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Wheat starch processing by-products as rearing substrate for black soldier fly: does the rearing scale matter?

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ABSTRACT

Rearing scale may influence black soldier fly (**BSF**) larvae traits when they are fed on a single diet, but different feeding substrates have not been tested yet. This study evaluated the effects of wheat starch processing by-products-based diets on growth performance, bioconversion efficiency (**BER**), and nutritional profile of BSF larvae reared in different scales. Four diets (D1 and D2 [isonitrogenous, isolipidic and isoenergetic]; D3 and D4 [displaying 1:1 and 1:2 as protein to carbohydrate ratios, respectively]) were tested at 3 rearing scales (4 replicate boxes/diet, with a constant volume [0.84 cm^3]/larva and feed [0.7 g]/larva): 1) small (S; 12 × 12 cm, substrate height: 4 cm, 686 6-day-old larvae (**6-DOL**)/box), 2) medium (M, 32 × 21 cm, substrate height: 7 cm, 5 600 6-DOL/box), and 3) large (L, 60 × 40 cm, substrate height: 7 cm, 20 000 6-DOL/box). Larval weight was recorded at the beginning of trial and every 4 days, and growth rate (**GR**), specific growth rate (**SGR**), feed conversion ratio (**FCR**), survival, BER corrected for residue, reduction rate (**RR**), and waste reduction index (**WRI**) calculated at the end of larval growth (frass DM ≥ 55%). Substrate pH, T and height were measured at the beginning, every 4 days, and end of trial. Larval proximate composition was analysed at the end of trial. Data were analysed by generalised linear mixed model (SPSS software, $P < 0.05$). The D1 larvae showed higher weight, GR, SGR and WRI (along with higher substrate T) than D2 at M scale, while increased SGR and FCR – as well as decreased survival, RR and WRI – were observed in D2 larvae at S scale ($P < 0.05$). Larval CP and ether extract (**EE**) contents were influenced by M and L scales only, being higher in D2 group than in D1 ($P < 0.001$). Differently, decreased ash was recorded in D2 larvae when reared at S and M scales, while L scale revealed higher ash in D2 group than D1 ($P < 0.001$). The D3 larvae displayed greater weight, SGR, survival, RR and WRI (along with greater substrate T) than D4 at M scale, with increased survival and substrate T being also highlighted in L scale ($P < 0.05$). The D3 larvae also showed lower DM and EE – as well as higher CP – than D4 at all the rearing scales ($P < 0.001$). In conclusion, D1 and D3 led to better BSF larval growth performance, BER and nutritional profile mainly at M and L scales, as a consequence of their ability to facilitate larval aggregation and, in turn, allow achieving a higher substrate T.

Implications

Insect rearing faces two main challenges: (1) the choice of suitable by-products as feeding substrates for the larvae and (2) the difficulty in scaling up the production. This research identifies wheat starch processing by-products as potential, novel rearing substrates for black soldier fly larvae, highlighting that medium- and large-sized rearing boxes are preferable when compared to small-sized ones in terms of growth performance, BER, and nutritional profile. These outcomes suggest opting for larger scales than smaller ones when testing novel by-products as feeding substrates for black soldier fly larvae, thus potentially facilitating the translation from research to industrial applications.

Introduction

Starch is a natural polysaccharide that is known for several, practical properties, such as its high availability, abundance and renewability, remarkable ability in being biologically degraded, and low allergenicity and costs, which make it having a primary role in food and chemical industries (Podgorbunskikh et al., 2022). On the one hand, starch can be fermented and distilled to obtain the so-called "grain neutral spirits" or "grain neutral alcohols", products with a minimum ethanol content of 95–96% v/v that lack distinctive aroma and taste (Black and Walker, 2023).

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On the other hand, starch may be enzymatically hydrolysed to produce glucose syrup, by means of gelatini sation, liquefaction and saccharification processes (Bueno-Zabala et al., 2020). However, both the production processes generate huge amounts of waste by-products, such as distillery stillage (**DS**) and filtration aid. Indeed, the distillery industry generates 15 L of DS per each L of alcohol produced, which are characterised by a low pH, a high content of hard-to-degrade organic substances and nitrogen, and dark brown pigments (Kharayat, 2012; Ratna et al., 2021). In parallel, glucose syrup can furtherly be clarified by applying conventional solid–liquid separation techniques – such as sedimentation, centrifugation, and filtration – that generate the so-called "filtration aid" as the final by-product (Castellanos Domínguez et al., 2004). Multiple valuable products can already be recovered from these cereal processing waste (DS especially) – such as polyphenols, tetrahydrofurfuryl alcohol, and biochar (Awasthi et al., 2020) –, but their high production volumes (about 1 755 billion litres of DS can be estimated from alcohol production in 2020 (Sonnichsen, 2021)) still require new strategies to manage and valorise them within a circular economy perspective.

Nowadays, insect biorefinery emerges as a novel, environment-friendly and sustainable strategy for waste management and resource production due to the capability of insects in digesting and valorising heterogenous organic by-products into proteins and fats via larval production. Among all the insect species, *Hermetia illucens* (also commonly known as black soldier fly – **BSF**) seems to be the most promising to promote circular economy, as its saprophagous nature allows it easily feeding on decaying organic matter (Kee et al., 2023). When compared to other rearing substrates, DS have previously been reported to lead to the worst BSF larval yield and BER, as a consequence of its high amounts of cellulose (Yurina and Karagodin, 2018). However, the utilisation of analogous by-products such as dried distiller's grains with solubles (**DDGS**) – which derive from grain ethanol fermentation – is able to support BSF larval growth (Bava et al., 2019) and to increase its lipid and protein contents as well (Tschirner and Simon, 2015; Howdeshell and Tanaka, 2018), thus suggesting that cereal processing by-products could be suitable rearing substrates for BSF.

Along with the well-known influence of the rearing substrate (Rehman et al., 2023), the growth and nutritional profile of BSF larvae may also be impacted by abiotic factors, such as temperature and rearing scale. Indeed, higher substrate temperatures have recently been observed in larger rearing scales when compared to smaller ones, with the maximum larval growth and lipid content being, in parallel, achieved (Yakti et al., 2022). Better growth, survival, and biomass conversion have also previously been identified in BSF reared in industrial containers when compared to bench-top-sized ones (Yang and Tomberlin, 2020). Such findings lay the first groundwork for translating the research to an industrial scale, as most of the published studies are based on small-scale (bench-top) experiments (Miranda et al., 2020), and scaling up from small laboratory studies to large industrial studies may not be necessarily linear (Scala et al., 2020). So far, manure, fruits, brewery spent grains, and restaurant waste represent the first by-products tested as rearing substrates for BSF on a large-scale (Miranda et al., 2020; Scala et al., 2020; Yang and Tomberlin, 2020), but a clear comparison between small- and large-scale outcomes was explored by farming BSF larvae on single diets only (Yang and Tomberlin, 2020; Yakti et al., 2022).

Therefore, based on the above-mentioned background, the present study aims to evaluate the suitability of wheat starch processing by-products (DS and filtration aid) as rearing substrates for BSF farming at three different rearing scales (small, medium and large), in terms of larval growth performance, BER, and nutritional profile.

Material and methods

Colony

The BSF larvae used for the trial belonged to the colony of the experimental facility of the Department of Agricultural, Forest and Food Sciences of the University of Turin, where the larvae are fed Gainesville diet (GA; Hogsette, 1992) and reared in a climatic chamber ("breeding chamber", MONTI & C. – Tecnologie del Freddo S.r.L.; Potenza, Italy) set at 28.5 °C, 65% RH, and 0:24 L:D conditions. The adult flies are kept in a second climatic chamber ("reproduction chamber", MONTI & C. – Tecnologie del Freddo S.r.L.; Potenza, Italy) with artificial lighting, and set at 30.5 °C, 80% RH, and 12:12 L:D conditions.

By-products and experimental diets

A total of three wheat starch processing by-products were provided from a local supplier located in the Piedmont region of the North of Italy: (1) dry DS, obtained after centrifugation and drying of DS and mainly composed of insoluble fractions (i.e., insoluble proteins, fibres), (2) liquid DS, obtained after DS centrifugation and mainly composed of soluble fractions (i.e., soluble proteins, organic acids, glycerol, hemicelluloses), and (3) filtration aid, obtained after filtration of glucose syrup and mainly composed of lipids and minerals. All these by-products were analysed according to the methods reported in the "Chemical analyses" section, and their proximate composition is displayed in Table 1. Any sample replications with CV values greater than 5 were reanalysed.

A total of 4 experimental diets were formulated based on the proximate composition of the collected by-products (Table 2): (1) D1, containing a higher inclusion level of liquid DS than that of filtration aid, (2) D2, containing a higher inclusion level of filtration aid than that of liquid DS, (3) D3, characterised by a protein:carbohydrate (P:C) ratio of 1:1 and a P + C sum of 40%, and (4) D4, characterised by a P:C ratio of 1:2 and a P + C sum of 47%. The D1 and D2 diets were formulated to be isonitrogenous, isolipidic and isoenergetic, and to contain a constant % of dry DS in a lower amount when compared to that of liquid DS and filtration aid. Differently, the D3 and D4 diets were formulated to display different contents in CP and non-structural carbohydrates (**NSCs**), without any constrains in relation to the inclusion levels of the by-products. As a final aspect to consider, all the diets were formulated to contain a constant % of wheat bran (Mangimi Monge, Torre San Giorgio [CN], Italy – DM, 86.34; CP, 18.72% DM; ether extract (**EE**), 2.89% DM; ash, 6.88% DM; NDF corrected for residual ash [aNDFom], 56.21; NSC, 15.75% DM; gross energy, 14.65 MJ/kg DM) and about 35% of DM, in order to counteract the low water holding capacity of the filtration aid observed in the preliminary trial (data not shown).

Table 1
Proximate composition and gross energy of the by-products considered for black soldier fly rearing.

Proximate composition (%, DM)	Dry DS	Liquid DS	Filtration aid
DM	91.36	28.16	66.99
CP	38.27	5.48	5.93
EE	7.82	0.24	22.62
Ash	3.31	2.77	29.94
aNDFom	49.75	10.42	16.01
NSC[1]	0.85	81.09	25.50
GE (MJ/kg DM)	20.66	4.51	13.03

Abbreviations: DS = distillery stillage; EE = ether extract; NSCs = non-structural carbohydrates; aNDFom = NDF corrected for residual ash; GE = gross energy.
[1] Calculated as 100 – (CP + EE + Ash + aNDFom).

Table 2
Ingredients and proximate composition and gross energy of the by-products based diets considered for black soldier fly rearing.

Item	D1	D2	D3	D4
By-products (%, as is)				
Dry DS	5.00	5.00	5.50	0.10
Liquid DS	14.50	9.50	12.50	27.80
Filtration aid	12.00	11.85	12.50	13.50
Wheat bran	20.00	20.00	20.00	20.00
Water	48.50	53.65	49.50	38.60
Total	100	100	100	100
Proximate composition (%, DM)				
DM	36.14	32.74	36.74	37.47
CP	21.50	21.06	20.33	18.37
EE	9.43	9.62	9.86	11.82
Ash	14.80	13.55	14.55	16.71
aNDFom	28.82	33.56	34.52	25.25
NSC[1]	25.45	22.22	20.75	27.85
GE (MJ/Kg)	19.12	19.48	19.34	19.03

Abbreviations: DS = distillery stillage; EE = ether extract; NSCs = non-structural carbohydrates; aNDFom = NDF corrected for residual ash; GE = gross energy.
Values are reported as mean of duplicate analyses.
[1] Calculated as 100 – (CP + EE + Ash + aNDFom).

Larvae rearing conditions

After an oviposition period of 24 h, eggs were collected and let hatching without substrate in the reproduction chamber. The 1-day-old larvae (1-DOL) were collected within 24 h and distributed in plastic rearing boxes (19 × 13 × 6 cm) located in the breeding chamber, where they were fed the GA diet (1 g of 1-DOL/1 kg of wet feed [30% DM, 70% water]) till 6 days of age. After GA feeding, the 6-days-old larvae (**6-DOL**) from all the plastic boxes were sieved through a 0.8 mm sieve to remove the digested substrate, mixed in a single box, and estimated in number and weight (10 samples with a CV < 10%; Kern & Sohn GmbH, Balingen, Germany; d = 0.001 (Deruytter et al., 2023)). A 4 × 3 factorial design was considered, with the 4 dietary treatments being tested in 3 different rearing scales as follows (four replicate boxes/scale): (1) small (S, rearing boxes of 12 × 12 × 5 cm), (2) medium (M, 32 × 21 × 8 cm), and (3) large (L, 60 × 40 × 10 cm). To keep both the volume/larva (0.84 cm3) and the feed/larva (0.7 g) constant in all the rearing scales, either the number of the estimated 6-DOL or the quantity of feeding substrates varied depending on the rearing boxes as follows: (1) 686 6-DOL and 480.2 g of substrate for the S scale, (2) 5 600 6-DOL and 3 920 g of substrate for the M scale, and (3) 20 000 6-DOL and 14 000 g of substrate for the L scale, respectively. All the rearing boxes were filled with the weighed (SB16001 DeltaRange, Mettler Toledo, Milano, Italy; d = 0.1) feeding substrate (one batch feeding) and placed in the breeding chamber to acclimatise (1 h). After that, the estimated 6-DOL biomass was weighed (SB16001 DeltaRange, Mettler Toledo, Milano, Italy; d = 0.1) and inoculated in the substrate. All the rearing boxes were daily checked, along with the microclimatic conditions of the breeding chamber.

Growth performance and BER

Larval average weight was determined every 4 days by taking 5 subsamples of larvae and substrate from all the rearing boxes (one from the centre and one from each of the four corners). The five samples were mixed into a single sample, and all the larvae were manually counted. If the number of larvae was ≥ 100, the sample was considered representative of the rearing box, and all the larvae were washed with warm tap water, dried with paper tissue, and weighed together (Kern & Sohn GmbH, Balingen, Germany; d = 0.001). If the number of larvae was < 100, the procedure was repeated in order to obtain a representative sample. Considering that weight recording was not destructive, larvae were put back in the box. At the end of the larval growth – considered when the frass DM was ≥ 55% (Moisture Analyser; Kern & Sohn GmbH, Balingen, Germany; d = 0.001) – all the rearing boxes were sieved through a 5 mm sieve for 1–5 min (depending on the box size), and the weights of larval biomass and digested substrate were recorded (SB16001 DeltaRange, Mettler Toledo, Milano, Italy; d = 0.1). As larvae were not perfectly sieveable from the frass, they were estimated in number and weight, and the digested substrate was estimated by difference (three samples with a CV < 10%; Kern & Sohn GmbH, Balingen, Germany; d = 0.001 (Deruytter et al., 2023)). The estimated weight of the frass was added to the recorded weight of the frass after sieving to obtain the definitive frass weight and to calculate the sieveability percentage (SP) as follows:

$$SP = (\text{weight of frass after sieving [g]} \times 100) / \text{definitive weight of frass (g)}$$

Survival and growth indices were calculated on as−is basis as follows (Veldkamp et al., 2021; Seyedalmoosavi et al., 2022):

- Growth rate (**GR**) = (final larval average weight [g] – initial larval average weight [g]) / days of feeding.
- Specific growth rate (**SGR**) = ((ln(final larval average weight [g]) – ln(initial larval average weight [g]) / days of feeding) × 100.
- Feed conversion ratio (**FCR**) = feed intake (g) / weight gain (g);
- Survival rate (**SR**, %) = (number of larvae at the end of the trial / number of larvae at the beginning of the trial) × 100.

The BER of the larvae was also calculated on a DM basis as follows (Bosch et al., 2020):

- BER corrected for residue (BER, %) = ((larval biomass at the end of the trial [g] – larval biomass at the beginning of the trial [g]) / (distributed substrate [g] – residual substrate [g])) × 100.
- Reduction rate (RR, %) = ((distributed substrate [g] – residual substrate [g]) / distributed substrate [g]) × 100.
- Waste reduction index (WRI) = RR / days of feeding.

Nutritional profile

At the beginning of the trial, 6-DOL from the same batch that was used for the growth trial were collected and devitalised at −80 °C to characterise their initial proximate composition. Larvae were also collected from each rearing box at the end of the trial, washed with warm tap water, dried with a paper tissue, and devitalised following the same procedure. All the larvae were analysed according to the methods reported in the "Chemical analyses" sec-

tion. Any sample replications with CV values greater than 5 were reanalysed.

Substrate parameters

At the beginning of the trial and every 4 days, pH and T of the substrate were recorded in two opposite corners of the rearing box (HI 99161, volume precision 0.01; Hanna Instruments Italia S.r.L., Padova, Italy). The substrate height was also manually measured with a ruler (d = 1 mm) in three standardised areas of the rearing box (half of the two longest sides and centre). The average substrate pH, T and height values were then calculated.

Chemical analyses

All the by-products, wheat bran, experimental diets and larvae were submitted to analysis of their proximate composition. All the samples were stored at −20 °C and freeze-dried, with the only exception of the by-products with a DM over 60% (dry DS, filtration aid and wheat bran) that were analysed as is. The larvae samples were ground as frozen (GM 200; Retsch, Haan / Duesseldorf, Germany) and freeze-dried. The DM (AOAC #934.01), the CP (AOAC #984.13; conversion factor for ingredients and diets N × 6.25, for larvae N × 4.67) and the ash (AOAC #942.05) were determined by the International AOAC (2000), while the EE (AOAC #2003.05) by the International AOAC (2003). The aNDFom – the NDF assayed with a heat−stable amylase and expressed exclusive of residual ash – was determined by Mertens et al. (2002). The gross energy was determined using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). The NSCs were calculated as follows: 100 – (CP + EE + Ash + aNDFom). Any sample replications with CV values greater than 5 were reanalysed.

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics software (V20.0.0; IBM, Armonk, NY, United States). Outliers lying over 1.5 IQRs below the first quartile (Q1) or above the third quartile (Q3) were first detected and removed from the statistical analysis. Considering the different diet formulation approaches herein adopted, D1 was compared to D2 only, whereas D3 and D4 were exclusively compared to each other. The normality or non-normality distribution of the residuals was determined by Shapiro-Wilk test. The experimental unit for all the analysed parameters was the rearing box. Larval weight, as well as substrate pH, T and height, were analysed by fitting a generalised linear mixed model that allowed them to depend on three fixed effects (diet, rearing scale and time [plus, accordingly, their interaction]) through a gamma *P* distribution with a non−linear link function (log). The replicate was included as a random effect to account for repeated measurements on the same box. Differently, a generalised linear model evaluating the influence of two fixed effects (diet and rearing scale [plus, accordingly, their interaction]) through a gamma *P* distribution with a non−linear link function (log) was fitted to analyse larval growth performance, BER, and nutritional profile. The interactions between the levels of the fixed factors were evaluated by means of pairwise contrasts. A likelihood-ratio test was also performed in case of not significant interaction terms, and, when necessary, a model simplification was applied by removing them from the statistical models. The results were expressed as least square mean and pooled SEM. *P*-values ≤ 0.05 were considered as statistically significant.

Results

Growth performance and BER

Larval weights are summarised in Table 3 and Fig. 1. When comparing the D1 and D2 data, larval weights were influenced by rearing scale, time, and interaction between diet and rearing scale (P < 0.01, Table 3). In particular, the M scale only allowed to highlight that the D1 performed better when compared to the D2 (*P* = 0.015, Fig. 1A), while the two diets led to analogous larval weights in the different sampling times (*P* > 0.05, Fig. 1B). Independently on diet or rearing scale, larvae at the end of their growth were also characterised by greater weights than the 10-DOL (*P* < 0.001, Table 3). Differently, in relation to the comparison between the D3 and D4, larval weights depended on all the considered variables (*P* < 0.05, Table 3). The S and M scales only allowed observing that the D3 led to higher larval weights when compared to the D4 (*P* = 0.001, Fig. 1C). Furthermore, the D3-fed 10-DOL were heavier than those fed the D4, while the opposite trend was highlighted for the end of the larval growth (*P* < 0.001, Fig. 1D).

Table 4 and Fig. 2 display the larval growth performance. When comparing the D1 and D2 data, the GR and SGR were influenced by rearing scale and the interaction between diet and rearing scale (*P* < 0.05, Table 4). In particular, the D1 showed higher GR and SGR than the D2 at the M scale (*P* = 0.022 and *P* = 0.001, respectively; Fig. 2A and 2B), while lower SGR was observed in the D1-fed larvae when compared to the D2 at the S scale (*P* = 0.049, Fig. 2B). Differently, all the considered variables affected the FCR (*P* < 0.01, Table 4), with the D1 leading to lower values than the D2 at the S scale only (*P* < 0.001, Fig. 2C). The SR depended on all the considered variables as well (*P* < 0.01, Table 4), with the D1 being characterised by higher SR when compared to the D2 at the S scale only (*P* < 0.001, Fig. 2D). The days of growth (from 8 to 9 [14–15 DOL at harvest]) were also influenced by all the considered variables (*P* < 0.001, Table 4), as the D2-fed larvae took more time to complete their growth than those fed the D1 at the M scale only (*P* < 0.001, Fig. 2E). In relation to the comparison between the D3 and D4, all the considered variables influenced the GR and SGR (*P* < 0.05, Table 4). The D3 led to lower GR than the D4 at the M and

Table 3
Effects of diet, rearing scale, time and their corresponding interactions on black soldier fly larva weights.

Item	Diet (D)		Scale (S)			Time (T)		SEM			*P*-value				
	D1	D2	S	M	L	10-DOL	END	D	S	T	D	S	T	D × S	D × T
Larval weight (g)	0.090	0.093	0.091[b]	0.103[a]	0.082[c]	0.079	0.106	0.002	0.002	0.002	0.202	<0.001	<0.001	0.009	0.796
	Diet (D)		Scale (S)			Time (T)		SEM			*P*-value				
	D3	D4	S	M	L	10-DOL	END	D	S	T	D	S	T	D × S	D × T
Larval weight (g)	0.084	0.078	0.088[a]	0.085[a]	0.070[b]	0.055	0.117	0.002	0.002	0.003	0.021	<0.001	<0.001	0.006	<0.001

Abbreviations: S = small-scale; M = medium-scale; L = large-scale; 10-DOL = 10-days-old larvae; END = end of larval growth. Least square means with superscript letters (a, b, c) identify significant differences among the rearing scales (*P* < 0.05).
Experimental unit: rearing box (n = 4). Applied statistical model: general linear mixed model (GLMM), with five fixed factors (diet, scale, time, interaction between diet and scale, and interaction between diet and time), replicate as a random effect, and pairwise contrasts to compare least square means. Least square means per each single fixed effect (diet, scale and time) are displayed.

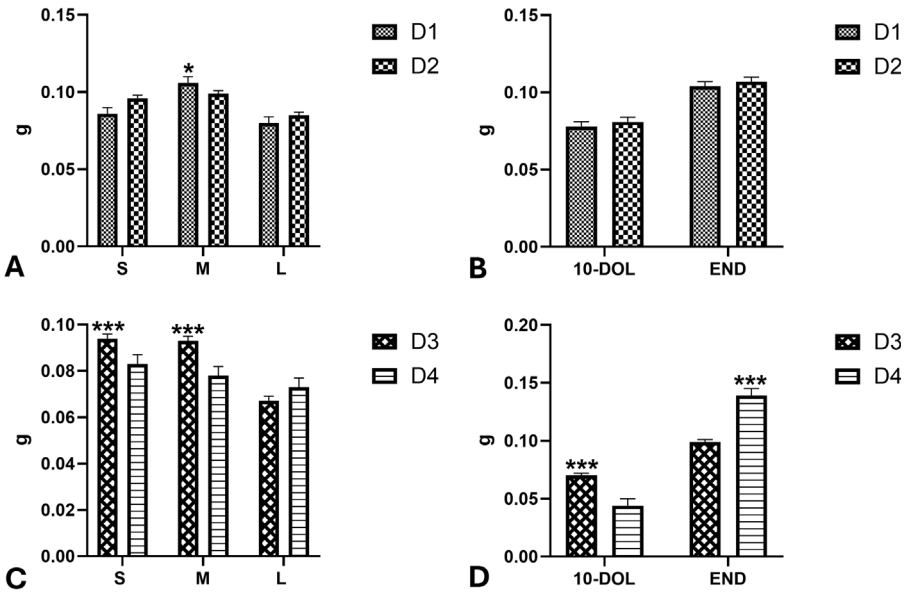

Fig. 1. Black soldier fly larva weights recorded for the different rearing substrates depending on the rearing scale (A, C) and the time (B, D). D1 and D2, isonitrogenous, isolipidic and isoenergetic diets; D3 and D4, diets displaying 1:1 and 1:2 as protein to carbohydrate ratios; S, small-scale; M, medium-scale; L, large-scale; 10-DOL, 10-days-old larvae; END, end of larval growth. * = $P \leq 0.05$; *** = $P \leq 0.001$. The asterisks identify significant differences between the diets within the single rearing scale or time.

Table 4
Effects of diet and rearing scale and their interaction on black soldier fly larva growth performance.

Item	Diet (D)		Scale (S)			SEM		*P*-value		
	D1	D2	S	M	L	D	S	D	S	D × S
GR (g/day)	0.012	0.012	0.012^{b}	0.015^{a}	0.011^{b}	0.001	0.001	0.929	<0.001	0.013
SGR (%/day)	37.50	37.02	37.87^{a}	38.11^{a}	35.83^{b}	0.55	0.67	0.534	0.028	<0.001
FCR	6.66	7.27	10.02^{a}	5.40^{b}	6.23^{c}	0.17	0.20	0.009	<0.001	0.004
SR (%)	73.55	66.06	57.64^{c}	70.52^{b}	83.31^{a}	1.86	2.29	0.004	<0.001	<0.001
Days of growth (day)	8.00	8.24	8.00^{b}	8.37^{a}	8.00^{b}	0.05	0.06	<0.001	<0.001	<0.001
	Diet (D)		Scale (S)			SEM		*P*-value		
	D3	D4	S	M	L	D	S	D	S	D × S
GR (g/day)	0.011	0.014	0.013^{a}	0.014^{a}	0.011^{b}	0.000	0.001	<0.001	<0.001	<0.001
SGR (%/day)	36.72	34.22	38.91^{a}	34.56^{b}	33.13^{b}	0.71	0.86	0.012	<0.001	0.016
FCR	8.56	6.24	9.82^{a}	5.86^{c}	6.78^{b}	0.24	0.30	<0.001	<0.001	0.067
SR (%)	59.59	48.92	48.94^{b}	49.20^{b}	65.37^{a}	1.96	2.43	<0.001	<0.001	<0.001
Days of growth (day)	8.16	9.50	8.00^{c}	9.67^{a}	8.83^{b}	0.18	0.21	<0.001	<0.001	<0.001

Abbreviations: S = small-scale; M = medium-scale; L = large-scale; GR = growth rate; SGR = specific growth rate; FCR = feed conversion ratio; SR = survival rate. Least square means with superscript letters (a, b, c) identify significant differences among the rearing scales ($P < 0.05$).
Experimental unit: rearing box (n = 4). Applied statistical model: general linear mixed model (GLMM), with three fixed factors (diet, scale, and interaction between diet and scale), replicate as a random effect, and pairwise contrasts to compare least square means. Least square means per each single fixed effect (diet and scale) are displayed.

L scales ($P < 0.020$ and $P < 0.001$, respectively; Fig. 2F), while higher SGR was observed in the D3-fed larvae when compared to the D4 at the M scale ($P = 0.001$, Fig. 2G). Differently, the FCR depended on diet and rearing scale ($P < 0.001$, Table 4), with the highest FCR being highlighted in the D3 and at the S scale, respectively ($P < 0.001$, Table 4). However, the two diets led to similar results at all the rearing scales ($P > 0.05$, Fig. 2H). The SR was influenced by all the considered variables ($P < 0.001$, Table 4), with the D3 displaying greater values than the D4 at the M and L scales ($P < 0.001$, Fig. 2I). However, the opposite trend was observed at the S scale ($P = 0.036$, Fig. 2I). The days of growth (from 8 to 11 [14–17 DOL at harvest]) also depended on all the considered variables ($P < 0.001$, Table 4), as the D3-fed larvae took less time to complete their growth than those fed the D4 at the M and L scales only ($P < 0.001$, Fig. 2J).

Larval BER is summarised in Table 5 and Fig. 3. When comparing the D1 and D2 data, the BER corrected for residue depended on diet and rearing scale ($P \leq 0.001$, Table 5). In particular, the highest BER corrected for residue was observed in the D2 and at the M scale, respectively ($P \leq 0.001$, Table 5), but the two diets led to analogous results at all the rearing scales ($P > 0.05$, Fig. 3A). Differently, the RR and WRI were influenced by all the considered variables ($P < 0.05$, Table 5), with greater values being highlighted in the D1 than the D2 at the S scale (RR; $P < 0.001$, Fig. 3B), and at both the S and M scales (WRI; $P < 0.001$ and $P = 0.002$, respectively; Fig. 3C). The SP depended on diet and the interaction between diet and rearing scale ($P < 0.01$, Table 5), with the D1 showing lower values when compared to the D2 at the S scale only ($P = 0.001$, Fig. 3D). In relation to the comparison between the D3 and D4, the BER corrected for residue depended on diet and rearing scale ($P \leq 0.001$, Table 5). In particular, the highest BER corrected for residue was observed at the D4 and M scale, respectively ($P < 0.001$ and $P < 0.05$, Table 5), but the two diets led to similar results at all the rearing scales ($P > 0.05$, Fig. 3E). Differently, the

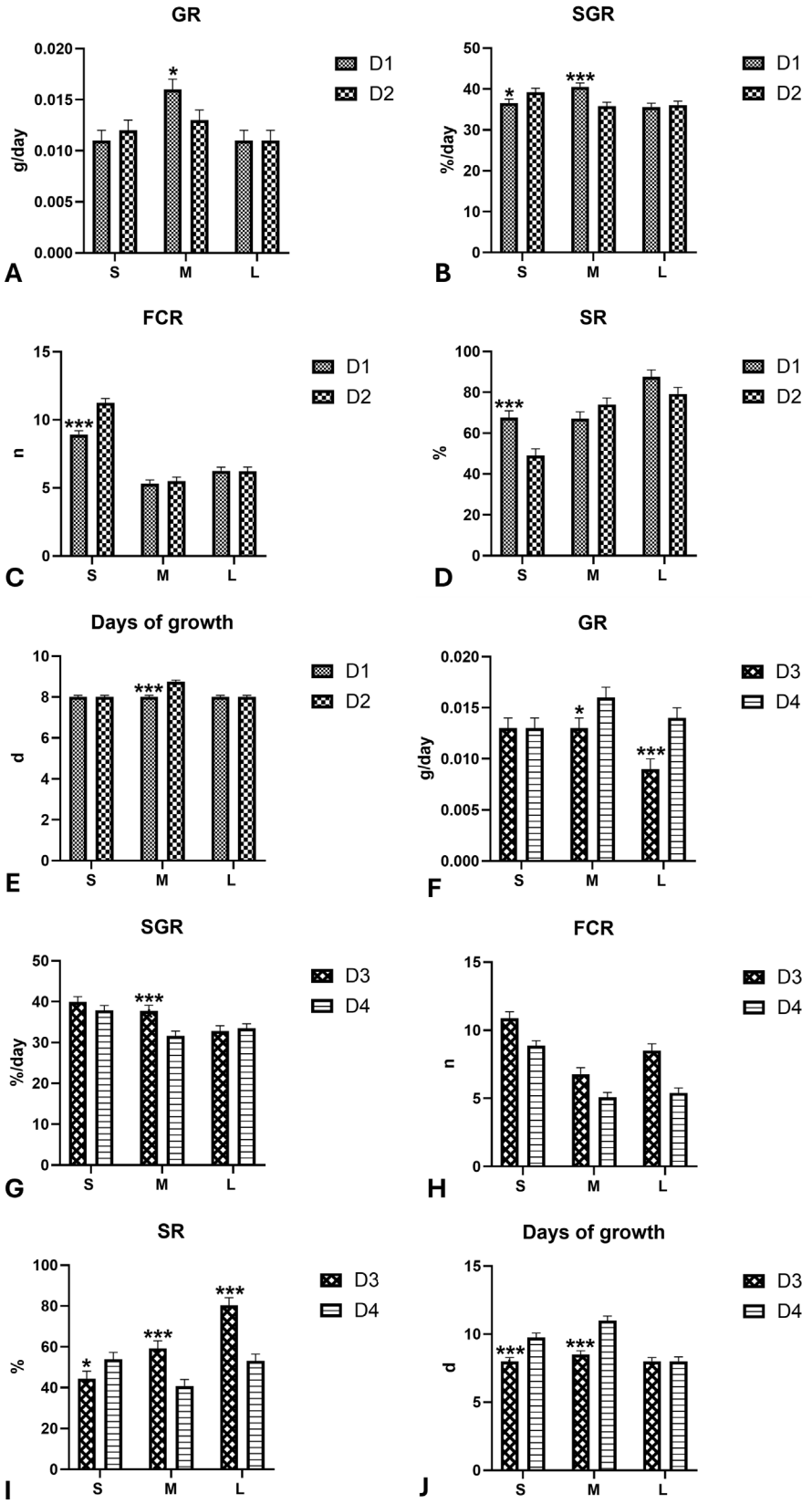

Fig. 2. Black soldier fly larva growth performance recorded for the different rearing substrates depending on the rearing scale. D1 and D2, isonitrogenous, isolipidic and isoenergetic diets; D3 and D4, diets displaying 1:1 and 1:2 as protein to carbohydrate ratios; S, small-scale; M, medium-scale; L, large-scale; GR, growth rate; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate. * = $P \leq 0.05$; *** = $P \leq 0.001$. The asterisks identify significant differences between the diets within the single−rearing scale.

Table 5
Effects of diet and rearing scale and their interaction on black soldier fly larva BER.

Item	Diet (D)		Scale (S)			SEM		*P*-value		
	D1	D2	S	M	L	D	S	D	S	D × S
BER (%)	20.10	22.78	15.33^{c}	26.46^{a}	24.16^{b}	0.58	0.72	0.001	<0.001	0.327
RR (%)	38.45	34.56	36.78^{b}	39.63^{a}	33.23^{c}	0.65	0.79	<0.001	<0.001	0.014
WRI	4.81	4.20	4.60^{a}	4.74^{a}	4.15^{b}	0.09	0.10	<0.001	<0.001	0.038
SP (%)	81.89	82.64	70.65^{c}	85.29^{b}	92.40^{a}	0.93	1.12	0.567	<0.001	0.001
	Diet (D)		Scale (S)			SEM		*P*-value		
	D3	D4	S	M	L	D	S	D	S	D × S
BER (%)	17.01	22.18	13.17^{c}	26.02^{a}	21.40^{b}	0.90	1.12	<0.001	<0.001	0.398
RR (%)	31.09	30.36	37.63^{a}	25.48^{c}	30.24^{b}	1.04	1.28	0.623	<0.001	<0.001
WRI	3.81	3.07	4.70^{a}	2.63^{c}	3.22^{b}	0.12	0.15	<0.001	<0.001	<0.001
SP (%)	83.90	67.63	68.09^{c}	74.97^{b}	83.73^{a}	1.23	1.51	<0.001	<0.001	0.066

Abbreviations: S = small-scale; M = medium-scale; L = large-scale; BER = bioconversion efficiency corrected for residue; RR = reduction rate; WRI = waste reduction index; SP = sieveability percentage. Least square means with superscript letters (a, b, c) identify significant differences among the rearing scales ($P < 0.05$).
Experimental unit: rearing box (n = 4). Applied statistical model: general linear mixed model (GLMM), with three fixed factors (diet, scale, and interaction between diet and scale), replicate as a random effect, and pairwise contrasts to compare least square means. Least square means per each single fixed effect (diet and scale) are displayed.

RR was influenced by the rearing scale and the interaction between diet and rearing scale ($P < 0.001$, Table 5), with the D3 displaying higher values when compared to the D4 at the M scale only ($P < 0.001$, Fig. 3F). The WRI depended on all the considered variables ($P < 0.001$, Table 5), with greater values being highlighted in the D3-fed larvae than the D4 at the M scale only as well ($P < 0.001$, Fig. 3G). The SP was influenced by either the diet or the rearing scale ($P < 0.001$, Table 5), but the two diets led to analogous results at all the rearing scales ($P > 0.05$, Fig. 3H). The D3 was characterised by higher SP when compared to the D4, with the greatest value is also observed at the L scale ($P < 0.001$, Table 5).

Nutritional profile

Table 6 and Fig. 4 display the larval nutritional profile. When comparing the D1 and D2 data, the DM was influenced by both the diet and the rearing scale ($P < 0.05$, Table 6). In particular, the D1-fed larvae showed higher DM than those fed the D2 ($P < 0.05$, Table 6), but the two diets led to similar results at all the rearing scales ($P > 0.05$, Fig. 4A). Independently on the diet, the highest DM was also observed on the S scale ($P < 0.001$, Table 6). The CP, EE and ash depended on all the considered variables ($P < 0.001$, Table 6). The D1 led to lower CP and EE contents when compared to the D2 at the M and L scales only ($P < 0.001$, Fig. 4B and 4C). Differently, higher ash contents were highlighted in the D1-fed larvae than those fed the D2 at the S and M scales, while the L scale displayed the opposite trend ($P < 0.001$, Fig. 4D). In relation to the comparison between the D3 and D4, the whole proximate composition was influenced by all the considered variables ($P < 0.001$, Table 6). In particular, the D3 led to lower SS and EE contents than the D4 at all the rearing scales, with the opposite trend being observed for the CP ($P < 0.001$, Fig. 4E, F and G). Differently, the D3-fed larvae displayed higher ash contents when compared to those fed the D4 at the M and L scales only ($P < 0.001$, Fig. 4H).

Substrate parameters

Table 7 and Fig. 5 summarise the substrate parameters. When comparing the D1 and D2 data, pH depended on both the rearing scale and the time ($P < 0.01$, Table 7). In particular, the lowest substrate pH was observed at the L scale, while the highest one was at the end of the larval growth ($P < 0.01$, Table 7). However, the two diets led to analogous results at all the rearing scales and for all the sampling times ($P > 0.05$, Fig. 5A and B). Differently, the T was influenced by all the considered variables ($P < 0.05$, Table 7). The D1 led to higher substrate T than the D2 at the M and L scales only ($P < 0.001$, Fig. 5C), while greater substrate T was observed in the D1-fed larvae when compared to those fed the D2 at the end of larval growth exclusively ($P = 0.011$, Fig. 5D). The height depended on diet, rearing scale, time and the interaction between diet and rearing scale ($P < 0.01$, Table 7). In particular, the D1 led to lower substrate height than the D2 at the S scale only ($P < 0.001$, Fig. 5E). The lowest substrate height was also highlighted at the end of larval growth ($P < 0.001$, Table 7), but the two diets led to analogous results for all the sampling times ($P > 0.05$, Fig. 5F). In relation to the comparison between the D3 and D4, the pH depended on either the time or the interaction between diet and time ($P < 0.001$, Table 7), but the two diets led to similar results at all the rearing scales ($P > 0.05$, Fig. 5G). The D3 showed higher substrate pH than the D4 at the end of larval growth only ($P = 0.017$, Fig. 5H). Differently, the T was influenced by all the considered variables ($P < 0.01$, Table 7). The D3 led to higher substrate T than the D4 at the M and L scales only ($P < 0.001$, Fig. 5I), while greater substrate T was observed in the D3-fed larvae when compared to those fed the D4 at 10 days of age exclusively ($P < 0.001$, Fig. 5J). The height depended on diet, rearing scale and time ($P < 0.001$, Table 7). In particular, the highest substrate height was highlighted in the D3 and at the M scale, while the lowest one was at 10 days of age and at the end of the larval growth ($P < 0.001$, Table 7). However, the two diets led to similar results at all the rearing scales and for all the sampling times ($P > 0.05$, Fig. 5K and L).

Discussion

The present study allowed identifying the best wheat processing by-products-based diets for BSF in selected rearing scales only, as a significant diet*rearing scale interaction was observed for most of the investigated parameters. Nevertheless, a clear effect of both the diet and the rearing scale was also observed independently on their interaction, thus providing general considerations about the suitability of wheat processing by-products and the optimal rearing scales for BSF farming.

Growth performance and BER

When comparing the D1 and D2 diets, D1 overall led to better growth performance (in terms of higher larval weight, GR and SGR, and less days of growth) and BER (in terms of higher WRI) at the medium-scale rearing. This can reasonably be attributed to the achieved higher substrate T, as previously reported (Yang and Tomberlin, 2020; Yakti et al., 2022). The substrate T commonly reflects its microbial activity, since the presence of BSF larvae in

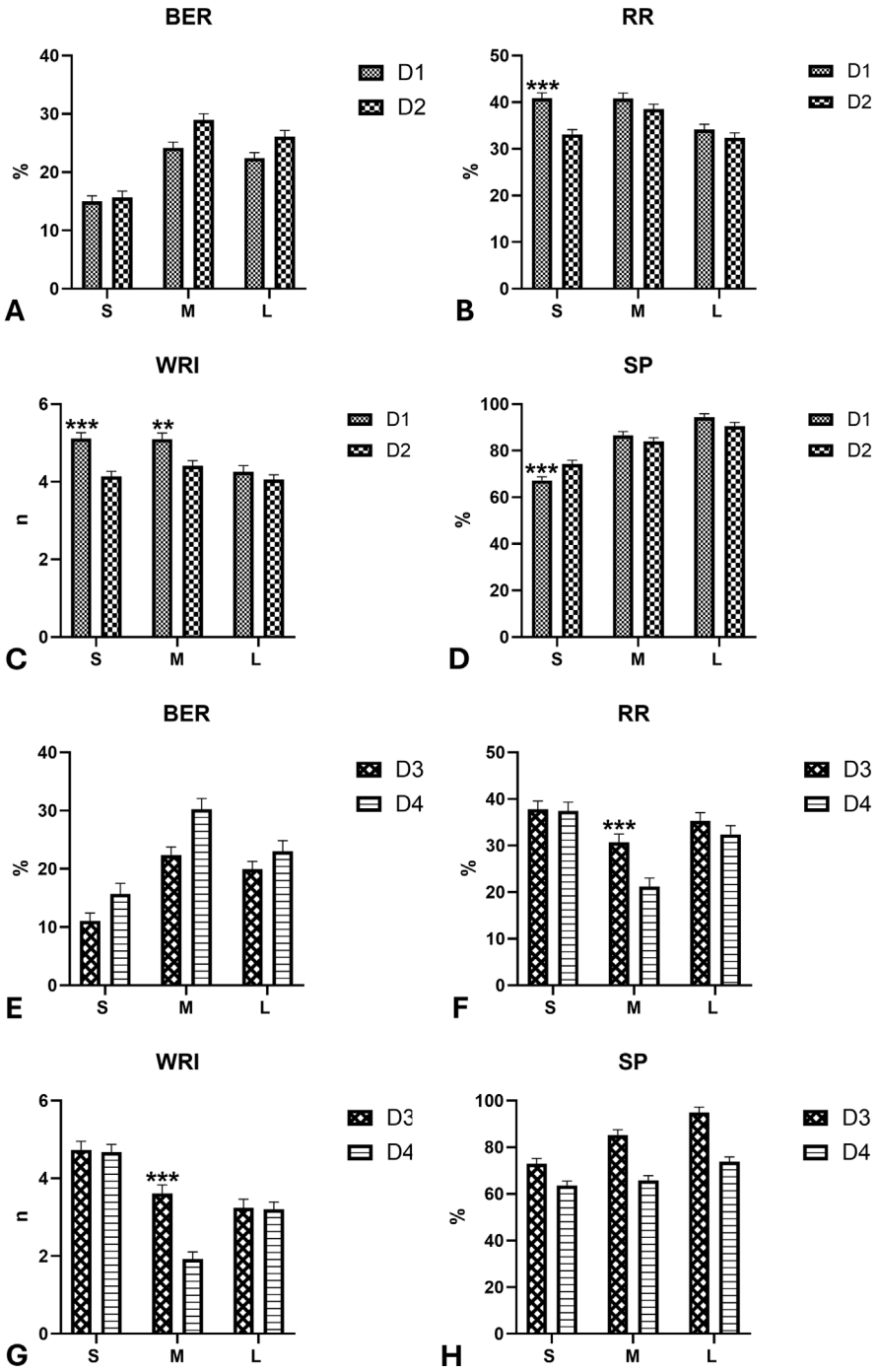

Fig. 3. Black soldier fly larva BER recorded for the different rearing substrates depending on the rearing scale. D1 and D2, isonitrogenous, isolipidic and isoenergetic diets; D3 and D4, diets displaying 1:1 and 1:2 as protein to carbohydrate ratios; S, small-scale; M, medium-scale; L, large-scale; BER, bioconversion efficiency corrected for residue; RR, reduction rate; WRI, waste reduction index; SP, sieveability percentage. ** = $P \leq 0.01$ *** = $P \leq 0.001$. The asterisks identify significant differences between the diets within the single−rearing scale.

the feeding substrate is associated with the proliferation of mesophilic (in the first 3 days) and thermophilic (since day 4) bacteria that are responsible for degrading simple compounds such as sugars, amino acids, proteins – necessary for the growth and development of the larvae – and decomposing fats and cellulose, hemicellulose and lignin (Bloukounon-Goubalan et al., 2019). The identification of higher substrate T after 4 days of rearing (10-DOL) and at the end of larval growth seems to confirm these dynamics, with D1-fed larvae further increasing it during the last days of rearing. As BSF larvae tend to aggregate to achieve a more efficient feed consumption (Shishkov et al., 2019; Shishkov and Hu, 2020), and aggregation of dipteran larvae may lead to heat-related shorter development time (Charabidze et al., 2011), it is reasonable to hypothesise that larger rearing scales could give BSF larvae higher opportunities to aggregate and, in turn, perform a higher feed bioconversion and increase the substrate T. Furthermore, Bloukounon-Goubalan et al. (2019) underlined that fibre-rich rearing substrates are accompanied by lower substrate T and mass loss, as a consequence of the low breakdown of carbon chains during the biodegradation process. Considering that the D2 diet was

Table 6
Effects of diet and rearing scale and their interaction on black soldier fly larva proximate composition.

Item	Diet (D)		Scale (S)			SEM		*P*-value		
	D1	D2	S	M	L	D	S	D	S	D × S
DM (%)	28.70	28.00	29.82[a]	28.57[b]	26.74[c]	0.21	0.26	0.020	<0.001	0.065
CP (%, DM)	37.54	38.64	40.68[a]	37.54[b]	36.35[c]	0.08	0.10	<0.001	<0.001	<0.001
EE (%, DM)	15.95	17.56	11.17[c]	22.25[a]	18.86[b]	0.11	0.13	<0.001	<0.001	<0.001
Ash (%, DM)	9.73	10.41	9.50[b]	8.77[c]	12.25[a]	0.08	0.10	<0.001	<0.001	<0.001
	Diet (D)		Scale (S)			SEM		*P*-value		
	D3	D4	S	M	L	D	S	D	S	D × S
DM (%)	26.28	31.84	27.98[b]	30.57[a]	28.29[b]	0.37	0.44	<0.001	<0.001	<0.001
CP (%, DM)	38.85	34.41	40.37[a]	34.25[c]	35.35[b]	0.14	0.16	<0.001	<0.001	<0.001
EE (%, DM)	15.70	21.09	10.75[c]	24.49[a]	22.87[b]	0.15	0.19	<0.001	<0.001	<0.001
Ash (%, DM)	10.47	9.21	10.06[b]	7.91[c]	11.89[a]	0.11	0.13	<0.001	<0.001	0.066

Abbreviations: S = small-scale; M = medium-scale; L = large-scale; DM = dry matter; CP = crude protein; EE = ether extract. Least square means with superscript letters (a, b, c) identify significant differences among the rearing scales ($P < 0.05$).
Experimental unit: rearing box (n = 4). Applied statistical model: general linear mixed model (GLMM), with three fixed factors (diet, scale, and interaction between diet and scale), replicate as a random effect, and pairwise contrasts to compare least square means. Least square means per each single fixed effect (diet and scale) are displayed.

characterised by a greater amount of aNDFom than D1 (as the filtration aid is richer in fibres when compared to the liquid DS), the nutritional profile of the rearing substrate also concurred to the better D1-related larval growth performance and BER. Despite the less consistent results, the S scale confirms the better suitability of the D1 diet than the D2 for BSF rearing as well, since lower FCR and higher SR, RR and WRI were obtained. However, the identification of lower SGR and SP may suggest an opposite trend. Nevertheless, the lower SGR may be attributable to the higher SR – as the D2-fed larvae had more feed available to consume –, while the lower SP can be more related to the small-scale rearing rather than the D1 diet itself, as the former was clearly characterised by reduced SP when compared to the other rearing scales in both the dietary treatments.

In relation to the comparison between the D3 and D4 diets, D3 overall led to better growth performance (in terms of higher larval weight, SGR and SR, and less days of growth) and BER (in terms of higher RR and WRI) at the M (mainly) and L scales. As already observed for the D1 diet, the lower larval GR detected for the D3 derived from their higher SR, thus not representing a misleading outcome. The achieved higher substrate T was analogously considered as the main driver of such an improvement, with the increased T detected after 4 days of rearing (mainly for D3) and at the end of the trial reflecting the physiological substrate dynamics as well (Bloukounon-Goubalan et al., 2019). However, the comparison between D3 and D4 diets also suggests that diets characterised by a P:C ratio of 1:1 and a P + C sum of 40% seem to be preferable. Diets with P:C ratios of 1:2 and 1:4 have previously been reported to lead to the best BSF larval and adult performance, with 1:1 being, instead, not able to allow larval surviving (Barragán-Fonseca et al., 2018 and 2021). The same authors also underlined that BSF performance was more affected by the P + C sum than the P:C ratio, identifying the optimal P + C concentrations at 25 and 50% (Barragan-Fonseca et al., 2021). Therefore, the results of the present study seem to disagree with this scenario. However, despite being nutritionally more suitable than the D3, the D4 substrate was characterised by a reduced ability to retain water (in the first 4 days of the cycle) and a high tendency to agglomerate and dry (in the final phases of larval growth), as demonstrated by the overall low larval weight, SR and SP. Therefore, the poor outcomes of the D4 diet were attributable to texture issues rather than its nutritional profile, which were, in turn, related to the highest inclusion levels of both the filtration aid – mainly responsible for the already mentioned low water holding capacity (as watery substrates may reduce mobility by preventing larvae from anchoring their bodies (Goldstein and Jeffreys, 1929)) – and the liquid DS – which, being rich is NSC, was reasonably responsible for the substrate agglomeration (as starch is a well-recognised thickening agent (Li et al., 2018)). The better suitability of the D3 diet when compared to D4 was also confirmed by the less days of growth highlighted at the small-scale rearing, while the apparently contrasting lower SR was, however, more related to the small scale rather than the D3 diet itself. Indeed, similarly to what was already observed for the D1-D2 comparison, the S scale was clearly characterised by reduced SP, thus suggesting a less feed consumption by BSF larvae.

Nutritional profile

When comparing the D1 and D2 diets, D1 leads to decreased larval CP and EE contents at the M and L scales. As already highlighted for growth performance and BER, the predominant identification of significant outcomes in these scales may be related to the achieved higher substrate T. Considering that the D1 diet was characterised by higher NSC content than the D2, an increase in larval EE content could, however, have been expected, as excess carbohydrates are commonly transformed into lipid reserves (Li et al., 2016). In parallel, a decrease in CP content could have been hypothesised as well, as fat and protein accumulation seem to be negatively correlated to each other (Scala et al., 2020; Yakti et al., 2022). However, the D1-fed larvae took less time to complete their growth when compared to the D2 at the medium-scale rearing, thus partially explaining the less accumulated lipids and proteins. Furthermore, the D1 diet led to numerically higher SR than the D2 at the L scale, thus potentially leaving to lower feed availability for the individuals (Yakti et al., 2022). Ash content was higher in the D1-fed larvae in comparison with the D2 at the small- and medium-scale rearing, but the large scale displayed the opposite outcome. The D1 diet was characterised by greater ash content than the D2, thus logically being reflected in the increased larval ash detected at the S and M scales – as an increase in ash levels in the rearing substrate has recently been reported to increase the ash content in BSF larvae as well (Alifian et al., 2022). However, the inverse trend observed in the L scale deserves further investigations.

In relation to the comparison between the D3 and D4 diets, D3 leads to increased CP content, as well as decreased DM and EE levels, at all the rearing scales. The reduced NSC content characterising the D3 diet logically explained the larval lower EE levels (Li et al., 2016) and, in turn, the lower DM (as lipids are hydrophobic substances) and the higher CP content (Scala et al., 2020; Yakti et al., 2022). However, despite the higher dietary ash content,

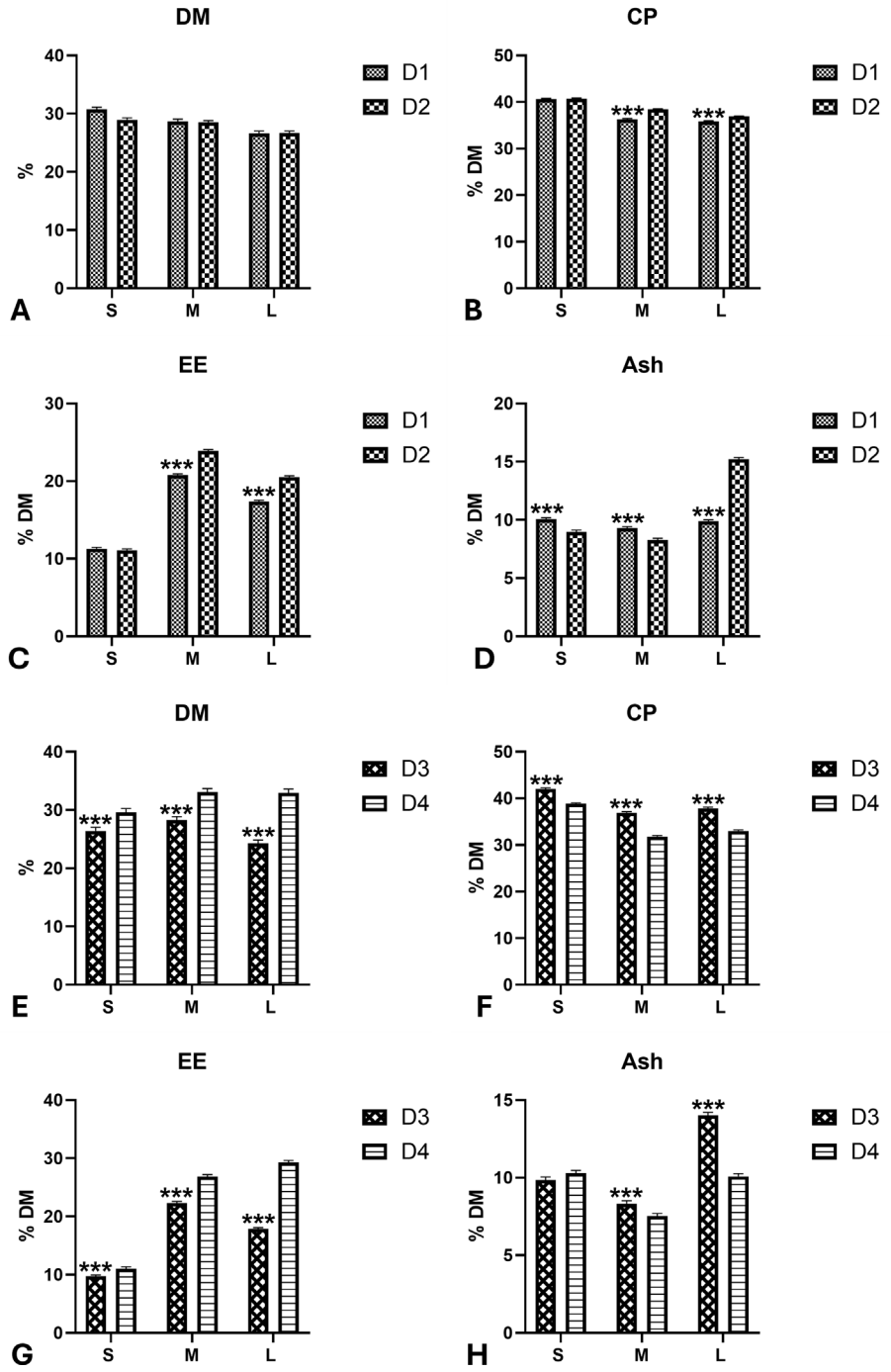

Fig. 4. Black soldier fly larva proximate composition recorded for the different rearing substrates depending on the rearing scale. D1 and D2, isonitrogenous, isolipidic and isoenergetic diets; D3 and D4, diets displaying 1:1 and 1:2 as protein to carbohydrate ratios; S, small-scale; M, medium-scale; L, large-scale; EE, ether extract. *** = $P \leq 0.001$. The asterisks identify significant differences between the diets within the single−rearing scale.

lower ash levels were highlighted in the D3-fed larvae when compared to D4 at the M and L scales. As high larval densities with lower feed volumes have previously been suggested to increase mineral concentrations in the rearing substrate (Yakti et al., 2022), the higher SR observed in the D3 diet in the same rearing scales could potentially be the reason behind such findings.

Substrate parameters

As already discussed before, the optimal wheat processing by-products-based diets were mainly identified at the medium- and large-scale rearing, as a consequence of the achieved higher substrate T. This confirms that larger scales are able to allow the substrate generating and maintaining high temperatures, thus overall improving larval growth (Yakti et al., 2022). Differently, the present study did not highlight any key role of substrate pH or height changes, as no significant diet*rearing scale interaction was overall highlighted for these parameters, with the only exception of the D1 substrate height being lower than the D2 at the S scale. This could be related to the higher WRI displayed by the D1-fed larvae when compared to D2, as feed was more easily and efficiently consumed. Furthermore, since numerically lower substrate heights were

Table 7
Effects of diet, rearing scale, time and their corresponding interactions on black soldier fly substrate parameters.

Item	Diet (D)		Scale (S)			Time (T)			SEM			*P*-value				
	D1	D2	S	M	L	6-DOL	10-DOL	END	D	S	T	D	S	T	D × S	D × T
pH	5.68	5.70	5.83^{a}	5.77^{a}	5.48^{b}	4.82^{c}	5.37^{b}	7.11^{a}	0.04	0.07	0.07	0.825	0.003	<0.001	0.522	0.138
T	32.41	30.89	29.00^{b}	32.82^{a}	33.29^{a}	22.71^{c}	38.28^{a}	36.44^{b}	0.16	0.20	0.40	<0.001	<0.001	<0.001	0.010	0.011
Height	5.37	5.71	3.51^{c}	7.76^{a}	6.24^{b}	6.74^{a}	5.60^{b}	4.50^{c}	0.07	0.09	0.10	<0.001	<0.001	<0.001	0.004	0.115
	Diet (D)		Scale (S)			Time (T)			SEM			*P*-value				
	D3	D4	S	M	L	6-DOL	10-DOL	END	D	S	T	D	S	T	D × S	D × T
pH	5.36	5.28	5.35	5.30	5.31	4.59^{c}	5.11^{b}	6.43^{a}	0.04	0.05	0.07	0.156	0.751	<0.001	0.616	<0.001
T	30.69	27.27	27.19^{b}	29.68^{a}	30.01^{a}	19.16^{b}	35.49^{a}	35.61^{a}	0.22	0.26	0.43	<0.001	<0.001	<0.001	<0.001	0.003
Height	6.11	5.25	3.73^{c}	7.77^{a}	6.28^{b}	6.51^{a}	5.43^{b}	5.14^{b}	0.10	0.09	0.11	<0.001	<0.001	<0.001	0.760	0.961

Abbreviations: S = small-scale; M = medium-scale; L = large-scale; 6-DOL = 6-days-old larvae; 10-DOL = 10-days-old larvae; END = end of larval growth. Least square means with superscript letters (a, b, c) identify significant differences among the rearing scales and larval ages ($P < 0.05$).
Experimental unit: rearing box (n = 4). Applied statistical model: general linear mixed model (GLMM), with five fixed factors (diet, scale, time, interaction between diet and scale, and interaction between diet and time), replicate as a random effect, and pairwise contrasts to compare least square means. Least square means per each single fixed effect (diet, scale and time) are displayed.

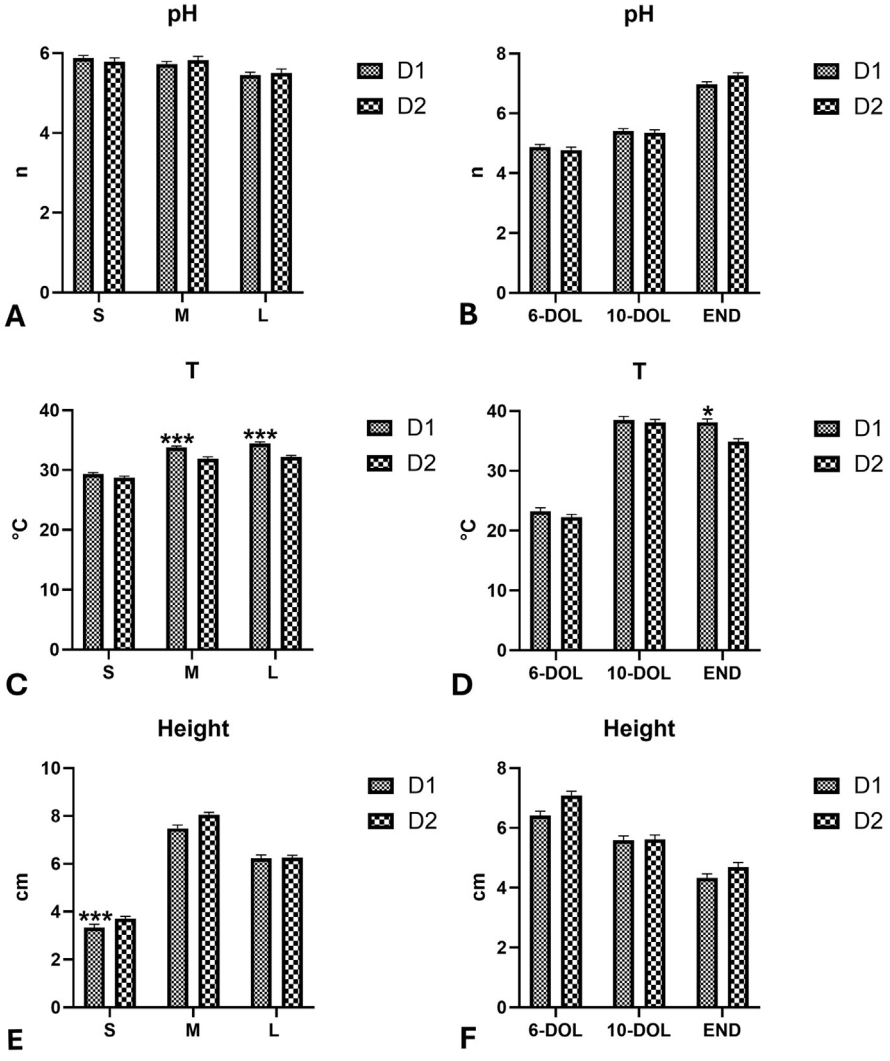

Fig. 5. Substrate parameters recorded for the different rearing substrates depending on the rearing scale (A, C, E, G, I and K) and the time (B, D, F, H, J and L). D1 and D2, isonitrogenous, isolipidic and isoenergetic diets; D3 and D4, diets displaying 1:1 and 1:2 as protein to carbohydrate ratios; S, small-scale; M, medium-scale; L, large-scale; 6-DOL, 6-days-old larvae; 10-DOL, 10-days-old larvae; END, end of larval growth. * = $P \leq 0.05$; *** = $P \leq 0.001$. The asterisks identify significant differences between the diets within the single−rearing scale or time.

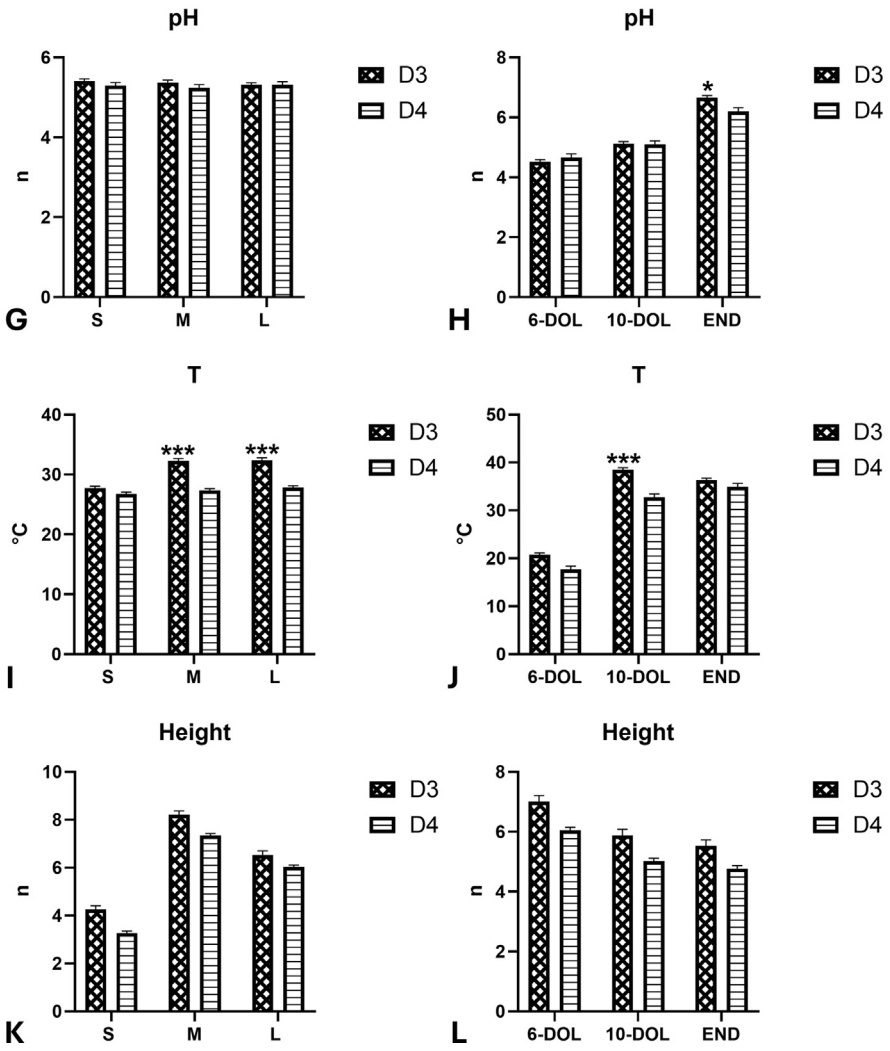

Fig. 5 (*continued*)

detected in the D1 substrate of the medium- and large-scale rearing as well, this outcome appears to be more related to the dietary characteristics rather than the adoption of different rearing boxes. On the contrary, pH and height mainly varied on varying larval age, as pH increased with time, while height displayed the opposite trend. Both these outcomes reflect the BSF physiological rearing cycle, since substrate pH evolves from acid to alkaline during larval colonisation and digestion for the release of ammonium ions and ammonia (Ma et al., 2018), and substrate height reasonably decreases as a consequence of substrate reduction and drying.

General remarks

Suitability of wheat processing by-products as rearing substrates for black soldier fly

Even if the D1 and D3 diets were characterised by better productive outcomes when compared to the D2 and D4, all the wheat starch processing by-products-based diets acted as sub-optimal rearing substrates for BSF larvae in terms of overall low final weights (0.099–0.139 g), SR (49–74%), RR and WRI (30–38% and 3–5, respectively), and SP (68–84%). These poor outcomes can potentially be attributed to three different aspects. Firstly, all the diets were formulated to contain slightly higher DM contents (33–38%) than the recommended one (25–30% (Cheng et al., 2017)), in order to counteract the reduced water–holding capacity of the lipid-rich filtration aid observed in the preliminary trial (data not shown). This may have accelerated the drying of the substrate, thus not allowing the BSF larvae to assimilate enough nutrients before the end of their growth. As a second aspect to consider, all the rearing substrates displayed a moderate to high tendency to agglomerate in the last days of larval growth, as a reasonable consequence of including the NSC-rich, viscous liquid DS. Indeed, increased substrate viscosity may require larvae to expend more energy during movement to overcome the increased drag (Goldstein and Jeffreys, 1929). However, soluble fibres have also been reported to increase substrate viscosity in relation to their different physical structures (McRorie and McKeown, 2017). Therefore, as moderate to high aNDFom contents (25–35%) characterised all the diets tested in the present study, fibre can reasonably be considered the third main driver of the poor suitability of the wheat starch processing by-products as rearing substrates for BSF larvae. All these physico-chemical features were partially reflected by the substrate T, as it did not decrease for all the tested diets when larvae reached the end of their growth. Indeed, increased amounts of heat are commonly generated as larvae age, but then, the substrate T begins to decrease when they start losing weight (Li et al., 2023).

A proper comparison between the findings of the current research and the available scientific literature appears to be challenging, as only Yurina and Karagodin (2018) tested the DS for

BSF larvae rearing, similarly highlighting low larval biomass yield/kg of substrate (0.084 kg) and substrate conversion (52.7%) and attributing these poor outcomes to the presence of cellulose as limiting factor. However, DDGS – a by-product of bioethanol fermentation that uses the dry milling technology for starch-rich grains such as corn, wheat, and barley (Iram et al., 2020) – may represent an interesting benchmark for their nutritional and processing similarities with DS. Indeed, previous research assessing the suitability of DDGS as feed for BSF larvae interestingly revealed low SR (21.70–73.00%), RR (37.80%) and WRI (3.22) as well (Tschirner and Simon, 2015; Bava et al., 2019). Differently, larval final weights (0.197–0.270 g) – as well as their DM (30.2–38.5%), CP (44.6–53.4% on DM) and EE (29.9–38.6%) contents – resulted to be higher when compared to the findings of the present study. Nevertheless, these incongruencies may be attributed to the different CP contents of the rearing substrates (29.5% (Bava et al., 2019) vs 18.37–21.50% [current research]) or to the very low SR (Tschirner and Simon, 2015).

Optimal rearing scales for black soldier fly

Despite the S scale partially allowing to confirm the best wheat starch processing by-products-based diets clearly outlined by the M and L scales, it overall led to sub-optimal larval performance. The small-scale rearing showed higher FCR (around 10) and lower BER corrected for residue (13–15%) when compared to the medium and large scales (5–6 and 21–26%, respectively), thus agreeing with the reduced conversion efficiency previously highlighted in benchtop-sized containers (Yang and Tomberlin, 2020) and the smallest scale (Yakti et al., 2022). Therefore, the findings of the present study confirm that the application of benchtop data to an industrial production facility could remarkably underestimate production levels and conversion rates (Yang and Tomberlin, 2020). The BSF larvae reared at the small scale also displayed lower SR (49–58%) than medium- and large-scale rearing (49–83%), as already reported by the above-mentioned research (Yang and Tomberlin, 2020; Yakti et al., 2022). This could be related to the sub-optimal population size (686 larvae vs 5 600 [M] and 20 000 [L]), as smaller groups of larvae in smaller containers previously resulted in less ideal survivorship (Yang and Tomberlin, 2020; Yakti et al., 2022). As a consequence of the lower BER and survival, a reduced SP was also observed for the S-reared larvae when compared to those reared in the M and L boxes. No previous studies have assessed the frass sieveability, but this parameter could be an additional, useful indicator to quantify the amount of unprocessed substrate. The nutritional profile of the larvae reared at the small scale significantly differed from that of the medium- and large-scale rearing as well in terms of higher CP (40 vs 34–38%) and lower EE (11 vs 19–25%). This is in agreement with the findings from Yakti et al. (2022), which attributed the higher CP content to the higher larval mortality – thus leading to leading to higher feed availability for the individuals and a reduction in larval density – and the lower EE content to its negative correlation with CP availability.

Apart from the already discussed role of the substrate T in allowing the M and L scales obtaining the best productive outcomes, the substrate height has to be considered as well, since higher heights characterised both the medium- and large-scale rearing when compared to the small scale. Dortmans et al. (2017) previously suggested that depths greater than 5 cm may not allow larvae to process the material at the bottom of the containers. Indeed, limitation in the substrate depth build-up seems to determine an optimal biomass conversion ratio and material reduction and, in turn, achieving the optimal residue DM (Lalander et al., 2020). Furthermore, increasing substrate depth (from 1 to 5.7 cm) has recently been reported to decrease BSF larval BER and substrate reduction (Guidini Lopes et al., 2023). However, as the initial substrate heights in the M and L rearing boxes were higher than 5 cm (7–10 cm), the findings of the present study seem to disagree with this scenario. These different outcomes can mainly be related to the different larval density (0.84 cm^3/larva vs 2.4 (Lalander et al., 2020) and 3.42–7.80 (Guidini Lopes et al., 2023)), as Opare et al. (2022) recently suggested that a combination of temperature and larval density gives a stronger response in terms of larval growth and development, thus highlighting the need of taking into account multiple parameters in order to achieve an optimal substrate bioconversion. Therefore, they confirmed that higher larval densities allow for larval aggregations and, in turn, enhance the larval ability to digest food (Opare et al., 2022), as both herein and previously suggested (Yang and Tomberlin, 2020; Yakti et al., 2022).

As a final, interesting aspect to underline, despite the absence of significant differences between the substrate T, the medium-scale rearing overall led to more pronounced outcomes in terms of larval performance and BER when compared to the large scale. This could reasonably be related to the longer time the M−reared BSF larvae took to complete their growth, as a consequence of the longer time the substrate required to dry. This delay in the drying process could have, in turn, exerted a significant influence on the maintenance of a greater substrate height in the M scale in comparison with the L one, since increasing substrate water contents (in the presence of constant feeding rates) have been associated with higher substrate depths (Lalander et al., 2020).

Conclusions

In conclusion, D1 and D3 diets performed better than D2 and D4 mainly at the medium- and large-scale rearing, as a consequence of the achieved higher substrate T. However, wheat starch processing by-products acted as sub-optimal rearing substrates for BSF farming in terms of larval growth and BER, reasonably due to their physical properties (reduced water holding capacity and increased viscosity). As a final aspect to consider, the medium- and large-scale rearing seem to be preferable to test new by-products for BSF rearing when compared to the small scale, as a consequence of their ability to allow optimal larval density and, in turn, growth performance and substrate bioconversion. Considering the very limited amount of information currently available in relation to both the wheat starch processing by-products and the rearing scales for BSF farming, further research is strongly recommended to assess the suitability of such by-products in association with other by-products and/or waste, also evaluating if the scale-related response may vary in varying the feed characteristics.

Ethics approval

None.

Data and model availability statement

The dataset analysed in this study is available on request to the corresponding author. None of the data were deposited in an official repository.

Declaration of Generative AI and AI-assisted technologies in the writing process

None.

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Declaration of interest

None.

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