Effect of different food by-products on *Tenebrio molitor* growth in an industrial environment

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Effect of different food by-products on *Tenebrio molitor* growth and development in an industrial environment

Effekten av olika sidoströmmar av foder på Tenebrio molitors tillväxt i en industriell miljö.

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Abstract

Due to an increasing need for high-quality protein around the world, eyes are turning towards alternative methods of farming, such as insect farms. As insects can eat what humans cannot, e.g. food by-products, they can be a part of sustainable food systems. In this thesis I experimentally test food by-products on growth rates as well as maturation rates on one of the most reared insects species, the mealworm *Tenebrio molitor*. I experimentally reared *T. molitor* on four different types of feed while controlling other environmental variables. The feed used was gluten free crispbread, spent brewers' grain, a combination of the two, and wheat bran, all of which are considered by-products. This means they are usually considered "waste" and brought to either a landfill or to a biogas plant, usually at a cost for the producing company. My study showed that larval growth rate increased when fed with gluten free crispbread (average weight 0.1g per larvae after 10 weeks) as well as larvae fed with gluten free crispbread in combination with spent brewers' grain (0.09g per larvae after 10 weeks) compared to individuals fed only spent brewers' grain or wheat bran and water. Feeding larvae with only spent brewers' grain (0.07g per larvae) or wheat bran combined with water (0.05g per larvae) also make *T. molitor* grow, but not as rapidly as with the other two feeds. I performed a follow-up test on the maturation rate of larvae to pupae to adult and I found indications that gluten free crispbread combined with spent brewers' grain as well as only feeding spent brewers' grain had a faster pupation rate but it is difficult to justify this with very few sampling points. These indications are noteworthy however as it differs from normal larvae-larvae morphosis, requiring further analysis on what amino-acid combinations are required for pupation and emergence as adults It is positive that all by-products nurtured growth and development of the species and because of this, the study shows the possibilities of by-products as an investment into insect rearing. If companies want to make a real impact I think they need to focus on breeding insects as sustainable as possible. A perfect way to do this is by looking into agricultural by-products that are otherwise considered waste and using it as high-quality feed for insects. After reading all the sources for this paper it is my firm belief that this is an industry that will only grow stronger in the future and if we are to feed the growing population on earth it is essential that this is done in a sustainable way.

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Abbreviations

FCR	Feed conversion ratio
TM	*Tenebrio molitor*
SLU	Sveriges lantbruksuniversitet
MC	Moisture content

1. Introduction

The planet's growing human population requires novel solutions in terms of both food and feed. One solution is industrial production of insects that offer proteins, fats and minerals for both humans and other animals reared for consumption (Siemianowska et al. 2013). Insect rearing requires less space than the rearing of cattle, pigs and chicken and have a better feed conversion ratio (FCR) than these. The FCR value tells how much feed is required for animal growth. A low value means high conversion efficiency, e.g., if 2 kg feed is needed for a 1 kg growth the FCR is 2:1 = 0.5 (Thévenot et al. 2018). Standard FCR values for beef, pork and poultry are 10:1, 5:1 and 2.5:1 respectively, however this can vary depending on nutrition, genetics and of course how you manage the animals (Vaclav, 2002). For an insect such as a cricket, the FCR value is around 1.7:1 (Dickie et al. 2019) and for yellow mealworms one study has found an FCR value of 2.2:1 (Dennis et al. 2012). Further, an additional positive aspect of insect rearing is that insects can consume a variety of feeds. This opens up to feeding insects agricultural residues from side-streams such as spent brewers' grain from breweries. Also, wheat bran, the layer around the core of the wheat, can be such a feed (Naser El Deen et al. 2021). Agricultural side-streams can be sustainable in several different ways, for example it is possible to produce energy in biogas facilities. However, this usually has a cost of disposal for the company producing the side-stream (Al-Wahaibi et al. 2020). This is the reason why by-products are interesting from a sustainability point of view, there are plenty of these resources (Lindberg et al. 2016) and insect rearing companies may utilise this product free and use it to feed their animals, resulting in a win-win option for everyone. This of course depends on the quality, volumes, transportation costs and regulations of the side-stream. Together with agricultural by-products there are also animal by-products, these are regulated by the EU and are divided into three different categories:

1. Highest risk category. An example of these are zoo and circus animals, specified risk materials as well as carcasses used in experiments. This category may be used for disposal in the form of incineration or as fuel in an approved combustion plant. (EU No. 1069/2009. 2009).
2. High risk category. An example of these are unhatched poultry who died in the shell. Carcasses of dead livestock and digestive tract content. These by-products

can be used as landfill (after sterilization) and safe technical uses. (EU No. 1069/2009. 2009).

3. Low risk category. An example of these are by-products from slaughterhouses that are fit for humans, domestic catering waste, eggs and eggshells (EU No. 1069/2009. 2009). With further research being done in reared insects it likely will be easier to use side-streams in a safe and sustainable way in the future, whether it be agricultural or animal biproducts.

The mealworm beetle *Tenebrio molitor* is one of the main insects currently used in the insect rearing industry (Cadinu et al. 2020). For *T. molitor* it is common that wheat bran is used in production as both a substrate and as dry feed, generally it is also combined with some sort of wet feed (Kröncke et al. 2023). *T. molitor* is very good as a sustainable food product, as one can use of the whole insect as it is (not only use separate parts). In addition to the insect, there is also a possibility to use the frass (insect excrement) as fertiliser with biostimulative properties through the chitin and its inherent chitinase in the frass (Watson et al. 2021).

Mealworms are generally considered hardy organisms in the sense that they manage to survive on only dry-feed such as wheat bran (Johnsen et al. 2021), infected wheat bran, such as wheat bran that has suffered from Fusarium head blight (Sanabria et al. 2019), and even plastics such as polystyrene (Wang et al. 2022). However, when *T. molitor* receives wet-feed, in the form of for example vegetables, there is a large increase in body growth as well as a decrease in generation time. This indicates that on an industrial scale, wet-feed is needed to reach a large production volume in a shorter period of time (Deruytter et al. 2021). Even though *T. molitor* are hardy organisms, measuring carbon dioxide is important as it has been shown that hypoxia (low oxygen levels) lowers larval survival rate to approximately 20% while normoxia (normal oxygen levels) and hyperoxia (higher oxygen levels) shows larval survival rates around 96% (Greenberg et al. 1996), assuming there are no other major gases in the cell. Temperature is directly correlated to metabolism since *Tenebrio molitor* (TM) are ectothermic, meaning they need to rely on heat produced from external sources (Dahl Bjørge et al. 2018). Humidity is correlated to larval survival rate, specifically to young larvae where humidity levels below 40% can severely hinder growth or in some cases be lethal (Mirzaeva et al. 2020). It is known that fewer individuals per rearing group will be important for the pre-pupal stage (approximately week 11-13), as a previous study has found that isolation has a larger impact on maturation than feed due to larvae mechanically stimulating each other inhibiting pupation (Connat et al. 1991).

In this thesis I describe experiments conducted on the effect of different by-product feeds on the growth of *T. molitor* in industrial rearing. In the study I used four different feed types (sole or mixed feeds) to evaluate their impact on bodily growth. The main questions in the study were:

- Will the growth rate differ significantly between treatments?
- If they differ, which type of feed promote most growth in the larval stage of *T. molitor*?
- Is it the same feed that promotes the most growth in all life-stages?

With the null-hypothesis that there would be no significant difference in growth rate no matter the treatment the individuals are fed and my alternative hypothesis was that there would be a difference in growth depending on what is used as feed in all stages. I also think that a mixture of feed would promote the most growth as there would be a wider array of amino acids readily available for different needs in any point of the insects' development (Wu, 2010; Finke et al. 2014).

2. Material and methods

2.1 Study species

Tenebrio molitor is a species that belongs to the family Tenebrionidae and are often called mealworms or yellow mealworms. These insects have the capability to survive in a wide array of environments and are distributed all over the world. In domestic setting, they can be found in pantries or other places where grain, cereals, corn or breadcrumbs are kept and it is believed that they spread throughout the world because of human activity (GBIF, 2023). *T. molitor* has a life cycle of 75 days in optimal rearing conditions with 27-30 °C and a humidity level around 70% (Spencer and Spencer. 2006). In rearing conditions that are not optimal, such as ambient temperatures of 15 °C, the life cycle can take up to 600 days (Cotton. 1927).

2.2 The experiment

The animal rearing

In order to ensure the same basis for all individuals that were going to be used in the tests, all individuals were taken from the same "batch" hatched within the company Tebritos' facility, a facility used for production of mealworms as a future source of protein. Since cages are stacked upon each other in the rearing facility an equal number of cages in the bottom of the stack were used as in the top of the stack in the experiment in case there would be a difference in microclimate within the cages. In the experiments the individuals were housed in the same type of cages used in the regular production. The cages were 600 x 400 x 145 mm in size and have edges, except for the corners that have openings on the sides to ensure air circulation. Cages have no lids, but since they are stacked on top of each other, the bottom part of one cage acts as a lid for the cage below. The only cage that is not covered is the one at the top of the stack. The top cage switches position with the bottom cage at every feeding in order to reduce effects from the cage's position in the stack. This stacking of cages during the experiment mirrored normal production routines.

Feeding in production systems

In regular production the cycle starts with only eggs in the cage together with a substrate layer of wheat bran. The eggs hatched into larvae live in the wheat bran for four weeks (week 0 – 3), before they receive their first wet feed. Before the first wet feeding, the larvae receive a refill of wheat bran due to them having consumed parts of the initial wheat bran. The wet feed is then added on the first, third and fifth day of each week (Week 4-10), before the individuals being harvested at week 11. I decided to mirror this type of feeding to a large degree in the experiment, partly in order to mimic the practical routines performed in the industry but also for practical reasons (this way I could perform feeding tests at the same time as feeding was done in normal production). The reason that the "control" treatment consists of only wheat bran and water is that this is often the regular and basic feed for individuals. In the experiment each cage receives an initial feeding of dry feed in the form of 3.5 L (0.98 kg) wheat bran, this is in order to ensure that larvae have enough substrate to survive until wet feeding starts in the fourth week of the larvae's life. In addition to this, initiating wet feed before the fourth week would increase the likelihood of mould in the cages due to over-feeding, as there is not knowledge on the amount of feed eaten at this early stage.

The experimental feeds

The different feed types in the experiments all belong to the category food by-products. They were: 1) gluten free crispbread from a company that produces crispbread in a circular shape. In order to get the circular shape they need to cut off the edges which are unusable for the producing company. These parts has the potential to be used as feed for insects. 2) Spent brewers' grain (also known as 'draff') from a local brewery that produces beverages. The spent brewers' grain generally has a high protein content and is therefore often used as either petfood or in biogas facilities (Heuzé et al. 2015). 3) Wheat bran which is the outermost part or shell of a wheat kernel, is usually removed during the processing of wheat. Wheat bran has several uses, but it is still considered an agricultural by-product (Balandrán-Quintana et al. 2015). 4) Gluten free crispbread mixed with spent brewers' grain.

I fed the individuals the four different feeds mixed with water: gluten free crisp bread mixed with water, spent brewers' grain (no added water as it already had ca 70% moisture content (MC), wheat bran mixed with water and a combination of gluten free crispbread, spent brewers' grain and water (Table 1). The ingredients and their proportions in the gluten free crispbread and the spent brewers' grain are not available since the companies producing them mix crispbread by-products from several different bread productions and brewing processes. The crispbread could be from chia, oats, flax or rosemary in different proportions, for this reason the mixing was slightly different each time in order to get a good consistency, this means that the proportions between spent brewers´ grain and crispbread were not always exactly 50/50 due to some parts of the crispbread being more easily mixed than others.

Table 1. Feed treatments in the experiments

Treatment	Feed type
Control	Wheat bran + water
Draff	Spent brewers' grain
Crispbread	Gluten free crispbread + Water
Draff + Crispbread	Gluten free crispbread + Spent brewers' grain + Water

The feeding in the experiment

The different feeds that was part of the trial gluten free crispbread, spent brewers' grain and wheat bran with water were prepared in a container and manually handfed to each cage. To ensure the larvae were not crushed by large pieces of feed and to maximise the area that the larvae could feed from, the feed was carefully spread out over the whole area of the cage (Figure 1, 2). The feeding of the different treatments followed the normal production schedule where the first week of wet feeding for the larvae (Week 4) entailed a total of 150 g of feed per larvae cage, the second week (Week 5) had a total of 300 g feed per larvae cage and finally the following weeks (Week 6-10) had a total of 600 g feed per larvae cage (Table 2). I increased the feeding over time as insects, just like most other animals, are able and require feeding more as they increase in size. The feeding experiments were terminated at week 11. At this time there were individuals pupating in all test-groups except for the control test-group.

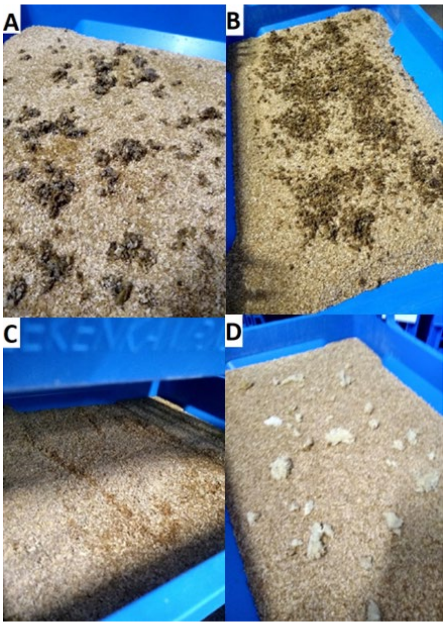

Figure 1. Feed treatment in cages for larvae that are four weeks old. A. spent brewers' grain and gluten free crispbread mix, B. spent brewers' grain, C. wheat bran and water, D. gluten free crispbread.

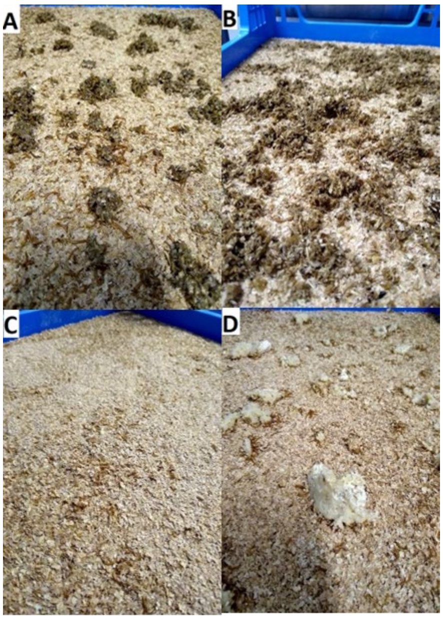

Figure 2: Feed treatment in cages week 5. A. spent brewers' grain and gluten free crispbread, B. spent brewers' grain, C. wheat bran and water, D. gluten free crispbread.

Water content

I measured the moisture in the feed pre-experiment using a HC103 moisture analyser (Mettler Toledo). The analyser dries feed material samples, absorb the moisture content that evaporates and by that calculates the % moisture content (MC). This allowed me to test and retest the different feeds to acquire as similar moisture contents as possible. For example, before retesting, gluten free crispbread had 49.12 % MC, while spent brewers´ grain had 75.2 % MC. As the moisture analysis takes significant time per analysis, I decided that a moisture content between 70 and 75% was an acceptable value for each feed. Water was added to the feed to reach this level using a micropipette. 2-10 ml were added to the feed until a MC percent of around 70-75% was achieved. This was done to make sure all feeds had approximately the same percent MC in order to minimise the effect of the water itself.

2.3 Methods

The experimental animals

I collected beetles from normal production at Tebrito and from the same starting batch. The beetles were selected through sieving using a 4.0 mm sieve which means that smaller individuals and pupae fall through the sieve while larger individuals stayed in the sieve and were collected. These beetles laid eggs in 60 cages for 1 week, where each cage started off with 110 g of beetles. At this stage the beetles were in their fifth week of their adult stage. I chose this time period to ensure that all beetles had a chance to mate and lay eggs. The morphological fitness of the beetles were on the same level in the sense that there were no deformities and they were all of the approximate same size. It is difficult to know how many eggs were laid in each cage as eggs are hard to visually count and measure (Froonickx et al. 2022). Instead, it was estimated from normal production numbers that it was approximately 90 000 larvae in the experiment in total. The eggs were collected from the adults by sieving them using a 3.5 mm sieve, this ensured that only the wheat bran and eggs were allowed to pass through the netting (together with a few smaller adult individuals who were then removed with forceps). The cages which now had eggs and wheat bran only were then marked with labels into different test-groups. This means that the 60 cages were split into 4 different categories with 15 cages each. The labels were to make it possible to discern the cages and treatments from each other.

Table 2. Amount of wet-feed over time for each cage (control n=15, draff n= 15, Crispbread n=15, Draff + crispbread n= 15.

Feed	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10

	g/cage	g/cage	g/cage	g/cage	g/cage	g/cage	g/cage
Control	150	300	600	600	600	600	600
Draff	150	300	600	600	600	600	600
Crispbread	150	300	600	600	600	600	600
Draff + Crispbread	150	300	600	600	600	600	600

The growth measurements

To get a measure of individual larval weight I collected larvae from each cage (Figure 3) and weighed 10 g once a week. This was done over 6 weeks (week five to week ten). The samples were collected using forceps and individuals were collected from different corners of the cage every time in order to keep the sampling as randomised as possible. I calculated the average individual weight of each test-group by counting the number of larvae required to reach 10 g from each cage. I then divided the total amount in grams by the total amount of larvae counted for the whole batch. For example, larvae that had received the crispbread treatment and were in week ten of their life had a total of 1494 larvae counted in order to reach 150 g in total weight. By using the following equation: $\frac{150}{Counted\ larvae\ in\ batch} = \frac{150}{1494} = 0.1$ we see that the average individual larvae weight at week ten for crispbread treated larvae is 0.1 g. Ten g from each cage were collected in order to ensure there were no outliers in the sense that one cage had vastly fewer larvae to reach 10 g than any other cage. After the counting I returned all larvae to their respective cages, this was so that there would not be an ever decreasing amount of larvae per opportunity of counting.

Figure 3: Counting of larvae at week 5 in one of their cages, wheat bran had to be sieved in order to properly count larvae.

Measuring maturation rate

To examine if feed affected the frequency of pupation, I counted the number of pupae every time I added feed during the experiment; every first, third and fifth day of the week for 2 weeks. This resulted in the number of pupae counted a total of 6 times during the experiment. Additionally, to see if larvae fed a specific feed hatched faster into adults after their pupae stage, I counted the number of beetles emerging from pupae in the different treatment groups. To further examine the effect of different feeds on pupation rate at the end of the feeding test, I gathered 100 g of larvae and pupae from every cage. I put these individuals in their 'home' cage after the rest of the larvae were terminated. The individuals stayed in this cage until pupation. I used a smaller number of larvae for this part of the experiment, as pupation is heavily dependent on larvae not stimulating each other mechanically (Connat et al. 1991). Even if individuals that are close to pupation are not as affected by starvation as younger individuals I still ensured that the larvae were fed *ad libitum* by feeding on day one, three and five of each week. That is the same way as they were fed from week 4-10 but with an amount of 100 g per cage as they were now fewer individuals.

Environmental variables

Carbon dioxide, temperature and relative humidity was analysed on day two and four of each week. These variables were measured using an in-house climate control system that has the capabilities to measure temperature, carbon-dioxide and relative humidity levels, these are logged overtime which allowed me to keep an eye on any irregularities. This was done in order to observe if there were any environmental variables that could explain irregularities in either the growth or maturation rate of the larvae.

Statistical analyses

To examine if the different feeds had any effect on larval growth and at what capacity. I used an anova for multiple groups. I performed a Tukey's honest significant difference (HSD) to investigate how the mean of every sample compares to the mean of all other samples. All analyses were carried out using R-studio (R-studio 4.2.2).

3. Results

All types of tested feed led to larval growth, as weight increased over time for all feeds. There were differences in rate of growth depending on what type of feed that was consumed. There was a difference between treatments on average individual weight. There was a significant difference between the crispbread treatment and the control treatment with a p-value <0.05 using Tukey's honest significant difference test (Table 3) as well as between draff combined with crispbread and the control (Table 3).

Table 3: A significant difference between the control and crispbread as well as between spent brewers´ grain (draff), crispbread and control is shown using Tukey HSD. Significance level: p <0.05

Tukey HSD	Difference	Lower	Upper	p-value
Crispbread – Control	*-144.7*	*-237.4*	*-52.0*	***0.0005***
Draff – Control	*-76.3*	*-169.0*	*16.46*	*0.1478*
DraffCrispbread – Control	*-108.7*	*-201.5*	*-16.0*	***0.0141***
Draff – Crispbread	*68.4*	*-24.3*	*161.1*	*0.2280*
DraffCrispbread – Crispbread	*35.9*	*-56.8*	*128.7*	*0.7491*
DraffCrispbread - Draff	*-32.5*	*-125.2*	*60.3*	*0.8028*

Larvae fed gluten free crispbread were heavier than the individuals that were fed other types of feed throughout the whole experiment (Figure 4). In fact, all other feed by-products tested (spent brewers´ grain, gluten free crispbread, spent brewers´ grain and gluten free crispbread mix) produced heavier individuals than the control feed (wheat bran). At the end of the experiment at week 10, the average weight per individual was for wheat bran 0.05 g, spent brewers' grain 0.07 g, crispbread 0.1 g and spent brewers' grain and crispbread 0.09 g (Figure 4).

Figure 4: Comparison of larvae size in the different feeding trials at week 10: wheat bran: 0.05 g per larva, spent brewers' grain: 0.07 g per larva, gluten free crispbread: 0.1 g per larvae, spent brewers' grain + gluten free crispbread: 0.09 g per larvae.

All feed led to pupation of the individuals, but for individuals fed control feed, this was approximately one week later than for the other feeds. Fastest maturation was seen in draff (spent brewers' grain) with 235 pupae in total in the 13th week of the individuals' life (Figure 5).

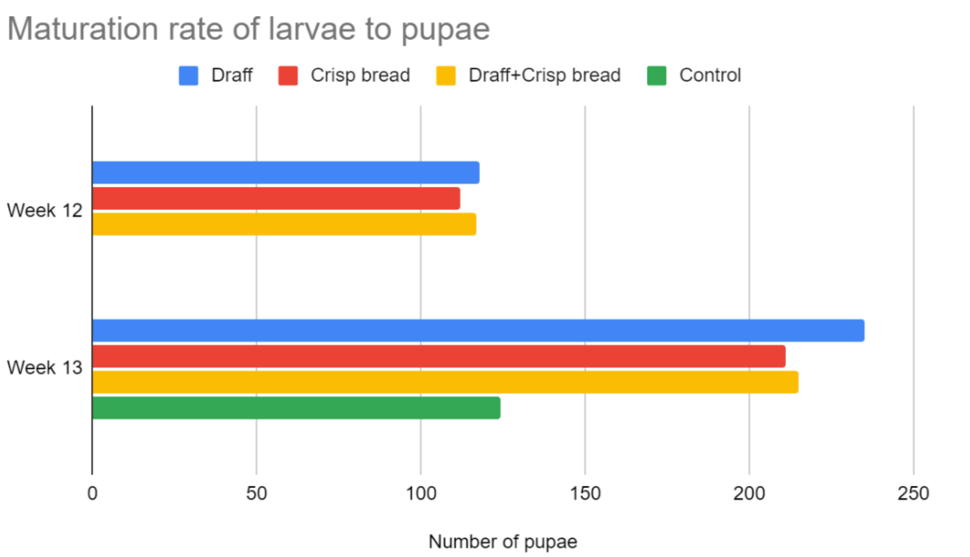

Figure 5: Maturation rate of larvae to pupae over a two-week period (week 12 – 13). Control has no pupae at week 12. Draff (spent brewers' grain) has more pupae than the rest (235 pupae).

There was an average decrease of 122 larvae per week (Table 4). Using the same linear model as was used in table 4 but instead basing it on the average weight per individual we get an overall significant interception ($p =< 2.2e^{-16}$). We see that growth differs from each other (Table 5). This can also be seen if it is plotted (Figure 6).

Table 4: Every week the number of counted individuals decreases by an average of 122 individuals.

Linear model	Estimate	Std. error	t-value	Pr(>|t|)
(Intercept)	*1336.1*	*29.9*	*44.7*	$\underline{\boldsymbol{< 2e^{-16}}}$
Feed Crispbread	*-144.7*	*17.5*	*-8.3*	$3.03e^{-15}$
Feed Draff	*-76.3*	*17.5*	*-4.4*	$1.77e^{-5}$
Feed Draff Crispbread	*-108.7*	*17.5*	*-6.2*	$1.54e^{-9}$
Week	***-122.5***	*3.6*	*-33.8*	$< 2e^{-16}$

Table 5: Significance shows that the slopes are different for the treatments, indicating a difference in growth rate.

Linear model (Combined to look at slopes)	Estimate	Std. error	t-value	Pr(>|t|)
Feed Crispbread : Week	*0.01*	*0.065*	*14.713*	$\underline{\boldsymbol{< 2e^{-16}}}$
Feed Draff : Week	*0.002*	*0.065*	*3.596*	$\underline{\mathbf{0.0004}}$
Feed Draff Crispbread : Week	*0.007*	*0.065*	*11.323*	$\underline{\boldsymbol{< 2e^{-16}}}$

4. Discussion

Effects on growth and size

The hypothesis that feed does indeed influence larval growth and size can be confirmed through these results. There are significant differences between the different feeds and the control when looking at the crispbread treatment and the draff + crispbread treatment. This means that null hypothesis can be rejected in exchange for the alternative hypothesis that feed type does indeed influence larval growth rate. The largest effect on average individual weight can be seen in larvae fed gluten free crispbread (Figure 6).

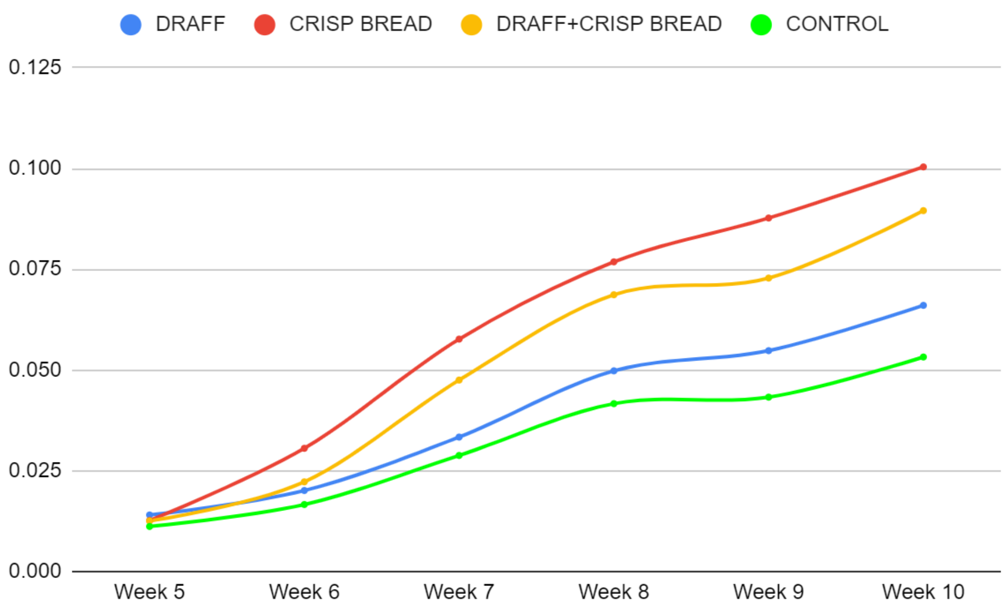

Figure 6: T. molitor larval weights over the 10-week trial using feed from 3 different by-products and control feed (g). Draff i.e. spent brewers' grain treatment in the figure legend.

Surprisingly we see a larger increase in a shorter period of time for larvae fed only gluten free crispbread than gluten free crispbread in combination with spent brewers' grain and water, spent brewers' grain or wheat bran and water. The larger growth rate was expected compared to the control (wheat bran + water) but surprising compared to the other treatments as one hypothesis was that the broader range of amino acids from the mix of all feeds would result in the largest growth (Wu, 2010; Finke et al. 2014). Instead, we see that gluten free crispbread has a faster growth rate between the fifth and sixth week. The individuals fed gluten free crispbread seems to not be as affected by a “dip” in growth-rate between week 8 and 9 as seen for the other feeds. All test groups experience some sort of decrease in their growth rate at this time, and there might be something in the larval development or the environment that causes this (Mirzaeva et al. 2020, Greenberg et al. 1996). The increasing carbon dioxide levels were an anomaly during week 5 and 6. Potentially this could affect growth rate later, but this is unclear (Greenberg et al. 1996). Lack of studies on stage-specific growth changes indicate that there might be environmental affects during the experiment that might have caused this pattern.

Since all test-groups were in cages that had wheat bran it was deemed too difficult to look at mortality as a variable due to young larvae being too small to see in the substrate, this means that in theory there could be few individuals that are very heavy and many individuals that are very light. Since all feeding and counting was done manually it is still notable that there were no obvious visual discrepancies in the number of larvae in the different cages.

Effect on development and maturation

I found that spent brewers’ grain caused the fastest maturation. The reason behind this may be that larva-pupa or pupa-adult has a different amino-acid requirement than simple moulting from larva-larva. However due to only a small amount of data collected combined with the mold-outbreak which led to the termination of the experiment after two weeks, there is not much evidence to support this statement other than my own observations (Figure 5). Disregarding the small amount of data, this result contradicts my hypothesis that a larger mix of feed would result in the fastest maturation but it also indicates that feed does have an effect on maturation. There are other studies that support that different life-stages in many cases require different arrangements of amino-acids due to, for example different gene-expressions (Wu, 2010). At the same time there are other articles noting that: *“prediction of an insect´s amino acid profile based on species or diet provided is difficult at best”* (Finke et al. 2014) which further complicate the matter. Finally, there is a study discussing the use of developmental models and optimal temperature for development and pupation (Abbas, 2019) which is something that was largely ignored in this study but would be interesting to look further into in future studies.

Future studies

It should also be noted that that all the beetles that were allowed to lay eggs at the start of the experiment, came from the same "batch" from normal production. This was done in order to ensure similarity of genetic and environmental similarities of the studied individuals. However, looking at potential variation between "batches" during the egg-laying phase could have been informative. This would be very interesting to focus on in future studies. It would also be very interesting in the future to find the exact nutritional requirements for each larval instar in order to maximise growth in each stage as well as what is different when the larvae reaches the point where it starts to pupate and emerge as adults. When it comes to adults and there is no more moulting you would instead be interested to look into what stimulates reproduction most and what feed leads to more eggs in the following generation. Finally, as mentioned in the introduction, it is very difficult to approximate the number of eggs that are in each cage. In this day and age when AI and image recognition software is developed at a rapid pace, I think it would be good for a future study to look into software that is able to count the eggs and in doing so, try to pinpoint the "perfect" density of adults that leads to the most larvae in the following generation. This could also be helped a lot by theoretical ecology if future studies start looking more into life history traits such as fecundity at different weeks in the adult beetle's life. If we could pinpoint the timeframe where fecundity is the highest and ensure that all other environmental variables as well as feed is available at this point, then you would very likely produce a large amount of eggs. I also think that future studies should look more into the relationship between water and nutrients in the larvae to check what ratios are considered optimal for breeding. It would perhaps be undesirable to produce very large larvae very fast if they end up only containing water and almost no nutrients.

Future sustainable feed

The manual mixing of feed worked relatively well as it was mixed in small proportions before every feeding opportunity. The biggest issue was mixing the draff + crisp bread treatment as the spent brewers' grain (i.e. draff) has a larger particle size than the crispbread, but with enough mixing it turned into a homogenous substance and was therefore still possible to use in a small-scale experiment like this was. It would likely work to mix feed in an industrial-grade feed blender. It is unlikely that there are no other interesting by-products in the world that might also require processing before feeding to insects such as *T. molitor*. These should be tested in a smaller scale before being applied to a larger industrial production from the perspective of biosafety (EU No. 1069/2009. 2009), so it is not dangerous for the insects or those consuming the insects, but also from an economical perspective. It may not be profitable if you need to process the by-product by grinding, heat-treating or freezing it before feeding the insects.

The results of these experiments could be useful for the industry as a whole in order to validate the functionality of food by-products as high-quality feed for the industry. Depending on the size of the company and its geographic location there may be different types, quantities and qualities of food by-products available. Considering this experiment only tested three different types (gluten free crispbread, spent brewers' grain and wheat bran) there needs to be more studies done on different kinds of food by-products to find something that increases the growth rate substantially. In this experiment we can observe an almost 100% increase in average larval weight between gluten free crisp bread and the control (Figure 6) during the same time span. I imagine this could be increased further if we can find the exact nutritional values required for the different instars of the larval lifespan and try to find the best match in terms of food by-product.

Even if not all food by-products increase average larval weight, it could still be an interesting alternative if, for example it decreases the time for larvae to pupate and emerge as adults. This would lead to a possibility to have different kinds of wet-feed for different stages in *T. molitors* life. One could for example use gluten free crispbread in order to get a high average individual larval weight faster than the 75 days that is considered optimal (Spencer and Spencer. 2006) and then switch to another feed that may increase pupation rate and amount of eggs laid for adults. If this could be done, it is very likely that the production would become incredibly efficient and cost-effective over a long period of time. Assuming of course that the trade-off is worth it when you logistically and economically need two different sources of feed to work with. This is not an easy question, and it depends heavily on what resources are available as well as what research has been done.

Another food side-stream that could be useful for producing *T. molitor* is agricultural products infected by *Fusarium*. This fungus has a negative impact on crops as they cause a disease known as *Fusarium* head blight which decreases both yield and the quality of yield. Along with this, *Fusarium* also produces secondary metabolites known as mycotoxins (Sanabria et al. 2019) which cause mycotoxosis in livestock consuming the grain. This is however not a problem for *T. molitor* as the mealworm is able to digest mycotoxins without sequestering it and there has even been indications that *T. molitor* prefers infected wheat over healthy wheat (Sanabria et al. 2019). This means that mealworms can consume something that would otherwise simply be discarded (Sanabria et al. 2019). Recently it was discovered that *T. molitor* can degrade polystyrene foam through the usage of its gut microbes (Wang et al. 2022). It is believed that using only plastics as feed may be insufficient for the larvae in the long run and could lead to growth retardation. By mixing it into the regular feed, or by using feed that is susceptible to contain microplastics would be an interesting topic to pursue in order to further expand the positive aspects of breeding *T. molitor*.

It can be quite difficult to clearly define an ecological perspective when it comes to artificially creating an ecological system to rear insects. You need to ensure you fulfil as many of the requirements as possible for your specific species of insect while still making it logistically viable to rear and harvest. These requirements, as mentioned in the introduction can be environmental variables such as humidity, temperature and oxygen. All of which can be commercially costly to keep at a stable level. Because of this I firmly believe that agricultural and food by-products is the way to go when it comes to this type of rearing as it makes it possible for start-up companies with a smaller financial status to still be able to compete with regular farming and animal rearing by creating a sustainable artificial ecological system. I am overall positive to the future of the industry as more and more research is done and becomes available for industries to make informed choices when it comes to investing in food by-products for insect rearing.

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Popular science summary

Mänskligheten har gått från att vara jägare/samlare till att bruka jorden och sedan vidare till ett industriellt jordbruk. Denna typ av jordbruk ses som det absolut bästa sättet för att mänskligheten ska kunna fortsätta växa och expandera. Det verkar dock som att vi närmar oss en topp på vad vi kan producera genom traditionellt jordbruk. Det kommer inte vara möjligt att fortsätta som vi gör just nu för att ha föda till den växande befolkningen utan att permanent skada den biologiska mångfalden. Om den mänskliga befolkningen ska fortsätta öka så behöver vi hitta alternativa livsmedel som innehåller de näringsämnen vi behöver och vill ha, med en svag miljöpåverkan, samt är möjligt i en stor skala. *Tenebrio molitor* är en insekt som vi kallar för mjölmask eller mjölbagge och är en av de främsta kandidaterna för framtidens utfodring. Är det möjligtvis dags att gå tillbaka till att äta insekter?

Vi vet alla om att klimatet på vår planet blir sämre och sämre ur ett mänskligt perspektiv, detta på grund av utsläpp av exempelvis koldioxid. Betesmarker öppnas upp för att hålla boskap istället för skogar, fyllda av koldioxid-absorberande träd. Allt detta går att lösa genom att byta ut konsumtionen av kött från boskap till kött från insekter. Ett lägre ekologiskt fotavtryck samt ett sänkt utsläpp av exempelvis, ammoniak. Mjölmask är otroligt effektiva för att vara så pass små, detta betyder att även då man behöver stora mängder mjölmask för att komma upp i samma mängd kött som det mesta boskap så är det mer miljövänligt att använda sig av just mjölmask för att producera behovet av protein. Mjölmask innehåller stora mängder fett och protein men även essentiella aminosyror samt mineraler som behövs för våra dagliga aktiviteter. Detta i motsvarande eller till och med bättre mängder än både rött och vitt kött.

För att undersöka möjligheten att använda olika olika reströmmar från livsmedelsproduktionen i Sverige utfördes ett experiment på mjölmask, en av de vanligaste insekterna som föds upp globalt. Reströmmar kostar ofta pengar att göra sig av med för producenterna, då det inte finns något lämpligt användningsområde för dem. Av dessa reströmmar användes glutenfritt knäckebröd i pulverform som blivit över då knäckebrödet delas i dess olika försäljningsformer, drav som är en restprodukt från ett bryggeri, vetekli, som är höljet runt kärnan på sädesslaget vete och som inte används. De olika födotyperna användes för att studera hur de påverkade tillväxten samt mognad hos mjölmasken. Jag fann en hög tillväxt hos larver som matats med glutenfritt knäckebröd blandat med vatten där individerna blev nästan 100% tyngre än individerna som endast fick vetekli och vatten.

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Helena Bylund and Gabriel Nordström for examining the results.

Appendix 1

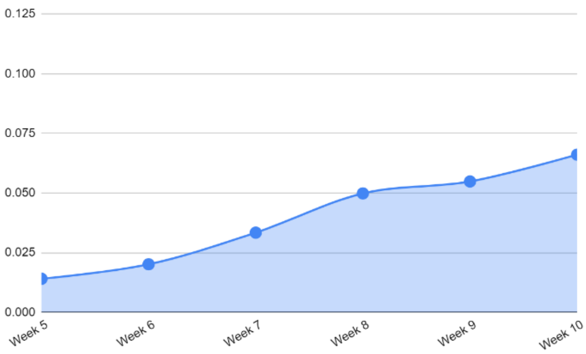

Figure 7: Graph showing larval growth rate of larvae fed spent brewers' grain. Average individual larvae weight on the Y-axis (g), time in larval life on the X-axis

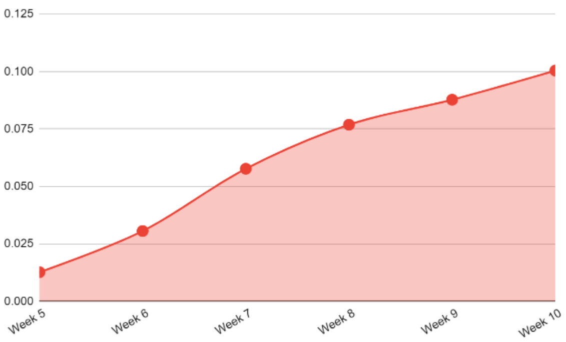

Figure 8: Graph showing larval growth rate of larvae fed gluten free crispbread. Average individual larvae weight on the Y-axis (g), time in larval life on the X-axis

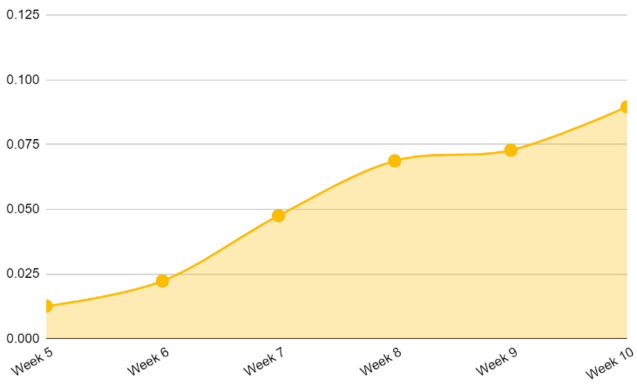

Figure 9: Graph showing larval growth rate of larvae fed spent brewers' grain and gluten free crispbread. Average individual weight on the Y-axis (g), time in larval life on the X-axis

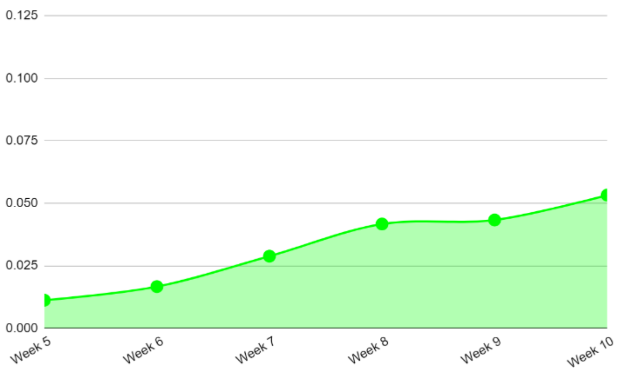

Figure 10: Graph showing larval growth rate of larvae fed wheat bran and water. Average individual weight on the Y-axis (g), time in larval life on the X-axis.

```
Call:
lm(formula = count ~ feed, data = bugs)

Residuals:
   Min     1Q Median     3Q    Max
-252.0 -173.9 -113.2  129.5  625.6

Coefficients:
                    Estimate Std. Error t value
(Intercept)           417.03      25.40  16.417
feedcrispbread       -144.68      35.92  -4.027
feeddraff             -76.27      35.92  -2.123
feeddraffcrispbread  -108.73      35.92  -3.027
                    Pr(>|t|)
(Intercept)          < 2e-16 ***
feedcrispbread       6.9e-05 ***
feeddraff            0.03444 *
feeddraffcrispbread  0.00265 **
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 241 on 356 degrees of freedom
Multiple R-squared:  0.04728,	Adjusted R-squared:  0.03925
F-statistic: 5.889 on 3 and 356 DF,  p-value: 0.000623
```

Figure 11: Summary of the linear model used to compare the different feed-types

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