



WELFARE OF THE MEALWORM (*TENEBRIO MOLITOR*) BREEDING WITH REGARD TO NUTRITION VALUE AND FOOD SAFETY

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ABSTRACT

Livestock welfare is an important condition for obtaining high-quality and safe food. According to the legislation edible insects are classified as livestock; and for this reason it is necessary to comply with the edible insect welfare conditions. This article focuses on selected welfare conditions for mealworm (*Tenebrio molitor*) breeding, with special focus on the fat content influenced by different breeding temperature (17 °C, 23 °C and 28 °C). Maximum fat content 24.56% was observed at 23 °C. To obtain maximum fat content this appears to be the optimal breeding temperature. Another evaluated aspect was the nutritional stress and a way of killing, and their impact on fat content, which showed to decrease with the nutrient stress. The most decline was detected towards the end of the observation period. The analysis showed that in terms of preservation of the fat content, the best way is killing by freezing, due to the metabolism slowdown. We also analysed the content of heavy metals in a mealworm larvae using cyclic voltammetry with subsequent evaluation. In the measured sample concentrations of heavy metals did not exceed the maximum allowable concentration of heavy metals in this commodity. From this point of view mealworm appears to be a safe food.

Keywords: welfare; *Tenebrio molitor*; nutritional stress; way of killing; heavy metal

INTRODUCTION

Animal welfare is a condition, when the animal organism tries to deal with the surrounding (Broom, 1986). Welfare is defined as a state of fulfilment of all material and immaterial conditions, which are prerequisites for the health of the animal, and are also in accordance with its environment (Angelovičová and Poláčková, 2015; Doležal, Bílek and Dolejš, 2004). Webster (2016) described the five freedoms of animal welfare. It is freedom from hunger, thirst, discomfort, pain, injury, disease, fear and distress, and the expression of normal behaviour (van Huis et al., 2013). According to the Regulation (EC) No. 1069/2009 insect breeding is considered as breeding of common livestock. Because of this it is necessary to follow the rules of insect welfare as well as with other livestock. That's why Eisemann et al. (1984) suggested, that insect should be given the benefit of the doubt (van Huis et al., 2013).

Among the species with the greatest potential as food and feed within the European Union are mealworm (*Tenebrio molitor*), giant mealworm (*Zophobas atratus*), lesser mealworm (*Alphitobus diaperinus*), greater wax moth (*Galleria mellonella*), silkworm (*Bombyx mori*), house cricket (*Acheta domestica*) and African migratory locust (*Locusta migratoria migratorioides*) (EFSA, 2015).

Mealworm (*Tenebrio molitor*) in general belongs to the food pests (Schroeckenstein, Meier-Davis and Bush, 1990; Wang, Liao and Chen, 2012), however, thanks to its nutritional values, easy breeding and minimal environmental burden it has a potential to become a fully-fledged source of protein and fat for mankind (Wang, Liao and Chen, 2012). Nutritionally, it is important in terms of protein and fat, and there is a presumption of good use in the food industry (Park et al., 2014). Another mealworm benefit could be biodegradation of organic waste to proteins (Veldkamp et al., 2012). Based on the above advantages, possibilities for rearing this species in space are being explored (Katayama et al., 2008).

From the economical point of view, mealworm breeding is cheap and fast (Wang, Liao and Chen, 2012). According to Li, Zhao and Liu (2013) its life cycle is short, egg stage lasts 3 – 9 days, larval stage 26 – 76 days and pupal stage 5 – 17 days. Rearing usually takes place in breeding tanks with feed at the bottom (often cereal meal, milk powder and bone meal) (Hůrka, 2005). Growth rate and size of individuals is affected by several factors, including temperature, humidity, light intensity, composition of the feed and density of specimens within the breeding tanks (Wu et al., 2009).

One of the main factors influencing the growth speed is the temperature (Xu et al., 2012). With growing

temperature the live cycle shortens. In 20 °C the length of the life cycle was 97 days, and in 32 °C only 60 days (Xu et al, 2012). However, with growing temperature the weight of pupae and adult specimens tends to decrease from certain temperature level (Kim et al., 2015).

Another factor is the insect feed. Suitable feed is necessary for maintaining good health and optimal specimen performance (Dussutour et al., 2016). However, freely living animals do not necessarily have the access to the feed that reflects their nutritive needs. The influence of the nutritional composition of feed on the nutritional value of insects had been investigated (Li, Zhao and Liu, 2013). Nutritive stress or small breeding space leads to cannibalism (Wu et al., 2009) and decreases the breeding yield. Killing is carried out in different ways, most commonly by freezing, direct grinding, or cooling and subsequent killing by boiling. Methods of killing insects, however, should be chosen so as to avoid insect suffering (van Huis, 2013). Prior to the slaughter, dead individuals must be sorted out.

Due to inadequate safety review of edible insects as a novel food for humans in the EU, it is necessary not only to know the rules of breeding and provide sufficient welfare, but also to know the effect of eating insects to human health and safety risks (EFSA, 2015). EFSA (2015) draws attention to the lack of safety reviews of the insect consumption and various risks, including the contents of heavy metals (mainly Cd, Pb, Hg, and As) and calls for setting limits for safe consumption.

This article focuses on the impact of nutrient stress as a welfare aspect, and ways of killing on the nutritional value (fat content) of the final product. Furthermore, the article deals with the safety of edible insects in terms of the content of heavy metals (Cd and Pb), which have a significant impact on human health.

MATERIAL AND METHODOLOGY

Material

Insect

Larvae of mealworm (*Tenebrio molitor*) were used for the analysis (Figure 1). Samples were bought in the pet supplies store Krmiva Hostovice.



Figure 1 Larvae of mealworm (*Tenebrio molitor*)

Feed

Wheat brans with the following nutritional values were used as feed (values for 100 g of product): Energy: 1210 kJ / 292kcal, Fat 5.3 g, of which saturated fatty acids 0.88 g, carbohydrates 24.9 g, of which sugars 2.2 g, fibre 40.2 g a protein 16.2 g, salt 0.1 g. Company: Country Life, s.r.o., Beroun 1.

Chemicals

- Petrol ether 40/65, p.a., CAS No.: [8032-32-4], Ing. Petr Švec – PENTA s.r.o., Praha,
- HNO₃ 65%, p.a., CAS No.: [7697-37-2], Ing. Petr Švec – PENTA s.r.o., Praha,
- H₂O₂ 30%, p. a., CAS No.: [7722-84-1], Ing. Petr Švec – PENTA s.r.o., Praha,
- CdCl₂ p. a. – Mr. 183,32, CAS No.: [10108-64-2], Fluka analytical, Sigma Aldrich,
- PbCl₂ p. a. – Mr. 278,11, Lachema, n. p., Brno, CZ,
- Deionized water – 18.2 MOhm cm, Milli-Q, Millipore.

All chemicals were of analytical reagent grade or equivalent analytical purity.

Methods

Insect processing

In the first experiment, mealworm larvae were divided into three experimental groups. These experimental groups were placed in a plastic boxes in a thermostat (HS 62A, Chirana) by rearing temperature of 17 °C, 23 °C and 28 °C. The groups were fed ad libitum. Before analysis, larvae in the last and penultimate instar development (the full length of the body just before pupation) were collected from breeding. Subsequently the larvae were left to starve for 24 hours and then killed with boiling water (100 °C). The prepared samples were dried at 105 °C, homogenized and stored in a cold storage box at 4 – 7 °C until analysis.

For the second experiment, two groups of mealworms were bred in room temperature 22 °C ± 2 °C and divided by the nutrient stress. The first group was fed wheat brans ad libitum during the whole test. The other group was in nutritional stress, which means without feed for 27 and 39 days. Before analysis, larvae in the last and penultimate

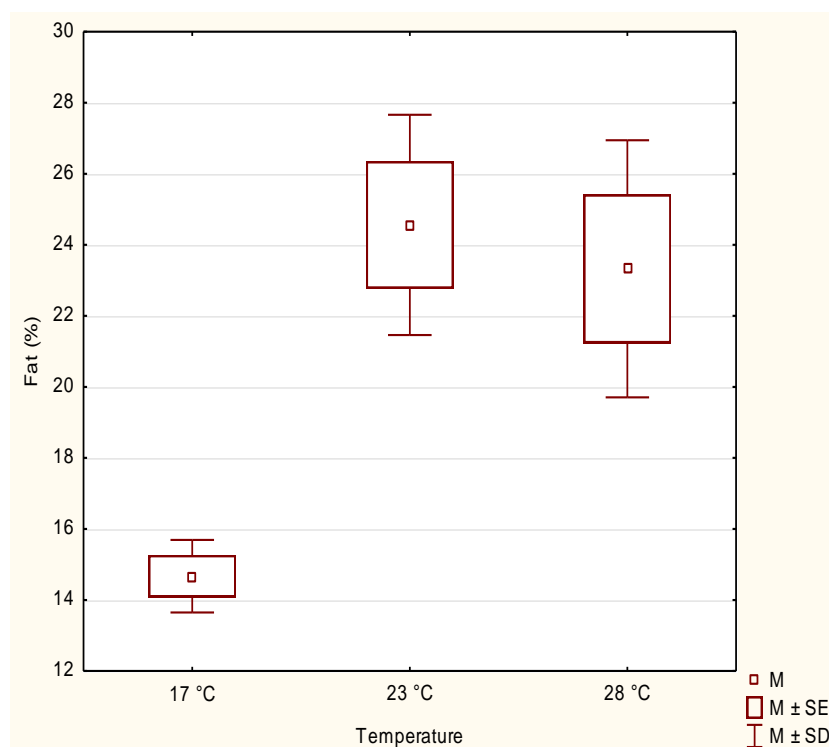


Figure 2 Fat content in mealworm (*Tenebrio molitor*) -dependency on the breeding temperature (boxplot).

Table 1 Fat content in mealworm (*Tenebrio molitor*) and its dependency on the breeding temperature.

Sample	Fat (%)
TM 17 °C	14.67
TM 23 °C	24.56
TM 28 °C	23.32

instar development (the full length of the body just before pupation) were collected from breeding. Subsequently the larvae were left to starve for 24 hours. Each group was then divided into two sub-groups. The first sub-group was killed by freezing (-18 °C) in the freezer and the second one was killed by boiling water (100 °C). Dead larvae were dried at 105 °C, homogenized and stored in a cold storage box at 4 – 7 °C until analysis.

Fat content determination

The fat content determination was performed by extraction using Soxhlet method (Soxhlet, 1879) on the Gerhardt Soxtherm SOX414 (C. Gerhardt GmbH & Co. KG, Germany). Approximately 5 g of dried and homogenized samples (with the accuracy of 0.0001 g) were put into extraction thimbles and extracted with 150 ml of petroleum ether via cold water extraction (program: 70 °C for 120 minutes). The extraction flask was then dried at 103 °C and weighed until a constant sample weight was attained.

Heavy metals content

0.1 g of homogenized sample was put into a vial, and then 2 mL HNO₃ with 65% concentration was added. Metals were being extracted for 24 hours at room temperature and then heated to reach the temperature 110 °C for 1 hour. Subsequently, 200 µL 30% H₂O₂ was added and the sample was kept warm for another 30 minutes. After cooling the sample was diluted 5 times with deionized water (v/v) (18.2 MOhm cm, Milli-Q,

Millipore). Prior to the analysis the sample was diluted 10 times using an acetate buffer (v/v).

Detection of selected metals (Cd, Pb) was carried out using cyclic voltammetry within the range $U = <-1000; +400 > mV$ with scanning speed $10 mV s^{-1}$. Accumulation time was $t_{cond} = 45 s$ with potential $E_{cond} = -1000 mV$. To evaluate metals, analytic electrochemical station µAUTOLAB, Type III/ FRA 2 was used with NOVA 1.11 application. Ag/AgCl Reference Electrode Metrohm AG, Switzerland was used as a reference electrode. Pt Rod Electrode Metrohm AG, Switzerland, was used as auxiliary electrode. Working electrode was created using the thick layer method on a ceramic substrate (Al₂O₃). Sensor size was 25.4 mm × 7.25 mm × 625 µm. The silver duct outcome was created by ESL 9562-G paste (ESL Electroscience, England). Working electrode with 3 mm diameter was created using DuPont 7102 paste (DuPont, USA). ESL 4917 paste (ESL Electroscience, England) was used to create the protective cover layer. Measured characteristics were derived and analysed in Excel 2013 (Microsoft Corporation, USA).

Statistical analysis

Data obtained from chemical analysis was processed using MS Excel (Microsoft Corporation, USA), to calculate basic percentage of fat content. These data was then processed using the Statistica 12 (StatSoft, Inc., USA) application.

RESULTS AND DISCUSSION

Breeding temperature is one of the most important factors affecting the development speed (Xu et al., 2012). In our study, we examined the fat content dependency on the temperature (17 °C, 23 °C and 28 °C). Table 1 and Figure 2 show that the fat content at 17 °C is lower than at 23 °C, but further increase of the temperature to 28 °C did not lead to greater change. These are, however, the initial results, which will be further verified.

While different studies show the general influence of temperature on the edible insect nutritional value, available sources do not state any specific dependence of nutritional values on the breeding temperature (Ooninx and van der Poel, 2011). Xu et al. (2012) investigated the optimum temperature for breeding mealworm (*Tenebrio molitor*) and compared five different temperatures (20 °C, 23 °C, 26 °C, 29 °C a 32 °C). Their results showed that the mealworm development gets faster with growing temperature. Also the numbers of laid eggs grew (max. at 29 °C) (Xu et al., 2012), however, the weight of pupa and adult decreased (Kim et al., 2015). The change in weight and size of specimens in different temperatures can suggest the change of their nutritive value. Role of nutrition is to provide the needed nutrients into the body via the feed, in order to preserve life, growth, reproduction, good health and performance. The body can relieve the lack of food (nutritional stress) by drawing energy from adipose tissue, mineral saturation from skeletons, nitrogenous substances saturation or more economical excretion of certain elements. The depletion of certain nutrient without refilling may result in death of the individual. Lack of fats reduces the viability, slows

growth, reduces reproduction, performance and leads to diseases; long-term deficiency has lethal effects (Horniaková et al., 2010). To observe the effect of nutritional stress, insect was divided into two experimental groups. The first one was fed ad libitum; the other was in nutritional stress by starving for 27 and 39 days. In the second group the cannibalism occurred more often, as also mentioned by Erens et al. (2012). Subsequently, both groups of starving insects were divided into sub-group killed by freezing (-18 °C) and boiling water (100 °C). The results of the fat content show that with the length of starving the fat content decreases (see Figure 3). A difference was detected between the groups for both ways of killing. This trend became more significant towards the end of the experiment. It's supposed that the reason is decreasing of the fat deposits in insect bodies due to their consumption by basal metabolism (Nation, 2015).

Changes in fat content in insect bodies as well as insect behaviour were observed also by (Dussutour, 2016) in ants. Decrease of the fat content was detected after reducing the nutrient concentration in the diet, when the ratio of proteins to carbohydrates was increased, and when the ants were starving. Regression model shows that the fat content was determined almost exclusively by the carbohydrate intake. There was also a change in the fat content among various castes (Dussutour, 2016). However, nutritional stress can be caused not only by the lack of feed as a whole, but also by the lack of a specific nutrient. For example Simpson a Raubenheimer (2012) state that excessive consumption of carbohydrates leads to obesity when feed is low in protein with ratio to carbohydrates, while the lack of carbohydrates in the diet leads to a lack of energy.

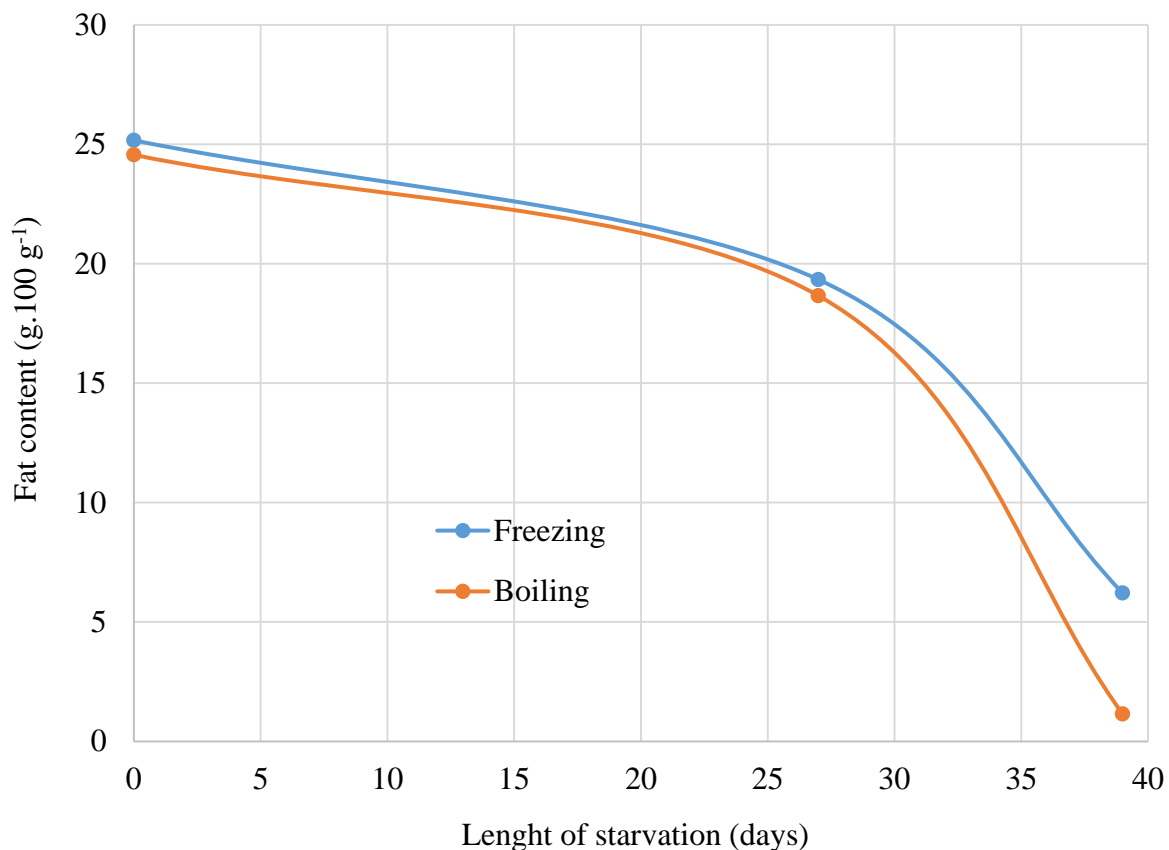


Figure 3 Mealworm (*Tenebrio molitor*) fat content dependency on the length of starvation and the way of killing.

Some ways of killing insect, described by Erens et al. (2012), are considered humane, but as he states, some processors reject them for financial reasons. Benefit of freezing, for example, is that by putting insect into the freezer, its metabolism slows down (hibernation), until it freezes. Freezing should be carried out quickly to prevent tissue damage (Drdák, 1989). Considering the nutritional stress, there was no difference between the ways of killing. However, Figure 3 shows that the group killed by freezing had a higher fat content than the group killed by boiling water. We suppose that the loss of fat in the second group may be caused by fat extraction into the boiling water. When using the fat for protection from the cold and freeze, the losses were smaller than with boiling. Heavy metal concentration in food and feed for animal breeding is an important topic of health safety and has an impact on the consumers' health (Chovancová et al., 2014; Lukáčová et al., 2014). In our research we focused on the detection of cadmium and lead in edible insect using carbon electrode created with the technology of thick layers. After creating the calibration curves the cadmium and lead concentration was evaluated in mealworm larvae (*Tenebrio molitor*) in last and penultimate instar development, bred in 23 °C and fed by wheat brans ad libitum. Both cadmium and lead concentration was below the limit of detectability of the used sensor (detection limit is 2 µg.L⁻¹ for lead and 3 µg.L⁻¹ for cadmium). The sensor can be used to detect the levels of cadmium and lead, stated as a maximum limit in Regulation (EC) No. 1881/2006 (Pb 0.5 mg.kg⁻¹ that is 2.41 µg.L⁻¹ and Cd 4.46 µg.L⁻¹). Although these are initial results, gained with using sensor with detection limits slightly below the maximum allowable concentration in this commodity, the content of cadmium and lead seems to be safe for using insect as human food.

Poma (2017) evaluated nine mineral elements in his study. In mealworm (*Tenebrio molitor*) the content he detected was: Cd 0.06 mg.kg⁻¹ and content of Pb was lower than the detection limit. The level of heavy metals evaluated in this study was lower than requested by the Regulation (EC) No. 1881/2006 and lower or comparable with other commodities of animal origin.

CONCLUSION

Our work was focused on the impact of welfare on the nutritional value and safety of the mealworm (*Tenebrio molitor*), as a potential food source. As an analysis of fat content indicates, breeding temperature – as one of the welfare factors – significantly affects the fat content. Other evaluated welfare factor was nutritional stress, while the experimental group loaded with nutritional stress had lower fat content. It can be concluded, that nutritional deprivation affects not only the animal welfare, but also nutritional yield with a further impact on the economic aspects of breeding. For commercially raised livestock the moment of death is an important part of animal welfare, having an impact on the nutritional value and quality of the meat obtained. The way of killing also influenced the nutritional values of mealworm larvae in our study. From the viewpoint of fat yield, killing by freezing can be recommended.

Although the question of heavy metals content is not part of welfare, it is an important aspect of the food safety.

Based on our study, we can conclude that heavy metal content of the analysed species was lower than the maximum permitted levels of heavy metals in food. From this point of view, the consumption of mealworm larvae is safe.

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