TIP Revista Especializada en Ciencias Químico-Biológicas, 29: 1-8, 2026.
https://doi.org/10.22201/fesz.23958723e.2026.789

Artificial Diet Development for *Tenebrio molitor* Under Laboratory Mass Rearing Conditions

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Abstract

Tenebrio molitor (Coleoptera: Tenebrionidae), commonly named yellow mealworm, far from simply being an important pest for stored grains, also has the potential to be used as a food source for animals and humans, for biodiesel production and more importantly as a valuable biological model for entomological research as it is possible to alter nutritional value and food habits of the larvae. The aim of this study was to develop an artificial diet for mass-rearing *T. molitor* larvae under laboratory conditions. For this, three artificial diets were assessed (fresh carrot as a control, gelled and encapsulated diet). Larval Weight Gain (LWG), Feed Conversion Rate (FCR), mortality rate, protein and moisture content into the flour were evaluated. The results showed that an encapsulated diet offers an LWG average of 0.209 mg but also presented the lowest FCR with 3.69 kg and the highest protein content with a 65.28 % in the composition of the insect. Not likewise the gelled diet whose results showed even a lower performance than the control (LWG=0.83 mg FCR=5.7 kg) with an LWG of 0.60 mg and FCR of 8.78 kg. With no significant difference (p=0.05) in either moisture or mortality, the encapsulated diet proved to be the most efficient food source for *T. molitor* mass-rearing larvae under laboratory conditions.
Keywords: coacervation, encapsulation, jellification, yellow mealworm.

Desarrollo de una dieta artificial para *Tenebrio molitor* bajo condiciones de cría masiva en laboratorio

Resumen

El gusano de la harina o *Tenebrio molitor* (Coleoptera: Tenebrionidae), es una plaga importante de los silos, su potencial está en ser un suplemento alimenticio para animales y humanos, utilizado en la producción de biodiésel y un modelo biológico muy valorado en la investigación entomológica por la capacidad de cambiar sus hábitos alimenticios y transformar los nutrimentos que consume al alimentarse. El objetivo de esta investigación se basó en el implemento de una dieta artificial, destinada a la cría masiva de larvas de *T. molitor*, consistente en: zanahoria fresca como control, una dieta encapsulada y otra gelificada. Se evaluó el Incremento de Peso Larval (IPL), el Índice de Conversión Alimenticia (ICA), los contenidos proteico y de humedad y la tasa de mortalidad. Al finalizar el experimento, se observó que con la dieta encapsulada la composición corporal de las larvas mostró un mayor IPL (209 mg), un menor ICA (3.69 kg), y mayor contenido de proteína (65.28 %). Por otro lado, con la dieta gelificada los resultados fueron menores a los obtenidos con el control (IPL=0.83 mg FCR=5.7 kg), un IPL de 0.60 mg y un FCR de 8.7 kg. De igual manera no se observó diferencia significativa (p=0.05) en la humedad ni en la tasa de supervivencia. La investigación demostró que la dieta encapsulada es la fuente de alimento más eficiente para la cría masiva de larvas de *T. molitor* en condiciones de laboratorio.
Palabras clave: coacervación, encapsulación, gelificación, gusano de la harina.

Artículo recibido el 25 junio del 2025.
Artículo aceptado el 03 de marzo del 2026.

Introduction

Insect class is the most diverse and widespread group around the world, representing nearly 66 % of the species in the animal kingdom (Jankielsohn, 2018). Not just for their role as pests causing losses up to 70 % of storage products, but also for contributing to pollinating over 75 % of the 111 most important crops worldwide (Miñarro-Prado, García-García & Martínez-Sastre, 2018). Additionally, insects have been used as biological models since the earlies of 20th century (Wilson-Sanders, 2011). In this sense, their high insect reproduction rate and handleability, had led them to take an important part in numerous disciplines and research areas, ranging from cleaner biodiesel production, insect-base flours and even as an innovative tool for ecological pest control (Djouadi, Sales, Carvalho & Raymundo, 2022).

Tenebrio molitor is a cosmopolitan insect, brought to America by European colonizers in the 15th century. With a lifespan of 10 weeks to five months this holometabolous species is well known as a secondary pest of stored grains such as soy, wheat, corn, and rice. More importantly the larval composition is up to 41 % protein (Gkinali, Matsakidou, Vasileiou & Paraskevopoulou, 2022). Their high fiber content along with other vitamins, minerals and amino acids make them a viable feed alternative for farm animals and even humans. Additionally, they can be used as a source for chitin and chitosan, bioactive extracts and compounds or even for biodiesel production. Rearing *T. molitor* has become so popular that only in the USA, its productions exceed the milk and poultry industries (Mariod, Mirghani & Hussein, 2017).

Meanwhile cattle rearing has a significant impact on greenhouse gas production, conversely, the mass-rearing of insects (MRI) provides an alternative for the protein industries exploding fewer resources and generating less waste. This technique has the potential to enhance the nutritional value for both livestock and humans (Gahukar, 2016). While insects can thrive on natural diets, artificial diets have significant potential to drastically improve larval quality and nutritional content (Moruzzo, Riccioli, Espinosa Diaz, Secci, Poli & Mancini, 2021).

Therefore, feed intake has played a significant role in MRI, making possible the reproduction with natural diets and even more complex feed matrices to ensure large-scale production. For this, growth conditions must be as similar as possible to natural conditions in a controlled environment. Consequently, initial approaches utilized natural diets, based on observations of insect dietary habits and adapting primarily to the mouthparts of the target species (Aceituno & Hernández, 2020).

Nevertheless, in some cases it is necessary to improve feed matrices by using artificial diets which are defined as a cheap, safe and accessible feed option, to guarantee healthy insects with enhanced performance for their targeted applications. (Huynh, Shelby & Coudron, 2021). According to Behmer (2009), the feed matrix primarily depends on the diet composition and secondarily, on its water content. These matrices can be classified into several types. A holidic diet is composed of chemically well-defined ingredients, while a meridic diet is formed from at least one well-defined ingredient. An oligidic diet, on the other hand, is composed of raw organic ingredients. Liquid diets are ideal for lepidopteran insects, facilitating cleaner rearing and reducing waste. However, they are prone to drying out before consumption, limiting their use to smaller-scale reproduction. Semi-solid diets are often used as starter diets; their high-water content efficiently dissipates heat produced in rearing chambers. Using agar as a gelling agent in semi-solid diets offers advantages over organic ingredients due to its low cost and antifungal properties. Additionally, solid diets use texturizers to provide a more stable structure for chewing mouthparts. In addition to mitigating metabolic heat accumulation caused by improper handling, solid diets promote healthier insect reproduction by reducing humidity within the colony.

Not only the nutrients to produce healthy subjects must be provided through any diet, but the water intake also plays a more important role for successful MRI. Therefore, encapsulation offers a solution for humidity control. Allowing for a more controlled release of moisture within the insect enclosure (Johnsen, Andersen & Offenberg, 2021). This technique consists of coating the feed matrix with a semi-permeable membrane that protects it from volatilization. Since the 1930s a lot of encapsulation methods have been evaluated, but simple coacervation has remained the most used procedure for coating essential oils, active ingredients or feed matrices in the pharmaceutical and food industries (Napiórkowska & Kurek, 2022). Simple coacervation consists of separating a polymer into tiny drops or "coarcervate" which will coat the matrix. As the polymer becomes insoluble, it will begin to deposit on the core wall, resulting in supersaturated capsules that harden at the appropriate pH or salinity levels (Agnihotri, Mishra, Goda & Arora, 2012). The objective of this research was to develop an artificial diet intended for *T. molitor* larvae mass-rearing under laboratory conditions.

Materials and Methods

Tenebrio molitor larvae

Neo-nate larvae (F3) were randomly selected from a mature colony established at the Laboratory of Natural Insecticidal Compounds at the Autonomous University of Querétaro for their further experimental treatments.

Artificial diets assessment

An encapsulated diet was prepared using a Nutritive solution (NS) formulated with 4.5 g of dry, milled and sterile carrot (*Daucus carota*), 7.5 g of grounded wheat (*Triticum* sp.), 1 g of yeast (*Saccharomyces cerevisiae*) (MDC Labs®, Mexico

City, México), methyl 4-hydroxybenzoate, monopotassium phosphate, ascorbic acid, formaldehyde (Golden Bell®, México City, México), neomycin trisulfate salt hydrate (Merck®, Darmstadt, Germany) and a commercial B complex pill (Farmacias del Ahorro®, Jalisco, México). All ingredients were mixed in 50 mL of distilled water (Ecopura®, Querétaro, México). The mixture was stirred in a magnetic stirring plate until it became a homogenous solution, prior to be stored at four °C in the fridge. Additionally, a 0.08 % w/v sodium alginate solution (Golden Bell®, México City, México) was prepared in 150 mL of distilled water and stirred with a magnetic stirrer. Once homogeneous, the NS was mixed forming an alginate-NS matrix which was dripped into a 2 % saturated calcium chloride solution for 15 min to obtain the diet capsules (Golden Bell®, México City, México). Furthermore, the diet capsules were washed three times with distilled water.

A gel diet was also prepared and assessed. The feed matrix was formulated by milling 100 g of wheat and 75 g of dried and sterile carrot. To this, potassium phosphate and yeast were added. This mixture was stirred using a magnetic stirrer along with 150 mL of distilled water until homogeneous. Subsequently, a 1 % dextrose solution was prepared as described by Cerna-Mendoza, Coronado, Doria-Bolaños, García-Gonzáles & Fachin-Ruiz (2021), to which ascorbic acid, neomycin and a commercial B complex pill were added. Following this, 40 % formaldehyde and ethanol (Ecopura®, Querétaro, México) were incorporated. Simultaneously, 12 g of agar-agar (BD Bioxon®, México City, México) were dissolved in 250 mL of distilled water, stirred and heated to 80 °C on a hot plate. The wheat and carrot mixture was then added to the agar solution and the heat was turned off. When the temperature dropped to 45 °C, the dextrose solution was added while stirring. Once thoroughly mixed, the solution was placed in refrigeration to set.

For the assessment, three replications of 30 larvae were used for each treatment. A control group was also evaluated, consisting of fresh and unpeeled carrots which were replaced every 48 h until the end of the experiment. Each group received 1 g of diet, which was changed and weighed every 48 h to record food intake. Additionally, larvae weight was recorded every 10 d. This methodology allowed to evaluate the FCR (Equation 1), which represents the feed intake per unit of body weight gained (Deruytter & Coudron, 2021). Furthermore, the mortality rate was also evaluated. After 100 d of experimentation, larvae reached the ninth instar. Subsequently, they were maintained under starvation conditions for 24 h, recording their final weight prior to their preservation at 0 °C in a freezer until milled.

Crude protein and humidity determination of insect meal

In order to determine the nitrogen, crude protein and humidity of the insect, a larvae meal was prepared according to the methodology described by Melgar-Lalanne, Hernández-Álvarez & Salinas-Castro (2019). *T. molitor* larvae were rinsed sequentially with distilled water during 1, 10 and 5 min. After each rinse, the larvae were allowed to settle for 5 min. Subsequently, they were dried in an oven (Norsa®, México City, México) at 55 °C for 24 h and then milled to obtain the *T. molitor* flour. The crude protein content of the flour was determined according to the AOAC (2002) 2001.11 official method (Equation 2 and 3). On the other hand, the humidity content was also analyzed following the NOM-147-SSA1-1996 guidelines (Equation 4 and 5) to obtain the dry matter content (Equation 5).

$$FCR = \frac{Feed\ intake}{Weigth\ gain}$$

Equation 1. FCR equation (Bordiean, Krzyżaniak & Stolarski, 2022).

$$N\% = \frac{(V_S V_B) \times M \times 14.01}{W \times 10}$$

Equation 2. Nitrogen determination equation according to AOAC.

$$Crude\ protein = N\% \times F(6.25)$$

Equation 3. Crude protein determination equation according to A.O.A.C.

$$\%Humidity = \frac{(Fresh\ weight(g) - Dry\ weight(g)) \times 100}{Sample\ weight(g)}$$

Equation 4. Flour humidity determination equation according to NOM-147-SSA1-1996.

$$\%Dry\ matter = 100 - \%Humidity$$

Equation 5. Dry matter equation.

Statistical analysis

Data from the experiment was subjected to a one-way analysis of variance (ANOVA) to identify statistically significant differences between treatments ($p<0.05$). Subsequently, a Tukey test was performed to compare the treatment groups to the control group.

Results

Larval Weight Gain

During the initial 10 d of experiment, the gel diet exhibited the lowest increase larval weight gain, showing a 42.86 % decrease compared to the control treatment (Figure 1). Subsequently, by day 30, the encapsulated diet showed a significant increase in larval weight, exhibiting a 46.88 % relative to the control. The

https://doi.org/10.22201/fesz.23958723e.2026.789

encapsulated diet maintained the highest overall increase after 100 d (Figure 2), which achieved a 138 % increase in larval weight compared to the control treatment. In contrast, the gel diet had the lowest larval weight gain, which resulted in a 25 % decrease compared to the control.

Feed Conversion Rate

Regarding the FCR, the control treatment exhibited the lowest value after the initial 10 d, differing significantly from both the encapsulated and gel diets (Figure 3). By day 20, the encapsulated diet began to show an FCR similar to the control treatment. However, it was until day 30 that the encapsulated diet demonstrated a lower conversion rate compared to the other treatments, a trend that persisted until the end of the trial, where it maintained the lowest values. At the end of the experiment, the encapsulated diet showed the significantly lowest conversion rate, indicating that larvae fed with this diet require less diet to gain weight. Conversely, the gel diet generally showed the highest FCR values across most measurement points.

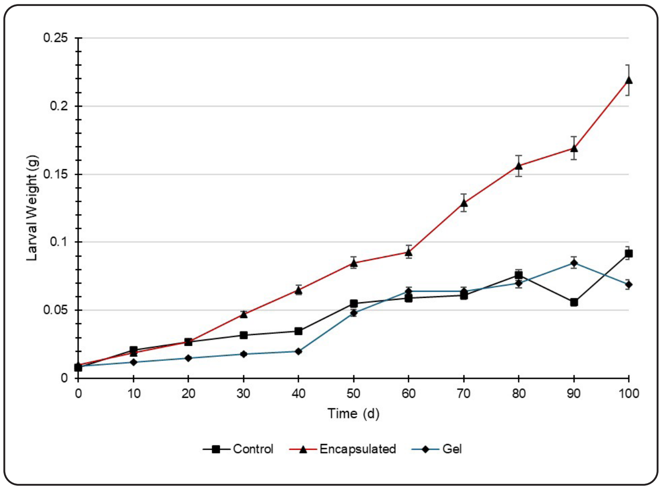

Figure 1. ***Tenebrio molitor*** **larval weight gain. Data is the average of 90 measurements ± standard error.**

Tenebrio molitor **larvae viability**

Throughout the experimental period, no significant differences in larval viability were observed between any of the treatments (Figure 4). However, at the conclusion of the experiment, the encapsulated diet demonstrated the highest viability with a 90 %, followed by gel diet with 88.9 %, and finally, the control treatment with 87.8 %.

Tenebrio molitor **flour protein analysis**

Following the assessment of the artificial diets, the nitrogen and crude protein content of the insect meals were determined (Table I). The encapsulated diet exhibited statistical similarity regarding the nitrogen content, showing a slight increase of 3.72 % compared to the control. In contrast, the gel diet demonstrated a significant decrease, with a reduction of 64.49 % compared to the control diet. Regarding protein

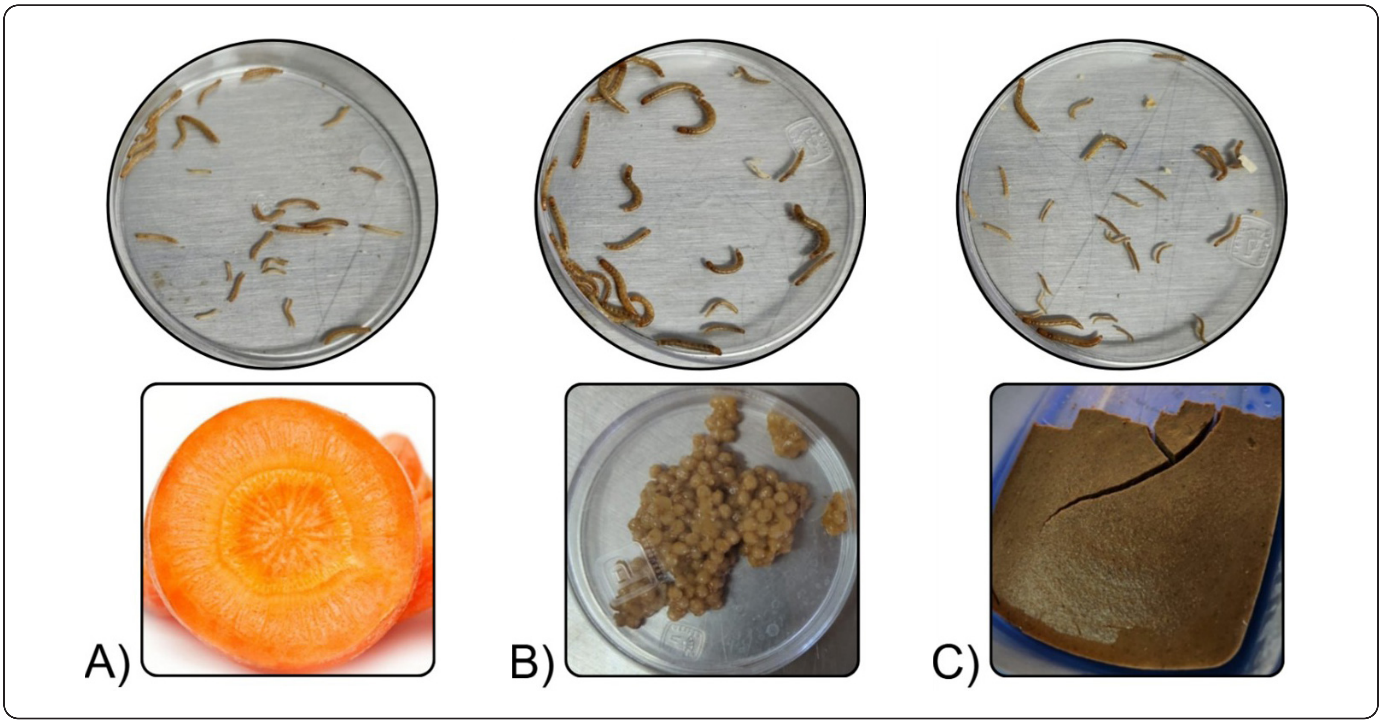

Figure 2. Larval growth of *Tenebrio molitor* at 100 d fed with three different synthetic diets. A) Control, B) Capsules and C) Gel.

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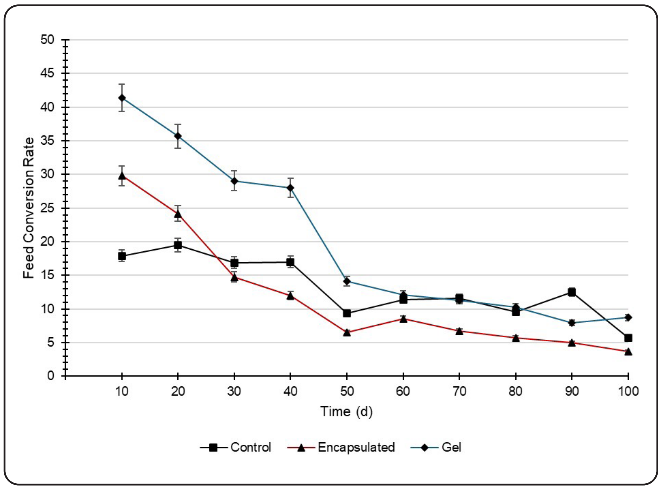

Figure 3. *Tenebrio molitor* larvae FCR fed with artificial diet. Data is the average of 90 measurements ± standard error.

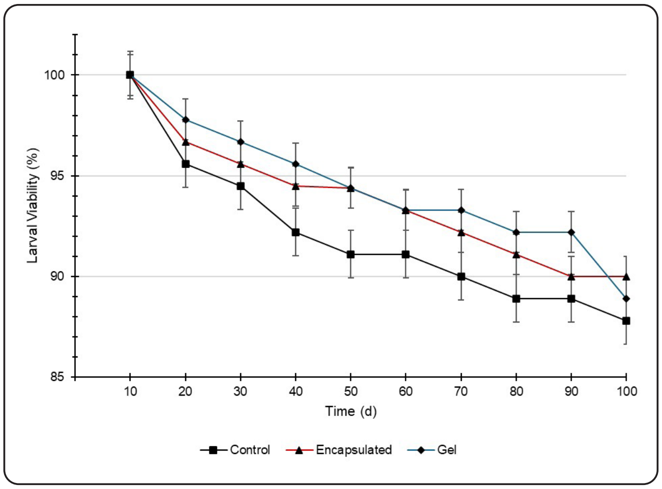

Figure 4. *Tenebrio molitor* larval viability. Data is the average of 90 measurements ± standard error.

content, the encapsulated diet did not differ significantly from the control, but it showed a 3.20 % increase. Conversely, the gel diet presented the lowest protein content, being 64.48 % lower than the control diet.

Dry matter content in *T. molitor* flour

After analyzing the humidity content of the insect flour from all diet treatments, the gel diet proved most effective at producing solid mass (Table II). It showed significant increases compared to the other diets, being 9.19 % higher than the control value. In contrast, the encapsulated diet produced the lowest dry matter, with a 1.43 % decrease compared to the control diet.

Table I. *Tenebrio molitor* flour nitrogen and protein content.

Treatment	% Nitrogen (g/100 g)	% Protein (g/100 g)
Control	10.39 ± 0.54^{A}	64.99 ±3.41^{A}
Capsules	10.73 ± 0.34^{A}	67.07 ± 2.13^{A}
Gel	3.69 ± 0.59^{B}	23.09 ± 3.69^{B}

Data is the average of 3 determinations ± standard deviation. Different letters indicate significant differences between treatments.

Table II. *Tenebrio molitor* flour dry matter content.

Treatment	Dry matter %
Control	29.857 ± 0.002^{B}
Capsules	29.433 ± 0.061^{C}
Gel	32.601 ± 0.008^{A}

Data is the average of 3 determinations ± standard deviation. Different letters indicate significant differences between treatments.

Discussion

The encapsulated diet showed itself to be the most effective diet for larval weight gain among all the treatments. There are research insights about the assessment of different ingredients in diets for *T. molitor*. In this sense, Zim, Sarehane & Bouharroud (2022) evaluated different crop residues with wheat bran and found that the diet composed of 50 % citrus and 50% wheat bran had a weight gain of 0.014 g after 45 d. While the treatments in the present study showed a higher weight gain, being the encapsulated more than four times effective than the reported in the previous work. Furthermore, Rumbos, Bliamplias, Gourgouta, Michail & Athanassiou (2021) assessed various diets based on lupine, triticale, oat and alfalfa, where they observed a weight gain of 0.130, 0.108, 0.090 and 0.080 g, respectively. While in the present study, the weight gain of all the treatments was lower at 30 d. The encapsulated diet was almost half effective compared to the alfalfa-based diet reported by Rumbos *et al.* (2021). Thus, the encapsulated diet can be used for *T. molitor* larvae weight gain. However, future research can explore different formulations to enhance larval weight gain.

Overall, the encapsulated diet yielded the lowest FCR throughout the experimental period. This diet required two kg less food than fresh carrots to gain one kg in larval mass. Other studies have reported varying FCR values based on different dietary constituents. For instance, Rumbos, Karapanagiotidis, Mente, Psofakis & Athanassiou (2020) evaluated cereal flours, legumes and cereal commodities, to name a few, on

T. molitor larval development. They observed that wheat bran resulted in the highest FCR (14.9) among all assessed feeding substrates after a 70 d trial. This value is more than twice the FCR obtained in this study. Furthermore, Bordiean *et al.* (2022) found that *T. molitor* larvae fed a 3:1 mixture of rapeseed meal and chicken feed exhibited an FCR of 1.53 until pupation (82 d). This value is more than three times lower than the observed in our study at 80 d, indicating greater efficiency in converting feed into larval mass. The encapsulated diet described herein demonstrated its utility for *T. molitor* larval weight gain. However, incorporating constituents identified in previous research could enhance its efficiency.

Throughout the entire experimental period, no significant differences in larval viability were observed between treatments. This was an expected outcome, as it is desirable that the diet does not induce mortality in the tested population. Mahmoud, Abotaleb & Zinhoum (2025) observed a similar behavior with the evaluation of diet consisting of chickpea flour, wheat bran and yeast where they observed a viability of 89.46 % until the larvae pupation. This treatment had no significance compared to the control group (wheat bran). Also, this survival rate is very similar to the obtained in this study. In the other hand, there are dietary constituents that have proven to be deleterious in the development of *T. molitor* larvae. In this sense, Mlček *et al.* (2021) demonstrated the diets based of raw potatoes, dry buttermilk and wheat bran had survival rated of 75, 60 and 55 %, respectively. These findings suggest that all the diets tested in this study maintained the viability of this insect.

Regarding the protein content on the *T. molitor* flour, the encapsulated diet showed the highest protein yield (67.7%). Other dietary components have shown slightly lower protein development on this insect. In this sense, a diet composed of wheat flour, chicken feed, apple and carrot peels exhibited a protein yield of 51.20 % on *T. molitor* larvae flour (Djouadi *et al.*, 2022). Similarly, López-Gámez, del Pino-García, López-Bascón & Verardo (2024) found a protein content of 50.19 % generated on *T. molitor* larvae fed with a diet composed of ecological tomatoes and wheat bran in a 1:1 ratio. On the other hand, Harsányi *et al.* (2020) evaluated different organic wastes as feeding substrates for *T. molitor*, In their study, chicken feed was used as control and produced a crude protein content of 47.18 %. Nonetheless, the chicken feed mixed with vegetable waste (mixed peels of 10 % onion, 10 % cucumber, 25 % potato, 25 % sweet potato and 30 % carrot) in a 1:9 ratio yielded a 46.30 % of crude protein content, being the nearest value compared to the control.

The gel diet showed the highest dry matter production in *T. molitor* meal (32.60 %). In comparison, Polovinski-Horvatović *et al.* (2024) obtained dry matter contents of 90.88 % and 91.86 % of larvae fed with wheat bran and oat flakes, respectively, nearly three times higher than the obtained by the gel diet in this study. By the other way, Kröncke & Benning (2023) assessed different flours with wheat bran in comparison with wheat bran solely as control. They observed that the mixture of cassava flour (10 %) and wheat bran (90 %) yielded the highest dry matter with 41.6 %, while the lowest value was produced by the mixture of pea protein flour (80 %) and wheat bran (20 %) which reached a 19.7 % of dry matter. In comparison, the wheat bran solely had a 31.4 % yield of dry matter, the nearest value compared to the obtained in the present study.

According to the findings of the present study and insights from previous works, the varying dietary formulations significantly influenced *T. molitor* larval development. Notably, the encapsulated diet consistently yielded the highest larval weight gain, FCR and crude protein content in the insect flour. However, all tested diets showed no significant differences in larval viability, which was an expected outcome. Conversely, while the gel diet exhibited the highest dry matter percentage, it was associated with the lowest protein content. Consequently, the encapsulated diet demonstrated significant potential as an optimized rearing diet for *T. molitor.*

Conclusions

Two artificial diets were successfully developed for *T. molitor* mass-rearing under laboratory conditions. Among these, the encapsulated diet represented the best alternative for this purpose. This diet resulted in higher larval weight gain compared to the gel diet and the control. Additionally, it showed a lower FCR, representing higher efficiency in converting feed into biomass. However, both the gel and encapsulated diets were statistically equal to the control in terms of larval viability. Moreover, the encapsulated diet was comparable to the control regarding the protein content of the *T. molitor* flour. Finally, the encapsulated diet showed the lowest dry matter content, while the gel diet showed a higher content compared to the control.

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