



Do Happy Mealworms Make Happy Meals?

Analysis of fat content as an indicator of stress response in *Tenebrio molitor* in captivity to be used as live feed.

Rys Howells



Introduction

Many zoos across the UK rear their own YML to use for live feed for their collections, Barrett et al (2023) estimated 300 billion individuals are reared every year for these purposes. YML are a very popular source of protein and fat, 15% in fresh YML (Zhao et al, 2016) and 28% fat in dry larve (Langston et al, 2023). Furthermore, they provide 206 kcal/100g (Kouřimská & Adámková, 2016).

Despite being recognised as livestock, they are not protected by European Animal welfare directive for farmed insects (Lähteenmäki-Uutela et al, 2017).

The stress response effects metabolism by the release of different stress hormones. Mench et al in 2000 discussed that there is a positive correlation between magnitude of stress and the change in metabolism in invertebrates. Mench et al (2000) related this to adipose tissue which ss a main component in invertebrate physiology (Barnes et al, 2006) is the first to loose priority for nutrient allocation when under stress. It therefore follows that mealworms raised in a less stressful environment will maintain their content for longer.

This would lead to the implication that fatter mealworms are happier mealworms and will therefore be more nutritionally valuable.

Methods

500 grams of mealworms were divided between habitat 1 which was to represent a typical enclosure (Figure 1), with a wheat bran substrate and 2 egg cartons. And Habitat 2 which would be considered an enriched enclosure (see figure 2), having an oat and wheat bran substrate, with egg cartons and cardboard tubes with fresh fruit and vegetables put in periodically.

A baseline sample was taken before being separated into the habitats. The subjects were checked on everyday and fruit and vegetables were replaced if necessary. 60 grams from each habitat was taken on day 2, day 4, and day 6 and were humanely killed by placing in a freezer. The frozen samples were prepared for fat extraction via Soxtherm by being crushed to a paste consistency, then dried in an oven overnight.

Data analysis was performed using the Mann-Whitney test and the Spearman's correlation test in R studio.

Figure 1
Picture of Habitat 1

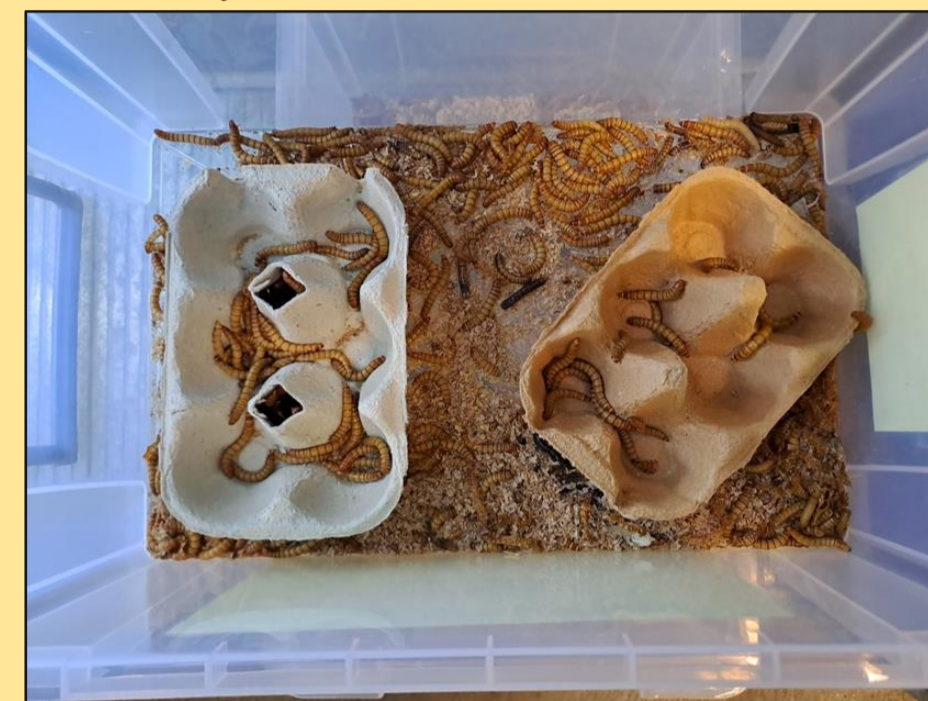


Figure 2
Picture of Habitat 2



Results

Figure 3

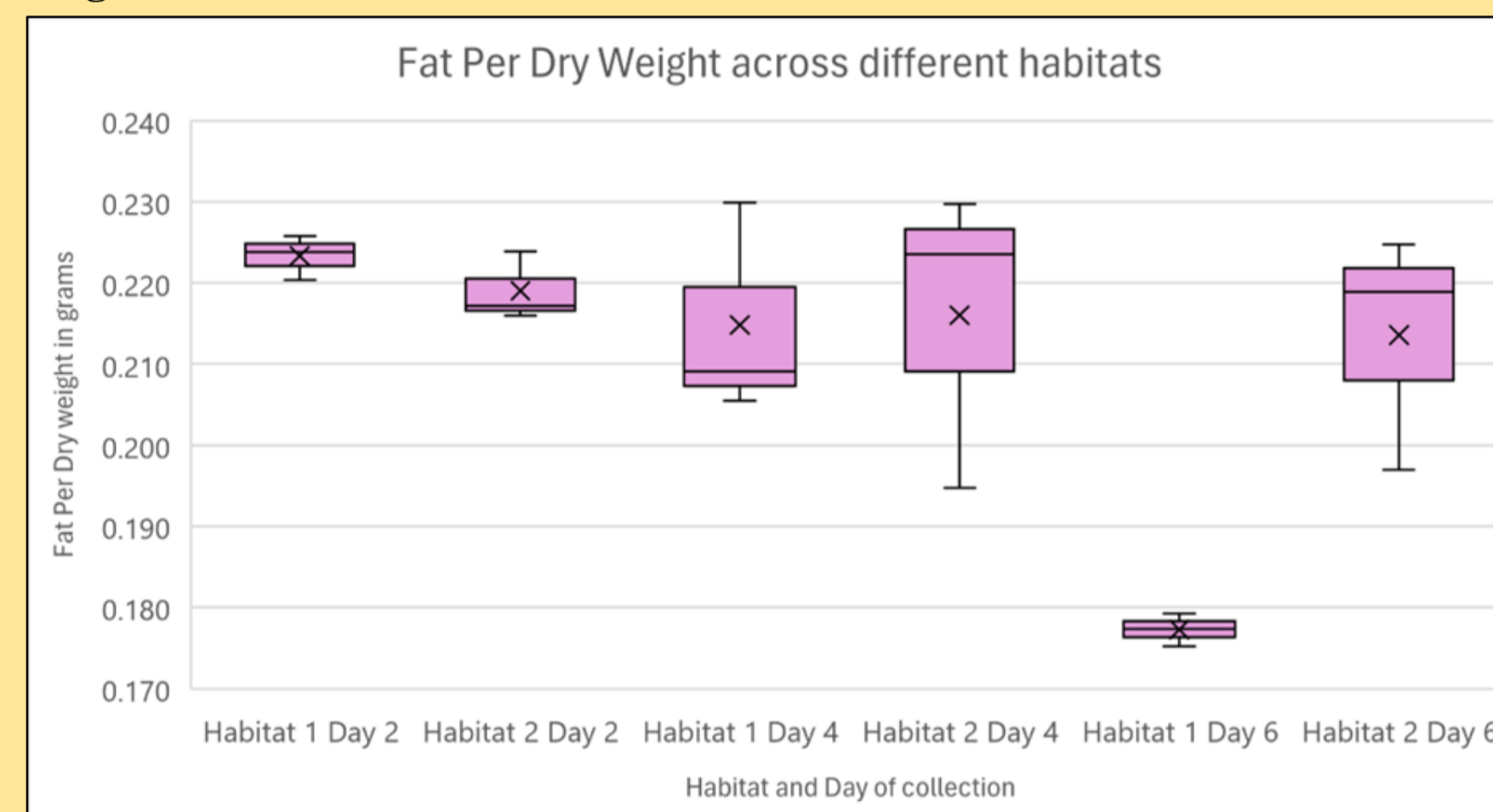
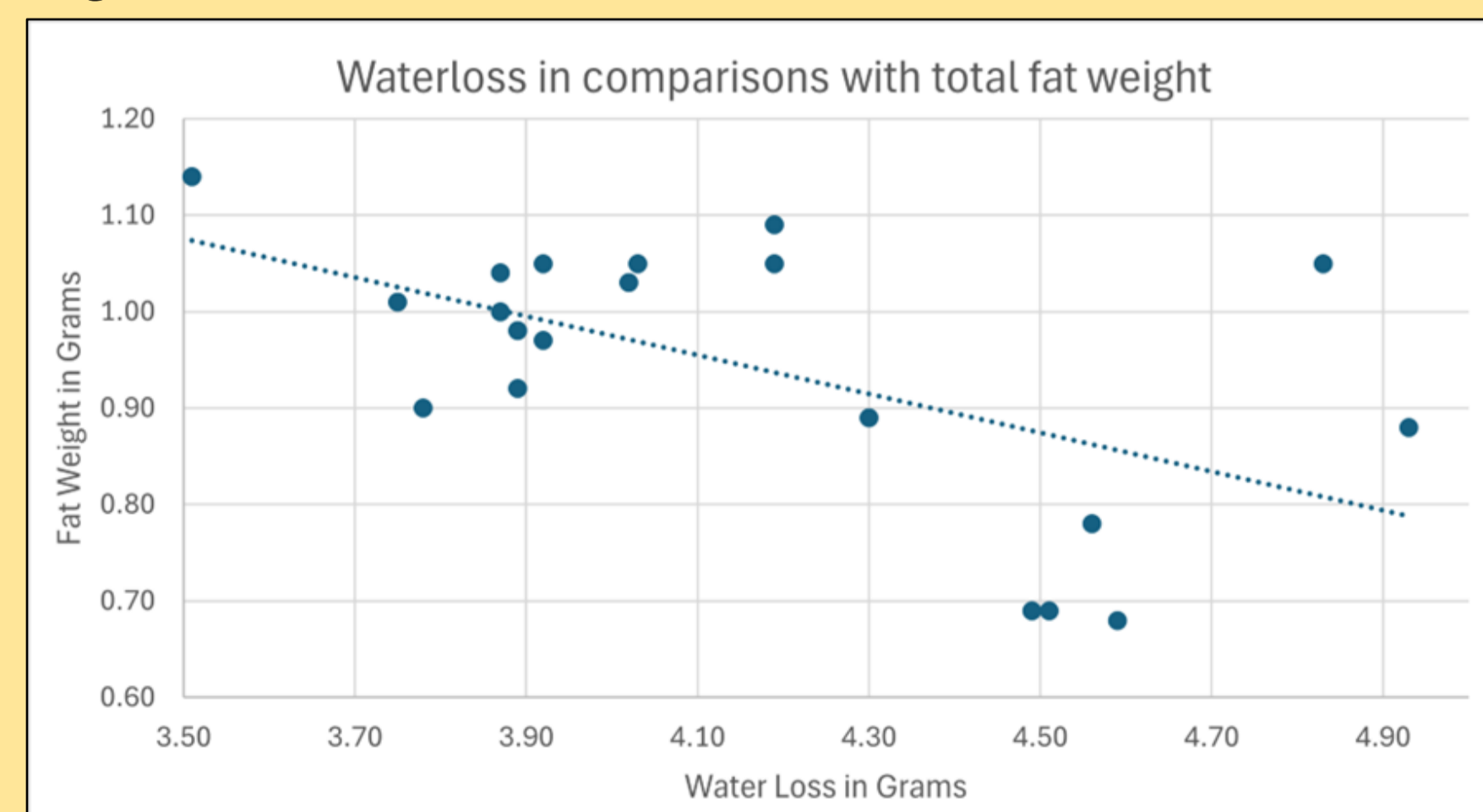


Figure 4



Results (cont.)

Using the Mann-Whitney U test, habitat 1 and 2 was compared on each of the collection days to look for a difference. Day 2 collection had a p value = 0.505, day 4 p=1, day 6 while not significant was approaching significance with p= 0.059. Figure 3 shows the grams of fat per dried weight across all collection days and both habitats, however, habitat 1 on day 6 it is obvious that there is an outlier, and this may be the source of the near significance.

The water lost and the fat weight was tested to look for a correlation. The Spearman's rank correlation was used and is another approaching significant p value = 0.06547 and rho = -0.419. This relationship is illustrated by Figure 4

Discussions and Conclusion

YML are very high in fat and are rich in nutrients as they are in their larvae state, this is due to the presence of the juvenile hormone along with biogenic amines and dopamine which increases trehalose and glucose content (Bobrovskikh & Gruntenko, 2023). As stated before, there are many studies about how to raise fatter YML, one of the key environmental factors for YML keeping is temperature. Renault et al (2002) found that if at the correct temperature, the larvae could survive longer when being starved, although the glycogen levels did decrease by 78%. The diet of the larvae seems to be a less important aspect of YML keeping, many research papers discuss substrate and diet to rear and breed YML rather than the larvae that are being kept as feed (Langston et al, 2023, Bordiean et al, 2022, Ooninx et al 2019).

The stress response in invertebrates is a complex and under researched area, and the effects on fat and lipid content differ from paper to paper. On one hand the stress response uses up energy stores from the fatty body of the insect to prepare for fight or flight (Bobrovskikh and Gruntenko 2023) but on the other, octopamine which is the main insect stress hormone increases lipid content (Adamo, 2010). Whether or not stress increases or decrease fat content may rely on if the stressor is short term or long term and would require more research to be done in order to determine this relationship.

These results may not be significant but portray the beginning of a relationship between different habitat types and weight of fat per gram. This study would be better served as a precursor to further study by using more subjects for a longer period. This would be supported by Fasel et al (2017) who conducted a study to look into how diet affected the fatty acid composition and found that significant differences were only found after nine days in captivity.

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