0.12*ab*	7.11 ± 0.29*a*
Arginine	Substrate	5.85 ± 0.12*a*	5.68 ± 0.17 *ab*	5.54 ± 0.02*ab*	5.28 ± 0.03*a*
	BSFL	4.94 ± 0.19*a*	4.02 ± 0.11*b*	4.53 ± 0.17*ab*	4.93 ± 0.14*a*
Proline	Substrate	6.88 ± 0.18*a*	6.90 ± 0.11*a*	6.49 ± 0.09*a*	5.67 ± 0.05*b*
	BSFL	6.24 ± 0.21	6.18 ± 0.21	6.04 ± 0.09	6.41 0.13

[a] Capronic (6:0), caprylic (8:0), undecanoic (11:0), pentadecenoic (15:1), margaroleic (17:1), linoleilaid (18:2 n-6 *trans*), γ-linolenic (18:3 n-6), erucic (22:1 n-9), eicosatrienoic (20:3 n-3), tricosanoic (23:0), arachidonic (20:4 n-6), docosadienoic (22:2) acids were measured but not detected neither in substrates nor in BSFL (limit of detection = 0.005%). [b] Taurine and hydroxyproline were measured but not detected neither in substrates nor in BSFL. Tryptophan was not measured.

observed by El-Dakar *et al.* (2021)[57] and may be due to a metabolic adaptation connected to the low percentage of carbohydrates in the diet.[56] The same explanation may also be adopted for oleic acid as significantly higher concentrations of this fatty acid ($P < 0.001$) have been found in BSFL raised on substrates containing high percentages of PUFA n-3 and low amount of carbohydrates.[41,55] Metabolization and oxidation of PUFA n-3 might on the other hand be the main reason for the higher concentration of linoleic acid in BSFL from substrate 75ASW ($P < 0.001$). Several studies have indeed observed that PUFA n-3 are only accumulated during the first hours of life of BSFL, while a progressive degradation of these fatty acids was noticed when longer rearing times were applied.[52,53,58]

Although statistical analyses showed some variability on amino acid profiles of BSFL (Table 4), differences between samples were much lower than between substrates. No correlation was indeed found between levels of amino acids in larvae and substrates (ESI S3-D†), suggesting that the rearing substrate did not affect the amino acid profile of the larvae. Such an observation is in accordance with Oonincx and Finke (2021),[59] who stated that amino acids in edible insects are independent of the diet. On the other hand, recent studies have shown the possibility of manipulating some amino acids such as methionine, arginine, leucine, threonine, lysine, valine and tryptophan.[42,60] The use of a uniform starter feed during the first 6 days of life might be the explanation for the absence of difference in the amino-acid profile in larvae from different substrates. BSFL are indeed known for synthesizing amino-acids at the beginning of their life, keeping the composition stable during the later stages.[61]

Microbial evaluation of BSFL

Microbial analyses performed in the present study showed that anaerobically digested ASW is an important source of

Table 5 Microbial counts (log CFU g^{-1}, average ± standard error, $n = 3$) of experimental diets and larvae growth on the respective experimental substrate. Values within the same column and referred to the same material (substrate or BSFL) followed by different letters are significantly different ($P < 0.05$)

Treatment		Total viable count	Yeasts & moulds	Enterobacteriaceae	*Escherichia coli*	*Clostridium perfringens*	Coagulase positive Staphylococci
0ASW	Substrate	8.18 ± 0.02*c*	5.49 ± 0.03*a*	7.96 ± 0.06*ab*	3.18 ± 0.02*a*	1.30 ± 0.47*c*	5.30 ± 0.10
	BSFL	8.39 ± 0.05	4.47 ± 0.13	8.14 ± 0.20	3.73 ± 1.25*b*	5.61 ± 0.88*a*	7.76 ± 0.11
25ASW	Substrate	8.74 ± 0.04*b*	5.23 ± 0.19*a*	8.16 ± 0.05*a*	3.39 ± 0.14*a*	5.45 ± 0.01*b*	5.24 ± 0.14
	BSFL	8.86 ± 0.31	5.57 ± 0.83	8.24 ± 0.14	5.23 ± 0.43*a*	2.94 ± 0.53*b*	8.00 ± 0.18
50ASW	Substrate	8.84 ± 0.04*ab*	3.47 ± 0.31*b*	7.87 ± 0.12*ab*	1.00 ± 0.33*b*	6.07 ± 0.07*a*	5.68 ± 0.13
	BSFL	8.99 ± 0.38	2.85 ± 0.95	8.40 ± 0.23	5.62 ± 0.68*a*	4.92 ± 0.16*a*	7.73 ± 0.38
75ASW	Substrate	8.97 ± 0.01*a*	3.49 ± 0.09*b*	7.75 ± 0.08*b*	<1	6.52 ± 0.03*a*	5.65 ± 0.07
	BSFL	9.28 ± 0.12	>5	7.72 ± 0.16	>5	>5	7.31 ± 0.17

microorganisms, especially Enterobacteriaceae and anaerobic bacteria (ESI S1-D†). Occurrence of *L. monocytogenes* was observed in pure ASW, while no *Salmonella* spp. was found in ASW. Mixing chicken feed with ASW led to a progressive dilution of these microorganisms, although total viable counts, as well as coagulase positive Staphylococci, were not severally affected (Table 5). Interestingly, while *L. monocytogenes* was detected in one of the three samples from substrate 75ASW, *Salmonella* spp. was only found and confirmed in all the samples from substrate 0ASW (ESI S4†).

According to the microbial contamination of the rearing substrate, high total viable counts were also detected in all the obtained insects with no apparent effect of the rearing substrate (Table 5). Such high contamination, which is in line with previous studies,[62,63] can be explained by the absence of an inactivation treatment and by the presence of the gut.[64,65]

No significant differences were observed in Enterobacteriaceae and coagulase positive Staphylococci counts, while increasing counts of *E. coli* were measured for BSFL raised on substrates with higher ASW levels (Table 5). Such microorganisms have already been reported in edible insects[66] and may raise safety concerns if not properly considered. However, while occurrence of *Staphylococcus* spp. was not confirmed in any samples, *E. coli* was clearly identified (ESI S4†). The presence of this species, which has been often found in edible insects, might be explained by the absence of a post-harvest sanitisation treatment.[64]

Presumptive *C. perfringens* were observed in all the samples (Table 5), with contamination levels extremely variable between the treatments ($P < 0.001$). However, MALDI-ToF analyses did not allow any confirmation (ESI S4†), leading us to conclude that *C. perfringens* was not present in the studied material. No *Salmonella* spp. were detected in any larvae sample, although these species were found in the 0ASW substrate. This result is in accordance with Wynants *et al.* (2019)[62] and Osimani *et al.* (2021),[63] who also did not observe the presence of *Salmonella* spp. in fresh larvae reared on organic waste. On the other hand, De Smet *et al.* (2021)[67] and Grisendi *et al.* (2022)[68] recorded high *Salmonella* spp. counts in BSFL reared on chicken feed artificially contaminated with *Salmonella*. Since microbial contamination of BSFL as well as their antimicrobial activity is extremely variable and highly affected by the rearing substrates and the environmental conditions,[62,69] we cannot conclude that *Salmonella* spp. will never be detected in BSFL reared on ASW. Microbial inactivation treatments should therefore be always carried out before allowing any use of BSFL, regardless of their rearing substrate.

L. monocytogenes was detected on BSFL harvested from substrate 50ASW. However, although MALDI-ToF analyses confirmed the occurrence of *Listeria* spp., it did not offer any confirmation at the species level (ESI S4†), leaving room for discussion. Indeed, while several non-pathogenic *Listeria* species have already been detected in edible insects, *L. monocytogenes* has never been detected.[70] To the best of the authors' knowledge, the only studies where the occurrence of *L. monocytogenes* was reported consisted of challenge experiments using artificially contaminated substrates.[68] Since the ASW used in the present study was naturally contaminated with *L. monocytogenes*, it cannot be assumed that this species is completely absent in the harvested insects, although previous studies suggest low chances of occurrence.

Conclusions

Although the current European Union legislation does not allow to rear insects on aquaculture waste, the present study clearly shows that anaerobically digested ASW can be managed by BSFL. However, compared with previous studies where fresh ASW was used, lower conversion ability was observed, suggesting that treatment of fresh ASW should be preferred. The main reason for the lack of insect growth observed in pure ASW (substrate 100ASW) was attributed to the anaerobic digestion, which resulted in low lipid and carbohydrate levels and high ash content, while the protein level remained constant. Addition of an amending material acting as the main source of carbohydrates and lipids has been shown to improve BSFL growth and enhance bioconversion performances, although the environmental sustainability of the overall process might be significantly reduced. Mixing ASW and CF in a 1 : 1 ratio (substrate 50ASW) did not show any significant reduction in larval growth, although lower feed assimilation and substrate reduction was recorded. Obtained materials (*i.e.* insect biomass and frass) might be valorised in several sectors, including feed

production or extraction of molecules with bioactive properties to be applied in pharmaceutical, medical, industrial or agriculture. BSFL reared on the substrate containing increasing concentrations of ASW displayed acceptable nutritional quality with regard to protein and lipid contents, although levels of crude protein and fat were lower than in insects reared on pure chicken feed. Amino-acid and fatty acid profiles were not severally affected, albeit significant differences were computed for insects raised on substrate 75ASW. High microbial counts were detected in all the produced insects, regardless of the rearing substrate. No pathogens were detected; however, their presence could not be neglected. Therefore, a sanitisation treatment is advised prior to further use of larvae.

Data availability

The data supporting this article have been included within the article and additionally as part of the ESI.†

Author contributions

Giacomo Rossi: conceptualization, data curation, investigation, methodology, project administration, validation, visualization, writing – original draft, writing – review & editing. Shikha Ojha: methodology, supervision, writing – review & editing. Julia Hankel: data curation, methodology, validation, writing – review & editing. Oliver K. Schlüter: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), Grant Number 2819ERA01M and the BlueBio ERA-NET COFUND on the Blue Bioeconomy—Unlocking the Potential of Aquatic Bioresource and the European Commission (AquaTech4Feed project) within the Horizon 2020 program. The authors would like to thank Mrs Giovanna Rehde from the Leibniz Institute for Agriculture Engineering and Bioeconomy and Mrs Julia Hausmann from the University of Veterinary Medicine Hannover for supporting the chemical analyses as well as Mrs Jessica Wegner and Mrs Beate-Kristin Kröck from the Leibniz Institute for Agriculture Engineering and Bioeconomy for coordinating the microbial investigation.

References

1 M. C. Bianchi, F. Chopin, T. Farme, N. Franz, C. Fuentevilla, L. Garibaldi and A. L. Laurenti, *The State of World Fisheries and Aquaculture*, Food and Agriculture Organization of the United Nations, Rome, 2014.
2 D. P. Bureau and K. Hua, *Aquacult. Res.*, 2010, **41**, 777–792.
3 S. B. Kurniawan, S. R. Abdullah, M. F. Imron, A. Ahmad, N. S. Mohd Said, N. F. Mohd Rahim, M. Mohammad Alnawajha, H. Abu Hasan, A. R. Othman and I. F. Purwanti, *Aquacult. Res.*, 2021, **52**, 2954–2962.
4 A. B. Dauda, A. Ajadi, A. S. Tola-Fabunmi and A. O. Akinwole, *Aquacult. Fish.*, 2019, **4**, 81–88.
5 Y. Wu and K. Song, *Environ. Eng.*, 2021, **147**(3), 03120012.
6 A. L. Ahmad, J. Y. Chin, M. H. Z. M. Harun and S. C. Low, *J. Water Process Eng.*, 2022, **46**, 102553.
7 E. Schmitt, I. Belghit, J. Johansen, R. Leushuis, E. J. Lock, D. Melsen, R. K. R. Shanmugam, J. Van Loon and A. Paul, *Animals*, 2019, **9**, 189.
8 J. Klein, A. Schüch, P. Sandmann, M. Nelles, H. W. Palm and A. Bischoff, *Sustainability*, 2023, **15**, 7429.
9 J. A. Cammack, C. D. Miranda, H. R. Jordan and J. K. Tomberlin, *J. Insects Food Feed*, 2021, **7**, 605–619.
10 S. A. Siddiqui, I. A. Harahap, J. Osei-Owusu, T. Saikia, Y. S. Wu, I. Fernando, R. Perestrelo and J. S. Câmara, *Process Saf. Environ. Prot.*, 2024, **187**, 1–25.
11 T. Liu, T. Klammsteiner, A. M. Dregulo, V. Kumar, Y. Zhou, Z. Zhang and M. Kumar Awasthi, *Sci. Total Environ.*, 2022, **833**, 155122.
12 H. Čičková, G. L. Newton, R. C. Lacy and M. Kozánek, *J. Waste Manage.*, 2015, **35**, 68–80.
13 A. Mertenat, S. Diener and C. Zurbrügg, *J. Waste Manage.*, 2019, **84**, 173–181.
14 R. Salomone, G. Saija, G. Mondello, A. Giannetto, S. Fasulo and D. Savastano, *J. Cleaner Prod.*, 2017, **140**, 890–905.
15 N. S. Liland, M. Sorensen, I. Belghit, F. Perera Willora, A. Torrissen and O. Torrissen, *J. Insects Food Feed*, 2023, **9**, 885–892.
16 A. Isibika, P. Simha, B. Vinneras, C. Zurbrügg, O. Kibazohi and C. Lalander, *Sci. Total Environ.*, 2023, **858**, 159985.
17 G. Rossi, S. Ojha, A. Müller-Belecke and O. K. Schlüter, *Sci. Rep.*, 2023, **13**, 20982.
18 N. Mirzoyan, R. C. McDonald and A. Gross, *J. World Aquacult Soc.*, 2012, **43**, 238–248.
19 S. F. Fu, D. H. Wang, Z. Xie, H. Zou and Y. Zheng, *Sci. Total Environ.*, 2022, **830**, 154654.
20 M. Oteri, A. R. Di Rosa, V. Lo Presti, F. Giarratana, G. Toscano and B. Chiofalo, *Sustainability*, 2021, **13**, 5447.
21 L. Van Moll, J. De Smet, A. Paas, D. Tegtemeier, A. Vilcinskas, P. Cos and L. Van Campenhout, *Microbiol. Spectrum*, 2022, **10**, e0166421.
22 K. Mohan, P. Sathishkumar, D. K. Rajan, J. Rajarajeswaran and A. R. Ganesan, *Sci. Total Environ.*, 2023, **859**, 160235.
23 Z. Hopper, B. Desbrow, S. Roberts and C. Irwin, *J. Food Drug Anal.*, 2023, **31**, 232–243.
24 D. A. Peguero, M. Gold, A. Endara, N. Mutian, C. Zurbrügg and A. Mathys, *J. Waste Manage.*, 2023, **160**, 123–134.
25 G. Lepage and C. C. Roy, *J. Lipid Res.*, 1986, **27**, 114–120.
26 A. Fröhling, S. Bussler, J. Durek and O. K. Schlüter, *Front. Microbiol.*, 2020, **11**, 884.
27 R-CoreTeam, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2020.

28 L. C. Sousa, B. S. Moromizato, V. do Nascimento Santana de Almeida, C. T. Miasaki, L. S. Takahashi and J. Dalbello Biller, *ACS Food Sci. Technol.*, 2020, **262**, 114382.
29 A. E. Cioabla, N. Pop, G. Trif-Tordai and D. G. Calinoiu, *J. Therm. Anal. Calorim.*, 2017, **127**, 515–523.
30 R. G. Chiquito-Contreras, L. Hernandez-Adame, G. Alvarado-Castillo, M. d. J. Martinez-Hernandez, G. Sanchez-Viveros, C. J. Chiquito-Conteras and L. G. Hernandez-Montiel, *Sustainability*, 2022, **14**, 7257.
31 T. Spranghers, M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq and S. De Smet, *J. Sci. Food Agric.*, 2017, **97**, 2594–2600.
32 S. B. Oddon, I. Biasato, A. Resconi and L. Gasco, *Ital. J. Anim. Sci.*, 2024, **23**, 331–341.
33 C. Lalander, S. Diener, C. Zurbrügg and B. Vinnerås, *J. Cleaner Prod.*, 2019, **208**, 211–219.
34 L. Pinotti and M. Ottoboni, *J. Insects Food Feed*, 2021, **7**, 585–596.
35 K. Bohm, G. A. Hatley, B. H. Robinson and M. J. Gutierrez-Gines, *Resour., Conserv. Recycl.*, 2022, **180**, 106149.
36 K. U. Rehman, A. Rehman, M. Cai, L. Zheng, X. Xiao, A. A. Somroo, H. Wang, W. Li, Z. Yu and J. Zhang, *J. Cleaner Prod.*, 2017, **154**, 366–373.
37 K. U. Rehman, M. Cai, X. Xiao, L. Zheng, H. Wang, A. A. Soomro, Y. Zhou, W. Li, Z. Yu and J. Zhang, *J. Environ. Manage.*, 2017, **196**, 458–465.
38 D. A. Cheon, T. Jang and K. P. Lee, *J. Insects Food Feed*, 2022, **8**, 481–493.
39 A. S. P. Paz, N. S. Carrejo and C. H. G. Rodríguez, *Waste Biomass Valorization*, 2015, **6**, 1059–1065.
40 K. Riekkinen, K. Väkeväinen and J. Korhonen, *Insects*, 2022, **13**, 590.
41 K. M. Eggink, I. Lund, P. B. Pedersen, B. W. Hansen and J. Dalsgaard, *PLoS One*, 2022, **17**, e0275213.
42 I. Yandi, R. C. Öztürk, M. Kocabas, I. Z. Kurtoglu and I. Altinok, *J. Insects Food Feed*, 2023, **9**, 557–567.
43 L. L. Wang, S. Wang, R. Yang, B. Zhang, L. Xu, Q. Hu, Z. Zhao and Z. Cao, *Sci. Total Environ.*, 2024, **912**, 169579.
44 K. Barragan-Fonseca, J. Pineda-Mejia, M. Dicke and J. J. A. van Loon, *J. Econ. Entomol.*, 2018, **111**, 2676–2683.
45 M. A. El-Dakar, R. R. Ramzy and H. Ji, *Sci. Total Environ.*, 2021, **772**, 145528.
46 K. B. Barragan-Fonseca, G. Gort, M. Dicke and J. J. A. van Loon, *J. Insects Food Feed*, 2021, **7**, 51–61.
47 A. Scala, J. A. Cammack, R. Salvia, C. Scieuzo, A. Franco, S. A. Bufo, J. K. Tomberlin and P. Falabella, *Sci. Rep.*, 2020, **10**, 19448.
48 A. Giannetto, S. Oliva, C. F. Ceccon Lanes, F. de Araujo Pedron, D. Savastano, C. Baviera, V. Parrino, G. Lo Paro, N. C. Spano, T. Cappello, M. Maisano, A. Mauceri and S. Fasulo, *J. Biotechnol.*, 2020, **307**, 44–54.
49 M. Tschirner and A. Simon, *J. Insects Food Feed*, 2015, **1**, 249–259.
50 K. C. Surendra, J. K. Tomberlin, A. van Huis, J. A. Cammack, L. H. L. Heckmann and S. K. Khanal, *Waste Manage.*, 2020, **117**, 58–80.
51 E. L. Fitriana, E. B. Laconi, D. A. Astuti and A. Jayanegara, *Bioresour. Technol.*, 2022, **18**, 101061.
52 F. G. Barroso, M. J. Sanchez-Muros, M. Segura, E. M. A. Torres, R. Ramos and J. L. Guil, *J. Food Compos. Anal.*, 2017, **62**, 8–13.
53 N. Ewald, A. Vidakovic, M. Langeland, A. Kiessling, S. Sampels and C. Lalander, *J. Environ. Manage.*, 2020, **102**, 40–47.
54 N. S. Liland, I. Biancarosa, P. Araujo, D. Biemans, C. G. Bruckner, R. Waagbo, B. E. Torstensen and E. J. Lock, *PLoS One*, 2017, **12**, e0183188.
55 D. G. A. B. Oonincx, S. Laurent, M. E. Veenenbos and J. J. A. van Loon, *Insect Sci.*, 2020, **27**, 500–509.
56 B. Hoc, M. Genva, M. L. Fauconnier, G. Lognay, F. Francis and R. Capparos Megido, *Sci. Rep.*, 2020, **10**, 11916.
57 M. A. El-Dakar, R. R. Ramzy, M. Plath and H. Ji, *J. Cleaner Prod.*, 2021, **278**, 123570.
58 D. P. Rodrigues, R. Calado, M. Pinho, M. R. Domingues, J. A. Vazquez and O. M. C. C. Ameixa, *J. Waste Manage.*, 2022, **141**, 183–193.
59 D. G. A. B. Oonincx and M. D. Finke, *J. Insects Food Feed*, 2021, **7**, 639–659.
60 A. Fuso, S. Barbi, L. I. Macavei, A. V. Luparelli, L. Maistrello, M. Montorsi, S. Sforza and A. Caligiani, *Foods*, 2021, **10**, 1773.
61 X. Liu, X. Chen, H. Wang, Q. Yang, K. U. Rehman, W. Li, M. Cai, Q. Li, L. Mazza, J. Zhang, Z. Yo and L. Zheng, *PLoS One*, 2017, **12**, e0182601.
62 E. Wynants, L. Frooninckx, S. Crauwels, C. erreth, J. De Smet, C. Sandrock, J. Wohlfahrt, J. Van Schelt, S. Depraetere, B. Lievens, S. Van Miert, J. Claes and L. Van Campenhout, *Microb. Ecol.*, 2019, **77**, 913–930.
63 A. Osimani, I. Ferrocino, M. R. Corvaglia, A. Roncolini, V. Milanovic, C. Garofalo, L. Aquilanti, P. Riolo, S. Ruschioni, E. Jamshidi, N. Isidoro, M. Zarantoniello, L. Cocolin, I. Olivotto and F. Clementi, *Food Res. Int.*, 2021, **140**, 110028.
64 S. Ojha, S. Bussler, M. Psarianos, G. Rossi and O. K. Schluter, *J. Insects Food Feed*, 2021, **7**, 877–900.
65 O. K. Schlüter, B. Rumpold, T. Holzhauser, A. Roth, R. F. Vogel, W. Quasigroch, S. Vogel, V. Heinz, H. Jäger, N. Bandick, S. Kulling, D. Knorr, P. Steinberg and K. H. Engel, *Mol. Nutr. Food Res.*, 2017, **61**, 1600520.
66 C. Garofalo, V. Milanovic, F. Cardinali, L. Aquilanti, F. Clementi and A. Osimani, *Food Res. Int.*, 2019, **125**, 108527.
67 J. De Smet, D. Vandeweyer, L. Van Moll, D. Lachi and L. Van Campenhout, *Food Res. Int.*, 2021, **149**, 110692.
68 A. Grisendi, F. Defilippo, C. Lucchetti, V. Listorti, M. Ottoboni, M. Dottori, A. Serraino, L. Pinotti and P. Bonilauri, *Foods*, 2022, **11**, 2208.
69 H. Vogel, A. Müller, D. G. Heckel, H. Gutzeit and A. Vilcinskas, *Dev. Comp. Immunol.*, 2018, **78**, 141–148.
70 D. Vandeweyer, J. De Smet, N. Van Looveren and L. Van Campenhout, *J. Insects Food Feed*, 2021, **7**, 807–822.