



Bioconversion of aquaculture waste blended with vegetable by-products using *Hermetia illucens* larvae: Process parameters and larval quality

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ABSTRACT

The constantly increasing aquaculture production generates high amounts of biowaste worldwide, which must be properly treated in order to keep aquaculture's footprint low. This study aimed at evaluating the bioconversion of aquaculture waste streams (RAS sludge, fish trimmings, and harvest macroalgae waste) using black soldier fly (*Hermetia illucens*, BSF) larvae and the quality of the resulting larval biomass. The study was conducted in a modified shipping container simulating a large-scale setting and the diets were formulated using brewery spent grains and cabbage as base, mixing in aquaculture waste, aiming to treat the highest inclusion rate possible. A bioconversion efficiency of above 20 %_{DM} and a material reduction generally above 55 %_{DM} were observed, while generating a larval biomass that was rich in protein (> 35 %_{DM}), essential amino acids and fatty acids. The larvae reared on fish trimmings (from an anchovy processing plant) had the highest crude fat content (29.0 ± 1.1 %_{DM}) in relation to other treatments, while the macroalgae waste dietary inclusion generated larvae with low fat content (14.7 ± 1.5 %_{DM} on average). Interestingly, it was observed that the addition of aquaculture wastes, even in small inclusion levels (between 15 % and 25 % on wet basis), reduced the concentration of saturated fatty acids in the larvae (especially lauric acid and pentadecanoic acid). It was concluded that BSF larvae are able to bioconvert varied aquaculture waste streams and it is possible to produce tailored larval biomass by adding such waste streams in their diets, enabling the production of a protein ingredient with specific traits to be used in aquafeeds.

1. Introduction

The global aquaculture production has been steadily increasing over the past decades and for the first time it surpassed the total volume of capture fisheries in 2022, reaching 94.4 million tonnes against 91.0 million tonnes (live weight equivalent) (FAO, 2024). This is an important milestone for this category of livestock production, which is advocated to have a lower carbon footprint in comparison to other livestock production, such as beef, dairy cows, pigs and sheep farming, due to lower water and land use and lower feed conversion ratios that result in reduced use of feed (Froehlich et al., 2018; MacLeod et al., 2020; Tsakiridis et al., 2020). As aquacultural production increases, waste generation follows at the same pace, rising public concern and threatening the sustainability of the sector if not properly managed, as thoroughly discussed by Dauda et al. (2019). Those authors identified the main sources of aquaculture waste, including solid waste (e.g. feed leftovers, animal carcasses and processing waste) and dissolved waste fractions (e.

g. sludge from closed systems and wastewater), and proposed strategies to mitigate the risks associated with these waste materials. As highlighted by Liu et al. (2024), special attention should be given to the treatment of aquaculture wastewater, as it might contain hazardous pollutants, including drugs, chemicals (e.g. antibiotics, anaesthetics and disinfectants), heavy metals and biological contaminants (e.g. enterotoxigenic *Escherichia coli* and *Salmonella* spp.). Additionally, the treatment of carcasses - including parts of animals after slaughter and dead individuals during the production - is of paramount importance, as they might also carry pathogens and other contaminants (Bao et al., 2019).

Distinct technologies are well-established for the treatment of both solid and dissolved wastes from aquaculture, including thermophilic composting for the treatment of carcasses, pond-derived solid wastes and sludges (Lopes et al., 2019; Tom et al., 2021) and anaerobic digestion for the same types of waste materials (Choudhury et al., 2022). However, an emerging technology that has been proven effective for the treatment of varying waste streams from aquaculture, including salmon

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carcasses (Lopes et al., 2020), shrimp carcasses (Hu et al., 2024), bio-flocs from shrimp production (Zhang et al., 2023), macroalgae waste (Liland et al., 2017) and sludge from a recirculating aquaculture system (RAS) (Rossi et al., 2023) is the bioconversion of waste with larvae of the black soldier fly (*Hermetia illucens*, BSF).

Despite current legal barriers existing in several regions around the world on the use of animal waste as feed substrate for BSF larvae rearing, scientific knowledge on the bioconversion of these wastes has been advancing significantly, showcasing the viability of this process in transforming waste into valuable feedstock (Belghit et al., 2024). Lopes et al., (2020b) demonstrated the capacity of BSF larvae to inactivate pathogenic *Salmonella* spp. and *Escherichia coli* in contaminated salmon carcasses, possibly as a result of the production of antimicrobial peptides produced by the larvae. Schmitt et al. (2019) demonstrated that, even though there is a risk of heavy metal accumulation in the larval biomass (especially cadmium), aquaculture sludge can be a feasible substrate for rearing BSF larvae, in case risks are mitigated; e.g. by mixing sludge with other feed substrates or blending the obtained larvae meal with other feed ingredients in order to produce feeds within the acceptable limits for certain compounds. These and other studies demonstrate that this technology safely transforms potentially contaminated waste into animal feed, seen that biological and chemical risks are normally highly mitigated after larval bioconversion, which should be considered when evaluating the risks associated with its adoption.

Despite the evidence of the efficiency of the process described above, it is noteworthy that most of the studies conducted with aquaculture waste streams were carried out on laboratory scale. In addition, several of these studies had established goals that were not directly related to the efficiency of the bioconversion process itself, but rather distinct topics, such as nutritional quality of the larval biomass and its effects in the growth of livestock when included as part of the animals' diets. Following these findings, this study was designed with the goal of evaluating the process efficiency of the bioconversion of various aquaculture waste streams (anchovy trimmings from a processing plant, post-harvest waste of two species of macroalgae and RAS sludge) with BSF larvae and the nutritional quality of the obtained larval biomass, in a setting that mimics a large-scale production (stacked 60 × 40 cm boxes with controlled ventilation and temperature). It is currently known that it is not possible to treat aquaculture waste alone as a single substrate for BSF larvae, but that the presence of such wastes, even at low concentrations, can improve process efficiency and larval proximate composition - as demonstrated by Lopes et al. (2020a) and Ewald et al. (2020) using rainbow trout (*Oncorhynchus mykiss*) carcasses. In addition, Liland et al. (2017) demonstrated that at high inclusion levels (> 50 % wet basis) of a brown macroalgae in the larval diet, the process efficiency was compromised as a whole. Thus, in this study it was hypothesized that aquaculture waste streams, including sludge, anchovy carcasses and algae waste, when provided as part of the feed substrate for BSF larvae, would result in an improvement of the process as a whole, especially in relation to the proximate composition of the larval biomass.

2. Materials and methods

2.1. Waste materials and larvae

Waste streams from selected aquaculture production sites in Sweden were used in this study. Anchovy trimmings were collected at the fillet production site at Orkla foods in Sweden (containing heads, bones and viscera); fish sludge was sampled in an Atlantic salmon RAS and provided by Smögen Lax Aquaculture AB; and freshly harvested algae (*Ulva lactuca* and *Saccharina latissima*) were provided by Nordic Seafarm®. In addition to the aquaculture-derived waste streams, two waste materials were also used in this study, namely cabbage from a local vegetable processing industry (Grönsakshallen Sorunda) and brewery spent grains from a local brewery (Tempel Brewery) located in Uppsala, Sweden. Whilst the spent grains and fish trimmings were used as collected from

their respective sources, the cabbage was ground in a fruit grinder fitted with 10 mm mesh, and the algae waste was processed in a manual grinder. Thus, the cabbage and algae were reduced to a particle size of approximately 10 mm before being used in the experimental diets.

The BSF larvae used in this experiment were obtained from a BSF rearing unit that has been continuously running since 2015 at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. Eggs were collected on a daily basis in the unit, to ensure seed larvae are the same age when used in experiments. As a standard procedure, eggs were collected and placed on top of a plastic box containing feed (a blend of chick feed and water with an approximate 70 % moisture content). Newly hatched larvae were then reared in this substrate for approximately five days, separated from the processed feed by sieving (1 mm mesh) and batch-counted for obtaining an estimation of the weight and number of seed larvae. In this way, it was possible to start the bioconversion process with precise numbers of larvae of the same age.

2.2. Experimental design

Each aquaculture waste stream was used in combination with brewery spent grains and cabbage to formulate diets aiming at the best possible treatment outcome. Therefore, four experimental diets were designed with each aquaculture waste (RAS sludge - RAS; fish trimmings - FsT; *U. lactuca* - ULV; *S. latissima* - SAC) mixed with cabbage and spent grains, in addition to one control diet that contained only spent grains and cabbage (CT). The amounts of each waste stream used in the larvae's diets are presented in Table 1. A pilot study was carried out to evaluate larval growth on some of the aquaculture waste streams (RAS sludge and fish trimmings) mixed with cabbage and spent grains. The algae waste was not used in the pilot study due to the low availability of those, which were preferentially used in the actual study. Based on the observations made in that trial (Supplementary Data), diets containing aquaculture waste were formulated with the aim of including as much aquaculture wastes as possible, while still maintaining an initial moisture content of around 70–75 % in the blends, a larval volatile solid (VS) feeding dose of around 0.1–0.2 g of VS per larva and a substrate depth of less than 6 cm. As demonstrated by Guidini Lopes et al. (2023), maintaining these parameters within specific ranges is crucial for an efficient bioconversion of organic materials by BSFL. Diets were provided in all treatments at a feed dose of 0.1125 g VS larva⁻¹, seen that in the pilot study (in which each larvae received 0.15 g VS) it was established that a substrate depth greater than around 6.5 cm prevented the substrate to substantially dry towards the end of the process. This is due to the bulky nature of brewery spent grains.

2.3. BSFL conversion process

The experiment was conducted inside a climate-controlled room with average temperature of 28.0 ± 1.5 °C and humidity of 55.0 ± 10.0 %. The bioconversion process was carried out in plastic boxes (60 × 40 × 12 cm), with larval densities ranging between 4.16 larvae cm⁻² (sludge-based diet, totalling 10,000 larvae box⁻¹) and 6.25 larvae cm⁻² (other substrates and CT diet, without aquaculture waste, totalling 15,000 larvae box⁻¹). Three replicates were assembled per treatment. The treatment containing sludge had a limitation in the amount of sludge that was possible to add into the box. If a density of 6.25 larvae cm⁻² would have been adopted (and consequently more waste would need to be provided), the moisture content of those boxes would have been > 90 %, which would likely compromise the treatment (Lalander et al., 2020), and thus a lower larval density was used. Boxes were placed in racks with a space of approximately 3 cm between them, and were randomly re-distributed every second day of experiment to ensure similar conditions over time across all experimental units. This approach was used to minimize variations in humidity and temperature within the experimental site, which could otherwise affect the bioconversion process in specific replicates depending on their position in the

Table 1

Summary of the treatments established in the study and the total amount of each waste stream (wet basis), including brewery spent grains, cabbage, sludge from a RAS system, fish trimmings, and harvest waste from *U. lactuca* and *S. latissima* production. The moisture contents of these biowastes were 74.9%, 85.9%, 98.4%, 55.6%, 80.7% and 89.0%, respectively.

	Brewery spent grains	Cabbage	RAS sludge	Fish trimmings	<i>U. lactuca</i> harvest waste	<i>S. latissima</i> harvest waste	Total feed load
CT	5652 g	3768 g	-	-	-	-	9420 g
RAS	4039 g	1009 g	1683 g	-	-	-	6731 g
FsT	3967 g	1803 g	-	1443 g	-	-	7213 g
ULV	4523 g	3166 g	-	-	1357 g	-	9046 g
SAC	4710 g	3297 g	-	-	-	1413 g	9420 g

CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* algae; SAC: diet assembled with *Saccharina latissima* algae.

racks. The total feed load was divided into two equal portions, provided at the first and fourth days of bioconversion. After the bioconversion was completed (9 days for the treatments CT, RAS and FsT and 10 days for ULV and SAC), larvae were separated from frass by manually sieving using different mesh sizes (2–10 mm), and the total larvae and frass biomass were recorded. The treatment was considered complete when the resulting frass (larvae faeces and digested substrate) was dry, and it appeared that larvae could be easily sieved from the frass. This explains the one-day difference in treatment time between CT, RAS and FsT, and ULV and SAC.

The maximum depth of the feeding substrates was observed immediately after the second feeding event, at which point it was measured with a ruler. It was recorded to be 3.6 ± 0.1 cm in CT, 3.4 ± 0.2 cm in RAS, 4.4 ± 0.2 cm in FsT, 5.9 ± 0.2 cm in ULV, and 5.5 ± 0.3 cm in SAC.

2.4. Sampling and analysis

All waste streams were sampled in triplicates for physical and chemical characterization. Samples were dried in an oven at 65 °C until constant weight for dry matter (DM) determination and then the dry samples were burned in a muffle oven in which the temperature was increased to 250 °C, over 30 min, held constant for 2 h and then increased to 550 °C for 4 h to determine their ash and VS content. In addition, the waste streams were analysed for their proximate composition in a credited laboratory, following the recommendations of EC Regulation 152/2009.

At each feeding event, one sample of 10 larvae were collected in each replicate to monitor larval growth. On the first and last day of experiment, the pH and electrical conductivity (EC) were measured in the material using a pH-meter (pH meter 913, Metrohm®) and a conductivity-meter (Conductometer 912, Metrohm®). For that purpose, 5 g of the substrate were dissolved into 20 mL of deionized water, mixed thoroughly for 1 min and left at room temperature for 1 h prior to analysis. One representative sample of larvae and frass was collected from each replicate (approximately 10 g) and evaluated for DM and VS content, according to the procedures described in section above. In addition, 300 g samples of larvae were collected, frozen (-20 °C) and sent to an accredited laboratory (Eurofins AB, Sweden) for analysis. Both the waste streams and the larvae were analysed for crude protein (Kjeldahl method) and crude fat (Soxhlet method using light petroleum as extraction solvent), according to the EC Regulation 152/2009. In addition, the waste materials were analysed for crude fibre according to the sequential method using neutral and acid detergents, while the larval biomass was evaluated for amino acids (following the ISO 13903:2005) and fatty acids (following the ISO 12966–2 and 12966–4). The crude protein of larvae was calculated using a conversion factor of 4.76, as suggested by Janssen et al. (2017).

2.5. Process efficiency assessment

The following process parameters were evaluated and calculated according to Guidini Lopes et al. (2023): bioconversion efficiency (BCE), which is a representation of how much waste was converted into larval

biomass (Eq. 1); material reduction (MatRed), which is the total waste biomass reduction after the bioconversion process (both expressed on dry matter basis) (Eq. 2); larval survival; and total yield of larval biomass and frass per experimental unit (expressed as kg of larvae/frass m⁻²).

Bioconversion efficiency on a dry matter basis (BCE_{DM}) was calculated as:

$$BCE_{DM} = 100 \times \frac{mDM_{larvae}}{mDM_{feedstock}} \quad (1)$$

where mDM_{larvae} and $mDM_{feedstock}$ is dry mass of larvae at the end of the experiment and of the initial feed substrate, respectively.

Material reduction on a dry matter basis (MatRed_{DM}) of the substrate was calculated as:

$$MatRed_{DM} = 1 - \frac{mDM_{frass}}{mDM_{feedstock}} \quad (2)$$

where mDM_{frass} and $mDM_{feedstock}$ is total dry mass of the final residue (frass) and the initial feedstock, respectively.

2.6. Statistical analysis

The normal distribution of parameters related to process efficiency (BCE_{DM}, MatRed_{DM}, survival, yields of frass and larvae, and larval final weight) and proximate composition of the larval biomass (DM, crude protein, crude fat and ash content) were evaluated using Shapiro-Wilk's test with a 5 % significance level. Homoscedasticity of variances was assessed using Levene's test, considering a 5 % significance level. As the data met the assumptions of normality and homogeneity of variance, a one-way ANOVA was performed. When $p < 0.05$, Tukey's *post-hoc* test was applied at a 5 % significance level to identify specific treatments with significant differences among each other. Considering that the amino acid and fatty acid composition of the larval biomass of each treatment was analysed as a single pooled sample from all replicates from respective treatments, no statistical analysis were conducted with that data, which were then simply presented graphically. All statistical analyses and graphical visualisations were done in R-studio (RStudio Team, 2024).

3. Results

The aquaculture-derived waste streams used in this study were characterized prior to assembling the experimental diets, in which they were combined with cabbage and spent grains. All organic materials were evaluated for DM content, crude protein, fat and fibre and ash (Table 2).

All the feed substrates were bioconverted by the BSFL within 9 (CT, RAS and FsT) or 10 (ULV and SAC) days. The larvae were easily sievable from the remaining frass, even though the presence of big particles (e.g. fibrous components of the algae) remained undigested and slightly hindered the sieving process in the ULV and SAC treatments. There was no significant difference ($p = 0.269$) in the pH of the initial mixtures (day 1) in the treatments with brewery spent grains and cabbage, with or

Table 2

Proximate composition of the blended waste materials used as feedstock for rearing black soldier fly larvae.

	Dry matter %	Crude protein %	Crude fat %	Crude fibre % _{DM}	Ash %	Volatile solids %
Fish trimmings	44.1 ± 1.2	25.8 ± 1.2	40.9 ± 0.9	1.4 ± 0.0	24.3 ± 1.9	75.5 ± 2.3
Sludge	3.2 ± 0.0	15.0 ± 0.3	6.8 ± 2.5	18.7 ± 4.4	73.9 ± 0.3	25.9 ± 0.3
<i>U. lactuca</i> waste	19.6 ± 4.1	9.4 ± 2.6	2.4 ± 1.1	6.6 ± 1.4	33.4 ± 1.5	65.2 ± 2.7
<i>S. latissima</i> waste	11.2 ± 0.5	14.6 ± 0.5	< DL	9.56 ± 1.0	30.0 ± 1.4	69.3 ± 1.7
Brewer spent grains	23.5 ± 0.6	24.4 ± 0.6	7.9 ± 0.1	35.3 ± 6.0	2.6 ± 0.4	97.4 ± 0.4
Cabbage waste	11.0 ± 0.3	19.9 ± 1.0	2.7 ± 1.2	-	7.7 ± 0.4	92.3 ± 0.4

DL: detection limit (0.1 g/100 g)

without aquaculture waste streams (4.97 ± 0.37 on average) (Fig. 1a). The pH increased to above 7.0 in all experimental units, although significant differences between treatments ($p = 0.0085$) was demonstrated (Fig. 1c). There was a significant difference in EC already on the first day ($p < 0.0001$), with the highest EC being observed in the FsT treatment ($3.80 \pm 0.18 \text{ mS cm}^{-1}$) (Figure b). At the end of the experiment, the

same treatment remained with the highest EC, reaching $23.20 \pm 2.09 \text{ mS cm}^{-1}$, while the EC was substantially lower in the other treatments; $9.01 \pm 1.87 \text{ mS cm}^{-1}$ on average in SAC, ULV, RAS and CT (Fig. 1d).

The bioconversion efficiency on a DM basis was similar among treatments, with a single significant difference being observed between SAC ($20.7 \pm 0.6 \%_{\text{DM}}$) and FsT ($24.5 \pm 1.3 \%_{\text{DM}}$) (Fig. 2a). The total material reduction was higher in the treatment assembled with RAS sludge ($69.2 \pm 3.6 \%_{\text{DM}}$), while still not being statistically different from the control (CT) ($63.6 \pm 1.0 \%_{\text{DM}}$). The lowest reduction was achieved in the ULV treatment $56.7 \pm 2.4 \%_{\text{DM}}$ (Fig. 2b). Considering these parameters on a wet basis, the highest bioconversion efficiency ($p < 0.05$) was observed in the FsT treatment ($20.1 \pm 0.9 \%$) compared to the other treatments, while material reduction did not differ significantly among treatments (Table S3). As for the yield of larval biomass, the RAS treatment resulted in the lowest yield per m^2 ($4.6 \pm 0.2 \text{ kg larvae m}^{-2}$), while there was no significant difference in the larval yield of the other treatments (on average $6.0 \pm 0.3 \text{ kg larvae m}^{-2}$) (Fig. 2c).

As expected, an opposite trend to the material reduction was observed for the frass yield, which was lower in the RAS treatment that generated $3.9 \pm 0.1 \text{ kg frass m}^{-2}$ (fresh basis). The highest frass yield was observed in the control (CT) ($7.9 \pm 0.5 \text{ kg frass m}^{-2}$) (Fig. 2d). The average survival was high ($94.5 \pm 7.3 \%$) with no significant difference ($p = 0.0906$) in the between any of the treatments (Fig. 2e). The final larval weight was higher for larvae reared in RAS ($118 \pm 7 \text{ mg larva}^{-1}$) in relation to FsT ($99.1 \pm 0.0 \text{ mg larva}^{-1}$) and ULV ($99.1 \pm 0.0 \text{ mg}$

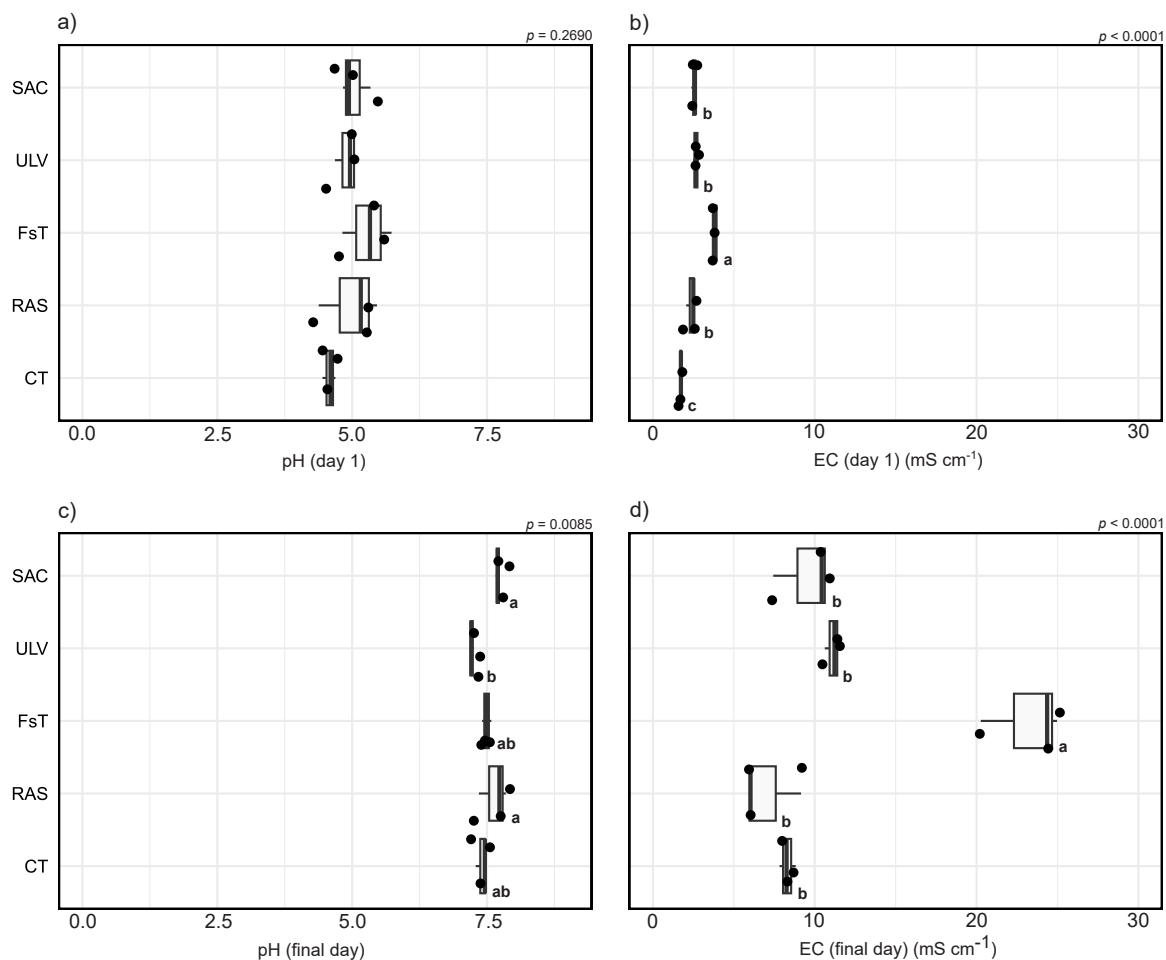


Fig. 1. The pH (a) and electrical conductivity (EC) (b) in the initial treatment mixtures (day 1) and the pH (c) and EC (d) in the harvested frass at the end of the treatment (day 9 for CT, RAS and FsT and day 10 for ULV and SAC). Different letters indicate significant differences between treatments for the same day of analysis, according to Tukey's *post-hoc* test at a 5 % probability level ($p < 0.05$). CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* algae; SAC: diet assembled with *Saccharina latissima* algae.

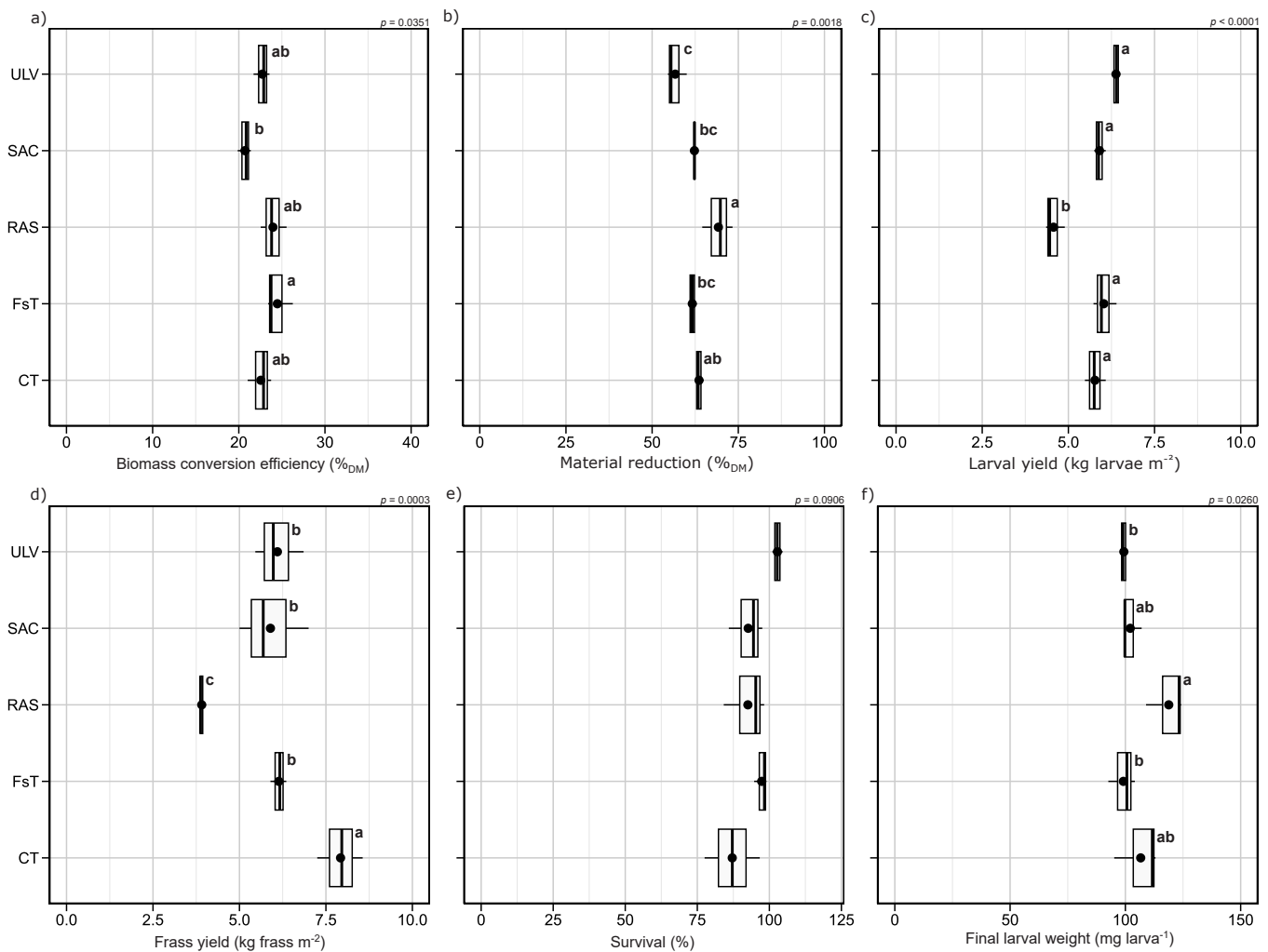


Fig. 2. Boxplots of the process efficiency parameters of the bioconversion of aquaculture waste streams with black soldier fly larvae: a) biomass conversion efficiency on a dry matter (DM) basis (%_{DM}); b) material reduction on a dry matter basis (%_{DM}); c) larval yield per treatment cycle (kg larvae m⁻²); d) frass yield per treatment cycle (kg frass m⁻²); e) larval survival (%); and f) final larval weight (mg larva⁻¹). The vertical lines in boxplots indicate median, the black point indicate the mean value, the left edge the 25th percentile and the right edge the 75th percentile. Different letters within the same variable indicate significant differences among treatments ($p < 0.05$). CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* algae; SAC: diet assembled with *Saccharina latissima* algae.

larva⁻¹), albeit not statistically different to the weight of larvae reared in SAC and CT (Fig. 2f).

Table 3

Proximate composition of black soldier fly larvae fed distinct diets containing aquaculture waste streams. Data are presented as mean \pm standard deviation. Different letters within the same variable indicate significant differences among treatments ($p < 0.05$). CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* algae; SAC: diet assembled with *Saccharina latissima* algae.

	Dry matter %	Crude protein % _{DM}	Crude fat % _{DM}	Ash % _{DM}
CT	29.4 \pm 0.2 ^a	41.5 \pm 0.4 ^a	21.3 \pm 1.7 ^b	7.2 \pm 0.1 ^b
RAS	24.7 \pm 0.3 ^b	39.1 \pm 0.3 ^b	17.3 \pm 1.1 ^{bc}	9.6 \pm 0.5 ^a
FsT	30.8 \pm 0.6 ^a	35.8 \pm 1.1 ^c	29.1 \pm 1.3 ^a	7.2 \pm 0.1 ^b
ULV	26.5 \pm 0.1 ^b	38.9 \pm 0.9 ^b	15.0 \pm 2.3 ^c	10.6 \pm 0.9 ^a
SAC	25.4 \pm 0.4 ^b	38.6 \pm 0.6 ^b	14.4 \pm 1.1 ^c	9.4 \pm 0.4 ^a

CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* algae; SAC: diet assembled with *Saccharina latissima* algae.

The proximate composition of the BSFL reared in different aquaculture wastes differed significantly (Table 3). Both the CT and FsT treatments resulted in larvae with higher DM contents (31.9 \pm 1.3 % on average) in relation to other treatments (27.5 \pm 2.3 % on average). Regarding crude protein, larvae reared in the CT treatment had the highest crude protein content (41.5 \pm 0.3 %_{DM}), while the larvae in FsT displayed the lowest 35.8 \pm 0.9 %_{DM}. Interestingly, the fat content of the larvae changed significantly depending on the aquaculture waste provided. Larvae fed fish trimmings had the highest fat content (29.0 \pm 1.1 %_{DM}), while the larvae in both the algae treatments had very low fat content (14.7 \pm 1.5 %_{DM} on average). As for the ash content, the larvae in treatments ULV, SAC and RAS had similar ash content (9.9 \pm 0.7 %_{DM} on average) and higher than both FsT and CT (7.2 \pm 0.1 %_{DM} on average) (Table 3).

The control treatment, with no aquaculture waste streams (CT), had the highest crude protein and that was also reflected in the amino acid composition of the obtained larval biomass, where several amino acids - including tyrosine, asparagine, leucine, lysine and arginine - were found in higher concentrations in the control larvae (Fig. 3). Nevertheless, the presence of fish trimmings in the larvae diet (FsT) seemed to favour the accumulation of proline and alanine and methionine. The generally lowest concentrations of amino acids were observed in the ULV

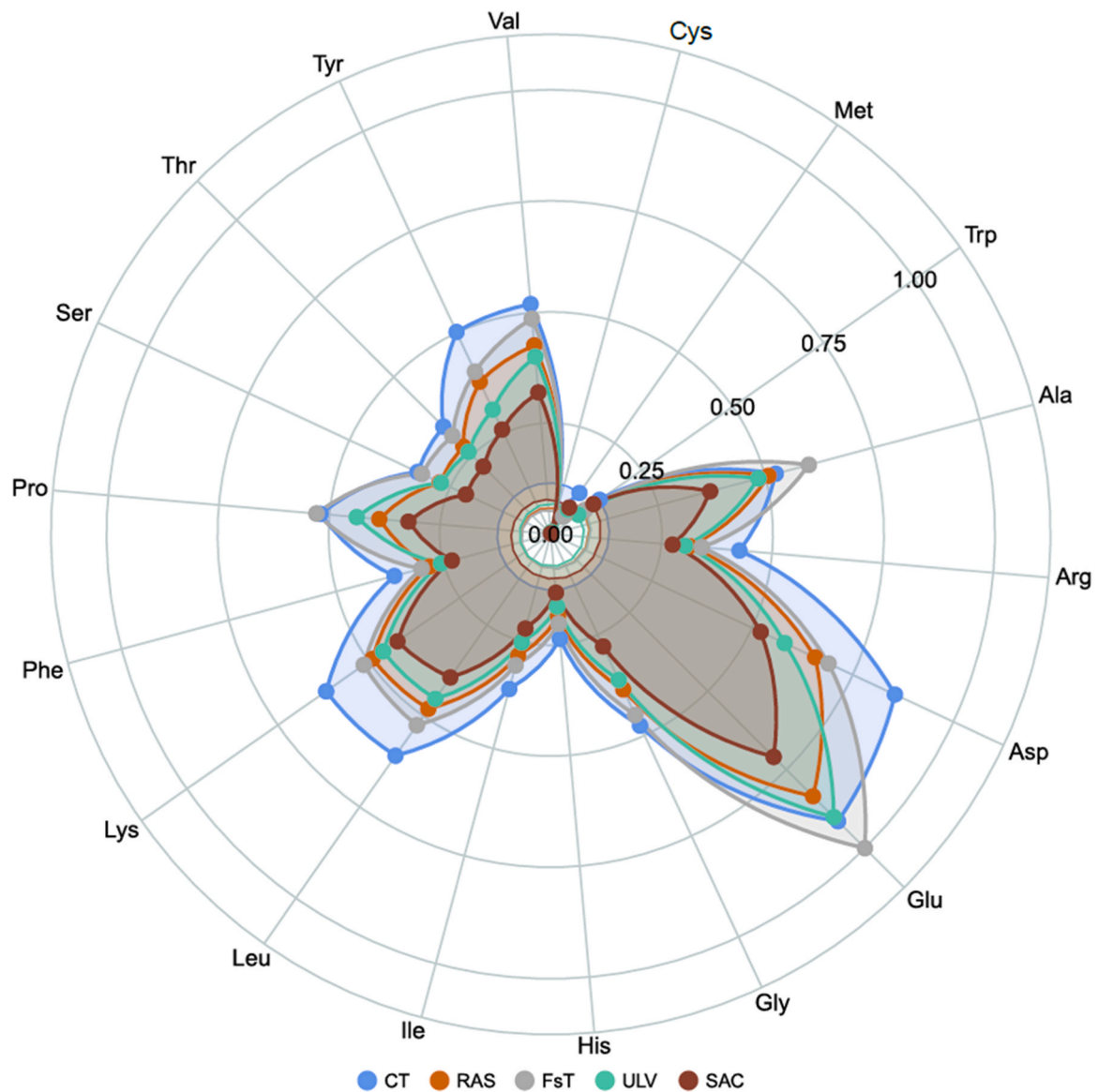


Fig. 3. Spider chart displaying the relative variation in amino acid concentrations of larvae across distinct treatments. The data for each amino acid was scaled to a range from 0 to 1 to facilitate the comparison and the plot thus represents the relative abundance of each amino acid within treatments. **Ala:** alanine; **Arg:** arginine; **Asp:** asparagine; **Cys:** cysteine; **Glu:** glutamic acid; **Gly:** glycine; **His:** histidine; **Ile:** isoleucine; **Leu:** leucine; **Lys:** lysine; **Met:** methionine; **Phe:** phenylalanine; **Pro:** proline; **Ser:** serine; **Thr:** threonine; **Trp:** tryptophan; **Tyr:** tyrosine; **Val:** valine. **CT:** control treatment with only brewery spent grains and cabbage as part of the larvae's diet; **RAS:** diet assembled with sludge from a recirculating aquaculture system; **FsT:** diet assembled with fish trimmings; **ULV:** *Ulva lactuca* waste; **SAC:** diet assembled with *Saccharina latissima* waste.

treatment, followed by SAC.

Regarding the concentration of fatty acids in the larvae, it was possible to observe a predominance of lauric (C12:0), palmitic (C16:0) and linoleic acid (C18:2_(n-6)) in all larvae, regardless of the diet they received. A reduction in the concentration of lauric acid seemed to have occurred when aquaculture waste was included in the larvae diets, as well as an increase in pentadecanoic acid and oleic acid (Fig. 4a). Although the fatty acid composition seemed to differ amongst treatments, all larvae had a higher proportion of saturated fatty acids (PUFA) in relation to unsaturated ones in their composition, while the concentration of saturated fatty acids seemed to be slightly reduced in the presence of aquaculture waste as part of their diets (Fig. 4b). Generally, larvae of the control treatment displayed the lowest proportion of several identified fatty acids, with some exceptions, such as lauric acid (C12:0) and myristic acid (C14:0). Larvae of the FsT treatment appeared to have a higher content of oleic (C18:1) and palmitoleic acid (C16:1_(n-7)). Regarding the class of fatty acids (Fig. 4b), the larvae from the FsT

treatment had a higher content of monounsaturated fatty acids (MUFA) and omega-6 (ω -6), as well as a lower ω -3/ ω -6 ratio. All other treatments appeared to have very similar content of other fatty acid classes (Fig. 4b).

4. Discussion

All diets formulated in this study, whether containing aquaculture waste streams (RAS, FsT, SAC and ULV) or not (CT), had an acidic pH of 5.1 ± 0.4 on average at the start of BSFL rearing. This is possibly due to the fact that the spent grains were fermented during the production of beer. The pH of the frass resulting for each aquaculture waste increased by an average of 48 %, reaching values above 7.1 in all treatments, as normally observed during waste bioconversion using BSF larvae (Gärtling and Schulz, 2022). In the study by Meneguz et al. (2018), bioconversion of the Gainesville diet (containing a mixture of grains) was initiated at different pH levels (4.0, 6.1, 7.5 and 9.5). The authors

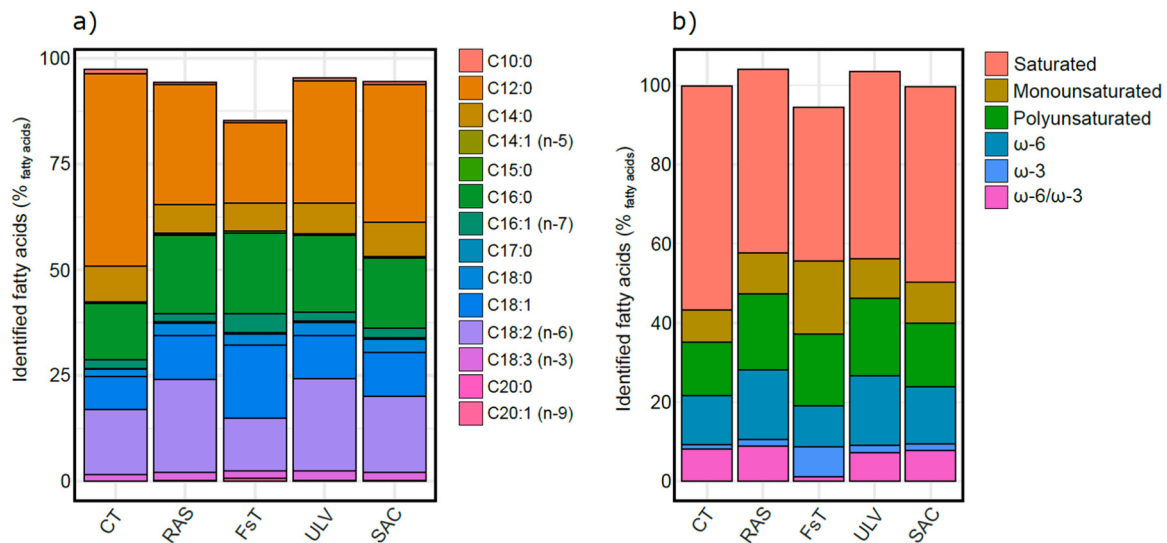


Fig. 4. Stacked bar plots showing a) the composition of specific fatty acids and b) the composition of fatty acid groups identified in the larval biomass of larvae reared in the different treatments. Each fatty acid (seen in a) and the fatty acid groups (seen in b) are presented as percentage of identified fatty acids. CT: control treatment with only brewery spent grains and cabbage as part of the larvae's diet; RAS: diet assembled with sludge from a recirculating aquaculture system; FsT: diet assembled with fish trimmings; ULV: *Ulva lactuca* waste; SAC: diet assembled with *Saccharina latissima* waste.

observed no impact on larval growth by the end of the process, and the pH increased to a steady state between day 5 of bioconversion and the end of the study (day 11). In contrast, Ma et al. (2018) reported negative effect on larval growth and prepupal weight when larvae were fed a low-pH diet (pH 2.0 or 4.0) composed of wheat bran and corn flour. In this case, pH remained low and did not significantly increase by the end of the 17–21 day long bioconversion process. It is thus reasonable to assume that the pH did not affect larval growth during the course of the present study.

A similar trend was observed for the EC of the initial diets and the resulting frass, which increased on average from $2.84 \pm 0.58 \text{ mS cm}^{-1}$ to values above 10.0 mS cm^{-1} . The treatment FsT displayed the highest EC among treatments ($23.19 \pm 2.09 \text{ mS cm}^{-1}$), due to unknown reasons. Such a high EC was expected to be observed in the RAS treatment, due to the fact that RAS sludge often contains high concentration of salts, as it concentrates several substances during fish rearing, including feed leftovers, fish faeces and dissolved solids in the system (Li et al., 2023). Even though the bioconversion process in the presence of fish trimmings did not seem to be compromised by this variable, a high EC observed in the frass resulting from the FsT treatment might have implications regarding the use of this frass in soils, seen that composts with high EC might be classified as phytotoxic, due to the fact that high concentrations of salts (causing high EC) near a plant's root zone might accumulate to a level at which the roots can no longer extract water from the soil in sufficient amounts for its metabolic maintenance (Gondek et al., 2020). In addition, substrate salinity has been reported to negatively affect BSF larval growth, particularly at concentrations above 3% (Cho et al., 2020). However, it is important to note that in the present study, the concentration of different salts in the waste streams and the resulting frass was not analysed. Therefore, the potential phytotoxicity of the frass and its possible impact on larval growth, remain unknown and should be addressed in future studies.

The efficiency of the bioconversion process was similar among treatments, with no statistically significant difference between the control (without aquaculture waste and the other treatments), with a single difference in BCE observed between the SAC treatment ($20.7 \pm 0.6 \%_{\text{DM}}$) in relation to FsT ($24.5 \pm 1.3 \%_{\text{DM}}$). It was demonstrated by Lopes et al. (2020a) and by Isibika et al. (2021) that it is not possible to bioconvert fish carcasses as a single waste source with BSF larvae, due to the physical and chemical nature of this waste stream. It is therefore feasible to assume that RAS sludge and the algae processing waste

streams could also not be treated alone, as also discussed by Liland et al. (2017), justifying the use of other waste sources (spent grains and cabbage) as part of the feed substrate adopted in this study. The presence of aquaculture waste streams did not seem to compromise the process efficiency, as evidenced by the statistically similar survival of the larvae among treatments, including the control. The MatRed was observed to be above $50 \%_{\text{DM}}$ in all treatments, in both wet and dry basis, which is in accordance with the studies of Lopes et al. (2020a) and Isibika et al. (2021). Lopes et al. (2020a) reported a MatRed ranging between 59% and 69% when using trout carcasses as feed substrate for BSF larvae. Isibika et al. (2021) demonstrated that wild-caught fish carcasses bioconverted with BSF larvae have a MatRed of $62.8 \pm 8.2 \%$ (VS basis), when the carcasses are used in inclusion levels between 10% and 75% in the larval diets. Even for shrimp carcasses, which contain significant amounts of shells, the MatRed of diets containing 20% - 100% of this waste ranged between $55 \%_{\text{DM}}$ and $65 \%_{\text{DM}}$ (Hu et al., 2024).

The BSF larvae displayed an average individual wet weight of approximately 105 mg in all treatments, but the larvae from the RAS treatment having the highest mean weight of $119 \pm 7 \text{ mg larvae}^{-1}$. This is likely due to the fact that in this treatment, the larval density adopted ($4.16 \text{ larvae cm}^{-2}$) was lower in relation to other treatments ($6.25 \text{ larvae cm}^{-2}$). It has been shown that a lower areal density of larvae can result in higher final larval weight (Guidini Lopes et al., 2023; Guillaume et al., 2023). This lower larval density was due to the limitation of the amount of RAS sludge that could be added to the boxes, as described in Section 2.3. This could be overcome by partially drying the sludge to achieve a moisture content that would be feasible for the larvae to digest. Belghit et al. (2024), mixed 13 different aquaculture sludge samples with varying moisture contents, achieving a final dry matter content of approximately 30%, which was effectively converted by BSF larvae into protein and fat, reaching up to 39.6% crude protein and 22.8% crude fat in the larval biomass, on dry basis. In this study, the larval biomass of the RAS treatment had a protein and fat content of $39.1 \pm 0.3 \%_{\text{DM}}$ and $17.3 \pm 0.9 \%_{\text{DM}}$, respectively, which are close to the values reported by Schmitt et al. (2019), Rossi et al. (2023) and Belghit et al. (2024). The results obtained in this study on larval growth and process performance are in accordance with the findings of Cattaneo et al. (2024), who suggested that providing omnivorous diets in quantities slightly higher to the ones there were provided in this study could support adequate growth in terms of protein and fat accumulation in the larval biomass, ensuring that no nutrient catabolism would occur and other possible

signs of discomfort. But it is important to highlight that increasing the larval feed dose to obtain larger larvae poses several challenges. For example, the bulky nature of the brewery spent grains result in excessive substrate depth and the high moisture content of the blended feedstock hinders sufficient moisture removal, making it difficult to ensure complete consumption of the substrate and achieve dry harvesting of BSF larvae from the frass. One potential solution could be to identify other biowaste streams, without these specific limitations, to blend with the aquaculture-derived waste.

It is noteworthy how the type of aquaculture waste affected the crude fat content of the larval biomass in this study. While the larvae fed fish trimmings (FsT) had the highest crude fat content close to 30 %_{DM}, larvae fed algae waste (both SAC and ULV) had only about 15 %_{DM}. When feeding rainbow trout carcasses and bread to BSF larvae, Ewald et al. (2020) observed a high crude fat content of approximately 47 %_{DM} in the larval biomass, which was partially due to the high proportion of carbohydrates used in that diet due to the high bread inclusion. In that same study, another set of diets was elaborated with bread and mussels, with inclusion levels of 10–50 % mussels, and the more mussels were included (*i.e.* the lower the carbohydrate inclusion level), the lower the crude fat content of the larval biomass, demonstrating a fat accumulation due to the presence of bread. After the brewing process, most of the sugars and other easily available carbohydrates are significantly reduced, and the spent grains are then composed mostly of fibres (around 70 %) and proteins (around 20–30 %) (Mussatto et al., 2006; Chia et al., 2018); therefore, it is likely that the low concentration of carbohydrates caused the larvae to accumulate less fat even in the presence of high concentrations of spent grains in this study. As highlighted by Barragan-Fonseca et al. (2017), high-fat larval biomass could affect the digestibility and palatability of BSF larvae meals for other animals. Therefore, producing low-fat larvae (SAC and ULV treatments) could be advantageous from an feed industry perspective, since such larvae may be suitable for direct use without requiring defatted prior to inclusion in feed.

In the larval biomass fed different aquaculture waste streams, the highest proportion of the type of fatty acid was composed of saturated fatty acids such as lauric (C12:0) and palmitic acid (C16:0), followed by polyunsaturate and monounsaturated ones. These findings are in line with other studies on the composition of BSF larval biomass reared on different (Ameixa et al., 2023; Ewald et al., 2020; Knudsen et al., 2022; Li et al., 2022). Interestingly, the composition of groups of fatty acids of even specific ones did not seem to change significantly according to the type of waste stream provided to the larvae, except for the ratio between ω -6 and ω -3 fatty acids in the FsT treatment, which was reduced in relation to other treatments, due to a greater proportion of ω -3 observed in that treatment. It has been suggested that it is possible to manipulate the composition of fatty acids in BSF larvae, by providing diets with distinct compositions. For instance, Ewald et al. (2020) demonstrated that, within certain limits, it is possible to stimulate the accumulation of docosahexaenoic acid and eicosapentaenoic acid, which are fatty acids of great interest in aquaculture, by providing different proportions of a carbohydrate-rich (bread) and a protein-rich (mussels) substrate combined. Similarly, Ameixa et al. (2023) demonstrate that it is possible to significantly reduce the concentration of lauric acid while proportionally increasing the concentration of oleic acid (C18:1) by providing olive pomace in increasing concentrations in the larvae diet. In that same study, the authors also demonstrated *de novo* synthesis of fatty acids by the BSF larvae. This was evidenced by the absence of lauric and myristic acids in the experimental diets, while both fatty acids were present in the larval biomass. Similarly, Knudsen et al. (2022) reported a linear effect in the fatty acid composition of BSF larvae when fed increasing amounts of fish oil. Based on the proportions of fatty acids observed in this study, it is reasonable to assume that using fish trimmings alone could lead to a different fatty acid composition —specifically, an increasing concentration of ω -3 fatty acids and reduced ω -6/ ω -3 ratio.

It is noteworthy that the presence and type of aquaculture waste

stream impacted on the concentration of amino acids in the larval biomass in this study. Furthermore, the larvae from the control treatment with no aquaculture waste had the highest concentration of most amino acids, with the exceptions of alanine, glutamine and proline, which were found in higher concentrations in the larvae reared on FsT. Fish trimmings is a commonly used waste for the production of amino acid-rich animal feed ingredients and agricultural inputs, such as silage (Vidotti et al., 2003) and liquid fertilizers (Ahuja et al., 2020); thus, it was expected that some amino acids would be present at higher concentrations in the larval biomass. Conversely, the algae-derived biomass (SAC and ULV treatments) resulted in the lowest accumulation of amino acids. To the best of the authors' knowledge, a limited number of studies have investigated the use of marine algae to feed insect larvae. In the study of Swinscoe et al. (2020), the authors fed several powdered algae species (including *U. lactuca*) to BSF larvae and mentioned nutritional benefits for the larval biomass, even though none of these benefits were disclosed. Warwas et al. (2024) fed larvae of the bristly-legged seaweed fly (*Coelopa frigida*) with the algae *Fucus vesiculosus* and *S. latissima*, demonstrating the feasibility of algae bioconversion with an insect larva, resulting in a processed larval meal with 60 %_{DM} crude protein and significant accumulation of amino acids, similar to a commercial BSF larvae meal, but containing approximately 19 % less amino acids than a commercial fishmeal. These results put together demonstrate that this aquaculture waste stream can be a feasible substrate for rearing insects, in a circular economy perspective.

The use of insect meal in general as an aquafeed ingredient has been proposed as a way of reducing the environmental impacts of aquaculture production. Quang Tran et al. (2022) demonstrated how the use of BSF larvae meal in replacement of fishmeal or soybean could reduce water, land and energy use, as well as eutrophication and the global warming potential as a whole in aquaculture production. The authors further highlighted that in case the larvae are fed waste streams instead of standard substrate (*e.g.* chicken feed and distillers' grains), the environmental impacts could be even more reduced, especially by reducing the need for the currently mostly used protein ingredient that is fishmeal, from extractive fisheries. This was also discussed by Bosch et al. (2019), who highlighted that the use of currently banned substrates (*e.g.* fish carcasses, food waste and manures) is the way forward for insect biomass production, seen that when the currently allowed substrates are used for insect rearing (*e.g.* pre-consumer food waste), the environmental impact of the obtained insect meal is comparable to that of fishmeal or soybean. In this study, currently allowed substrates that could be associated with a higher environmental impact if used for BSFL rearing were also included. Brewery spent grains could be used directly as animal feed, although some challenges with the direct use as feed has been raised (Bianco et al., 2020). The vegetable cuttings likely have too high moisture content and low nutrient content to be of interest for feed purposes. Even so, including aquaculture waste streams lower the use of the currently allowed waste streams and thus the associated environmental impact could be expected to decrease. It is currently known that from a nutrition perspective, insect meals provide essential protein, amino acids and fatty acids for fish and in relation to fishmeal, enables comparable growth of several fish species, regardless of the substrate used for rearing them (Fantatto et al., 2024). Thus, future studies investigating the safety aspects of feeding insects with different waste materials, including the aquaculture-derived waste streams used in the present study are needed.

One major finding of this study regards the possibility of modifying the fatty acid profile of the larval biomass by adding aquaculture waste. It seems that the presence of any of the four waste streams in the larvae diets resulted mainly in a reduction of lauric acid (C12:0) and slightly increase of pentadecanoic acid (C15:0), both saturated fatty acids, a group that generally seemed to be reduced when aquaculture wastes were used in the diets, as well as a small increase in the unsaturated oleic acid (C18:1). Changing the larval biomass fatty acid profile can have major implications in the use of this biomass in fish feed production,

considering that a biomass with a lower concentration of saturated fatty acids might have a lower melting point, which in turn might ease feed processing and enable the use of BSF oil in new food and feed applications (Bogevik et al., 2022), as well as improve the digestibility of the feed by several fish species (Menoyo et al., 2003). A very similar trend of lauric acid reduction and oleic acid increase was observed by Ameixa et al. (2023) when feeding BSF larvae with olive pomace-rich diets, and by Ewald et al. (2020) when feeding the larvae with a mixture of varying inclusion rates of fish and bread, demonstrating that producing tailored larval biomass is possible with minor inclusion levels of selected waste streams.

5. Conclusions

The results presented in this study demonstrated that several aquaculture waste streams (fish trimmings, sludge from a recirculation aquaculture system, and macroalgae post-processing waste) can be effectively bioconverted by BSF larvae. The bioconversion efficiency of diets containing these waste streams were always above 20 %_{DM}, while the material reduction was always above 50 %_{DM}, and the larval biomass yield was generally above 5 kg larvae m⁻². The larvae biomass obtained with this bioconversion is rich in protein (>30 %_{DM}) and variable in relation to crude fat content. While the use of fish trimmings resulted in high fat larvae (close to 30 %_{DM}), diets containing macroalgae as part of the feed substrate resulted in low fat larvae (< 15 %_{DM}). Additionally, it was concluded that the fatty acid profile of the larval biomass changes when aquaculture waste is provided as part of the feed substrate even in small amounts, leading to a reduction of saturated fatty acids, especially lauric acid and pentadecanoic acid, which might have significant implications in the use of this biomass in fish feed production and digestibility of the diets. These put together demonstrates the possibility of obtaining tailored larval biomass for inclusion in the diets of livestock in general. These results demonstrate that promoting circularity in aquaculture is possible through the use of BSF larvae as bioconverting tools for distinct waste streams. However, it should be noted that the use of animal-derived waste - including fish trimmings and sludge from systems like RAS - is still prohibited in the European Union and many other regions. This regulatory restriction limits this waste bioconversion technology to reach its full potential, particularly in terms of economic and environmental sustainability. Aquaculture production could benefit greatly from the recirculation of its waste streams in fully circular systems, for example by using BSF larvae to simultaneously treat waste and produce protein.

CRedit authorship contribution statement

Ívã Guidini Lopes: Investigation, Formal analysis, Methodology, Writing – original draft, Conceptualization. **Viktoría Wiklicky:** Methodology, Conceptualization, Writing – review & editing, Investigation. **Cecilia Lalander:** Conceptualization, Supervision, Writing – review & editing, Project administration, Investigation, Resources, Methodology, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.aqrep.2025.102961](https://doi.org/10.1016/j.aqrep.2025.102961).

Data availability

Data will be made available on request.

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