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# Growth of Black Soldier Fly Larvae Reared on Organic Side-Streams

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**Abstract:** Black soldier fly (BSF) larvae may play a role in a circular economy by upcycling low-value organic streams into high value biomass. In this paper, the capacity of BSF larvae to process 12 organic side-streams (mono-streams) and two standard substrates (chicken start mash and Gainesville diet) was investigated. Survival, larval mass, feed conversion ratio, and waste reduction were evaluated in relation to the proximate composition of the side-streams used. Survival rates larger than 80% were observed for 10 of the organic mono-streams and the two standard substrates. Maximum mean larval weight ranged from 38.3 mg up to 176.4 mg regardless of high survival and was highly correlated with substrate crude protein content. Feed conversion ratio (range 1.58–8.90) and waste reduction (range 17.0–58.9%) were similar to values reported in other studies in the literature. On low protein substrates (e.g., apple pulp), survival rates remained high, however, possibly due to protein deficiency, limited larval growth was observed. It is concluded that several low value organic side-streams can successfully be processed by BSF larvae, thereby opening the possibility of lowering the costs of BSF farming. Potentially mixing nutritionally distinct mono-streams into a mixed substrate might improve BSF performance. However, more research is needed for optimizing diets to guarantee production of BSF larvae of constant yield and quality.

**Keywords:** black soldier fly; feed conversion ratio; side-streams; nutritional composition; waste reduction

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## 1. Introduction

The continuous growth in the global population and the inevitable increase in demand for natural resources have had a negative impact on all life on Earth. Annual global waste generation has reached approximately 17 billion tons and is expected to rise to 27 billion tons by 2050. Currently, waste production and improper management is responsible for the production of 1.6 billion tons of $CO_2$ emissions [1]. The depletion of natural resources, the increasing use of valuable land surface for human activities, and the generation of waste are detrimental to our planet. More sustainable alternatives to guarantee a sufficient supply of food, feed, and biomaterials are needed.

In the last decades, an evolution toward organic, sustainable, and more environmental friendly systems has emerged. This necessary evolution is reflected by the Sustainable Development Goals (SDG) of the United Nations [2]. Additionally, the European Green Deal, which aims to implement the United Nation's SDG, provides objectives and an action plan to move to a clean, circular economy that restores biodiversity and cuts pollution [3]. In line with this, EC Directive No. 2008/98 establishes the order of priority in the choice of by-product treatments ranging from prevention, preparing for re-use, recycling and other recovery, down to disposal [4]. Many initiatives in diverse domains have been undertaken with the aim to implement sustainable processes for (a) generating food and feed alternatives; (b) energy; (c) reducing waste production; (d) recycling materials; (e) implementing renewable feedstock; and (f) generating biomaterials. Many people consider plants as sustainable and environmental-friendly sources for food, feed, and biomaterials. However, this perception is not entirely correct as plants require massive amounts of land, which results in land-use for agriculture, leading to deforestation and a loss of biodiversity [2]. Additionally, in the case of plant products derived from tropical regions, transport to EU countries also imposes an environmental impact. The use of plant-based biomaterials for feed and technical applications also counters opposition, especially when there is competition to implement it in food products [5].

One of the alternatives that may hold an interesting position in a circular economy is the implementation of insects [6]. As insects have proven to be able to efficiently convert low-value biomass into their own biomass consisting of high-quality components (i.e., proteins, fats and chitin [7]), they have the potential to help tackle the societal challenges [8]. Using insects for food, feed, biomaterial production, and to valorize side-streams is a strategy that has gained increased interest. Insects are believed to have a lower ecological impact than current livestock. Moreover, research has shown that insects produce less greenhouse gases and emit significantly less ammonia [9]. In addition, insects convert feed into biomass more efficiently than conventional livestock [8] because they are ectothermic and therefore use little energy to maintain their body temperature [10]. Furthermore, insects can be produced in vertical farming systems, making them more productive per $m^2$ [11] and they generally require less water [12]. Therefore, insects might be able to play a key role in the circular economy, meeting the requirements for a more sustainable society.

In particular, *Hermetia illucens* (Diptera: Stratiomyidae), better known as the black soldier fly (BSF), is a very interesting insect species that is intensively investigated to implement as a waste-converter [13,14]. It is a non-pest insect species that can convert diverse (non-value) organic waste streams into biomass that can be used for the production of feed [15], biomaterials for biodiesel production [16], technical applications such as surfactants [17], and protein-based bioplastics [18]. BSF larvae (BSFL) can play an important role in organic waste reduction and renewable biomaterial production, providing a useful addition to a circular economy, and can contribute to the EU Green Deal strategy of reducing food waste by 50% by 2030 [3]. However, several aspects to improve the sustainable production and economic viability of BSFL and their derived biomaterials need to be investigated [19]. LCA analyses indicate that the production of BSFL can be sustainable and economically viable if they can be reared on low quality biomass such as manure or mixed organic wastes [20].

BSFL have been shown to thrive on a wide variety of substrates including food waste, agri-industry co-products, animal waste, and meat [19,21]. However, there are differences in the growth, survival, and bioconversion efficiency of BSFL grown on different substrates. One of the conceivable factors that influence this variation is their nutrient content. Indeed, varying macronutrient content (i.e., the protein, fat, or carbohydrate content) has a significant influence on the performance and composition of BSFL [22,23]. Several studies have been published in which BSF is reared on waste streams of which the macronutrient content is also investigated [22,24–32]. These studies show that the macronutrient content influences BSFL growth performance and composition, however, diets with similar macronutrient composition show differences that may be due to other factors not analyzed such as texture or nutrient quality, vitamins, and minerals [22]. Often, streams classified as the same type of side-stream vary greatly in nutritional content as demonstrated by Gold and coworkers [14]. These differences emphasize the need to evaluate the potential of local side-streams.

Here, we evaluate the potential of BSFL to process a selection of low-value side-streams available in Flanders, Belgium, in order to evaluate the larval performance on each of these. By analyzing the macronutrient content (proteins, lipids, non-fiber carbohydrates), fibers (cellulose, hemicellulose, and lignin), and the mineral content of the side-streams, we investigated their influence on larval performance and bioconversion.

## 2. Materials and Methods

### *2.1. Selection and Preparation of the Feed Substrates*

#### 2.1.1. Side-Streams

Twelve side-streams were selected for breeding trials: apple pulp (pulp left behind after apple-juice production), beer draff (side-stream from beer-brewing), industrial food waste (supermarket and restaurant waste), chicken manure (mix of wood-pulp bedding and chicken manure), corn meal (ground corn), forced chicory roots (matured roots of chicory), fruit puree (fruit overproduction, mixed into a slurry), grain middlings 1 and 2 (side-stream from wheat-industry collected in either July 2020 or January 2021), household food waste (organic food waste picked up from household containers, mixed into a slurry), tomato leaves (ground tomato leaves and stalk leftovers after tomato harvesting), and vegetable overproduction (Overproduction from auctions, mixed into a slurry). The side-streams were selected on the basis of their availability in the Flanders region, Belgium. Only streams that are currently destined for biogas or composting were selected. These streams are often not allowed to be used as animal feed (legislation) or are currently just not used for this purpose due to overproduction or unsuitable nutritional composition.

#### 2.1.2. Control Substrates

Two diets were used as control substrates: chicken feed (chicken start mash, AVEVE, Belgium) and Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% corn meal [33]).

#### 2.1.3. Feed Preparation

Upon arrival, the side-streams were ground (Robot Coupe blixer 23, robot-coupe, The Netherlands) and frozen at −20 °C. Dry matter content of the side-streams was determined on a sample of the fresh side-stream by oven-drying at 105 °C until constant weight. Prior to the experiment, side-streams were defrosted and dry matter content was adjusted by adding water or by oven-drying (UF55, Memmert, Germany) at 60 °C until 30% dry matter was obtained. Prior to feeding, the side-streams were brought to 27 °C.

### *2.2. Substrate Analysis*

#### 2.2.1. Pretreatment of the Substrates for Chemical Analysis

A sub-sample of 1 L was taken from the fresh, pre-treated side-streams for chemical analysis. The samples were oven-dried at 60 °C, milled with a stainless-steel mill (IKA, Tube mill 100, Staufen, Germany) and sieved through a 1-mm screen before analysis.

#### 2.2.2. Determination of the Proximate Analysis

Dry matter content (DM) was determined in an oven at 105 °C for 24 h (Heraeus, UT 6420). Fixed mineral residue or crude ashes (CA) was determined by incineration in a muffle furnace at 550 °C for 4 h (L9/11/SKM, Nabertherm, Germany).

Lipid extraction or ether extract (EE) was performed with petroleum ether (40–60 °C, BP) using Soxhlet equipment following NBN EN ISO 11,085 [34] without hydrolysis. Petroleum ether was evaporated and recuperated at 50 °C using a rotary evaporator (Rotavapor R-300, Büchi, Switzerland).

Crude protein content (CP) was calculated by the determination of nitrogen present in the samples using the Kjeldahl method (Kjeldatherm Vapodest 20 s, Gerhardt, Germany) following NBN EN ISO 5983-1 (2005) [35]. Appropriate conversion factors were used. In general, a nitrogen to crude protein factor of 6.25 was used. However, for vegetable/fruit side-streams, a factor of 4.39 was applied [36], for plant materials 4.23 [37] and for poultry manure 3.11 [36]. Therefore, in this study, a factor of 4.39 was applied for apple pulp, fruit puree, and vegetable overproduction auction. A factor of 4.23 was used for tomato leaves and forced chicory roots, and for chicken manure, a factor of 3.11 was applied. For the control substrates and the remaining side-streams, the general nitrogen to crude protein factor of 6.25 was used.

Fiber concentration was analyzed as the amylase-treated neutral detergent fiber content (aNDF), following the extraction protocol of Gerhardt (Fibretherm, Gerhardt, Germany) based on the Van Soest method [38].

Non-fiber-carbohydrates (NFC) were calculated using aNDF according to the following formula:

$$NFC\% = 100\% - (CP\% + aNDF\% + EE\% + Ash\%) \quad (1)$$

Gross energy (GE) was calculated based on the Atwater conversion factors [39]:

$$GE\ (kcal/100\ g\ DM) = 9 \times EE\% + 4 \times CP\% + 4 \times NFC\% \quad (2)$$

#### 2.2.3. Determination of Mineral Composition and Fiber Profile

Minerals were determined by ICP-OES (Optima 4300™ DV ICP-OES, Perkin Elmer, Massachusetts, USA) after acid digestion of the samples, following CMA/2/IV/6 5.3 [40]. The macro-elements P, Na, K, Mg, and micro-elements Zn, Cu, and Fe were determined.

Fiber profile included the determination of amylase-treated neutral detergent fiber (aNDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) following the extraction protocols of Gerhardt (Fibretherm) based on the Van Soest method [38]. Based on ADF, ADL, and aNDF, cellulose (ADF-ADL) and hemicellulose (aNDF-ADF) could be calculated.

### *2.3. Rearing Experiment*

#### 2.3.1. *H. illucens* Origin and Maintenance

*H. illucens* is continuously propagated by the RADIUS group (Research group at Thomas More University of applied sciences, Belgium). Facilities are in place to rear and process insects at laboratory and pilot level. For the feed experiments in this study, 1 g of eggs (deposited in a timespan of 24 h) was collected on a plastic weighing dish. The dish was put into a plastic container (diameter 95 mm) on a mixture of 100 g chicken feed (Chicken Start Mash 259, AVEVE, Belgium) and 100 mL tap water (total dry matter content of 45%). The container with the eggs was incubated in a climate chamber at 27 °C and 60% RH. On day 3 after egg collection, the weighing dish was removed and the chicken feed containing newly hatched larvae was gently mixed using a table spoon. At day 5, 50 mL of tap water was added and the substrate was gently mixed. Seven days after harvesting the eggs, larvae were separated from the residue by sieving and by forceps.

#### 2.3.2. Feed Experiment

For each sample, 500 7-day-old larvae were put into a plastic container (17.5 × 11.9 × 5.9 cm). Larvae were fed four times, being on days 1, 2, 3, and 6 of the experiment. On days 1 and 2, larvae received 50 g of substrate. On day 3 and day 6, 150 g was given. Growth of the larvae was monitored by daily weighing 10 larvae for each sample (except for days 4 and 5). At the end of the experiment on day 9, larvae were separated from the remaining residue by forceps. Larvae were counted, weighed, and dry matter content was determined. After harvesting the larvae, total mass and dry matter content of the residues was determined.

The feeding trials were executed in either one or two rounds, depending on the amount of side-stream that was sampled. In the first round, every side-stream was tested as a feed substrate in order to evaluate larval growth. In case enough material was present for a second feed trial, the side-stream was tested again in round 2 to evaluate reproducibility. For each of these rounds, feed experiments were performed in triplo for every substrate.

### *2.4. Calculations*

Larval survival was determined by counting larvae at the start and end of the feed experiments.

Mean larval weight for plotting the growth curve was determined by randomly selecting and weighing 10 BSF larvae each day starting at day 1 until day 9 of the experiment.

Bioconversion efficiency (BE), feed conversion ratio (FCR), and waste reduction (WR) were calculated at harvest as:

$$BE = (L_{end,\ dry\ matter} - L_{start,\ dry\ matter})/D \times 100\% \quad (3)$$

$$FCR = D/(L_{end,\ fresh\ larvae} - L_{start,\ fresh\ larvae}) \quad (4)$$

$$WR = ((D - R)/D) \times 100\% \quad (5)$$

where $L_{end}$ is the larval biomass at the end and $L_{start}$ is the larval biomass at the start. D is the amount of diet provided and R the residue left at harvest, both in g dry matter.

### *2.5. Statistical Analyses*

All statistical analysis and graphical illustrations were drafted using the JMP Pro 15.1.0 software package from SAS (Buckinghamshire, UK). In order to compare maximal larval weights, bioconversion efficiencies, feed conversion ratios, waste reduction ratios, and larval survival ratios, normal distribution of the residuals was checked and also equal variances were tested using Levene's test. If normal distribution and equal variances were confirmed, then one-way ANOVA and Tukey HSD post hoc tests were used, applying a $p$-value of 0.05.

Principal component analysis (PCA) was used to identify the most important factors explaining the data variance. A standard least square regression model was drafted describing both the main effects and interaction effects of the substrate protein, fat, and non-fiber carbohydrate contents on the maximal larval weight. Non-significant effects ($p < 0.05$) were excluded from the model, while keeping significant effects on mean larval growth.

## 3. Results

### *3.1. Substrate Analysis*

#### 3.1.1. Proximate Analysis

The results of the proximate analyses of the different substrates are presented in Table 1. Gainesville diet (GVD) and chicken start mash (CSM) are frequently used standard diets for BSFL rearing. Both have low crude fat contents (under 5%) and crude protein contents over 15%. Non-fiber carbohydrate contents were respectively 33.1% and 48.2%. The gross energy content of CSM was 315 kcal/100 g DM, which was 44% higher compared to GVD (219 kcal/100 g DM).

**Table 1.** Proximate analysis of standard feed and side-streams with dry matter content (DM), fat content or ether extract (EE), crude protein content (CP), crude ash content (CA), amylase-treated neutral detergent fiber content (aNDF), non-fiber carbohydrate content (NFC), and gross energy content (GE). Values are reported as mean ± standard deviation ($n$ = 3).

| Substrate | DM [1] | EE [2] | CP [2] | CA [2] | aNDF [2] | NFC [2] | GE [3] |
|---|---|---|---|---|---|---|---|
| Gainesville diet | 90.5 ± 0.0 | 2.6 ± 0.2 | 15.7 ± 0.3 | 6.9 ± 0.3 | 41.7 ± 0.3 | 33.1 ± 1.1 | 219 |
| Chicken start mash | 90.6 ± 0.1 | 4.6 ± 0.4 | 20.4 ± 2.4 | 6.0 ± 1.3 | 20.9 ± 1.2 | 48.2 ± 5.3 | 315 |
| Apple pulp | 25.9 ± 0.1 | 4.6 ± 0.1 | 3.4 ± 0.0 | 1.9 ± 0.1 | 43.3 ± 1.0 | 47.0 ± 1.2 | 242 |
| Beer draff | 29.4 ± 0.5 | 5.5 ± 0.2 | 19.4 ± 0.6 | 4.5 ± 0.1 | 50.6 ± 0.4 | 20.0 ± 1.3 | 207 |
| Industrial Food Waste | 17.8 ± 0.7 | 10.9 ± 0.6 | 18.5 ± 0.1 | 9.2 ± 0.0 | 7.8 ± 0.4 | 53.5 ± 1.1 | 386 |
| Chicken manure | 67.3 ± 3.2 | 3.3 ± 0.1 | 13.1 ± 0.2 | 13.0 ± 0.1 | 41.5 ± 0.6 | 29.1 ± 1.0 | 198 |
| Corn meal | 87.0 ± 0.0 | 8.1 ± 0.1 | 9.5 ± 0.2 | 2.3 ± 0.1 | 32.8 ± 2.0 | 47.3 ± 2.4 | 300 |
| Forced chicory roots | 15.7 ± 0.2 | 0.9 ± 0.0 | 4.6 ± 0.1 | 31.0 ± 0.9 | 14.2 ± 0.5 | 49.3 ± 1.5 | 224 |
| Fruit puree | 6.2 ± 0.1 | 4.5 ± 0.1 | 10.0 ± 0.1 | 12.6 ± 0.2 | 33.3 ± 0.1 | 39.9 ± 0.5 | 239 |
| Grain middlings 1 | 90.2 ± 0.1 | 2.9 ± 0.1 | 14.1 ± 0.1 | 7.9 ± 0.2 | 22.0 ± 0.9 | 53.1 ± 1.3 | 295 |
| Grain middlings 2 | 87.8 ± 0.1 | 1.4 ± 0.0 | 9.6 ± 0.4 | 19.7 ± 0.7 | 45.9 ± 0.6 | 23.4 ± 1.7 | 144 |
| Household food waste | 23.9 ± 0.7 | 14.6 ± 0.2 | 16.7 ± 0.6 | 8.4 ± 0.3 | 15.5 ± 0.1 | 44.8 ± 1.2 | 377 |
| Tomato leaves | 88.4 ± 0.0 | 1.2 ± 0.1 | 12.1 ± 0.3 | 29.0 ± 0.2 | 21.2 ± 2.2 | 36.5 ± 2.8 | 205 |
| Vegetable overproduction auction | 8.1 ± 0.2 | 2.0 ± 0.3 | 10.5 ± 0.4 | 15.3 ± 0.1 | 30.2 ± 1.1 | 42.0 ± 1.9 | 228 |

[1] g/100 g fresh matter; [2] g/100 g dry matter; [3] kcal/100 g dry matter.

The gross energy content of apple pulp is 242 kcal/100 g DM, which is higher than GVD. Both the crude fat content and the non-fiber carbohydrate content were similar to CSM, however, a low crude protein content (3.4% DM) was present in this side-stream. This was 4.6 times lower than GVD and 6.0 times lower than CSM.

For beer draff, both the crude fat and crude protein content were comparable to CSM. The non-fiber carbohydrate content was only 20.0%, as the extraction of that fraction is the main aim in the brewing processes [41]. This results in a lower gross energy content than CSM, however, the gross energy is still comparable to GVD.

Industrial food waste has a very high gross energy content of 386 kcal/100 g DM. A relatively high fat content of 10.9% is present, which is more than double compared to CSM. The protein content is comparable to CSM. A very high non-fiber carbohydrate content is present in this stream.

The crude fat content of chicken manure was 3.3%, which is lower than CSM, but higher than GVD. The protein content was still 13.1%, which is relatively close to GVD. Additionally, the non-fiber carbohydrate content was slightly lower, but still close to that of GVD. The gross energy content was about 10.6% lower compared to GVD.

Corn meal had a relatively high crude fat content compared to the controls of 8.1%. The crude protein content was only 9.5%, which was significantly lower than the control feeds. The non-fiber carbohydrate content was comparable to CSM, resulting in a gross energy content of 300 kcal/100 g dry matter.

Forced chicory roots contain very low fat contents of 0.9%. Additionally, the protein content was only 4.6%. The gross energy was still 224 kcal/100 g DM, which is mainly due to the high non-fiber carbohydrate content of 49.3%.

Fruit puree has a similar fat content to CSM and a crude protein content of 10.0%, being significantly lower than CSM and GVD. The non-fiber carbohydrate content was 39.9%. Therefore, the gross energy content was still higher than GVD.

Grain middlings have relatively low fat contents. The crude protein content of the first sampling round was comparable to GVD, however, on the second sampling round, the protein content was remarkably lower (9.6%). The biggest difference between the two sample rounds was found in the non-fiber carbohydrate content. In the first sampling round, a higher content (53.1%) was measured than in CSM. This content was much lower in the second sampling round (23.4%). This resulted in a gross energy of 295 kcal/100 g dry matter for sampling round 1 and only a 144 kcal/100 g dry matter for sampling round 2.

Household food waste turned out to be a very rich substrate with a very high crude fat content of 14.6%. This was 33.9% higher than industrial food waste and 217% higher than CSM. The protein content and non-fiber carbohydrate content were both higher than GVD, but lower than CSM. The high gross energy content of 377 kcal/100 g DM was similar to that of industrial food waste.

Tomato leaves have a very low crude fat content of 1.2%. The crude protein content was 12.1%, which was only 29.8% lower compared to GVD. The non-fiber carbohydrate content was comparable to GVD. Additionally, the total gross energy content was similar to GVD.

Vegetable overproduction has a low fat content of 2.0% and a relatively low protein content of about 10.5%. The gross energy content was still 228 kcal/100 g DM, which is mainly due to the non-fiber carbohydrate content of 42.0%.

#### 3.1.2. Determination of Minerals and Fiber Profile

The macro-element profiles of the substrates are presented in Table 2. Phosphorus contents ranged from 0.18 to 1.11 g/100 g DM, with GVD, CSM, and chicken manure being the highest. Magnesium ranged between 0.01 and 1.06 g/100 g DM. Potassium contents ranged between 0.16 and 4.41 g/100 g DM. The highest amount of K was found in fruit puree, however, high contents were also present in tomato leaves, vegetable overproduction, and chicken manure. Both industrial and household food waste have very high sodium contents of 1.30 and 0.55 g/100 g DM, respectively. Calcium contents had a wide range between 0.00 and 8.14 g/100 g DM.

**Table 2.** Determination of macro-elements of standard feed and substrates (g/100 g DM). Values are reported as mean ± standard deviation (*n* = 3).

| Substrate | P | Mg | K | Na | Ca |
|---|---|---|---|---|---|
| Gainesville diet | 1.11 ± 0.02 | 0.33 ± 0.02 | 1.61 ± 0.12 | 0.01 ± 0.00 | 0.60 ± 0.04 |
| Chicken start mash | 0.85 ± 0.09 | 0.25 ± 0.02 | 0.82 ± 0.06 | 0.13 ± 0.02 | 1.14 ± 0.50 |
| Apple pulp | 0.14 ± 0.01 | 0.11 ± 0.00 | 0.61 ± 0.01 | 0.01 ± 0.00 | 0.11 ± 0.00 |
| Beer draff | 0.48 ± 0.04 | 0.16 ± 0.01 | 0.15 ± 0.01 | 0.00 ± 0.00 | 0.18 ± 0.01 |
| Industrial food waste | 0.56 ± 0.03 | 0.16 ± 0.01 | 1.36 ± 0.04 | 1.30 ± 0.03 | 1.14 ± 0.05 |
| Chicken manure | 0.93 ± 0.02 | 0.62 ± 0.01 | 2.76 ± 0.03 | 0.33 ± 0.00 | 1.05 ± 0.02 |
| Corn meal | 0.68 ± 0.02 | 0.22 ± 0.07 | 0.63 ± 0.18 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Forced chicory roots | 0.18 ± 0.01 | 0.20 ± 0.01 | 2.04 ± 0.06 | 0.29 ± 0.01 | 0.98 ± 0.01 |
| Fruit puree | 0.65 ± 0.01 | 0.38 ± 0.02 | 4.41 ± 0.12 | 0.28 ± 0.01 | 0.57 ± 0.02 |
| Grain middlings 1 | 0.59 ± 0.01 | 0.16 ± 0.00 | 0.66 ± 0.01 | 0.08 ± 0.00 | 2.29 ± 0.11 |
| Grain middlings 2 | 0.30 ± 0.01 | 0.13 ± 0.01 | 0.68 ± 0.01 | 0.00 ± 0.00 | 0.68 ± 0.04 |
| Household food waste | 0.50 ± 0.01 | 0.15 ± 0.03 | 1.15 ± 0.09 | 0.55 ± 0.02 | 1.24 ± 0.09 |
| Tomato leaves | 0.57 ± 0.02 | 1.06 ± 0.06 | 3.29 ± 0.16 | 0.05 ± 0.00 | 8.14 ± 0.12 |
| Vegetable overproduction auction | 0.58 ± 0.02 | 0.29 ± 0.01 | 3.09 ± 0.08 | 0.06 ± 0.01 | 1.22 ± 0.01 |

Micro-element composition of the substrates is shown in Table 3. Zinc contents ranged between 0.002 and 0.041 g/100 g DM. The highest content, by a large margin, was found in chicken manure. Little variation was found for Cu, as all streams ranged between 0.001 and 0.010 g/100 g DM. The highest iron content was found in chicory roots, being 0.567 g/100 g DM. Additionally, high contents were present in grain middlings 2 (0.308 g/100 g DM). All other side-streams had iron contents ranging between 0.003 and 0.091 g/100 g DM).

**Table 3.** Determination of micro-elements of standard feed and substrates (g/100 g DM). Values are reported as mean ± standard deviation (*n* = 3).

| Substrate | Zn | Cu | Fe |
|---|---|---|---|
| Gainesville diet | 0.005 ± 0.000 | 0.001 ± 0.000 | 0.016 ± 0.001 |
| Chicken start mash | 0.012 ± 0.003 | 0.002 ± 0.000 | 0.016 ± 0.002 |
| Apple pulp | 0.001 ± 0.000 | 0.001 ± 0.000 | 0.003 ± 0.000 |
| Beer draff | 0.006 ± 0.000 | 0.001 ± 0.000 | 0.008 ± 0.001 |
| Industrial food waste | 0.015 ± 0.000 | 0.010 ± 0.001 | 0.091 ± 0.000 |
| Chicken manure | 0.041 ± 0.001 | 0.009 ± 0.000 | 0.073 ± 0.002 |
| Corn meal | 0.004 ± 0.000 | 0.000 ± 0.000 | 0.006 ± 0.000 |
| Forced chicory roots | 0.004 ± 0.000 | 0.002 ± 0.000 | 0.567 ± 0.024 |
| Fruit puree | 0.006 ± 0.000 | 0.001 ± 0.000 | 0.011 ± 0.000 |
| Grain middlings 1 | 0.007 ± 0.000 | 0.002 ± 0.000 | 0.018 ± 0.002 |
| Grain middlings 2 | 0.006 ± 0.000 | 0.001 ± 0.000 | 0.308 ± 0.037 |
| Household food waste | 0.004 ± 0.000 | 0.001 ± 0.000 | 0.048 ± 0.003 |
| Tomato leaves | 0.004 ± 0.000 | 0.001 ± 0.000 | 0.010 ± 0.001 |
| Vegetable overproduction auction | 0.002 ± 0.000 | 0.001 ± 0.000 | 0.060 ± 0.003 |

The fiber profiles of the standard feeds and substrates are presented in Table 4. Crude fiber contents ranged between 0.5 and 25.7% for all substrates. Highest crude fiber contents were determined for apple pulp (25.7%), grain middlings 2 (20.9%), beer draff (18.4%), fruit puree (17.6%) chicken manure (14.2%), and tomato leaves (13.7%). ADL displays the lignin content, which was highest for apple pulp (14.3%) and vegetable overproduction (9.9%). All other substrates contained lignin contents of 6.0% or lower. After determination of ADF and ADL, cellulose contents can be calculated. Cellulose contents in all substrates varied between 5.3 and 21.5%. Streams containing high cellulose contents were apple pulp (21.5%), fruit puree (20.7%), vegetable overproduction (19.8%), tomato leaves (18.0%), chicken manure (17.9%), beer draff (16.9%), grain middlings 2 (15.9%), forced chicory roots (15.0%), and GVD (13.7%). All other streams contained a cellulose content lower than 10%. Hemicellulose contents up to 27.7% were calculated in the side-streams. Low hemicellulose contents of under 10% were present in vegetable overproduction, tomato leaves, household food waste, industrial food waste, fruit puree, forced chicory roots, and apple pulp. Hemicellulose contents are calculated based on the aNDF and ADF content. A negative value was displayed for forced chicory roots. A possibility is that the wood-like texture, compact structure, or even the particle size of chicory roots lead to a partial digestion and subsequently to an over-estimation of ADF, resulting in a negative calculated hemicellulose content.

**Table 4.** Determination of the fiber profile of standard feed and substrates (g/100 g DM). Values are reported as mean ± standard deviation ($n$ = 3).

| Substrate | CF | aNDF | ADF | ADL (Lignin) | Cellulose | Hemicellulose |
|---|---|---|---|---|---|---|
| Gainesville diet | 5.1 ± 0.1 | 41.7 ± 0.3 | 17.9 ± 0.3 | 4.2 ± 0.0 | 13.7 | 23.8 |
| Chicken start mash | 12.0 ± 0.1 | 20.9 ± 1.2 | 6.7 ± 0.3 | 1.1 ± 0.2 | 5.7 | 14.1 |
| Apple pulp | 25.7 ± 0.1 | 43.3 ± 1.0 | 35.7 ± 1.0 | 14.3 ± 0.3 | 21.5 | 7.5 |
| Beer draff | 18.4 ± 0.4 | 50.6 ± 0.4 | 22.9 ± 0.4 | 6.0 ± 0.2 | 16.9 | 27.7 |
| Industrial food waste | 3.2 ± 0.1 | 7.8 ± 0.4 | 6.7 ± 0.1 | 1.3 ± 0.1 | 5.3 | 1.2 |
| Chicken manure | 14.2 ± 0.1 | 41.5 ± 0.6 | 21.4 ± 0.3 | 3.5 ± 0.1 | 17.9 | 20.1 |
| Corn meal | 4.6 ± 0.1 | 32.8 ± 2.0 | 6.6 ± 0.0 | 0.7 ± 0.1 | 5.9 | 26.2 |
| Forced chicory roots | 8.7 ± 0.3 | 14.2 ± 0.5 | 16.9 ± 0.3 | 1.9 ± 0.0 | 15.0 | -2.7 |
| Fruit puree | 17.6 ± 0.1 | 33.3 ± 0.1 | 24.5 ± 0.3 | 3.8 ± 0.0 | 20.7 | 8.8 |
| Grain middlings 1 | 6.6 ± 0.7 | 22.0 ± 0.9 | 6.8 ± 0.1 | 1.1 ± 0.1 | 5.8 | 15.2 |
| Grain middlings 2 | 20.9 ± 0.2 | 45.9 ± 0.6 | 18.7 ± 0.4 | 2.8 ± 0.2 | 15.9 | 27.2 |
| Household food waste | 6.6 ± 0.1 | 15.5 ± 0.1 | 12.6 ± 0.4 | 3.5 ± 0.1 | 9.1 | 2.9 |
| Tomato leaves | 13.7 ± 0.4 | 21.2 ± 2.2 | 18.8 ± 0.1 | 0.8 ± 0.1 | 18.0 | 2.4 |
| Vegetable overproduction auction | 8.6 ± 0.2 | 30.2 ± 1.1 | 29.8 ± 0.7 | 9.9 ± 0.2 | 19.8 | 0.4 |

CF: crude fiber; aNDF: amylase-treated neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

### *3.2. Rearing Experiment*

As displayed in Table 5, high survival rates of over 90% were obtained for larvae grown on GVD, CSM, apple pulp, beer draff, chicken manure, forced chicory roots, fruit puree, grain middlings 2, vegetable overproduction, and corn meal. Survival rates for larvae grown on industrial food waste and household food waste were below 90% (88.8% and 83.2%, respectively), however, the survival rates of larvae reared on these side-streams were not significantly different. Only 50.0% of larvae reared on grain middlings 1 survived, which was significantly lower compared to the ones above-mentioned. On tomato leaves, none of the larvae survived.

**Table 5.** Survival rate, maximal larval weight, and time to reach the maximal weight of black soldier fly larvae. Values are reported as mean ± standard deviation (replicates indicated in the table as n).

| Substrate | $n$ | Survival Rate (%) | Maximal Mean Larval Weight (mg) | Time to Reach Maximum Weight (day) |
|---|---|---|---|---|
| Gainesville diet | 6 | 97.2 ± 1.8 $^{a}$ | 83.8 ± 13.8 $^{a,f,g}$ | 8 |
| Chicken start mash | 6 | 96.3 ± 1.9 $^{a}$ | 148.4 ± 21.8 $^{a,b}$ | 7 |
| Apple pulp | 6 | 95.5 ± 3.5 $^{a}$ | 38.3 ± 4.4 $^{h}$ | 8 |
| Beer draff | 6 | 95.2 ± 3.1 $^{a}$ | 130.9 ± 19.6 $^{b,c,d}$ | 7 |
| Industrial food waste | 5 | 88.8 ± 3.2 $^{a}$ | 176.4 ± 15.3 $^{a}$ | 9 |
| Chicken manure | 6 | 97.7 ± 1.4 $^{a}$ | 134.9 ± 11.8 $^{b,c}$ | 8 |

**Table 5.** *Cont.*

| Substrate | *n* | Survival Rate (%) | Maximal Mean Larval Weight (mg) | Time to Reach Maximum Weight (day) |
|---|---|---|---|---|
| Corn meal | 6 | 91.8 ± 4.0 [a] | 110.4 ± 13.2 [c,d,e] | 9 |
| Forced chicory roots | 3 | 98.0 ± 1.7 [a] | 46.7 ± 4.9 [g,h] | 8 |
| Fruit puree | 3 | 99.0 ± 1.0 [a] | 80.1 ± 8.0 [a,f,g] | 9 |
| Grain middlings 1 | 6 | 50.0 ± 25.5 [b] | 98.5 ± 26.3 [d,e,f] | 9 |
| Grain middlings 2 | 6 | 97.5 ± 2.1 [a] | 78.0 ± 12.5 [a,g] | 8 |
| Household food waste | 6 | 83.2 ± 8.0 [a] | 65.3 ± 13.9 [g,h] | 8 |
| Tomato leaves | 3 | 0.0 ± 0.0 c | 3.1 ± 0.0 [i] | 1 |
| Vegetable overproduction auction | 3 | 98.0 ± 0.4 [a] | 88.1 ± 5.8 [d,e,f,g] | 7 |

[a–i]: Values not connected by the same letter are significantly different.

Maximal mean larval weights between 38.3 mg and 176.4 mg were obtained on the side-streams (other than tomato leaves). Low larval growth was observed for larvae grown on apple pulp (38.3 mg), forced chicory roots (46.7 mg), and household food waste (65.3 mg). Highest larval growth weights were observed when larvae were fed with industrial food waste (176.4 mg), CSM (148.4 mg), chicken manure (134.9 mg), and beer draff (130.9 mg). All remaining side-streams had maximal mean larval weights in the range between 78.0 and 110.4 mg.

A growth curve was made based on the data acquired during the rearing experiment (Figure 1). Most larvae reached their maximal weight after seven to eight days (Table 3). The decrease in mean larval weight is due to larvae going into the prepupal stage, in which the larvae stop eating and spend energy to either pupate or search for a suitable place to pupate. As can be seen in the growth curve in Figure 1, larvae grown on industrial waste and grain middlings 1 potentially did not reach their maximal weight yet on day 9 of the experiment.

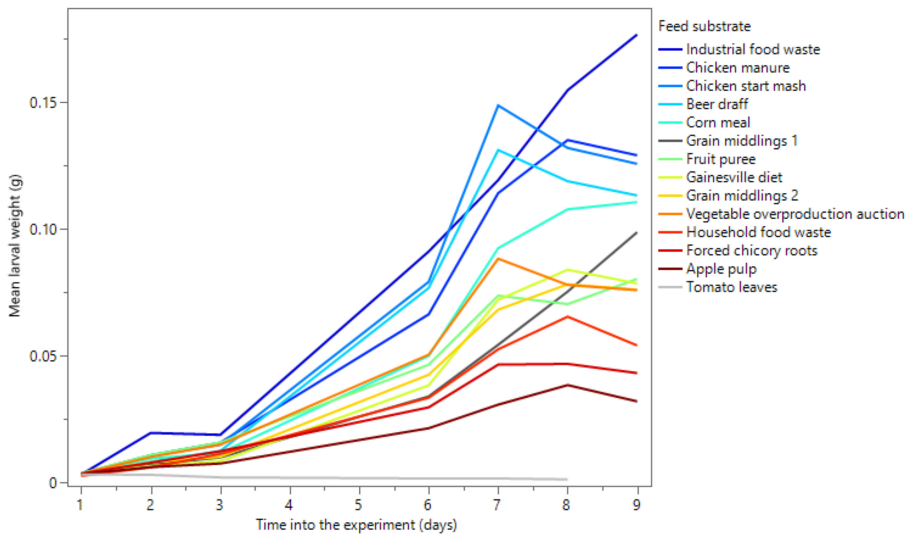


**Figure 1.** Daily mean larval weight (g) for each of the substrates over the course of the experiment. No measurements were carried out on days 4 and 5 of the experiment.

Bioconversion efficiencies are displayed in Table 6. This efficiency represents the percentage of dry substrate that is converted into dry larval biomass. The highest bioconversion efficiencies were found for larvae reared on industrial food waste (20.71%), CSM (17.56%), corn meal (15.61%), chicken manure (15.43%), and beer draff (14.4%). The lowest bioconversion efficiencies were calculated for apple pulp (3.59%), grain middlings 1 (3.68%), and forced chicory roots (4.49%). Another way to display the efficiency of converting feed into biomass is by using the feed conversion ratio (FCR). This ratio displays the amount of dry substrate required for the production of 1 kg of biomass (based on wet matter). Larvae reared on industrial food waste had the most optimal FCR of 1.58. The results of FCR are relatable to the results of bioconversion efficiency, meaning that high bioconversion efficiency corresponds to a low FCR and vice versa. Waste reduction rates (omitting tomato leaves) ranged from 17.0 up to 58.9%. Vegetable overproduction showed relatively poor growth, however, a very high waste reduction ratio was observed (53.9%). The lowest waste reduction ratios were measured for apple pulp (17.0%) and household food waste (18.6%), which are streams that also showed relatively poor growth.

**Table 6.** Bioconversion efficiency, feed conversion ratio, and waste reduction. Values are reported as mean ± standard deviation (replicates indicated in the table as *n*).

| Substrate | *n* | Bioconversion Efficiency (%) | Feed Conversion Ratio (FCR) | Waste Reduction (%) |
|---|---|---|---|---|
| Gainesville diet | 6 | 7,72 ± 1.46 [d] | 3.43 ± 0.74 [c,d,e] | 45.9 ± 5.7 [a,b] |
| Chicken start mash | 6 | 17.56 ± 3.37 [a,b] | 2.08 ± 0.35 [d,e] | 49.9 ± 9.6 [a,b] |
| Apple pulp | 6 | 3.59 ± 0.39 [a] | 8.90 ± 0.80 [a] | 17.0 ± 7.5 c |
| Beer draff | 6 | 13.91 ± 0.40 [b,c] | 2.32 ± 0.17 [d,e] | 46.2 ± 10.4 [a,b] |
| Industrial food waste | 5 | 20.71 ± 2.71 [a] | 1.58 ± 0.19 [a] | 58.9 ± 4.8 [a] |
| Chicken manure | 6 | 15.43 ± 1.65 [b] | 2.00 ± 0.33 [d,e] | 36.4 ± 13.1 [b,c] |
| Corn meal | 6 | 15.61 ± 2.24 [b] | 2.49 ± 0.30 [d,e] | 42.5 ± 7.2 [a,b] |
| Forced chicory roots | 3 | 4.49 ± 0.10 [d,e] | 6.20 ± 0.14 [a,b,c,d] | 30.2 ± 9.3 [b,c] |
| Fruit puree | 3 | 9.32 ± 0.56 [c,d] | 3.19 ± 0.32 [b,c,d,e] | 39.3 ± 5.3 [a,b,c] |
| Grain middlings 1 | 6 | 3.68 ± 1.59 [a] | 7.35 ± 4.95 [a,b] | 36.6 ± 10.5 [b,c] |
| Grain middlings 2 | 6 | 7.06 ± 1.75 [d,e] | 3.53 ± 0.77 [c,d,e] | 35.5 ± 21.6 [b,c] |
| Household food waste | 6 | 6.47 ± 2.52 [d,e] | 6.32 ± 2.44 [a,b,c] | 18.6 ± 4.9 c |
| Tomato leaves | 3 | - | - | - |
| Vegetable overproduction auction | 3 | 7.59 ± 0.69 [d,e] | 3.43 ± 0.42 [b,c,d,e] | 53.9 ± 3.4 [a,b] |

[a–e]: Values not connected by the same letter are significantly different.

Principal component analysis (Figure 2) was conducted to identify the factors contributing to the data variance. The maximal larval weight, bioconversion efficiency, and waste reduction were highly correlated. From the substrate macronutrients (Figure 2a), the fat and NFC contents closely correlated with the substrate gross energy content. The substrate protein content was the only factor closely correlated with the maximal larval weight, bioconversion efficiency, and waste reduction. In Figure 2b, a PCA was drafted using the substrate fiber contents. It was observed that maximal larval weight was negatively correlated with ADL (lignin) and cellulose. No clear correlation between maximal larval weight and hemicellulose content was observed.

As displayed in Table 7, a predictive regression model was generated, describing the effect of substrate protein content ($p < 0.0001$), substrate NFC content ($p = 0.0268$), substrate fat content ($p = 0.0063$), substrate fat content × substrate NFC content ($p = 0.0005$), substrate protein content × substrate fat content ($p = 0.0035$), and substrate protein content × substrate fat content × substrate NFC content ($p = 0.0476$) on maximal larval weight. The model had a $R^2$ value of 0.63 and $p < 0.0001$.

In Figure 3, the predicted interaction effects based on the data acquired in this study are displayed. The prediction showed maximal larval weight when substrate protein content was highest, with minimal substrate fat content. Additionally, it could be observed that when both substrate fat and protein content were high, lower maximal larval weight was observed. Dots display the side-streams plotted in this model.

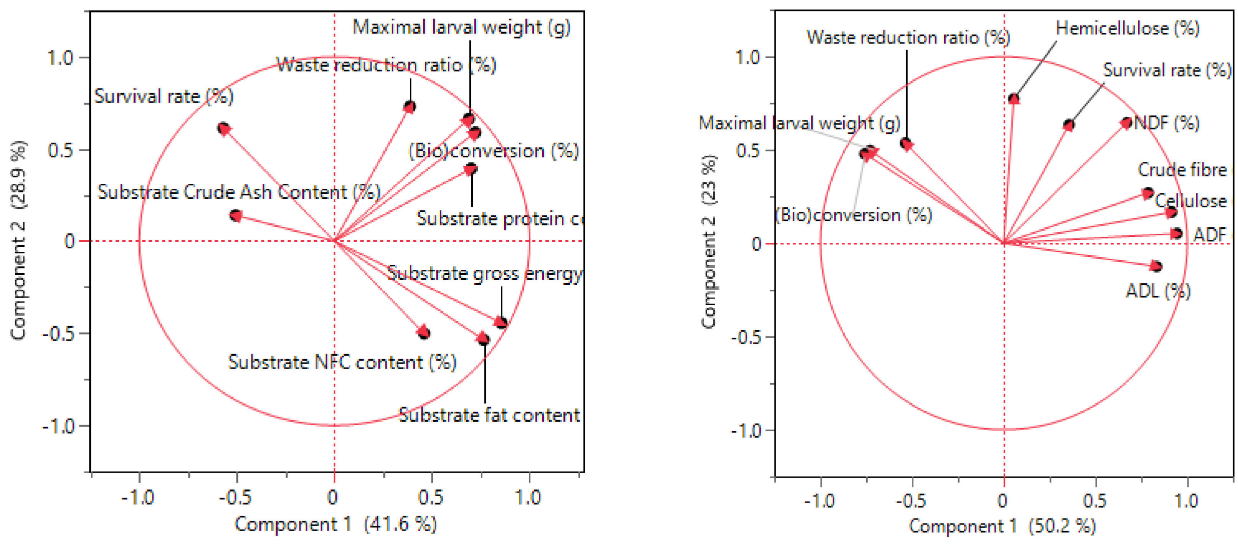


**Figure 2.** PCA analysis using correlations performed on larval maximal weight, survival, bioconversion efficiency, waste reduction, and (**a**) macronutrient concentrations and (**b**) substrate fibers.

**Table 7.** Parameter estimates determined for maximal larval weight prediction model.

| Term | Estimate | Std Error | t Ratio | Prob > \|t\| |
|---|---|---|---|---|
| Intercept | 0.00996 | 0.02202 | 0.45 | 0.6527 |
| Substrate protein content (%) | 0.00508 | 0.00085 | 5.97 | <0.0001 |
| Substrate fat content (%) | −0.00393 | 0.00138 | −2.84 | 0.0063 |
| Substrate NFC content (%) | 0.00105 | 0.00046 | 2.27 | 0.0268 |
| (Substrate protein content (%) − 13.1387) × (Substrate fat content (%) − 5.5629) | −0.00117 | 0.00039 | −3.05 | 0.0035 |
| (Substrate fat content (%) − 5.5629) × (Substrate NFC content (%) − 39.0081) | 0.00054 | 0.00015 | 3.68 | 0.0005 |
| (Substrate protein content (%) − 13.1387) × (Substrate fat content (%) − 5.5629) × (Substrate NFC content (%) − 39.0081) | 0.00007 | 0.00004 | 2.03 | 0.0476 |

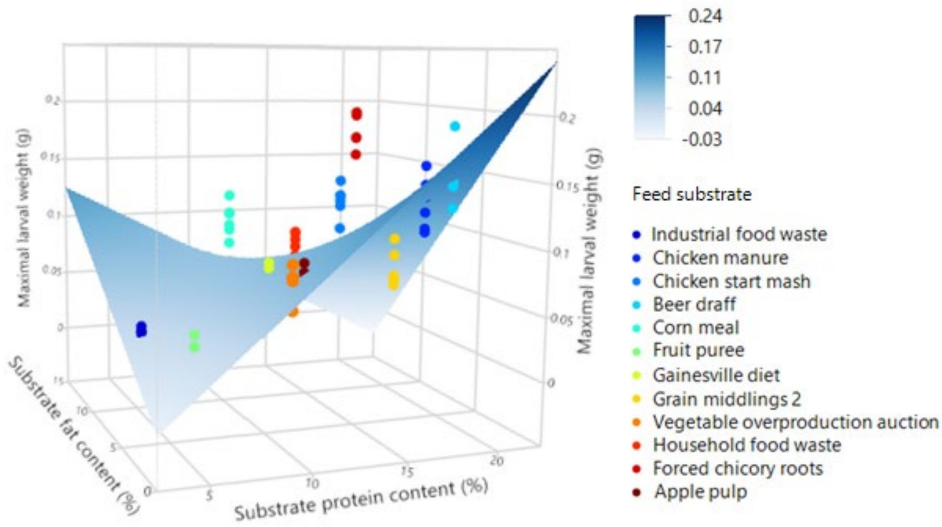


**Figure 3.** Surface plot displaying the predicted interaction effects between substrate fat content and substrate protein content on maximal larval weight.

## 4. Discussion

We evaluated the survival and growth of black soldier fly larvae reared on a set of 12 low-value organic side-streams (mono-streams) and two standard diets. Black soldier fly larvae have been shown to thrive on a wide variety of substrates including food waste, agri-industry co-products, animal waste, and meat, with a minimal need for pre-treatment. This confirms that BSFL are able to upcycle low-value side-streams into valuable biomass [19,21]. For this research, side-streams were selected that are mainly used for biogas production. Some of these side-streams are not valorized as feed due to legal restrictions. Moreover, some of the side-streams tested (household food waste, industrial food waste, and chicken manure) are currently also not allowed to be used as feeding substrates for BSF larvae [19]. Other side-streams are not used because they are of low interest as feed due to low quality or overproduction.

Several parameters influence the survival and growth of BSF larvae, making it difficult to compare different studies in the literature. There are differences between laboratories in breeding conditions (light, temperature, humidity, larval density, time of harvesting, . . . ), substrate compositions (that differ between laboratories or between batches within the same laboratory), and physico-chemical properties of substrates may be different or the used BSF strain may be different. Otherwise, differences in study setup and in analytical techniques used to determine the nutritional composition of substrates might also cause variation. In addition, there are inconsistencies regarding the calculation of bioconversion and waste reduction (fresh weight, dry weight bases, and combinations thereof) and other parameters. A recent paper by Bosch et al. also focused on the possible sources of the variability observed among BSF feeding experiments and argues for the development of procedures to improve harmonization and reproducibility among studies [19]. For these reasons, an international working group was established during the EAAP 2019 conference, aiming at the standardization of methods, parameters, and terminology in future insect research [42].

As expected, relatively high survival rates of 97.2% and 96.3%, respectively, were obtained for larvae reared on control diets. Similar high survival rates (>95%) on standard substrates have been observed in the literature [24,43–45]. The average weight of larvae reared on CSM was 148.4 mg, which is significantly higher than larvae reared on GVD ($p < 0.001$). This is possibly due to the higher gross energy as well as lower lignin and cellulose content present in CSM compared to GVD. Larvae reared on apple pulp had a relatively high survival rate of 95.5%, however, the average larval weight was only 38.3 mg. This is remarkably low, especially since the gross energy content of apple pulp is higher than GVD. Possibly the low protein content of 3.4% was the reason for the limited larval growth. Liland et al. [46] also observed that BSF larvae grown on seaweed substrates with low protein content had similar survival rates but lower weights compared to the control substrate. They hypothesize that this was also due to limiting amounts of proteins in the substrates and that a 7% protein content in rearing substrate is advisable for proper growth of BSF [46]. Another factor might be the high crude fiber content of 25.7% or the high cellulose content of 21.5% present in apple pulp, impairing the digestive processes. In addition, it must be noted that the apple pulp was remarkably acidic, with a pH of 3.7 (data not shown). This could potentially also have been a factor hindering growth. Similar to apple pulp, larvae grown on fruit puree had a very high survival rate of 99.0%. However, the average larval weight was 80.1 mg. Fat contents in this stream were comparable to CSM and the protein content was also rather low (10.0%), however, a relatively high non-fiber carbohydrate content of 39.9% was present. A possible explanation for the lower average larval weight is the relatively high crude fiber content of 17.6%, a lignin content of 3.5%, and a cellulose content of 20.7%. These fiber contents might result in this stream being more difficult to digest. The adverse effect or negative correlation (especially of lignin) on larval growth could also be observed by studying principal component analysis (Figure 2b), which was conducted using the data acquired in this study. With the exception of Scala et al. [47], who observed higher larval weight (150 mg) in larvae grown on apple substrate, several studies have been published that also indicate lower larval weight of BSF larvae reared on fruit substrates [29,48,49]. Scala et al. indicate that the rearing of BSF larvae at the industrial scale, in contrast to lab scale rearing, might have positively influenced their development performance [47].

Larvae reared on beer draff had a survival rate of 95.5% and an average larval weight of 130.9 mg. The protein and fat contents were comparable to CSM. The non-fiber carbohydrate content was only 20.0%, which is remarkably low and the crude fiber content was 18.4%. This, however, did not halt their growth, as the average larval weight was only 11.8% lower than those reared on CSM.

Larvae reared on industrial food waste had a survival rate of 88.8% and a maximal mean larval weight of 176.4 mg, which was the highest mean larval weight measured in this study. The high larval weight can be explained by the very high gross energy amount of 386 kcal/100 g DM due to a very high fat content of 11.9%, a protein content of 19.4%, and non-fiber carbohydrate content of 50.6%. Even though the survival rate was only 88.8%, statistical data analysis showed that this was not significantly different from that measured in control substrates. Interestingly, high sodium content was present in this stream (1.30%). Little information is known about insect sodium requirements and limitations. For other farm animals such as poultry, more information is available. Poultry feed requires sodium contents ranging from 0.10 to 0.25% [50]. Sodium contents of 0.40% lead to significantly higher water intake in broilers, while sodium contents of 0.9 to 1.2% lead to high mortality rates [51]. Both industrial and household food waste contain high sodium contents of 1.30 and 0.55 g/100 g DM, respectively. However, larvae still managed to grow well on these sodium-rich substrates without a significant negative effect on larval survival rates. For household food waste, similar results to industrial food waste were expected, since the nutritional composition is relatively similar. Even though a survival rate of only 83.2% was achieved for larvae reared on this stream, the survival rate was not significantly lower from the control substrates. The average maximal larval weight, however, was significantly lower, being only 65.3 mg. This low average weight was unexpected, since a gross energy content of 377 kcal/100 g DM was present. The stream had a fat content of 14.6%, which is relatively high, as this was even 33.9% higher than the industrial food waste, being the stream with thee second-highest fat content. However, research has shown that canteen waste or poultry slaughterhouse waste with a fat content of 34.9% and 42.9%, respectively, are still suitable as a substrate for rearing black soldier fly larvae [24], indicating that the high fat content is probably not the reason for the poor larval growth and low survival rates. The protein and non-fiber carbohydrate contents of household food waste were comparable to CSM. The fiber contents of household food waste were relatively low. Furthermore, no outliers in mineral contents were found for this stream. Since it is household food waste, the possible presence of other harmful substances cannot be excluded, which could be a reason for the larvae performing poorly on this nutrient-rich side-stream.

Larvae reared on chicken manure did remarkably well, as 97.7% of all larvae survived and a maximal mean larval weight of 134.9 mg was measured, which is a little bit higher than, but comparable to beer draff. The gross energy content of this stream was slightly lower than GVD and comparable protein, fat, and non-fiber carbohydrate contents were measured. This stream contained the highest zinc concentration (0.041 mg/100 g DM). High zinc contents were expected, as the 'Poultry NRC' recommends 35 mg of zinc per kg of feed in the diets of laying hens as it is an essential trace mineral in poultry diets, which is required to regulate bone resorption and DNA replication [52,53]. The high substrate pH of 8.2 (data not shown) did not have a visible adverse effect on larval growth. The results were similar to Rehman et al. [54], who also observed high survival of larvae on chicken manure, but lower larval weights of 97 mg were observed in their study.

Larvae reared on corn meal had a survival rate of 91.8% and an average maximal larval weight of 110.4 mg, which was higher than the larvae reared on GVD, but lower than the larvae fed with CSM. A possible reason of the mean maximal larval weight being lower than larvae reared on CSM is the relatively low protein content of 9.5%, which might be the growth-limiting nutrient in this side-stream.

Forced chicory roots was a stream with a gross energy content of 224 kcal/100 g DM, however, a very low fat content of 0.9% as well as a very low protein content of 4.6% was present. Most of the gross energy was due to a non-fiber carbohydrate content of 49.3%.

Larvae reared on this stream had a survival rate of 98.0%, however, larvae had stunted growth as the maximal average larval weight was only 46.7 mg. A similarly low protein content also led to a low maximal average larval weight in apple pulp. In this forced chicory root stream, a remarkably high iron content of 0.567 ± 0.024 g/100 g DM was present, but it is not known whether this might have had an adverse effect on larval growth.

Larvae reared on grain middlings 1 had a survival rate of only 50.0%, which is significantly lower than all other tested side-streams (with the exception of tomato leaves). Out of six feeding trials respectively 74, 122, 243, 344, 387, and 328 of 500 larvae survived. The fat content of 4.5% was comparable to CSM, a protein content of 14.1% was comparable to GVD and a non-fiber carbohydrate content of 53.1% was also relatively high, resulting in a gross energy content of 295 kcal/100 g DM, which was similar to CSM. No extreme values in fiber contents were found. A relatively high calcium content of 2.29 g/100 g DM was measured. The effect of high calcium contents on larval survival is still unknown.

Grain middlings 2 contained a significantly higher amount of fibers and the gross energy content was only 144 kcal/100 g DM, which was only half compared to grain middlings 1. Only 1.4% fat content was present, a protein content of 9.6% was present, and the non-fiber carbohydrate content was only 23.4%. However, a survival rate of 97.5% was measured with an average maximal larval weight of 78.0 mg, which is not significantly different from larvae reared on GVD.

Tomato leaves were not suitable as a substrate for black soldier fly larvae, as all larvae died quickly and no notable growth was observed. The protein content, however, was 12.1%, the fat content was 1.2%, and the non-fiber carbohydrate content was 36.5%. This resulted in a gross energy content of 205 kcal/100 g DM. This makes the stream comparable to GVD, on which larvae did manage to grow well. Tomato leaves may contain insecticidal compounds such as glycoalkaloids, as already described in recent research, where tomato leaf extract is used as an insecticide against aphids [55]. Additionally, during the determination of the mineral contents, the addition of nitric acid to the ashes leads to $H_2S$ production, which implies high sulfur contents present in the stream. This might be due to the use of a sulfur evaporator, which is commonly used in tomato growing to prevent mildew and other fungal diseases [56]. The high amounts of sulfur may have had a toxic effect on the larvae. Moreover, a calcium content of 8.14 g/100 g DM was measured for tomato leaves, which was remarkably high compared the other substrates. However, as already described, the effects of high calcium content on larval growth is still unknown.

The final substrate tested for the growth of black soldier fly larvae was vegetable overproduction from auctions. The larvae survived well (98.0% survival rate) and the growth was not significantly different from larvae grown on GVD (mean maximal average weight of 88.1 mg). This stream had a relatively low protein content of 10.5%, a relatively low fat content of 2.0%, and a non-fiber carbohydrate content of 42.0%, resulting in a gross energy content of 228 kcal/100 g DM, which is similar to GVD.

The aim of this study was to evaluate whether the growth of larvae was possible on each of the selected side-streams, in order to evaluate the potential of different side-streams for black soldier fly rearing. However, by also conducting a chemical analysis to determine nutritional composition, fiber contents, and micro/macro-element contents of each of the side-streams, we attempted to find out 'why' some side-streams would work and some would not. However, this study showed that side-streams are complex matrices and not all relevant parameters could be determined within this study. Compounds such as insecticidal residues, amino/fatty acid profiles, water-retention properties of substrates, microbial loads, hemicellulose profiles, and the exact non-fiber carbohydrate composition, etc. were not included in this study. However, these might have played an important role in modeling larval growth. In this study, for example, we were not able to specifically declare why tomato leaves resulted in a 0% survival rate or why only 50% of all larvae grown on grain middlings 1 survived. Additionally, when comparing the nutritional composition of industrial food waste and household food waste, the result is that those compositions were relatively similar. Both side-streams had high protein, fat, and NFC contents. However, the maximal larval weight of larvae grown on household food waste is significantly lower compared to industrial food waste. Even though most fiber contents were higher in household food waste than in industrial food waste, these fiber contents were not higher than, for example, chicken manure. However, even though chicken manure is a nutritionally poorer side-stream, the maximum larval weight of larvae grown on chicken manure was significantly higher. This highlights an issue when working with side-streams in order to build a prediction model, as it is extremely difficult to control every single parameter. Another interesting parameter is substrate dry matter content. In this study, we brought each substrate to a dry matter content of 30%, however, the dry matter content of the residues had a lot of variation when the experiment started. This means that over the course of the experiment, the gradual change in substrate dry matter content was not constant for each side-stream. Therefore (even though the dry matter contents at the start of the experiment were equal), the amount of accessible moisture during the course of the experiment might have had an influence on larval growth. Moreover, there is room for discussion whether substrate dry matter content is the right parameter to standardize, as depending on the matrix, 30% DM can result in either a soaking wet, a saturated, or a dry substrate. Therefore, subsequent research is required to determine whether it is better to standardize substrate moisture content based, for example, on dry matter content or on maximal water binding capacity. The rate in which water is leaving the substrate (for example by evaporation) should potentially also be described, as this turned out not to be constant for all substrates. Exploring methods to monitor loss of water and to maintain adequate moisture content throughout the entire experiment should be explored. Even though these issues are present, this experiment might still provide potentially interesting correlations. A standard least square regression model was built describing both the main effects and interaction effects of substrate protein, fat, and NFC contents on the maximal larval weight. As main parameters, substrate protein, fat, and NFC content were used to describe the model as these contribute to the gross energy content of the substrate. It was decided to exclude side-streams with survival rates being significantly lower compared to the control substrates. Therefore, data from grain middlings 1 and tomato leaves were excluded from the model. A proximate component analysis was run (Figure 2a) using the remaining substrates, which showed that no correlation between survival rate and maximal larval weight was present. This may indicate that larval density during this experiment was low enough and that the larvae did not hinder each other's growth. In Figure 2b, it can also be observed that larval survival rate is positively correlated with substrate fibers. This is potentially due to the substrate structure or water retention properties of the substrate fibers [57].

The model predicted maximal larval weight when protein content was maximized (between the borders of protein concentrations tested in this study) and when the substrate fat content was low (0.9%). Previous studies also show that final weight of larvae, bioconversion rate, and feed conversion rate positively correlated with the amount of protein in the rearing substrates [31,58]. For larvae grown on apple pulp and chicory roots, it was observed that low protein contents led to poor larval growth. Not only the protein content itself, but also the amino acid composition and/or digestibility of proteins is important. If essential amino acids are limited or digestibility is low, the potential for larval growth is also limited [14].

The model also includes the influence of NFC (%) on maximal larval weight, however, leverage of the predicted influence of substrate NFC content was way lower compared to the substrate protein and fat content. The low influence of substrate NFC content is remarkable, however, it can be verified by comparing the nutritional composition of beer draff to chicken start mash. The protein contents were similar (19.4% vs. 20.4%), as were the ether extracts (5.5% vs. 4.6%), however, the NFC content of beer draff was much lower compared to CSM (20.0% vs. 48.2%). However, both larval survival and maximal larval growth did not significantly differ. This comparison supports the findings described by the model above.

Another possibly important set of factors playing a role in larval growth is substrate fiber. As observed in the principal component analysis (Figure 2b), a negative correlation is potentially present between mainly ADL (lignin), ADF, and cellulose contents. Not only may fibers make the side-stream harder to digest, but high fiber contents also leave less room for proteins, fats, and carbohydrates, thus lowering the gross energy content of the side-stream.

The bioconversion efficiency, feed conversion ratio, and waste reduction of the substrates are important parameters when evaluating BSF larvae as potential bioconversion technology. Several studies have evaluated these parameters and a lot of variability is observed, which is influenced by BSF strain, rearing circumstances including larval density, feeding rates and amounts, nutritional composition of the substrates, pretreatment of the substrates, [19,24]. Diener et al., for example, showed that waste reduction is highly dependent on the amount of diet that is provided [54]. For chickenfeed, this ranged between 39.7% and 26.2% when providing 12.5 mg or 200 mg chicken feed per larva and day. As discussed previously, calculations of bioconversion efficiency, feed conversion ratio, and waste reduction also differed between studies. In this study, all conversion efficiencies were calculated on a DM basis as recommended by Bosch et al. [59].

Larvae reared on CSM had a mean bioconversion efficiency of 17.56%. This is in line with the bioconversion efficiencies of other studies that are also using chicken feed as a control diet, having a bioconversion efficiency between 12% and 21% [24,44]. The waste reduction for CSM was 49.9%. Similar studies calculating waste reduction for chicken feed had report numbers ranging between 30.9% and 80.4% [25,28,47]. Larval density and the amount of diet provided had a negative influence on waste reduction [60,61]. The variation in density and provision of diet between these studies does indeed vary and hampers comparison. The second control diet GVD is less often used as a control diet although it may differ less between studies as it is prepared in a similar manner between labs (GVD is composed of 50% wheat bran, 20% maize, and 30% alfalfa) and could overcome differences that are currently seen between commercial chickenfeed coming from different providers. [33]. Bioconversion efficiency and waste reduction were respectively 7.72% and 45.9% for GVD, which is similar to previous studies [62]. For side-streams in this study, bioconversion efficiencies between 3.59% and 20.71% were calculated and waste reductions were measured in the range between 17.0% and 53.9%, which illustrates the larval ability to both efficiently convert organic waste into larval biomass and to significantly reduce the waste amounts. As indicated from the PCA analysis, maximal larval weight and bioconversion efficiency were closely correlated, which is to be expected when having a similar survival rate. These values are similar to those obtained by other authors for a diversity of organic streams [19,24,44].

## 5. Conclusions

In this study, 12 side-streams were chemically analyzed and their suitability as feed substrates for *H. illucens* larvae was evaluated. From this and other studies [28,29,63], it is clear that the composition of feed substrates has an important impact on the survival, growth, and performance of black soldier fly larvae. Apart from tomato leaves, larval growth was observed for all substrates tested in this study. For the other substrates, a clear correlation between larval growth and substrate protein content could be established. On low protein substrates (apple pulp and forced chicory roots), survival rates remained high, but limited larval growth was observed. This poor growth could be due to protein deficiency, perhaps these streams have potential when mixed with a stream high in protein. Mixing of side-streams would allow producers to optimize the nutritional content of the diet and reduce variations. Several studies have been published in which improvement in survival and larvae mass is observed when streams are mixed compared to the individual mono-streams [24,54,64].

Although agricultural side-streams might be available over a substantial period of time, this research showed that their nutritional value might not be constant over time.

This was observed for grain middlings, which were sampled on two different dates. The nutritional value changed between the two sampling dates, resulting in significant differences in larval growth, larval survival, and bioconversion efficiency. This may have important implications for industrial scale BSF rearing, where a steady and homogenous supply of the substrate is preferred to guarantee a homogenous output of qualitative BSF larvae. In this respect, it should also be noted that the substrates that are supplied to the BSF larvae may influence the nutritional composition of the BSF larvae and thus also the potential applications [30].

Although the macronutrient contents (crude proteins, lipids, non-fiber carbohydrates), fibers (cellulose, hemicellulose, and lignin) and the mineral contents of the side-streams were analyzed, it is clear that other factors not measured in this study have a major influence on performance and bioconversion for the tested side-streams. This highlights the need to further investigate different factors influencing BSFL under controlled conditions.

The economic feasibility of the commercial use of insects depends largely on cost effective and readily available organic waste streams and sustainable production capacity [65,66]. Here, we show that a selection of several low value organic side-streams can be successfully processed by black soldier fly larvae, thereby opening the possibility of lowering the costs of BSF farming, but more research is needed for optimizing diets.

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