Nutritive Value of Wheat Bran Diets Supplemented With Fresh Carrots and Wet Brewers’ Grains in Yellow Mealworm

B. Fasce, L. Ródenas, M. C. López, V. J. Moya, J. J. Pascual, and M. Cambra-López

1Feedect, Calle de la Buitrera, 6, 46180 Valencia, Spain, 2Institute of Animal Science and Technology, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain, and 3Corresponding author, e-mail: macamlo@upvnet.upv.es

Abstract

We evaluated the effect of supplementation of a wheat bran (WB) diet with fresh carrots (FC) or wet brewer’s grains (WBG) on the growth performance and chemical composition of Tenebrio molitor larvae. Additionally, a digestibility trial was performed to determine the nutritional value of the raw materials used. The control diet was based on WB-only. Two other diets were formulated in which WB was supplemented with FC (FC diet) or with WBG (WBG diet). The experiment was conducted in trays (12 per treatment) and lasted 90 d. Larval weight, feed intake, and excreted feces were controlled in each tray the experiment. The digestibility trial was performed from 48 to 62 d post-hatch. Results showed digestibility coefficients of ashes, crude protein, and gross energy were significantly higher in FC diet compared with the other diets. Consequently, both digestible energy (DE) and digestible protein (DP) contents of FC were also significantly higher than those obtained for WB and WBG diets (on av. +1.12 megajoule [MJ] DE and +9.15 g DP per kg dry matter [DM]; P < 0.003). Mealworms fed FC diet showed significantly higher final weight and average daily gain than those fed the WBG diet (+12.4%), being higher in WBG than in WB diet (+3.5%). Dietary treatment did not affect DM, ashes, ether extract, and crude protein content of mealworms obtained. Some dietary effects on amino acid and fatty acid composition were observed. This study provides novel data and a unique experimental approach to assess the nutritional value of raw materials in mealworms.

Key words: digestibility, feeding, growth, insects, protein

The global demand for animal protein will be double in 2050 (FAO 2006). Production of animal protein has a high environmental footprint (IPPC 2019), and thus, there is need to search for alternative protein sources which can co-exist with the main sources of protein used nowadays. van Huis (2020) reviewed the potential of insect products to reduce the major environmental burdens associated with the global food system. Insect species have an adequate nutritional and amino acid profile to be used in human and animal nutrition (Ramos-Elorduy et al. 1997, Agbidye et al. 2009, Rumpold and Schlüter 2013). Payne et al. (2016) reported crickets and mealworms have a nutritional value similar to beef, chicken, or pork meat.

Yellow mealworm, Tenebrio molitor L. (Coleoptera: Tenebrionidae), is a small beetle that can be reared for animal protein production. Tenebrio molitor has a high protein content (approximately 550 g/kg) and possesses a balanced amino acid and lipid profile, rich in mono- and polyunsaturated fatty acids (Oonincx et al. 2015, van Broekhoven et al. 2015, Shokooh et al. 2018).

Tenebrio molitor is omnivorous, like more than other 40 families of insects (Coll and Guershon 2002). It is a usual pest in stored grains and milled products and hence can feed on cereal meal, flour, bran, and grains (Ghaly and Alkoak 2009). Most mealworms producers use wheat bran (WB) for feeding these insects. This by-product fits adequately their feeding behavior and is usually self-selected by T. molitor to balance their nutritional requirements (Morales-Ramos et al. 2011). Besides, WB is easy to obtain and manage and can be found at a low price. However, feeding T. molitor with a single food increases the risk of nutritional imbalances that may limit their growth. Therefore, it is frequently recommended to supplement adult and larval diets with additional fresh feed (Baek et al. 2015, Deruytter et al. 2020), like chopped vegetables (carrots and potatoes, amongst others) and yeast, which can provide them with additional water and complementary amino acids, trace elements, and vitamins.

In this framework, there is need to develop complete diets that can fulfill all T. molitor larvae nutritional requirements. Some authors have demonstrated that the quantity and quality...
of protein (Morales-Ramos et al. 2010, van Broekhoven et al. 2015) and starch (Mereiles et al. 2009) can impact on the growth of mealworms. To optimize growth and adjust feed formulations for mealworms, it is essential to determine the nutritional value of the most common ingredients (raw materials) used in their diets. However, to the best of our knowledge, the nutritional value of feed ingredients in mealworms has seldom been studied and there are no digestibility studies evaluating different raw materials for these insects.

The main aim of this work was to evaluate the adequacy of supplementing mealworm’s diets based on a single raw material (using WB) with additional fresh food (fresh carrots, FC and wet brewers’ grains, WBG). To this end, we evaluated the effect of iso-nutritive supplementation of a WB diet with FC or WBG on the growth performance and chemical composition of larvae. Additionally, we designed and conducted an experimental approach to assess the nutritional value of raw materials in mealworms for the first time. A digestibility trial was performed to determine the nutritional value of the diets and the raw materials used in this study.

Material and Methods

Insects

*Tenebrio molitor* imagos and their larvae used in this work were obtained from one generation; bred and supplied by Feedect Entogroup, S.L. (Benaguasil, Valencia, Spain). Insects were reared on WB substrate, in polystyrene plastic trays kept in an environmental room at a temperature from 26 to 29°C and a relative humidity from 50 to 70%. Air was constantly renovated using ventilator fans and the room was kept dark except during feeding or other maintenance activities.

Raw Materials and Diets

WB, FC, and WBG were used to formulate the experimental diets. Table 1 shows the chemical composition of these raw materials. All raw materials were obtained at the start of the experiment from a single batch. WB bags were stored at room temperature, whereas FC and WBG were weighed and packed in daily doses and stored at −20°C.

Experimental diets were formulated from the chemical composition of the raw materials shown in Table 1. The control diet was a simple diet based on WB-only. From this diet, two other diets were formulated in which WB was supplemented with FC (FC diet) or with WBG (WBG diet). All diets were formulated to be iso-nutritive.

Table 2 shows similar dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and starch contents of the experimental diets. Fresh water was daily added to WB and WBG diets, so that the amount of water supplied was similar amongst treatments. Water was sprayed onto each tray. The amount of water added generated no mold growth. The average detailed chemical composition of the experimental diets is presented in Table 2. On average, diets presented DM, CP, and ADF content of 148, 140, and 122 g/Kg DM, respectively, as well as 19.0 MJ of gross energy (GE) per kg DM. The protein level used in our study was close to the dietary protein level recommended for mealworms in van Broekhoven et al. (2015).

### Experimental Procedure

Initially, a total of 12 polystyrene plastic trays were used for egg laying. To start the rearing process, 150 g of fertile imagos (7 d old from their emergence from the pupae) were introduced into each clean plastic tray along with 215 g of WB. Then, 20 g of FC were daily supplied. Every 7 d for 21 d (3 egg extractions), wheat bran with the eggs of the imagos was separated by means of manual shaking and sieving using a galvanized steel mesh (3,068 mm mesh size, Central de Enrejados, Spain), obtaining three trays with eggs from each tray containing the imagos. After the three-imago egg laying periods, imagos were frozen at −20°C.

Using this experimental approach, a total of 36 trays with eggs were obtained in two batches. The larval growth period lasted 90 d. All trays with their eggs and WB, were daily sprayed with water for 24 d until the larvae were large enough (2–4 mm) to start the administration of the experimental diets. From that moment, the experimental diets were provided. The 36 trays were distributed among the three dietary treatments (12 per treatment), including in each treatment 4 trays of each laying period (first, second, and third). Diets were fed during these 90 d as described in Table 3.

Performance traits were controlled once the feed had been almost completely consumed by the mealworms. During the experiment, the excreted feces were removed from the trays by means of a screening process and the exuvia (containing chitin amongst others) was separated using a fan. Total weight of the existing larvae, weight of the excreted feces, and weight of the refusal of feed (not consumed feed, used to calculate feed intake) in each tray

### Table 1. Chemical composition of the evaluated raw materials (dry matter [DM] basis; g/kg DM)

| Chemical composition      | Wheat bran | Fresh carrots | Wet brewers' grains |
|---------------------------|------------|---------------|---------------------|
| Dry matter                | 900        | 90            | 136                 |
| Ashes                     | 61.8       | 88.9          | 46.0                |
| Crude protein             | 171        | 109           | 284                 |
| Ether extract             | 51.6       | 7.5           | 80.4                |
| Starch                    | 123        | 103           | 80                  |
| Neutral detergent fiber   | 490        | 128           | 526                 |
| Acid detergent fiber      | 142        | 94            | 166                 |
| Acid detergent lignin     | 30.2       | 0.0           | 24.4                |
| Total dietary fiber       | 488        | 259           | 437                 |
| Soluble fiber             | 53         | 141           | 0                   |
| Gross energy (KJ/kg DM)   | 19.1       | 17.3          | 21.0                |

**Diets**

| Chemical composition      | WB          | FC          | WBG         |
|---------------------------|-------------|-------------|-------------|
| Dry matter (DM)           | 414         | 425         | 406         |
| Ashes                     | 61.8        | 65.2        | 61.0        |
| Crude protein             | 154         | 140         | 151         |
| Ether extract             | 51.6        | 46.1        | 53.1        |
| Starch                    | 123         | 121         | 121         |
| Neutral detergent fiber   | 490         | 445         | 492         |
| Acid detergent fiber      | 142         | 136         | 143         |
| Acid detergent lignin     | 30.2        | 26.5        | 29.9        |
| Total dietary fiber       | 488         | 460         | 485         |
| Soluble fiber             | 53.0        | 63.9        | 50.3        |
| Gross energy (KJ/kg DM)   | 19.1        | 18.9        | 19.2        |

WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer's grains, and water.
Chemical Analysis

Raw materials and experimental diets were analyzed for DM, ashes, CP, ether extract (EE), starch, neutral detergent fiber (NDF), ADF, and GE. Feces were analyzed for DM, ashes, CP, NDF, ADF, and GE. Larvae were analyzed for DM, ashes, EE and CP, as well as for starch, protein, and GE. Feces were analyzed for DM, ashes, CP, NDF, ADF, and GE. The intermediate interval was chosen because it was assumed to be representative of the growing period and of the digestive/feeding performance of the larvae. A total of 30 trays were used (10 trays per treatment). Diets were offered on day 48 within the experiment and 14 d after the last weighing day, the larvae obtained were weighed, sampled, grounded, and stored until further analysis.

The amino acid content was determined after acid hydrolysis with hydrochloric acid 6 N at 110°C for 23 h as previously described by Bosch et al. (2006), using a Waters (Milford, MA) HPLC system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters), and a temperature control module. Aminobutyric acid was added as internal standard. The amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18 reverse-phase column Waters AcQ Tag standard after hydrolyzation. The amino acids were identified by comparing their retention times with those of a pat-tern of authentic standards.

The fatty acid methyl esters of the samples were analyzed in a gas chromatograph Focus gas chromatograph (Thermo, Milan, Italy) equipped with a split/splitless inlet and a flame ionization detector. Separation was performed on a capillary column SPTM 2560 (Supelco, PA) (100 m × 0.25 mm × 0.2 mm film thickness) with a flow rate of 1.1 ml Helium min⁻¹, according to the following temperature gradient: 140°C initial temperature for 5 min, gradually increasing along a linear gradient of 4°C min⁻¹ to 240°C, maintaining this temperature for 30 min, to finally return to the initial conditions. The injector and detector were maintained at 260°C. Fatty acids were identified by comparing their retention times with those of a pattern of fatty acid methyl esters (47885-U) from Supelco (PA, USA) and quantified using C13:0 as internal standard (O’Fallon et al., 2007). Total saturated, monounsaturated, polyunsaturated, and unsaturated fatty acids were calculated as saturated fatty acid (SFA) = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0, monounsaturated fatty acids (MUFA) = C14:1 + C16:1

Table 3. Daily provision of ingredients per tray of the experimental diets

| Daily provision (g/d) | WB | Water | WB | FC | WB | WBG | Water |
|-----------------------|----|-------|----|----|----|-----|-------|
| 0–24 d                | 4.5| 0.0   | 4.5| 0.0| 4.5| 0.0 | 0.0   |
| 24–34 d               | 4.8| 3.6   | 4.5| 4.0| 4.5| 1.6 | 2.3   |
| 34–41 d               | 3.3| 10.8  | 4.5| 12.0| 4.5| 3.0 | 8.3   |
| 41–48 d               | 5.6| 16.3  | 4.5| 18.0| 4.5| 5.0 | 12.1  |
| 48–62 d               | 21.1| 32.5 | 18.9| 36.0| 18.9| 9.0 | 25.0  |
| 62–69 d               | 49.6| 65.1 | 45.0| 72.0| 45.0| 18.3| 49.7  |
| 69–76 d               | 58.4| 86.8 | 52.1| 96.0| 52.1| 24.0| 66.7  |
| 76–83 d               | 78.9| 108.5| 71.4| 120.0| 71.4| 30.0| 83.3  |
| 83–90 d               | 152.0| 130.2| 142.9| 144.0| 142.9| 36.5| 99.6  |
| Global: 0–90 d        | 32.2| 37.9 | 29.6| 41.9| 29.6| 10.6| 29.0  |

WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer’s grains, and water.
Table 4. Apparent fecal digestibility coefficients, expressed per gram of nutrient ingested, of main nutrients for the experimental diets in Tenebrio molitor larvae from 48 to 62 d post-hatch (n = 30 trays; degrees of freedom: 2 for diets and 1 for batch)

| Nutrient             | WB    | FC    | WBG   | SEM   | F-value | P-value |
|----------------------|-------|-------|-------|-------|---------|---------|
| Dry matter (DM)      | 0.390 | 0.406 | 0.368 | 0.006 | 12.01   | <0.001  |
| Ashes                | 0.095 | 0.146 | 0.053 | 0.009 | 26.71   | <0.001  |
| Crude protein        | 0.399 | 0.471 | 0.373 | 0.011 | 20.06   | <0.001  |
| Neutral detergent fiber | 0.139 | 0.147 | 0.144 | 0.009 | 0.84    | 0.4432  |
| Acid detergent fiber | 0.062 | 0.053 | 0.052 | 0.013 | 0.19    | 0.8251  |
| Gross energy         | 0.399 | 0.458 | 0.384 | 0.005 | 54.35   | <0.001  |
| Nutritive value      |       |       |       |       |         |         |
| Digestible energy (MJ/kg DM) | 7.61  | 8.61  | 7.38  | 0.10  | 42.65   | <0.001  |
| Digestible protein (g/kg DM) | 68.4  | 76.0  | 66.5  | 2.0   | 7.51    | 0.0027  |

 WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer’s grains, and water (see Table 3). SEM: standard error of the means.

Means not sharing letter for the same period were significantly different at P < 0.05.
and threonine, but higher tyrosine (−2, −15, −15 and +2%, respectively; \(P < 0.05\)). As regards fatty acid composition, body fat of mealworms fed the FC diets was characterized by the highest MUFA levels (+2.2 percentage units; \(P < 0.05\)), mainly due to their higher content on C16:1, and specially C18:1n9c content (+2.1 percentage units; \(P < 0.05\)). On the contrary, both WB and WBG showed the

Fig. 1. Effect of dietary treatment on daily feed intake per tray of *Tenebrio molitor* larvae. Each tray included the eggs generated by 150 g of fertile imagos for one week. WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer’s grains and water (see Table 3). Standard error of the mean: 0.23 g dry mater (DM)/tray day. **Means not sharing letter for the same period were significantly different at \(P < 0.05\).

Table 5. Larval weight (g/tray), average daily gain (g/tray d), and feed conversion rate (g DM/g larvae) of *Tenebrio molitor* larvae from 48 to 62 d post-hatch with the different experimental diets (n = 36 trays; degrees of freedom: 2 for diets, 5 for days post-hatch, and 165 for dietary comparison at each day post-hatch)

|             | WB       | FC       | WBG       | SEM | F-value | \(P\)-value |  
|-------------|----------|----------|-----------|-----|---------|-------------|  
| Larval weight |          |          |           |     |         |             |  
| 48 d        | 45.37    | 65.84    | 52.61     | 4.41| 1.26    | 0.0574      |  
| 62 d        | 119.1a   | 154.1b   | 125.9a    | 9.4 | 1.55    | 0.0013      |  
| 69 d        | 224.9b   | 267.4a   | 236.4b    | 10.3| 1.72    | <0.001      |  
| 76 d        | 354.3a   | 414.9b   | 372.5a    | 14.1| 1.72    | <0.001      |  
| 83 d        | 519.2a   | 603.9b   | 541.3a    | 20.9| 1.72    | <0.001      |  
| 90 d        | 760.7a   | 884.6c   | 787.0b    | 32.5| 1.72    | <0.001      |  
| Average daily gain |          |          |           |     |         |             |  
| 0–48 d      | 0.945a   | 1.372c   | 1.096b    | 0.026| 1.72    | <0.001      |  
| 48–62 d     | 5.264b   | 6.307a   | 5.233c    | 0.307| 1.35    | 0.0189      |  
| 62–69 d     | 15.12    | 16.18    | 15.79     | 0.72 | 1.07    | 0.3085      |  
| 69–76 d     | 18.50b   | 21.07b   | 19.45a    | 0.80 | 1.31    | 0.0309      |  
| 76–83 d     | 23.53    | 27.00    | 24.11     | 1.62 | 1.17    | 0.1402      |  
| 83–90 d     | 34.50b   | 40.10b   | 35.10b    | 1.96 | 1.29    | 0.0419      |  
| Global: 0–90 d | 8.452a | 9.828c   | 8.745b    | 0.080| 1.52    | 0.0021      |  
| Feed conversion ratio |          |          |           |     |         |             |  
| 0–48 d      | 4.54c    | 3.26a    | 3.84b     | 0.07 | 1.72    | <0.001      |  
| 48–62 d     | 3.44     | 3.22     | 3.41      | 0.14 | 1.08    | 0.2893      |  
| 62–69 d     | 2.96     | 2.91     | 2.80      | 0.13 | 1.04    | 0.4032      |  
| 69–76 d     | 2.83     | 2.63     | 2.63      | 0.12 | 1.10    | 0.2514      |  
| 76–83 d     | 3.00     | 2.81     | 3.04      | 0.21 | 1.03    | 0.4302      |  
| 83–90 d     | 3.44     | 3.21     | 3.29      | 0.23 | 1.01    | 0.4801      |  
| Global: 0–90 d | 3.19b | 2.92c    | 2.99b     | 0.07 | 1.37    | 0.0149      |  

WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer’s grains and water (see Table 3). SEM: standard error of the means.

\* a, bMeans not sharing letter for the same period were significantly different at \(P < 0.05\).
highest PUFA, n-6, and PUFA/SFA levels (on av. +2.2 and +2.1 percentage units and +0.10 ratio points, respectively; \( P < 0.05 \)), mainly due to their higher content on C18:2n6c (on av. +2.1 percentage units; \( P < 0.05 \)).

### Discussion

The digestive physiology of mealworm’s is very similar to that of other Coleoptera larvae (Terra et al. 1985). The digestion of the
main carbohydrates begins in the foregut, where there is an optimal secretion of carbohydrases, and the digestion of proteins is only relevant in the posterior region of the midgut. As far as we known, there are no previous studies where the digestibility of feed has been evaluated in mealworms.

In general terms, the digestibility coefficients obtained in this study are low, with an average DM digestibility of 0.39. The low DM digestibility obtained in mealworms may be related to the fact that approximately half of the ingested feed is fiber (476 g NDF/kg DM), being the digestibility coefficient of this fibrous fraction considerably low (0.15). In fact, the NDF digestibility of WB is higher in monogastric animals compared with mealworms (0.40–0.44 in pigs, Chabauti et al., 1991; 0.25–0.43 in rabbits, Blas et al., 2000).

Cellulose degradation capacity of many insects seems to be limited and only comes from fungi, which can colonize insect’s gut (Martin, 1983). Terra et al. (1983) observed a very low cellulolytic activity in mealworms fed WB. The breakdown of cellulose is mainly restricted to the anterior part of the digestive tract, shown to be related with the activity of intrinsic cellulases produced through fermentative symbioses with microbes and fungi (Karasov and Douglas, 2013). These findings indicate that, although WB is a widely used raw material in mealworm production, WB should be complemented with concentrate foods to reduce the fiber content and to increase diet’s utilization in mealworms. This would probably result in improved productive performance.

On the other hand, the values obtained for the CP and GE digestibility of WB in mealworms are also low (both equal to 0.40). It is well known that fibre-rich diets can reduce the digestibility of dietary protein and energy (Le Goff and Noblet, 2001) due to the low digestibility of fiber and possible negative interactions with other nutrients. Fiber increases the rate of passage in the whole tract, reducing the time available for enzyme degradation of the rest of the nutrients (Gidenne, 1992). Data obtained in our study constitute a necessary first step in the nutritional assessment of raw materials for mealworms. A WB, as that used in this study (142 g ADF, 171 g CP, and 123 g starch per kg DM), would have 7.61 MJ of DE and 68 g of DP per kg DM. Consequently, our data will contribute to provide the necessary information for a precise and adequate diet formulation in this insect species.

The supplementation of WB with FC, frequently used in mealworm’s feed (Liu et al. 2020), significantly improved the digestibility coefficients of ashes, CP, and GE of the diet. Consequently, both DE and DP contents of the diet were considerably improved (+1 MJ and +8 g per kg DM, respectively). The introduction of FC, rich in soluble fermentable fiber and soluble sugars, with low NDF and lignin content could explain the improvement in the utilization of main nutrients. The protein in FC, although scarce, could be more accessible to digestive enzymes of mealworms than in WB. WBG supplementation is also frequent in mealworms diets (Kim et al. 2017). However, its dietary inclusion at 6% (on a dry basis) did not improve the digestibility coefficients of the main nutrients evaluated in our study. In fact, the inclusion of WBG reduced DM and ashes digestibility, probably due to the high ADF content in WBG.

As regards growth performance, in the present work, we observed a high survival rate of the larvae between 48 and 90 d post-hatch (survival rate equal to 99%). Diet did not affect survival rate. Kim et al. (2017) observed that larval losses (mortality) during the first week accounted for about 30%, but survival was close to 100% from that moment until the end of growth period (12 wk post-hatch). van Broekhoven et al. (2015) observed an average survival rate of 80% between days 28 and 112 of larval growth. These authors reported that larval survival could be affected by the dietary protein and starch content.

As expected, the evolution of feed intake and larval growth was exponential between 0 and 90 d post-hatch. Exponential growth curves were also observed by Kim et al. (2017) with diets based on combination of WB, brewer’s spent grain, and/or distillers dried grain during 112-d feeding period. These results are also in agreement with van Broekhoven et al. (2015) feeding mealworms with organic by-products originating from beer brewing, bread/cookie baking, potatoe processing, and bioethanol production.

The inclusion of FC (14% on a dry basis) on an iso-nutritive WB diet led to an increase in the average daily intake of the larvae, as well as a higher growth (+14%) and an improved feed conversion rate (–8.5%). The supplementation with FC is very common in mealworm feeding, as carrots constitute an optimal way to supply water, macro and micro minerals, vitamins, and soluble sugars to the larvae. In non-isonutritive diets, where growth of mealworms fed WB (50 g) was compared to those fed with WB and FC (50 + 20 g, respectively), Liu et al. (2020) observed that larvae supplemented with FC showed a clear higher growth (+40%) for 4 wk. Similarly, Oominxc et al. (2015) observed that supplementation of commercial mealworm diets with FC shortened the growth period 55 d and improved the conversion rate of DM. These results could indicate that the inclusion of carrots could supply essential micronutrients for the development of mealworms. Hence, when using simple diets based on single raw materials (i.e., WB-only), micronutrients could be insufficient to meet larvae requirements and would not be covering correctly all nutrient requirements, limiting growth and nutrient utilization. Our results indicate that there is further need to obtain data on nutrient requirements in T. molitor larvae. This can be done through rearing mealworms in the presence of FC. In this material, we would observe the larval growth and development in the presence of FC.

The inclusion of WBG (5.8% on a dry basis) on an iso-nutritive WB diet led to an improvement in both growth (+3.5%) and feed conversion ratio (–6.3%), whereas it did not increase DM intake of mealworms. However, these changes were lower than when FC were introduced. Mancini et al. (2019), using diets based only on brewery spent grains (with a lower CP: 18% in DM than those in this study), observed that mealworms need 150 d to reach adult weight and an optimal feed conversion ratio (2.35). When these authors included 50% of a cookie diet, the time needed to reach adult weight increased (190 d) and feed conversion factor decreased (2.90) compared with brewery grains. In fact, when diets did not include brewery spent grains, mealworms did not reach the pupae stage. This fact was attributable to the low protein content of these diets. When a large part of the protein comes from yeast, some studies have reported positive relation between dietary protein, growth rate, and final weight in mealworms (Morales-Ramos et al. 2010, van Broekhoven et al. 2015). Nevertheless, research indicated that there is not always a direct relationship between dietary protein content and growth. Kim et al. (2017), comparing the growth of mealworms fed 100% WB, 100% brewer’s spent grains, and a mixture of these at 50% (with a dietary CP content of 12, 22, and 16% in DM, respectively), observed that the larvae fed the mixture with 16% CP were those that reached faster adult weight.

In conclusion, previous research shows that growth performance of T. molitor larvae depends on the raw materials used in feed, the level of protein, and the energy to protein ratio of the diet. Precise nutritional assessment of the raw materials that are frequently used in their feed is key to design complete and adequate diets that meet their nutritional requirements.

Finally, mealworm’s body composition data showed adult larvae of T. molitor are a good source of protein (54% in DM) and also...
provide a non-negligible amount of fat (33% in DM). This chemical composition agrees with that observed by other recent studies (Ravzanada et al., 2012; van Broekhoven et al. 2015; Ruschioni et al., 2020). However, in our study, body composition was not affected by the type of diet during larvae developmental stage. Literature has shown that it is difficult to change the proximal composition of mealworms through feeding only. van Broekhoven et al. (2015), using diets varying in protein content (from 11 to 39% in DM), did not observe changes in the protein content of mealworms. Similarly, Ruschioni et al. (2020), comparing diets that differed in their EE content (from 1 to 7% in DM), did not observe significant changes in the fat content of mealworms.

Regarding the amino acid profile of the mealworms, their protein was especially rich in glutamic acid, aspartic acid, alanine, leucine, tyrosine, and proline, amongst others. These results are quite similar to those recently obtained by other authors in mealworms (Jajić et al. 2020, Ruschioni et al. 2020; Wu et al. 2020).

In the present study, the inclusion of protein from FC (rich in aspartic acid and iso-leucine; Tran et al., 2016) at expenses of WB (rich in glutamic, proline, and arginine; Heuzé et al., 2015) resulted in a reduced content of histidine, phenylalanine, and tyrosine, as well as an increase in methionine content in mealworms. The reduction in phenylalanine and tyrosine could be related to the lower availability of glutamine (which participates in the biosynthesis of aromatic amino acids; Lehninger, 1987). Dietary inclusion of WBG increased tyrosine while decreased arginine, alanine, and threonine in mealworm protein. The increase in tyrosine and the lower content in arginine could be related to the higher and lower content of these amino acids in WBG, respectively (Heuzé et al. 2017), compared with WB. Nevertheless, changes in the whole amino acid profile shown in Table 6 are difficult to explain and the available information so far is not enough to establish unequivocal relationships between dietary protein content and body protein of the larvae. In fact, Ruschioni et al. (2020), changing the level and source of protein, observed changes in the dietary amino acid content that did not correspond to the changes observed in the mealworms’ protein. In general terms, our results show WB protein would have a protein composition that matches closely that of mealworms (both rich on glutamic acid, aspartic acid, alanine, and leucine), constituting a suitable protein base for them.

The fatty acid composition of the mealworm’s larvae in this study, in SFA (22.5 ± 0.1%), MUFA (34.7 ± 0.6%) and PUFA (42.8 ± 0.6%) agrees with those reported by Dreassi et al. (2017) and Fasel et al. (2017). Several works indicate that the proportion of fatty acids in mealworms can be easily changed depending on the main source of fat used (Fasel et al. 2017, Wu et al. 2020). Most research coincides that when using diets based on cereal by-products, the SFA/UFA ratio is low and close to that obtained in this study (0.290 ± 0.002). High levels of PUFA could increase the rancidity during storage, which requires a proper handling of the larvae, meals, and oils obtained from T. molitor. The main difference observed in the fat composition of the larvae with the different experimental diets was that those fed with WB and WBG diets showed a higher level of linoleic acid (+2.1 percentage points) at the cost of a reduction in the level of oleic acid (+2.1 percentage points) compared with those fed the FC diet. These changes were mainly due to the relatively high level of linoleic and low oleic acid (56 and 14% of total fatty acids, respectively) in WB and WBG (INRA-CIRAD-AFZ, 2022).

To summarize, common simple diets based on a single raw material, such as WB, do not cover adequately all nutrient requirements of mealworms, limiting their growth and nutrient utilization. The supplementation of wheat bran diets with FC and WBG improves access to certain essential nutrients, significantly improving growth parameters. Thus, precise nutritional assessment of the raw materials that are frequently used in T. molitor larvae feed is key to design complete diets that meet their macro and micronutrient requirements and optimize larval production and the economic return of insect production. This study provides novel data and a unique experimental approach to assess the nutritional value of raw materials in mealworms. Using this approach, the nutritive value of WB in mealworms was 7.61 MJ of DE and 68 g of DP per kg DM. This approach is a valuable first attempt towards a rational formulation of diets for mealworms that cover all their requirements and allows them to express their full growth potential.

Acknowledgments

We thank Juan Cañamero (Production Manager of Feedect) for his contribution maintaining the colony and collaborating in data collection. Dr. Enrique Blas (Universitat Politècnica de València) is also thanked for contributing with his ideas, opinions, and support during the design of the experiment.

Author’s Contributions

BF: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Writing – original draft. LR: Investigation; Methodology. MCLL: Investigation; Methodology. VJM: Investigation; Methodology. JJP: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Writing – original draft; Writing – review & editing. MCL: Conceptualization; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing.

References Cited

Agbiyide, F. S., T. I. Ofuya, and S. O. Akindele. 2009. Marketability and nutritional qualities of some edible forest insects in Benue State, Nigeria. Pak. J. Nutr. 8: 917–922. doi:10.3923/pjn.2009.917.922
Alagon, G., O. N. Arce, E. M. Martinez-Paredes, L. Ródenas, V. J. Moya, E. Blas, C. Cervera, and J. J. Pascual. 2016. Nutritive value of distillers dried grains with soluble from barley, corn and wheat for growing rabbits. Anim. Feed Sci. Technol. 222: 217–226. doi:10.1016/j. Feedsci.2016.10.024
Association of Official Analytical Chemist. 2019. Official methods of analysis, 21st ed. AOAC International, Gaithersburg, MD.
Baek, S., A. E. Perez, R. M. Turcotte, J. B. White, F. Adedipe, and Y.-L. Park. 2015. Response of Tenebrio molitor (Coleoptera: Tenebrionidae) adults to potato: implications for monitoring and sampling. J. Stored Prod. Res. 60: 5–10. doi:10.1016/j.jspr.2014.11.002
Batyé, I. L. 1982. Starch analysis using thermostable alpha-amylases. Starch/ Stärke 34: 125–128. doi:10.1002/star.198203404047
Blas, E., J. Fernández-Carmona, C. Cervera, and J. J. Pascual. 2000. Nutritive value of coarse and fine wheat brans for rabbits. Anim. Feed Sci. Technol. 88: 239–251. doi:10.1016/S0377-8401(00)00212-1
Bosch, L., A. Alegría, and R. Farré. 2006. Application of the 6-aminoquinolyl-N-hydroxysuccinimidy carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods. J. Chromatogr. B Analys. Sci. Technol. Biomed. Life Sci. 831: 176–183.
Chabeauti, E., J. Noble, and B. Carré. 1991. Digestion of plant cell walls from four different sources in growing pigs. Anim. Feed Sci. Technol. 32: 207–213. doi:10.1016/0377-8401(91)90024-M
Coll, M., and M. Guershon. 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. Annu. Rev. Entomol. 47: 267–297.
Deruytter, D., C. L. Coudron, and J. Claeyss. 2020. The influence of wet feed distribution on the density, growth rate and growth variability of Tenebrio molitor. *J. Insects Food Feed* 7(2): 141–149. doi:10.3920/JIFF2020.0049

Dresassi, E., A. Cito, A. Zanfìni, L. Materozzi, M. Botta, and V. Francardi. 2017. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm Tenebrio molitor (Coleoptera: Tenebrionidae). *Lipids* 52(3): 285–294. doi:10.1007/s11745-016-4220-3

FAO. 2006. World agriculture towards 2030/2050. Prospects for food, nutrition, agriculture and major commodity group—interim report. Available at: https://www.fao.org/3/ap010e/ap010e.pdf

Fasel, N., L. Mene-Saffrane, I. Ruczynski, F. Komar, and P. Christe. 2017. Diet induced modifications of fatty-acid composition in mealworm larvae (*Tenebrio Molitor*). *J. Food Res.* 6: 22–31. doi:10.539/jfr.6a5p22

Ghaly, A. E., and F. N. Alkoaik. 2009. The yellow mealworm as a novel source of protein. *Am. J. Agric. Sci.* 4: 319–331.

Gidenne, T. 1992. Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *Br. J. Nutr.* 67: 133–146.

Heuzé, V., Tran, G., Baumont, R., Noblet, J., Renaudeau, D., Lefèvre, M., and Lebas, F. 2015. Wheat bran. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO, France. Available at: https://www.feedipedia.org/node/726.

Heuzé, V., Tran, G., Sauvant, D. and Lebas, F., 2017. Brewers grains. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO, France. Available at: https://www.feedipedia.org/node/74.

INRAE-CIRAD-AFZ. 2022. Composition and nutritive value of feeds for cattle, sheep, goats, pigs, poultry, rabbits, horses and salmonids. Available at: https://www.feedtables.com.

IPCC. 2019. Summary for Policymakers. In: *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Available at https://www.ipcc.ch/srccl.

Jajić, I., A. Popović, M. I. Urošević, S. Krsatović, M. Petrović, D. Guliša, and M. Samardžić. 2020. Fatty and amino acid profile of mealworm larvae (*Tenebrio molitor* L.). *Biotechnol. Anim. Husb.* 36: 167–180. doi:10.2298/BAH2002167

Karasov, W. H., and A. E. Douglas. 2013. Comparative digestive physiology. *Compr. Physiol.* 3: 741–783.

Kim, S. Y., H. G. Kim, H. J. Yoon, K. Y. Lee, and N. J. Kim. 2017. Nutritional analysis of alternative feed ingredients and their effects on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Entomol. Res.* 47: 194–202. doi:10.1111/1748-5967.12236

Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *J. Anim. Sci.* 79: 2418–2427.

Lehninger. 1987. *Principles of biochemistry*. CBS Publishers and Distributors, New Delhi.

Litell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76: 1216–1231.

Liu, C., J. Masri, V. Perez, C. Maya, and J. Zhao. 2020. Growth performance and nutrient composition of mealworms (*Tenebrio molitor*) fed on fresh plant materials-supplemented diets. *Foods* 9: 151. doi:10.3390/foods9020151

Mancinoni, S., F. Fratini, B. Turchi, S. Mattioli, and A. y Dal Bosco. 2019. Former foodstuff products in *Tenebrio Molitor* rearing: effects on growth, chemical composition, microbiological load and antioxidant status. *Animals* 9: 484. doi:10.3390/an9080484

Martin, M. M. 1983. Cellulose digestion insects. *Comp. Biochem. Physiol.* 75A: 313–324. doi:10.1016/1096-9263(83)90088-9

Mereilles, E. A., C. N. B. Carneiro, R. A. DaMattá, R. I. Samuels, and C. P. Silva. 2009. Digestion of starch granules from maize, potato and wheat by larvae of the Yellow mealworm, *Tenebrio molitor* and the Mexican bean weevil, *Zabrotes subfasciatus*. *J. Insect Sci.* 9: 43.

Mertens, D. R. 2002. Grainometric determination of amylase-treated neutral detergent fiber in feeds with refining in beaks or cricules: collaborative study. *J. AOAC Int.* 85: 1217–1240.