Influence of Different Substrates on The Production and Nutritional Composition of *Tenebrio Molitor* Larvae

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Abstract

To address one of the challenges facing the poultry sector, novel feed ingredients such as insect larvae particularly *Tenebrio molitor* are emerging as viable alternatives to traditional protein sources. This research investigated which substrate (wheat bran, corn bran, and their combination) best supports the production of *Tenebrio molitor* larvae. During the egg-laying and survival phases, 2 g of each substrate and a 1:1 mixture (1 g of each) was used. The effects of these substrates on the lifespan and oviposition of female *Tenebrio molitor*, as well as the growth and average weight of the larvae, were evaluated under ambient conditions (temperature: 37 °C; relative humidity: 60%). The results showed that wheat bran yielded significantly higher survival (28.8 ± 0.58 individuals) and oviposition rates (150 ± 1.5 eggs per female) compared to other substrates. Developmental parameters including pupation, pre-pupation, one-month survival, and adult emergence were also superior in wheat bran, with adult emergence reaching 96.4%. Based on the results, it can be concluded that wheat bran is the most effective substrate for promoting female survival and oviposition in *Tenebrio molitor*. Additionally, the combined use of wheat and corn bran contributes positively to larval development, suggesting its potential as a balanced substrate for mealworm production.

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Keywords: Insect larvae, Novel ingredients, Nutrition, Production substrates, Protein sources, *Tenebrio molitor*

Introduction

The poultry industry plays a vital role in human livelihoods, serving as a key source of high-quality protein, generating substantial market value, creating employment opportunities, and contributing significantly to economic growth. Among meat-producing animals, broiler chickens are particularly efficient, reaching market weight (2.2–2.5 kg) within just 6–8 weeks (Métayer-Coustard *et al.*, 2019; Essen *et al.*, 2024a) [9].

Despite these advantages, the sector faces persistent challenges most notably the limited availability and inconsistent quality of protein sources, ration deficiencies, and the high cost of conventional ingredients such as soybean meal (SBM) and fish meal. Additionally, competition between humans and livestock for these feed resources continues to intensify. SBM, in particular, is both the most expensive component of poultry feed due to its high protein content and global demand, and the second most included ingredient after maize (Pomalégni *et al.*, 2017; Essen *et al.*, 2025) [12, 41]

In response to these challenges, researchers are exploring alternative feedstuffs that are nutritionally adequate, economically viable, locally available, and less competitive with human food sources. Insects especially those with high reproductive substitutes for traditional animal protein sources (Garcia-Lópezet al., 2023; Rashidi et al., 2025) [15, 45]. Among these, *Tenebrio molitor* larvae (yellow mealworms) have gained attention for their nutritional value and adaptability in poultry diets (van Huis, 2024) [58].

Mealworms are known for their rich nutritional profile. For instance, Li *et al*. (2013) reported protein and fat contents of 76.14% and 6.44%, respectively, while carbohydrate levels have been shown to range between 5.02% and 16.21% depending on diet and rearing conditions (Ghaly and Alkoaik, 2009; Rashidi *et al*., 2025) [44]. Rashidi *et al*. (2025) [44]emphasized that the nutritional composition of *T. molitor* larvae is strongly influenced by the type of substrate and environmental conditions, with high-protein diets yielding larvae with elevated protein content.

Studies have demonstrated the feasibility of using plant waste and agricultural by-products as substrates for *T. molitor* larvae. Li *et al*. (2013) observed fresh and dry weight increases of 56.15% and 46.76%, respectively, when larvae were reared on plant waste. Other research has confirmed the potential of insects as complementary or primary protein sources in poultry production (Schiavone *et al*., 2019) [49]. Biasato *et al*. (2017) [5], for example, reported improved growth performance and enhanced gut morphology such as increased villus height and crypt depth in broilers fed mealworm-based diets, with no adverse histological effects. indicating safety of inclusion.

Sustainable insect production relies heavily on substrate quality. Materials such as water-soaked bran, chicken and small ruminant droppings, wheat bran, corn flour, brewer's yeast, oats, fruits and vegetable peel have been used to enhance egg-laying and larval growth (Morales-Ramos *et al*., 2010; Sankara *et al*., 2021) [31, 48] Van Broekhoven *et al*. (2015) [57, 58] found that substrates with high protein and low moisture content promote faster pupation. Keil *et al*. (2020) [20]. reported that wheat bran supports larval fresh weights of 1.6–1.8 mg, while Sankara *et al*. (2021) [48] observed yields of 23.05 g, 45.16 g, and 148.20 g when larvae were reared on chicken and small ruminant droppings. Ghaly and Alkoaik (2009) noted that *T. molitor* females lay eggs on flour-like substrates, and Purushothaman *et al*. (2012) [42] highlighted how moisture-rich substrates can alter nutrient profiles. Oonincx *et al*. (2015) [37] further demonstrated that dry substrates promote higher growth rates and stabilize nutritional composition.

Corn flour, rich in phenolic antioxidants, iron (269%), zinc (11%), and fiber, is a viable substrate for larval production (N'Guessan *et al*., 2016) [34]. Wheat bran, abundant in carbohydrates, proteins, fats, minerals, bioactive compounds, and vitamins, also supports robust larval development (Slavin, 2003) [52]. Various substrates including palm powder (*Acrocomia aculeata*), plant leaves, fungi, probiotics, straw, cocoa seed husks, chickpea bran, and animal droppings have been explored for their effects on *T. molitor* growth, with promising results. However, to the best of our knowledge, there is limited documentation on the use of wheat bran and corn bran either individually or in combination for rearing *T. molitor* larvae in the southwestern part of the Maritime Region of Lomé, Togo. This study hypothesizes that incorporating wheat bran, corn bran, and their mixture into *T. molitor* diets will significantly influence larval growth, rates, efficient organic matter conversion, and minimal greenhouse gas emissions are emerging as promising nutritional profile, survival rate, oviposition, hatchability, and overall production efficiency. It is expected that the bran mixture will outperform single-substrate diets, offering an optimal balance of nutrients. Ultimately, this research aims to evaluate the impact of these substrates on the production performance, hatchability traits, nutritional composition, and reproductive success of *T. molitor* larvae providing the poultry industry with a cost-effective and locally sourced alternative protein solution.

Materials and Methods

Experimental site

This research study was conducted at two locations. The production of *T. molitor* larvae took place at the Research Laboratory on Agro-resources and Environmental Health (LARASE), while the bromatological analyses was carried out at the Togolese Institute for Agricultural Research (ITRA), University of Lomé, Togo**.** This facility is situated 17 km northeast of Lomé, in the Maritime Region of Togo. The study sites are positioned at latitude 6°13′ N and longitude 1°22′ E**,** at an altitude of 21.63 meters above sea level. The region lies within the tropical rainforest zone of southwestern Togo, characterized by average annual rainfall of 1300–1485 mm, Relative humidity during rainy season**:** 67–89%, and Environmental temperature range of 27–30°C (UL, 2024; AccuWeather, 2024) [1].

Experimental Ethical Statement

All experimental protocols were reviewed and approved by the Ethics Committee of the Regional Centre of Excellence in Avian Science (CERSA) at the University of Lomé, Lomé Togo, a branch of the National Ethics Committee for the control and supervision of experiments on animals. All the authors complied with the ARRIVE guidelines.

Preparation of the insect production unit for *T. molitor*

The breeding site was prepared to ensure optimal conditions for the development and reproduction of *Tenebrio molitor*. The production centre was cleaned and disinfected prior to use to minimize contamination and disease risk. All equipment including rearing trays, breeding boxes, and handling tools was sterilized using a mild disinfectant and dried thoroughly.

Environmental conditions such as temperature, humidity, and ventilation were monitored and adjusted to meet the species' requirements. The site was organized into separate zones for egg laying, larval development, pupation, and adult emergence to facilitate efficient management and data collection.

Substrates such as wheat bran and corn bran were stored in clean, dry containers and weighed accurately before distribution into rearing units. Moisture sources, including orange slices, were prepared and added according to experimental design. The entire setup was inspected to ensure consistency across treatments and replicates.

Experimental materials and biological samples

The substrates used in this study (wheat bran and corn bran) were obtained from a reputable milling and agro-processing company, CIMTOGO Officiel, located in Lomé, Maritime Region, Togo. Each substrate was purchased in 50 kg bags

and stored in the laboratory under cool, dry ambient conditions (temperature: 20°C–25°C, relative humidity: 30%–50%). At the start of the experiment, 200g of wheat bran and 200g of corn bran were measured into two separate plastic plates and refrigerated at 4°C for 12 hours. The substrates were later used in their dry state for insect rearing. Fresh orange slices, peeled and cut into small pieces (50g) were used as a supplement and energy source for the insects. These were also stored at 4°C prior to use.
The biological material consisted of pupae and adult *Tenebrio molitor* insects, including both males and females. The insect colony used for larval production was imported from the United States in 2021 and has since been maintained at the Agro-Ressource and Environmental Health (LARASE) Laboratory.

Collection and rearing of *Tenebrio molitor* pupae and adults

Tenebrio molitor pupae were collected from production trays using metal tweezers and transferred into plastic containers measuring 7.5 cm in height and 16 cm in diameter. These containers were kept in open-air conditions for 5 to 11 days to allow the emergence of adult insects.
Newly emerged adults were placed in lightly covered plastic trays with dimensions of 37.5 cm (length) × 28 cm (width) × 16.5 cm (height). Each tray was pre-filled with a layer of wheat bran and corn bran as a substrate. Additionally, 10 grams of orange slices were distributed to the insects, serving as a source of moisture and energy.

Egg production and lifespan of female *Tenebrio molitor*

To assess egg-laying capacity and lifespan, two male and three female *T. molitor* insects, aged 5 to 11 days, were selected. The insects were transferred into breeding boxes containing: 2g of each substrate (wheat bran and corn bran) for individual treatments and 1g of each substrate (wheat bran + corn bran) for the combined treatment. The number of eggs laid per female and the lifespan of each female were monitored daily. The experiment was conducted using six (6) replicates for each substrate condition.

Determination of hatching rate in *Tenebrio molitor*

The hatching rate was assessed using three substrate treatments: 5g of wheat bran, 5g of corn bran and 2.5g of a mixture of wheat and corn bran. Each treatment was replicated six times. After a two-week oviposition period in their respective substrates, the collected eggs were transferred into breeding boxes. To monitor the hatching of eggs into larvae, observations were conducted daily. The hatching rate for each substrate was calculated using the following formula:

$$Hatchability\ (\%) = \left(\frac{Total\ number\ of\ larvae\ hatched}{Total\ number\ of\ eggs\ laid}\right) x\ 100$$

Assessment of developmental parameters in *Tenebrio molitor*

Data collection on developmental parameters including weekly number and weight of larvae, total number of pupae, and total number of adults began one month after egg hatching.
For each substrate treatment, 60 larvae of the same age were reared, divided into groups of 10 larvae per rearing box.
The following substrate quantities were used: 10g of wheat bran, 10g of corn bran and 5g of wheat bran + 5g of corn bran for the mixed treatment Larvae were reared until the emergence of new adults, with substrates changed weekly. The following developmental rates were calculated:

$$Survival\ rate\ (\%) = \left(\frac{\text{Number of larvae alive}}{\text{Number of larvae introduced}}\right) x\ 100$$

$$Pupation\ rate\ (\%) = \left(\frac{\text{Number of pupae formed}}{\text{Number of larvae introduced}}\right) x\ 100$$

$$Adult\ emergence\ rate\ (\%) = \left(\frac{\text{Number of adults emerged}}{\text{Number of pupae formed}}\right) x\ 100$$

Larval production of *Tenebrio molitor* for growth and nutritional analysis

Larval production of *Tenebrio molitor* was initiated to support growth and nutritional studies. A total of 20 males and 30 early-age adult females (5 to 11 days old) were selected for mating. The insects were placed in breeding boxes containing one of the following substrates: 100g of wheat bran, 100g of corn bran, 50g of wheat bran + 50g of corn bran.
After one week of oviposition, eggs were collected and transferred into breeding trays, which were maintained for four weeks. Upon hatching, larvae were monitored regularly over a period of three months.
To provide moisture and energy, orange slices were added to each rearing tray once per week. Prior to distribution, the orange slices were refrigerated for 12 hours, then left at room temperature for 15 minutes to defrost.

Monthly renewal of rearing substrates in *Tenebrio molitor* production

To maintain optimal rearing conditions, the production substrates in each tray were manually renewed on a monthly basis. Prior to substrate replacement, larvae were carefully collected and transferred to clean trays. Fresh substrates were added to the trays in measured quantities of 100g per tray, according to the three treatment groups.
Waste substrates removed during this process were packaged in sterile bags and stored at 4°C in a refrigerator for subsequent nutritional analysis. Prior to analysis, the samples were milled using a 1 mm screen, following the protocols outlined in the *Official Methods of Analysis* (AOAC, 2023; Ferri *et al.*, 2025) [3]. Daily visual inspections of the production trays were conducted to monitor larval health and to remove any dead individuals. This maintenance routine was consistently applied throughout the entire production period.

Manual separation and harvesting of *T. molitor* larvae using the strainer method

Mealworm larvae (*Tenebrio molitor*) were harvested at two and three months of age, coinciding with the emergence of the first pupae. The strainer method was employed to extract larvae from the production substrate. This involved pouring the entire mixture comprising larvae and substrate into a handheld strainer positioned over a plastic bucket. The contents were sifted manually for 5–7 minutes to separate active larvae from the substrate. Immobile larvae, pre-pupae, and pupae were then carefully isolated using metal forceps.

Post-harvest processing and nutritional evaluation of yellow mealworms and their production substrates

After harvest, *Tenebrio molitor* larvae were fasted for 24 hours to clear their digestive tracts. The sample were then oven-dried at 65°C for 72 hours. After drying, the larvae were cooled at room temperature for 15–20 minutes and packaged in bags lined with allure paper. Samples were sent to the Togolese Institute of Agronomic Research (ITRA) for bromatological analysis.

Both the larvae and their production substrates were analyzed after rearing durations of 2 and 3 months. The dried samples were ground using a GRINDOMIX GM 300 blender (manufactured by Retsch GmbH, Germany).

Nutritional analyses focused on determining protein, moisture, lipid, carbohydrate, and ash content, following standardized methods described in the previous section. This comprehensive approach ensured accurate profiling of the nutritional value of both the larvae and their rearing substrates.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 9.1.1; GraphPad Software Inc., San Diego, California, 2022). Data were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure. Mean comparisons were evaluated using Tukey's post hoc test, with statistical significance set at $p < 0.05$. All charts and graphical representations were generated using GraphPad Prism.

The one-way statistical model is given as follows:

$$Yij = \mu + Ti + eij$$

Where;

Yij = Single observation;

μ = Over all mean;

Ti = Treatment effect; and

eij = Random error.

Results and Discussion

Results

Effect of rearing substrates on the life cycle of adult female *Tenebrio molitor* insects

Figure 1 illustrates the variation in lifespan duration of adult female *Tenebrio molitor* reared on different substrates. Significant differences were observed across treatments. Females raised on wheat bran exhibited a notably longer lifespan *(P= 0.0001)* compared to those reared on the wheat–corn bran mixture and corn bran substrate. The shortest lifespan was observed in the group reared on corn bran.

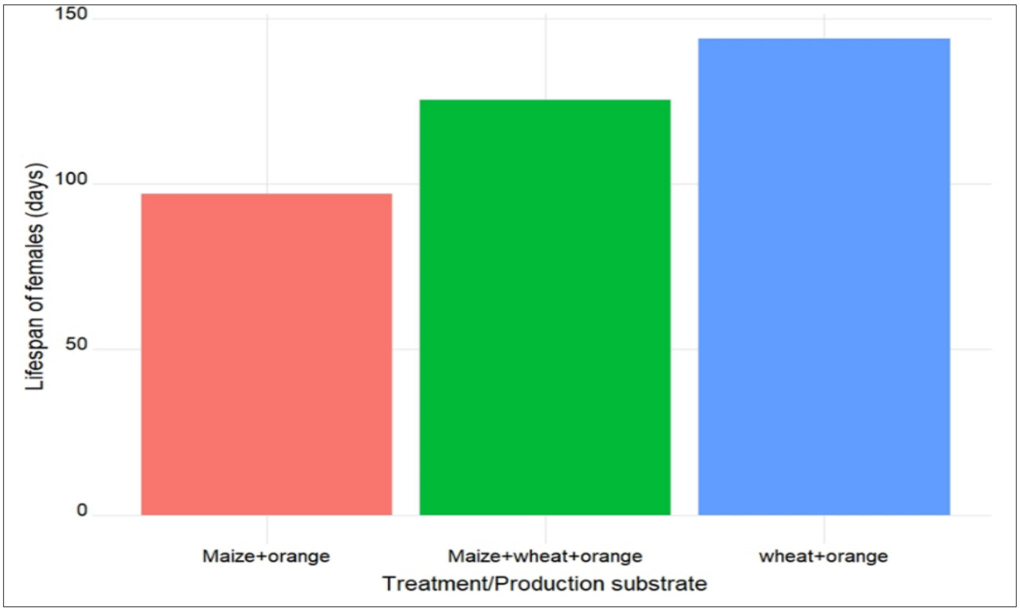

Fig 1: Effect of rearing substrates on the life cycle of adult female *Tenebrio molitor insects.* The values are expressed as the mean ± standard error. Asterisks with different superscripts indicate significantly different means, as follows: (*P<0.0001).* The different treatment groups include: (a) Corn substrate (red); (b) Wheat–corn substrate (green); and (c) Wheat substrate (blue).

Effect of rearing substrates on egg production in female *Tenebrio molitor* insects

Figure 2 illustrates the effect of different rearing substrates on egg production in female *Tenebrio molitor*. The number of eggs laid was significantly higher in females reared on wheat bran *(P = 0.0001)* compared to those raised on the wheat–corn bran mixture and corn bran.

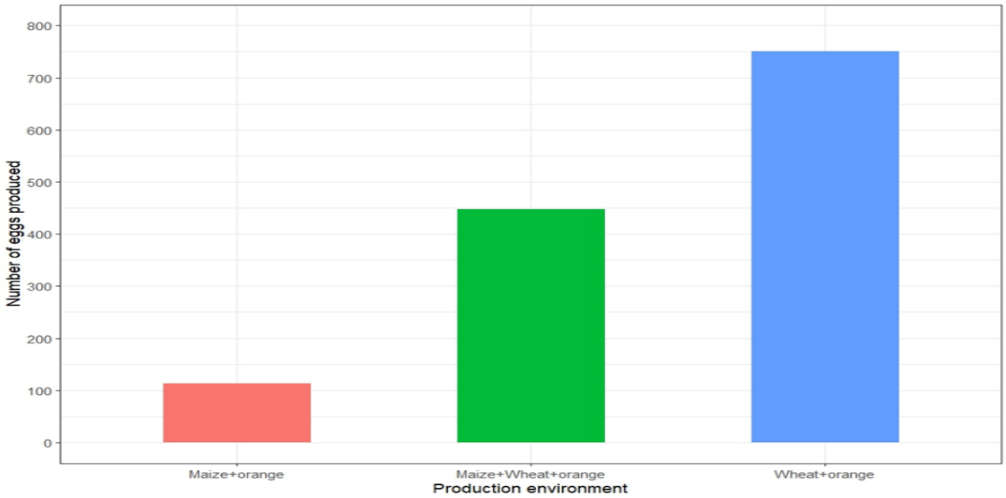

Fig 2: Effect of rearing substrates on egg production in female *Tenebrio molitor* insects
The values are expressed as the mean ± standard error. Asterisks with different superscripts indicate significantly different means, as follows: *(P<0.0001)*. The different treatment groups include: (a) Corn substrate (red); (b) Wheat–corn substrate (green); and (c) Wheat substrate (blue).

Effect of rearing substrates on larval development and growth performance in *Tenebrio molitor* insects

Figure 3 presents key developmental parameters of *T. molitor* insects, including survival rate before pupation, pupation rate, survival rate at one month, and adult emergence rate. Larvae reared on wheat bran showed significantly higher *(P<0.05)* values across these metrics compared to those raised on the corn bran and wheat–corn bran mixture. In contrast, egg hatching was notably significant with the wheat–corn bran combination. Moreover, none of the production stages were correlated with the corn bran substrate.

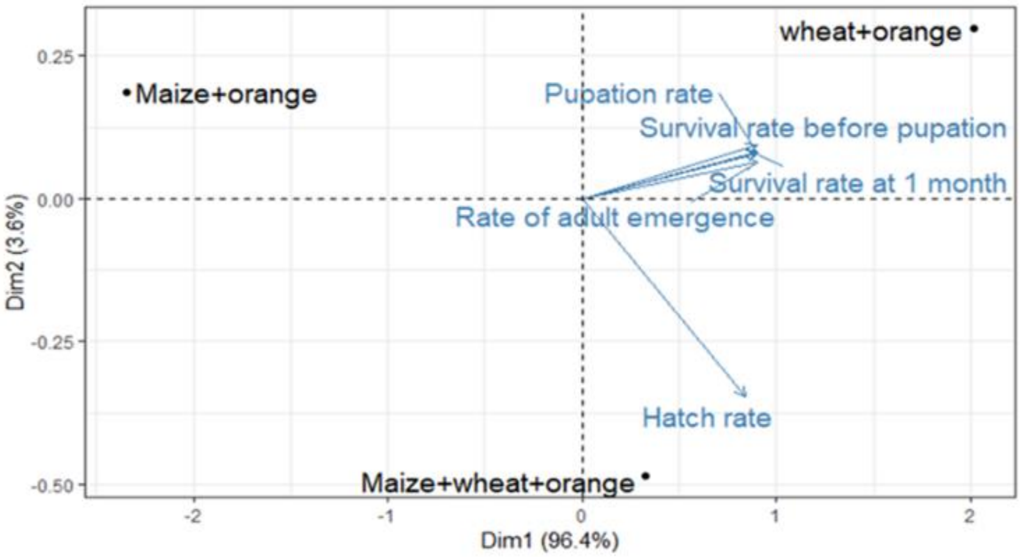

Fig 3: Principal component analysis showing the effect of rearing substrates on larval development and growth performance in *Tenebrio molitor* insects.

Effect of rearing substrates on the growth dynamics of *Tenebrio molitor* larvae

Figure 3 shows the variation in average larval weight of *Tenebrio molitor* across different ages and rearing substrates. Larval growth was significantly greater *(P = 0.016)* in the wheat–corn bran mixture compared to the other treatments, across all stages of development. The lowest larval growth was observed in the corn substrate group.

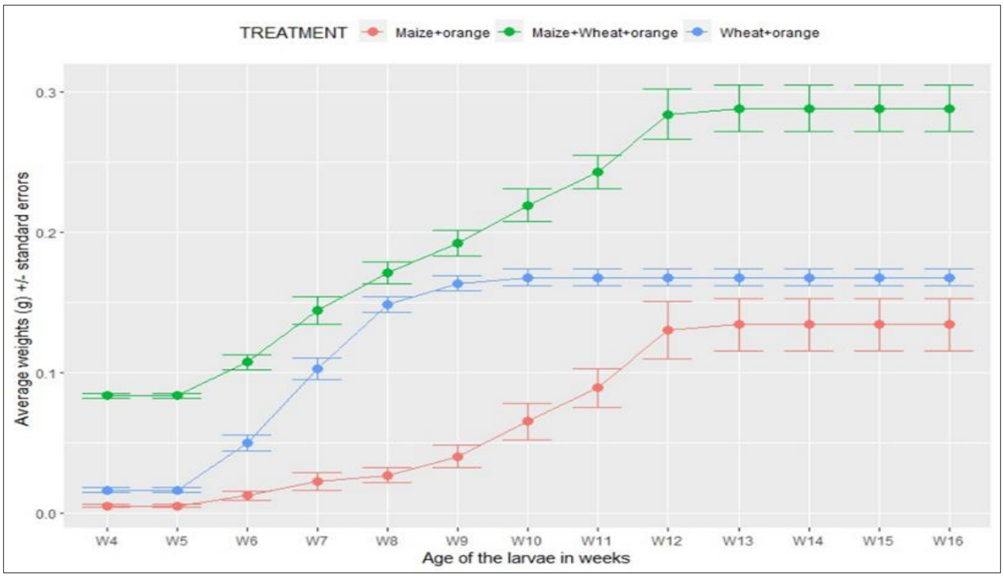

Fig 4: Variation in average larval weight (g) by age and rearing substrate. Asterisks with different superscripts indicate significantly different means, as follows: *(P = 0.016)*. The different treatment groups include: (a) Corn substrate (red); (b) Wheat–corn substrate (green); and (c) Wheat substrate (blue).

Variation in average dry weight of *Tenebrio molitor* larvae at 2 and 3 months of age across different rearing substrates

Table 1 summarizes the average fresh and dry weights of *T. molitor* larvae reared on different substrates at 2 and 3 months of age. The values represent both pre-drying (fresh) and post-drying measurements, illustrating how substrate composition influences larval biomass accumulation over time. Statistically significant differences *(P<0.05)* were observed across the treatment groups, with the wheat–corn bran mixture group recording the highest values and the corn bran group showing the lowest.

Table 1: Variation in average fresh and dry weight of *Tenebrio molitor* larvae at 2 and 3 months of age across different rearing substrates

Treatments	Age (months)	Average weight		*P-value*
		Fresh	Dry	
WCB	2	46.50±0.22^{a}	36.40±0.34^{b}	<0.001
WB	2	43.59±0.57^{a}	34.12±0.88^{b}	0.015
CB	2	42.36±1.00^{a}	34.94±0.95^{b}	0.024
WCB	3	56.76±0.48^{a}	41.38±0.46^{b}	<0.001
WB	3	52.15±0.32^{a}	41.24±0.63^{b}	<0.001
CB	3	49.34±0.41^{a}	35.91±0.72^{b}	<0.001

a,b,c, values within the same row followed by different subscripts differ significantly *(P<0.05)*. Mean ± SD, n = 3. *WCB* = Wheat–corn bran mixture; *WB* = Wheat bran; *CB* = Corn bran

Effect of rearing substrates on the proximate composition (%) of *Tenebrio molitor* larval meal at 2 and 3 months of age

Table 2 summarizes the effect of different production substrates: wheat bran, corn bran, and a wheat–corn bran mixture on the proximate composition of *T. molitor* larval meal at 2 and 3 months of age.

At both time points, larvae reared on corn bran exhibited significantly higher moisture and carbohydrate contents compared to those raised on other substrates (*P = 0.0089* at 2 months; *P<0.0001* at 3 months). In contrast, protein levels were significantly greater in larvae reared on wheat–corn bran mixture and wheat bran than those on corn bran *(P<0.0001)*. Fat content was highest in larvae raised on wheat–corn bran mixture at both ages *(P<0.0001)*. No significant differences were observed in ash content across substrates at either time point *(P = 0.0840* at 2 months; *P = 0.0555* at 3 months).

Table 2: Effect of rearing substrates on the proximate composition (%) of *Tenebrio molitor* larval meal at 2 months of age

Parameters	Treatments			*P-value*
	WCB	WB	CB	
Moisture	3.40±0.03^{b}	3.70±0.02^{ab}	4.50±0.37^{a}	0.0089
Protein	46.95±0.06^{a}	46.80±0.17^{a}	42.95±0.35^{b}	<0.0001
Ash	3.73±0.13	3.904±0.15	3.50±0.02	0.0840
Fat	34.22±0.03^{a}	31.34±0.46^{b}	26.53±0.01^{c}	<0.0001
Fibre	4.60±0.07^{b}	5.10±0.02^{a}	5.00±0.01^{a}	<0.0001
Carbohydrates	7.10±0.01^{c}	9.16±0.01^{b}	17.52±0.01^{a}	<0.0001

a,b,c, values within the same row followed by different subscripts differ significantly *(P<0.05)*. Mean ± SD, n = 3. *WCB* = Wheat–corn bran mixture; *WB* = Wheat bran; *CB* = Corn bran

Table 3: Effect of rearing substrates on the proximate composition (%) of *Tenebrio molitor* larval meal at 3 months of age

Parameters (%)	Treatments			*P-value*
	WCB	WB	CB	
Moisture	2.53±0.03[b]	2.39±0.08[c]	3.05±0.05[a]	<0.0001
Protein	52.09 ±0.02[a]	46.56±0.13[b]	43.73± 0.21[c]	<0.0001
Ash	3.29±0.14	3.52±0.06	3.62±0.55	0.0555
Fats	31.44±0.36[a]	30.53±0.47[b]	29.82±0.03[b]	<0.0001
Fibre	4.10±0.08[a]	5.22±0.16[a]	4.59±0.55[a]	0.035
Carbohydrates	6.55±0.46[c]	11.78±0.52[b]	15.19±0.08[a]	0.0002

a,b,c, values within the same row followed by different subscripts differ significantly *(P<0.05)*. Mean ± SD, n = 3. *WCB* = Wheat–corn bran mixture; *WB* = Wheat bran; *CB* = Corn bran

Proximate composition of substrates used for *T. molitor* larvae production (%)

Table 3 presents the proximate (nutritional) composition of the substrates used for rearing *Tenebrio molitor* larvae. The moisture content in the wheat bran–corn substrate and corn bran was significantly higher than that of wheat bran *(P = 0.0001)*. Conversely, the protein content in the wheat bran substrate was significantly greater than in the other two treatments *(P = 0.0001)*. Additionally, the fat content in wheat-corn bran and corn bran (SM) was significantly higher than in the wheat bran substrate *(P = 0.0001)*. The ash content was significantly higher in the wheat bran substrate and the least values were observed in corn bran substrate. Same trend occurred in the carbohydrate content were corn bran substrate recorded significant higher value *(P = <0,0001),* followed by wheat-corn bran and the least was noticed in the wheat bran.

Table 4: Proximate composition of substrates used for *T. molitor* larvae production (%)

Parameters (%)	Treatments			*P-value*
	WCB	WB	CB	
Moisture	10.28±0.06[a]	9.53±0.08[b]	10.19±0.04[a]	<0.0001
Protein	14.31±0.24[b]	17.94±0.19[a]	9.47±0.19[c]	<0.0001
Ash	4.10±0.06[b]	5.86±0.04[a]	2.29±0.02[c]	<0.0001
Fat	6.22 ±0.21[a]	3.43±0.01[c]	5.27±0.01[b]	<0.0001
Fibre	5.70±0.01[c]	5.40±0.01[b]	6.10±0.06[a]	< 0.0001
Carbohydrates	59.39±0.11[b]	57.84±0.09[c]	66.68±0.16[a]	<0.0001

a,b,c, values within the same row followed by different subscripts differ significantly *(P<0.05)*.
WCB = Wheat–corn bran mixture; WB = Wheat bran; CB = Corn bran

Discussion

The factors influencing the growth, development and overall performance of all living organisms depend on the quantity and quality of food consumed (Rashidi *et al*., 2025; Essen *et al*., 2025) [12, 44]. The present study indicates that *T. molitor* larvae can be produced on different production substrates, viz wheat bran (WB), corn bran (CB), and a wheat–corn bran mixture (WCB). Evaluation of these substrates for adult insect rearing revealed that female *T. molitor* exhibited the longest lifespan on the wheat bran diet, ranging from 16 to 31 days. This was significantly higher than the lifespan observed on the WCB (16 to 27 days) and CB (16 to 23 days) substrates. These findings are consistent with the report by Kanwal *et al*. (2023) [19], which concluded that wheat bran alone was the most effective substrate across all measured parameters, including lifespan. The superior performance of female *T. molitor* on WB can be attributed to its rich content of nutrients and abundance of bioactive compounds, as highlighted by Slavin (2003) [52]. This observation aligns with the findings of Cortes Ortiz *et al*. (2016), who reported that *T. molitor* can obtain all the essential nutrients required for optimal growth, development, and reproduction from high-quality wheat substrates. The higher nutritional composition of wheat bran, compared to other tested substrates, likely accounts for its effectiveness in promoting development and extending lifespan. Additionally, Myung and Kwang (2025) [33]investigated the impact of dietary protein and carbohydrate intake on the lifespan and reproduction output of *Tenebrio molitor* beetles. Their study reported a significantly longer lifespan of 183–197 days, which exceeds the findings of the present study. This extended longevity was attributed to the balanced composition and adequate quantity of nutrients provided in the diet.

The present study revealed that substrate composition significantly influenced the egg-laying yield of *Tenebrio molitor*. The highest yield was recorded on the WB substrate, with a mean value of 150 ± 1.5 g, which was statistically higher than the yields obtained on the WCB (89.4 ± 1.2 g) and CB (22.6 ± 1.08 g). This superior performance on WB can be attributed to its nutritional richness, particularly when supplemented with orange slices, which served as an additional source of moisture and energy. The combination likely created an optimal microenvironment for female reproductive activity. These findings align with those of Rumbos *et al*. (2020) [46] who evaluated 44 agricultural by-products and reported that protein-rich substrates supported the highest reproductive rates in *T. molitor*. Similarly, Rashidi and Akmali (2025a) assessed seven bran-based diets and found that diets containing higher proportions of wheat bran resulted in better overall performance, including enhanced egg-laying potential. Furthermore, Juan *et al*. (2024) emphasized the importance of substrate quality and moisture content in influencing reproductive success, reinforcing the conclusions drawn from this study

The present study demonstrated that *Tenebrio molitor* reared on WB exhibited superior developmental outcomes compared to those reared on WCB and CB. Specifically, the WB substrate yielded the highest survival rate before pupation, pupation rate, survival rate at one month, and adult emergence rate. These findings suggest that wheat bran provides optimal nutritional support across multiple larval

This observation is consistent with the results of Liu *et al.* (2020) [25]. who reared *T. molitor* on wheat bran supplemented with red cabbage, carrot, and orange. Their study reported high pupal survival rates (78.8%, 73.9%, 75.1%, and 77.7%) and pupation rates (15.4%, 12.7%, 15.7%, and 16.2%) over a four-week period. Similarly, Paloukas *et al.* (2020) [39] in their work on black soldier fly (*Hermetia illucens*) production using manure-based substrates, recorded comparatively lower developmental metrics, including larval survival rates of 3–9%, nymph rates of 22–48%, and adult emergence rates of 18–42%.

The high survival rate of one-month-old *T. molitor* larvae observed in the present study further supports the findings of Morales-Ramos *et al.* (2013) [30]. who emphasized the importance of dietary supplementation in improving insect growth and development. Additionally, the results align with those of Rashidi and Akmali (2025b), who evaluated seven bran-based diets using pure wheat bran as a control and combinations of barley, chickpea, and corn brans. Their study reported a survival rate before pupation of up to 98.8% on wheat bran, a pupation rate of 43.3% by the 13th instar, and significantly higher adult emergence compared to diets containing corn bran (32.9% pupation by the 15th instar).

In a similar trend, Shah *et al.* (2023)[51] found that larval mortality was lowest on wheat bran, while adult emergence and sex ratio were most favorable under wheat bran diets. Kanwal *et al.* (2023) [19]. reared *Tenebrio molitor* using a range of growth-promoting diets, with wheat bran serving as the standard control substrate. Their study concluded that larvae reared on wheat bran exhibited no pupal mortality, highlighting its effectiveness in supporting healthy development through the pupation stage. Furthermore, Leyo *et al.* (2021) [23], in their study on *Musca domestica* (housefly) larvae reared at varying egg loads (1.25, 2.5, 5, and 10 mg) across three substrates (wheat bran, millet bran, and cow dung), reported the highest survival rate from egg to final larval instar on wheat bran. Adult emergence was also significantly influenced by both substrate type and egg load, reinforcing the conclusion that wheat bran consistently enhances key developmental parameters across insect species.

The present study revealed that the hatching rate of *Tenebrio molitor* eggs was highest on the WCB substrate, exceeding other substrate treatments by a margin of 3.6%. This suggests that the combination of corn bran with wheat bran positively influenced egg hatchability. Interestingly, this finding contrasts with the results of Koo *et al.* (2013) [21], who reported a hatching rate above 70% when *T. molitor* eggs were reared on wheat bran supplemented with Chinese cabbage.

During larval development, the average weight of *T. molitor* larvae was significantly higher on the WCB substrate (0.08–0.29 g) compared to WB alone (0.02 ± 0.17 g) and CB alone (0.05–0.13 g), indicating that the synergistic effect of wheat and corn bran enhanced larval growth. These findings diverge from those of Chelsea *et al.* (2019) [7]. and Bordiean *et al.* (2022) [6]. who reported larval weights of 0.30 mg and 0.20 mg, respectively, when wheat bran was combined with *Silybum marianum* cake for rearing *T. molitor* and black soldier fly larvae.

Moreover, the average larval weight recorded on wheat bran alone in the present study (0.02–0.17 g) is notably lower than the values reported by Mancini *et al.* (2019) [26], who achieved average weights of 0.87 g and 0.95 g when *T. molitor* larvae were fed biscuit and bread-based diets over a one-year period. In contrast, Kanwal *et al.* (2023) [19]. evaluated various growth-promoting diets for *T. molitor*, using wheat bran as the standard control. Their findings showed that the wheat bran-only group produced the heaviest larvae (65.03 mg) and pupae (107.55 mg), highlighting its efficiency in promoting growth. Similarly, Rashidi *et al.* (2025a) reported that wheat bran-based diets yielded superior growth performance and nutritional outcomes.

Further supporting these observations, Jamaa *et al.* (2021) assessed six affordable livestock feed substrates—including dairy cow feed, calf starter, chick starter/grower, and wheat bran—and found that wheat bran alone resulted in the highest larval mass gain, growth rate, and feed consumption. Additionally, Ferri *et al.* (2024) [14] investigated the effects of supplementing wheat bran with chestnut shell and concluded that such supplementation enhanced larval health and bioactivity. Collectively, these findings underscore the nutritional value and versatility of wheat bran either alone or in combination with other substrates in promoting the development and physiological performance of *T. molitor* larvae.

The results of this study showed a consistent pattern in the average fresh and dry weights of *Tenebrio molitor* larvae reared on different production substrates. Larvae raised on the wheat–corn bran (WCB) substrate exhibited significantly higher weights at both the second and third months of rearing compared to those reared on other substrates. Specifically, the average dry weight ranged from 34.12 to 41.38 g, while the fresh weight ranged from 42.36 to 49.34 g during this period.

These values, although indicative of improved growth on the WCB substrate, remain lower than those reported by Li *et al.* (2013), who observed fresh and dry weight increases of 56.15% and 46.76%, respectively, when *T. molitor* larvae were reared on plant waste. The discrepancy may be attributed to differences in substrate composition, nutrient density, and bioavailability. Plant waste substrates, depending on their origin and processing, may offer higher levels of fermentable carbohydrates, micronutrients, or microbial activity that enhance larval metabolism and biomass accumulation.

In this study, we examined how different production substrates such as wheat bran (WB), corn bran (CB), and their mixture (WCB) influenced the chemical composition of *Tenebrio molitor* larvae over a three-month rearing period. The composition and quality of an insect's diet are known to significantly affect growth rates and overall production performance (Papastavropoulou *et al.*, 2024) [40].

Moisture plays a fundamental role in enzymatic activity, nutrient absorption, and physiological processes, all of which impact larval development and quality. Live mealworms typically contain 60–75% moisture, which reduces their energy density compared to dried mealworms that contain approximately 5% moisture (Selaledi and Mabelebele, 2021; Rashidi *et al.*, 2025) [44, 50]. This difference highlights the nutritional concentration achieved through drying.

Our results showed that larvae reared on CB had significantly higher moisture content at both two and three months (4.50% and 3.05%, respectively) compared to those reared on WCB (3.40%; 2.53%) and WB (3.70%; 2.39%). This suggests that the CB substrate, likely due to its lower amino acid concentration, promoted higher moisture retention in the larvae, consistent with findings by Davis (1974) [8] and Rashidi *et al*. (2025) [44].

However, these values differ from those reported by Rumpold and Schlüter (2013) [47], who found a moisture content of 64% in yellow mealworms reared on wheat bran for four weeks. Other studies have reported even higher moisture levels (4.75%; 55.34%), likely due to differences in rearing conditions, diets, or processing methods (Son *et al*., 2021; Rashidi *et al*., 2025) [44, 53].

Ash content reflects the mineral availability essential for larval development and production performance (Umeoka, 2024; Essen *et al*., 2025) [12, 56]. In our study, ash levels remained relatively consistent across all substrates and time points, ranging from 3.29% to 3.94%. Although no significant differences were observed, higher ash values (e.g., 4.125%) have been reported elsewhere, possibly due to variations in diet composition and environmental conditions (Son *et al*., 2021) [53].

Protein is a critical nutrient in insect rearing, and our findings revealed that larvae reared on WCB had the highest crude protein content at both two and three months (46.95% and 52.09%, respectively), followed by WB (46.80%; 46.56%) and CB (42.95%; 43.73%). This indicates that combining WB (a protein source) with CB (an energy source) enhances protein accumulation in *T. molitor* larvae.

The protein values obtained in this study (42.95–52.09%) are higher than those reported for black soldier fly (BSF) larvae reared on palm kernel meal, poultry manure, and agro-by-products (35–44%) (Rachmawati *et al*., 2010; Wang and Shelomi, 2017; Oonincx *et al*., 2015) [37, 43, 59] However, our results align with those of Nguyen *et al*. (2015) [35] and Hogsette (1992) [17], who reported protein contents of 47.9% and 48%, respectively, in BSF larvae fed on chicken feed and a mixture of wheat bran, alfalfa flour, and corn.

Mealworm protein levels typically range from 19.18% to 38.13% (Rashidi *et al*., 2025) [44], and can be influenced by various agricultural by-products such as cabbage, potato, wheat bran, barley bran, chickpea bran, corn bran, and rice bran. It has been noted that dietary fats and carbohydrates may affect larval composition more than protein content (Noyens *et al*., 2024) [36]. For instance, potato waste diets have been shown to increase fat while reducing protein levels compared to wheat bran (Musembi *et al*., 2024) [32]. Overall, our findings support the potential of dietary optimization to enhance the nutritional value of *T. molitor* larvae, especially given the high cost of protein in animal feeds (Zirari *et al*., 2024; Essen *et al*., 2025) [9].

Fat content is another key indicator of nutritional quality. Larvae reared on WCB had significantly higher fat levels (34.22%; 31.44%) than those reared on WB (31.34%; 30.53%) and CB (26.53%; 29.82%). This suggests that the combined substrate promoted fat accumulation in the larvae, enhancing their energy density and feed value.

Our findings are consistent with previous studies showing that dietary protein and fat levels influence larval composition (Rumbos *et al*., 2020; Morales-Ramos *et al*., 2020; Rashidi *et al*., 2025) [29, 44]. However, our findings contrast with Rumpold and Schlüter (2013) [47], who reported a fat content of only 2.5% in yellow mealworms reared on wheat bran. Conversely, our results agree with Wang and Shelomi (2017) [59], who found fat levels of 28–35% in BSF larvae reared on poultry manure.

Fiber is essential for digestive health and nutrient absorption. In our study, fiber content was lowest in WCB (4.60%; 4.10%) and highest in WB (5.10%; 5.22%) across both time points. These variations reflect the influence of substrate composition on digestive efficiency (Essen *et al*., 2025).

Our results align with studies showing that substrate type affects fiber levels. For example, Baena and Cardona (2012) reported 8.66% soluble fiber in cocoa seed husks. Crude fiber levels of 6.10% (Son *et al*., 2023) and 6.30% (Rashidi *et al*., 2025) have also been reported in mealworms. The higher fiber content reported by Rashidi *et al*. (2025) in a 50% wheat and 50% corn diet does not align with our WCB values, suggesting differences in substrate formulation. However, our fiber values (4.10–5.22%) are higher than the 3.14% reported by Arong and Eyo (2017) [4]. and comparable to values reported by Ossey *et al*. (2014) [38] Ukanwoko and Olalekan (2015) [55], and Aniebo and Owen (2010) [2].

Carbohydrate content varied significantly across substrates and time points. CB yielded the highest carbohydrate levels (17.52%; 15.19%), followed by WB and WCB (7.10%; 6.55%). Mealworms naturally contain approximately 11.5–16.21% carbohydrates (Lee *et al*., 2016; Rashidi *et al*., 2025) [44], and our findings confirm that diet composition—especially inclusion of chickpea or corn bran can elevate carbohydrate levels.

Corn bran, with a carbohydrate content of 65–86% depending on moisture, is primarily an energy source rather than a protein contributor. Its inclusion in the diet promoted higher carbohydrate accumulation in *T. molitor* larvae. This contrasts with findings by Melo *et al*. (2011) [27] who reported only 1–4% carbohydrate content in substrates rich in protein such as wheat bran and lupine straw.

The proximate analysis of the production substrates (wheat bran, corn bran, and their mixture) used for *Tenebrio molitor* larvae rearing revealed the presence of moisture, protein, fiber, ash, fat, and carbohydrates. These findings confirm that the substrates contain essential nutrients that may enhance the growth and performance of the larvae.

Overall, the substrates exhibited relatively high levels of moisture, protein, and carbohydrates, while ash, fat, and fiber contents were comparatively lower. The moisture and fiber values recorded in this study were higher than those reported by Essen *et al*. (2024b) [9]. who analyzed commercial starter/finisher chicken feed and found moisture and fiber contents ranging from 3.44–4.82% and 2.65–3.80%, respectively. In contrast, the protein, ash, fat, and carbohydrate values observed in our study were comparable and fell within the ranges reported by the same authors: 14.09–19.91% for protein, 5.05–5.25% for ash, 4.45–4.95% for fat, and 61.77–69.83% for carbohydrates.

These results suggest that the tested substrates are suitable as dietary nutrient sources in animal nutrition. Furthermore, analyzing the spent substrates after larval rearing provides insight into the extent of nutrient utilization by the insects. This approach allows for the assessment of nutritional efficiency, comparison of nutrient retention and depletion across substrates, detection of residual nutrients, and monitoring of substrate transformation during the rearing process.

Conclusion

This study demonstrates that the lifespan and female reproductive traits of *Tenebrio molitor* are significantly influenced by the type of production substrate used. Wheat bran enhanced survival rates and supported a moderately high egg-laying capacity, while corn bran alone was less effective. However, the combination of wheat and corn bran (WCB) emerged as the most favorable substrate, improving both hatching rates and larval weight. Therefore, incorporating wheat bran ensures reproductive success, while the addition of corn bran promotes larval growth and development.

Further research is recommended across diverse insect species to evaluate whether similar substrate combinations yield comparable or superior outcomes in terms of lifespan, reproduction, growth rate, hatchability, and survival. We hope this study opens new avenues for establishing *T. molitor* as a valuable model organism in lifespan and aging research. Moreover, the findings highlight the potential of mealworm larvae as a sustainable protein source and underscore the critical role of dietary composition in shaping nutritional profiles and production performance. The use of cereal brans—particularly in balanced combinations—significantly affects food composition, oviposition, growth, survival, and hatchability in *T. molitor*.

Ultimately, this research offers valuable insights into optimizing larval diets to enhance protein yield and overall health. These findings contribute to the development of efficient and sustainable insect-based protein production systems, offering a viable alternative to conventional livestock

Data Availability Statement

All data generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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