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# Changes in the chemical composition of the yellow mealworm (*Tenebrio molitor* L.) reared on different feedstuffs

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**ABSTRACT.** The aim of this study was to investigate the chemical composition of powdered mealworm larvae (*Tenebrio molitor* L.) reared on different nutritional substrates. Wheat bran was used as the control substrate, while, barley (whole grain), oats (whole grain), oats and barley whole grain mixture (50:50), buckwheat, and mixture of oat and barley sprouts (50:50) were selected as experimental substrates. A proximate analysis and mineral content determination were carried out for all substrates and larvae. Since insects are becoming an attractive alternative protein source for poultry, pigs and fish as a "novel" and natural feed material, lysine, methionine, and threonine levels have also been determined. Furthermore, in addition to fat content, the fatty acid profile was also determined. It was found that wheat bran was the most suitable substrate in terms of high protein yield in larvae (71% dry weight) with lowest fat content (7% dry weight). Linoleic acid content was the highest in the larvae fed wheat bran, while the highest α-linolenic acid content was obtained in the larvae reared on a mixture of oat and barley sprouts (50:50). Moreover, linear regression analysis demonstrated a weak correlation of substrate and larval protein content for all selected substrates. The highest content of each mineral was also obtained in the larvae reared on wheat bran (except iron and manganese, which were the second highest). Based on the experimental results, it can be concluded that meals from *T. molitor* larvae are an excellent feed material for use in livestock diets, especially those reared on wheat bran.

## Introduction

The demand for animal-based food protein is expected to increase in the near future due to exponential growth of the global population, which is projected to reach 9 billion by 2050 (Caparros Megido et al., 2016). In order to reduce the negative impact of food production activities on the environment, while meeting the demand of the population, new alternative sources of protein and foods are proposed (Wegier et al., 2018). Insects can convert agriculture and food waste residues into protein of high biological quality (Bordiean et al., 2020). Commercial mass production of insects as a protein source involves only a few insect species, including the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Small-scale producers have been rearing it for animal feed, fish bait, or human consumption (Ribeiro et al., 2018).

According to recent studies, *T. molitor* is one of the most widely insects in Europe (Sogari et al., 2019; Bordiean et al., 2020). It is also one of the most promising insect species for the food and feed sectors due to its low rearing requirements, high

industrial-scale productivity and rich nutritional composition (Mancini et al., 2020). The chemical composition of mealworms is balanced in terms of protein (approx. 50% dry matter) and lipid contents (approx. 30–35% dry matter) (Mancini et al., 2020) and they are a good source of essential amino acids, vitamins, and minerals (Finke, 2015; Mancini et al., 2020). To maintain low environmental impact, edible insects are reared on sustainable feeds, such as by-products that do not meet the nutritional requirements of other farmed animals (Pinotti et al., 2019). Considering both economic and environmental issues, recent efforts have been made to valorise agricultural raw materials, wastes, side streams, and by-products of the agro-industry through their utilization as substrates for rearing mealworm larvae (Kim et al., 2014).

*T. molitor* may convert many substrates originating from the agricultural and food industries (Oonincx et al., 2015; van Broekhoven et al., 2015). Furthermore, this species has been extensively studied to confirm its nutritional value and resistance to harmful compounds (mycotoxins, pesticides, heavy metals, etc.) (Bordiean et al., 2020). It seems likely that the yellow mealworm will be used on a large scale as food in the near future (Bordiean et al., 2020). The European Union regulated the production of animal protein from insects for use as food and feed (Regulation (EU) 2015/2283, 2015; Commission Regulation (EU) 2017/893, 2017). The most recent effort has been made in the legislation area thanks to the positive opinion of the EFSA Panel on Nutrition, Novel Foods and Food Allergens (Truck et al., 2021). The Panel noted that the levels of contaminants in the yellow mealworm as a novel food (NF) depend on the occurrence of these substances in insect feed. The Panel stated that there were no safety concerns regarding the stability of NF, as well as its toxicity. The Panel also noted that the consumption of NF was not nutritionally disadvantageous. The mealworm is commonly reared on starchy substrates, such as wheat, spent grains, bread and cookie leftovers and other former foodstuffs (Oonincx et al., 2015; van Broekhoven et al., 2015). It should be noted that *T. molitor* larvae are considered scavengers (Rees, 2004), however, they are capable of consuming a wide variety of organic materials and wastes (Ramos-Elorduy, 2002). Thus, research into other possible feedstocks for *T. molitor* cultivation is warranted (Stull et al., 2019). Large-scale production of the yellow mealworm is expected to be significantly improved by screening alternative raw materials for use as low cost, high nutritional value feedstuffs (Melis et al., 2019). On the one hand, this would improve the sustainability of the supply chain, while on the other, it would likely improve the nutritional properties of this edible insect species relative to more traditional diets (Melis et al., 2019). Although the nutritional requirements of *T. molitor* have been studied in some detail (Heckmann et al., 2018), large-scale production of such insect species could be improved by focusing on the exploitation of low-cost by-products as dietary components (Melis et al., 2019). Van Broekhoven et al. (2015) found that *T. molitor* larvae exhibited extended survival and shorter development time on diets higher in protein, while lower survival and longer development time on the LPHS (low in protein and high in starch) diet compared to the control diet used by commercial mealworm producers (mixed grain diets). Similar to the protein source, larval performance could be influenced by the source of starch rather than the its absolute amount (van Broekhoven et al., 2015).

While *T. molitor* has been extensively studied, the nutritional value of the larvae reared on different diets and under variable conditions is less understood (Stull et al., 2019). Therefore, the aim of this study was to investigate the chemical composition of powdered mealworm larvae reared on different nutritional substrates.

## Material and methods

### Rearing of insects

Insects were obtained from the Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia. Mealworm cultures were maintained in an incubator under controlled conditions (27 ± 1 °C, 55% relative humidity in the dark) in 12-l plastic containers (20 cm × 40 cm × 15 cm). Since wheat bran is the most common diet in the mealworm industry and laboratory rearing facilities (Ribeiro et al., 2018), it was selected as the control substrate (diet) for insect rearing. Barley (whole grain) (S1), oats (whole grain) (S2), oat and barley whole grain mixture (50:50) (S3), buckwheat (S4), and mixture of oat and barley sprouts (50:50) (S5) were used as the experimental substrates. Whole grain substrates were ground in a laboratory hammermill, model SM100 rostfrei (Retsch GmbH, Haan, Germany). A 2-mm sieve was used to prepare fine granulation for easier consumption by young larvae. Throughout the breeding process, carrot pieces were spread

four times a week over the food mixture to provide additional moisture to the insects. Before the next step, larvae were separated from feed and frass debris and subsequently fasted for 24 h to eliminate residual frass contained in the gastrointestinal tract. All experiments were performed in triplicate.

### Preparing insects for drying and cooking

Insects were sieved (2.5 mm pore diameter) and the remaining insect parts were removed with a weak air flow produced by a hair dryer. The sieved larvae were transferred to a sieve with smaller holes and remains of insect bodies were removed with a weaker airflow. Afterwards, the cleaned larvae were transferred into a 2-l plastic container and gently washed under a stream of water. Subsequently, the insects were placed in a container with boiling water and cooked for 180 s. The entire content of the cooking pot was then filtered through a sieve to remove water, and the larvae were spread in a thin layer on filter paper to evaporate excess water for 24 h. The dried insects were collected and placed on a new filter paper and allowed to dry for another 24 h.

### Chemical analysis

Chemical analysis of nutrient substrates and larvae was conducted using the same analytical methods. Proximate analysis was carried out using standard methods. Dry matter content (DM) was determined after drying (AOAC Official Method 934.01; AOAC International, 2005). Crude protein (CP) was analysed according to the standard Kjeldahl method (AOAC Official Method 2001.11; AOAC International, 2009) while crude fat content (EE) was determined as petroleum ether extract (AOAC Official Method 991.36; AOAC International, 2006). Ash content was determined in a furnace at 600 °C (AOAC Official Method 942.05; AOAC International, 2012). Crude fibre (CF) was determined using an ANKOM$^{2000}$ Fibre Analyser (ANKOM Technology, Macedon, NY, USA) by applying the AOCS method (AOCS, 2017). NFE was calculated by subtracting the sum of moisture, CP, EE, ash, and CF contents from 100% of whole sample. Amino acids were determined after acid hydrolysis in 6 M HCl containing 0.1 phenol at 150 °C for 6 h. Detection was carried out using an Agilent Technologies 1260 series high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) and previously established analytical conditions (Jajić et al., 2013). Fatty acid composition was determined on a Thermo Scientific TRACE 1300 gas chromatograph equipped with a flame ionization detector (Thermo Scientific, Waltham, MA, USA) using a TR-FAME (length 30 m, inner diameter 0.32 mm, film thickness 0.25 μm) column (Thermo Scientific, Waltham, MA, USA). The injector and detector temperatures were 200 °C. Helium was used as the carrier gas with a flow rate of 1.3 ml/min. The sample and standard were diluted in n-heptane (analytical grade). Precisely 1 μl of the sample was injected into the injector. Fatty acid composition was calculated based on the peak areas. Prior to gas chromatography (GC) analysis, fat was extracted from the samples using a Soxhlet extractor. Approximately 20 mg of fat was weighed in a 5-cm$^3$ v-vial (Sigma-Aldrich, Buchs, Switzerland) and 0.5 ml of 0.5 M NaOH was added. The vial was then heated to 70 °C for 10 min and cooled to room temperature. Subsequently, 0.5 ml of boron trifluoride (Sigma-Aldrich, Buchs, Switzerland) was added and again heated to 70 °C for 10 min and cooled to room temperature. Finally, 1 ml of saturated NaCl solution and 1 ml of n-heptane was added and gently mixed. The upper (heptane) layer was transferred into a 1-ml tube containing anhydrous sodium-sulphate. After incubation for 30 min, the heptane layer was transferred to a GC vial and analysed. All analyses were carried out in duplicate.

### Statistical analysis

Data analysis was performed using Statistica software version 13.5.0.17 (TIBCO Software Inc., 2018, Palo Alto, CA, USA). One-way ANOVA and Dunnett's multiple comparison test were used to compare the obtained data, while simple linear regression was used to determine the relationship between nutritional substrates and larvae in terms of nutrient contents. The $P$ values $< 0.05$ were considered statistically significant.

## Results and discussion

A total of six nutritional substrates were used for the rearing of *T. molitor* larvae; their chemical composition is presented in Tables 1–5. The highest protein (22.6%) and fat content (9.6%) was determined in S5. A similar protein content (20.9%) was observed in the control substrate (S0). Protein content in other substrates (S1–S4) ranged from 11.4 to 15.9%. The lowest fat content was found in S1 (1.8%). Crude fibre content varied among substrates, ranging from 3.8 (S5) to 16.7% (S4). On the other hand, ash content was less variable and ranged

**Table 1.** Proximate analysis of nutritional substrates, dry weight (n = 3)

| | Dry matter, % | Protein, % | Fat, % | CF, % | Ash, % | NFE, % |
|---|---|---|---|---|---|---|
| S0 | 90.4 ± 0.3 | 20.9 ± 0.0 | 5.2 ± 0.0 | 7.4 ± 0.4 | 4.5 ± 0.1 | 62.1 ± 0.0 |
| S1 | 91.7 ± 0.0 | 11.4 ± 0.1 | 1.8 ± 0.0 | 5.3 ± 0.2 | 2.5 ± 0.0 | 79.1 ± 0.2 |
| S2 | 90.8 ± 0.1 | 15.9 ± 0.1 | 5.1 ± 0.0 | 12.7 ± 0.3 | 3.6 ± 0.0 | 62.7 ± 0.3 |
| S3 | 91.4 ± 0.2 | 13.9 ± 0.1 | 3.4 ± 0.0 | 10.1 ± 0.0 | 3.2 ± 0.1 | 69.5 ± 0.1 |
| S4 | 89.2 ± 0.0 | 12.2 ± 0.3 | 2.5 ± 0.2 | 16.7 ± 0.3 | 2.2 ± 0.0 | 66.4 ± 0.2 |
| S5 | 92.7 ± 0.0 | 22.6 ± 0.2 | 9.6 ± 0.0 | 3.8 ± 0.0 | 3.7 ± 0.0 | 60.3 ± 0.2 |

S0 – wheat bran, S1 – barley (whole grain), S2 – oats (whole grain), S3 – oats and barley (50:50), S4 – buckwheat, S5 – mixture of oat and barley sprouts (50:50), CF – crude fibre, NFE – nitrogen-free extract

from 2.2 (S4) to 4.5% in the control substrate. Nitrogen-free extract (NFE) content, representing starchy carbohydrates was the highest in S1 (79.1%) and the lowest in S5 (60.3%).

Mineral composition of nutritional substrates (Tables 2, 3) indicated high calcium levels in S2, phosphorus in the control substrate and S5, while sodium levels ranged from 0.011 to 0.029%. Potassium content was the highest in S2 and S0.

**Table 2.** The content of macro-minerals in nutritional substrates, dry weight (n = 3)

| | Ca, % | P, % | Na, % | K, % |
|---|---|---|---|---|
| S0 | 0.059 ± 0.005 | 0.874 ± 0.024 | 0.029 ± 0.005 | 0.257 ± 0.023 |
| S1 | 0.047 ± 0.005 | 0.236 ± 0.005 | 0.029 ± 0.005 | 0.167 ± 0.011 |
| S2 | 0.103 ± 0.005 | 0.316 ± 0.019 | 0.015 ± 0.005 | 0.230 ± 0.006 |
| S3 | 0.062 ± 0.005 | 0.277 ± 0.010 | 0.029 ± 0.005 | 0.195 ± 0.013 |
| S4 | 0.060 ± 0.005 | 0.333 ± 0.011 | 0.019 ± 0.005 | 0.226 ± 0.018 |
| S5 | 0.081 ± 0.005 | 0.749 ± 0.027 | 0.011 ± 0.003 | 0.186 ± 0.011 |

S0– wheat bran, S1 – barley (whole grain), S2 – oats (whole grain), S3 – oats and barley (50:50), S4 – buckwheat, S5 – mixture of oat and barley sprouts (50:50)

**Table 3.** The content of micro-minerals in nutritional substrates, dry weight (n = 3)

| | Fe, mg/kg | Zn, mg/kg | Cu, mg/kg | Mn, mg/kg |
|---|---|---|---|---|
| S0 | 115.3 ± 3.4 | 54.4 ± 4.6 | 7.8 ± 0.1 | 16.5 ± 2.1 |
| S1 | 94.3 ± 3.4 | 41.3 ± 0.8 | 2.3 ± 0.1 | 21.1 ± 1.3 |
| S2 | 98.6 ± 2.5 | 58.9 ± 3.1 | 3.3 ± 0.1 | 19.9 ± 2.0 |
| S3 | 101.0 ± 8.2 | 50.4 ± 5.6 | 2.1 ± 0.1 | 11.3 ± 1.1 |
| S4 | 108.6 ± 4.8 | 67.7 ± 3.2 | 2.2 ± 0.1 | 13.7 ± 1.8 |
| S5 | 60.3 ± 3.3 | 54.8 ± 4.5 | 5.7 ± 0.1 | 7.1 ± 0.9 |

S0 – wheat bran, S1 – barley (whole grain), S2 – oats (whole grain), S3 – oats and barley (50:50), S4 – buckwheat, S5 – mixture of oat and barley sprouts (50:50)

Iron content was more consistent, ranging from 94.3 to 115.3 mg/kg, except for S5 (60.3 mg/kg). Zinc was the least variable mineral and its levels varied from 50.4 to 67.7 mg/kg. Copper content was low (2.1 to 7.8 mg/kg), while that of manganese was slightly higher (7.1–21.1 mg/kg). Considering the findings of Klasing et al. (2000), who showed that supplementing *T. molitor* diets with calcium increased calcium content in the larvae, it could be expected that mineral contents in the substrates might affect their levels in the larvae.

The fatty acid profile of nutritional substrates (Table 4) revealed a similar n-6/n-3 fatty acid ratio, ranging from 12.1 to 12.9. The substrates were rich in linoleic acid (C18:2), especially S0, S1 and S3. These substrates also contained high levels of α-linolenic acid (C18:3). A significant level of eicosapentaenoic acid (C20:5), which belong to polyunsaturated fatty acids, was found in S2 (1.30 g/100 g fat).

Threonine, methionine and lysine contents in nutritional substrates are presented in Table 5.

Proximate analysis of *T. molitor* larvae (Table 6) revealed the highest protein content in the larvae grown on the control substrate, significantly higher ($P < 0.05$) than protein content in the larvae reared on all experimental substrates. It was found that the larvae showed a 2- to 4-fold increase in crude protein content relative to the substrate, which was consistent with the results of van Broekhoven et al. (2015). In contrast to protein, fat content was the lowest in L0, and it was significantly lower ($P < 0.05$) in comparison to L1–L5. Crude fibre content in L0 was significantly higher ($P < 0.05$) than in the larvae from the experimental substrates, while NFE content did not show significant differences between the control and experimental larvae.

The regression coefficients ($R^2$) showed a weak correlation of main nutrients in the nutritional substrates and their levels in the larvae (Table 7). The relationship was particularly weak in terms of protein content ($R^2 = 0.3169$). Rumbos et al. (2020) reported similar findings, as they obtained a regression coefficient of $R^2 = 0.36$, but emphasized that despite this weak correlation, large larvae were produced. Contrary to our results, Ramos-Elorduy et al. (2002) and Gao et al. (2010) observed small differences in *T. molitor* protein content when grown on different diets.

**Table 4.** Fatty acid profile (g/100 g fat) of nutritional substrates (n = 3)

| | S0 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|
| C14:0 | ND | ND | ND | ND | ND | 0.16 ± 0,04 |
| C16:0 | 16.29 ± 0.09 | 19.27 ± 0.12 | 18.25 ± 0.13 | 19.68 ± 0.12 | 12.98 ± 0.20 | 15.94 ± 0.30 |
| C18:0 | 1.11 ± 0.04 | 1.42 ± 0.02 | 1.75 ± 0.01 | 2.00 ± 0.04 | 2.05 ± 0.00 | 1.01 ± 0.10 |
| C18:1 | 16.17 ± 0.14 | 16.27 ± 0.01 | 32.09 ± 0.76 | 23.37 ± 0.94 | 35.26 ± 0.09 | 30.03 ± 0.05 |
| C18:2 | 55.32 ± 0.68 | 52.79 ± 0.10 | 40.00 ± 0.66 | 49.27 ± 0.52 | 33.28 ± 0.07 | 43.45 ± 0.31 |
| C18:3 | 4.15 ± 0.07 | 4.28 ± 0.01 | 1.96 ± 0.04 | 3.41 ± 0.02 | 2.54 ± 0.01 | 2.89 ± 0.02 |
| C20:0 | 0.22 ± 0.01 | 0.31 ± 0.01 | ND | ND | 1.66 ± 0.01 | 0.12 ± 0.01 |
| C22:0 | 0.25 ± 0.00 | 0.32 ± 0.03 | ND | 0.26 ± 0.01 | 1.85 ± 0.01 | 0.16 ± 0.02 |
| C20:5 | 0.37 ± 0.03 | 0.09 ± 0.01 | 1.30 ± 0.00 | 0.65 ± 0.03 | 0.04 ± 0.00 | 0.70 ± 0.07 |
| C24:0 | ND | ND | ND | ND | ND | ND |
| C22:6 | ND | ND | ND | ND | ND | ND |
| ω6/ω3 | 12.3 | 12.1 | 12.3 | 12.1 | 12.9 | 12.1 |

S0 – wheat bran, S1 – barley (whole grain), S2 – oats (whole grain), S3 – oats and barley (50:50), S4 – buckwheat, S5 – mixture of oat and barley sprouts (50:50), ND – not determined, C14:0 – myristic acid, C16:0 – palmitic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2 – linoleic acid, C18:3 – α-linolenic acid, C20:0 – arachidic acid, C22:0 – behenic acid, C20:5 – eicosapentaenoic acid, C24:0 – lignoceric acid, C22:6 – docosahexaenoic acid

**Table 5.** Amino acid content in nutritional substrates, dry weight (n = 3)

| | S0 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|
| Thr, mg/g protein | 50.4 ± 0.7 | 53.2 ± 3.6 | 31.6 ± 2.0 | 43.9 ± 2.1 | 56.9 ± 1.1 | 77.4 ± 1.6 |
| Met, mg/g protein | 23.8 ± 0.5 | 26.7 ± 2.0 | 20.0 ± 1.5 | 20.5 ± 2.0 | 22.3 ± 3.0 | 40.1 ± 1.2 |
| Lys, mg/g protein | 35.4 ± 0.3 | 59.0 ± 1.9 | 27.9 ± 0.4 | 22.2 ± 1.8 | 40.9 ± 1.0 | 70.7 ± 1.3 |

S0 – wheat bran, S1 – barley (whole grain), S2 – oats (whole grain), S3 – oats and barley (50:50), S4 – buckwheat, S5 – mixture of oat and barley sprouts (50:50), Thr – threonine, Met – methionine, Lys – lysine

**Table 6.** Proximate analysis of *Tenebrio molitor* larvae, dry weight (n = 3)

| | Dry matter, % | Protein, % | Fat, % | CF, % | Ash, % | NFE, % |
|---|---|---|---|---|---|---|
| L0 | 77.2 ± 0.7 | 71.2 ± 1.1 | 6.1 ± 1.1 | 10.4 ± 0.2 | 7.5 ± 0.16 | 4.8 ± 0.3 |
| L1 | 64.7 ± 0.4* | 38.9 ± 0.2 | 45.2 ± 0.4* | 6.3 ± 0.1* | 3.48 ± 0.02** | 6.1 ± 0.4 |
| L2 | 75.7 ± 0.2* | 66.4 ± 0.7* | 12.1 ± 0.3* | 9.8 ± 0.4* | 6.63 ± 0.08* | 5.0 ± 0.9 |
| L3 | 69.2 ± 1.0* | 48.2 ± 0.7* | 34.0 ± 1.0* | 7.4 ± 0.3* | 4.50 ± 0.08* | 6.0 ± 0.5 |
| L4 | 65.8 ± 0.7* | 51.4 ± 0.7* | 34.0 ± 0.4* | 6.7 ± 0.1* | 3.63 ± 0.11* | 4.3 ± 0.4 |
| L5 | 62.7 ± 0.6* | 51.2 ± 0.2* | 34.9 ± 1.1* | 6.5 ± 0.2* | 3.58 ± 0.12* | 3.7 ± 1.2 |

L0 – wheat bran, L1 – barley (whole grain), L2 – oats (whole grain), L3 – oats and barley (50:50), L4 – buckwheat, L5 – mixture of oat and barley sprouts (50:50), CF – crude fibre, NFE – nitrogen-free extract; *symbol indicates statistically significant difference when compared to the control group $P < 0.05$ by Dunnett's multiple comparison test

**Table 7.** Regression coefficients ($R^2$) illustrating correlation of the substrate composition with protein and fat contents of *Tenebrio molitor* larvae after linear regression analysis

| | | Substrate | | | |
|---|---|---|---|---|---|
| | | Protein | Fat | CF | NFE |
| Larvae | Protein | 0.3169 | 0.1148 | 0.0444 | 0.5711 |
| | Fat | 0.2508 | 0.0650 | 0.0368 | 0.4458 |

CF – crude fibre, NFE – nitrogen-free extract

Furthermore, our results indicated a correlation between NFE content in the substrate and protein in the larvae, with a regression coefficient of $R^2 = 0.5711$. When comparing the protein to carbohydrate (NFE) ratio, increased larval protein content coincided with a lower ratio in almost all experimental combinations (except for the mixture of oat and barley sprouts). The highest content was obtained in the case of wheat bran at the protein to NFE ratio of approximately 1:3, while the lowest was recorded in the larvae grown on barley – a ratio of approximately 1:7. These findings were in line with previous research of Rho and Lee (2016), who reported that the optimal protein-to-carbohydrate ratio for lifespan and lifetime reproductive success was 1:1. In our study, higher protein content, indicating good larval development, was obtained with substrate ratios closer to 1:1.

It should be noted that we determined protein as crude protein using the Kjeldahl method, and calculated the protein-to-nitrogen ratio using the conversion factor for meat and feed samples (6.25), which is still most widely applied by researchers (Finke, 2015; van Broekhoven et al., 2015; Stull et al.,

2019). Alternatively, Janssen et al. (2017) suggested a conversion factor of 4.76 for *T. molitor*. Whichever conversion factor is used it did not affect the correlation between protein in the substrate and larvae, although it affected the protein-to-carbohydrate ratio.

Regarding fat content, van Broekhoven et al. (2015) noted that larval fat content and the fatty acid profile were more strongly affected by the diet. We found that larvae L1, grown on substrate S1, with the lowest protein and the highest NFE content, contained the highest fat content (45.2%). This was in line with the findings of van Broekhoven et al. (2015) who noted that insects could synthesize lipids from different dietary components, such as carbohydrates. On the contrary, Arrese and Soulages (2010) reported that larvae reared on the LPHS diet had a lower fat content.

In terms of the mineral composition of the larvae (Tables 8, 9), the highest content of all macronutrients was found in control group L0. The second highest macronutrient contents were recorded for larvae L2, although all (except calcium) were significantly lower ($P < 0.05$) compared to the control group.

**Table 8.** The effect of different nutritional substrates on the mineral composition of macronutrients in *Tenebrio molitor* larvae, dry weight (n = 3)

| | Ca, % | P, % | Na, % | K, % |
|---|---|---|---|---|
| L0 | 0.207 ± 0.011 | 1.429 ± 0.014 | 0.337 ± 0.024 | 1.929 ± 0.031 |
| L1 | 0.134 ± 0.008* | 0.664 ± 0.019* | 0.155 ± 0.001* | 0.644 ± 0.044* |
| L2 | 0.198 ± 0.014 | 1.299 ± 0.017* | 0.291 ± 0.013* | 1.656 ± 0.015* |
| L3 | 0.144 ± 0.013* | 0.876 ± 0.021* | 0.149 ± 0.006* | 1.039 ± 0.074* |
| L4 | 0.132 ± 0.007* | 0.724 ± 0.029* | 0.106 ± 0.001* | 0.678 ± 0.028* |
| L5 | 0.117 ± 0.009* | 0.696 ± 0.011* | 0.096 ± 0.001* | 0.728 ± 0.020* |

L0 – wheat bran, L1 – barley (whole grain), L2 – oats (whole grain), L3 – oats and barley (50:50), L4 – buckwheat, L5 – mixture of oat and barley sprouts (50:50); *symbol indicates statistically significant difference when compared to the control group $P < 0.05$ by Dunnett's multiple comparison test

**Table 9.** The effect of different nutritional substrates on mineral composition of micronutrients in *Tenebrio molitor* larvae, dry weight (n = 3)

| | Fe, mg/kg | Zn, mg/kg | Cu, mg/kg | Mn, mg/kg |
|---|---|---|---|---|
| L0 | 143.3 ± 10.5 | 183.7 ± 24.7 | 65.0 ± 45.1 | 11.3 ± 1.7 |
| L1 | 96.8 ± 8.5* | 82.3 ± 1.2* | 21.8 ± 1.9 | 3.3 ± 1.1* |
| L2 | 163.3 ± 32.5 | 134.2 ± 5.6* | 31.7 ± 3.3 | 4.5 ± 2.5* |
| L3 | 132.3 ± 16.6 | 109.4 ± 1.7* | 20.7 ± 2.0 | 6.3 ± 0.8* |
| L4 | 91.20 ± 13.2* | 117.4 ± 1.8* | 20.7 ± 2.8 | 8.1 ± 0.3 |
| L5 | 102.5 ± 11.6 | 121.4 ± 2.5* | 58.4 ± 75.3 | 13.5 ± 0.3 |

L0 – wheat bran, L1 – barley (whole grain), L2 – oats (whole grain), L3 – oats and barley (50:50), L4 – buckwheat, L5 – mixture of oat and barley sprouts (50:50); *symbol indicates statistically significant difference when compared to the control group $P < 0.05$ by Dunnett's multiple comparison test

All macronutrient concentrations in other larvae were significantly lower ($P < 0.05$) in comparison to the control group.

Klasing et al. (2000) noted positive correlation between calcium suplemented diet and its content in the larvae. Unfortunately, we were not able to confirm that the findings, as it seems that this correlation could can only be achieved when supplementing *T. molitor* diets with calcium. On the other hand, we obtained a 1.4-fold (L5) to 3.5-fold (L0) increase in calcium in the substrate. A significant increase in the substrate was recorded for sodium (19-fold) and potassium (more than 7-fold). Moreover, Klasing et al. (2000) obtained higher calcium levels in the larvae cultured on wheat bran. In contrast, Finke (2002) and Wu et al. (2020) found notably lower calcium, sodium and potassium levels, but these authors did not indicate the nutritional substrate.

The highest iron content was found in larvae L2 (163.3 mg/kg), although it was not significantly higher ($P > 0.05$) than in the control group. Iron content in larvae L3 and L5 was not significantly different from the control group, while the remaining groups (L1 and L4) contained significantly lower iron levels than the control group ($P < 0.05$). Zinc level was the highest in the control group (183.7 mg/kg), while other larvae (L1–L5) contained significantly lower concentrations of this element ($P < 0.05$). Groups L0 and L5 were the richest in copper, although statistically none of the larvae differed from control ($P > 0.05$). Manganese content was the highest in L5 (13.5 mg/kg), but it was not significantly different ($P > 0.05$) from control (11.3 mg/kg). Larvae L1–L3 had significantly lower levels of this element when compared to the control group ($P < 0.05$). As regards the micromineral increase in the substrate, only copper (up to 10-fold) and zinc (up to 3-fold) showed considerably higher values in the larvae. Unlike for macronutrients, Wu et al. (2020) found similar levels of micronutrients compared to our results. On the other hand, Finke (2002) indicated lower levels of all micronutrients tested. Such differences in the larval mineral composition could be due to different nutritional substrates used for insect rearing.

The fatty acid profile was analysed to determine the nutritional quality of fat in the larvae (Table 10). The results showed higher levels of unsaturated FA in comparison to saturated FA, which was consistent with previous studies (Tzompa-Sosa et al., 2014; Melis et al., 2019; Mattioli et al., 2021). With respect to unsaturated FA, the highest level of linoleic acid (C18:2) was found in larvae L1 (28.56%

**Table 10.** The effect of different nutritional substrates on the fatty acid (% in fat) profile of *Tenebrio molitor* larvae (n = 3)

| | L0 | L1 | L2 | L3 | L4 | L5 |
|---|---|---|---|---|---|---|
| C14:0 | 2.79 ± 0.04 | 4.20 ± 0.39* | 4.44 ± 0.61* | 3.55 ± 0.29 | 2.73 ± 0.22 | 2.75 ± 0.00 |
| C16:0 | 12.31 ± 0.69 | 18.63 ± 0.84* | 13.20 ± 0.67 | 14.52 ± 0.39* | 14.73 ± 1.07* | 15.69 ± 0.17* |
| C18:0 | 3.80 ± 0.43 | 3.21 ± 0.95 | 2.72 ± 0.07* | 2.65 ± 0.11* | 2.63 ± 0.23* | 2.41 ± 0.08* |
| C18:1 | 40.39 ± 1.29 | 48.36 ± 4.60* | 53.08 ± 1.47* | 54.85 ± 0.48* | 52.26 ± 5.20* | 46.39 ± 0.14 |
| C18:2 | 24.12 ± 1.56 | 28.46 ± 10.61 | 17.25 ± 0.05 | 17.20 ± 0.56 | 12.90 ± 1.59* | 25.57 ± 0.50 |
| C18:3 | 0.78 ± 0.04 | 0.68 ± 0.41 | 0.50 ± 0.47 | 0.26 ± 0.03 | 0.26 ± 0.09 | 0.97 ± 0.03 |
| C20:0 | 0.23 ± 0.04 | 0.06 ± 0.05* | 0.12 ± 0.10 | 0.15 ± 0.01 | 0.23 ± 0.02 | 0.10 ± 0.01* |
| C22:0 | 0.11 ± 0.02 | ND | 0.02 ± 0.03* | 0.03 ± 0.00* | ND | 0.03 ± 0.01* |
| C20:5 | 0.14 ± 0.13 | ND | 0.12 ± 0.13 | 0.10 ± 0.03 | 0.17 ± 0.08 | 0.03 ± 0.01 |
| C24:0 | ND | ND | ND | ND | ND | ND |
| C22:6 | 0.50 ± 0.16 | 0.05 ± 0.05* | 0.33 ± 0.26 | 0.15 ± 0.04* | 0.14 ± 0.00* | 0.07 ± 0.02* |
| ω6/ω3 | 16.9 | 38.6 | 18.1 | 34.2 | 22.5 | 24.0 |

L0 – wheat bran, L1 – barley (whole grain), L2 – oats (whole grain), L3 – oats and barley (50:50), L4 – buckwheat, L5 – mixture of oat and barley sprouts (50:50), ND – not determined, C14:0 – myristic acid, C16:0 – palmitic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2 – linoleic acid, C18:3 – α-linolenic acid, C20:0 – arachidic acid, C22:0 – behenic acid, C20:5 – eicosapentaenoic acid, C24:0 – lignoceric acid, C22:6 – docosahexaenoic acid; *symbol indicates statistically significant difference when compared to the control group $P < 0.05$ by Dunnett's multiple comparison test

fat), although similar levels were determined in control (24.12% fat) and larvae L5 (25.57% fat). A significantly lower level ($P < 0.05$) of linoleic acid in comparison to control was found only in larvae L4. When compared to control, the content of α-linolenic acid (C18:3) was higher only in the case of group L5, however, none of the differences were significant ($P > 0.05$). High levels of polyunsaturated fatty acids (C20:5 and C22:6), were found in control. However, groups L1–L5 were not significantly different ($P > 0.05$) from control in terms of C20:5 content. On the other hand, all larval samples, except for L2, contained significantly lower levels of C22:6 ($P < 0.05$) compared to the control group; in addition, a high level of C20:5 was detected in group L4. This was rather surprising as neither of these two polyunsaturated fatty acids was found in the substrates. On the contrary, Tzompa-Sosa et al. (2014) quantified C20:5 and C22:6 in feed, but did not detect them in *T. molitor* larvae. Furthermore, our results concerning the FA profile were consistent with the study of Melis et al. (2019) with respect to wheat bran both in the substrate and in the larvae.

In terms of predominant FA, our results were in line with the findings of van Broekhoven et al. (2015) who determined that the major fatty acids in the larval body were palmitic acid, oleic acid, and linoleic acid, which together accounted for 72–91% of total fatty acids. In our study, the abovementioned three fatty acids constituted from 76.8 to 95.5% of total fatty acids.

In the present work, the n-6/n-3 ratio in the substrates did not differ significantly and ranged from 12.1:1 to 12.9:1. However, this ratio was highly variable in the larvae, ranging from 16.9:1 in the control group to 38.6:1 in the group grown on barley (L1). This was rather unexpected, although it could be explained by the previous findings of Tzompa-Sosa et al. (2014) who believed that high carrot consumption contributed to the high n-6/n-3 ratio in mealworms, since the carrot n-6/n-3 ratio exceeded 50:1. Moreover, the same authors obtained the n6/n-3 ratio of about 27:1 in *T. molitor* fed a mixed diet combined of wheat, wheat bran, oats, soy, rye, corn, carrot and beer yeast. Mattioli et al. (2021) reported in turn the n-6/n-3 ratio of 19.77:1 for *T. molitor* larvae fed spent grains, and both cited reports were consistent with our results.

Threonine, methionine, and lysine amino acid levels were also determined in the larvae (Table 11). For easier interpretation of the results, amino acid content was given per protein, since protein levels varied considerably between the samples. Threonine levels ranged from 30.1 mg/g protein in group L0 up to 75.0 mg/g protein in group L1. Methionine and lysine levels were also the lowest in the control group – 15.7 mg/g protein and 43.9 mg/g protein, respectively. However, methionine in groups L1–L5 was not significantly different ($P > 0.05$) from control. On the other hand, threonine and lysine contents in groups L1, L3 and L5 were significantly higher ($P < 0.05$) when compared to control. The highest level of lysine was found in group L1 (105.6 mg/g protein), while methionine content was the highest in groups L3 and L5. When comparing amino acid concentrations between individual substrates (Table 5) and corresponding larvae (Table 11), lysine content was increased in the larvae in both the experimental and control groups. Threonine content was increased in three samples from the

**Table 11.** The effect of different nutritional substrates on amino acid content in *Tenebrio molitor* larvae, dry weight (n = 3)

| | L0 | L1 | L2 | L3 | L4 | L5 |
|---|---|---|---|---|---|---|
| Thr, mg/g protein | 30.1 ± 1.1 | 75.0 ± 4.6* | 36.6 ± 1.4 | 54.0 ± 0.3* | 38.8 ± 1.0 | 48.5 ± 1.1* |
| Met, mg/g protein | 15.7 ± 0.7 | 24.4 ± 4.2 | 17.6 ± 0.9 | 25.2 ± 0.7 | 20.7 ± 0.7 | 25.2 ± 0.5 |
| Lys, mg/g protein | 43.9 ± 0.7 | 105.6 ± 3.5* | 51.5 ± 2.0 | 74.9 ± 1.0* | 65.4 ± 2.7 | 87.9 ± 0.7* |

L0 – wheat bran (control), L1 – barley (whole grain), L2 – oats (whole grain), L3 – oats and barley (50:50), L4 – buckwheat, L5 – mixture of oat and barley sprouts (50:50), Thr – threonine, Met – methionine, Lys – lysine; *symbol indicates statistically significant difference when compared to the control group $P < 0.05$ by Dunnett's multiple comparison test

experimental groups of larvae (L1–L3), while in the control group and groups L4 and L5, it was lower than in the corresponding substrates. Methionine content was increased in the larvae only in group L3, while other larvae contained less methionine than the corresponding substrates. This may indicate that an increase in crude protein content does not necessarily mean an increase in actual protein, but also some other nitrogen compounds. On the other hand, lysine content was increased in all groups of larvae, which is promising as it is the first limiting amino acid in pig diets (Boisen, 2003).

Similar levels of amino acids were determined in a study by Janssen et al. (2017). Unfortunately, the latter authors did not indicate what kind of feed was applied, and thus such a comparison could not be made. Janssen et al. (2017) found 6.14 ± 0.08 g lysine, 1.52 ± 0.04 g methionine and 4.52 ± 0.03 g threonine in 100 g protein. The values expressed as mg/g protein were: 61.4 (lysine), 15.2 (methionine) and 45.2 (threonine), and although these values were similar to our results for larvae grown on some of the substrates tested in the present study, they could not be fully related to any of the substrates. The results of Ghosh et al. (2017) were consistent with our findings regarding threonine and lysine contents in the larvae reared on wheat bran; however the latter authors could not detect methionine. In addition, Zielińska et al. (2015) found slightly lower results for all three amino acids, but did not indicate the nutritional substrate for the larvae.

## Conclusions

This study has confirmed that *T. molitor* is a very rich source of all nutrients for animals, especially protein and fat, with a very good fatty acid profile and high content of the most important amino acids. However, the nutrient substrate used for growing *T. molitor* was also found to be important, as it affected the nutritional composition of the larvae. We have found that among the five substrates tested, wheat bran should be the best option to obtain larvae with the highest levels of protein and crude fibre, but also the lowest fat content. Based on the experimental results, it can be concluded that meals from *T. molitor* larvae have great potential for use as a feed material in livestock diets, especially when the larvae are reared on wheat bran.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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