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ORIGINAL RESEARCH ARTICLE

Protein content in mealworms (*Tenebrio* sp.) fed with different food by-products

Gloria Sciuto[1] · Douan Bleu Gondo[2] · Francesca La Terra[3] · Giovanni Farina[3] · Iris Schadt[3] · Margherita Caccamo[3] · Cinzia L. Randazzo[1,4] · Cinzia Caggia[1,3,4] · Koua Kouakou Hervé[5]

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Abstract
The edible insect market has grown significantly due to their high nutritional value, digestibility, palatability, and functional properties. They are straightforward to cultivate and present a low environmental impact, keeping a high protein content regardless of diet. In this study, *Tenebrio guineensis* larvae were fed a mixture of various local food by-products (FBPs) at 33.3% and a combination of maize and bran flour for about two weeks. A control group was fed only a conventional flour mixture. Every two days, the weight, total length, and head width were monitored, and at the end of the trial, the mortality rate, moisture content, protein content, and protein productivity were assessed. Results showed that all the FBP groups showed better larval growth than the control, while the cabbage-based diet yielded the most favourable overall performance. Larvae fed cabbage (group 3) reached the highest final weight (0.122 g/larva), total length (2.15 cm), head width (2.15 mm), moisture content (54.39%), and estimated protein productivity (1.78 mg/larva/day), together with one of the lowest mortality rates (12.5%). Although the control group showed the highest final protein percentage (39.04%), all FBP-fed groups achieved higher estimated protein productivity than the control (0.83–1.78 vs 0.32 mg/larva/day). These results suggest that locally available food by-products may represent a promising approach to support larval growth performance under preliminary rearing conditions.

✉ Cinzia Caggia
cinzia.caggia@unict.it

[1] Department of Agriculture, Food and Environment, University of Catania, Via S. Sofia, 100, 95123 Catania, Italy

[2] Department of Animal Biology, University Peleforo Gon Coulibaly, Po Box 1328, Korhogo, Côte d'Ivoire

[3] Consorzio Per La Ricerca Nel Settore Della Filiera Lattiero-Casearia E Dell'agroalimentare (CoRFiLaC), 97100 Ragusa, Italy

[4] ProBioEtna Srl, Spin Off University of Catania, Via S. Sofia 100, 95123 Catania, Italy

[5] Department of Zoology, Animal Biology and Ecology, University Felix Houphouët-Boigny, 22 Po Box, Abidjan, Côte d'Ivoire

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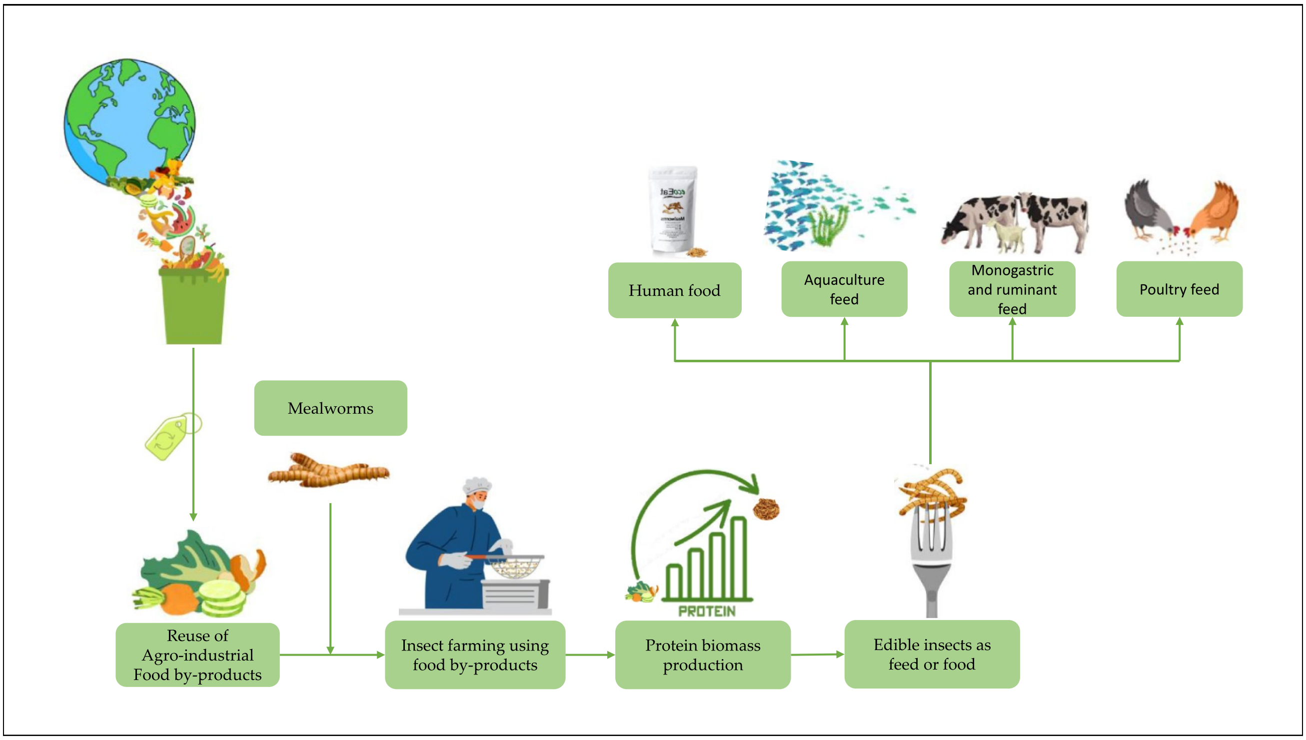
Graphical Abstract



Keywords Alternative protein · *T. guineensis* larvae · Food by-products · Circular economy

Introduction

Ensuring safe, nutritious food for the global population is a major challenge for current and future food systems (Abbasi 2025a, b). In this scenario, the search for alternative protein sources with lower environmental impact than conventional livestock is receiving increasing attention (Karaca et al. 2022; Gasc 2024). Among the various green alternatives, edible insects have already been proposed as a valuable protein source for both animal feed and human consumption, due to their nutritional composition and their potential role in more sustainable production systems (Huis et al. 2013; Hong et al. 2020; Mlček et al. 2021; Aiello et al. 2023; Abbasi 2025a, b). In different countries, the consumption of insects as food is an old practice: Asia, South America, and Africa are the countries where eating insects has long been a cultural tradition. However, in countries such as Canada, the United States, and the European Union (EU), the consumption of insects is less common and meets various regulatory and cultural challenges (Yi et al. 2013; Selaledi and Mabelebele 2021). Among edible insects, mealworms of the genus *Tenebrio* are of particular interest because of their good adaptability to rearing conditions and their ability to grow on different plant-based substrates. In addition, the use of food by-products (FBPs) as feed ingredients for insects has been proposed as a useful strategy to valorize agro-food waste and generate protein-rich biomass within a circular economy perspective (Poissant 2020; Dongo & Della Penna 2023; Kotsou et al. 2024). This aspect is particularly relevant considering the large amount of food waste and agro-food residues generated worldwide every year. Most of the studies have focused on *Tenebrio molitor*, while much less information is available on *Tenebrio guineensis*, especially regarding its growth performance under diets supplemented with locally available food by-products. This lack of information is even more relevant in local tropical contexts, where both insect species and feeding substrates are often selected based on their cultural availability, rather than on the species and substrates more commonly investigated in the literature (Lienhard et al. 2023). Therefore, the present study aimed to evaluate the effects of five locally available food by-products, used individually or in combination, on the growth performance and biomass composition of *T. guineensis* larvae. In particular, weight increase, total length, head width, mortality rate, moisture content, protein content, and estimated protein productivity were assessed under preliminary rearing conditions.

Materials and methods

Tenebrio guineensis larvae growth conditions

In the present study, the mealworms used were the larval stage of tenebrionid beetles collected from Korhogo, Northern Ivory Coast, Africa. The beetles were morphologically identified (Supplementary Figure S1) as *T. guineensis* (Ihoff, 1843; Coleoptera: Tenebrionidae) according to the taxonomic criteria of Delobel & Tran (1993), conducted at the Zoology laboratory of the University of Mons (Belgium). The *T. guineensis* larvae were part of the insect farm collection at Pelefofo Gon Coulibaly University in Korhogo, Ivory Coast, Africa. Images of the larval, pupal, and adult stages, along with detailed views of key anatomical features, are shown in Fig. 1.

The larvae were reared at room temperature (32 ± 2 °C) on a standard substrate (maize bran) until they reached the ideal size, approximately 1.3 ± 0.1 cm in length and 0.03 g in weight per larva. Early-stage larvae were selected to better assess growth changes during this phase. They were manually harvested using tweezers and placed into five different plastic boxes (26 × 15x7.5 cm), corresponding to different local FBPs. For each treatment, three independent rearing boxes were used, with 20 larvae placed in each box under identical experimental conditions. The boxes were fitted with lids with holes covered with metal mesh for ventilation. FBPs were manually chopped before being offered to the larvae; particle size was not standardized across treatments. The experimental design was inspired by previously reported mealworm feeding strategies, based on substrates supplemented with vegetable or fruit by-products (Oonincx and de Boer 2012; Liu et al. 2020). Five FBPs Groups were set up: zucchini (Group 1), carrots (Group 2), cabbage (Group 3), oranges (Group 4), and a mix of these (Group 5). The control (Group 6) was fed a flour mixture without any addition of FBPs. The nutritional composition of the dietary treatments is reported in Table 1. These nutritional values were derived from source-based food composition databases (USDA 2018) and should therefore be considered approximate reference data that may not exactly match the real composition of the experimental diets.

In each box, larvae were provided with a total of 160 g of feed composed of 40 g of FBP and 120 g of a maize and bran flour mixture (60 g of each). The FBPs were used as a replacement for 12 g of the corresponding fresh portion every 2 days to reduce mold growth. During the experimental period, humidity and room temperature were monitored to record environmental conditions in the box (Mengshen, MS-TH03).

Growth parameters of *Tenebrio guineensis* larvae

The larvae's growth was monitored every 2 days for 14 days, measuring weight, total length, head width, and mortality rate. Weight was measured using a digital precision food balance (KUBEI, 1 kg/0.01 g, UPC 741311086641). Total length and head width were measured using millimeter paper as a reference; larvae were placed in a petri dish to prevent escape and then manually measured.

Larval mortality rate

Larval mortality was determined by counting the number of dead larvae at each time point and expressing it to the initial number of larvae in each group (20 larvae *per* group), according to the following formula: mortality (%) = $(n_2/n_1) \times 100$, where n_2 is the final number of dead larvae and n_1 is the initial number of larvae.

Protein content, moisture content, and productivity

After 17 days, the larvae were killed via low temperature (at 4 °C overnight) and then placed in a drying oven at 105 ºC until constant weight (Mancini et al. 2019). Before cold treatment and after drying, the fresh and dry weights of the samples were recorded, respectively, to calculate moisture loss, using the standard formula as follows:

$Moisture(\%) = [(Fw - Dw)/Fw] \times 100$, where Fw is the fresh weight (g) before drying, and Dw is the dry weight (g) obtained after oven-drying.

Once dried, the larvae protein content was detected by the Kjeldahl method ISO 1871:2009 adapted to the matrix, as follows: 0.5 g of dried larvae were placed into 20 mL glass tubes and mineralized using 12 g of K_2SO_4, 1 mL of catalyst, and 20 mL of H_2SO_4. The samples were placed in an automatic digester (Digestor™ 2508 and 2520, Foss) at 430 ºC for 2 h. Subsequently, the distillation was performed using an automatic distiller (Distiller Kjeltec™ 9, FOSS Analytical). The content of total nitrogen was determined by titration with HCl 0.2 N. Multiplying the nitrogen percentage by a factor of 4.76 (Janssen et al. 2017; Costa et al. 2020), the protein percentage was obtained.

Protein productivity, expressed as milligrams of protein per larva per day (mg/larva/day), was calculated to estimate the average daily accumulation of protein by each larva throughout the experiment by the following formula: $Pp\ (\%) = [(W_f - W_i)\ x\ (\%P/100)]/t \times 1000$, where W_f and W_i are the final and initial individual weights (g), *%P* is the final protein content, *t* is the number of experimental days and the factor *1000* is used to convert grams to milligrams. This

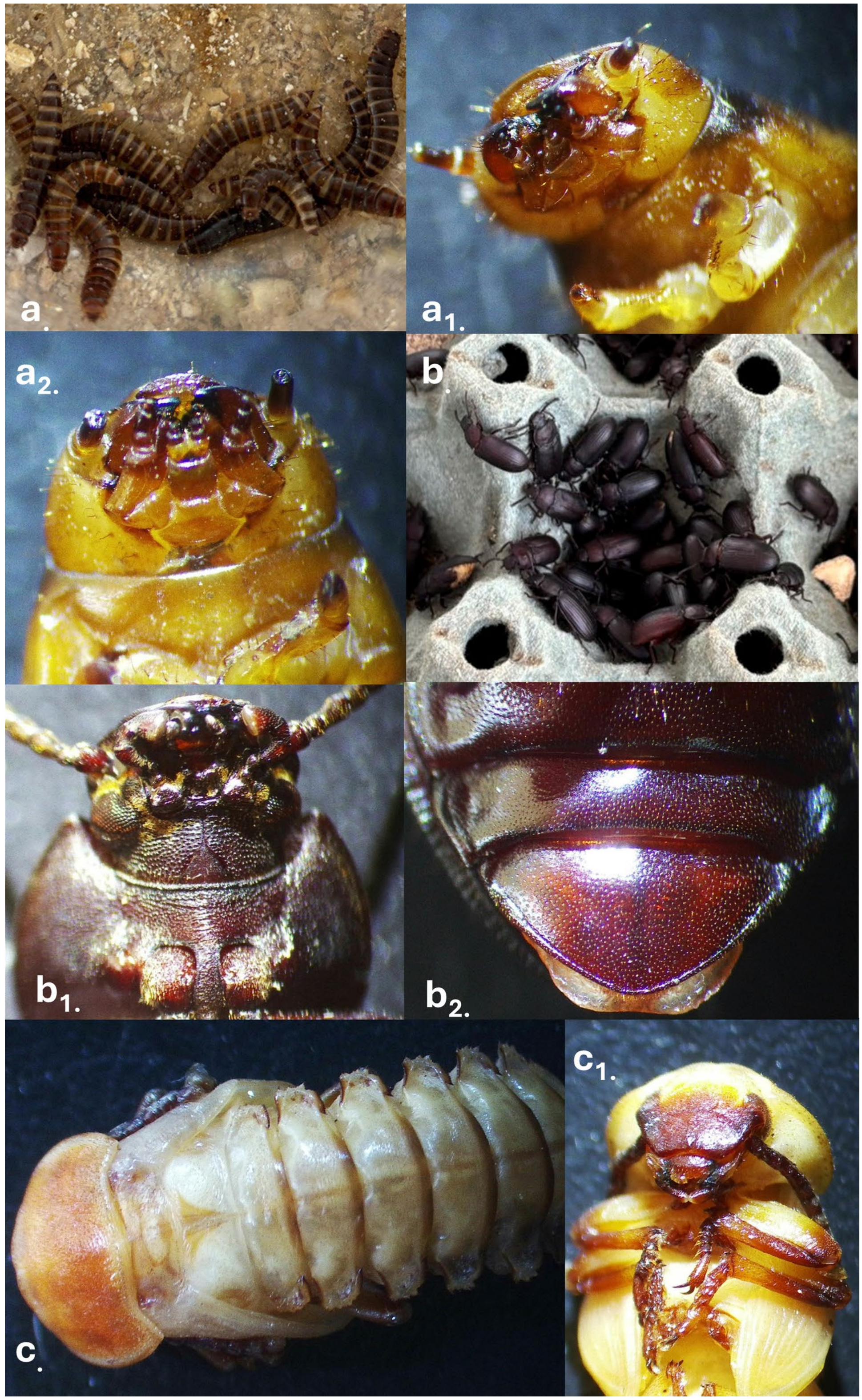


Fig. 1 Morphological stages of *T. guineensis*. (**a**) larvae; ($a_{1,}$ $a_{2.}$) details of larval head and thorax; (**b**) adult beetles; ($b_{1,}$ $b_{2.}$) details of adult head and abdomen; (**c**) pupa (dorsal view); ($c_{1.}$) details of pupal head and abdomen

parameter was used as a comparative index among treatments and should be interpreted as an indirect estimate rather than a direct measurement of protein deposition over time.

Statistical analyses

The data were analyzed using StatSoft software, version 10.0.228.8. For time-course data, One-way analysis of

Table 1 Diet treatments and approximate nutritional content

	Food ingredients (g)						Basic nutrients (g/100 g) *			
Groups	Wheat bran	Maize bran	Zucchini	Carrot	Cabbage	Orange	Protein	Starch	Sugars	Lipids
1	60	60	40	0	0	0	17.20	0	2.97	4.72
2	60	60	0	40	0	0	17.06	0	3.89	4.73
3	60	60	0	0	40	0	17.23	0	3.29	4.70
4	60	60	0	0	0	40	17.08	0	2.69	4.72
5	60	60	10	10	10	10	17.14	0	3.70	4.71
6	80	80	0	0	0	0	22.23	36.39	2.01	4.68

* Nutritional values are approximate reference data derived from source-based food composition databases, including USDA FoodData Central (fdc.nal.usda.gov) and NutriData (www.nutridata.com). Group 1: zucchini, Group 2: carrot, Group 3: cabbage, Group 4: orange, Group 5: mix, Group 6: control

variance (ANOVA) followed by Tukey's post-hoc test was applied separately to compare dietary groups at each sampling time and to evaluate temporal differences within each dietary group. For endpoint parameters, one-way ANOVA was used to test for significant differences among groups, and $p < 0.05$ was considered statistically significant.

Results & discussion

Given the limited scientific literature on *T. guineensis*, in the present study, the *T. molitor*, the most extensively studied mealworm species, was used as a reference. However, some differences may be present due to potential physiological and nutritional differences between the two species.

Survey of environmental conditions

The environmental conditions (such as temperature and humidity) were recorded during the experimental trial, and the results are reported in Table 2. Environmental conditions recorded during the trial showed average temperatures between 31.3 and 34.7 °C and relative humidity values between 41 and 60%. Since this was a preliminary study, larvae were reared under ambient conditions, following a realistic approach rather than strictly controlled laboratory settings. Although these values are not in line with the ranges generally reported as most favourable for *T. molitor* rearing, they remained within conditions compatible with larval development. Previous studies indicate that temperatures of 25–28 °C and relative humidity between 60 and 75% are generally more suitable for larval growth, with optimal development often reported around 27 °C and approximately 70% relative humidity. Despite these environmental conditions, which should be seen as a practical and sustainable rearing approach rather than an optimized system, the FBP-fed groups still showed better biological performance than the control, suggesting that diet supplementation has partially supported larval development under real local ambient conditions.

Tenebrio guineensis larvae growth performance

Larval weight, total length and head width increases

Overall, all FBPs treatments showed significantly higher larval growth than the control group (Group 6). As reported in Table 3, the highest final larval weight was observed in Group 3 (cabbage), reaching 0.122 g *per* larva, showing a significantly higher value than the control group (0.045 g). Considering that the initial larval weight was approximately 0.03 ± 0.005 g, Group 3 showed the greatest weight increase during the experimental period. Group 2 (carrot) and Group 5 (mix) also showed relatively high weights, reaching 0.092 and 0.104 g *per* larva, respectively. By contrast, the lowest final weight among FBP groups was recorded in Group 1

Table 2 Temperature (°C) and relative humidity (%) values detected in the insect farm during the experimental trial

Days	Temperature	Min	Max	Humidity	Min	Max
	Expressed as °C			Expressed as %		
0	31.3	25.7	48.4	58	20	69
2	34.7	25.6	48.4	41	20	67
4	32.5	26.5	48.4	50	20	69
6	34.2	25.6	48.4	60	20	69
8	34.1	25.6	48.4	57	20	69
10	32.9	26.2	48.7	47	20	69
12	33.6	25.6	49.2	48	20	69
14	32.1	26.4	49.2	57	20	67

For each sampling day, mean values as well as minimum and maximum values are reported

Table 3 Growth performance as weight increases per larva (g) during the experimental trial

Groups	Time (days)							
	0	2	4	6	8	10	12	14
1	0.036 ± 0.001^{aE}	0.037 ± 0.007^{aE}	0.039 ± 0.003^{cdE}	0.046 ± 0.003^{dD}	0.064 ± 0.005^{bC}	0.065 ± 0.003^{dC}	0.073 ± 0.004^{dB}	0.085 ± 0.001^{dA}
2	0.034 ± 0.002^{aG}	0.037 ± 0.002^{aG}	0.041 ± 0.001^{eF}	0.059 ± 0.002^{bcE}	0.064 ± 0.00^{bcD}	0.068 ± 0.00^{cC}	0.086 ± 0.003^{cB}	0.092 ± 0.002^{cA}
3	0.0325 ± 0.001^{aH}	0.038 ± 0.001^{aG}	0.047 ± 0.001^{bF}	0.067 ± 0.001^{aE}	0.08 ± 0.003^{aD}	0.0965 ± 0.002^{aC}	0.103 ± 0.00^{aB}	0.122 ± 0.003^{aA}
4	0.031 ± 0.003^{aE}	0.041 ± 0.004^{aD}	0.053 ± 0.001^{aC}	0.055 ± 0^{eC}	0.063 ± 0.004^{bB}	0.069 ± 0.001^{bcA}	0.07 ± 0.00^{dA}	0.072 ± 0.003^{eA}
5	0.0350 ± 0.004^{aG}	0.0390 ± 0.001^{aG}	0.0460 ± 0.001^{bF}	0.0590 ± 0.001^{bE}	0.0655 ± 0.001^{bD}	0.0725 ± 0.004^{bC}	0.0880 ± 0.001^{bB}	0.104 ± 0.00^{bA}
6	0.034 ± 0.002^{aC}	0.037 ± 0.002^{aBC}	0.037 ± 0.002^{eBC}	0.037 ± 0.002^{eBC}	0.039 ± 0.001^{eB}	0.045 ± 0.001^{eA}	0.045 ± 0.001^{eA}	0.045 ± 0.001^{fA}

Group 1: zucchini, Group 2: carrot, Group 3: cabbage, Group 4: orange, Group 5: mix, Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters within the same column (between groups at the same sampling time) indicate significant differences ($p<0.05$), whereas different uppercase superscript letters within the same row (within the same group over time) indicate significant differences ($p<0.05$)

(zucchini) and Group 4 (orange), with values of 0.085 and 0.072 g *per* larva, respectively.

Results related to the total length are reported in Table 4. Group 3 (cabbage) consistently showed the highest performance, with a final total length of 2.15 cm, which was significantly greater than the control group (1.40 cm). The other FBP groups also showed an increase in total length, with final values of 1.80 cm in Group 2 (carrot), 1.90 cm in Group 5 (mix), 1.73 cm in Group 1 (zucchini), and 1.75 cm in Group 4 (orange), although differences among them were limited. In general, the FBP-fed groups showed a more regular and homogeneous increase over time, whereas the control (Group 6) remained almost unchanged over time, showing a single significant increase before the 4th day, and remaining quite stable until the 14th day.

Results of head width (Table 5) showed a pattern consistent with that observed for the weight and total length parameters. Group 3 (cabbage) exhibited the highest final value, reaching 2.15 mm, while the other FBP groups generally approached 2.00 mm during the experimental period. Group 5 (mix) reached this value earlier, within the 6th day, whereas the remaining groups in the last days of the experimental period. Except for these small differences, head width remained quite similar among the treatments. The control (Group 6) also approached 2.00 mm, but with greater variability among replicates.

The growth-related parameters considered in the present study showed a coherent trend, with all the FBP-fed groups performing better than the control, which instead showed the lowest efficiency of larval development under the tested conditions. In particular, cabbage (Group 3), carrot (Group 2), and the mixed diet (Group 5) supported the best overall performance, whereas zucchini (Group 1) and orange (Group 4) resulted in moderate results, although still better than the control. Similar observations were reported by Liu et al. (2020), who showed that the supplementation of bran-based diets with fresh plant materials improved larval growth compared with dry substrates alone. From a nutritional point of view, this trend may be explained, at least in part, by the combined effect of a higher nutrient intake and a greater water supply through the feed. Fresh substrates may provide moisture, but also additional micronutrients, including vitamins and antioxidant compounds, which have been suggested to contribute to larval physiological performance in mealworms and other invertebrates (Moret et al. 2019; Liu et al. 2020; Knežić et al. 2024). At the same time, moisture appears to be one of the most important factors explaining the better performance of the FBP-fed groups. Mealworm larvae generally perform better when water is provided through fresh, high-moisture feed sources rather than through dry substrates alone (Ribeiro 2017; Lee et al. 2024). In particular, Lee et al. (2024) showed that increasing

Table 4 Growth performance as total length increases per larva (cm) during

	Time (days)							
Groups	0	2	4	6	8	10	12	14
1	1.20 ± 0.00^{aE}	1.20 ± 0.00^{bE}	1.38 ± 0.04^{bD}	1.45 ± 0.07^{cdC}	1.50 ± 0.00^{cC}	1.50 ± 0.00^{cC}	1.60 ± 0.00^{cB}	1.73 ± 0.04^{cA}
2	1.10 ± 0.00^{bE}	1.30 ± 0.00^{aD}	1.40 ± 0.00^{bC}	1.65 ± 0.07^{bB}	1.65 ± 0.07^{bB}	1.65 ± 0.07^{bB}	1.70 ± 0.07^{bB}	1.80 ± 0.00^{cA}
3	1.25 ± 0.07^{aD}	1.30 ± 0.00^{aD}	1.5 ± 0.00^{aC}	1.88 ± 0.04^{aB}	1.90 ± 0.00^{aB}	1.93 ± 0.04^{aB}	1.93 ± 0.04^{aB}	2.15 ± 0.07^{aA}
4	1.28 ± 0.04^{aD}	1.35 ± 0.07^{aD}	1.35 ± 0.07^{bD}	1.55 ± 0.07^{bcC}	1.60 ± 0.00^{bBC}	1.60 ± 0.00^{bBC}	1.70 ± 0.00^{bAB}	1.75 ± 0.07^{cA}
5	1.20 ± 0.00^{aF}	1.33 ± 0.04^{aE}	1.43 ± 0.04^{abD}	1.50 ± 0.00^{cD}	1.65 ± 0.07^{bC}	1.65 ± 0.07^{bC}	1.75 ± 0.07^{bB}	1.90 ± 0.00^{bA}
6	1.25 ± 0.07^{aD}	1.30 ± 0.00^{aBC}	1.35 ± 0.07^{bAB}	1.35 ± 0.07^{dAB}	1.40 ± 0.00^{dA}	1.40 ± 0.00^{dA}	1.40 ± 0.00^{dA}	1.40 ± 0.00^{dA}

Group 1: zucchini, Group 2: carrot, Group 3: cabbage, Group 4: orange, Group 5: mix, Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters within the same column (between groups at the same sampling time) indicate significant differences ($p<0.05$), whereas different uppercase superscript letters within the same row (within the same group over time) indicate significant differences ($p<0.05$)

Table 5 Growth performance as head width increases per larva (mm) during the experimental trial

Time (days)								
Groups	0	2	4	6	8	10	12	14
1	1.15 ± 0.01^{abD}	1.25 ± 0.07^{bC}	1.60 ± 0.00^{cB}	1.65 ± 0.07^{bB}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}
2	1.18 ± 0.00^{abE}	1.30 ± 0.00^{bD}	1.78 ± 0.04^{bC}	1.93 ± 0.04^{aB}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}
3	1.10 ± 0.00^{bE}	1.50 ± 0.00^{aD}	1.75 ± 0.07^{bC}	2.00 ± 0.00^{aB}	2.00 ± 0.00^{aB}	2.00 ± 0.00^{aB}	2.00 ± 0.00^{aB}	2.15 ± 0.00^{aA}
4	1.18 ± 0.00^{abD}	1.28 ± 0.04^{bC}	1.75 ± 0.07^{bB}	1.75 ± 0.07^{abB}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}
5	1.15 ± 0.07^{abC}	1.30 ± 0.07^{bB}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}
6	1.23 ± 0.04^{aC}	1.50 ± 0.00^{aBC}	1.78 ± 0.00^{dB}	1.80 ± 0.32^{abAB}	2.00 ± 0.28^{aAB}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}	2.00 ± 0.00^{aA}

Group 1: zucchini, Group 2: carrot, Group 3: cabbage, Group 4: orange, Group 5: mix, Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters within the same column (between groups at the same sampling time) indicate significant differences ($p < 0.05$), whereas different uppercase superscript letters within the same row (within the same group over time) indicate significant differences ($p < 0.05$)

water availability through the diet further improved larval growth, with bean sprouts leading to greater weight gain than lettuce or milk, thus highlighting the importance of both the type and the amount of the moist feed source. Similar evidence was also reported by Zafeiriadis et al. (2024), who found that gel-based wet feeds could effectively support larval growth and shorten development time in *T. molitor*. Under the tested experimental conditions, the better biological performance observed in the FBP-fed groups may therefore suggest a more efficient conversion of feed into biomass than in the control group, although no direct measurement of feed intake or feed conversion efficiency was performed (Van Broekhoven et al. 2015). For this reason, these interpretations, although supported by the literature and coherent with the observed results, should still be considered preliminary. Even if wheat bran is often described as one of the most suitable substrates for mealworm growth under optimized dry rearing conditions (Riaz et al. 2023), in the present study the control group showed the least favourable performance, suggesting that, under practical and locally realistic conditions, the partial inclusion of fresh food by-products may represent a more suitable strategy to support larval development in *T. guineensis*. Representative images of *T. guineensis* larvae at the end of the feeding trial are shown in Fig. 2.

Larval mortality rate

The effect of diet supplementation with different FBPs on larval mortality is reported in Fig. 3. The control group (Group 6) showed the highest mortality rate, equal to 22.5%, whereas all FBP groups showed lower values, ranging from 12.5 to 15%. The higher mortality rate in the control group (Group 6), along with its markedly slower growth could be attributed to reduced nutritional diversity and, above all, to the lower moisture content of the feed substrate.

Liu and co-workers (2020) reported slightly different results on larval survival and mortality; they observed survival rates of 91.7% in the control group, 92.5% in the cabbage group, 89.3% in the carrot group, and 89.7% in the orange group. The discrepancies between our results and those of Liu and co-workers (2020) may be related to several factors, including differences in the proportion of fresh feed as well as in rearing conditions such as temperature, humidity, and photoperiod. Nevertheless, our findings agree with those reported by Lienhard and co-workers (2023), who observed that *T. molitor* larvae fed with wheat bran enriched with other agro-industrial by-products, such as pea peels or sugar beet pulp, showed lower mortality and improved growth performance. According to these authors, the inclusion of these by-products increased both the nutritional

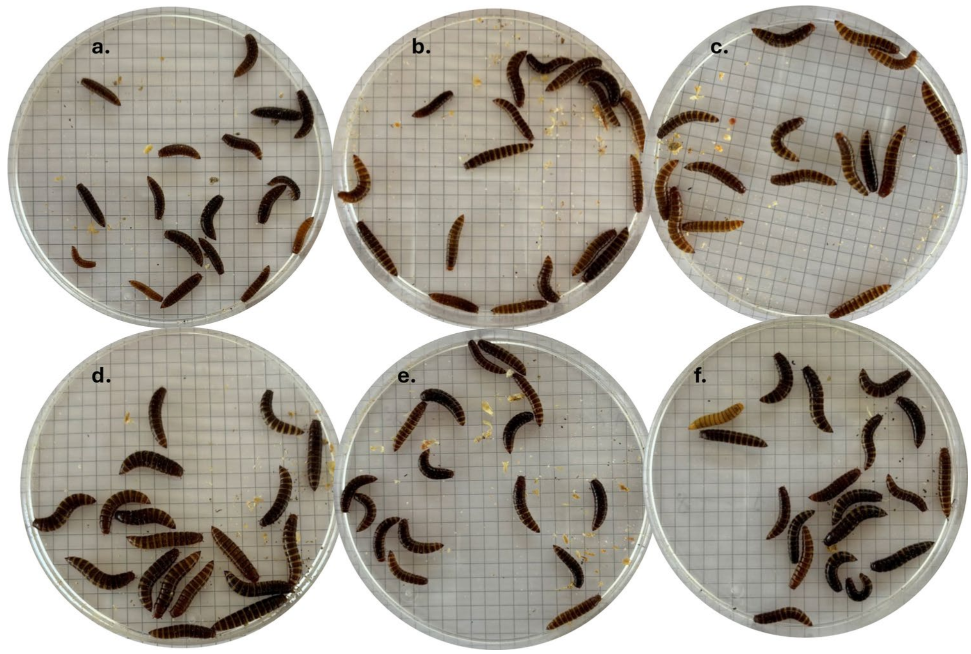


Fig. 2 *T. guineensis* larvae at the end of the feeding trial (**a**) Control Group; (**b**) zucchini Group; (**c**) carrot Group; (**d**) cabbage Group; (**e**) orange Group; (**f**) mix Group

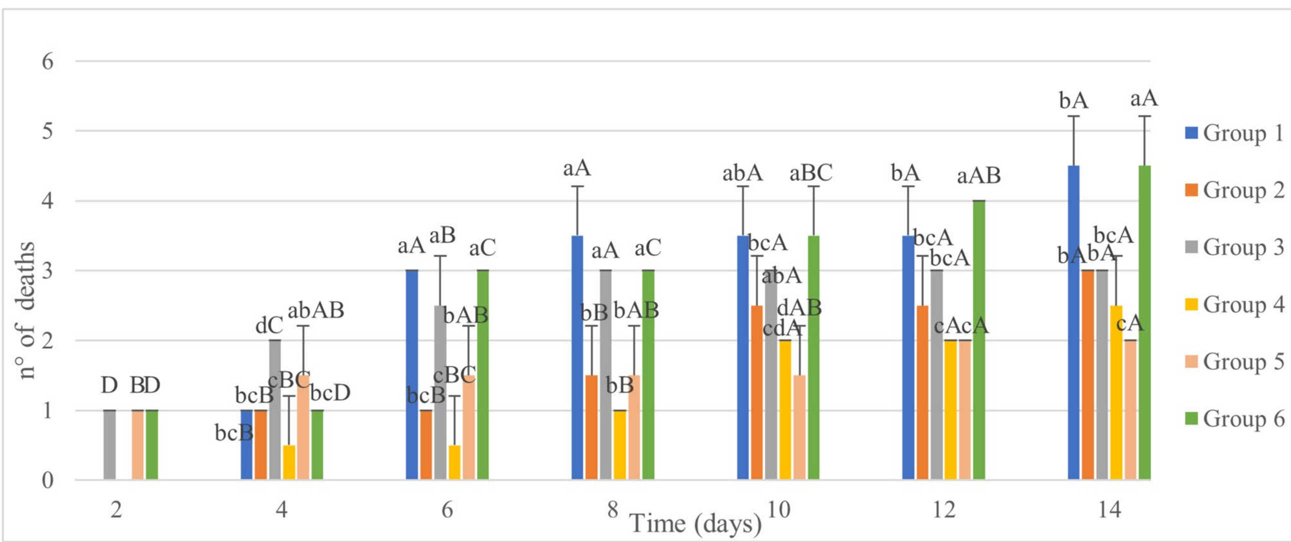


Fig. 3 Number of dead larvae recorded at each sampling time in the different dietary groups. Group 1: zucchini; Group 2: carrot; Group 3: cabbage; Group 4: orange; Group 5: mix; Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters within the same column (between groups at the same sampling time) indicate significant differences ($p < 0.05$), whereas different uppercase superscript letters within the same row (within the same group over time) indicate significant differences ($p < 0.05$)

value and the moisture content of the diet, which may have contributed to better larval development.

The results of the present study suggest that supplementing wheat bran with selected food by-products may have a beneficial effect on larval growth and survival. However, since no direct measurements of feed intake or micronutrient utilization in relation to *T. guineensis* larval mortality were performed, these interpretations, although supported by the scientific literature, should be considered preliminary.

Larval moisture content

Moisture content percentage, at the end of the experimental trial, is reported in Fig. 4. The highest moisture value was recorded in Group 3 (cabbage), reaching 55.96%, whereas the lowest value was observed in the control group (Group 6), with 48.26%. Groups 1, 2, and 4 showed similar intermediate moisture values, equal to 53.79%, 53.77%, and 53.73%, respectively, while Group 5 showed a slightly

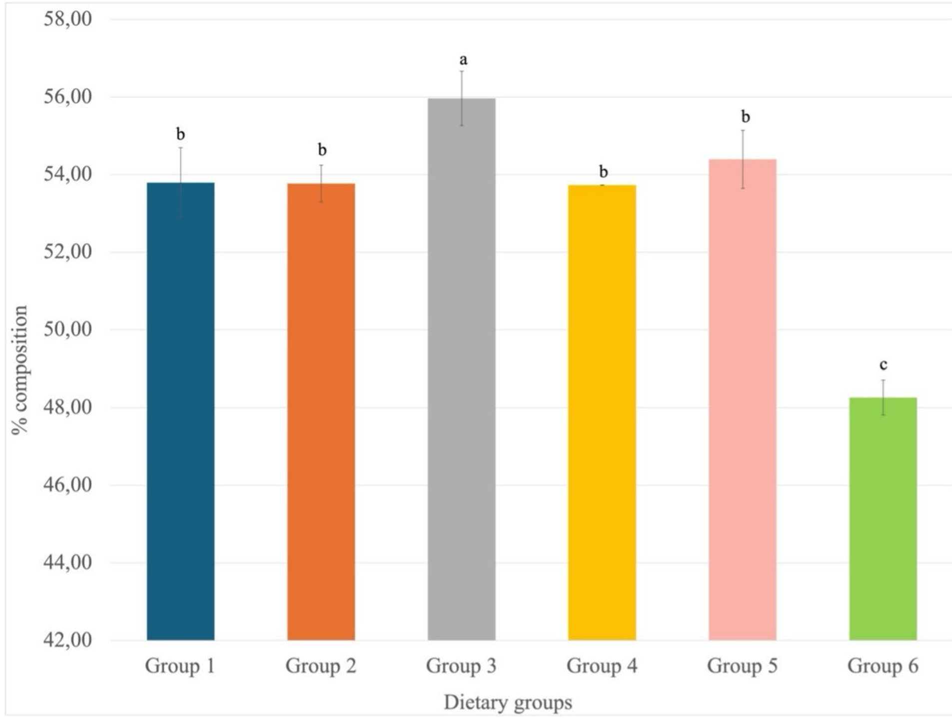


Fig. 4 Moisture content of *T. guineensis* larvae at the end of the experimental trial. Group 1: zucchini; Group 2: carrot; Group 3: cabbage; Group 4: orange; Group 5: mix; Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters indicate significant differences among groups ($p < 0.05$)

higher value (54.39%). All FBP groups showed higher moisture content than the control, whereas no marked differences were observed among the FBP groups, which all displayed broadly similar moisture values around 50–55%. Similar trends have been reported in previous studies on mealworms fed fresh or moist feed sources, in which a greater availability of water supplied through fresh feed was reflected in higher larval moisture content and associated with improved growth performance under the tested rearing conditions (Rovai et al. 2021; Zafeiriadis et al. 2024). In this sense, the moisture data provide further support for the interpretation that fresh FBPs contributed to larval development, improving water availability within the substrate and consequently, biomass production. At the same time, a greater water content in the larvae does not necessarily correspond to a proportional increase in nutrient concentration within the biomass.

Larvae protein content and estimated protein productivity

The protein content of *T. guineensis* larvae fed differently is reported in Fig. 5. Protein content ranged from 26.85% to 31.03% in the FBP-fed groups, whereas the control group showed the highest value of 39.04%. Among the FBP-fed groups, Group 1 (zucchini) showed the highest protein percentage, with a final value of 31.03%, followed by Group 5 (mix), Group 4 (orange), Group 3 (cabbage), and Group 2 (carrot), with final values of 29.67%, 28.21%, 27.87%, and 26.85%, respectively. These findings indicate that the inclusion of FBPs did not directly increase final protein concentration in larval biomass. Previous studies on *T. molitor* have generally reported higher protein percentages than those observed in the present study, with values typically ranging from 40 to 60% on a dry matter basis (Rumbos et al. 2020; Mariod 2020), and even higher values, up to 70–74%, have been reported when larvae were reared on diets enriched with pea or rice protein (Kröncke and Benning 2022). However, these comparisons should be interpreted with caution, since the present study was carried out on *T. guineensis*. Therefore, the observed differences may reflect not only dietary and methodological factors, but also species-specific variation in body composition.

In addition, larval body composition may vary with developmental stage and nutritional conditions, and because the present study covered only an initial phase of larval development, the final relative protein proportion observed here may not reflect the body composition that would be reached at the end of the larval cycle (Heckmann et al. 2018; Kröncke et al. 2019; Yu et al. 2021). Several factors may therefore have influenced the protein-related results, including the nutritional quality of diets, growth rate, substrate moisture, and the physical characteristics of the feed. In this regard, feed particle size may affect larval intake and nutrient assimilation (Hooper-Bui et al. 2002; Naser El Deen et al. 2022; Jankauskienė et al. 2024). Notably, in the present study, fresh feeds were hand-chopped without strict standardization, which may have introduced additional

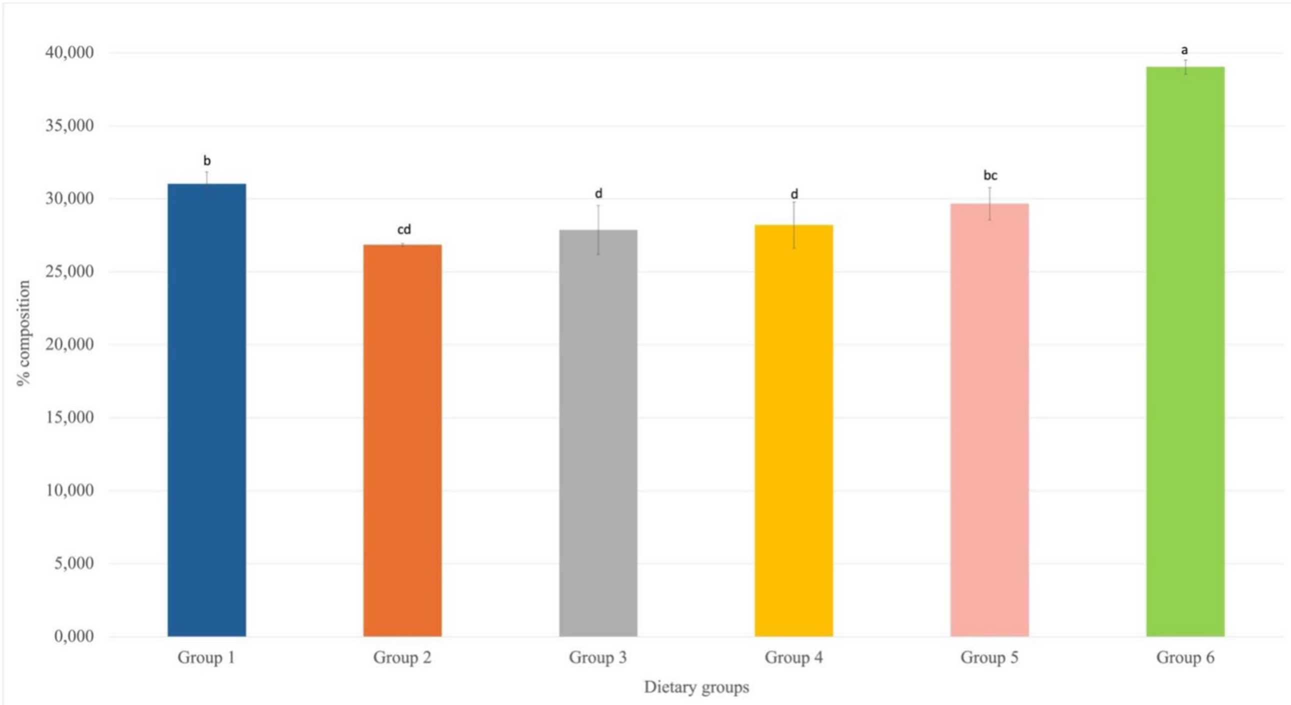


Fig. 5 Protein content of *T. guineensis* larvae expressed as percentage on a dry-matter basis. Group 1: zucchini; Group 2: carrot; Group 3: cabbage; Group 4: orange; Group 5: mix; Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters indicate significant differences among groups ($p < 0.05$)

variability in consumption and nutrient utilization. This aspect should therefore be considered when interpreting the present results.

A further important point to be discussed is protein productivity, since several authors have emphasized that relative protein content alone does not fully describe the actual protein yield obtained during larval growth (Makkar et al. 2014; Morales-Ramos et al. 2020; Lu et al. 2024). For this reason, protein productivity was also evaluated as an indirect comparative estimate based on biomass gain and final protein content. Table 6 reports the estimated protein productivity (mg/larva/day) calculated for each dietary group. Among the FBP-fed larvae, Group 3 (cabbage) recorded the highest value, reaching 1.78 mg/larva/day, followed by Group 5 (mix) reaching 1.46 mg/larva/day. Group 2 (carrot) and Group 1 (zucchini) showed intermediate values of 1.11 and 1.09 mg/larva/day, respectively, whereas Group 4 (orange) reached 0.83 mg/larva/day. The control group, by contrast, showed the lowest protein productivity, corresponding to 0.32 mg/larva/day. Overall, these results indicate that, despite their lower final protein percentage, the FBP-fed groups yielded a higher estimated protein output over time than the control, mainly because of their greater larval growth. From a production perspective, this aspect is particularly relevant, since higher estimated protein productivity reflects a greater protein yield obtained within a shorter experimental period (Morales-Ramos et al. 2020). Although this parameter should be interpreted as an indirect estimate rather than as a direct measure of feed conversion efficiency, it still provides useful preliminary information on the productive outcome achieved under the tested conditions.

These results are particularly relevant because they were obtained for *T. guineensis*, a local species that, before this work, had been only scarcely investigated from this perspective. Since edible insects are increasingly considered promising alternative protein sources (Oonincx & de Boer 2012; Van Huis et al. 2013; Halloran et al. 2016; Abbasi 2025a, b; Abbasi 2026), the present study may represent a preliminary indication that this species deserves further investigation, especially within locally adapted rearing strategies based on food by-product valorization.

Table 6 Protein productivity expressed as milligrams of produced protein (mg/larva/day)

Groups	1	2	3	4	5	6
Productivity	1.09 ± 0.004^{c}	1.11 ± 0.003^{c}	1.78 ± 0.06^{a}	0.83 ± 0.11^{d}	1.46 ± 0.08^{b}	0.32 ± 0.09^{e}

Group 1: zucchini; Group 2: carrot; Group 3: cabbage; Group 4: orange; Group 5: mix; Group 6: control. Results are reported as mean values ± standard deviation of three biological replicates. Different lowercase superscript letters indicate significant differences among groups ($p < 0.05$)

Conclusions

This study provides preliminary evidence that including 33.3% fresh FBPs in the diet of *T. guineensis* larvae may support growth, survival, biomass production, and estimated protein productivity. Among the tested substrates, the cabbage-based diet showed the most favorable overall performance, particularly in terms of final weight (0.122 g), total length (2.15 cm), head width (2.15 mm), low mortality rate (12.5%), moisture content (55.96%), and estimated daily protein yield (1.78 mg/larva/day). Although the control group showed the highest final protein percentage (39.04%), it was also associated with the lowest overall performance, including lower final weight (0.045 g), lower total length (1.40 cm), higher mortality (22.5%), and the lowest moisture content (48.16%) and estimated protein productivity (0.32 mg/larva/day). From this perspective, the present findings are particularly relevant because they were obtained for *T. guineensis* larvae, a poorly investigated local species, using locally available FBPs under practical conditions. Therefore, this study may be considered a first step toward the evaluation of locally adapted insect-rearing strategies based on food by-product valorization. Further studies should extend the evaluation over the full larval developmental cycle, improve substrate standardization, and include direct assessments of feed intake and biomass composition.

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All authors have read and agreed to the published version of the manuscript.

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Data Availability Data available on request from the authors.

Declarations

Competing interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Furthermore, C.C. and C.L.R. declare that they are members of ProBioEtna, a spinoff of the University of Catania, Italy. In addition, the authors declare that they do not have any personal, financial, professional, political, or legal interests with a significant chance of interfering with the performance of their ethical or legal duties.

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