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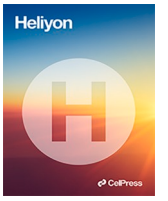

Research article

Bio-upcycling of cheese whey: Transforming waste into raw materials for biofuels and animal feed

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ABSTRACT

Cheese whey (CW), by-product of cheese production, has potential as a valuable resource due to its nutritional composition. Although options for CW degradation have been explored, a biological treatment with black soldier fly larvae (BSFL) has not been reported. This study evaluated the growth and composition of BSFL in four experimental diets with CW under different conditions. Results show that the use of CW allows larval development and weight gain, also, the conversion into larval biomass was up to 0.215. Diets ED3 (fresh CW, 38 °C) and ED4 (fresh CW, room temperature) allowed higher weight accumulation (final weight up to 0.285 g); the highest fat accumulation (12 % higher than control) was observed in ED3 (up to 45.57 %), which had less protein. Moreover, higher amounts of saturated fatty acids are generated. This study highlights the importance of an appropriate pretreatment designed for a specific waste to control desired by-products.

1. Introduction

Cheese whey (CW) is a by-product of cheese production. This waste, of which about 145 million tons are generated annually, is of interest because of the management required, since only nearly half of this waste is currently reused [1,2]. Improper handling of CW can cause adverse environmental impacts due to its high organic load and nutrient content [3]. CW mainly comprises lactose (40–50 g/l), proteins (6–8 g/l), lipids (4–5 g/l), and mineral salts (8–10 % dried extract). It also has a high chemical oxygen demand [4], and its pH varies depending on whether it is sweet whey (pH 6.2–6.4) or acid whey (pH 4.6–5.0) [2]. All these features give this waste contaminating characteristics that, if improperly disposed of, can affect water bodies and soils [3]. However, this waste has potential as a valuable resource if adequately treated.

Although the treatment of CW depends on its origin and application, among the options reported for its treatment are wetland treatment, coagulation, flocculation, filtration, activated sludge, and the most widely used, anaerobic digestion [5–7]. From the latter, methane can be obtained and used as biofuel [4]. Alternatively, microalgae treatment has been proposed for generating nutrient-rich biomass that can be processed into proteins, oils, and pigments [8]. However, the mentioned treatments fail to degrade biomass fully. In contrast, biological treatments involving insects have gained attention for their high efficiency and ease of waste conversion. Specifically, the treatment with black soldier fly larvae (BSFL) has stood out for its efficiency in treating large amounts of waste, with

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reported reductions of up to 80 % [9]. After transforming the waste into larval biomass, BSFL can be collected for animal consumption or for extracting their oil, which can be converted into biodiesel [10]; furthermore, it is important to consider the acceptability of BSFL technology for social acceptance [11].

Although BSFL have shown the ability to grow in a variety of wastes such as kitchen [12], brewery, manure [13] restaurant [14], and agro-industrial wastes [15], accumulating significant amounts of fat and protein, their rearing in cheese whey, which is rich in nutrients, holds promise as an alternative for their treatment. In this way, not only is bioconversion of the material achieved, but also a reduction in the impact of this waste.

It has been reported that the composition and other factors of the substrate play a crucial role in the optimal development of BSFL. The nutrients present in the substrate where larvae are reared directly influence their growth and composition [16,17], as well as external factors such as temperature, pH, or humidity [18–21]. For example, a study by Ma et al. [22] investigated how different initial pH levels affected BSF larval production, development time, and adult longevity. Larvae were raised on diets with different pH values (2.0, 4.0, 6.0, 8.0, and 10.0, with a control at 7.0). Larval and prepupal weight were highest at neutral or basic pH, while the lowest weights were observed at acid pH (2.0 and 4.0). Larval development time was shortest at pH 8.0. pH levels generally stabilized around 5.7 and 8.5, except for pH 2.0 and 4.0 diets, which remained slightly acidic [22]. Another study by Pang et al. [19] concludes that a pH 3.0 treatment is not practical. However, increasing the pH of food waste could improve carbon and nitrogen recycling while minimizing environmental impact. Regarding the temperature and humidity, a study made by Salam et al. [23] describes that the recommended humidity range is of 70%–75 %, as sieving becomes impractical when moisture content exceeds 80 %. Temperature is also a critical factor in the development and survival of BSFL, with an optimal range typically between 25 °C and 35 °C. Extreme temperatures, either above 40 °C or below 10 °C, can significantly impact their growth, metabolism, and overall development. For example, survival rates drop dramatically above 47 °C. Thus, maintaining the correct temperature range is vital for successful BSFL production [23].

Therefore, understanding how to adjust the substrate composition and conditions to ensure an optimum environment for larval development could improve their efficiency in terms of waste management and production of valuable by-products.

Although it has been reported that these larvae can grow in a significant number of wastes [10,23–26], to our knowledge, there are no reports of the use pf CW as a substrate for BSFL nor the ideal conditions for its inclusion and treatment with this organism. Therefore, the novelty of this study lies in the application of an innovative biological approach to treat cheese whey using black soldier fly larvae. This method has not been widely explored in the scientific literature to date. Furthermore, this approach could have environmental benefits by reducing the amount of cheese whey discharged into the environment, helping to mitigate the pollution associated with its improper handling. It is also crucial to investigate the pre-treatment of the waste to ensure the proper growth of the larvae and the accumulation of fat in them, which would allow for effective control of the production of the desired products. Therefore, this work aimed to compare the effect that CW management and its degradation can have on the composition and growth of the larvae as well as the generation of possible products of interest and biofuels.

2. Material and methods

2.1. Study area

The experiment was conducted at the Amazcala Campus of the Faculty of Engineering at the Autonomous University of Querétaro, located on Chichimequillas Highway, s/n km 1, El Marqués, Querétaro, Mexico, 76265.

Fig. 1. Initial 5-day old BSFL.

2.2. BSFL and cheese

BSFL were obtained from a fly colony at the Autonomous University of Queretaro located at the Amazcala Campus, El Marqués, Querétaro, Mexico. After hatching, they were kept in a rearing substrate consisting of a Gainesville diet (70 % water and 30 % a mixture of bran, corn, and rabbit feed) for approximately five days before transferring them to the experimental diets. Fig. 1 shows the 5-day larvae (average weight 0.0082 g), which were separated by sieving (1-mm mesh).

Cheese whey was obtained from the Dairy Pilot Plant at the Autonomous University of Queretaro located at the Amazcala Campus, El Marqués, Querétaro, Mexico after the production of panela cheese. Gainesville diet grains were mixed with CW (25:75) in each experimental diet [27].

2.3. Experimental diets

To evaluate the effect of feedstock on BSFL composition, growth, process efficiency, as well as generation of products, four experimental diets, and a control diet were proposed. Table 1 shows each diet with its particular characteristics. The characteristics were selected considering several management scenarios. These management scenarios involve: (1) the production and storage of CW without refrigeration (ED1); (2) refrigerated CW (at - 4 °C) (ED2); (3) freshly produced CW (ED3); and (4) transported freshly produced CW (allowing its temperation) (ED4). The main differences involve variations in the storage and transport temperatures of CW, which affect its composition and quality, as well as the effectiveness of its use in feeding black soldier fly larvae. The specific whey conditions were considered as the independent variable, while larval growth and composition were considered as dependent variables.

2.4. Experimental setup

The setup consisted of three plastic containers (34.6 cm × 21 cm x 12.4 cm) per experimental diet and control placed in a room with a continuous temperature of 24–30 °C [28] and humidity of 65–70 % [29]. Each replicate consisted of a plastic container, 1,500 larvae, and 1.0 kg of their respective experimental diet treatment [30]. The larvae were not individually counted; instead, the weight of 100 larvae was measured and extrapolated to estimate the total weight of 1,500 larvae.

The experimental diets were prepared fresh, heated with the aid of an electric grill, and administered three times during the experiment [1], distributed on days 1, 3 and 6 for ten days [29]. This feeding schedule was implemented to accommodate the larvae's increasing appetite as they grow. On the eleventh day, larvae were separated from the residue with sieves; both were weighed and placed in previously labeled bags. The larvae were placed in a freezer for death, where they remained until further analysis.

2.5. Cheese whey analysis

Cheese whey was analyzed using a Lactoscan Milk Analyzer [31]. Non-fat solids, density, conductivity, freezing point, salts, fat, protein, and lactose were determined. An Ohaus ST Series Pen Meter was used to measure the temperature and pH levels. The acidity was assessed by carrying out a titration using 0.1 N sodium hydroxide (NaOH), while employing phenolphthalein (1 % v/v) as an indicator [32].

2.6. Growth and process efficiency of BSFL

Records of the weight of 300 larvae from each replica were taken before feeding. At the end of the experiments, both total larvae and total residual material (frass) were weighed. Length and width were recorded at the beginning and end of the trial as a standard practice for collecting comprehensive data [33].

To evaluate the rate of CW consumption, two indices were calculated: the waste reduction index (WRI) (Equation (1)) and the efficiency of conversion of digested diets (ECD) to larval biomass, which was determined with Equation (2); larval growth rate (GR) was calculated with Equation (3). The equations used were proposed by Diener et al. [34].

$$WRI = (W–R/W)/\text{days of experiment (d)} \times 100 \quad (1)$$

Where:

Table 1
Composition and characteristics of experimental diets.

Diet	Gainesville diet (%)	Cheese whey (%)	Water (%)	Cheese whey characteristics
ED1	25	75	0	Obtained the day of its generation and stored without refrigeration.
ED2	25	75	0	Obtained the day of its generation and refrigerated. Tempered before use.
ED3	25	75	0	Obtained each day of its generation and heated up to 38 °C before being use.
ED4	25	75	0	Obtained each day of its generation.
Control	30	0	70	–

ED1L: experimental diet 1 larvae, ED2L: experimental diet 2 larvae, ED3L: experimental diet 3 larvae, ED4L: experimental diet 4 larvae, and Control: control larvae.

W = total amount of feed provided, g.
R = residual amount, g

$$ECD = B/(W-R) \quad (2)$$

where:
B = larvae pupal biomass, g.
W = total amount of feed provided, g.
R = residual amount, g

$$GR = (FW\text{-}IW)/\text{days of the experiment} \quad (3)$$

Where:
FW = final body weight, g.
IW = initial body weight, g.

2.7. Proximate composition of substrates and BSFL biomass

The CW used for the first feeding was the same for all experimental diets, so an initial sample of the mixture and the control were taken for comparison. Before the analysis, substrates and BSFL biomass were dried at 65 °C for 72 h; subsequently, samples were grounded. Ash determination was done by gravimetric method, and the anthrone method was utilized to estimate total carbohydrates [35]. Lipids were extracted with a Soxhlet system. About 25 g of dried BSFL were used with 250 mL hexane solvent for 6 h. Their quantity was calculated using the AOAC [35] method. Total Protein Kit, Micro Lowry Sigma-Aldrich protein assay was employed to determine the total protein content [36].

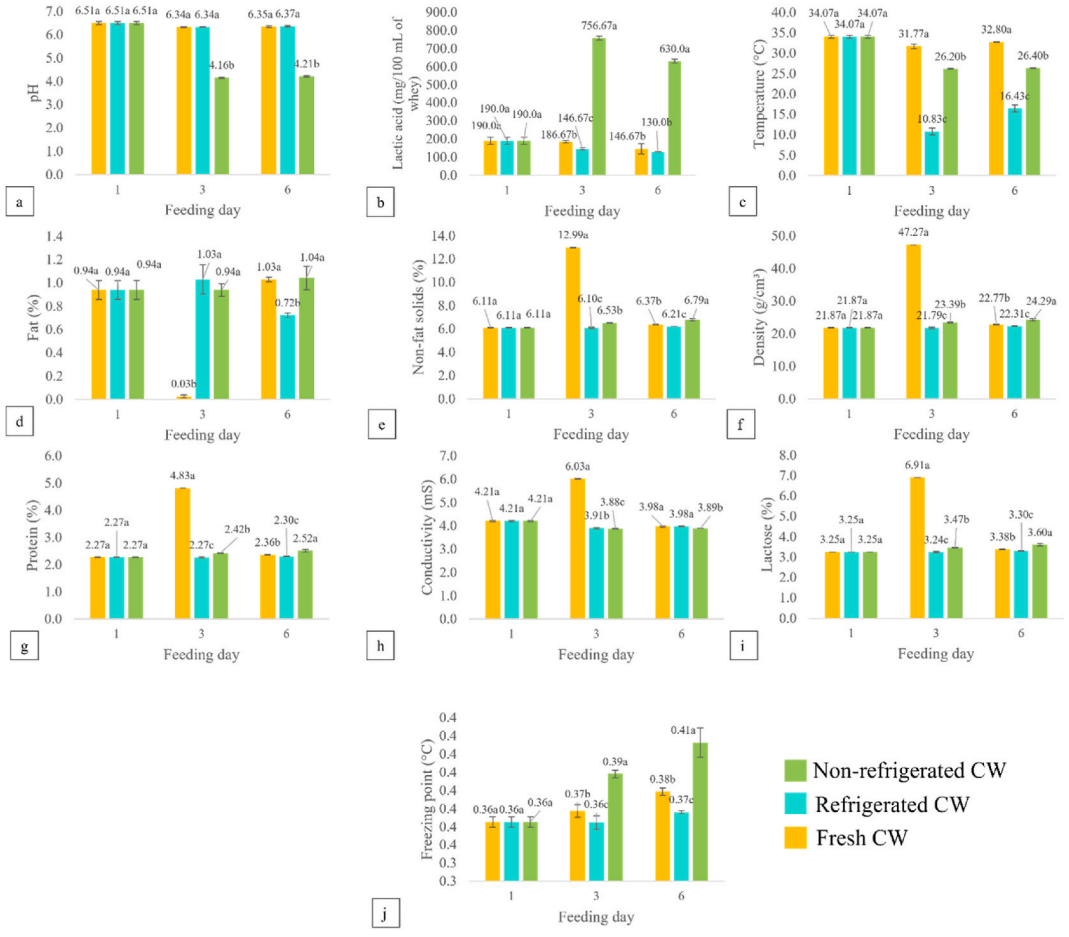

Fig. 2. Cheese whey analysis. (a) pH, (b) lactic acid, (c) temperature, (d) fat, (e) non-solid fats, (f) density, (g) protein, (h) conductivity, (i) lactose, and (j) freezing point. *All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

2.8. Fatty acids profile by GC–MS of BSFL lipids

Fatty acid methyl esters (FAMEs) were synthesized from the previously extracted lipids of BSFL biomass. The synthesis process involved a basic-acid transmethylation method. Initially, 50 mg of the lipids were weighed and combined with 400 μl of NaOH solution (1.25 M in methanol). The mixture was stirred for 1 min and then subjected to 5 min of sonication at 40 kHz. Subsequently, 400 μl of H_2SO_4 solution (1.75 M in methanol) was added, and the mixture was stirred for an additional minute, followed by another 5 min of sonication. To complete the process, 800 μl of hexanes were added, and the mixture was stirred for 30 s before being centrifuged at 10,000 g for 5 min. The supernatants were collected, and one μL of each sample was injected into an Agilent gas chromatograph (GC) series 7890A, equipped with an HP-88 capillary column (30 m × 0.25 mm inner diameter × 0.25 μm). The GC was coupled with a single quadrupole mass spectrometer (MS) detector (Agilent 5975C). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}, and the injector temperature was set at 250 °C with a split ratio of 1:10. The content and composition of the FAMEs were determined by comparing them with a standard Supelco® 37 Component FAME Mix. Data processing was carried out using Chemstation software from Agilent Technologies.

3. Results and discussion

3.1. Cheese whey analysis

The CW was analyzed on feeding days (1, 3, and 6) before mixing with the grains. No refrigerated CW was used for ED1, refrigerated CW was used for ED2, fresh CW was used for ED3 (analyzed and then heated), and ED4. Fig. 2(a–j) summarizes the physicochemical analysis determined for the experimental cheese wheys. These data reveal significant differences between all the analyzed parameters that can be attributed to the conditions under which they were stored.

Non-refrigerated whey had a lower pH (Fig. 2a) due to the production of lactic acid by lactic acid bacteria (Fig. 2b) [37]; in contrast, the refrigerated whey did not show this increase because refrigeration inhibits bacterial growth given that low temperature slows enzymatic reactions [38]. Since CW is classified into two types according to the pH (6.0–6.5 for sweet whey and 4.5 for acid whey) [39], the experimental wheys used in these trials started as sweet whey, and only the non-refrigerated acidified through the days. Temperature and fat were also different (Fig. 2c and d) due to the storage conditions. Fat percentage was lower in the refrigerated CW; this difference shows consistency with the expected since milk fat undergoes a slight contraction upon solidification as milk is cooled [40]. In contrast with other reports (0.4–0.5 %) [41], the fat percentage is higher in the experimental CW (0.72–1.04 %), since the milk utilized for cheese production differs from the norm applied by other producers, as it is not skimmed.

Non-fat solids quantity affects density, and since the first was affected by the storage conditions, both of them showed lower values (Fig. 2e and f). However, the obtained data displayed similar values to what is reported: for total solids (6–6.2 %) [42] and for goat milk density (31.19 g/cm^3) [31].

While protein content showed differences (Fig. 2g), the obtained values (2.30–2.52 %) fall within the reported range (1.0–10.8 %) [43]. Conductivity or salinity in CW depends on the quantity of NaCl added during cheese production. The experimental wheys (Fig. 2h) displayed conductivity values within 3.88 and 6.03 mS, which are low compared to an average conductivity of 8 mS [44] for other wheys.

Lactose content in whey is important since it is considered one of the main pollutant components as it is a carbohydrate that serves as food for various microorganisms, which increases its biological and chemical oxygen demand [45]. Although the lactose content varied in the different experimental wheys (Fig. 2i), the values remained within the range reported in other works [8]. The last analyzed parameter, the freezing point enables the determination of adulterated milk; if it contains a higher water content than the standard, it indicates potential adulteration. The experimental cheese wheys present a lower freezing point (0.36–0.41 °C) (Fig. 2j) than the freezing point from cow milk (0.53 °C) [46]. This difference between results presumably relates to the different compositions of whey, since the main constituents of milk were taken for cheese production.

It is crucial to analyze the parameters in the whey, since it has been shown that pH, temperature, humidity, other environmental conditions, and the composition of the substrate in which they are grown affect the growth and development of BSFL [47].

Table 2
Proximate composition of initial Control and Experimental Diets (ED) (% dry matter unless stated).

Component	Control (70 % water and 30 % Gainesville diet)	ED (75 % cheese whey and 25 % Gainesville diet)
Carbohydrates	38.15 ± 2.53^a	34.87 ± 1.20^b
Lipids	3.76 ± 0.03^b	8.11 ± 0.70^a
Protein	13.47 ± 0.80^a	15.06 ± 2.79^a
Ash	5.16 ± 0.04^b	5.51 ± 0.02^a
Calories (cal/g)	3382.33 ± 148.65^b	4114.93 ± 28.56^a
Reference	–	–

All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

3.2. Proximate composition of substrates

Table 2 presents the findings from the proximate composition of both the initial control and experimental diet. The experimental diet has fewer carbohydrates as it contains a lower amount of the grain mixture; however, it shows a higher fat and ash content due to the addition of the CW, as it contains lipids and minerals. The increase in calorie content is associated with a higher fat content.

3.3. Growth and process efficiency of BSFL

Initial larval weights (0.0082 ± 0.0004 g) were not significantly different between treatments. Fig. 3 shows the weight gain of larvae with the experimental diets during the days the experiment was maintained.

The proposed experimental diets affected the weight gain, growth, and process efficiency of BSFL. The initial and final weight, width, and length are presented in Fig. 4(a–c). The highest weights were recorded in ED3 and ED4, which contained fresh whey obtained on the day of its generation. This is consistent with reports that show that temperature [21] and pH are critical factors in larval development [18].

A lower pH (<6.0) could result in lower final BSFL weight, since it affects the BSFL gut microbes [19]. Therefore, the lower weight from ED1L can be attributed to the feed's lower pH as whey started acidification. Regarding temperature, it has been described that a higher temperature (around 45 °C, which is the optimum for proteolytic activity) contributes to increasing the metabolism of the BSFL and, thus, their bioconversion [20]. Since most of the parameters analyzed in experimental wheys were within the reported data, it can be assumed that pH and temperature were the main factors that affected length and width. ED3L, ED4L, and control showed the highest values in both measurements.

BSFL have shown the ability to be reared in a vast number of substrates showing in the same way that the composition of this substrate affects BSFL's final composition. For example, diets rich in protein and fiber cause larvae to develop more slowly than diets balanced with cereals [48]. This is supported by the obtained data since BSFL weight, length, and width values are higher than what other authors have reported (Table 3).

The reported values that most closely resemble those obtained are those that contain grains [21,49], are balanced diets [50], or have undergone pretreatment with microorganisms [51]. The values that differ the most come from diets rich in vegetables [33] and fiber [26] (Jucker et al., 2020). The CW appears to contribute several nutrients to the Gainesville diet that allow for better development in the larvae [18]. This difference can be due to the diet composition on which they were reared. The CW analysis showed that this waste gives the Gainesville diet an additional 6 % of carbohydrates, protein, and fat. Therefore, the experimental diets, which are a mix of cereals and CW, have nutrients that differ from the ones used in other reports.

Table 4 presents the findings regarding the impact of experimental diets on larval growth and conversion efficiency, along with values from other reported studies. All the tested diets supported the growth and development of BSF larvae, but its condition and composition impacted their performance. All treatments, including control, achieved a high waste reduction index, which describes the ability of BSFL to reduce feeding substrates. Therefore, CW did not affect BSFL ability, which can be attributed to the similarity in composition since CW only has 6 % more solids than the water used for control. Regarding the efficiency of conversion of the digested (ECD) food and the larvae growth, ED3L was the diet that outperformed the others. A high ECD value indicates that the feed or substrate is highly assimilated into BSFL biomass. The larvae growth rate is related to the latter index since better assimilation of the feed; the higher growth can be expected. These results demonstrate that, as mentioned in section I, temperature and pH play an important role in larval development [19,20]. It is significant to mention that the substrate condition and composition did not affect the frass generation, but affected the assimilation or metabolic efficiency of the substrate by BSFL.

Comparing the WRI obtained with those reported by other authors, it is observed that balanced diets such as fruit and vegetable mixtures or diets rich in cereals have a higher index [49,53]. This difference may be due to the number of days of experimentation, which is a value that directly affects the calculation of this index. In this work, BSFL were in contact with the substrate for 10 days, while in the listed works, they stayed for between 15 and 24 days [49,52,53]. This difference in days would allow the larvae to degrade

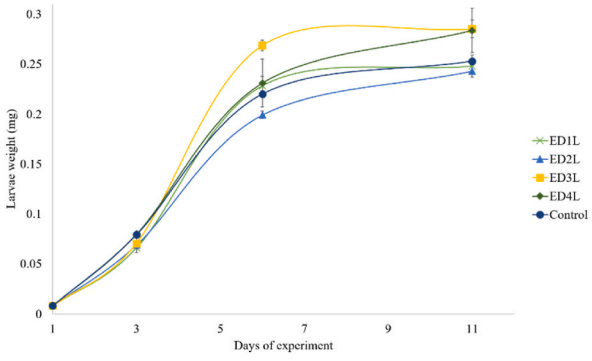

Fig. 3. BSFL weight (mg) during the days of experimentation. ED1L: experimental diet 1 larvae, ED2L: experimental diet 2 larvae, ED3L: experimental diet 3 larvae, ED4L: experimental diet 4 larvae, and Control: control larvae.

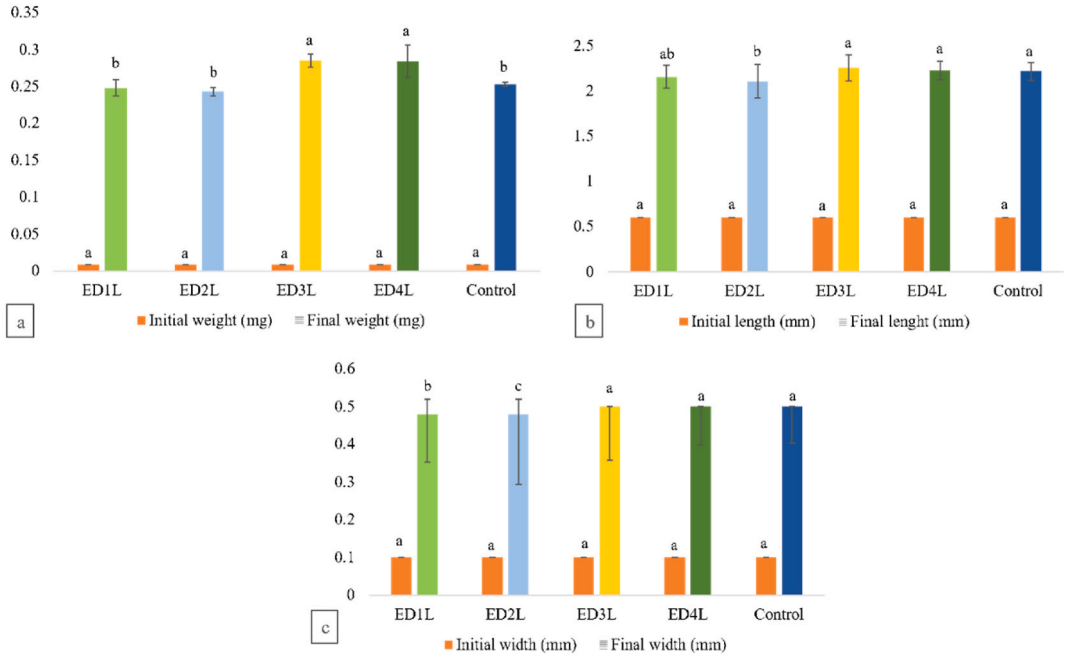

Fig. 4. Initial and final (a) weight, (b) length, and (c) width of the BSFL used in this work. ED1L: experimental diet 1 larvae, ED2L: experimental diet 2 larvae, ED3L: experimental diet 3 larvae, ED4L: experimental diet 4 larvae, and Control: control larvae *All values shown are mean ± standard deviation. Different letters indicate significant differences between treatments ($p \leq 0.05$).

Table 3
Final weight, length, and width of the obtained and reported larvae biomass.

Diet and conditions	Weight (g)	Length (cm)	Width (cm)	Reference
ED1	0.248 ± 0.011^{b}	2.30 ± 0.135^{ab}	0.5 ± 0.04^{b}	–
ED2	0.243 ± 0.006^{b}	2.10 ± 0.197^{b}	0.5 ± 0.04^{c}	–
ED3	0.285 ± 0.009^{a}	2.30 ± 0.151^{a}	0.5 ± 0.00^{a}	–
ED4	0.284 ± 0.022^{a}	2.33 ± 0.107^{a}	0.5 ± 0.00^{a}	–
Control	0.253 ± 0.003^{b}	2.30 ± 0.103^{a}	0.5 ± 0.00^{a}	–
Brewer's spent grains and yeast (37 °C)	0.216	NR	NR	Chia et al. [21]
Brewer's spent grains and water (35 °C)	0.168	NR	NR	Chia et al. [21]
Gainesville diet	0.153 ± 0.113	1.25	NR	Meneguz et al. [18]
Banana peels	0.12 ± 0.06	1.691 ± 0.399	0.437 ± 0.112	Rahmi et al. [33]
Cassava peels	0.09 ± 0.05	1.436 ± 0.318	0.367 ± 0.072	Rahmi et al. [33]
Banana peels (50 %) mixed with cassava peels (50 %)	0.06	1.506 ± 0.392	0.396 ± 0.103	Rahmi et al. [33]
Hen diet	0.229 ± 0.020	NR	NR	Bava et al. [49]
Maize distiller (ethanol production waste)	0.197 ± 0.014	NR	NR	Bava et al. [49]
Okara (tofu production waste)	0.138 ± 0.006	NR	NR	Bava et al. [49]
Brewer's grains	0.098 ± 0.001	NR	NR	Bava et al. [49]
Abattoir waste mixed with 50 % fruit & vegetables	0.252 ± 0.013	NR	NR	Lalander et al. [50]
Undigested sludge	0.070 ± 0.005	NR	NR	Lalander et al. [50]
Banana peels pre-treated with ammonia, followed by microbial pre-treatment	0.231	NR	NR	Isibika et al. [51]
Food waste	0.141	NR	NR	Lindberg et al. [26]
Broccoli and cauliflower trimmings	0.108	NR	NR	Lindberg et al. [26]
Cricket waste	0.0304 ± 0.00029	1.578 ± 0.010	NR	Jucker et al. [52]
Locus waste	0.0198 ± 0.00033	1.464 ± 0.0092	NR	Jucker et al. [52]

NR: not reported, ED1: experimental diet 1, ED2: experimental diet 2, ED3: experimental diet 3, ED4: experimental diet 4, and Control. *All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

Table 4
Performance of larval growth and conversion efficiency in experimental and reported diets.

Substrate	Waste Reduction Index % (g/d)	The efficiency of conversion of the digested food (ECD)	Larvae growth rate (LGR) (g/d)	Reference
ED1L	2.72710 ± 0.00002^{a}	0.1617 ± 0.0004^{c}	0.0218 ± 0.001^{b}	–
ED2L	2.72709 ± 0.00001^{a}	0.1824 ± 0.0097^{b}	0.0214 ± 0.001^{b}	–
ED3L	2.72696 ± 0.00003^{c}	0.2155 ± 0.0190^{a}	0.0252 ± 0.001^{a}	–
ED4L	2.72705 ± 0.00002^{b}	0.1837 ± 0.0159^{b}	0.0250 ± 0.002^{a}	–
Control	2.72709 ± 0.00001^{a}	0.1496 ± 0.0101^{c}	0.0223 ± 0.0003^{b}	–
Cricket waste	3.12 ± 0.53	0.19 ± 0.05	NR	Jucker et al. [52]
Locus waste	1.79 ± 0.10	0.40 ± 0.02	NR	Jucker et al. [52]
70 % vegetable and 30 % fruit waste	3.2 ± 0.26	0.07 ± 0.009	0.006 ± 0.0018	Meneguz et al. [53]
100 % fruit waste	3.2 ± 0.41	0.05 ± 0.011	0.007 ± 0.0007	Meneguz et al. [53]
Brewery by-product	5.3 ± 1.05	0.06 ± 0.002	0.006 ± 0.0009	Meneguz et al. [53]
Winery by-product	2.4 ± 0.32	0.14 ± 0.034	0.014 ± 0.0009	Meneguz et al. [53]
Hen diet	4.46 ± 0.36	0.27 ± 0.02	0.0051 ± 0.0007	Bava et al. [49]
Maize distiller (ethanol production waste)	3.22 ± 0.21	0.27 ± 0.02	0.0056 ± 0.0001	Bava et al. [49]
Okara (tofu production waste)	4.90 ± 0.07	0.36 ± 0.02	0.0021 ± 0.0.000	Bava et al. [49]
Brewer's grains	3.01 ± 0.06 a	0.25 ± 0.01	0.0014 ± 0.0000	Bava et al. [49]

ED1L: experimental diet 1 larvae, ED2L: experimental diet 2 larvae, ED3L: experimental diet 3 larvae, ED4L: experimental diet 4 larvae, and Control: control larvae. *All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

the substrate longer and convert it into biomass, which is likewise confirmed by a higher ECD. Interestingly, however, concerning LGR, the experimental diets exceeded the reported values; this would indicate that larvae reared on CW could convert and utilize a greater amount of waste into larval biomass per day.

3.4. Proximate composition of BSFL biomass

The results of the proximate composition from the larvae reared in the experimental diets are shown in Table 5. The fat content in BSFL is important since it functions as a raw material for biodiesel production. In this sense, the analysis of lipids in the BSFL biomass obtained with the different experimental diets shows a higher amount in ED3L. This could be attributed to the fact that this diet was prepared with fresh CW with a higher temperature and constant amount of fat during the experimental days than ED1L and ED2L. ED1L and ED2L showed a significantly lower amount due to the lower pH and temperature, respectively. BSFL grown on the control substrate showed the lowest values of lipids, which may be attributed to the difference in fat between substrates due to the inclusion of CW.

The amounts of fat obtained from the experimental larvae in the present study (35.95–45.57 % dry matter) are similar to those reported by various authors. Some of these works show larvae with a higher fat content than those of the present study. Ewald et al. [17] reported a crude fat content of up to 46.7 % for larvae reared on trout (*Onchorhynchus mykiss*) mixed in a 5:1 ratio with wheat bran; in another study, Scala et al. [16] described obtaining larvae with 36.1 % fat by being reared on apple; Danieli et al. [54] mentioned that up to 46.9 % could be generated in BSFL reared on a high carbohydrate diet; similarly, Meneguz et al. [53] reported that using fruit waste as substrate, larvae with 40.7 % can be obtained; finally, Jucker et al. [55] reported a fat percentage of 38.67 in larvae reared on cricket waste. However, there are also reports that are outperformed by the results presented in this study. Shumo et al. [56] obtained larvae with an amount of 30.1 %, which were reared on chicken manure; Meneguz et al. [53] reported up to 26.28 % fat in larvae reared on a mixture of vegetables and fruits; larvae reared on Okara generated 31.2 % [49]. Finally, Danieli et al. [54] reported a content of 31.9 % in larvae reared on a high fiber diet.

Table 5
Proximate composition (% dry matter) of larvae reared in experimental diets.

	ED1L	ED2L	ED3L	ED4L	Control
Ash	7.86 ± 0.412^{b}	7.53 ± 0.040^{c}	7.49 ± 0.051^{c}	7.65 ± 0.103^{bc}	8.34 ± 0.032^{a}
Lipids	36.70 ± 1.02^{b}	35.95 ± 1.88^{b}	45.57 ± 0.61^{a}	37.35 ± 0.80^{b}	33.01 ± 0.21^{c}
Protein	28.85 ± 0.06^{b}	27.67 ± 1.59^{bc}	26.28 ± 0.46^{c}	33.26 ± 0.44^{a}	29.27 ± 1.51^{b}
Carbohydrates	0.98 ± 0.14^{a}	1.04 ± 0.20^{a}	1.35 ± 0.05^{b}	1.37 ± 0.15^{b}	1.24 ± 0.17^{b}
Undetermined compounds	25.61	27.82	19.31	20.36	28.14

ED1L: experimental diet 1 larvae, ED2L: experimental diet 2 larvae, ED3L: experimental diet 3 larvae, ED4L: experimental diet 4 larvae, and Control: control larvae. *All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

Regarding protein, a lower amount was observed in ED3L. This is caused to the fact that, as previously mentioned, larval metabolism is affected by temperature [57]. Thus, temperature promoted in ED3L the conversion of nutrients into lipids rather than protein. In contrast, a higher amount of protein was observed in ED4L. This difference may be due to the proper balance of the substrate and its conditions (fresh CW); therefore, the nutrients provided were converted into similar proportions of fat and protein. The other experimental diets showed no differences, which indicates that CW does not negatively affect the amount of protein generated. Still, on the contrary, it can increase if the waste conditions are modified.

In other studies, in which different substrates were used, protein amounts similar to those described in this work were obtained (26.28–33.26 %). Some of the substrates reported are chicken manure (41.1 % dry matter) [56], apple (31.12 %) [16], hen diet (52.8 %), maize distiller (53.4 %), Okara (51.2 %), brewer's grains (54.1 %) [49], high fiber diet (22.2 %) [54], fruit waste (22.97 %), fruit and vegetable mixture waste (31.29 %) [53] cricket waste (37.06 %) and locust waste (49.18 %) [55]. Again, the effect of substrate composition on larval composition is observed. It is highlighted that the substrates that generated larvae with a similar amount of protein to those obtained in this work were apple, high fiber diet, fruit waste, and vegetable and fruit waste, which are rich in carbohydrates and are assimilated as fat. It is important mentioning that the protein quantification methods are different; thus, discrepancies may be due to this.

The reported information and what was obtained in the present article confirm that the amount of nutrients will depend on the composition of the substrate. Since they are mainly composed of carbohydrates and protein, the experimental diets generated in BSFL a significant amount of fat, it is important to highlight that CW (regardless of its conditions) used as a substrate can generate larvae with a greater or equal amount of fat than other wastes. Therefore, it is feasible to use these larvae as a treatment and their subsequent use to obtain biodiesel. It is important mentioning that among the compounds not determined in this study are fiber and/or chitin [58]. These components are of interest because BSFL can be used as feed for some animals [59]. It has been reported that both fiber and chitin, in some proportions, can affect digestibility or stimulate the immune system of some fish [60], respectively.

3.5. Fatty acids profile by GC–MS of BSFL lipids

The fatty acid profile of BSFL reared in the experimental diets and control is shown in Table 6. Regardless of the diet, BSFL presented a higher amount of saturated fatty acids (SFA), which agrees with what is reported in the literature. The fatty acid that composes the majority of saturated fats is lauric acid (C12:0), followed by palmitic acid (C16:0). Regarding unsaturated acids, a balanced composition of two fatty acids, oleic (C18:1 n-9) and linoleic (C18:2 n-6), was found. These proportions are similar to those reported by other authors [17,53,54,61–66].

ED4 was the diet with the highest content of SFA in larvae, followed by ED3 and Control. It was followed by ED3 and Control diets; those with the lowest amount of SFA were ED1 and ED2. Ewald et al. [17] found a positive correlation between larval weight and the percentage of total SFA. However, while this is true for ED1 and ED2, the Control diet generated a similar amount of these acids in larvae reared with ED3. Since the only difference between ED3 and ED4 was the increase in temperature, the analysis suggests that an increase in this factor negatively affects the synthesis of this type of fatty acids. Conversely, prioritizing and increasing the synthesis of SFA decreases the synthesis of monounsaturated fatty acids (MUFA). Regarding polyunsaturated fatty acids (PUFA), it has been described that it is likely that their accumulation in BSFL comes from the substrate [17]. Thus, the observed increase in ControlFA may be due to the fact that this diet contained a higher amount of grain mixture (30 %) than the other experimental diets (25 %). However, what is interesting is the minimal amount of these fatty acids found in ED4L, which should be similar to that reported in ED3L, considering that the only difference was temperature. This suggests that BSFL could metabolize PUFA, SFA, and MUFA, prioritizing the latter two.

It is important to know the fatty acid profile, as the potential use of these organisms will depend on it. For example, it has been reported that SFA, which are the most abundant in BSFL, are unfavorable in the diet because they increase low-density lipoproteins, which can cause thrombosis [56]. Similarly, the profile they have is similar to other oils, such as palm or coconut, which offers several opportunities for its use in both the cosmetic and biofuel industry [17]. Knowing how the substrate and its conditions affect the fatty acid profile of BSFL would allow the engineering of larvae for rearing, depending on their use.

4. Conclusions

The evaluation showed that the use of CW allows larval development and weight gain. Diets ED3 (fresh CW, 38 °C) and ED4 (fresh CW, room temperature) allowed higher weight accumulation in the larvae. The highest fat accumulation (up to 12 % more than the control) was observed in ED3, which had less protein. Higher amounts of SFA (which are suitable for biodiesel production) were generated in the larvae fed with CW. This study highlights the importance of an appropriate pretreatment designed for a specific waste to control desired by-products. Finally, it was demonstrated that cheese whey can be used as a valuable resource to feed black soldier fly larvae, which could have significant implications for the sustainable treatment of dairy industry by-products and the production of nutrient-rich larval biomass. The results emphasize the importance of considering the design of appropriate treatments for different types of waste, which could lead to a more efficient production of larval biomass with potential applications in the food and biofuel industries.

Data availability

Data available on request.

Table 6
Content of identified fatty acids in the experimental diets and control biomass larvae (dry mass).

Identified FA (mg/g)	ED1FA	ED2FA	ED3FA	ED4FA	ControlFA
C 10:0	0.89 ± 0.03^{b}	0.90 ± 0.01^{b}	1.01 ± 0.07^{b}	1.49 ± 0.22^{a}	1.44 ± 0.35^{a}
C 12:0	22.83 ± 1.59^{cd}	21.74 ± 0.71^{d}	26.47 ± 2.10^{c}	41.07 ± 4.93^{a}	35.16 ± 6.11^{b}
C 14:0	9.92 ± 0.10^{d}	10.79 ± 0.16^{a}	10.25 ± 0.03^{c}	10.67 ± 0.28^{ab}	10.49 ± 0.37^{bc}
C 16:0	18.62 ± 0.35^{ab}	19.02 ± 0.19^{a}	19.84 ± 0.60^{a}	16.91 ± 2.21^{b}	14.82 ± 2.41^{c}
C 16:1 (n7)	3.57 ± 0.11^{b}	3.81 ± 0.09^{b}	4.56 ± 0.14^{a}	2.56 ± 0.37^{d}	3.22 ± 0.16^{c}
C 18:0	6.43 ± 0.24^{a}	6.59 ± 0.08^{a}	4.78 ± 0.40^{b}	5.12 ± 0.99^{b}	2.05 ± 1.79^{c}
C 18:1 (n9)	17.96 ± 0.46^{a}	17.91 ± 0.31^{a}	16.40 ± 0.72^{a}	12.26 ± 2.37^{b}	11.79 ± 1.88^{b}
C 18:2 (n6)	17.77 ± 0.70^{b}	17.21 ± 1.10^{b}	15.83 ± 0.47^{c}	9.60 ± 0.65^{d}	19.21 ± 1.17^{a}
C 18:3 (n3)	2.01 ± 0.09^{a}	2.02 ± 0.21^{a}	0.87 ± 0.04^{b}	0.32 ± 0.27^{c}	1.82 ± 0.32^{a}
SFA	58.70 ± 0.97^{c}	59.04 ± 1.07^{c}	62.34 ± 1.33^{b}	75.26 ± 3.27^{a}	63.96 ± 2.11^{b}
MFA	21.53 ± 0.45^{a}	21.72 ± 0.33^{a}	20.96 ± 0.85^{a}	14.82 ± 2.71^{b}	15.01 ± 2.01^{b}
PUFA	19.78 ± 0.79^{b}	19.23 ± 1.31^{b}	16.70 ± 0.50^{c}	9.92 ± 0.64^{d}	21.03 ± 1.43^{a}

ED1FA: experimental diet 1 fatty acids, ED2FA: experimental diet 2 fatty acids, ED3FA: experimental diet 3 fatty acids, ED4FA: experimental diet 4 fatty acids, ControlFA: control fatty acids, SFA: saturated fatty acids, MFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids. *All values shown are mean ± standard deviation (SD; n = 3). Different letters indicate significant differences between treatments ($p \leq 0.05$).

CRediT authorship contribution statement

Valeria Caltzontzin-Rabell: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing – original draft. **Ana Angélica Feregrino-Pérez:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Claudia Gutiérrez-Antonio:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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. Supplementary data

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