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

B. Fasce,[1] L. Ródenas,[2] M. C. López,[2] V. J. Moya,[2] J. J. Pascual,[2] and M. Cambra-López[2,3,]

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

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

	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

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 2. Average chemical composition of the experimental diets

	Diets		
	WB	FC	WBG
Chemical composition	g/kg DM	g/kg DM	g/kg DM
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.

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

	Diets						
	WB		FC		WBG		
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	5.3	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	142.90	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.

were controlled at 0, 48, 62, 69, 76, 83, and 90 d post-hatch. In addition, the number of larvae per tray was also estimated on the same days. The number of larvae for a given weight was counted in each tray and then extrapolated to the total weight of larvae in that tray.

After each weighing day, the larvae were placed back in their previously cleaned trays and fresh diet was then added, according to their treatment and age. After the appearance of the first pupae in the trays (90 d post-hatch), larval growth period and data collection were finished. After the last weighing day, the larvae obtained in each tray were stored in 36 bags (12 sample bags per treatment), identified individually with the tray number (including dietary treatment, batch, and laying period), and frozen at –20°C. Frozen samples were mixed using a blender and then lyophilized in Petri dishes, grounded, and stored at –20°C until further analysis.

To determine the nutritive value of the experimental diets, a digestibility trial was performed from 48 to 62 d post-hatch. This 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 we waited until day 62 when all feed provided had been ingested (refusal of feed = 0). Feed offered and excreted feces during these 14 d were weighed, sampled, grounded, and stored until further analysis.

Chemical Analysis

Raw materials and experimental diets were analyzed for DM, ashes, CP, ether extract (EE), starch, neutral detergent fiber (NDF), ADF, acid detergent fiber (ADL), total dietary fiber (TDF), soluble fiber (SF), 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 amino acid and fatty acid composition.

Methods of the AOAC (2019) were used to determine DM (934.01), ash (942.05), CP (990.03, Dumas method, CN628 Elemental Analyzer, LECO, St. Joseph, MI), and EE (920.39, with acid-hydrolysis of samples prior to the extraction). Starch content was determined according to Batey (1982), by a two-step enzymatic procedure with solubilization and hydrolysis to maltodextrins with thermo-stable α-amylase followed by complete hydrolysis with amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the resulting D-glucose being measured by the hexokinase/glucose-6 phosphate dehydrogenase/NADP system (kit D-glucose-HK Megazyme Int. Ireland Ltd., Wicklow, Ireland). The total dietary fiber (TDF) content was determined by a gravimetric-enzymatic method, procedure 991.43 of the AOAC (2019), with α-amylase, protease, and amyloglucosidase treatments (Megazyme TDF R.30.K-TDFR-100A/200A), correcting for ash and CP. The NDF, ADF, and ADL fractions were analyzed sequentially according to Mertens (2002), procedure 973.18 of the AOAC (2019) and Robertson and Van Soest (1981), respectively, with a thermo-stable α-amylase pre-treatment and expressed exclusive of residual ash, by using a nylon filter bag system (Ankom, Macedon, NY). The SF content was determined as proposed by Van Soest et al. (1991), by subtracting the NDF corrected for CP from the TDF content. The GE was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, UK).

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 after hydrolyzation. The amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18 reverse-phase column Waters AcQ Tag (150 × 3.9 mm). Methionine and cystine were determined separately as methionine sulphone and cysteic acid, respectively, after performic acid oxidation followed by acid hydrolysis (Alagón et al. 2016).

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 + C15:00 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:00, monounsaturated fatty acids (MUFA) = C14:1 + C16:1

+ C17:1 + C18:1n7 + C18:1n9t + C18:1n9c + C20:1 + C22:1n9 + C24:1, polyunsaturated fatty acids (PUFA) = C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n3 + C20:4n6 + C20:5n3 + C22:2 + C22:4n6 + C22:5n3 + C22:6n3, and unsaturated fatty acids (UFA) = MUFA + PUFA, respectively. The atherogenic (AI) and thrombogenic (TI) indexes were calculated according to Ulbricht and Southgate (1991) as AI = [C12:0 + 4 × C14:0 + C16:00)/(MUFA + n-6 + n-3] and TI = [C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 + 3 × n-3 + (n-3/n-6)].

Statistical Analysis

Traits of apparent digestibility coefficients and larvae composition were analyzed using the GLM procedure of Statistical Analysis System (SAS, 2008). The model included as fixed effects the experimental diet (WB, FC, and WBG) and the batch (1 to 2) for the digestibility traits, as well as the laying period (1 to 3) for the composition traits. Preliminary analysis showed that the diet×batch interaction was not significant, so it was not included in the final model.

A mixed model (SAS, 2008), with a repeated measure design, was used to analyze growth performance data. The model considered the variation between trays and the co-variation within them. The covariance structure was estimated using the spatial power function, after objectively comparing among other covariance structures as suggested by Littell et al. (1998). The spatial power function is a direct generalization of first-order auto-regressive covariance function for equally time-spaced data, with the advantage of accounting for different lag times between two measurements. The model included the experimental diet (WB, FC, and WBG), the batch (1 to 2), the laying period (1 to 3), the monitoring period (0 to 90 d post-hatch), and their interactions. All models included the random effect of tray [p ~ N (0, σ^2_p)].

Results

Table 4 shows the apparent digestibility coefficients of main nutrients with the different experimental diets, as well as their nutritive value. Digestibility coefficients of ashes, CP, and GE were significantly higher in trays with FC diet respect to the other diets (on av. + 7.2, +8.5, and +6.7 percentage points, respectively; $P < 0.001$). Consequently, both digestible energy (DE) and digestible protein (DP) contents of FC diet were also significantly higher than those obtained for the other diets (on av. +1.12 MJ DE and +9.15 g DP per kg DM; $P < 0.003$). In addition, WBG diet showed the lowest digestibility coefficients of DM and ashes ($P < 0.001$).

Fig. 1 shows the evolution of daily DM intake of mealworms with the different diets. During the global period, and despite that the same amount of feed was offered to all the trays (both in fresh and dry basis), mealworms given FC diet showed a higher daily intake than those with WB and WBG diets (+6.4% and 10.2%, respectively; $P < 0.05$).

Average larval count on day 48 of the experiment (9144 individuals per tray) was close to that observed at 90 d post-hatch (9064 individuals per tray). Growth performance of the mealworms with the different experimental diets is shown in Table 5. From 0 to 76 d post-hatch, mealworms fed the FC diet showed significantly higher total larval weight per tray at 62, 69, and 76 d post-hatch than those fed WB and WBG diets (on av. +14.1% at 76 d; $P < 0.001$). This result was mainly due to the significantly higher average daily gain observed with FC diet compared with the rest from 0 to 48, 48 to 62, and 69 to 76 d post-hatch (on av. 5.46, 4.66, and 4.90 g/tray and day for FC, WB, and WBG diets from 0 to 76 d, respectively; $P < 0.001$). In fact, from 0 to 48 d post-hatch, mealworms fed the FC diet showed a lower feed conversion ratio than those fed WB and WBG diets (−1.28 and −0.58 g DM/g larvae, respectively; $P < 0.001$). From 76 to 90 d post-hatch, mealworms fed the FC diet showed a significantly higher average daily gain to those fed with WB diet, showing those with WBG diet intermediate values (on av. 33.6, 29.0, 29.6 g/tray and day, respectively; $P < 0.05$).

During the whole period (0–90 d), mealworms fed with FC diet showed significantly higher final weight and average daily gain than those fed the WBG diet (+12.4%), being final weight and average daily gain in WBG diet higher than those fed the WB diet (+3.5%). The lowest feed conversion ratio was obtained with diet WB (on av. −0.24 points lower than the average of the other treatments; $P < 0.05$).

Finally, Table 6 shows the chemical, amino acid, and fatty acid composition of mealworms after 90 d of growing period with the different experimental diets. Dietary treatment did not significantly affect DM, ashes, EE, and CP content of mealworms. However, some dietary effects on amino acid and fatty acid composition were observed. Compared with WB diet, mealworms fed the FC diet had lower arginine, histidine, phenylalanine, and tyrosine, but higher methionine content (−12, −9, −6, −2 and +7%, respectively; $P < 0.05$), whereas those fed the WBG had significantly lower alanine, arginine,

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)

	Diets					
	WB	FC	WBG	SEM	*F*-value	*P*-value
Apparent digestibility coefficients						
Dry matter (DM)	0.390[b]	0.406[b]	0.368[a]	0.006	12.01	<0.001
Ashes	0.095[b]	0.146[c]	0.053[a]	0.009	26.71	<0.001
Crude protein	0.399[a]	0.471[b]	0.373[a]	0.011	20.06	<0.001
Neutral detergent fiber	0.159	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[a]	0.458[b]	0.384[a]	0.005	54.35	<0.001
Nutritive value						
Digestible energy (MJ/kg DM)	7.61[a]	8.61[b]	7.38[a]	0.10	42.65	<0.001
Digestible protein (g/kg DM)	68.4[a]	76.6[b]	66.5[a]	2.0	7.51	0.0027

WB: diet including wheat bran andwater; 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,b,c]Means not sharing letter for the same period were significantly different at $P < 0.05$.

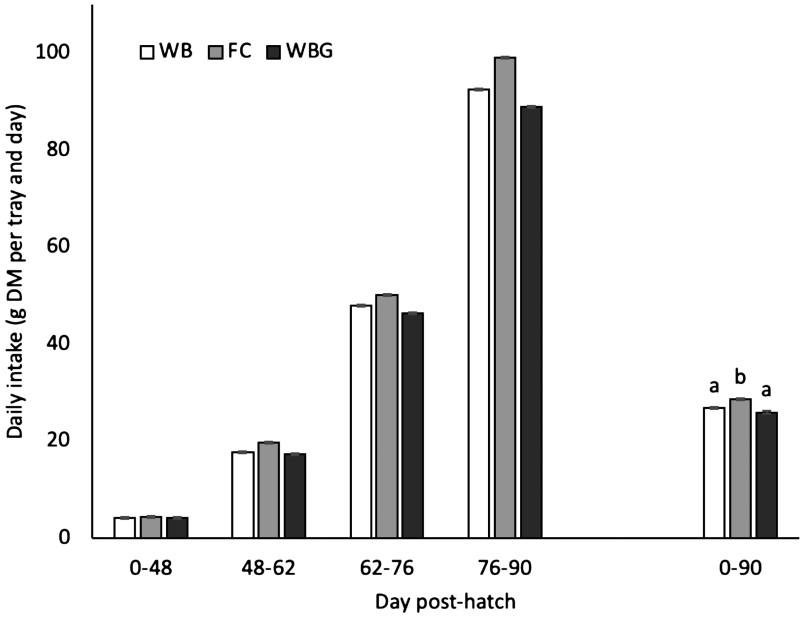

Fig. 1. Effect of dietary treatment on daily feed intake per tray of *Tenebrio molitor* larvae. Each tray included the eggs generated by 150 g of fertile imagos for one week. WB: diet including wheat bran and water; FC: diet including wheat bran and fresh carrots; WBG: diet including wheat bran, wet brewer's grains and water (see Table 3). Standard error of the mean: 0.23 g dry mater (DM)/tray day. [a,b] 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)

	Diets					
	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	119.1[a]	154.1[b]	125.9[a]	9.4	1.55	0.0013
69 d	224.9[a]	267.4[b]	236.4[a]	10.3	1.72	<0.001
76 d	354.5[a]	414.9[b]	372.5[a]	14.1	1.72	<0.001
83 d	519.2[a]	603.9[c]	541.3[b]	20.9	1.72	<0.001
90 d	760.7[a]	884.6[c]	787.0[b]	32.5	1.72	<0.001
Average daily gain						
0–48 d	0.945[a]	1.372[c]	1.096[b]	0.026	1.72	<0.001
48–62 d	5.264[a]	6.307[b]	5.235[a]	0.307	1.35	0.0189
62–69 d	15.12	16.18	15.79	0.72	1.07	0.3085
69–76 d	18.50[a]	21.07[b]	19.45[a]	0.80	1.31	0.0309
76–83 d	23.53	27.00	24.11	1.62	1.17	0.1402
83–90 d	34.50[a]	40.10[b]	35.10[a b]	1.96	1.29	0.0419
Global: 0–90 d	8.452[a]	9.828[c]	8.745[b]	0.080	1.52	0.0021
Feed conversion ratio						
0–48 d	4.54[c]	3.26[a]	3.84[b]	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	3.19[b]	2.92[a]	2.99[a]	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, b]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

Table 6. Chemical, amino acid, and fatty acid composition of *Tenebrio molitor* larvae at 90 d post-hatching with the different experimental diets (n = 36 trays; degrees of freedom: 2 for diets, 2 for laying period and 1 for batch)

	Diets					
	WB	FC	WBG	SEM	*F*-value	*P*-value
Chemical composition (g/100 g)						
Dry matter (DM)	35.42	34.44	35.42	0.38	2.21	0.1243
Ashes	3.58	3.62	3.64	0.09	0.10	0.9029
Ether extract	32.35	33.06	32.56	0.56	0.41	0.6698
Crude protein	54.71	53.80	54.80	0.42	1.68	0.2014
Amino acid composition (g/100 g DM)						
Alanine	4.012[b]	4.098[b]	3.916[a]	0.025	5.79	0.0066
Arginine	2.866[b]	2.534[a]	2.447[a]	0.076	4.52	0.0177
Aspartic acid	4.968	4.720	5.570	0.360	1.24	0.3014
Cysteine	0.514	0.534	0.503	0.029	0.30	0.7450
Glutamic acid	6.815	6.885	6.583	0.173	0.75	0.4785
Glycine	2.593	2.640	2.500	0.067	0.99	0.3830
Histidine	1.636[b]	1.482[a]	1.606[a b]	0.036	3.21	0.0520
Isoleucine	2.297	2.294	2.256	0.042	0.29	0.7506
Leucine	3.913	3.823	3.904	0.081	0.36	0.7002
Lysine	3.032	2.962	2.969	0.039	0.89	0.4213
Methionine	0.795[a]	0.848[b]	0.772[a b]	0.022	2.32	0.1128
Phenylalanine	1.930[b]	1.822[a]	1.907[a b]	0.025	3.26	0.0501
Proline	3.177	3.168	3.080	0.043	1.28	0.2893
Serine	2.601	2.529	2.802	0.173	0.62	0.5461
Threonine	2.120[b]	1.812[a]	1.798[a]	0.073	3.67	0.0354
Tyrosine	3.814[b]	3.735[a]	3.892[c]	0.016	8.04	0.0013
Valine	3.551	3.540	3.494	0.039	0.56	0.5762
Fatty acid composition (% total fatty acids)						
Lauric acid (C12:0)	0.131	0.147	0.131	0.008	1.14	0.3319
Myristic acid (C14:0)	1.678	1.719	1.660	0.065	0.21	0.8152
Pentadecanoic acid (C15:0)	0.167[b]	0.152[a]	0.168[b]	0.003	5.46	0.0085
Palmitic acid (C16:0)	16.82	16.98	16.97	0.11	0.69	0.5103
Palmitoleic acid (C16:1)	1.124[a]	1.220[b]	1.112[a]	0.013	7.23	0.0023
Heptadecanoic acid (C17:0)	0.214	0.210	0.228	0.011	0.65	0.5257
Cis-10-Heptadecenoic acid (C17:1)	0.221	0.161	0.182	0.016	2.30	0.1140
Stearic acid (C18:0)	3.270	3.229	3.298	0.049	0.46	0.6351
Elaidic acid (C18:1n9t)	0.045	0.048	0.044	0.002	1.01	0.3731
Oleic acid (C18:1n9c)	32.59[a b]	34.44[b]	32.09[a]	0.581	2.99	0.0628
Linoleic acid (C18:2n6c)	40.28[b]	38.35[a]	40.62[b]	0.49	3.73	0.0337
Arachidic acid (C20:0)	0.110	0.098	0.107	0.005	1.52	0.2323
G-Linolenic acid (C18:3n6)	0.049	0.051	0.051	0.003	0.15	0.8573
Cis-11-Eicosenoic acid (C20:1)	0.251[b]	0.223[a]	0.243[b]	0.003	7.57	0.0018
Linolenic acid (C18:3n3)	2.771	2.700	2.825	0.070	0.72	0.4955
Cis-11,14-Eicosadienoic acid (C20:2)	0.088	0.074	0.082	0.004	2.18	0.1280
Cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.095	0.097	0.100	0.003	0.55	0.5844
Arachidonic acid (C20:4n6)	0.096	0.086	0.091	0.005	0.91	0.4103
SFA	22.38	22.53	22.56	0.10	0.80	0.4592
MUFA	34.23[a b]	36.10[b]	33.67[a]	0.59	3.06	0.0594
PUFA	43.38[b]	41.37[a]	43.76[b]	0.55	3.37	0.0457
n-3	2.866	2.801	2.921	0.070	0.68	0.5147
n-6	40.43[b]	38.49[a]	40.76[b]	0.49	3.70	0.0345
n-6/n-3	14.11	13.75	13.96	0.19	0.78	0.4662
PUFA/SFA	1.938[b]	1.835[a]	1.939[b]	0.024	3.63	0.0367
SFA/UFA	0.288	0.291	0.291	0.002	0.80	0.4589
AI	0.305	0.310	0.307	0.003	0.78	0.4665
TI	0.473	0.479	0.476	0.003	0.97	0.3889

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,b,c]Means in the same row with no common superscripts differ significantly ($P < 0.05$). SFA,saturated fatty acids [C14:0 + C15:0 + C16:0 + C17:0 + C18:0]; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-3: Omega-3 fatty acids; n-6: Omega-6 fatty acids; PUFA/SFA: ratio PUFA/SFA; SFA/UFA: ratio SFA/(MUFA + PUFA); AI, atherogenic index; TI, thrombogenic index.

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, Chabeauti 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. (1985) observed a very low cellulosic 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, Oonincx 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* mealworm (macro and micronutrients) to formulate diets that can take into account all these factors to optimize larvae production and the economic return of the production of *T. molitor*.

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 (Ravzanaadii 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 isoleucine; 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 (INRAE-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 larvae 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.

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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.

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