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# Metabolic performance of black soldier fly larvae during entomoremediation of brewery waste

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## Abstract

This study aims to evaluate the metabolic performance, in terms of specific rates of growth and feed assimilation, as well as the cost of growth and maintenance of black soldier fly larvae, BSF, *Hermetia illucens* on brewery waste, a potential worldwide available resource for industrial scale insect production. Brewery waste lacks starch and thus has a nutritional profile substantially different from chicken feed, which is a well-established and excellent starchy food source for BSF larvae. It is therefore interesting to gain insight into how BSF larvae perform on brewery waste. Larvae of the BSF were reared on chicken feed, on brewery waste and on mixtures of the two. Measurements of the weight of the larvae and their respiratory $CO_2$ production were used to estimate metabolic performance on daily basis. The BSF larvae grew on all the substrates. They reached the highest weight on chicken feed, but their specific growth and feed assimilation rates were highest on the mixed substrates, in which the larvae also reached their maximal weight in the shortest time. Substrate-dependent costs of growth were not observed while maintenance rates tended to be only slightly lower on the mixed substrates. Overall, the BSF larvae converted the low-starch brewery waste and the starchy chicken feed into larval biomass about equally efficiently, although brewery waste led to smaller larvae and mixing of the two substrates enhanced feed assimilation and growth. Brewery waste seems thus a suitable resource for BSF larvae, comparable with chicken feed, with respect to their metabolic performance.

**KEYWORDS**
growth efficiency, *Hermetia illucens*, larval feed assimilation, larval growth, larval metabolism, larval respiration

## 1 | INTRODUCTION

The world population is rising and so are the demands for food and feed, as well as the generation of organic waste from households and industries. Entomoremediation, where insects in association with microorganisms treat organic waste, is being developed into a sustainable and economically attractive opportunity for the valorization of the waste (Gligorescu et al., 2020). Proteins, other organic components and inorganic nutrients are recovered in the insect biomass and used as raw materials for food, feed and biofuels, and the waste

is reduced in volume. Larvae of the black soldier fly, BSF, *Hermetia illucens* are particularly promising candidates for entomoremediation of a variety of organic materials into animal protein and lipid at industrial scale (Franco et al., 2021; Gold et al., 2020; Surendra et al., 2020). Sufficient knowledge of the ability and capacity of this species to utilize various organic resources, to meet the needs of industry, is not yet fully in place (Tettamanti et al., 2022).

Industrial production of BSF larvae depends on the availability of sufficient quantities of affordable, suitable and safe substrates (Gold et al., 2021), and conversion of waste products from food industries into BSF larvae has frequently been described (see, e.g. Scieuzo et al., 2022). Brewery waste is organic left-over from beer brewing and produced in large quantities in most parts of the world. It is a lignocellulosic resource holding 70% water. The rest consists of up to 70% fibre, 20–30% protein, 10% lipids, vitamins and minerals and occasionally bits of residual starch (Mussatto et al., 2006; Rachwal et al., 2020; Robertson et al., 2010). Brewery waste is mainly spent grain but includes also hot trub and yeast (Rachwal et al., 2020). It is already used in animal feed and foods, and for biogas production, but usage is challenged by the lignocellulosic components, the high water content, and that it is predisposed to microbial decomposition (Robertson et al., 2010). Entomoremediation of brewery waste by BSF larvae is an alternative to existing usages. Several studies have demonstrated that BSF larvae can grow on brewery waste and have characterized, for example, survival, development time, growth, final weight or size, lipid content and fatty acid composition of the larvae (Chia et al., 2018; Galassi et al., 2021; Jucker et al., 2019; Liu et al., 2018; Magee et al., 2021; Meneguz et al., 2018; Saadoun et al., 2020; Scala et al., 2020; Shumo et al., 2019). BSF larvae have also been reared on brewery waste in combination with other substrate sources (Ceccotti et al., 2022; Chia et al., 2020). In brewery waste, the proteins make up most of the nutritionally valuable components due to the low digestibility of fibres (Peguero et al., 2022). Brewery waste thereby differs from the more nutrient-dense, starch-rich substrates, such as commercial chicken feed blends that are commonly used as reference substrates in studies of BSF larvae. Chicken feed is dominated by starch (60–70% of the dry weight, DW) and supports excellent growth of BSF larvae (Bekker et al., 2021; Laganaro et al., 2021). Both starch and proteins are important feed components for BSF larvae. Both can be digested by the larvae (Bonelli et al., 2020), assimilated into their energy metabolism, used for growth (Beniers & Graham, 2019) or converted into fat (Hoc et al., 2020). Lipids can also be assimilated from the substrates, metabolized or stored as fat (Hoc et al., 2020; Knudsen et al., 2022). Some studies have found that BSF larvae grow well on brewery waste (Liu et al., 2018; Scala et al., 2020). Other studies report less favourable growth of BSF larvae on brewery waste compared with other substrates (Ceccotti et al., 2022; Galassi et al., 2021; Meneguz et al., 2018). In one study, growth was slow, but the BSF larvae grew larger on brewers' spent grain compared with other substrates (Saadoun et al., 2020). Different types of brewery waste may also give rise to differences in growth and larval size (Chia et al., 2018; Jucker et al., 2019).

Growth of BSF larvae depends on the availability and the quality of their feed sources, which in turn affects the rate and amount of feed assimilated into the body of the larvae. Growth rates as well as the weight of BSF larvae are reduced when the feed supply is restricted (Diener et al., 2009). It is when the BSF larvae transform into prepupae they reach their maximal weight (Gligorescu et al., 2019). Growth of BSF larvae also depends on the efficiency by which they convert assimilated feed components into new biomass (Padmanabha et al., 2020). Until the larvae reach the prepupal stage, the efficiency by which feed components are converted into new biomass depends on the specific rates of feed assimilation and growth, the cost of growth (the metabolic expenditures needed to convert substrate components into larval biomass) and the rate of their maintenance metabolism (the metabolic processes that are not linked to increasing larval weight). Altogether, these processes represent the metabolic performance of the larvae (Laganaro et al., 2021) and set the upper limit for the yield of larvae relative to the used amount of substrate. Actual yields of BSF larvae relative to substrate reduction (substrate conversion efficiencies) are obtained from mass balance approaches, comparing outputs of larvae and frass to substrate inputs. Results from different substrates show considerable variability (Bosch et al., 2019), but mass balances alone may not provide deeper insights into what determines the variability. Quantification of the metabolic performance in terms of the specific rates of feed assimilation, growth and respiration, and the distribution of resources between growth and maintenance may help explain differences in growth and yields of BSF larvae on different substrates (Laganaro et al., 2021) and thus elaborates observed larval yields and productivities from mass balance studies. This may help insect farmers to better understand their process and used as guidance for process optimization. The aim of this study is to compare the metabolic performance of BSF larvae reared on brewery waste, chicken feed and mixtures of the two substrates. The purpose is to provide insight into how BSF larvae perform metabolically on starch-deprived brewery waste, relative to starchy chicken feed.

## 2 | MATERIALS AND METHODS

### 2.1 | Cultivation of BSF larvae

BSF eggs were supplied by the company Entomass Aps (Løkken, Denmark). The eggs had been laid in a plastic sponge 2 days before the experiment. The sponge was placed above a nursery made of a cylindrical plastic container (radius = 5.5 cm, height = 13 cm) containing 300 g of a commercial chicken feed (Kyllekræs 3, Danish Agro, Karise, Denmark) with 65% moisture content. Eggs were allowed to hatch into the nursery during a 3 h period, whereafter the plastic sponge was moved to a second nursery for another 3 h. The nurseries were placed at 28°C. After 5 days, the larvae reached approximately 1 mg wet weight (WW) and were named 'starter larvae' (Parodi et al., 2020).

Cultivation experiments were carried out in triplicates in the same type of container as used for nurseries. Each container was supplied with substrates made from mixtures of chicken feed (starch content approximately 600 g $kg^{-1}$ DW, Knudsen et al., 2022) and brewery waste (starch content <1 g $kg^{-1}$ DW, measured in this study), supplied by Entomass Aps. A total of five different substrate mixtures of chicken feed: Brewery wastes (100:0, 75:25, 50:50, 25;75 and 0:100) were used in the experiments. On Day 0 of the cultivation experiments, each container was inoculated with 100 starter larvae (larval density of 1 $cm^{-2}$) and 104–106 g of substrate mixtures with moisture contents between 69% (100% chicken feed) and 79% (100% brewery waste). The larval cultures were placed in an incubator at 28°C. The air humidity in the incubator was around 37 %RH (measured by a Lascar EL-USB-2 datalogger, Whiteparish, UK), too low to prevent evaporation from the larval cultures. Another 104–106 g of newly mixed substrates were therefore added on Days 3, 6, 9 and 12 (on Day 6, substrate rations were raised to 208 g) to ensure feed sufficiency and to keep the substrates moist. It has previously been shown that the metabolic performance of BSF larvae stays the same within a broad interval of substrate moisture contents from 45% to 75% (Bekker et al., 2021). Variations in substrate moisture contents are thus not expected to have had effects on the experimental results in this study. Cultures were terminated after 14 days (larval age of 19 days), when growth had stopped and most larvae had become prepupae and darkened.

## 2.2 | Analytical procedures

Ten larvae were randomly selected and weighed daily. On Days 0–3, the larvae were weighed together whereafter their weights were measured individually. In addition, the DW contents of three larvae from each culture were determined on Days 5, 8, 11 and 14 after drying at 105°C for at least 24 h. Mean DW from two consecutive measurements was used as a conversion factor to predict larval DW based on WW measurements on days in between DW measurements.

From Days 3 to 15 and just before larvae were sampled, the temperature was measured halfway down in the substrate at three positions, at the centre of the container, at half radius distance from the wall and next to the wall.

$CO_2$ production rates were also measured on daily basis starting at Day 4. After being weighed, the larvae were transferred to a closed respiration chamber made from an airtight 50 mL plastic centrifugation tube placed in a water bath at 28°C. From Day 7, only five larvae were transferred to the respiration chamber. A precalibrated wireless $CO_2$ sensor PS-3208 (Pasco, Roseville, CA, USA) was inserted into the top of the tube and $CO_2$ production rates were determined from the linear increase in $CO_2$ concentration in the tube. Measurements were completed in 5 min, including 2 min for the sensor to stabilize (Laganaro et al., 2021). The larvae were returned alive to their original container.

Lipids were extracted and quantified in the dried larvae sampled on Day 11 (close to the point in time when the larvae reached maximal weight) using a modified protocol of Bligh and Dyer (1959). The larvae were ground in a mortar with a pestle, and about 50 mg of ground larvae was suspended in 12 mL $CHCl_3$:$CH_3OH$ (1:2 V/V) in 25 mL glass bottles sealed with Teflon-coated caps. The bottles were stirred for 24 h on a magnetic stirrer at room temperature, followed by the addition of 3 mL aqueous 0.9% NaCl, to separate the mixture into two phases. The $CHCl_3$ phase, containing lipids was collected and left in a fume cupboard until all $CHCl_3$ had evaporated, and the mass of the remaining oil extract was measured.

The starch content of brewery waste was estimated after enzymatic hydrolysis to glucose (Knudsen et al., 2022). Brewery waste (300 mg WW) was suspended in 30 mL 10 g $L^{-1}$ calcium chloride dihydrate and 19.5 g $L^{-1}$ MES hydrate buffer, pH 6.6. The suspension was heated to 95°C for 45 min and mixed at 500 RPM in a thermomixer, to solubilize the starch. After the solution had cooled to 75°C, 1 µL thermostable α-amylase and 1 µL amyloglycosidase solution (Termamyl and AMG 300 L, Novozymes, Bagsværd, Denmark) were added and incubated overnight in a Thermomixer at 75°C and 500 rpm. The solution was centrifuged at 3000 RPM for 10 min, and 250 µL of the supernatant was filtered through a 0.2 µm syringe filter, mixed with 750 µL of DNS solution (10 g $L^{-1}$ of 3,5-dinitrosalicylic acid, 200 g $L^{-1}$ potassium sodium tartrate and 20 g $L^{-1}$ NaOH), heated to 95°C for 15 min in a thermomixer and then cooled on ice for 20 min. The absorbance at 540 nm was read on a Tecan Infinite M1000 spectrophotometer and compared with glucose standards (0–2 g $L^{-1}$). Enzyme solution with no substrate was used as negative control. Brewery waste not treated enzymatically was used as negative control.

## 2.3 | Growth and feed assimilation

Growth and metabolic performance were evaluated and quantified as described by Laganaro et al. (2021). In brief, larval DW, $X_{DW}$ [mg] over time, $t$ was modelled by the Verhulst logistic growth model from Day 0 after inoculation and until they became prepupae and reached maximal weight, $X_{DW,max}$.

$$X_{DW} = \frac{X_{DW,max}}{1 + \left(\frac{X_{DW,max} - X_{DW,0}}{X_{DW,0}}\right) e^{-\mu_{max} t}} \quad (1)$$

$X_{DW,0}$ is DW of the starter larvae at $t = 0$, and $\mu_{max}$ the maximal specific growth rate [$day^{-1}$]. Equation 1 was fitted to the measured DW of the BSF larvae to find the combination of $X_{DW,max}$ and $\mu_{max}$ that resulted in the smallest difference (average mean error) between model and measured values. The specific growth rate, $\mu$ of different-sized larvae was then estimated from Equation 2

$$\mu = \mu_{max}\left(1 - \frac{X_{DW}}{X_{DW,max}}\right) \quad (2)$$

Costs of growth and maintenance rates were estimated based on measured rates of respiratory $CO_2$ production from larvae reared on the different substrate mixtures. If growth is balanced, the

relationship between specific respiratory $CO_2$ production rate, $q_{CO_2}$ [$day^{-1}$] and growth rate is expected to be linear

$$q_{CO_2} = Y\mu + m \quad (3)$$

*Y* and *m* represent the cost of growth [unitless] and specific maintenance metabolism [$day^{-1}$], respectively. Linear regression was used to estimate *Y* (slope of regression line) and *m* (intercept with *y*-axis) from relationships between measured specific $CO_2$ production rates and specific growth rates estimated from Equation 2.

The specific feed assimilation rate, *a* [$day^{-1}$] was calculated as

$$a = q_{CO_2} + \mu \quad (4)$$

where $q_{CO_2}$ is the specific $CO_2$ production rate, calculated by dividing $CO_2$ production rates by larval DW. The maximal specific rates of $CO_2$ production, $q_{CO_2,max}$ and feed assimilation, $a_{max}$ were estimated by substituting $\mu_{max}$ into Equations 3 and 4 and represent theoretical parameters, achievable only in infinitely small larvae (Laganaro et al., 2021).

## 2.4 | Conversion factors and statistical analyses

Growth, $CO_2$ production and feed assimilation were expressed as specific rates based on the mass fraction of carbon in the larvae, in the substrates and in $CO_2$. Thereby, the magnitudes of the rates could be compared in the same unit (Laganaro et al., 2021). Carbon was assumed to make up 49% of the organic fraction of the larvae and substrates, which is the carbon content in biomass of average elementary composition (Roels, 1980). The organic fraction of the larvae was estimated from measurements of the DW of the larvae, assuming 9% ash content, the average measured in this study.

Maximal weights and maximal specific growth rates of BSF larvae grown at different substrate moisture contents were compared by one-way ANOVA at 5% probability level. Individual larval DW followed Gaussian distributions throughout the growth phase.

# 3 | RESULTS

The BSF larvae grew on all substrate mixtures made from chicken feed and brewery waste. Growth was well described by the logistic model (Figure 1a–e), but substrate composition affected larval performances in several ways. The larvae used 10–11 days to reach their maximal weight on 100% chicken feed or 100% brewery waste (Figure 1a,e) but only 8–9 days on the mixed substrates (Figure 1b–d). Short growth periods were linked to high specific growth rates in the first 5–6 days, when the larvae increased exponentially in weight (Figure 2a). In this period, the specific growth rates, estimated from the first order rate constants, were 0.80–0.82 $day^{-1}$ on the mixed substrates, compared with only 0.70–0.72 $day^{-1}$ on chicken feed or brewery waste alone (Figure 2b). The specific growth rates were, however, statistically different only at 6% significance level. Maximal specific growth rates, estimated from Equation 1 (Figure 2c) were significantly higher on the mixed substrates (1.00–1.01 $day^{-1}$) than on the pure ones (0.79–0.85 $day^{-1}$), while the maximal DW of the larvae was significantly lowered from 90 to 58 mg, the higher the content of brewery waste (Figure 2d).

Individual $CO_2$ production rates depended on the weight of the larvae and their specific growth rates and peaked on Day 7. This was 1–2 days after the larvae had stopped growing exponentially (Figure 1a–f). The metabolic performance of the BSF larvae, in terms of cost of growth and maintenance (Equation 3), was estimated from the regression lines in Figure 1f. There was no statistically significant effect of substrate mixture composition on the cost of growth (Figure 2e). Maintenance tended to be lower on the mixed compared with the pure substrates (Figure 2f), although the differences in maintenance rates were also not statistically significant. Specific rates of growth, $CO_2$ production and feed assimilation predicted from Equations 2–4 are also shown in Figure 1a–e. Highest values were found in larvae reared on the mixed substrates and values remained high the first 4–5 days when the larvae were still small and grew exponentially. In this period, the specific $CO_2$ production rates were 0.45–0.5 $day^{-1}$ on the mixed substrates compared with 0.4 $day^{-1}$ on the pure substrates, predicted from Equation 3. The specific growth rates (Equation 2) were 0.9–1.0 $day^{-1}$ on the mixed substrates compared with 0.8 $day^{-1}$ on the pure ones. Thereby, the specific feed assimilation rates (Equation 4) were estimated to be 1.4–1.5 $day^{-1}$ on the mixed substrates compared with around 1.2 $day^{-1}$ on the pure ones. Maximal $CO_2$ production and feed assimilation rates are shown in Figure 2g,h. Both appeared to be slightly higher on the mixed than on the pure substrates, although these tendencies were not statistically confirmed.

The differences in substrate composition did not result in major differences in the biochemical composition of the BSF larvae. The DW content of the larvae increased as they became larger on all substrate mixtures while there were no apparent differences in the organic fraction of the dry biomass (Figure 2l). Lipid contents were measured on Day 11, when almost all larvae had become prepupae and made up 31.1%, 29.4%, 30.1%, 31.8% and 29.5% of larval DW on the substrates containing 0%, 25%, 50%, 75% or 100% brewery waste, respectively.

Average substrate temperatures in the larval cultures were between 26°C and 28°C, highest in the substrates containing the most brewery waste (Figure 3a). During most parts of the growth period, temperatures stayed in this interval but increased temporarily to 30°C on Days 7 and 8 in the substrates containing 75% and 100% brewery waste (Figure 3b). Most days, temperatures were 1–2°C higher at the centres of the containers, than at the walls.

# 4 | DISCUSSION

Brewery waste and chicken feed are substrates, which are poor and rich in starch, respectively. Nevertheless, the BSF larvae grew well on both. Specific growth rates increased when the two substrates were

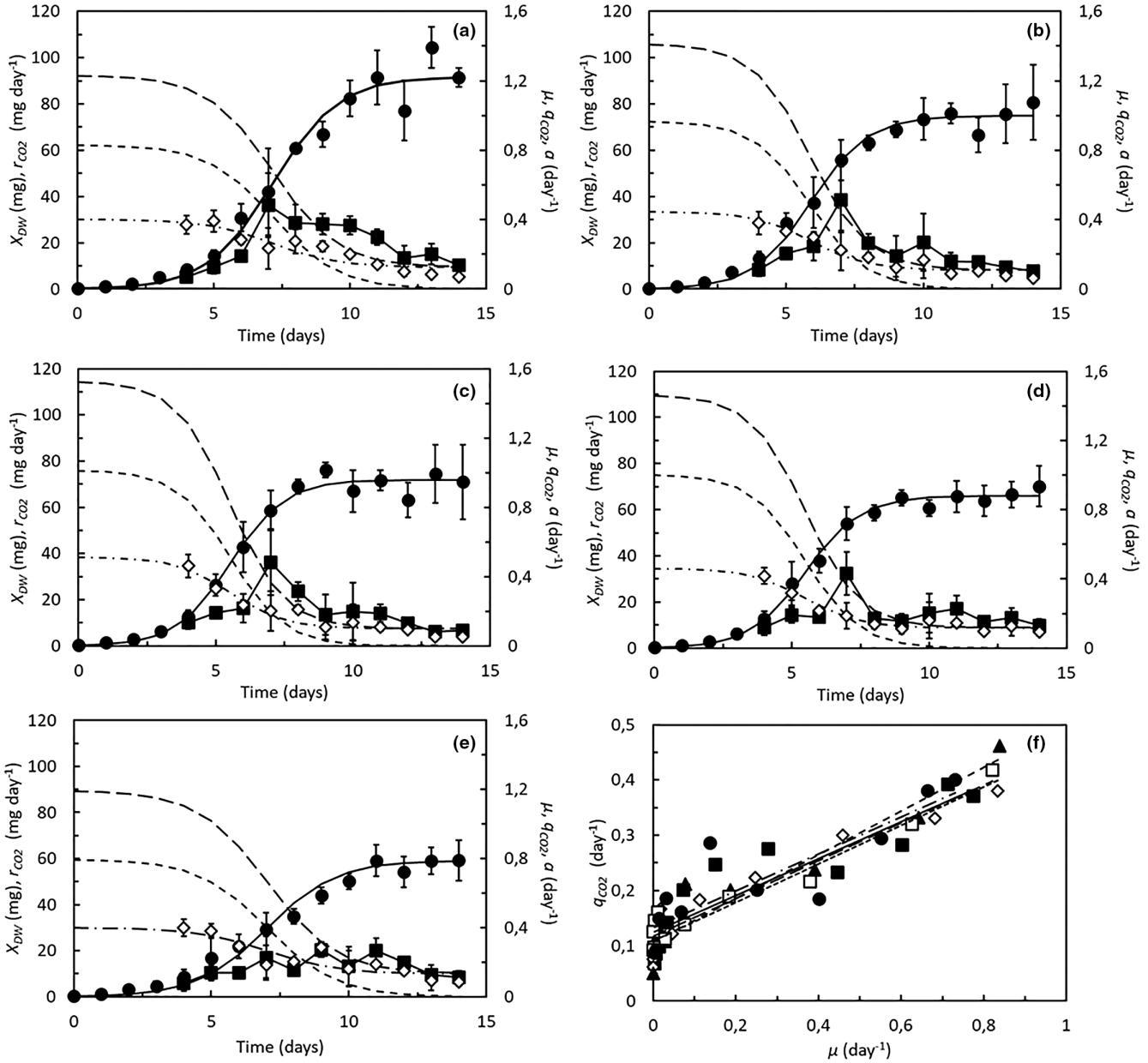


FIGURE 1 BSF larvae reared on different mixtures of chicken feed and brewery waste. (a) 100:0. (b) 75:25. (c) 50:50. (d) 25:75. (e) 0:100. Larval DW, $X_{DW}$ (●, growth curved modelled by Equation 1), individual $CO_2$ production rate, $r_{CO_2}$ (■), specific growth rate, $\mu$ (- - -, Equation 2), specific $CO_2$ production rate, $q_{CO_2}$ (◇, –·–, Equation 3) and specific feed assimilation rate, $a$ (– – –, Equation 4). Panel f shows relationships between specific $CO_2$ production rates and specific growth rates on mixtures of chicken feed and brewery waste at ratios 100:0 (■), 75:25 (◇), 50:50 (▲), 25:75 (□) and 0:100 (●). Regression lines used to estimate cost of growth, $Y$ and maintenance, $m$ by Equation 3.

mixed, while the larvae reached highest weights, the less brewery waste was in the substrate. Systematic differences in cost of growth on these substrates, as well as on mixtures of the two were not identified, indicating that there are no major differences in how efficient feed components taken up from the two substrates were converted into new biomass by the BSF larvae. Thereby, the differences in specific growth rate were largely related to differences in specific feed assimilation rate, which was highest on the mixed substrates. Maintenance metabolism tended to be lowest on the mixed substrates, leaving slightly more of the assimilated feed components available for growth.

One reason to compare the metabolic performance of BSF larvae on brewery waste and chicken feed is that results from earlier studies (Ceccotti et al., 2022; Chia et al., 2018, 2020; Galassi et al., 2021; Jucker et al., 2019; Liu et al., 2018; Meneguz et al., 2018; Saadoun et al., 2020; Scala et al., 2020; Shumo et al., 2019) have pointed in different directions in terms of how well brewery waste supports the growth of BSF larvae. The growth patterns of the larvae from all three cultures reared on chicken feed (Figure 1a) were as in previous studies, indicating that the rearing conditions had been appropriate. The dry weight of these larvae peaked at 90±2mg and

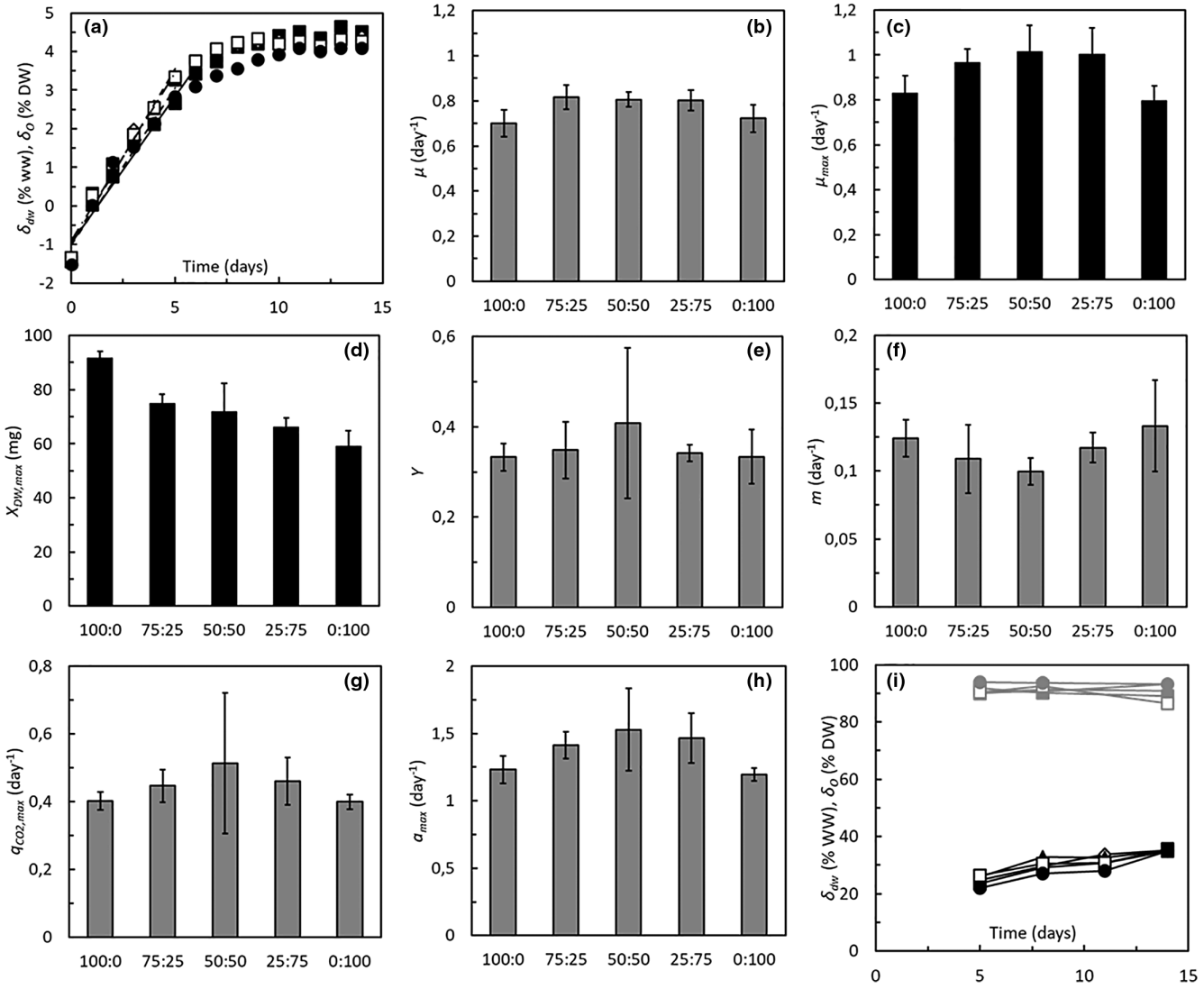


**FIGURE 2** Performance of BSF larvae BSF larvae reared in mixtures of chicken feed (0–100%) and brewery waste (0–100%). (a) *ln*-transformed biomass vs. time. Slopes of regression lines used to estimate the specific growth rate during the initial exponential growth phase. (b) Specific growth rate, $\mu$ estimated from regression lines in Panel a. (c) Maximal specific growth rate, $\mu_{max}$ estimated from Equation 1. (d) Maximal specific biomass DW, $X_{DW,max}$ estimated from Equation 1. (e) Cost of growth, $Y$ estimated from the slope of regression lines in Figure 1f. (f) Maintenance coefficient, $m$ estimated from Figure 1f. (g) Maximal specific $CO_2$ production rate, $q_{CO_{2,max}}$ estimated from Equation 3. (h) Maximal feed assimilation rate, $a_{max}$, estimated from Equation 4. (i) DW content of the wet biomass, $\delta_{DW}$, and organic fraction of the dry biomass, $\delta_O$ (light grey) of three larvae pooled from three replicate cultures. Error bars indicate standard deviation of larvae from three replicate cultures. Black bars indicate that effects of substrate mixture composition are significant at 5% probability level.

the specific growth rate during the exponential growth phase was $0.70 \pm 0.06\,day^{-1}$, which are within previous observed ranges on chicken feed of 84–114 mg (Bekker et al., 2021; Laganaro et al., 2021) and $0.50–0.92\,day^{-1}$ (Eriksen, 2022a), respectively. We found that the larvae performed notably alike on brewery waste and chicken feed. Considering the composition of brewery waste (Mussatto et al., 2006; Rachwal et al., 2020; Robertson et al., 2010), it thus seems that the BSF larvae utilized proteins and possibly lipids as rapidly and efficiently as starch to fulfil their metabolic needs for energy and growth. The specific feed assimilation rates estimated from Equation 4 in the period the larvae increased exponentially in weight ($1.2–1.5\,day^{-1}$) are well in line with earlier estimates of maximal feed assimilation rates in BSF larvae reared on chicken feed ($1.1–1.4\,day^{-1}$, Eriksen, 2022a). It has probably been the broader selection of nutritional compounds and maybe the ability of BSF larvae to adapt themselves physiologically to their diet (Bonelli et al., 2020) that enabled the larvae to assimilate feed and grow at highest specific rates when the two substrates were mixed. It can be noted that the metabolic performance is not always affected positively by mixing of substrates. In a previous study, degassed sludge was mixed into chicken feed, causing increased cost of growth and maintenance rates of BSF larvae (Laganaro et al., 2021). Although the sludge did not adversely affect feed assimilation rates, the increased allocation of resources for energy production lowered the specific growth rate and the size of the larvae.

The differences in maximal weight of the BSF larvae (Figure 2d) are not well explained by the experimental data and were not related

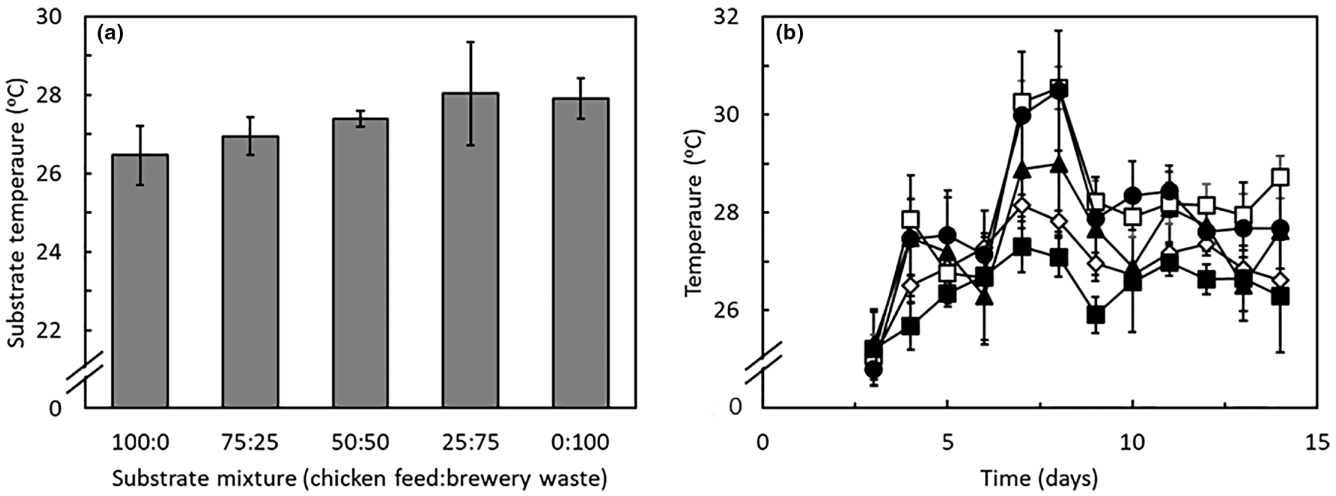


FIGURE 3 (a) Average substrate temperatures in cultures of BSF larvae reared on mixtures of chicken feed and brewery waste at ratios 100:0, 75:25, 50:50, 25:75 and 0:100. (b) Temperature profiles in same cultures, 100:0 (■), 75:25 (◊), 50:50 (▲), 25:75 (□) and 0:100 (●). Error bars indicate standard deviation of three replicate cultures.

to the specific growth rate, nor the time the larvae used to reach maximal weight. This is not as one would expect, although considerable variability in larval weight and lifetime can be observed also from other studies (Eriksen, 2022a). When insect larvae reach a certain critical weight, they initiate a hormone response that gradually terminates feeding and growth and leads to metamorphosis. Slow growth will expectedly result in small prepupae (Edgar, 2006). It has indeed been demonstrated that feed limitation results in slow growth and low weight, and delays the transformation of BSF larvae into prepupae (Diener et al., 2009). Other studies have, however, also observed that the fastest growth does not necessarily result in the largest BSF larvae (Saadoun et al., 2020). It has been suggested that concurrent microbial consumption of substrate components may gradually lead to reduced availability of nutritional compounds in the substrates and affect larval development (Bekker et al., 2021). In this study, the BSF larvae were therefore fed repeatedly to make feed of the predetermined composition available until they transformed into prepupae. Still, the elevated temperatures even after the larvae had stopped growing (Figure 3b) and the temperature differences between cultures (Figure 3a) do indicate microbial activities in the substrates, mostly in the brewery waste that became warmest. How these microbial activities may have affected the larvae is not clear. It turned out that the larvae became largest in the substrates with the lowest temperature. Systematic relationships between specific rates of growth, respiration, cost of growth or maintenance and temperature were, however, not identified. Finally, it seems that the weights of the larvae were not affected by substrate-dependent differences in their biochemical compositions. The organic content, the dry weight content and the lipid content were highly similar in the larvae reared on all substrate mixtures and within ranges measured before (Eriksen, 2022a). The dry weight content increased with time as the larvae grew larger on all substrates (Figure 3i). This indicates that the larvae became fatter with age and size, which is also what is normally seen in BSF larvae (Eriksen, 2022a). Earlier investigations of BSF larvae reared on substrates with different carbohydrate: protein ratios have also shown that the biochemical composition of the larvae is only little affected by the substrate composition (Beniers & Graham, 2019) while larval weight becomes highest when the carbohydrate content is high (Cammack & Tomberlin, 2017).

The cost of growth on all substrate mixtures was from 0.34 to 0.36, except for the larvae reared in equal mixtures of chicken feed and brewery waste, in which the cost of growth was estimated to be 0.44 (Figure 2e). This means that 0.34–0.44 gram of carbon from chicken feed or brewery waste was metabolized to $CO_2$ for each gram of carbon incorporated into the larvae. This is close to earlier estimates of the cost of growth of BSF larvae on chicken feed between 0.38 and 0.44 (Bekker et al., 2021; Laganaro et al., 2021). At the same time was 0.10–0.13 g of substate carbon metabolized daily for each gram of carbon present in the larval biomass to supply energy for maintenance purposes (Figure 2f). Earlier studies have estimated maintenance rates of BSF larvae in the order of 0.05–0.08 $day^{-1}$ (Bekker et al., 2021; Laganaro et al., 2021). This is less than the values estimated in this study. Temporary increases in specific respiration rates were observed on Day 10–11, at times where growth had almost ended. Therefore, the specific respiration rates showed variability at specific growth rates close to zero, causing uncertainties to the maintenance rates, estimated from the regression line intercepts with the *y*-axis in Figure 1f. If the maintenance rate is overestimated, the cost of growth will be underestimated. The slopes of the regression lines are not independent of the intercepts with the *y*-axis, and former estimates of the cost of growth of BSF larvae reared on chicken feed may also be more accurate. Still, the relationships between specific respiration and growth rates (Figure 1f) were comparable on all substrate mixtures, and nothing thus indicates that the BSF larvae should have performed notably different, metabolically on brewery waste compared with chicken feed.

## 5 | CONCLUDING REMARKS

High specific feed assimilation and growth rates, and low costs of growth and maintenance rates are requirements for efficient conversions of substrates into new biomass. A favourable metabolic performance is thus a prerequisite for achieving high feed conversion rates and an important criterium for evaluating the quality of substrates. In the BSF larvae examined in this study, the cost of growth and the maintenance rate on brewery waste were like those on chicken feed and feed assimilated from both sources was converted into larval biomass equally efficient. The BSF larvae also fed and grew almost as rapidly on brewery waste as on chicken feed, despite starch being the most available source for biosynthesis and energy in chicken feed but almost absent in brewery waste. These results demonstrate that brewery waste may make a good match to BSF larvae with respect to their metabolic performance. Starch-depleted feeds, in which proteins and to some degree lipids make up most of the nutritional value, may support metabolic performances comparable to the best-known starchy feeds.

## AUTHOR CONTRIBUTIONS

**Rasmus Juhl Hansen:** Investigation; methodology; writing – review and editing. **Signe Hannesbo Møller Nielsen:** Investigation; methodology; writing – review and editing. **Math Johansen:** Investigation; methodology; writing – review and editing. **Frederik Kjær Nielsen:** Investigation; methodology; writing – review and editing. **Freja Broholm Dragsbæk:** Investigation; methodology; writing – review and editing. **Oliver Schwarz Baden Sørensen:** Investigation; methodology; writing – review and editing. **Niels Thomas Eriksen:** Conceptualization; resources; supervision; writing – original draft; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Mendeley data https://data.mendeley.com/datasets/t82m6pcwff/1 (Eriksen, 2022b).

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