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Investigation of the production parameters, nutrient and mineral composition of mealworm (*Tenebrio molitor*) larvae grown on different substrates

Áron Molnár[1,2]* – Toviho Odunayo Abigeal[1,2] – Milán Fehér[1]

[1]University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Department of Animal Husbandry, Debrecen

[2]University of Debrecen, Doctoral School of Animal Science

*Correspondence: molnar.aron@agr.unideb.hu

SUMMARY

During the rearing of mealworm (Tenebrio molitor) larvae, the optimisation of the growing substrate has a particular importance. The application of the appropriate substrate is a fundamental pillar for intensive and safe production. The requirements for substrate include the lack of toxins, high nutrient and micro-macro element content. The aim of this study was the evaluation of the effects of different substrates on the production parameters, nutrient and mineral composition of the mealworm larvae. The experiment lasted through 14 days. 5 treatments were set up at, where the variable was the substrate. The test system consisted of 25 units (5 treatments and 5 replicates). 10 mealworm larvae per unit, (total of 250 larvae) were used at the beginning of the experiment. Regarding the substrates, our study included sweet potato (SP), may turnip tuber (MT) and may turnip leaf (ML). In addition to the plant by-products, wheat flour (WF) and wheat semolina (WS) were used as control substrates. Trace element uptake and production parameters of the larvae were determined at the end of the experiment. Regarding the production parameters, it can be stated that the wheat semolina (0.081 ± 0.005 g) and wheat flour (0.069 ± 0.007 g) substrate used as control gave the best results for the final body weight. In terms of plant raw materials and by-products, sweet potatoes (0.063 ± 0.007) can only be recommended as substrate, while may turnip tuber and may turnip leaf produced significantly lower final body weight results (MT=0.034 ± 0.002 g ; ML= 0.036 ± 0.002 g). The nutrient composition of the larvae was not affected by the substrate, these results confirmed the high protein and fat content reported in the literature. The results with the production parameters were contradicted by the micronutrient content. Larvae reared on may turnip leaf (ML) and tuber (MT) showed the highest values for most of the macro- and microelements (potassium, calcium, magnesium, iron, zinc, copper) tested.

Keywords: *Tenebrio molitor, substrate, by-product, sustainable*

INTRODUCTION

The world is facing a growing protein deficit due to overpopulation. Therefore, new alternative protein sources need to be introduced to cope with it. Insect protein is an excellent option. The larvae of the common mealworm (*Tenebrio molitor*) stand out among insects for their high nutritional value, besides that they are easy to breed its growth performance is also excellent. Larvae requires minimal space to grow and has no impact on the environment, but is a significant source of protein and fat (Elorduy et al., 2002; Ravzanaadii et al., 2012; Wang et al., 2012, Dreassi et al., 2017; Grau et al., 2017; Son et al., 2020; Costa et al., 2020; Cappelli et al., 2020; Shafique et al., 2021). Proteins and fats also play a key role in the diet, and mealworms are rich in these, which is why the food industry sees them as a potential raw material (Park et al., 2015). Besides the temperature and the processing method, the optimal substrate has also particular importance for proper production of mealworm. In the case of the substrate, in addition to good nutritional value, attention must be paid to sterile conditions, as various pathogens such as salmonella may be presented (Wynants et al., 2019).

During mealworm farming, a wide range of substrates are used in addition to traditional food raw materials, such as organic by-products or even food waste (Baiano, 2020). Several research groups have worked on testing different substrate for mealworm production (Hosen et al., 2004, Broekhoven et al., 2015; Rumbos et al., 2020). Olive pomace, a well-known organic by-product which is similar to sweet potato, was also found to be a suitable substrate in combination with wheat middling. They have a positive effect on growth rates in pupal and larval stages (Ruschioni et al., 2020). Many other organic by-products of agri-food industry may also be suitable as substrate for the mealworm (Melis et al., 2019). Even fungi as a substrate may be suitable for the mealworm at a certain percentage (Kim et al., 2014), for instance an edible fungi (*Lentitus edodes*) was mixed with 40% of the mealworm's substrate. The results of the experiment showed that this species can be used to partially supplement the mealworm's substrate (Li et al., 2020). Melis et al. (2019) investigated two different substrate as a possible meal for mealworm. Wheat bran and dried brewer's spent grain were compared as a possible nutrient for mealworm. Brewer's spent grain achieved better feed efficiency and markable lower fat content compared to wheat bran. Hosen et al. (2004) investigated several cereal flours as substrate on growth of lesser mealworm (*Alphitobius diaperinus*) in all the developmental stages. Their results shown that cereal flours has effect on the speed of growth. Cereal flours reduce the growth of larvae, pupa and adults in the end of the experiment. The substrate affects the fat content of mealworm larvae, according to Dreassi et al. (2017). They investigated the fat content of mealworm larvae by testing with 6 different fat content substrates. The substrate used were oats, corn flour, wheat flour, chickpea flour, bread and beer yeast. They kept the

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stock an average temperature of 27 ± 1 °C and the relative humidity was 40–50%. At the end of their experiment, they concluded that, despite using 6 different fat contents in the substrate, there was no change in fat content in either larval or pupal stages, with constant values in both larval and pupal stages (>34% in larvae and >30% in pupae). In the end, the authors point out that the pupal stage has a higher ratio of omega3/omega6 fatty acids, therefore worth using for human consumption in this stage.

In our experiment substrates are vegetables and raw materials that are commercially available. The sweet potato (*Ipomoea batatas* L.) is a well-known vegetable, it is indigenous to tropical America. Its root is rich in carbohydrates and β-carotene, furthermore its leaves has a high protein content. The roots has also beneficial component, such as vitamins C, B complex, and E, in addition potassium, calcium, and iron. It contains anthocyanins, which is an antioxidant (Lebot, 2010). It plays an important role in human nutrition and is also a common ingredient in animal feed (Hahn, 1977), it is the seventh most important food crop in terms of worldwide production (Loebenstein et al., 2003). May Turnip (*Brassica rapa subsp. rapa var. majalis*) is worldwide consumed vegetable and belongs to traditional Chinese medicine due to its high dietary fiber content (Gao et al., 2021). It contains vitamin C and antioxidants (Saeed et al., 2012).

The aim of our experiment was to test different natural substrates as sweet potato *(Ipomoea batatas* L.) and different parts of may turnip (*Brassica rapa subsp. rapa var. majalis*) on mealworm culture and to investigate the effect of plant raw materials and by - products on the production parameters, nutrient and mineral composition as well as dry matter content of the larvae.

MATERIALS AND METHODS

The test was carried out in the insect room of the Aquaculture Laboratory of University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management and lasted 14 days. Preparation of the substrate (plant samples): tubers and leaf greens of commercially purchased sweet potatoes (*Ipomoea batatas*) and may turnip (*Brassica rapa subsp. rapa var. majalis*) were sliced and dried in an industrial dehydrator (ALPFRIGO CFD 700) at 40 °C for 24 h. After drying, the plant parts were grated and used as culture substrate at the rate of 15 g per unit. The measured dry matter content of plant samples:

- sweet potatoes: 23.06%
- may turnip tubers: 6.30%
- may turnip leaves: 15.60%

The test system is consisted of 25 x 150 ml plastic containers. We applied 5 treatments with 5 replicates. The treatments are named after the substrate material.

- Treatment 1: edible semolina (WS)
- Treatment 2: wheat flour (WF)
- Treatment 3: sweet potato tuber (SP)
- Treatment 4: may turnip tuber (MT)
- Treatment 5: may turnip green leaf (ML)

10 mealworm larvae were stocked per unit, in a total of 250. The homogeneous stock was acquired from commercial sources. The mean individual body weight at the start of the experiment were 3.50 ± 0.33 mg. The following environmental parameters were determined every two days during the experiment.

- Air temperature: (PCE-THB 40 Thermo Hygrometer Barometer): 22.90 ± 2.32 °C
- Humidity (PCE-THB 40 Thermo Hygrometer Barometer): 46.50 ± 10.53%.
- Light intensity (PKT-5065 digital luxmeter): 3.10 ± 1.45 lux

The production and quality parameters of mealworm larvae were also determined.

- Survival: (number of live individuals/ released number of individuals) * 100)
- Body weight: individual body weights of larvae were determined one by one at the beginning and at the end of the experiment using a digital scale
- Initial biomass and final biomass
- Growth rate (% day^{-1}): (ln BWf - ln BWi) / t x 100, where: Wf: final body mass (g), Wi: initial body mass (g), t: number of days.

At the end of the experiment, larval samples were dried to constant weight in a drying oven to determine dry matter content, and then destruction with 2 x 4.0 ml of 65% (m m^{-1}) nitric acid and 1.0 ml of 30% (m m^{-1}) hydrogen peroxide. The samples were then placed in plastic tubes and made up to 10 ml. The elemental composition of the diluted samples was analysed using an AGILENT ICP-OES 5110 VDV (inductively coupled plasma atomic emission spectrometer) using a 5-point calibration linear (University of Debrecen Faculty of Science and Technology, Department of Inorganic and Analytical Chemistry, Atomic Spectroscopy Partner Laboratory).

We measured the protein content (MSZ ISO 937:20000), the ash content (MSZ ISO 936:20000) and finally the total fat content (MSZ ISO 1443:20000). The unit of measurement for these parameters was (m m^{-1}) % (University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Agricultural Laboratory Center).

At the end of the experiment, the survival, individual body weight, growth rate, and micro- and macroelement concentrations of the larvae were subjected to statistical analysis. IBM SPSS 20 software was used for the analysis. Data homogeneity was checked by Levene's test. One-way analysis of variance (ANOVA) was used to evaluate the results. Tukey test ($P<0.05$) was used to determine significant difference.

RESULTS AND DISCUSSION

Production parameters

Larval survival was good for all substrates tested. In the experiment, significantly lower survival, averaging 92%, was obtained with larvae reared on the green leafy substrate of may turnip (ML), while treatments MT, SP and WS showed 100% survival. WF treatment shown 98% survival rate. The experimental stock was homogeneous at the beginning of the study, with no

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significant difference in individual larval body weight between groups. At the end of the experiment, the highest individual body weight were measured for the groups fed with the wheat semolina substrate (WS), which was also found to be significantly the highest (*Table 1*). There was no significant difference between the WF and SP treatments, while the MT and ML treatments were statistically proven the lowest larval weights.

Table 1: **The production parameters of the mealworm larvae at the end of the experiment**

	ML	MT	SP	WS	WF
Survival (%)	92.000 ± 4.472[a]	100 ± 0.00[b]	100 ± 0.00[b]	100 ± 0.00[b]	98.000 ± 4.472[b]
Initial body weight (g)	0.036 ± 0.002	0.034 ± 0.002	0.035 ± 0.006	0.035 ± 0.005	0.034 ± 0.002
Final body weight (g)	0.029 ± 0.004[a]	0.035 ± 0.005[a]	0.063 ± 0.007[b]	0.081 ± 0.005[c]	0.069 ± 0.007[b]
Initial biomass (g)	1.778 ± 0.015	1.717 ± 0.017	1.759 ± 0.057	1.766 ± 0.050	1.721 ± 0.019
Final biomass (g)	1.328 ± 0.036[a]	1.753 ± 0.048[a]	3.138 ± 0.067[b]	4.048 ± 0.077[c]	3.385 ± 0.086[b]
Spec. growth rate (% day^{-1})	-2.085 ± 0.932[a]	0.151 ± 1.300[a]	4.134 ± 1.358[b]	5.927 ± 0.338[b]	4.834 ± 0.359[b]

At the start of the experiment, the average biomass of the treatments was 1.75 ± 0.03 g. After 14 days of the test, the biomass of larvae reared on may turnip green leaf medium (ML) showed a decrease, while the total biomass of larvae increased in the other groups. The most intensive increase in biomass was obtained by the larvae reared on wheat semolina substrate (WS). Significant biomass increase was observed also in larvaes reared on sweet potato substrate (SP) and wheat flour (WF), whereas the biomass increase was significantly lower in the case of may turnip tubers (MT) and may turnip leafs (ML) compared to other treatments. The development of the mealworm larvae is particularly influenced by the substrate, which is confirmed by our results and also Broekhoven et al. (2015). In their investigation they compared three different edible species belonging to the Tenebrionoidea family (*Tenebrio molitor* L., *Zophobas atratus Fab., Alphitobius diaperinus*), which were kept on different organic by-products during the experiment, investigating their specific growth rates and feed conversion ratio. The substrate was composed of the following ingredients: maize, beer yeast, bread remains, spent grains, potato steam peelings, cookie remains. The worm feed contained different percentages of these ingredients. The substrate containing cake had a strong odour of cinnamon and cloves during the experiment -substances that can be toxic to the insects- a hypothesis confirmed by the fact that the highest mortality rate was observed at the larval stage. The treatments with high protein diets consisted mainly of 40% beer yeast and produced the most favorable survival and growth performance at larval stage. Their results have shown that diet has an effect on growth, development and last but not least feed conversion efficiency and mealworm larvae can be effectively reared on organic by - products.

The nutrient composition

At the end of the experiment, we took an average sample of mealworm larvae reared on different substrate and determined the protein, ash, total fat and carbohydrate content of the larvae (*Table 2*). The dry matter content of larvae did not show significant differences (*Table 3*). The mean dry matter content of treatments was 41.84 ± 1.26%. In terms of results, the extremely high protein and fat content of mealworm larvae, which is in agreement with literature data, should be highlighted. Adámková et al. (2017) cultivated the larvae of the common mealworm (*Tenebrio molitor*) at three different temperatures, with the aim of determining the temperature at which the larvae's fat content is the highest. The room temperatures where the culture was conducted were 17 °C, 23 °C and 28 °C. The total fat content (24, 56%) was observed at 23 °C. Therefore, the authors suggest that it is advisable to maintain this temperature of 23 °C in our culture for optimal fat content. We measured higher total fat content (33.6%) in the larvae compared to their results, despite we set lower temperature (22.90 ± 2.32 °C) compared they set.

The test methods used and the results are summarized in *Table 2.*

Table 2: **The composition of the mealworm larvae at the end of the experiment**

Nutrient composition	Method	Concentration (dry-matter basis)
Dry matter (m m^{-1})%	MSZ ISO 1442:20000	41.84
Protein (m m $^{-1}$)%	MSZ ISO 937:20000	51.12
Ash (m m^{-1})%	MSZ ISO 936:20000	4.59
Total fat (m m^{-1})%	MSZ ISO 1443:20000	33.6
Carbohydrate (m m^{-1})%	calculated value	10.69

The micro and macro element content

Regarding the macro-element content of mealworm larvae in our experiment (*Table 3*), the concentration of calcium in larvae reared on may turnip green leaf substrate (ML) was significantly higher compared to the other groups (1151.17 ± 220.56 mg kg^{-1} Ca). We

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measured significantly higher potassium content for ML and MT treatments compared to the larvae reared on wheat semolina and wheat flour. The highest magnesium concentration was observed in groups reared on may turnip tuber (MT: 3262.17 ± 164.19 mg kg^{-1} Mg), while the highest sodium content was observed in groups reared on sweet potato (SP: 1692.77 ± 97.84 mg kg^{-1} Na). For the microelements, the only difference between the groups was not statistically proven for aluminium concentration. In terms of iron and zinc content in larvae, the ML treatment showed significantly the highest concentration (ML: 47.82 ± 2.88 mg kg^{-1} Fe and 111.28 ± 6.75 mg kg^{-1} Zn). Statistically verifiable higher concentrations of copper and manganese were measured in the MT (20.52 ± 0.87 mg kg^{-1} Cu) and WS (11.48 ± 0.94 mg kg^{-1} Mn) treatments, respectively. The mealworm larvae nutrional value is favorable, as confirmed by Siemianowska et al. (2013) results also. They investigated the nutritional parameters of mealworm larvae. Their culture substrate was oatmeal, various vegetables were added to the culture to cover the water requirements of the larvae. The fresh larvae contained 56% water, 18% protein, 22% fat and 1.55% ash. Minerals were present in very high levels in the larvae: magnesium (87.5 mg $100g^{-1}$), zinc (4.2 mg $100g^{-1}$), metal (3.8 mg $100g^{-1}$), copper (0.78 mg $100g^{-1}$) and manganese (0.44 mg $100g^{-1}$). The n-6/n-3 fatty acid ratio was also very favorable at 6.76. The authors consider the powdered larvae as an excellent source of nutrition, which can be easily added to food and feed.

Table 3: **The micro and macro element content of mealworm larvae at the end of the experiment on dry matter basis**

	ML	MT	SP	WS	WL
Dry matter %	40.59 ± 1.57	40.77 ± 3.05	39.56 ± 3.02	40.34 ± 1.49	42.94 ± 4.30
Al mg kg^{-1}	1.98 ± 0.76	1.42 ± 0.48	0.74 ± 0.30	0.25 ± 0.12	3.46 ± 2.64
Ca mg kg^{-1}	$1151.17±220.56^{b}$	$551.17±33.62^{a}$	$419.63±18.87^{a}$	$366.02±11.77^{a}$	$351.27±12.76^{a}$
Cu mg kg^{-1}	$17.23 ± 1.11^{b}$	$20.52 ± 0.87^{c}$	$18.08 ± 0.17^{bc}$	$17.06 ± 0.51^{ab}$	$14.10 ± 0.57^{a}$
Fe mg kg^{-1}	$47.82 ± 2.88^{b}$	$43.47±2.13^{ab}$	$41.95±2.29^{ab}$	$43.00±2.68^{ab}$	$35.90±2.08^{a}$
K mg kg^{-1}	$8462.5 ± 220.86^{b}$	$9008.83±283.59^{b}$	$8843.58±140.05^{b}$	$8263.5±204.29^{ab}$	$7380.36±310.26^{a}$
Mg mg kg^{-1}	$2864.28 ±177.4^{ab}$	$3262.17±164.19^{b}$	$2942.81±70.43^{ab}$	$2594.60±209.52^{a}$	$2446.38±112.16^{a}$
Mn mg kg^{-1}	$8.43 ± 0.24^{a}$	$7.59±0.22^{a}$	$7.45±0.50^{a}$	$11.48±0.94^{b}$	$7.80±0.65^{a}$
Na mg kg^{-1}	$1563.36±130.1^{ab}$	$1523.77±62.99^{ab}$	$1692.77±97.84^{b}$	$1252.35±82.56^{a}$	$1374.23±55.83^{ab}$
Zn mg kg^{-1}	$111.28±6.75^{b}$	$107.62±2.41^{ab}$	$99.78±1.70^{ab}$	$98.51±3.63^{ab}$	$92.53±2.48^{a}$

CONCLUSIONS

Regarding the production parameters of mealworm larvae, it can be stated that the use of wheat semolina and wheat flour as control substrate provided the best results. Regarding the tested plant raw materials and by-products as a substrate, only sweet potato can be recommended based on the results of growth and survival. Our results that plant raw materials and by-products such as sweet potatoes can be a suitable substrate for mealworm has been confirmed by several other research groups (Kim et al., 2014; Melis et al., 2019; Li et al., 2020; Ruschioni et al., 2020).

The dry matter content of the larvae was not affected by the substrate used. Contrary to the production parameters, larvae reared with green leaf and tuber of may turnip showed the highest values for most of the macro- and microelements (potassium, calcium, magnesium, iron, zinc, copper) tested. The protein, ash, total fat and carbohydrate contents of the average samples show a favorable picture for the conversion of various plant by-products into animal protein.

Based on these results, the mealworm larvae can be reared on organic by-products and it has a favorable micro- and macro element composition. However, these results are only valid for these tested substrate, and further production trials will be needed in the future for finding the optimal substrate for mealworm production.

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