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Rearing mealworm larvae with wheat, barley or maize grains as main source of nutrients in unbalanced or balanced substrates

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Feeding conditions of mealworm (*T. molitor*) larvae for livestock nutrition need to be optimised. The effects of the cereal offered as main nutrient source on growth performance and composition of *T. molitor* larvae were evaluated. In Experiment 1 (unbalanced diets), substrates included 80% of wheat (**W1**), barley (**B1**) or maize (**M1**) and 20% wheat straw. In Experiment 2 (balanced diets), substrates were formulated using wheat (**W2**), barley (**B2**) or maize (**M2**) as the main ingredient, combined with wheat bran or straw to contain similar N, starch and NDF contents (19.5, 520, and 270 g/kg DM, respectively). A control substrate based solely on wheat bran was also included in each experiment (**C1** and **C2**). Each treatment was replicated four times in trays containing 24 g of substrate and 60 larvae, and the experimental period lasted for 28 (Experiment 1) or 35 (Experiment 2) days. Larval weight and residual substrate were monitored weekly, and larval chemical composition was analysed at the end of each experiment. In Experiment 1, feed intake was greatest for B1 and W1, intermediate for C1 and lowest for M1 ($P < 0.001$). However, larval growth was greater for C1 than for B1 and W1, with the lowest rates observed for M1 ($P < 0.001$). Consequently, the feed to gain (F:G) ratio was lowest for C1 and highest for M1 ($P < 0.001$). Mortality was also greatest for M1 ($P < 0.001$). In Experiment 2, feed intake ($P < 0.001$) and larval growth ($P < 0.01$) decreased in M2 compared to B2 and W2, which in turn showed similar growth performance to C2 ($P < 0.001$). Larval composition followed a similar trend in both experiments, with the highest N content observed in larvae fed C1 and C2 ($P < 0.001$), while DM and ether extract contents were higher in larvae reared on barley ($P < 0.001$). In summary, maize-based substrates may compromise feed intake and growth performance of *T. molitor* larvae compared to those based on wheat or barley, regardless of the nutrient content of the diet. Apparently, larval performance does not respond to the substrate starch content but it might be influenced by the endosperm physical structure. Additionally, a lower N content in the substrate could impair growth performance, an effect that is alleviated once larval protein requirements are achieved. Larval composition was affected by the main dietary ingredient, with substrates based on wheat bran increasing the N content of larvae, whereas those based on barley resulted in increased DM and Ether extract contents.

Implications

The identification of the most suitable ingredients for insect production is essential to reduce costs. Cereal grains (wheat, barley and maize), used either as the sole source of nutrients or combined with other ingredients in nutritionally balanced substrates, were evaluated for rearing *T. molitor* larvae. In the initial approach, larval performance was reduced by the limited protein content of cereal grains, particularly maize. In the second approach, protein levels were standardised to meet the assumed nutritional requirements of the larvae, resulting in satisfactory performance for wheat and barley, although growth with maize as the primary ingredient remained lower. Results on larval composition were affected by the different ingredients tested.

Introduction

In recent decades, the production of insects has emerged as a promising source of protein for both humans and livestock (van Huis, 2020). Due to its high growth performance and adequate nutrient composition, *T. molitor* is one of the most widespread cultured species (Veldkamp et al., 2012; Khan, 2018). Under productive conditions, *T. molitor* larvae are generally reared on mixtures

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based on cereal products. In accordance with other animal productive species such as pigs and poultry, starch could be assumed as the most suitable energy source for *T. molitor* larvae. However, previous research (Stull et al., 2019; Ruschioni et al., 2020; Rumbos et al., 2020) has shown high growth performance in larvae reared on wheat bran substrates compared to other starchy ingredients. This finding suggests that *T. molitor* larvae can modulate feed intake to meet energy requirements through alternative dietary components, such as protein or fibre.

Most published research evaluates a wide range of agro-industrial co-products as insect feeds (Oonincx et al., 2015; Rumbos et al., 2022; Montalbán et al., 2022), whereas references comparing the nutritive value of cereal grains for rearing *T. molitor* are surprisingly scarce. Among several cereal sources, Rumbos et al. (2020) reported a greater growth performance in *T. molitor* larvae fed on durum wheat and barley grains compared to those given maize, rye or oats, whereas Pascual et al. (2024) reported similar digestibility values for barley and maize. In terms of larval composition, Jajić et al. (2022) and Ruschioni et al. (2020) reported higher CP (N × 6.25) and lower Ether extract (**EE**) contents in larvae reared with wheat bran compared to those reared with wheat grain diets, suggesting that variations in the ingredient composition of the substrate might modify the chemical composition of *T. molitor* larvae. However, most of the observed differences are difficult to explain due to variations in the procedures used among publications, and because there are factors related to cereal grains such as particle size, physical structure of the starch granules and amino acid profile that could potentially affect larval development and their preference for a given substrate. The lack of information on the nutritional value of cereal grains and products reaches major importance considering the notable increase in market prices in the last decade. Furthermore, the absence of data does not allow for an estimation of larval requirements and impedes the proper preparation of potential mixtures of ingredients to be used as industrial substrates for optimal growth, contrasting with the accurate commercial formulation of the feeds reached in other productive species.

Some authors evaluated combinations of feeds of variable nutrient composition to assess their suitability as substrates for larvae (Ramos-Elorduy et al., 2002; Rumbos et al., 2022). However, these studies often included diets with different CP and energy contents, which limits adequate discussion on the effects of the nutrient composition of the substrate on larval performance. Other studies evaluated different levels of CP and starch in the substrate (Oonincx et al., 2015; van Broekhoven et al., 2015; Montalbán et al., 2022), but these authors did not adjust the nutrients provided to the larval requirements and consequently, their results cannot be extrapolated to productive conditions. As a consequence of the practical limitations observed in previous research, the use of balanced substrates in terms of both CP and energy contents for rearing *T. molitor* larvae are not well documented and in practice, producers adapt the feeding programme to their own previous experience.

The initial hypothesis was that variations in nutrient composition and structure among cereal grains could influence larval performance and composition, and that these effects might be mitigated if nutrients are adequately balanced within the substrate. The objective of this work was to compare three whole cereal grains (wheat, barley and maize) as feed ingredients for *T. molitor* larvae in order to understand the effects on larval development when used as the unique source of nutrients in the diet or when included in iso-nutritive mixtures.

Material and methods

Experiment 1: unbalanced substrates

T. molitor larvae at an initial growth stage (approximately 6 weeks after hatching; from 0.5 to 0.7 mm length, 12.5 ± 0.92 m g weight) were obtained from our experimental farm and distributed in groups of 60 in 16 plastic trays (15 × 9 × 6 cm). Trays included 24 g of substrate made up of either 80% wheat (**W1**), barley (**B1**) or maize (**M1**) together with 20% wheat straw to facilitate larvae movement (4 replicates per treatment). Besides, a control treatment with wheat bran as the sole ingredient was included (**C1**). All ingredients were ground to 2 mm particle size and each treatment was replicated four times. Chemical composition of ingredients and feed substrates are shown in Tables 1 and 2, respectively.

The experimental period lasted for 28 days. Larvae were grown in darkness (except during sampling) and under controlled temperature (from 24 to 27 °C) and relative humidity (from 45 to 63%) conditions. Two agar cubes (3.37 ± 0.23 g, 0.97 water) were added to each tray twice a week as a source of water. Mortality was estimated by counting living larvae on each tray, and total weight of larvae and the remaining substrate were weekly recorded. From these data, average larval weight, larval growth (daily average individual larval weight gain, mg/d), larval mass gained (total increase in larval weight), feed intake (as the difference between initial substrate and final residue) and feed to gain ratio (calculated as the amount of substrate offered minus the remaining residue after 28 days per unit of weight gained, F:G), expressed both in fresh and DM basis, were calculated per tray. The appearance of pupae during the last week of the experiment (final average pupae proportion of 4.7% of initial larvae) was recorded, and the weight was considered for the estimation of total larval mass. At the end of the experiment, larvae were weighed, collected per tray and slaughtered by freezing at −80 °C. Then, larvae were lyophilised to determine the total production of DM, N and EE by considering the total mass gained per tray.

Experiment 2: balanced substrates

In Experiment 2, 60 larvae at approximately 5–6 weeks after hatching (from 0.4 to 0.7 mm length, 8.6 ± 0.14 mg weight) were distributed into 16 plastic trays. Four dietary treatments were

Table 1
Analysed chemical composition (g/kg fresh matter) of the ingredients included in feed substrates for *T. molitor* larvae in Experiments 1 and 2.

Item	Wheat bran	Wheat grain	Barley grain	Maize grain	Wheat straw
DM	905	878	892	891	934
Organic matter	850	863	871	879	863
Total N	25.5	17.6	15.2	11.5	4.6
Ether extract	25	12	16	29	5
NDF	448	92	165	84	713
Starch	146	645	596	664	13
Gross energy (kcal/kg DM)	4 324	4 368	4 290	4 338	4 261

formulated using wheat (**W2**), barley (**B2**) or maize (**M2**) as the main ingredient in the substrate combined with wheat bran and straw to have similar N, starch and NDF contents (Table 3). In order to minimise any potential ingredient effect on the response, wheat bran was used as a common ingredient to balance substrate composition. In addition, a treatment including wheat bran as the only ingredient was defined as a control (**C2**). Trays (four per treatment) included 24 g substrate. Environmental conditions for culturing larvae were controlled, maintaining temperature between 24 and 25 °C and relative humidity between 33 and 56%. The length of Experiment 2 was 35 days, and measurements controlled at weekly intervals and for the whole experimental period were similar to those indicated for Experiment 1.

Chemical and statistical analyses

Substrates and larvae were processed and analysed as indicated by Fondevila and Fondevila (2023). Ingredients and substrate feeds were ground through a 1-mm size sieve and analysed for DM, organic matter (OM), total N and EE (only substrates) following the AOAC (2004) procedures ref. 934.01, 942.05, 976.05 and 2003.05, respectively. Besides, the NDF concentration was determined as described by Mertens (2002) with an Ankom 200 Fibre Analyser (Ankom Technology, New York), using α–amylase and sodium sulphite, and the results were expressed exclusive of residual ashes. Total starch content was determined enzymatically from samples ground to 0.5 mm by using a commercial kit (Total Starch Assay Kit K-TSTA 07/11; Megazyme, Bray, Ireland). In addition, the gross energy of the diets was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, United States of America). The DM content of the larvae was determined after lyophilisation. Larvae were also analysed for total N, and EE by the procedures reported above.

Results on larval performance and composition in both experiments were statistically analysed as a completely randomise design by ANOVA, with the dietary treatment as the main source of variation, using the Statistix 10 package (Analytical Software, 2010). Weekly pattern of larval weight was tested as indicated by Littell et al. (1998) by the mixed procedure of SAS (SAS Institute, 2018), considering the sampling date as repeated measures. Four different models for variance structure were compared (compound symmetry and auto-regressive order, both considering homo and heterogeneous variances). A compound symmetry and heterogeneous variance were fitted as they showed the lowest BIC and AIC values. Data were explored for normality using the Shapiro-Wilk test prior to analysis, and results on larval mortality were contrasted by the Kruskall-Wallis non−parametric test. In all cases, the tray was considered as the experimental unit (n = 4). When significant differences were detected, average means were compared using the Tukey test. Mean differences at $P < 0.05$ were considered significant, and $0.10 < P < 0.05$ were considered as a trend for significance.

Table 2
Ingredients (g/kg fresh basis) and analysed chemical composition (g/kg DM) of substrates for *T. molitor* larvae made up of wheat bran (C1), wheat (W1), barley (B1) or maize (M1) in Experiment 1 (unbalanced diets).

Item	C1	W1	B1	M1
Ingredients				
Wheat bran	1 000	–	–	–
Wheat grain	–	800	–	–
Barley grain	–	–	800	–
Maize grain	–	–	–	800
Wheat straw	–	200	200	200
Chemical composition				
DM	905	889	901	899
Organic matter	939	971	966	974
Total N	28.1	17.0	14.6	11.3
Ether extract	27	12	15	27
NDF	496	236	301	228
Starch	161	590	538	599
Gross energy (kcal/kg DM)	4 324	4 370	4 299	4 341

Table 3
Ingredients (g/kg fresh basis) and analysed chemical composition (g/kg DM) of substrates for *T. molitor* larvae made up of wheat bran (C2), wheat (W2), barley (B2) or maize (M2) in Experiment 2 (balanced diets).

Item	C2	W2	B2	M2
Ingredients				
Wheat bran	1 000	150	200	420
Wheat grain	–	740	–	–
Barley grain	–	–	800	–
Maize grain	–	–	–	580
Wheat straw	–	110	–	–
Chemical composition				
DM	905	902	903	910
Organic matter	939	969	969	967
Total N	28.1	19.8	19.3	19.3
Ether extract	27	13	17	27
NDF	496	262	271	268
Starch	161	525	539	502
Gross energy (kcal/kg DM)	4 324	4 273	4 302	4 336

Results

Experiment 1: unbalanced substrates

The effects of the substrate used in Experiment 1 on larval performance are presented in Table 4. Larval mortality was higher for M1 than for C1, W1 and B1 ($P < 0.001$). There were no differences among treatments in initial larval weight ($P = 0.412$) but an interaction between treatment and date of experiment was detected for larval weight ($P < 0.001$), as indicated in Fig. 1. No differences among treatments on larval weight were observed up to 7 days of experiment but thereafter, larvae fed M1 showed the lowest BW at 14 and 21 days of experiment compared to the other treatments ($P < 0.01$). At the end of the experiment (28 days), larvae fed C1 were heavier than those fed W1 and B1, with the lowest larval weights observed for M1 ($P < 0.001$). As a result, the total mass gained per tray and individual larval growth from 0 to 28 days of experiment were highest for C1, intermediate for B1 and W1, and lowest for M1 ($P < 0.001$). Substrate intake expressed as either fresh or DM basis, was higher for B1 and W1 than for C1, with the lowest values recorded for M1 ($P < 0.001$). As a result, the F:G ratios in fresh and DM basis were highest for M1, intermediate for B1 and W1 and lowest for C1 ($P < 0.001$).

The effects of dietary treatment on larval composition are shown in Table 4. The DM content was highest for larvae grown on B1 and lowest for those grown on M1, with larvae fed C1 and W1 being intermediate ($P < 0.001$). In terms of N proportion, treatment ranked as follows: C1 > W1 > M1 > B1 ($P < 0.001$), whereas the EE content was highest for B1 and lowest for C1, with W1 and M1 being intermediate ($P < 0.001$). Combining total mass gain and nutrient composition per tray, the estimated total DM production was greater for C1 and B1 than for W1, with the lowest values observed for M1 ($P < 0.001$). Total N production was greater for C1 than for W1 and B1, with M1 showing the lowest value ($P < 0.001$). The total EE production according to treatment ranked as follows: B1 > W1 > C1 > M1 ($P < 0.001$).

Table 4
Mortality, growth performance, feed to gain ratio (F:G) and total nutrient production of *T. molitor* larvae grown on wheat bran (C1), wheat (W1), barley (B1) or maize (M1) for 28 days in Experiment 1 (unbalanced diets).

Item	C1	W1	B1	M1	SEM	*P*-value[1]
Mortality[2]	0.058b	0.046b	0.054b	0.179a	0.0155	< 0.001
Initial weight (mg)	12.3	12.2	12.3	13.2	0.46	0.412
Final weight (mg)	112a	98b	101b	54c	1.7	< 0.001
Mass gained (g/tray)	5.57a	4.86b	5.01b	1.84c	0.121	< 0.001
Larval growth (mg/day)	3.54a	3.05b	3.17b	1.44c	0.058	< 0.001
Intake (g/tray)	4.19b	5.14a	5.47a	2.44c	0.171	< 0.001
Intake (g DM/tray)	3.79b	4.57a	4.93a	2.19c	0.154	< 0.001
F:G (g/g, fresh basis)	0.752c	1.056b	1.092b	1.323a	0.0309	< 0.001
F:G (g/g, DM basis)	1.929c	2.665b	2.579b	3.589a	0.0803	< 0.001
Larval composition						
DM content	35.3b	35.2b	38.1a	33.2c	0.19	< 0.001
N content (mg/g DM)	83.1a	68.1b	61.5d	64.9c	0.50	< 0.001
EE content (mg/g DM)	271c	381b	441a	383b	4.0	< 0.001
Total production (mg/tray)						
DM	1 964a	1 713b	1 909a	612c	42.6	< 0.001
N	163a	117b	117b	40c	2.9	< 0.001
EE	533c	652b	841a	234d	20.9	< 0.001

EE = Ether extract.
Within rows, values not sharing a common letter are significantly different ($P < 0.05$).
[1] *P*-values from the ANOVA. A significant interaction between treatment × date of experiment was detected ($P < 0.001$) for larval weight (Fig. 1).
[2] Estimated as the ratio of dead larvae to the initial number of individuals.

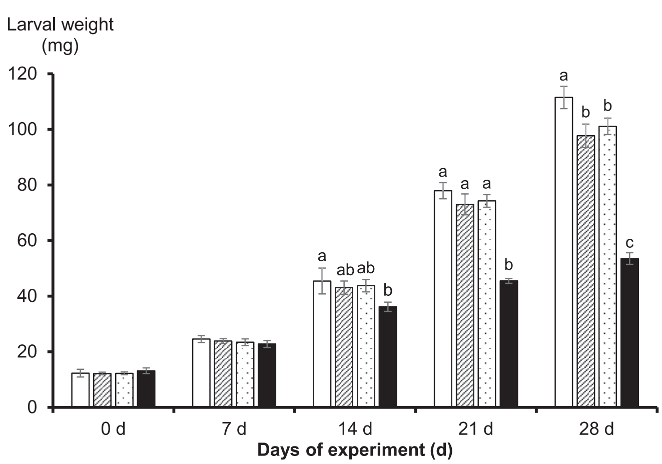

Fig. 1. Weight pattern (mg) of *T. molitor* larvae grown on wheat bran (C1, white bars), wheat (W1, diagonal lined bars), barley (B1, dotted bars) or maize (M1, black bars) along the experimental period in Experiment 1 (unbalanced diets). The interaction treatment × date was significant ($P < 0.001$; SEM = 0.98), and SD values for each column are given in bars. Columns not sharing a common letter are significantly different ($P < 0.05$).

Experiment 2: balanced substrates

The effects of the substrate given in Experiment 2 on larval performance are presented in Table 5. Larval mortality was low, with an average value of 0.040, and did not differ among treatments ($P > 0.10$). No treatment differences were detected for initial larval weight ($P = 0.259$) but an interaction between treatment and date of experiment was detected for larval weight (Fig. 2). From 0 to 7 days of the experiment, larval weight did not differ among treatments. However, from 14 days of the experiment onwards, the substrate fed to the larvae affected larval weight, with the lowest values observed for M2 at all times ($P < 0.01$), whereas larvae fed C2, W2 and B2 showed similar weights at the end of the experiment (35 days). As a result, the total mass gained per tray ($P < 0.001$) and individual larval growth ($P < 0.01$) from 0 to 35 days of the experiment were higher for C2, W2 and B2 than for M2. Substrate intake, either expressed as fresh or DM basis, was lower for C2 and M2 than for B2 and W2 ($P < 0.001$), and F:G ratio in fresh basis was highest for B2 and lowest for C2, recording intermediate values for W2 and M2 ($P < 0.001$). However, no treatment differences were recorded for F:G ratio when expressed on a DM basis.

The DM content of larvae reared on B2 was higher than those given C2, with larvae reared on W2 and M2 being intermediate ($P = 0.016$). The total N content was higher for C2 than for W2 and M2, with the lowest values observed for B2 ($P < 0.001$). In contrast, the opposite effect was observed for the EE content of larvae, which was greater for B2 than for W2 and M2, and lowest for C2 ($P < 0.001$). Total DM production per tray was higher for B2 than for M2, with C2 and W2 showing intermediate results ($P = 0.013$). Total N production tended to be lower in larvae fed M2 compared to the other treatments, although differences did not reach significance ($P = 0.053$). Total production of EE per tray was higher for B2 than for W2 and M2 and lowest for C2 ($P < 0.001$).

Discussion

Results observed in Experiment 1 showed that the cereal source included in the substrate as the unique source of nutrients in unbalanced diets affected *T. molitor* larvae performance. In this sense, larval growth and F:G ratio were better for C1 than for B1 and W1, whereas the poorest performance results were observed for M1. These results suggest a potential existence of a restriction in N and amino acid availability with respect to larval requirements when maize was used as the reference substrate. Compared to maize, productive performance was improved when substrates were made up of wheat bran (C1, 28.1 g N/kg DM, equivalent to 176 g CP/kg DM) or other cereal grains such as barley and wheat as the main source of nutrients, which resulted in 92 and 106 g CP/kg DM for the B1 and W1 diets, respectively (14.6 and 17.0 g N/kg DM, respectively). In this regard, results from our group showed that levels over 100 g CP/kg DM, which is equivalent to 16 g N/kg DM, should be necessary to allow for an adequate growth of *T. molitor* larvae (Fondevila and Fondevila, 2023). Similarly,

Table 5
Mortality, growth performance, feed to gain ratio (F:G) and total nutrient production of *T. molitor* larvae grown on wheat bran (C2), wheat (W2), barley (B2) or maize (M2) for 35 days in Experiment 2 (balanced diets).

Item	C2	W2	B2	M2	SEM	*P*-value[1]
Mortality[2]	0.025	0.025	0.063	0.046	0.0139	0.219
Initial weight (mg)	8.6	8.7	8.5	8.5	0.06	0.259
Final weight (mg)	97a	95a	94a	85b	1.8	< 0.001
Mass gained (g/tray)	5.15a	5.06a	4.84a	4.38b	0.097	< 0.001
Larval growth (mg/day)	2.51a	2.47a	2.45a	2.20b	0.050	0.003
Intake (g/tray)	3.95b	4.97a	5.35a	4.08b	0.114	< 0.001
Intake (g DM/tray)	3.35b	4.37a	4.76a	3.80b	0.109	< 0.001
F:G (g/g, fresh basis)	0.767c	0.981b	1.106a	0.932b	0.0123	< 0.001
F:G (g/g, DM basis)	2.165	2.486	2.487	2.337	0.1031	0.141
Larval composition						
DM content	32.5b	35.7ab	40.3a	36.3ab	1.43	0.016
N content (mg/g DM)	83.5a	75.1b	71.2c	74.3b	0.37	< 0.001
EE content (mg/g DM)	213c	285b	338a	287b	5.9	< 0.001
Total production (mg/tray)						
DM	1 670ab	1 808ab	1 948a	1 590b	67.0	0.013
N	140	136	139	118	54.2	0.053
EE	356c	515b	658a	458b	21.2	< 0.001

EE = Ether extract.
Within rows, values not sharing a common letter are significantly different ($P < 0.05$).
[1] *P*-values from the ANOVA. A significant interaction between treatment × date of experiment was detected ($P < 0.001$) for larval weight (Fig. 2).
[2] Estimated as the ratio of dead larvae to the initial number of individuals.

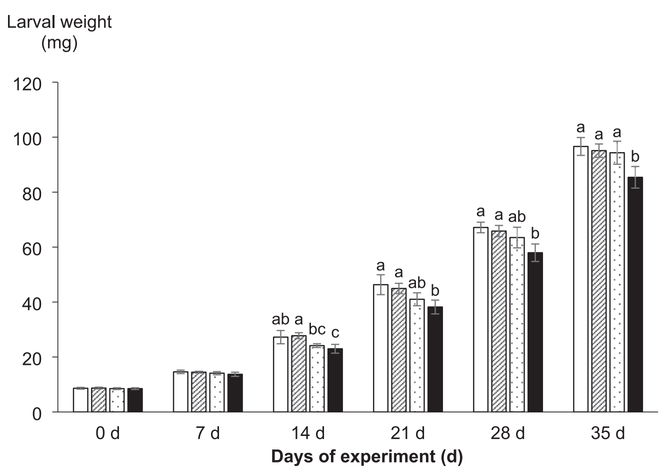

Fig. 2. Weight pattern (mg) of *T. molitor* larvae grown on wheat bran (C2, white bars), wheat (W2, diagonal lined bars), barley (B2, dotted bars) or maize (M2, black bars) along the experimental period in Experiment 2 (balanced diets). The interaction treatment × date was significant ($P < 0.001$; SEM = 0.85), and SD values for each column are given in bars. Columns not sharing a common letter are significantly different ($P < 0.05$).

Pascual et al. (2024) observed a lower larval growth and poorer feed conversion rate with maize than barley or wheat bran, where only the former feed had a CP content below 100 g/kg DM. Thus, the N content of M1 (71 g CP/kg DM, equivalent to 11.4 g N/kg DM) could not be enough for optimal larval growth. Furthermore, the limited N and amino acid availability could also be the cause of the higher mortality observed in larvae fed M1, as suggested by van Broekhoven et al. (2015). In this respect, Rumbos et al. (2020) compared different cereal sources and observed that larval weight decreased as the N content of the substrate was progressively reduced by the use of durum wheat, barley, oats, rye, and maize (151, 108, 94, 85, 77 g CP/kg DM, respectively). Wheat bran, a cereal co-product with low starch and high NDF contents (FEDNA, 2021), has been generally used as a reference substrate for *T. molitor* larvae, often complemented with cereal grains to increase energy input and to improve feed efficiency (Ramos-Elorduy et al., 2002; Ribeiro et al., 2018). The improved growth rate observed for C1 with respect to W1 and B1 in Experiment 1 suggests that substrates based on wheat or barley as the unique source of nutrients might not allow for an optimal larval performance. Furthermore, the improvements in growth performance followed a similar trend for the F:G ratio, which in turn improved with the increase of the N content of the substrate. This information supports that growth performance and feed efficiency in *T. molitor* larvae are impaired by reductions in the N content of the cereal used in the substrate, in agreement with the results reported herein.

In Experiment 2, the addition of wheat bran and straw was used to balance the N and starch contents in substrates based on cereal grains, using a common ingredient in all cases to minimise any ingredient effect on the response. Insects typically grow faster in high-protein substrates (Jensen et al., 2017) and differences in growth performance between substrates based on wheat bran (C2, 28.1 g N/kg DM) compared to those based on wheat or barley as main ingredients were minimised and became non-significant when the dietary N content of W2 and B2 was increased to 19.8 and 19.3 g N/kg DM (equivalent to a CP content of 124 and 121 g/kg DM), respectively. These findings support the previous assumption that 16 g N/kg DM are required to achieve maximum larval responses (Fondevila and Fondevila, 2023).

The increase in the N level of the substrate by the inclusion of wheat bran in the maize-based diet in Experiment 2 (19.3 g N/g DM for M2), although alleviated some response differences, did not result in a proportional increase in larval performance. In this respect, larvae fed M2 grew less and showed a poorer F:G ratio than those fed the other diets. Consequently, it could be assumed that the impairments observed in growth performance in larvae–fed maize diets might not only be associated with their N content but also with the nature of the carbohydrate fraction of grains. The physical structure of the starch from maize grains is more vitreous and contains a higher proportion of large starch granules compared to other cereal sources such as wheat or barley (Giuberti et al., 2013). The results reported in our research suggest that the physical structure of the particles present in maize might promote certain rejection for consumption, resulting in a reduction in feed intake. In this sense, although starch granules from cereals showed a similar pattern of insect enzymatic digestion *in vitro*, Meireles et al. (2009) suggested that mastication is an important step for their utilisation to allow for full access to nutrients.

Probably, the crystalline structure of the maize endosperm might impair larval feeding behaviour, showing an evident preference for other floury substrates such as wheat and barley. As a result, larval performance might be impaired due to the reduction in feed intake when maize is used as the main cereal source in the substrate. In any case, other factors related to maize composition, such as certain limiting nutrients, could also be partly responsible of the reduction in larval performance despite the total protein content of the maize-based diets was increased over the requirements in Experiment 2. In this respect, compared to wheat bran, maize protein is particularly deficient in lysine (6.1 and 2.2 g/kg DM for wheat bran and maize, respectively) and tryptophan (2.5 and 0.6 g/kg DM for wheat bran and maize, respectively), as well as in trace elements and vitamins such as K, S, Cu, Fe, Mn, Zn, biotin and choline (FEDNA, 2021). In contrast, the contents in essential amino acids are greater in wheat and barley compared to maize and consequently, differences in larval performance compared to wheat bran are expected to be lower. In fact, larval yield with these cereal grains was similar to wheat bran once the N content of the diet was increased over the larval requirements in Experiment 2. However, substrate intake was higher and F:G ratio was poorer in larvae fed W2 and B2 compared to C2, suggesting that *T. molitor* larvae increased their feed intake to compensate for the lower protein content of the substrate to maximise growth performance. However, intake and F:G results must be interpreted with caution, as the larval excreta mixed with residual substrate may bias the results both quantitatively and qualitatively, as discussed by Fondevila et al. (2024).

Impairments in F:G ratio may also result from deficiencies in certain essential amino acids, as suggested by the comparisons between the larval protein profile based on published research (Stull et al., 2019; Ruschioni et al., 2020; Montalbán et al., 2022) and that observed in the current ingredients (FEDNA, 2021). To date, no information is available in the literature on larval amino acid requirements and digestibility values. However, it appears that the amino acid profile of barley with respect to lysine is slightly poorer than that of wheat in terms of methionine, threonine, tryptophan, isoleucine, valine and arginine contents (45.6 vs 57.1, 91.9 vs 101.8, 33.3 vs 40.4, 97.2 vs 125, 136 vs 152 and 136 vs 173% lysine, respectively). These differences might help to explain the poorer F:C ratio observed in larvae fed B2 compared to those fed W2. The current results suggest that further research is required to better understand larval requirements in terms of amino acids.

Larval performance did not show a clear response to the starch content of the substrate in Experiments 1 and 2. Besides its higher N and NDF contents with respect to cereal grains, wheat bran has some extent of starch that can easily be used as an energy source by *T. molitor* larvae. However, compared to the wheat bran diets, the amount of starch in the substrates based on cereal grains was 3.3–3.7-fold higher in Experiment 1, and 3.1–3.4-fold higher in Experiment 2. It should be considered that larvae were reared under *ad libitum* conditions and consequently, larvae could select the proportions of particles and ingredients required to meet their energy requirements. However, the improvements in F:G ratio observed when the substrate was based exclusively on wheat bran suggest that larvae could efficiently obtain energy from other components such as protein and fibre. In this sense, protein could be used as a source of energy apart from providing amino acids for protein deposition. Furthermore, it could be speculated that protein levels exceeding the growth requirements might result in an extra amount of energy available without any evident detrimental effect on feed efficiency (Rumbos et al., 2020). In this respect, van Broekhoven et al. (2015) observed improvements in larval growth and survival rates in compound substrates with starch contents below 75 g/kg DM since the protein level was well above the expected requirements. On the other hand, Oppert et al. (2010) suggested that *T. molitor* larvae degrade cellulose by endogenous (insect produced) and exogenous (from digestive microbiota) fibrolytic enzymes. Besides, Yang et al. (2019) evaluated the ability of *T. molitor* larvae fed wheat bran and other cereal by-products to digest fibre polysaccharides and reported digestibility rates of cellulose, hemicelluloses and lignin that ranged from 0.27 to 0.53, 0.21–0.59 and 0.01–0.52, respectively. Fondevila et al. (2024) did not observe differences in larval performances among isonitrogenous wheat bran diets with NDF and starch contents ranging between 280 and 330 g/kg and 330 and 390 g/kg, respectively. In contrast, other authors proposed significantly lower fibre digestibility values in wheat bran diets, in the range of 0.14–0.16 (Fasce et al., 2022). Discrepancies among results might be partly attributed to methodological differences. In any case, the results of the current research suggest that, in contrast to the amount of N available in the substrate or the physical structure of the endosperm, the starch content of the diet could not be considered as a limiting factor for optimal performance of *T. molitor* larvae, provided that other sources of energy such as protein and structural carbohydrates are offered in sufficient amounts.

The results obtained in this study suggest that the chemical composition of larvae was strongly related with the substrate used. In Experiment 1, the DM content was highest in larvae fed B1, resulting in a total DM production similar to that observed for wheat bran. Furthermore, differences in DM content promoted by barley were maintained after the addition of wheat bran to balance nutrient content in the B2 diet in Experiment 2. The results given in the literature on the effects of feeding cereal products on larval DM content are variable and therefore do not allow to draw firm conclusions. Ruschioni et al. (2020) reported greater DM content in larvae fed wheat bran than in those fed wheat grain. However, the control period for larval analysis differed among treatments as larval pupation started earlier in larvae grown on wheat bran and consequently, the results obtained might be partially biased. Besides, Jajić et al. (2022) observed that the larval DM content increased with substrates based on wheat bran compared to barley or oats, although larval weight or age was not specified. The differences observed in the current research for the DM content of the larvae cannot be easily explained by substrate composition. However, the fact that the treatment differences detected in Experiment 1, although reduced, were maintained in Experiment 2 after balancing nutrient contents, suggests a potential ingredient effect on the DM content of the larvae, with greater values observed when barley was included as the main source of nutrients.

In both experiments, larvae given wheat bran showed the highest N content, probably as a response to the higher protein content of the substrate, in agreement with previous research (Heckmann et al., 2018; Rumbos et al., 2020; Langston et al., 2024). In this respect, a reduction in the N content of the substrate when cereal grains were used in Experiment 1 (17.0, 14.6 and 11.3 g N/kg for W1, B1 and M1, respectively) decreased linearly the N content of the larvae. Probably, when used as the main nutrient source, M1 could be considered as N deficient and B1 might be at the threshold. However, the lower N retention with barley remained significant in Experiment 2, when the substrates were balanced to an N content considered as sufficient to meet larval requirements (Fondevila and Fondevila, 2023). Consequently, the N content in the substrate could not be directly correlated to protein deposition in the larvae once the requirements are satisfied, suggesting an ingredient-related effect that could affect larval composition. In contrast, other authors suggested that the diet effect on the chemical composition of *T. molitor* larvae is generally lower for protein than for the lipid contents (van Broekhoven et al., 2015; Berezina, 2017). In the current research, the inverse relationship observed within treatments in both experiments between N and lipid larval retention seemed to be consistent. In Experiments 1 and 2, the inclusion of wheat bran or barley in the substrate increased the N and EE content of the larvae, respectively, in agreement with the results reported by Jajić et al. (2022). Differences in the carbohydrate availability (starch vs fibre) among cereal sources may promote differences in energy retention in the form of lipids instead of that of protein. However, most published research evaluating the larval response to different ingredients as feed does not include information on the nature of the carbohydrates present in the substrate or if so, this is limited to an estimation of the crude fibre and N-free extract contents, thus making difficult to draw firm conclusions on their effects on larval composition (Rumbos et al., 2020; Ruschioni et al., 2020; Jajić et al., 2022).

In summary, the cereal grain used in the substrate as the main source of nutrients altered the growth performance and chemical composition of *T. molitor* larvae. In this regard, larvae fed unbalanced diets based on maize reduced significantly their feed intake and growth performance compared to those fed barley and wheat, which in turn grew less than larvae fed wheat bran. The increase in the N content of the substrate by the inclusion of variable amounts of wheat bran reduced the differences in growth performance among substrates based on different cereal grains. In fact, larvae fed balanced diets based on wheat and barley yielded similar to that fed wheat bran. However, the impairments in growth performance in larvae given a substrate based on maize, although alleviated, were maintained when the diet was balanced in N, suggesting a larval preference for substrates based on wheat or barley. In terms of larval composition, the N content seemed to be inversely correlated to the lipid content. In this respect, substrates made of wheat bran increased the N content of the larvae, suggesting that protein deposition is related, to some extent, to the N content of the diet. In contrast, substrates based on barley as the main source of nutrients increased the DM and EE contents independently of the dietary level of N and starch.

Ethics approval

Not applicable, since the use of insects as laboratory animal does not require approval from an Ethical Commission. Anyway, the authors confirm that the study was conducted in an ethical and responsible manner, upon consideration of the 3 Ŕs.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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