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Recycling of Date Palm Seed Substrate: Utilisation as Feed Material for *Tenebrio molitor* Larvae (Coleoptera: Tenebrionidae)

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ABSTRACT

Background: Date palm seeds, a common waste product from date palm cultivation, have the potential to be repurposed as a feed material for *Tenebrio molitor* larvae, commonly known as mealworms.

Methods: This study investigates the feasibility of utilizing date palm seeds as a sustainable feed source for mealworm rearing. Different ratios of date palm seed (DS) powder to wheat bran (WB) were used in the feed: 100% DS, 75% DS/25% WB, 50% DS/50% WB and 25% DS/75% WB. These treatments were assessed for their effects on mealworm growth, survival, total protein and phenolic contents, comparing them to conventional feed sources.

Result: The analysis revealed that DS 25 and WB 75 exhibited the highest concentration of phenolic compounds among the examined extracts, measuring 0.91 mg GA/g. Conversely, WB 100 displayed the highest protein concentration at 51.69 mg/g, while DS 75 and WB 25 exhibited the lowest concentration at 38.62 mg/g. The total larval survival rates varied across different dietary compositions, with mealworms fed on DS 25: WB 75 achieving the highest survival rate of 95.50±0.95%. larvae fed with DS 25: WB 75 showed the highest weight gain compared to other dietary compositions, indicating optimal growth conditions for mealworm larvae in this ratio. Results indicate that date palm seeds can serve as a viable alternative feed material for *Tenebrio molitor* larvae, offering a sustainable solution for waste management in date palm farming while providing a nutritious feed source for insect rearing.

Key words: Antioxidant activity, Feed supplementation, Growth performance, Mealworm, Phenolic contents.

INTRODUCTION

Date palm, the most important tree in Saudi Arabia, produces considerable waste yearly through dried fruits, fibrous materials and seeds. Throughout its lifespan, date palm requires special care. The offshoot should be removed and dead or defective fronds must be removed yearly, generating about 20 kg of waste per year from only one date palm. Some studies have reported that Saudi Arabia generates more than 200,000 tons of date palm biomass annually (Zafar, 2021). Such waste is a source of excellent degradable biomass that can be used in numerous applications. That rich resource is burned yearly on date palm farms due to ineffective processing strategies (Faiad *et al*., 2022). Therefore, there is a need for alternative approaches, such as utilizing mealworms to recycle this biomass efficiently. This approach could address the environmental concerns associated with burning, contribute to sustainable waste management practices and potentially yield valuable by-products.

The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), is recognized as a valuable resource insect globally. Its larvae, renowned for their high protein content and nutritional value, serve as essential food sources in various contexts. Notably, they are extensively utilized as a dietary staple for insectivorous animals, including mammals, fish, amphibians, reptiles and birds, particularly in captive settings. Additionally, mealworm larvae are increasingly incorporated into human diets, contributing to their versatility and widespread use across different sectors (Morales-Ramos and Rojas, 2015; Ravzanaadii *et al*., 2012; Van Huis, 2020).

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While mealworms can survive solely on wheat bran, their diets are frequently supplemented with organic waste materials to enhance nutritional content. Previous research has investigated the impact of dietary supplementation on mealworms, examining factors such as survival, growth, development, fecundity, feed conversion and nutrient composition (Musembi *et al*., 2024; Van Broekhoven *et al*., 2015). Mealworms are utilized for food conversion due to their high nutritional value and efficient feed conversion into biomass. They are rich in protein, healthy fats, vitamins and minerals, making them a nutritious food source. Additionally, mealworms have a high feed conversion rate, meaning they can efficiently convert feed into edible biomass. This makes them a sustainable and environmentally friendly option for food production (Moruzzo *et al*., 2021; Spang, 2013).

The utilization of date palm seeds presents a cost-effective alternative for rearing *T. molitor* larvae, given their affordability compared to conventional feed sources and their ready availability from the date palm industry. While *T. molitor* larvae are primarily utilized as feed for poultry or fish in many regions, they are also directly consumed by humans (Van Huis, 2020). Concerns may arise regarding the safety of consuming *T. molitor* or animals fed with T. molitor reared on date palm seeds. However, it is noteworthy that date palm seeds are commonly roasted and utilized as a coffee substitute in Saudi Arabia, readily available in markets. Proper processing and storage of date palm seeds can mitigate potential food safety risks, mainly when provided as a dry feed to *T. molitor*. Given the anticipated increase in demand for animal protein and the environmental challenges posed by current animal production practices, edible insects like *T. molitor* offer a highly sustainable solution as a source of animal protein (Pelletier and Tyedmers, 2010; Van Huis, 2020).

The present study is the first to investigate the feasibility of utilizing date palm seeds as a substrate for rearing mealworm larvae. By evaluating the growth parameters of mealworms raised on date palm seeds, this research aims to determine the optimal proportion for substituting the potential of this alternative substrate as a more cost-effective and sustainable option as an addition to wheat bran substrate.

MATERIALS AND METHODS

Rearing of *Tenebrio molitor*

Yellow mealworm larvae were raised in the insect-rearing unit at King Saud University (Riyadh, Saudi Arabia), maintained at a temperature of 23±2°C, a relative humidity of 40±5% and subjected to 12-hour light-dark cycles. They were reared in plastic crates measuring 60×40×14 cm. The larvae were fed wheat bran as a dry feed and cabbage as a wet feed. The cabbage was purchased from a local grocery store in Riyadh, Saudi Arabia and stored in a refrigerator at 4°C until use.

Feeding trial

The control insects were provided with a diet of wheat bran. Date palm seeds were incorporated into wheat bran at varying ratios (100, 75%, 50% and 25% w/w). These seeds were cooked at 250°C, then ground into a fine powder using an electric grinder and frozen at -80°C for future use. Plastic insect breeding trays measuring 7×7×7 cm were utilized as experimental units for the feeding trials. Each tray contained 30 g of feed for 50 larvae. Four replicate trays were prepared for each treatment. The larvae were allowed to feed undisturbed in an incubator (Sanyo, Japan) set at 25°C and 50±5% relative humidity. On weeks 2, 4 and 6, the larvae underwent weighing using an analytical scale. The total weight of larvae was divided by the number of larvae in each replication to determine the average individual larval weight. On the 6 weeks, the larvae were harvested for further studies. Following harvesting, the larvae were subjected to a 24 h starvation period, after which they were divided into groups and weighed.

Sample preparation

Upon conclusion of the experiment, the larvae were euthanised by freezing at -80°C and subsequently subjected to drying in an oven at 50°C for a period of 48 hours. The resultant dried larvae were finely powdered and stored in glass vials at -80°C. For extraction, one gram with three replicates of oven-dried mealworm larvae was combined with 10 mL of hexane (Sigma, USA), followed by homogenization (IKA, Germany) and ultrasonication (Wise Clean, Korea) (100%, 10 min). Extraction was conducted at 25°C using a shaker (GFL Orbital Shaker 3005, Germany) at 250 RPM for 1 h. Following filtration through a single layer of filter paper and a 0.25 syringe filter (Eppendorf, Germany), the resulting supernatants underwent evaporation using a rotary evaporator (Heidolph, Germany) to assess extraction efficiency. The obtained extract was used to determine total phenolic content (TPC) and total antioxidant capacity (TAC).

Determination of total phenol content

The total phenolic content was determined using the Folin-Ciocalteu method in a 96-well plate. Each well contained 5 µL of the test samples, 20 µL of Folin-Ciocalteu phenolic reagent and 80 µL of 7.5% sodium carbonate solution. Absorbance readings were taken at 760 nm using a spectrophotometer. A standard curve was constructed using gallic acid with concentrations of 0.100, 0.200, 0.4, 0.6, 0.8 and 0.9 mg/mL. The results were expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/g DE).

Quantification of protein content

To assess the protein content, 1 gram of larvae from each dietary group was collected and homogenised in distilled water for 5 minutes, followed by sonication for an additional 5 minutes. The resulting extract underwent centrifugation at 4°C and 4000 ×g for 5 minutes. Protein content was assessed using the Bradford method (Bradford, 1976). Briefly, 5 µL of the supernatant (protein extract) was combined with 200 µL of Bradford's reagent (Sigma-Aldrich, Germany) and left to incubate at room temperature for 10 minutes. Absorbance was measured at 595 nm using a spectrophotometer. A blank solution was prepared using phosphate-buffered saline (PBS), while a standard curve was generated using known concentrations of bovine serum albumin (Sigma-Aldrich, Germany). Protein content calculations were performed using OriginPro 8.5 software.

DPPH radical scavenging activity of hexane extract

The radical scavenging activity of DPPH (Sigma, USA) was assessed following the method described by (Abutaha *et al*., 2022; Boulkenafet *et al*., 2023). A DPPH solution was prepared by dissolving 0.004 g of DPPH reagent in 100 mL of methanol. Subsequently, 290 µL of the solution was added to 10 µL of hexane in a 96-well plate. Following a 30-minute incubation period at room temperature in the absence of light, the absorbance at 515 nm was measured using a spectrophotometer. The percentage inhibition of DPPH was calculated using the formula:

$$\text{Inhibition\%} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

All statistical analyses were conducted using the SPSS software package. The collected data were subjected to a one-way analysis of variance (ANOVA). Significant differences between means were determined using Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

survival

Over the study duration, the total larval survival rates of mealworms fed on the control diet (WB only) and diets supplemented with different ratios of date palm DS100, DS 75: WB 25, DS 75: WB 25, DS 50: WB 50, DS 25: WB 75 and WB100 were 46.00±6.98%, 93.00±1.73%, 89.50±3.30%, 93.50±0.50% and 95.50±0.95%, respectively (Table 1). Within 4 and 6 weeks, only DS showed a significant difference ($p \leq 0.05$) in the number of live mealworm larvae detected between diet groups. This suggests that the mix of DS and WB has a notable impact on the survival rates of mealworm larvae over time. Similarly, larvae fed with DS100 exhibited a negative weight gain during all durations of treatment, suggesting a detrimental effect of this particular diet composition on larval growth. In contrast, larvae fed with other diet ratios showed varying degrees of weight gain, with some ratios demonstrating higher weight gain rates than others. Notably, larvae fed with DS 25 and WB 75 exhibited the highest weight gain compared to other dietary compositions, indicating optimal growth conditions for the mealworm larvae in this ratio. The average weight of mealworm larvae fed on wheat bran increased by 36.75% over six weeks (Fig 1). In week six, dietary supplementation with WB 25, DS 75: WB 25, DS 50: WB 50 and DS 25: WB 75 improved the growth rates to 11.86%, 25.51%, 31.75% and 36.75%, respectively. The negative impact of DS100 on survival and weight gain led to the decision not to conduct further assessments on antioxidant activity, total protein and total phenolic contents.

A previous study by Li *et al*. (2020) reported a significantly low survival rate of *T. molitor* larvae when exclusively fed with spent mushroom substrate, documenting a survival rate as low as 1.3% (Li *et al*., 2020). Our findings align with previous studies by Ghaly and Alkoaik (2009) and Kim *et al*. (2017), which similarly indicate the adverse impact of rice straw as a sole dietary component, resulting in a lower protein content ranging from 37.12% to 48.33%. Hence, it appears that spent mushroom substrate (SMS) may only serve as a partial substitute for conventional feed. Further comprehensive investigations are warranted to identify specific nutritional components within the seed that may influence the survival and developmental outcomes of *T. molitor* larvae.

Phenolic content

The TPC of the examined hexane extracts, determined using the Folin-Ciocalteu reagent, is expressed in gallic acid equivalent (GAE) per gram of extract, with a standard curve equation of y= 0.0053x - 0.0318 and anR^2 value of 0.985. The concentrations of phenols in the extracts of mealworm range from 0.76 to 0.91 mg GA/g. Specifically, the TPC values for DS 75 and WB 25, DS 50 and WB 50, DS 25 and WB 75 and WB 100 were 0.77±0.01, 0.83±0.03, 0.91±0.03 and 0.86±0.04 mg GAE/g, respectively. Analysis reveals that DS 25 and WB 75 exhibit the highest concentration of phenolic compounds among the examined extracts (Fig 2). The observed variations in TPC across the different feed ratios suggest that the diet composition significantly influences the mealworms' phenolic content. Phenolic compounds are known for their antioxidant properties, which can confer several health benefits, including reducing

Table 1: Survival (%) of *Tenebrio molitor* larvae (±SE) fed for 6 weeks with wheat bran (control) (WB), date seeds (DS) and bran fortified with different ratio of date seeds (DS75 and WB25), (DS50 and WB 50) and (DS25 WB75) (n= 5).

Diets	Survival (%)			
	After two weeks	After 4 weeks	After 6 weeks	Total
DS	97.00±1.50a	83.50±2.63b	70.50±4.57b	46.00±6.98b
DS 75 and WB 25	98.50±3.10a	100.00±0.00a	94.50±0.50a	93.00±1.73a
DS 50 and WB 50	94.50±0.95a	99.00±1.00a	96.00±0.82a	89.50±3.30a
DS 25 and WB 75	98.50±0.00a	97.50±0.95a	97.50±1.50a	93.50±0.50a
WB	97.00±1.50a	98.00±0.81a	97.50±1.50a	95.50±0.95a
dr	4	4	4	4
F	1.50	24.57	25.72	34.83

There are no statistically significant differences between wheat bran and bran fortified with date seeds, whereas significant differences were observed between both of them and date seeds alone at $p<0.05$.

DS= Date seeds, WB= Wheat bran.

benefits, including reducing oxidative stress and potentially extending the shelf life of mealworm-based products (Son *et al*., 2020).

The highest TPC levels were found in the DS 25 and WB 75 feed mixtures. This suggests that the highest phenolic content in mealworm larvae is found in a balance that favors wheat bran with a moderate amount of date seeds. The mealworms efficiently assimilate the inherent phenolic compounds present in wheat bran (Siddiqui *et al*., 2024). Conversely, while date seeds do contribute to the overall nutritional profile of the feed, their phenolic content appears less influential in boosting the TPC when they comprise a larger proportion of the diet.

The study suggests that optimizing feed mixtures can improve mealworm nutritional quality, especially in terms of phenolic content. High wheat-bran ratios can maximize antioxidant properties. Future research could explore phenolic uptake and metabolism mechanisms.

Protein content

The protein concentrations in the samples were determined using the Bradford method. The results were expressed in milligrams of protein per gram of sample (mg/g). The protein concentrations ranged from 38.63 to 51.69 mg/g. WB 100 exhibited the highest protein concentration, with a value of 51.69 mg/g, while DS 75 and WB 25 showed the lowest concentration at 38.62 mg/g. These findings suggest that WB 75 and WB 100 have a higher protein content than other mixed ratios (Fig 3). The lower protein content in the DS 75 and WB 25 diets could be attributed to the nutritional

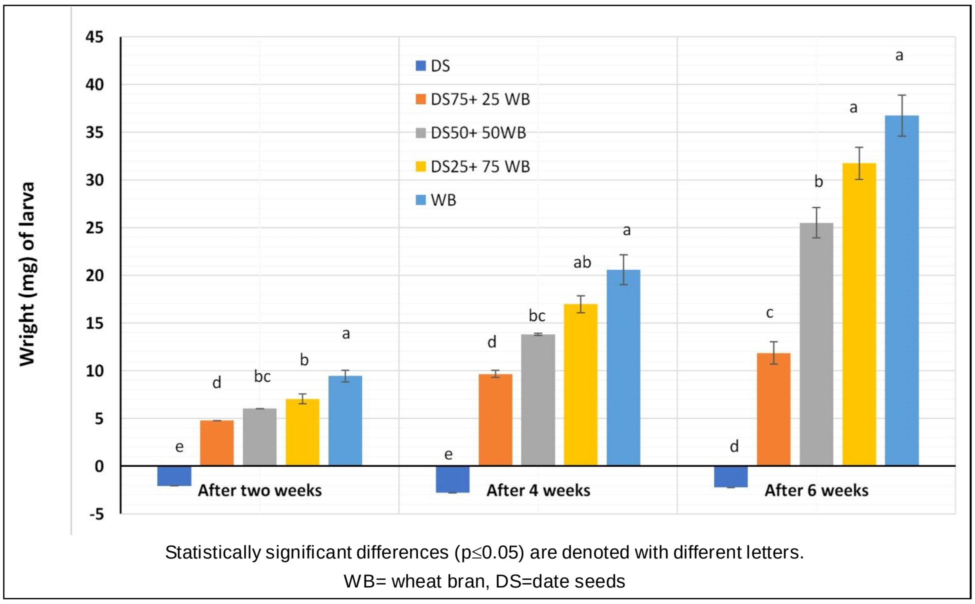


Fig 1: Individual weight (mg) of *Tenebrio molitor* larvae fed for 6 weeks with wheat bran (WB) (control), date seeds (LB) and bran fortified with different rates of date seeds (DS75 and WB25), (DS50 and WB 50) and DS25 and WB 75 (n= 5).

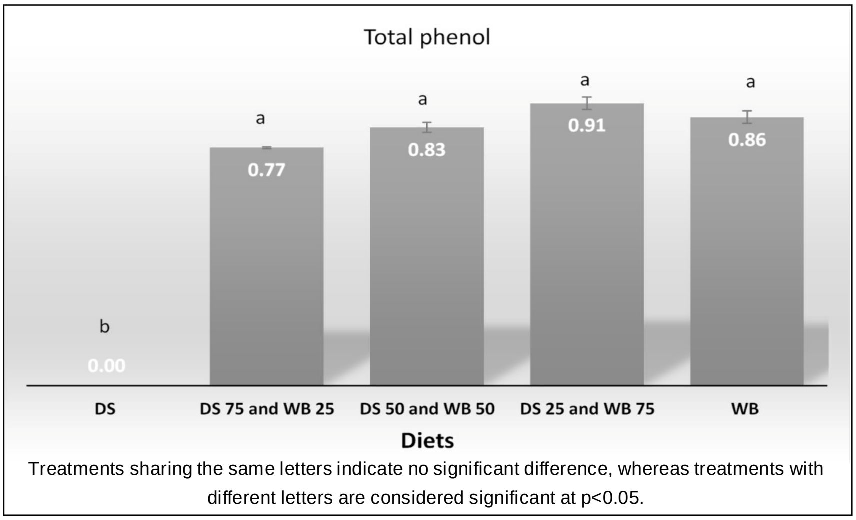


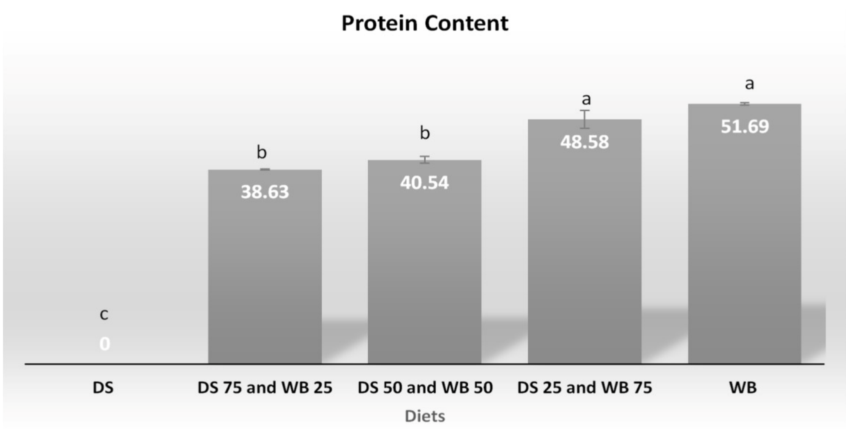
Fig 2: Total phenolic content of mealworm fed with varying ratios of date seed powder (DS) and wheat bran (WB).

be attributed to the nutritional composition of date seeds, which may lack certain essential amino acids or other nutrients necessary for optimal protein synthesis in mealworms (Langston *et al*., 2024). This underscores the importance of selecting appropriate feed ingredients to maximize the nutritional value of mealworm larvae, particularly for applications where high protein content is desired.

These results align with previous research highlighting the significant impact of diet composition on the nutritional quality of mealworms (Jankauskienë *et al*., 2024; Oliveira *et al*., 2024). The ability to manipulate protein levels through diet adjustments provides valuable insights for optimizing mealworm rearing practices, particularly for their use as a protein source in animal feed or human consumption.

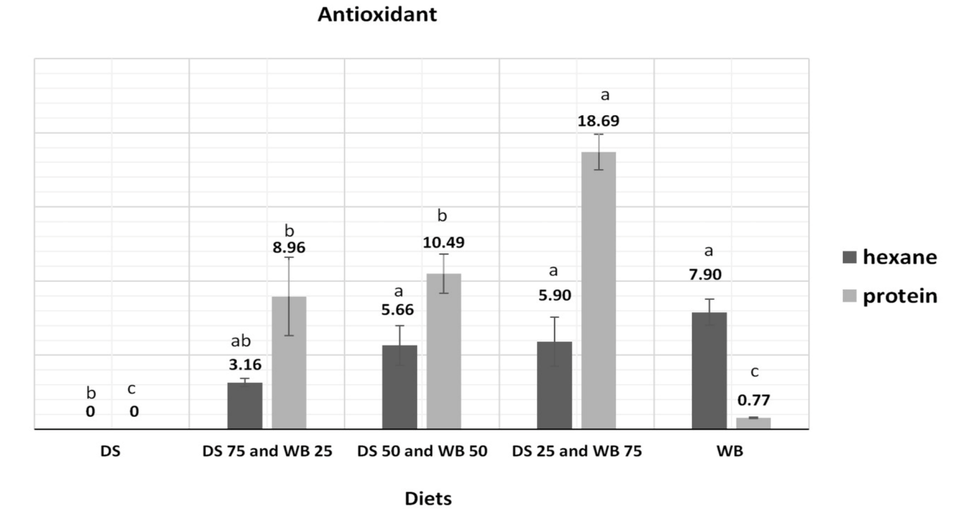
DPPH radical scavenging activity of hexane extract

The antioxidant potential of the samples was assessed using the DPPH method. Both the hexane extract and the protein extract displayed weak antioxidant activities. However, the protein extract exhibited higher activity compared to the hexane extract. Specifically, in the hexane extract, WB 100 demonstrated the highest antioxidant activity at 7.7%, whereas DS 75 and WB 25 showed the lowest antioxidant potential at 3.16%. Similarly, in the protein extract, DS 25 and WB 75 showed the highest antioxidant potential at 18.69% compared to other extracts, followed by DS 50 and WB 50 at 10.49% (Fig 4).



Treatments sharing the same letters indicate no significant difference, whereas treatments with different letters are considered significant at p<0.05.

Fig 3: Total protein contents of mealworm treated with varying ratios of date seed powder (DS) and wheat bran (WB).



Treatments with the same letters indicate no significant difference, while treatments with different letters are significant at p<0.05.

Fig 4: The percentage of DPPH inhibition (%) of hexane and protein extracts. The hexane extract data represent different ratios of date seed powder (DS) and wheat bran (WB).

No definitive pattern of antioxidant activity resulting from dietary supplementation was discernible. While the activity was notably low in the hexane extract, the protein extract exhibited greater potency. Interestingly, the highest WB100 ratio showed the lowest antioxidant activity in the hexane extract and the weakest in the protein extract. However, the antioxidant activity was the highest in DS25 and WB75. These findings suggest that the type of extract and the ratio of feed components significantly influence antioxidant activity. The higher antioxidant potential in the protein extracts of the DS 25 and WB 75 diets could be attributed to the synergistic effects of wheat bran and date seeds, which may enhance the availability or efficacy of antioxidant compounds in the protein fraction (Jankauskienë *et al*., 2024).

Proteins are antioxidants that can inhibit lipid oxidation through multiple pathways, including reduction of hydroperoxides, chelation of prooxidative transition metals, scavenging free radicals, inactivation of reactive oxygen species and alteration of the physical properties of food systems. How protein composition influences their ability to inhibit lipid oxidation is still poorly understood. Understanding the relationship between peptide composition and antioxidant activity could lead to the developing of a new class of antioxidants that could be used in many food applications (Elias *et al*., 2008).

This study demonstrates the potential of using date palm seeds as a substrate for rearing mealworm larvae, showing benefits in survival rates, weight gain, phenolic content and protein content. However, we need to conduct long-term and field studies, explore a broader range of organic waste materials and perform detailed nutritional and economic analyses. Addressing these areas will enhance the practical application and sustainability of using organic waste for insect rearing, contributing to better waste management and alternative protein production.

CONCLUSION

Date palm supplementation improved survival rates, with higher ratios showing better results. However, DS100 led to negative weight gain. DS 25 and WB 75 showed optimal weight gain. DS 25 and WB 75 also exhibited the highest concentration of phenolic compounds, potentially indicating antioxidant properties. Protein content varied across diets, with WB 100 having the highest concentration. Overall, diet composition significantly impacted the growth, survival and biochemical properties of mealworm larvae.

Authors' contribution

A. Nael and F.A. Al-Mekhlafi designed the study, conducted data analyses and Conducting experiments. A. Nael writing the manuscript. M.S. Al-Khalifa and M.A. Wadaan helped in writing the manuscript and conducted data analyses.

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Data availability statement

All the data is available within the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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