Effect of food shortage and metal contamination on the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Abstract. In this study we assessed the metabolic reaction of mealworms to toxic metals Cu and Pb alone or delivered with carrots as an additional source of water and minerals. We compared our results with metabolic reaction of mealworms to food shortage. We recorded the highest mortality and mass loss in culture contaminated with Pb (in relation to Cu contamination) proving the greatest impact of this metal on metabolism of mealworms. The great decrease in protein, carbohydrates and lipid content was observed in this variant (mealworms fed with food contaminated with Pb) as well. The slight positive effect of carrot addition was observed for larvae fed with both metals. Starvation had similar but slighter effect as Pb on metabolism as starved larvae were characterised by high, but not the highest decrease of protein, carbohydrates and lipid content. The studies proved that metal contamination could have great impact on metabolism of mealworms which is a key issue when we consider these insects as a source of proteins in place of vertebrates.

1 Introduction

Insects are considered as a good source of proteins which could partly replace conventional sources deriving from animals (vertebrates). The main advantage of insects over vertebrates is that they have a high feed conversion efficiency [1]. Additional advantage is that they can live on diet composed of organic by-products [2]. In general, the use of insects for food purposes is more sustainable than consumption of vertebrates as their demand of land and water is minimal comparing to vertebrates, as well as greenhouse gas emissions [3]. Larvae of yellow mealworm *Tenebrio molitor* is especially valued because of its high content of good quality protein, vitamins and minerals [4]. Therefore, it is important to know if they are not contaminated with heavy metals as chemical safety is the priority for insects consumption. Some studies proved that such contamination exist (from food they consume) and scientists investigated the potential accumulation of heavy metals in mealworms [5], although the effect of metal contamination on larval metabolism has never been studied. We have chosen two metals for our research: copper and lead which differ greatly.

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Copper is an essential trace element vital to the health of all living organisms. The circulatory fluids of most invertebrates contain hemocyanin for oxygen transport. Hemocyanin uses copper to bind to oxygen. Therefore, hemocyanin pathway interactions would be the first to occur for invertebrates as exposure increases. Whereas lead, is one of the most toxic elements not found naturally in organisms. It could cause serious changes in the body of living organisms.

The aim of the research was to study the metabolic responses for selected metals (Cu and Pb). Additionally, we observed the metabolic response of *T. molitor* to food contaminated with both metals but with addition of natural source of water and vitamins such as carrot. For the comparison we also study the reaction of mealworms for food shortage. To summarize: the research hypothesis assumes the negative effect of toxic elements such as copper, lead and starving on mealworms larvae.

2 Materials and methods

2.1 Materials

T. molitor, a beetle from Tenebrionidae family (Coleoptera, Insecta) was used for the studies. The life cycle lasts usually 280–630 days, however, its length varies depending on the humidity and temperature. The female lays eggs in a soft medium and after 10–12 days larvae start to hatch, then became pupae and after about 3 months, transform into adult individuals. Larvae and adult beetles feed on grain products in warehouses, pantries, poultry farms and dovecotes. Larvae are usually fed with flour or oatmeal but could consume vegetables or fruits to supply the water and vitamins [6]. In our research, *T. molitor* larvae came from insect breeding “Cricket farm” in Lublin, Poland.

2.2 Methods

2.2.1 Experiment design

To study the metabolic reaction of larvae to metal toxicity we chosen $\text{Cu}(\text{CH}_3\text{COO})_2$ and PbSO_4 . The experiment duration was 14 days. The cultures were fed with flour. Spiking was performed to reach the desired concentration of each element. 100 gram of feed was spiked with Cu and Pb to concentrations 20 mg/kg for copper acetate and 10 mg/kg for lead sulphate II. In some variants 0.5 g of carrot was added to each container and replaced 3 times per week allowing *ad libitum* consumption. Larvae were divided into 6 groups (in 3 replicates, 45 individuals each) depending on the type of food served (control, starved, Cu, Pb and both metals with the addition of carrot). We have chosen specimens of similar size (within the range of 0.04–0.06 g). The temperature was maintained at 28°C and the humidity at 70%. In order to record the weight loss, the mass of larvae was assessed 1 and 14 day of experiment. The variants used were as follows:

- control larvae fed with flour (C)
- larvae fed with copper (Cu)
- larvae fed with lead (Pb)
- larvae fed with copper with the addition of carrot (Cu + C)
- larvae fed with lead with the addition of carrot (Pb + C)
- starved larvae (S)

2.2.2 Biochemical analyses

At the end of the experiment, the larvae were homogenized by trituration in a mortar using ice-cold 0.1 M phosphate buffer pH 7.4, 1 mM EDTA (volume/weight ratio 1:10). In order to assess the content of proteins in the body of tested animals, prepared samples were centrifuged at 11,000 x g (5 min) and the Bradford method was applied [7], while sugars were assessed using the anthrone method [8]. For assessing the lipid content the homogenate was treated with chloroform-methanol (1:2 v/v) and then a vanillin reagent was used [9].

3 Results and discussion

3.1 Mortality of mealworms

The highest mortality was observed at the end of the experiment (14th day). We recorded 60 ± 2% of dead animals in the variant contaminated with Pb while the lowest rate was (2 ± 1%), as it was expected, in the control animals. For variant with Cu the mortality of mealworms was 47 ± 3%. Starved specimens accounted for 52 ± 2% of dead animals. Slightly lower mortality was observed in variants contaminated with Cu and Pb with the addition of carrot (45 ± 2% of mortality for Cu and 51 ± 1% for Pb respectively) (Fig. 1). Such results suggest that toxic impact of Pb influenced greatly the survival rate of studied culture. The impact of this metal on the population was greater than the influence of food shortage. The addition of carrots helped to survive the mealworms as the mortality rate was lower.

Fig. 1. Percentage of mortality after 14 days of the experiment (%) ± SD.

3.2 Mass loss of mealworms

The results of mass loss at the end of experiment (14th day) are quite consistent with the mortality observed. The highest loss was recorded for Pb contaminated variant ($21 \pm 2\%$ of mass loss) and for starved animals ($18 \pm 1\%$ of weight loss). We recorded $14 \pm 1\%$ of mass loss for Cu variant and for culture contaminated with copper + carrot (10% of mass loss), although 15% of mass loss was observed for the variant Pb + carrot. The increase in mass was observed for the control mealworms (+57%) (Fig. 2).

Fig. 2. Percentage of mass loss after 14 days of experiment for mealworms (%) $\pm$ SD.

3.3 Biochemical analyses

The results of metabolic studies revealed a negative effect of lead and starvation on mealworms. We observed rather stable content of protein in studied period. These compounds are very important building material for the body of insect and in the hydrolyzed form they could serve as a fuel for metabolism. Only slight changes in protein content in comparison to control larvae were recorded for Cu: 23.57, Pb + C: 20.26 and Cu + C: 21.34 $\mu\text{g}/\text{mg}$. Although, the lowest values were recorded for mealworms contaminated with Pb (16.25 $\mu\text{g}/\text{mg}$) and starved larvae (18.4 $\mu\text{g}/\text{mg}$). Carbohydrates provide most of the energy during insect life although the most common source of energy during starvation or stress period is the oxidation of fatty acids stored in the form of triglyceride. The total content of carbohydrates and lipid was also the lowest for Pb contaminated variant (lipid content: 14.73 $\mu\text{g}/\text{mg}$ and sugar: 36 $\mu\text{g}/\text{mg}$ of fresh mass). In copper + carrot variant the lowest decrease in the content of protein, sugar and lipid was observed in comparison to the control (total sugar: 59.08 $\mu\text{g}/\text{mg}$ and total lipid 28.63 $\mu\text{g}/\text{mg}$ of fresh mass), which confirms that carrots are good source of water and vitamins for

larvae. The metabolism of starved animals was highly affected e.g. protein content was 18.4 µg/mg, carbohydrates: 40.05 µg/mg and lipid: 17.34 µg/mg of fresh mass (Fig. 3, 4, 5).

Fig. 3. Protein content in larvae of mealworms (µg/mg of fresh mass) ± SD.

Fig. 4. Carbohydrates content in larvae of mealworms (µg/mg of fresh mass) ± SD.

The research was aimed at studying the metabolic responses of mealworm larvae to selected metals and starved conditions. Similar studies were performed by Vijver et al. [10] who investigated the influence of contaminated soil with heavy metals on *T. molitor* larvae. The negative effect of Pb had been shown, however the toxic effect of Cu had not been

demonstrated. All organisms survived studies which may indicate their resistance to heavy metals (in concentrations: 0.49 mmol Cd/ kg of dry soil and 9.08 mmol Zn/kg of dry soil). Our studies showed that the protein content does not decrease rapidly, while the content of other metabolites drops quite fast in reaction for toxic metals. Studies on the rate of insect metabolism were conducted by Renault et al. [11], but the authors investigated adult mealworms *Alphitobius diaperinus* belonging to the same family as *T. molitor*. The metabolic activity of this species was studied in starving conditions at various temperatures. After 35 days of starvation, it was shown that the level of protein and glycogen (sugar) did not change significantly, while the level of triglycerides (lipid) decreased sharply.

Fig. 5. Total lipid content in larvae of mealworms (µg/mg of fresh mass) ± SD.

The experiment showed a close relationship between temperature and the distribution of metabolites. It was found, that triglycerides are rapidly decomposed when the temperature reaches about 24 degrees Celsius [11]. Although, the protein synthesis could decrease during food deprivation [11], leading to a decrease in protein levels as it was observed in our study in starved larvae and mealworms fed Pb. Metabolic response of insects was also studied on *Drosophila* flies. Tests were carried out in conditions of drought and starvation and the content of protein, carbohydrates and lipid was examined. It was found, that carbohydrate metabolism is much higher during drought. It was also observed that in the initial stage of starvation insects use glycogen and then protein and lipid, and it has been shown that the content of fatty acids drops sharply [12]. Similar results were obtained in our studies as the highest decrease in lipid content was observed, while the lowest decrease was recorded for protein for all studied variants. In general, the majority of insects metabolize glycogen during the initial stages of starvation and stress, then switch to lipid and protein metabolism when carbohydrates are gone. Although, there are exceptions which rely on lipid metabolism during exposition to stress factors from the beginning [12, 13].

4 Conclusions

Studies showed a highly toxic impact of Pb on mealworms as we recorded the highest level of mortality and mass loss of animals and the most drastic decrease in the content of energy and backup compounds such as sugar and lipid. Carbohydrates are involved in the synthesis of protein and lipid, thus their metabolism is combined with the metabolism of other biochemical components. The positive effect of carrot addition was also recorded as a great source of vitamins and water, and therefore the larvae survived longer in unfavourable conditions. The lower mortality rate and mass loss was observed for larvae fed with copper which is probably connected with important function of copper in invertebrates. The studies gave evidence that metal contamination could have a great effect on metabolism of mealworms which is very important if these insects may replace vertebrates for food in the nearest future.

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