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# Growth performance, bioconversion efficiency, and nutritional quality of yellow mealworm larvae reared on formulated diets based on local agro-industrial by-products

Z. Loiotine [a], S. Bellezza Oddon [a,*], A. Resconi [a], D. Murta [b,c,d], T. Ribeiro [c], I. Biasato [a], L. Gasco [a]

[a] *Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO) 10095, Italy*
[b] *Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Caparica 2829-511 Almada, Portugal*
[c] *Ingredient Odyssey S.A.–EntoGreen, Rua Cidade de Santarém 140, 2005-079 Santarém, Portugal*
[d] *Thunder Foos S.A. – Rua Comendador José Júlio Eloy, lote 7, 2005-332 Santarém, Portugal*

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ABSTRACT

Studies on yellow mealworm (**YM**, *Tenebrio molitor*) have mainly used feeding substrates with heterogeneous nutritional composition. In this study, three multi-ingredient agro-industrial diets (YM1, YM2, YM3), formulated to be nutritionally comparable in terms of CP (19.0–19.6% DM), ether extract (**EE** 3.0–3.2% DM), and gross energy (17.7–18.5 MJ/Kg DM), were tested to evaluate their effects on larval growth, bioconversion, chemical composition, and economic affordability. Diet YM1 included wheat middlings, wafer dough cooked, dry distillery stillage, coffee silvery film, breading waste and feed waste. Diet YM2 was based on feed waste and breading waste, with minor inclusions of wafer dough cooked and sweet preparation, whereas YM3 consisted of feed waste, breading waste and rice by-products (rice husk, chaff and bran). Wheat bran (**WB**; CP 20.5%, EE 4.2%, gross energy 18.6 MJ/kg, DM) served as environmental control. Diet costs (€/ton) calculated from by-product inclusion level and market price were 87.80 (YM1), 83.30 (YM2), and 95.50 (YM3), when compared to €250.00/tonne for WB. Four-week-old YM larvae (10 000/tray) were reared on 3 kg of diet per replicate and sampled weekly until growth differences between consecutive samplings were < 50% (week 9 of age, 35 days). Agar (25 g/L) was supplied three times weekly. At the end of the larval growth and biomass, development time, survival, growth rate (**GR**, mg/day), specific growth rate (**SGR**, %/day), feed conversion ratio, efficiency of conversion of ingested food (%), feed intake and water intake (%) were recorded. Statistical analyses were performed using IBM SPSS (v.20.0; $P < 0.05$). Rearing tray was the experimental unit with four replicates per treatment. A one-way ANOVA with Tukey's post-hoc test was applied for all the tested parameters. At 9 weeks of age, larvae fed YM2 and YM3 showed significantly higher final average weight (139 mg for both YM diets), GR (3.90–3.91 mg), and SGR (11.90% for both) than those fed YM1 (118 mg; 3.31 mg–11.40%, respectively; $P < 0.001$), with no differences between YM2 and YM3 ($P > 0.05$). Larval composition varied across treatments ($P < 0.05$), with higher DM and EE in YM1 (36.50–37.90%, respectively) than YM3 (35.40–35.0%), while YM2 showed no significant differences (36.0–37.3%). Ash content was higher in YM3 (4.02%) than in YM1 and YM2 (3.34–3.45%; $P < 0.05$). In conclusion, nutritionally comparable multi-ingredient agro-industrial diets supported YM growth and bioconversion, while reducing feed costs when compared to the WB diet.

## Implications

Yellow mealworm larvae are commonly reared on heterogeneous substrates, which may obscure diet-specific effects on growth and bioconversion. The use of agro-industrial by-products to formulate nutritionally balanced diets was evaluated to compare larval performance under controlled macronutrient conditions. Results indicate that, despite comparable macronutrient composition, growth performance and bioconversion are influenced by ingredient interactions and qualitative nutrient composition (e.g. amino acids and fatty acids). The use of agro-industrial by-products in cost-effective diets is supported, with potential application in the livestock sector as a sustainable alternative protein source although variability in by-product composition may affect biological responses and economic efficiency.

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* Corresponding author.
*E-mail address:* sara.bellezzaoddon@unito.it (S. Bellezza Oddon).

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## Introduction

The transition of yellow mealworm (**YM**, *Tenebrio molitor* L.; Coleoptera: Tenebrionidae) from a parasitic pest of stored food products to a major global actor - following their approval and commercialisation as an alternative protein source for feed (EC, 2017; EC, 2021) and food (EFSA, 2021) - is a remarkable development that sets this species apart (Adamaki-Sotiraki et al., 2025). Nevertheless, the sector's competitiveness and the final cost of insect-derived raw materials are closely tied to farm-level management practices and, critically, to the cost of the rearing substrate, which represents a substantial share of overall production expenses (Leipertz et al., 2024).

In conventional livestock production systems, feed costs are commonly managed through targeted feed formulation strategies aimed at meeting species-specific nutritional requirements while minimising unnecessary nutrient oversupply (Akintan et al., 2024), as accurate knowledge of these requirements allows diet optimisation, reducing production costs, limiting losses of high-value nutrients such as proteins and amino acids, and ultimately decreasing the environmental footprint of animal production systems (Akintan et al., 2024). In contrast, for YM larvae, information on nutritional requirements remains limited, constraining the development of requirement-driven feeding strategies and slowing the transition towards economically efficient large-scale production systems.

Wheat bran (**WB**) is one of the most commonly used feeding substrates for the mass production of YM (Stull et al., 2019), and as the primary by-product of wheat milling, it accounts for up to 15% of the total grain weight (Yan et al., 2022; Islam and Khan, 2021). Its low moisture content (12%, DM), moderate protein levels (13–18%, DM), low fat content (3.5%, DM), and high carbohydrate concentration (56%, DM) make it particularly suitable for YM rearing (Adamaki-Sotiraki et al., 2025). Beyond insect farming, WB also represents an important feed ingredient in conventional livestock nutrition, being included in the diets of poultry (Salahi et al., 2025), pigs (Rosenfelder et al., 2013), sheep (Dhakad et al., 2002), and dairy cattle (Tahir et al., 2002; Islam and Khan, 2021), thus underscoring its dual relevance, for both insect farming and conventional animal production, underscores the strategic value of WB as a feedstuff.

In addition to its nutritional and functional properties, it is also essential to consider the wholesale market price of WB, which can fluctuate over time (https://www.to.camcom.it/borsa-merci-di-torino; https://dir.tridge.com/prices/wheat-bran) with prices ranging from a global average of €210.00/tonne in 2024 to a more recent local estimate of €250.00/tonne in 2025 (Italy, Turin). Such differences can be linked to seasonal variations (Fotschki et al., 2023; Aidos et al., 2002), thus reflecting the biological cycles that influence the growth and development of both animal and plant-derived organisms and their relative by-products, including WB.

From an economic perspective, a recent contribution on YM feeding substrates is provided by Langston et al. (2023), in which adult beetles were reared in South Africa on six alternative substrates - wheat, corn, oat and soybean meals, dog food and alfalfa pellets - compared to wheat bran, and larval output was assessed after 45 days, integrating both reproductive and larval growth performance. Wheat-based substrates resulted in high larval production, while alfalfa pellets and oat meal were associated with higher average larval weights, albeit with a lower number of larvae produced. The study identified corn and wheat meals with alfalfa pellets as economically competitive alternatives within the tested substrates, with respective diet costs of €630.00, €440.00, and €410.00 per tonne (Langston et al., 2023). Overall, beyond the relative ranking of substrates, these findings highlight that the feeding substrate costs are substantial and represent a key constraint in YM production systems, reinforcing the strategic importance of identifying alternative, locally available, and economically viable feeding substrates.

Despite the well-documented ability of YM larvae to grow on a wide range of single-component agro-industrial by-products (Syahrulawal et al., 2023; Rumbos et al., 2021; Rumbos et al., 2020), only a limited number of studies have assessed its bioconversion performance on mixtures of by-products (Ruschioni et al., 2020; Mancini et al., 2019). The research of Ruschioni et al. (2020) evaluated five feeding substrates, including two treatments based on single by-products (wheat meal and wheat middling) and three treatments consisting of mixtures based on wheat middlings and olive pomace, with the latter included at 25, 50, and 75%, respectively. The authors reported that YM larvae efficiently converted the mixture containing 25% olive pomace, which resulted in the highest larval weight, survival rate, shortest development time, and optimal larval chemical composition (Ruschioni et al., 2020). Similarly, Mancini et al. (2019) tested five treatments composed of three single by-products (brewer's spent grain, bread, and biscuits) and two mixtures (50% spent grain–50% biscuits; 50% bread–50% biscuits). Their results highlighted the remarkable plasticity of YM, which was able to grow successfully on all the substrates tested. At the end of the growth trial, average larval weights were high and comparable when spent grain was used alone or in combination with biscuits (approximately 160 mg). Notably, mixtures containing biscuits (spent grain–biscuits; bread–biscuits) yielded larvae with the highest DM content (37.53%) and the greatest fat concentration (11.77% on a fresh matter basis). These findings suggest that the use of multi-component by-product mixtures as feeding substrates for YM larvae not only supports the production of viable alternative protein sources but also contributes to the valorisation of agro-industrial by-products that would otherwise exacerbate the already significant problem of waste (Ruschioni et al., 2020; Mancini et al., 2019).

Based on the above background, the present study aims to investigate agro-industrial by-products that are easily available, local, not strongly affected by seasonality, and employed as a mixture in nutritionally comparable (i.e., isonitrogenous, isolipidic, and isoenergetic) feeding substrates. The objective is to assess YM larval growth performance, bioconversion efficiency and chemical composition, also providing preliminary insights into the cost description of the agro-industrial by-products employed, and its relative formulated feeding substrates tested.

## Material and method

### *Collection, description and processing of local agro-industrial by-products*

The trial was conducted at the Department of Agricultural, Forest and Food Sciences (DISAFA), within the Experimental Facility Tetto Frati located in the municipality of Carmagnola, approximately 30 km south of the city of Turin, Italy (44°50′52.80″N, 7°43′8.76″E). The selection of agro-industrial by-products was based on a survey of companies listed in the publicly available online registry ("Kompass database" – https://it.kompass.com/a/prodotti-alimentari/03/) from which the by-products tested were chosen according to the insect's feeding preferences (DM > 80%), the continuity of production (absence of seasonality and consistent availability), and the proximity to the experimental site (within 100 km from the experimental centre). The collected agro-industrial by-products were incorporated as multi-ingredient components in the feeding substrate treatments tested in the experimental trial, with each by-product characterised by its chemical composition, sourcing distance, and, when available, cost information directly provided by the suppliers (Table 1).

The “sweet preparation” refers to a sweet leavened dough base typically produced in Italy, especially during Christmas and Easter holidays, becoming a by-product available year-round at production facilities and originating from unsold batches close to expiration or affected by defects in shape, leavening, and/or baking. The “wafer dough cooked” is a by-product of traditional wafer production, generated during the baking process as leftover cooked dough, typically trimmed from wafer sheets during shaping. In the literature, the “breading waste” has already been investigated as a by-product (Samray et al., 2019), deriving from bread production residues later reused in food breading processes, where it can become enriched with additional nutrients. Similarly, “coffee silvery film” is another widely produced yet underutilised by-product (Garcia and Kim, 2021), obtained during coffee roasting and characterised by a high volume-to-weight ratio, representing approximately 4% of the total coffee bean weight (Garcia and Kim, 2021). Differently, the “feed waste” refers to leftover portions from compound feed production, typically representing the initial and final batches in feed lines for livestock species (pigs, poultry, rabbits); while, another tested and described by-product is the “dry distillery stillage,” obtained from industrial processes of fermentation, distillation, and hydrolysis of wheat starch for the production of neutral grain alcohols and glucose syrup (Biasato et al., 2024). Finally, the present work also included the main by-products collected from the wheat and rice industries, namely “wheat middlings” from the wheat processing (Prakash et al., 2024) and “rice chaff,” “rice husk,” and “rice bran” from the rice industry (Fiore et al., 2020).

Based on the diversity of agro-industrial by-products in terms of form, use, and size, the processing operations aimed to make each by-product suitable for larval ingestion by minimising moisture content (when needed) and finely reducing particle size, in order to best meet larval ingestion capability (Naser El Deen et al., 2022). Accordingly, “sweet preparation” residues were processed through a meat grinder (4.5 mm diameter – LABOR 32, Rheninghaus Factory; San Mauro Torinese, Italy), dried at 35 °C for 48 h (TAURO ESSICCATORI, B. MASTER-2023), and finally ground into a fine powder (< 2 mm) using a mill (Fimar, CUCL823050M; Italy). Similarly, “wafer dough cooked” and “breading waste” were first dried (35 °C for 48 h; TAURO ESSICCATORI, B. MASTER-2023) and then ground (Ceccato, Lucme 90; Italy) with a 2 mm mesh. The “coffee silvery film” and “rice husk” were processed using a jaw crusher (Retsch GmbH 5657-HAAN; Germany; 3 mm mesh), while the “feed waste” was ground (Ceccato, Lucme 90; Italy; 2 mm mesh) and then homogenised using a concrete mixer (TECH, 350 L). By-products that did not require any processing (wheat middlings, dry distillery stillage, rice chuff, and bran) were stored at room temperature (20 °C; 50% relative humidity, RH) and after processing, one sample of each by-product was collected for chemical analysis as described in the “Chemical Analyses” section.

### Diet formulation

The ten selected, processed, and analysed agro-industrial by-products (Table 1) were used to formulate three isonitrogenous, isolipidic, and isoenergetic diets (YM1, YM2, YM3), based on their chemical compositions (Table 2). Wheat bran (WB), employed as the environmental control following the use of the Gainesville diet for *Hermetia illucens* rearing (Bellezza Oddon et al., 2022a; 2022b) and routinely adopted both at the Experimental Facility and in the large-scale YM rearing (Deruytter et al., 2024), served as the chemical reference for the formulation of the experimental diets. The chemical composition values were obtained following the analytical methods described in the “Chemical analysis” section. Diets were considered nutritionally comparable when their main macronutrients (CP, ether extract, gross energy) varied by ≤ 5%. As WB exceeded this threshold, it was excluded from direct statistical comparison. Finally, the cost of each experimental diet (Table 2) was estimated by weighting the inclusion percentage of each by-product against its corresponding market price, as provided by the supplying industries (Table 1).

### Colony management

The trial was conducted using 4-week-old YM larvae of Belgian genetic origin (Research Facility, Inagro vzw – West Flanders, Belgium), maintained and mass-reared at the Experimental Facility under standardised conditions, with a weekly production of approximately 700 000 larvae. The adults used to produce the 4-week-old YM larvae for the experimental trial were allowed to reproduce in a climate-controlled chamber (28 ± 5 °C, 60 ± 5% RH, dark condition) for 4 days, using nine plastic boxes stacked vertically (60 × 40 × 14.5 cm - Beekenkamp Verpakkingen BV, Maasdijk, The Netherlands), each equipped with a mesh separator (3 mm of diameter). A total of 1 100 g of WB diet was added to each box, divided into 750 g placed below the mesh as a nutritional source for the emerging larvae, and 350 g placed above the mesh to feed 250 g of adults, while agar (20 g/L - Agar Agar INSECTAGAR TYPE ZN 5; B&V srl, Gattatico, Italy) was provided *ad libitum* as a water source in the form of 1 cm$^3$ solid cubes throughout the entire reproduction period. At the end of the reproduction phase, the mesh separators and adults were removed, and the nine boxes were left undisturbed in the environmental conditions previously described.

**Table 1**
Chemical composition of the processed and employed agro-industrial by-products tested as feeding substrates for *Tenebrio molitor* larvae, gross energy (MJ/Kg of DM), distance and cost.

| By-product | Chemical composition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| DM, % | DM | CP | EE | NDF | Ash | Gross energy | Distance (km) | Cost (€/ton) |
| Wheat middlings | 86.75 | 19.48 | 5.47 | 44.23 | 5.07 | 19.60 | 92 | 330.00 |
| Wafer dough cooked | 93.40 | 12.78 | 0.46 | 8.27 | 1.34 | 17.47 | 41 | 130.00 |
| Dry distillery stillage | 91.36 | 38.27 | 7.82 | 49.75 | 3.31 | 22.37 | 21 | 400.00 |
| Coffee silvery film | 91.24 | 16.67 | 5.04 | 58.59 | 7.36 | 19.70 | 59 | 0 |
| Rice husk | 90.50 | 2.36 | 0.55 | 78.35 | 18.13 | 16.58 | 74 | 110.00 |
| Rice chaff | 87.65 | 14.87 | 9.59 | 20.47 | 7.00 | 18.82 | 74 | 250.00 |
| Rice bran | 87.51 | 16.70 | 11.45 | 9.62 | 8.43 | 18.69 | 74 | 305.00 |
| Breading waste | 83.62 | 13.98 | 2.65 | 2.85 | 4.39 | 16.08 | 85 | 0 |
| Feed waste | 86.61 | 19.62 | 3.37 | 20.52 | 10.06 | 17.16 | 28 | 100.00 |
| Sweet preparation | 91.83 | 10.42 | 18.56 | 2.31 | 1.66 | 21.08 | 26 | 110.00 |

Abbreviation: EE, ether extract.

**Table 2**
Formulation, cost, and chemical composition of the experimental treatments for *Tenebrio molitor* larvae (n = 4).

| By-product | Treatments | | | |
|---|---|---|---|---|
| g/100 g | YM1 | YM2 | YM3 | WB |
| Wheat middlings | 5.0 | | | |
| Wafer dough cooked | 5.0 | 2.0 | | |
| Dry distillery stillage | 6.0 | | | |
| Coffee silvery film | 8.0 | | | |
| Rice husk | | | 3.0 | |
| Rice chaff | | | 2.0 | |
| Rice bran | | | 2.0 | |
| Breading waste | 37.0 | 18.0 | 12.0 | |
| Feed waste | 40.0 | 78.0 | 81.0 | |
| Sweet preparation | | 2.0 | | |
| Wheat bran | | | | 100 |
| Cost (€/ton) | 87.80 | 83.30 | 95.50 | 250.00 |
| Chemical composition (DM, %) | | | | |
| DM | 89.92 | 90.16 | 90.10 | 90.00 |
| CP | 19.02 | 19.58 | 19.45 | 20.51 |
| EE | 3.24 | 3.12 | 3.00 | 4.21 |
| NDF | 17.82 | 17.37 | 17.35 | 41.46 |
| Ash | 5.00 | 5.43 | 5.30 | 5.20 |
| Non-structural carbohydrates | 54.92 | 54.50 | 54.90 | 28.62 |
| Gross energy (MJ/Kg) | 18.51 | 17.69 | 17.73 | 18.62 |

Abbreviations: treatments: YM1, YM2, YM3, YM, yellow mealworm; WB, wheat bran; EE, ether extract.

### *Experimental set-up and sampling procedure*

The trial was initiated with 4-week-old YM larvae obtained from a homogeneous rearing batch, which were manually sieved using a 0.5 mm mesh to separate them from the residual substrate, and estimate their number. Based on the initial weight of the larval biomass (Kern & Sohn GmbH, Balingen, d = 0.1), a minimum number of three samples were collected after homogenisation, with each sample weighed (Kern & Sohn GmbH, Balingen, d = 0.001), and all the larvae within the sample extracted, counted and weighed (Kern & Sohn GmbH, Balingen, d = 0.001), as in Deruytter et al. (2024). A total of 10 000 YM larvae with an average individual weight of 2.2 mg ± 0.0218 were inoculated into 3 kg of experimental diet (0.3 g of feed per larva), with four replicates per treatment.

Agar was selected as the standard water source, being a cream-colored, odourless powder, characterised by 80% DM and 6.5% ash, with a pH ranging from 6 to 8. Gelation was achieved by mixing 20 g of agar powder per litre of water in a pot over moderate heat until the first bubbles appeared. The resulting liquid mixture was poured onto a suitable flat surface and allowed to cool completely to achieve full gelation, with preparation times depending on the required quantity (approximately 25 min for 80 g/4 L). Agar was weighed and administered to each replicate three times per week as in Deruytter et al. (2024) throughout the entire duration of the trial and provided in the form of solid cubes (1 $cm^3$, approximately 5 g per cube; Kern & Sohn GmbH, Balingen; d = 0.01 g). The schedule adopted for agar administration was designed to progressively increase the water supply over the course of the trial. Consequently, during the first 2 weeks (when larvae were 4 and 5 weeks old), 150 g of agar per replicate per week was provided. Larvae at 6 weeks of age received 200 g of agar per replicate per week. The amount was further increased to 350 g for 7-week-old larvae, and subsequently to 500 g and 700 g per replicate per week for 8- and 9-week-old larvae, respectively. Prior to each administration, any remaining agar was removed, weighed (Kern & Sohn GmbH, Balingen; d = 0.01 g) and recorded, while the residual fraction recovered at each sampling was used to calculate larval "water intake" expressed as the percentage ratio between the total amount of agar supplied and the fraction not consumed.

To monitor larval growth dynamics within each experimental treatment, a sample of larvae (at least 100 larvae per replicate) and substrate was collected weekly, beginning from the first day of the trial, and all sampling procedures were performed as suggested by Deruytter et al. (2024). For each dietary treatment, the average larval weight was recorded and represented through a "Growth curve" (Fig. 1). Accordingly, larval growth (and thus the experimental trial for a given treatment) was considered complete when the difference in average larval weight between two consecutive samplings was ≤ 50%. At the end of the trial, YM developmental time (days from start to end of the experiment) was recorded, the larvae were separated from the frass using a circular vibrating sieve with 2 mm mesh diameter (ERIMAKI s.n.c., Milan, Italy), and total larval biomass and frass weights were collected (Kern & Sohn GmbH, Balingen; d = 0.1 g) and registered to calculate the Feed Conversion Ratio (**FCR**), Efficiency of Conversion of Ingested food (**ECI**) and Feed Intake. For each replicate, three subsamples (each with at least 100 larvae) were collected from the final biomass

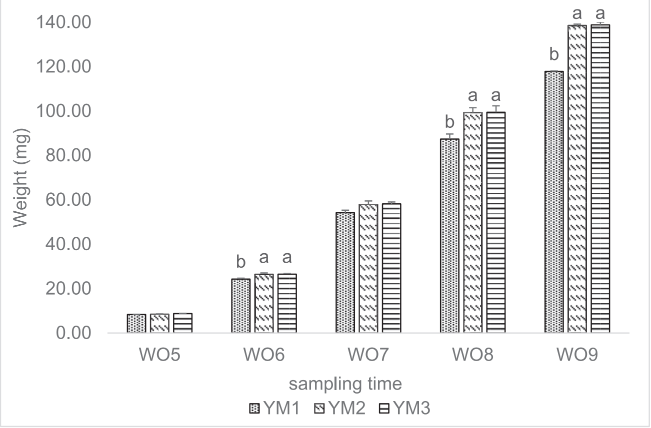

**Fig. 1.** Larval growth of *Tenebrio molitor* during the sampling times. Abbreviations: treatments: YM1, YM2, YM3; YM, yellow mealworm; sampling times, WO5, WO6, WO7, WO8, WO9; WO, week-old larvae; Means with different superscript letters (a, b) differ significantly ($P < 0.05$).

and sampled as in Deruytter et al. (2024) in order to estimate YM survival, as well as final average larval weight, which was used to compute the Growth Rate (**GR**) and Specific Growth Rate (**SGR**). All formulas used to determine performance indices at the end of the trial are reported below (Resconi et al., 2025).

- $\textit{Development time} = \textit{time spent by the larvae from the start of the trial to reach the end of growth}$
- $\textit{Survival}\ (\%) = (\textit{number of larvae at the end of the trial} / \textit{number of larvae at the beginning of the trial}) \times 100$
- $\textit{Feed Conversion Ratio (FCR)} = \textit{Diet} / (\textit{larval biomass at the end} - \textit{larval biomass at the start})$
- $\textit{Efficiency of Conversion of Ingested food (ECI)} = [(\textit{larval biomass at the end} - \textit{larval biomass at the start})/\textit{Diet}] * 100$
- $\textit{Growth rate (GR)} = (\textit{final larval average weight}\ [g] - \textit{initial larval average weight}\ [g]) / \textit{days of feeding}.$
- $\textit{Specific growth rate (SGR)} = ((\ln(\textit{final larval average weight}\ [g]) - \ln(\textit{initial larval average weight}\ [g]) / \textit{days of feeding}) \times 100.$

## Chemical analyses

All the agro-industrial by-products, WB, experimental diets, and YM larvae were analysed for proximate composition. Agro-industrial by-products were processed as described above and analysed in the same form in which they were used for diet formulations, while the experimental diets, prepared by mixing the processed by-products in defined proportions, were analysed to determine their overall nutritional profile. One sample of larvae per replicate was collected at the end of the trial, sieved, euthanised at −80 °C, and stored at −20 °C until analysis. Prior to chemical analyses, larvae were ground using a laboratory mill (GM 200; Retsch, Haan/Duesseldorf, Germany) and freeze-dried (LIO25FP freeze dryer – Cinquepascal S.r.L., Milano). For all the samples (by-products, diets, and larvae), the following parameters were determined: DM (AOAC #934.01), CP (AOAC #984.13; N × 6.25 for by-products and diets, N × 4.76 for larvae - Janssen et al., 2017), ether extract (**EE**; AOAC #2003.05), ash content (AOAC #942.05), and gross energy (using an adiabatic bomb calorimeter [C7000; IKA, Staufen, Germany]). The fibre content (NDF) of by-products and diets was analysed following Mertens et al. (2002) to determine amylase-treated neutral detergent fibre expressed on an organic matter basis, whereas for larvae, chitin content was assessed using a gravimetric method involving alkaline deproteinisation and acid demineralisation, according to Woods et al. (2020). In addition, the non-structural carbohydrates (% DM) were calculated as follows: 100 – (CP + EE + Ash + NDF).

## Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics (version 20.0.0; IBM, Armonk, NY, USA). The rearing box was considered the experimental unit for all the parameters. Since the WB diet served solely as an environmental control, it was excluded from the statistical comparisons. The normality of residuals was assessed using the Shapiro–Wilk test. Final larval weight, week-old (**WO**) weights, biomass weight, survival, GR, SGR, bioconversion indices and the water intake by the larvae, as well as their proximate composition, were analysed by one-way ANOVA after verifying the homogeneity of variances with Levene's test, followed by Tukey's post-hoc test for pairwise comparisons, and results are reported as mean. Statistical significance was set at < 0.05.

# Results

## Larval growth and bioconversion

Larval growth during the sampling times is shown in Fig. 1. At the first sampling (WO5), no significant differences were observed among the experimental treatments (YM1, 8.35; YM2, 8.45; YM3, 8.73 mg ± . 0.1205) ($P = 0.129$). At the second sampling (WO6), significant differences were detected ($P = 0.011$) with higher larval weights observed in YM2 and YM3 (26.50 mg for both) compared with YM1 (24.30 mg ± 0.4341). No significant differences among diets were found at WO7 (YM1, 54.20; YM2, 57.90; YM3, 58.20 mg ± 1.1862; $P = 0.08$). Differently, larvae fed the YM2 and YM3 diets showed significantly higher weights at both WO8 (99.40 mg for both YM diets; $P = 0.01$) and WO9 (139.0 mg for both YM2 and YM3; $P < 0.001$) compared with larvae fed YM1 (WO8: 87.3 mg ± 2.4455; WO9: 118.0 mg ± 0.7077).

The main growth performance parameters measured from the start of the experimental trial throughout the 35-day rearing period are presented in Table 3. Survival did not vary significantly among treatments ($P > 0.05$), ranging from 75.7% in YM2 to 80.8% in YM1. Most parameters, such as larval biomass, FCR, ECI, FI and water intake, showed no statistically significant differences ($P > 0.05$), with the exception of final larval weight, GR and SGR. For all the significant parameters, YM1 differed significantly (GR, 3.31 mg/day; SGR, 11.40% – $P < 0.05$) from YM2 and YM3 (GR, 3.90–3.91 mg/day) (SGR, 11.90%, for both YM2 and YM3 diets).

## Larval nutritional profile

Table 4 reports the nutritional composition of YM larvae analysed at the end of the rearing period. Larvae from the YM1 treat-

**Table 3**
Main growth and bioconversion indices of *Tenebrio molitor* larvae reared on the experimental treatments (fresh matter basis) (n = 4).

| | Treatments | | | | |
|---|---|---|---|---|---|
| Items | YM1 | YM2 | YM3 | SEM | *P-value* |
| Final larval weight (mg) | $118.0^{b}$ | $139.0^{a}$ | $139.0^{a}$ | 0.821 | <0.001 |
| Larval biomass (g) | 971.0 | 1074.0 | 1107.0 | 33.617 | 0.052 |
| Survival (%) | 80.8 | 75.7 | 78.0 | 3.076 | 0.538 |
| GR (mg/day) | $3.31^{b}$ | $3.90^{a}$ | $3.91^{a}$ | 0.022 | 0.001 |
| SGR (%/day) | $11.40^{b}$ | $11.90^{a}$ | $11.90^{a}$ | 0.016 | 0.001 |
| FCR | 3.18 | 2.86 | 2.77 | 0.105 | 0.070 |
| ECI (%) | 31.7 | 35.1 | 36.2 | 1.120 | 0.052 |
| Feed Intake (g) | 998.0 | 1135.0 | 1131.0 | 54.930 | 0.207 |
| Water intake (%) | 91.9 | 92.2 | 92.1 | 0.084 | 0.097 |

Abbreviations: treatments: YM1, YM2, YM3; YM, yellow mealworm; GR, growth rate; SGR, specific growth rate; FCR, feed conversion ratio; ECI, efficiency of conversion of ingested food. Values within a row with different superscripts differ significantly ($P < 0.05$).

**Table 4**
Final chemical composition (on DM basis, %) and gross energy (MJ/kg) of *Tenebrio molitor* larvae (n = 4).

| | Chemical composition | | | | |
|---|---|---|---|---|---|
| DM, % | YM1 | YM2 | YM3 | SEM | *P-value* |
| DM | $36.5^{a}$ | $36.0^{ab}$ | $35.4^{b}$ | 0.209 | 0.036 |
| CP | 39.2 | 40.4 | 40.4 | 0.430 | 0.179 |
| EE | $37.9^{a}$ | $37.3^{ab}$ | $35.0^{b}$ | 0.626 | 0.023 |
| Ash | $3.34^{b}$ | $3.45^{b}$ | $4.02^{a}$ | 0.129 | 0.010 |
| Chitin | 5.10 | 5.39 | 5.34 | 0.136 | 0.363 |
| Gross energy | 26.8 | 26.7 | 26.4 | 0.167 | 0.259 |

Abbreviations: treatments: YM1, YM2, YM3; YM, yellow mealworm; EE, ether extract. Values within a row with different superscripts differ significantly ($P < 0.05$).

ment exhibited higher DM content when compared to those from YM3 ($P < 0.05$), while YM2 larvae did not differ significantly from the other treatments ($P > 0.05$). Similarly, EE was lower in YM3 larvae when compared to YM1, whereas YM2 did not show significant differences from the other groups ($P > 0.05$). Conversely, ash content was significantly higher in YM3 larvae than in YM1 and YM2 ($P < 0.05$). Finally, no statistically significant differences were observed among the tested treatments for CP, chitin, and GE ($P > 0.05$).

## Discussion

Larval development results from a complex interaction of methodological, biological, physical and nutritional factors (Deruytter et al., 2025), among which the composition of the feeding substrate plays a decisive role in determining growth performance and nutrient conversion efficiency. In the available literature, several studies have investigated the use of various agro-industrial by-products as feeding substrates for YM larvae (Langston et al., 2023; Rumbos et al., 2020, 2021; Ruschioni et al., 2020; Mancini et al., 2019; Oonincx et al., 2015). Although these research works have significantly advanced the understanding of YM growth performance and bioconversion potential, the tested substrates often exhibited heterogeneous chemical compositions, as a lack of nutritional comparability, which may delay or even mask the interpretation of specific effects, with the consequent risk of over or underestimating the nutritional suitability of a given raw material or diet for YM growth simply due to its different initial chemical composition. More recently, a first attempt to formulate isonitrogenous feeding substrates for YM has been provided by Rumbos et al. (2022) and Adamaki-Sotiraki et al. (2024), who highlighted the importance of developing nutritionally standardised diets to ensure more reliable comparisons across treatments. Following this approach, the present study is among the first to formulate nutritionally comparable (isonitrogenous, isolipidic, and isoenergetic) diets for YM, allowing a more accurate interpretation of the observed differences in growth development, bioconversion efficiency and chemical composition of YM larvae.

Methodological factors and the physical characteristics of the substrate are known to influence insect growth and substrate bioconversion efficiency (Naser El Deen et al., 2022; Deruytter et al., 2025) and considering that all the treatments reached the experimental end-point (≤ 50% growth) at the same time (35 days) under identical rearing conditions, and that the feeding substrates presented a particle size suitable for larval development (Naser El Deen et al., 2022), the differences in average final larval weight among treatments (YM1, 118.0 mg; YM2, 139.0 mg; YM3, 139.0 mg) could be most likely explained by the qualitative composition of the feeding substrates. Feed waste (CP, 13.98% – EE, 3.37% DM) accounted for 40, 78, and 81% of the diet in the YM1, YM2, and YM3 formulations, respectively, and based on larval growth performance and in the absence of significant differences in survival rate ($P > 0.05$), feed waste derived from compound feed production for poultry, pigs, and rabbits appears to have played a key role in supporting larval growth, particularly in YM2 and YM3. Although this matrix is characterised by high physicochemical variability (Vrontaki et al., 2024; Riudavets et al., 2020), feed waste represents a nutritionally rich substrate and can reasonably be considered a major factor contributing to the improved larval growth observed among the treatments. Being composed of residues from feeds formulated for monogastric animals, it is plausibly enriched with essential amino acids specifically included to support growth, biological functions, and animal health (Spranghers et al., 2024). Therefore, although the amino acid composition of feed waste was not analysed in the present study, it is reasonable to assume that diets with high feed waste inclusion (YM2 and YM3) contained relevant levels of methionine, lysine, threonine, phenylalanine, and tryptophan, as some of the main essential amino acids commonly supplemented in livestock feed formulations (Spranghers et al., 2024), indicating that formulating isonutritional diets represents only the tip of the iceberg and highlights the need to further investigate the qualitative composition of the tested diets in order to balance micronutrients, optimise the actual value of by-products, and reduce waste.

A more detailed examination of the by-products used in the dietary formulation also helps to interpret the general lower performance reached in larvae fed the YM1 diet, which consisted of wheat middlings, wafer dough cooked, dry distillery stillage, coffee silvery film, breading waste, and feed waste. Excluding feed waste, already discussed above, and wheat middlings as widely recognised appreciated substrate for YM rearing (Adamaki-Sotiraki et al., 2025), particular attention should be paid to the presence of wafer dough cooked and breading waste, assigned to the broader category of bakery and cereal products (cookies, wafers, and bread residues), characterised by a high content of readily digestible starch. Previous studies evaluating the use of cookies a/o bread residues as feeding substrates for YM include those by Fondevila et al. (2024), Mancini et al. (2019), and van Broekhoven et al. (2015). Specifically, Fondevila et al. (2024) tested the inclusion of bread by-product (CP, 14.2% – EE, 4.0%, DM) at high levels, ranging from approximately 60–74% of the diet (fresh matter basis), in which context, the inclusion of protein and fibre-rich ingredients allowed larval growth to be maintained at levels comparable to a WB-based control diet. In contrast, Mancini et al. (2019) reported marked differences when bread and cookie-based by-products (CP, 11.49%–6.55%; EE, 0.32%–10.45% [DM], respectively) were used, with cookie-based substrates (alone or in mixtures) tending to delay growth and increase lipid deposition, whereas bread alone or in more balanced mixtures better supported larval development. Further evidence is provided by van Broekhoven et al. (2015), in which bread residues were typically included at low levels (10%) in most diets and only reached 50% in one formulation, while cookie residues were tested at very high inclusion levels (40–85%) as the main energy source (chemical composition not reported), resulting in reduced performance and increased mortality.

In the present study, wafer dough cooked (CP, 12.78% – EE, 0.46% DM) was included in YM1 at a relatively low level (5%), whereas breading waste (CP, 13.98% – EE, 2.65% DM) represented a much larger proportion of the diet (37%) with both ingredients characterised by a high starch content, generally considered a highly digestible carbohydrate source. However, the high inclusion of bakery by-products (42% of the diet) combined with the lower inclusion of feed waste (40%), which was approximately half of the quantity included in the other treatments, may have influenced substrate utilisation efficiency, potentially contributing to the lower growth performance observed in the YM1 larvae. In addition to bakery by-products, the YM1 diet also included coffee silvery film (8%) and dry distillery stillage (6%), two by-products characterised by a relevant chemical composition in the present study (CP, 16.67–38.27% DM; EE, 5.04–7.82% DM, respectively). Although coffee silvery film has not been extensively investigated as a feeding substrate for YM, evidence exists for related coffee-processing by-products, particularly spent coffee grounds (Kotsou et al., 2023), included in a WB-based diet at increasing levels (10, 25, 50, 75, and 100%) and showing that a 25% inclusion resulted in a favourable larval nutritional composition, while also highlighting the presence of potential antinutritional factors (Kotsou et al., 2023). In the present work, the inclusion of coffee silvery film at 8% was lower than the levels tested in the literature; nevertheless, compared to the spent coffee ground (Kotsou et al., 2023), the heterogeneous physical structure of the coffee silvery film by-product adopted in this experimental trial may have promoted selective ingestion by the larvae (Kröncke and Benning, 2022; Fondevila et al., 2024).

Finally, the YM1 diet included dry distillery stillage, a by-product derived from fermentation and distillation processes and compositionally comparable to distillers' grains (Biasato et al., 2024), while a recent study (Zhang et al., 2019) showed that, although distillers' grains can sustain YM development, they result in lower final weights and reduced feed conversion efficiency compared with WB control diets. Additional indirect evidence from *H. illucens* (Biasato et al., 2024) indicates that substrates derived from industrial processes can generate variable performance depending on formulation and rearing conditions, reinforcing the concept that fermentative or industrially processed ingredients are not nutritionally "neutral".

Overall, the lower performance observed in YM1 cannot be attributed to a single ingredient but rather to the combined effect of multiple by-products, including bakery products, coffee silvery film, and distillery-derived ingredients, together with the lower inclusion of feed waste, confirming that ingredient quality and their interactions play a decisive role beyond mere macronutrient comparability.

Although the feeding substrates were formulated to be nutritionally comparable, the biological response of the larvae in terms of proximate composition may still vary. Water, CP and EE are among the main components responsible for final larval weight, although their relative contribution may differ depending on diet composition and the physiological state of the larvae (Kröncke and Benning, 2022). In the present study, larvae showing the lowest average final weight (YM1, 118.0 mg) displayed higher DM and EE (36.5–37.9% DM, respectively) when compared to YM2 and YM3 (DM, 36.0–35.4%; EE, 37.3–35.0%, respectively), differences likely linked to the nutritional characteristics of the YM1 diet, which may have provided relatively more readily available carbohydrate in relation to protein content, potentially promoting lipid synthesis and storage as energy reserves (Vukadinović et al., 2026; van Broekhoven et al., 2015). Consequently, the higher EE content observed in YM1 larvae may also explain the higher DM values, due to the hydrophobic nature of lipids (Southall et al., 2002), while, the significantly higher ash concentration detected in larvae from treatment YM3 (4.03%) can be attributed to the inclusion of 7% rice-based by-products (rice husk, chaff, and bran), well recognised for their mineral richness (Esa et al., 2013).

As observed for larval weight and final chemical composition, the main bioconversion parameters analysed also appear to be influenced by the chemical composition of the dietary treatments and the quality of the by-products tested. In line with the higher average final larval weight observed for YM2 and YM3 (both 139 mg), total larval biomass followed the same pattern (1074.0–1107.0 g, respectively) compared with YM1 (118 mg per larva; larval biomass, 971.0 g; $P = 0.052$). These growth differences are further supported by the two growth-related parameters that showed statistically significant differences ($P < 0.001$), namely GR (mg/day) and SGR (%/day), with larvae reared on YM2 and YM3 exhibiting higher GR values (3.90 – 3.91, mg/day) and SGR values (11.90%/day for both diets) compared with those reared on YM1 (3.31 mg/day; 11.40%/day, respectively).

Considering that all the larvae started with the same initial average weight (2.2 ± 0.0218 mg) and completed their development within the same experimental period, these differences likely reflect the higher final larval weights recorded for YM2 and YM3, possibly linked to the higher inclusion of feed waste in these diets as a matrix potentially rich in essential amino acids that may have enhanced larval growth and substrate utilisation (Spranghers et al., 2024). Additional indices describing growth efficiency (Waldbauer, 1968), such as FCR and ECI, did not differ statistically among treatments ($P > 0.05$), although numerically more favourable values were observed for YM3 and YM2 compared to YM1, in agreement with the overall growth patterns observed among the YM treatments. Nevertheless, the economic perspective remains a key factor in determining the overall sustainability of YM farming.

In addition to biological and nutritional aspects, the economic dimension plays a crucial role in determining the efficiency and feasibility of production systems (Gasco et al., 2020). Even common raw materials such as WB may exhibit significant variability across regions, not only in their physicochemical composition (Deruytter et al., 2024) but also in market price (Vrontaki et al., 2024; Langston et al., 2023), with reported values ranging from €900/tonne in South Africa (Langston et al., 2023) to €150/tonne in Greece (Vrontaki et al., 2024) and €250/tonne in Italy (Piedmont region - https://www.to.camcom.it/borsa-merci-di-torino). Different approaches have been proposed to assess the economic efficiency of feeding substrates (Vrontaki et al., 2024; Langston et al., 2023), including the Economic Conversion Ratio, widely used in aquaculture (Vergara-Rubín et al., 2025) and applied by Vrontaki et al. (2024) to evaluate the cost efficiency of feed in producing a fixed amount of larval biomass, defined as the feed cost per kilogram of live larvae, calculated as the product of FCR and the feed price (€/ton). Similarly, Langston et al. (2023) estimated the cost of larval biomass as the ratio between the cost of 1 kg of substrate and the corrected larval yield obtained per kilogram of feed whereas, in the present work, the costs of the tested by-products were directly provided by the agro industries involved (see Table 1), and the overall diet costs were calculated according to the inclusion rate of each ingredient. Given the methodological and market differences among studies, direct comparisons remain difficult, and a standardised economic framework for YM rearing has yet to be established. In this case, the estimated costs of the formulated diets (YM1, €87.8/ton; YM2, €83.3/ton; YM3, €95.5/ton) appear to be economically affordable and competitive with values reported in other regions, regardless of larval growth performance. Nonetheless, wide margins for improvement persist, particularly regarding yield optimisation, ingredient procurement, and processing costs (e.g., drying, grinding, or transport), indicating that future studies should address not only the biological efficiency of diets but also their overall cost–benefit balance, integrating economic, logistical, and environmental factors to provide a realistic assessment of the economic sustainability of YM farming.

## Conclusion

The results demonstrate that the differences observed in YM growth performance cannot be attributed to methodological factors but are instead closely linked to the characteristics of the feeding substrates. Although the treatments were formulated to be nutritionally comparable (isonitrogenous, isolipidic, and isoenergetic), such formulation cannot overlook the interactions among individual by-products when combined as feeding substrates for larval growth and bioconversion. These interactions may represent the specific variables influencing larval growth development and their relative chemical composition, while the development of isonutritional diets represents one essential step within a broader effort to standardise insect rearing practices. The differences observed among treatments indicate that comparable macronutrient ratios alone are not sufficient to predict biological responses. Instead, larval performance appears to be influenced by the qualitative composition of the ingredients, as the presence of bioavailable compounds (amino acids, fatty acids, minerals) and potentially functional additives (e.g., probiotics, prebiotics, or other bioactive substances) naturally included in the feed waste fraction. Under isonutrient conditions, the higher inclusion of feed waste (78–81% in YM2 and YM3 vs. 40% in YM1) resulted in superior growth performance and bioconversion outcomes, supporting the potential of such by-products as viable rearing substrates, with the inclusion of different agro-food by-products emerging as a promising strategy that not only provides insights into the actual nutritional requirements of YM (Rumbos et al., 2020) but also contributes to the circular economy through by-products and waste re-utilzation. However, it should be recognised that by-products are inherently variable, as their composition depends on processing methods, origin, and local conditions, which may ultimately influence larval responses.

Furthermore, the economic assessment confirmed the affordability of the formulated diets (€83.3–95.5/ton), consistent with or even below values reported in other geographical contexts (Vrontaki et al., 2024; Langston et al., 2023). For this reason, it becomes crucial to systematically integrate economic data often considered secondary but in reality fundamental, in order to define the sustainability in all dimensions and growth potential of the sector.

## Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2026.101853) can be found at the foot of the online page, in the Appendix section.

## Ethics approval

Not applicable.

## Data and model availability statement

None of the data were deposited in an official repository.

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used language editing tools (ChatGPT) in order to perform proofreading. After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

## Author ORCIDs

**Z. Loiotine:** https://orcid.org/0000-0003-3137-7057.
**S. Bellezza Oddon:** https://orcid.org/0000-0001-8942-0682.
**A. Resconi:** https://orcid.org/0000-0002-2107-2383.
**D. Murta:** https://orcid.org/0000-0002-5583-485X.
**T. Ribeiro:** https://orcid.org/0000-0002-7616-1930.
**I. Biasato:** https://orcid.org/0000-0002-8855-4248.
**L. Gasco:** https://orcid.org/0000-0002-1829-7936.

## CRediT authorship contribution statement

**Z. Loiotine:** Writing – review & editing, Writing – original draft, Visualisation, Methodology, Investigation, Formal analysis, Data curation. **S. Bellezza Oddon:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation. **A. Resconi:** Writing – review & editing, Methodology, Investigation. **D. Murta:** Writing – review & editing, Software, Conceptualisation. **T. Ribeiro:** Writing – review & editing, Software, Conceptualisation. **I. Biasato:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualisation. **L. Gasco:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualisation.

## Declaration of interest

None.

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