Determining greenhouse gas production by edible insects

This report was written in context of the Interreg North-West Europe ValuSect project.

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Content

1 Introduction

This research is carried out as part of the Interreg NWE ValuSect project (work package 1: Quality improvement of the primary insect production process, activity 1: Testing greenhouse gas production by insects). The aim of this research is to improve the primary production process of insects addressing byproducts of food industry and agriculture. This investigation is related to the research of activity 2 of work package 1 (testing side streams as substrate for insects). The goal of both investigations is to obtain a more sustainable production of insect based food products, combining insect rearing on side streams with low emission of greenhouse gasses.

A literature review (work package 1, activity 3: Literature search to clarify questions from SMEs and companies regarding quality improvement of insect production process) was conducted prior to this research to obtain more knowledge about insect breeding on side streams and emissions related to insect rearing. This in order to be able to carry out applicable experiments during activity 1 and activity 2 of work package 1. The main output of this literature search, relevant for activity 1, is the selection of emissions to be measured during insect (*Tenebrio molitor, Acheta domesticus and Locusta migratoria*) production. The selection is based on the relevance for insect production. Following emissions will be measured during the insect rearing experiments: CO_2, ammonia and particulate matter. These emissions will be measured with sensors.

In the literature search, a number of questions were defined that should be answered during the research carried out within activity 1 of the project. The list below provides an overview of the questions to be answered. This list will be used as a guideline for the research to be performed.

1. How much of the selected greenhouse gases do insects emit when rearing on compound diets?

In order to investigate the emissions of insects produced on compound diets, greenhouse gases will be measured during the rearing experiments of activity 2. These experiments will be conducted on laboratory scale as well as on pilot scale. Diets with low overall emissions and good growth will be favoured over diets that produce more emissions to address a more sustainable insect production.

2. What methods will be used to measure the emissions?

In activity 3 it has already been broadly discussed which methods will be used to measure the selected emissions. However, in order to answer this question, more extensive research is needed. Detailed information on measuring methods, obtained from knowledge and literature, will be provided.

3. What measuring instruments will have to be developed or optimized during the project and how?

During the ValuSect project, measuring instruments will have to be developed or adjusted to make them suitable for the measurement of insect emissions. Extensive information on the optimization and the use of these instruments will be provided.

4. Optimization of the standard insect rearing protocol

The literature search has shown that the sustainable rearing of insects still needs to be optimized. A lot of questions on insect rearing rose during the conduction of activity 3 of the project. As output of the ValuSect project, the standard rearing protocol per insect species will be optimized to produce insects for food in a sustainable way. The standard rearing protocols will be complemented and adjusted with the knowledge obtained during this entire research.

The ValuSect project will contribute to the acceptance of insects in the human diet. This project focuses on 3 insect species, namely Tenebrio molitor (mealworm), Acheta domesticus (house cricket) and Locusta migratoria (migratory locust). For these insects a novel food dossier is submitted and under evaluation.

The aim of the project is, among others, quality improvement of the primary production process and the processing of insect products for food. This means that an attempt is made to obtain optimal rearing conditions for the insects in question, thereby demonstrating a positive environmental impact of these rearing conditions. This will lead to a sustainable and optimized insect production. Furthermore, efforts are being made to identify the nutritional value of insects and insect-based food products, as well as the factors that influence this. The project also focuses on the microbial load of insects and insect-based food products concerning food safety and shelf life. All this will initiate an insect-based food innovation.

2 Determining emissions by insects

2.1 Material and methods

2.1.1 Principle

Two devices were used to determine the emissions produced by the insects, a flowthrough chamber and an accumulation chamber (Figure 1).

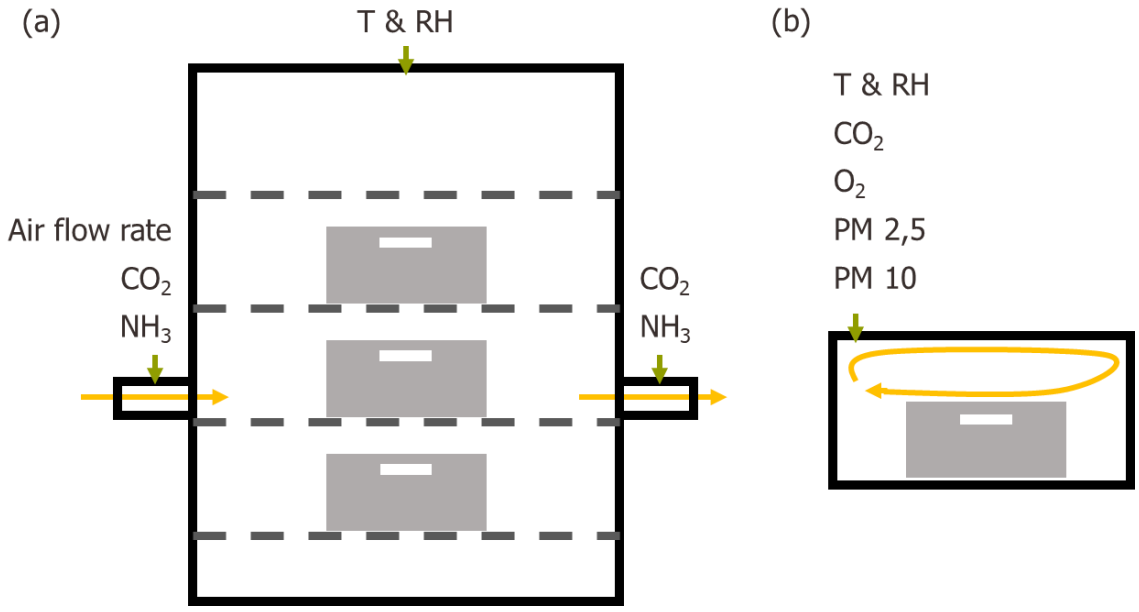


Figure 1: (a) Flowthrough chamber for determination of insect emissions CO2 and NH3, (b) Accumulation chamber for determination of CO2 production and O2 consumption. Two fractions of particulate matter (PM) are determined as well and there is the option to sample air.

The first device is a flowthrough chamber (Figure 1 (a)) measuring real time concentrations at the air inlet and at the outlet. The difference in concentration combined with the air flow rate and the amount of biomass inside the chamber, can be used to calculate the amount of emissions that are produced. The advantages and disadvantages of this system are discussed in Table 1.

The second device is an accumulation chamber (Figure 1 (b)). A batch of insects is placed inside the chamber and gasses are allowed to accumulate. The accumulation chamber collects a data point every pre-set time interval. From this timeseries data one coefficient is estimated. This is an estimate of the amount of CO_2 that is produced by one kg of live insects per hour. By repeating this several times throughout insect growth, the age dependent biomass specific CO_2 production can be determined. The advantages and disadvantages of this system are discussed in Table 1. The developmental process of the accumulation chamber is discussed in detail under paragraph 3.

Table 1: Advantages and disadvantages of both measuring devices.

	Flowthrough chamber	**Accumulation chamber**
Pros	• Emissions produced by insects can be monitored throughout their lifecycle • The insects are continuously in a stable climate	• The accumulated gasses are more easily detectable, allowing for less sensitive equipment. • Insects are only inside the chamber for short periods of time, allowing for more measurements per day.
Cons	• One measurement takes the entire production cycle, making it time consuming measurements • More parameters need to be measured, including air flow rate. Small measuring mistakes can have major implications on the final outcome	• The climate inside the accumulation is less stable. Temperature and humidity can start to deviate from the desired values. • The accumulated gasses might have an unknown effect on the behaviour of the insects (lethargic insects will have different emission patterns than active once).

2.1.2 Converting measurement data

All sensor data on gas concentration is registered in volumetric ppm. The following method was used to convert this to SI units:

Equation 1: converting volumetric ppm to SI units (g/m³). "X" is the gas concentration in ppm, "n" is the number of moles, "R" is the molar gas constant, "T" is the ambient temperature (K), "P" is the atmospheric pressure, "X_T" is the ambient temperature (°C).

$$X\ ppm_v = X\ \times 10^{-6}\frac{mol_{CO2}}{mol_{air}} \xrightarrow[P=101300\ Pa]{V=\frac{nRT}{P},\ R=8.31\frac{m^3 Pa}{Kmol}} X\ \times 10^{-6}\frac{mol_{CO2}}{\frac{mol_{air}\times R\ \times T}{P}}$$

$$= \frac{X\ \times 10^{-6}\ mol_{CO2}}{\frac{1\ \cancel{mol_{air}}\times 8.31\ \frac{m^3 \cancel{Pa}}{\cancel{K} mol_{air}}\times (X_T\ \cancel{K} + 273.15\ \cancel{K})}{101300\ \cancel{Pa}}}$$

$$= X \times \frac{10^{-6}}{\frac{8.31\times(T_{ambient}+273.15)}{101300}}\frac{mol_{CO2}}{m^3_{air}}$$

$$\xrightarrow{molar\ mass} X \times \frac{10^{-6}}{\frac{8.31\times(T_{ambient}+273.15)}{101300}} \times 44\frac{g_{CO2}}{\cancel{mol_{CO2}}}\frac{\cancel{mol_{CO2}}}{m^3_{air}}$$

$$= X \times \frac{44\times 10^{-6}}{\frac{8.31\times(T_{ambient}+273.15)}{101300}}\frac{g_{CO2}}{m^3_{air}}$$

2.1.3 Flowthrough chamber

The flowthrough chamber collects a measurement every 5 seconds of the gas concentration and the air flow rate at the inlet and outlet while keeping the inner climate stable at the desired temperature and humidity. Mealworms inside the chamber are kept undisturbed unless when monitored or when wet feed is supplied. In general the internal CO_2 concentration inside the flowthrough chamber is higher than the ambient atmospheric CO_2 concentration. When the mealworms are fed and the door is opened, the CO_2 concentration generally drops and needs some time to stabilise again. Therefore, it was decided to discard all measurements that were made between 9 am and 5 pm, the time when there was a chance that the chamber had been opened. At harvest, the live biomass of the insects is determined and the data is processed. Flow rate and gas concentration were combined. The difference between out and in gives us the net amount of gas that is produced in the chamber over a time interval. Measurement data was averaged per hour and a polynomial model was fitted on the data (using the method of least squares) to fill up for missing values (deleted data from during the day). By adding up the hourly gas emission rates (as shown in Equation 2) over the entire rearing process, the total gas emission can be calculated. This number is then corrected for the live biomass that was harvested.

Equation 2: Calculation of the total gas production by adding up the net (Δ) gas production for each consecutive time interval.

$$Total\ gas\ production = \Delta gas_{t_0} + \cdots + \Delta gas_{t_{n-1}} + \Delta gas_{t_n}$$

2.1.4 Accumulation chamber

Insects were grown under standard rearing conditions as described in the WP1.2 report: Testing side streams as substrate for insects. The accumulation chamber was left to

acclimatize inside the environment where the insects are reared so that all its components could reach the prevailing ambient temperature. Insect emissions were always measured on a rearing crate level, meaning that a rearing crate was placed integrally in the accumulation chamber with any amount of dry feed, wet feed, frass and live insects that were present at the time. Insect disturbance was avoided as much as possible prior to the measurements to reassure that the insects experienced no unnecessary stressors that could influence the measurements. A measurement inside the accumulation chamber would last until the internal CO_2 reached at least 1200 ppm. This could last from 20 minutes up to 15 hours, depending on the amount of live biomass that was inserted in the accumulation chamber. When the insects were taken out, the average weight of the insects was determined as well as the total mass of live insects and the number of individuals. Afterwards the insects were placed back under standard rearing conditions for future measurements.

Accumulation chamber data processing

The data collected by the accumulation chamber is the volumetric concentration (ppm) of a certain gas in the chamber and its evolution over time. It is observed that this evolution is initially linear. However, it is expected that during prolonged measurements certain accumulated gasses would inhibit normal metabolism of the insects which could lead to a stagnation of the increase of the measured gasses. As such, only data from the initial linear part of the accumulation curve as shown in Figure 2 is useable to assess normal emission rates. A linear model is fit on the data and the slope of this model is then used for further analysis.

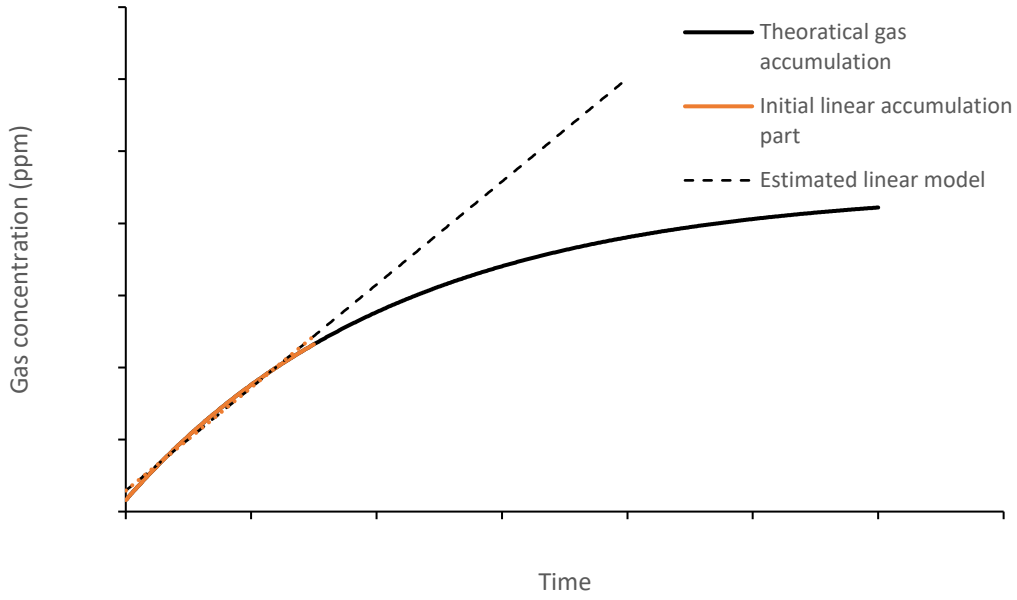


Figure 2: A hypothetical gas accumulation curve. The initial stage is approximated with a linear model.

2.2 Results and discussion

2.2.1 Mealworm (*Tenebrio molitor*) emissions

Carbon dioxide (CO_2)

The feed combinations that were tested in the accumulation chamber are shown in Table 2. In total 193 quality datapoints were collected. However, after closer inspection it was decided to drop the measurements that were done on the day of harvest. Crate conditions at harvest are characterised by the dry feed being almost entirely consumed and the absence of wet feed for the last several days. This group of datapoints is easily recognisable in the overall dataset as a cluster of points with remarkably lower CO_2 emissions than mealworms of similar size that still have plenty of feed as shown in Figure 3. The cause of this decreased emission pattern could be attributed to drier conditions in the crate which slows down microbial activity and associated CO_2 production or slowed down overall activity of the mealworms in the absence of feed.

Table 2: Number of measurements per feed combination in the accumulation chamber. "WB" is wheat bran, "HFM' is hydrolysed feather meal

Dry feed / **Wet feed**	**Wheat bran**	**Insectus**	**WB – HFM (% of WB replacement**			
			5%	**10%**	**15%**	**20%**
Agar	56		10	13	9	21
Cucumber foliage	21					
Fermented forced chicory roots	21	42				

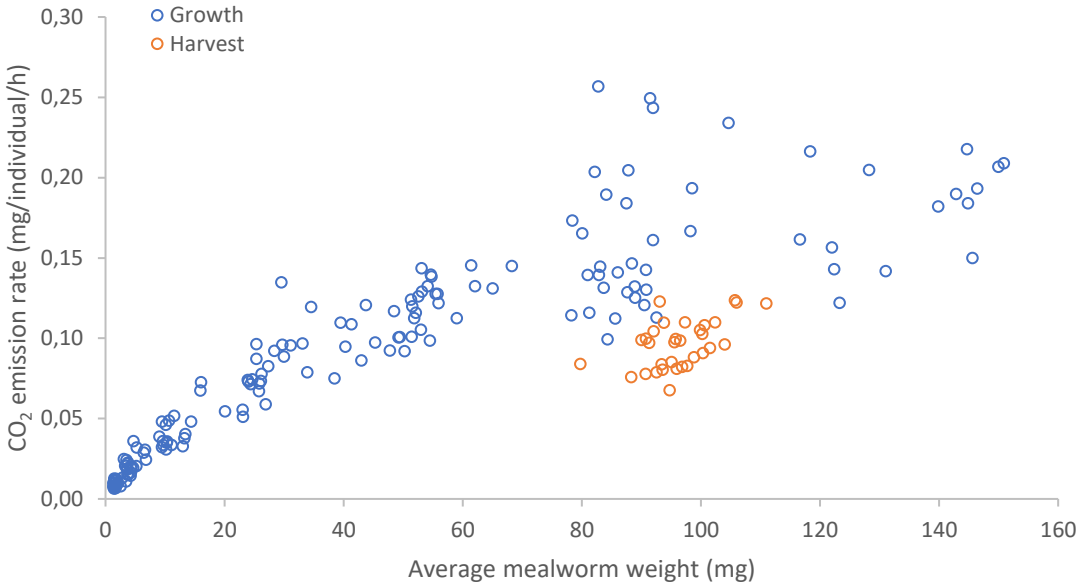


Figure 3: Pooled measured CO_2 emissions from mealworms of a specific average weight grown on different diets. Measurements during mealworm growth are shown in blue, measurements of mealworms ready for harvest are shown in orange.

The data was log-transformed to deal with the heteroscedasticity of the emission values and the higher density of measurements in the lower weight range. The transformed data is shown in Figure 4. A multiple linear regression was performed using the method of least squares to express the relationship between the average weight of a mealworm and the CO_2 production rate. The type of dry feed and wet feed were added to the model as dummy variables to look if they explained some of the variance. The full model (Equation 3) was further reduced by removing non-significant terms (on a 5% significance level) via backwards selection until only significant terms were remaining. This model also had the lowest AIC value. This ultimately resulted in an equation from which all feed-terms (dry and wet) were removed and only the average weight and average weight squared remained as predictors (Equation 4). This indicates that the analysis found no coefficients for the feed-terms that were significantly different from zero. Or in other words that the feed that the mealworms received, did not significantly affect the CO_2 production rate. The reduced model explained 96% of the observed variance (R^2 : 0.96, p-value: <2.2E-16). The model is visualised in Figure 5 with both the 95% confidence interval and prediction interval shown. For further analysis

the confidence interval will be used to express uncertainty related to total estimated CO_2 production.

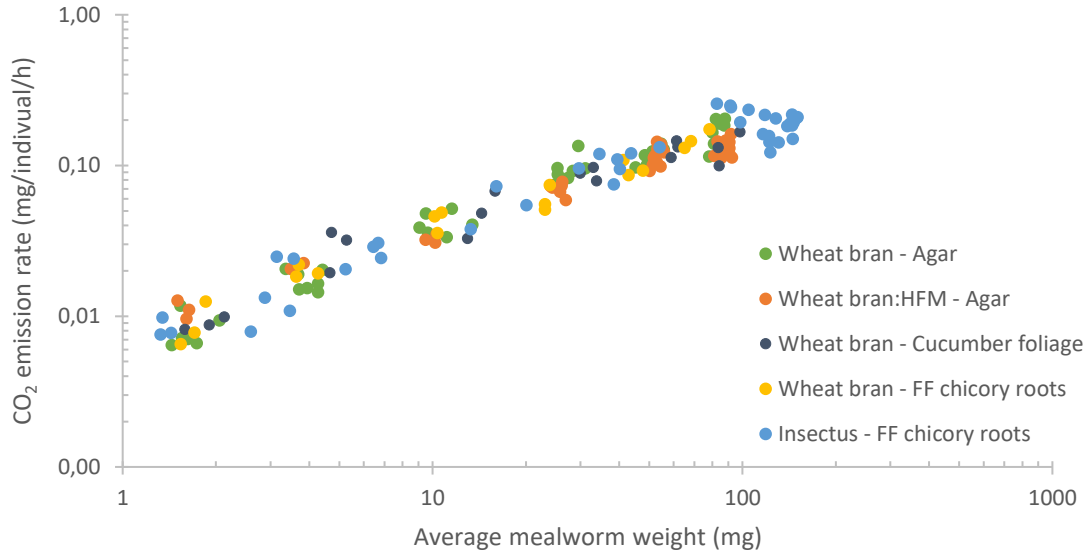


Figure 4: CO_2 emissions per hour from an individual mealworm in function of its average individual weight. "HFM" is hydrolysed feather meal, "FF" is fermented forced.

Equation 3: Full model with "CO_2" being the CO_2 production rate (mg/h) by a mealworm, "Avg" is the average weight of an individual mealworm (mg). "Dry feed" is a categorical variable with levels: "Insectus", "Wheat bran" and "Wheat bran : hydrolysed feather meal". "Wet feed" is a categorical variable with levels: "Agar", "Cucumber foliage" and "fermented forced chicory roots".

$$\log_{10}(CO_2) = Intercept + \log_{10}(Avg)^2 + \log_{10}(Avg) + Dry\ feed + Wet\ feed + \log_{10}(Avg) \times Dry\ feed + \log_{10}(Avg) \times Wet\ feed$$

Equation 4: Reduced model with "CO_2" being the CO_2 production rate (mg/h) by a mealworm, "Avg" is the average weight of an individual mealworm (mg).

$$\log_{10}(CO_2) = Intercept + \log_{10}(Avg)^2 + \log_{10}(Avg)$$

Table 3: Coefficient estimates of Equation 4, the Standard Error (SE), the t-value and its according probability are shown (R^2 : 0.96, p-value: <2.2E-16).

	Estimate	**SE**	**t-value**	**p-value**
Intercept	-2.26	0.028	-80.7	<2E-16
Log(Avg)²	-0.11	0.023	-4.76	4.79E-6
Log(Avg)	0.96	0.056	17.3	<2E-16

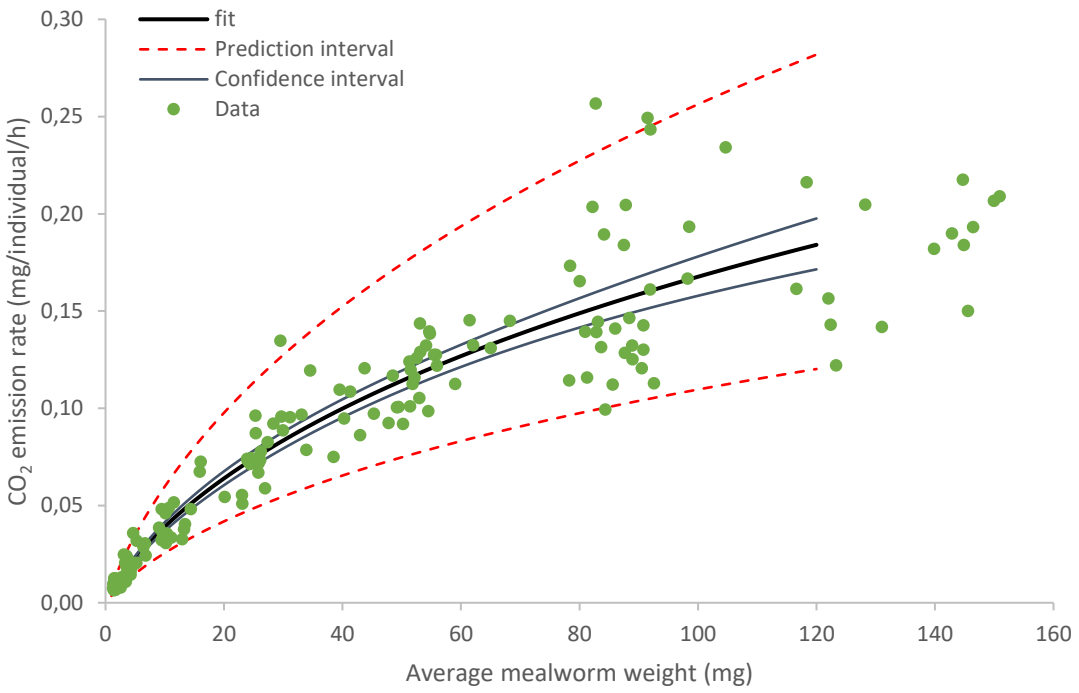


Figure 5: The final model based on Equation 4 and parameters shown in Table 3, including confidence interval and 95% prediction interval.

Although mealworms grown on different diets did not show significant differences in CO_2 emission rates, different types of feed can still lead to differences in total CO_2 produced throughout the entirety of the rearing process. This is more easily explained by giving an example. The model established above tells us that a mealworm of for example 10 mg will produce around 0.039 mg of CO_2 in one hour independent of the type of feed used. However, a mealworm grown on a diet characterised by slower growth will take longer to reach its harvestable size. This longer development time will lead to higher cumulative emissions although the rate at which these emissions are produced will be the same.

To calculate the total CO_2 production over the period where mealworms grow from their first instar to harvestable size, the emission rate model has to be combined with the growth model. Six growth models were selected from a historic data pool as shown in Figure 6. Two types of dry feed are compared, wheat bran and Insectus Mealworm Grow. For each dry feed three different types of wet feed were tested. For wheat bran agar gel, cucumber foliage and fermented forced chicory roots were tested. For Insectus agar gel, cucumber foliage and tomatoes were compared.

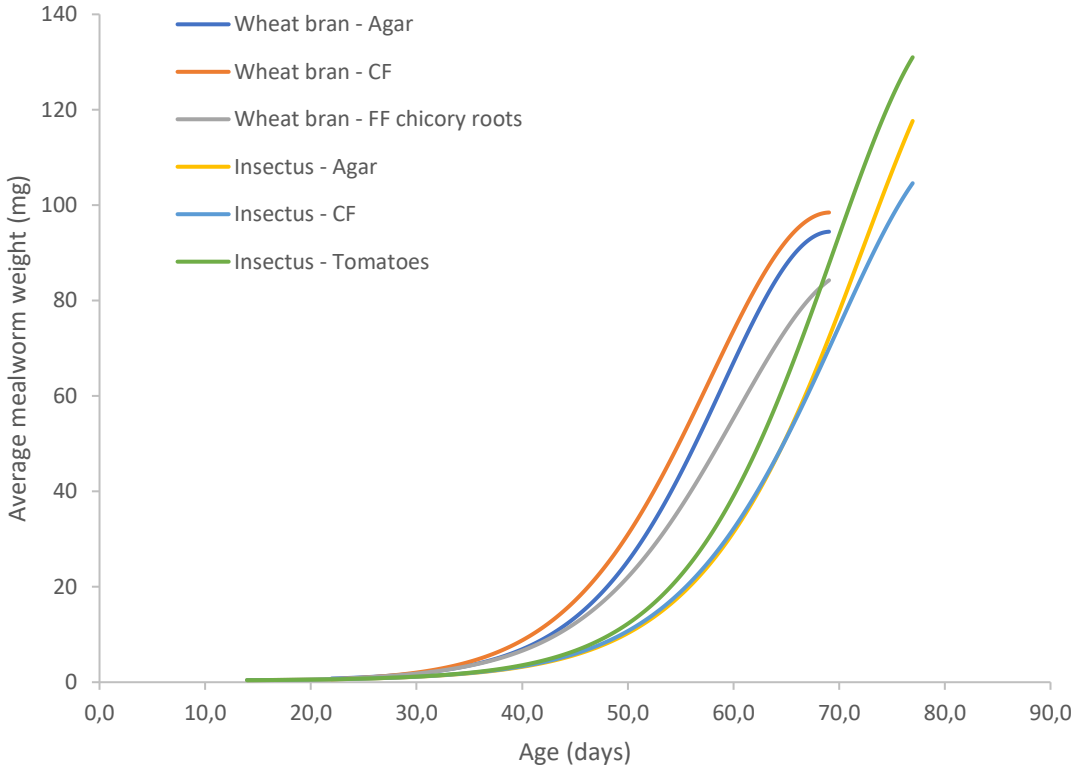


Figure 6: Growth curves for mealworms grown on different diets. "CF" is cucumber foliage, "FF" is fermented forced.

After combining these growth models with the CO_2 emission rate models the CO_2 production curves are established (Figure 7). If we calculate the surface below these curves we end up with the total amount of CO_2 that is produced over the entire production period. These results are shown in Figure 8. In general it can be concluded that the type of feed can affect overall CO_2 production, but in a somewhat unexpected way. Mealworms that were grown on Insectus had the longest development time and it would be expected that they would have the highest overall CO_2 production. On the contrary, wheat bran had around 15% higher total CO_2 production. This can be explained by looking at growth rate. In this trial the mealworms grown on Insectus seemed to have a slow start and they were more or less a week behind in development due to a week delay in starting to supply them with wet feed. However, at this point their very small size lead to negligible amounts of CO_2 produced. When exponential growth finally kicked in they grew from 10 mg to 100 mg in 23 days compared to 38 days when they grew on wheat bran. This slower growth rate ultimately lead to higher amounts of total CO_2 production. Finally the average weight at harvest is another important parameter as it determines how many mealworms that are needed for 1 kg of live harvest. The fewer mealworms are needed, the lower the emission rate will be as is seen in the difference between mealworms grown on fermented forced chicory roots and agar gel with a 135 g difference in CO_2 produced.

The total CO_2 production results are quite similar with what was found during the literature search where Oonincx et al. (2010) reported CO_2 emissions of 1031 ± 349 g per kg of mass gain for mealworms. The difference between this study and the reported values in literature are 21.5% with overlapping error bars. The difference seems minimal and could be explained as Oonincx only looked at mealworms in their 5th instar, although it is not stated what the average weight of the mealworms was, it is likely they were still quite small as mealworms

can have up to 17 instars (Park et al., 2014). Smaller mealworms have higher CO_2 emission rates proportional to their body size than older instars. As a consequence an overestimation by Oonincx et al. (2010) is likely.

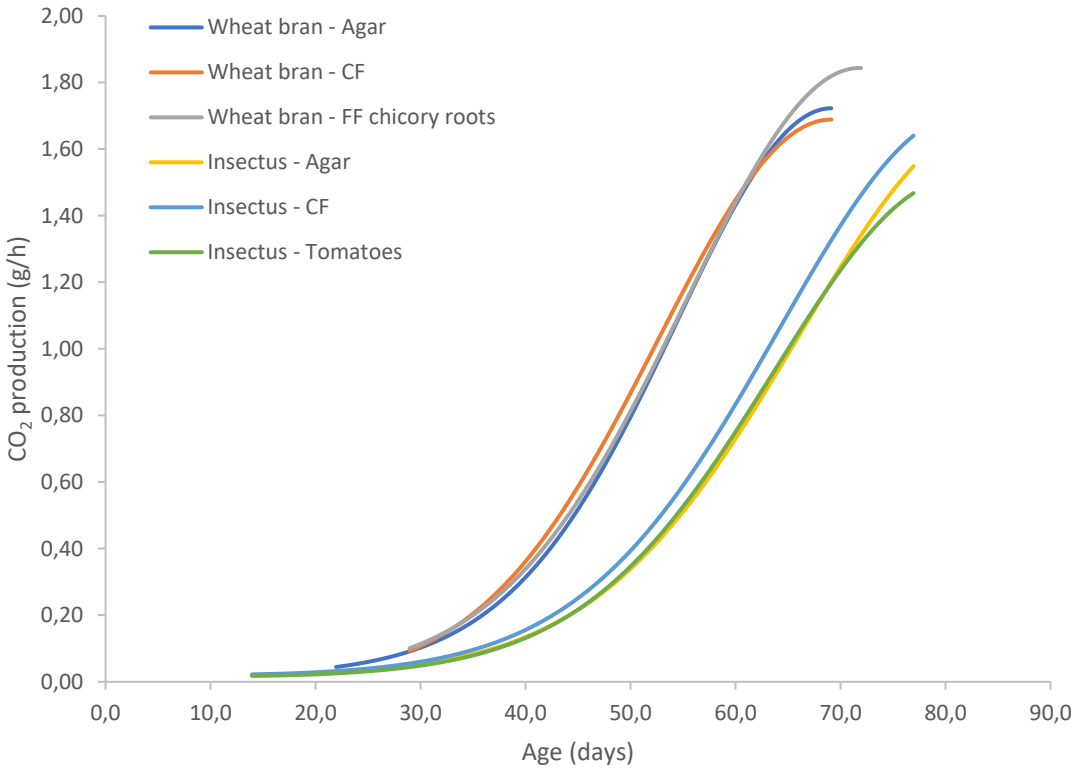


Figure 7: CO_2 production rate per hour for growing mealworms that will yield 1 kg of live mealworms at harvest. "CF" is cucumber foliage, "FF" is fermented forced.

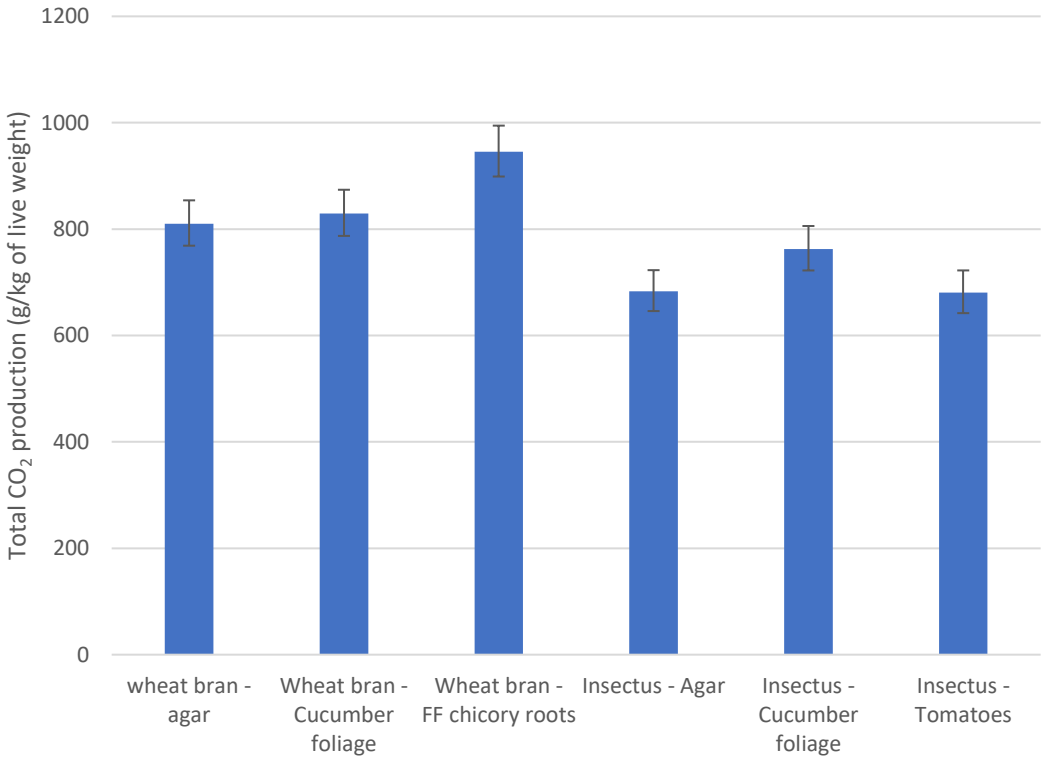


Figure 8: cumulative CO_2 production of mealworms from hatching until harvest for mealworms grown on different diets. "FF" is fermented forced. The error bars shown on the graphs are the 95% confidence interval.

Measurements in the flowthrough chamber were done on the mealworms grown on the control diet which was wheat bran as a dry feed and agar-agar as a wet feed. The growth of the mealworms was monitored at regular intervals for which the crates needed to be removed from the flowthrough chamber. This event was combined with a measurement in the accumulation chamber. The results of these measurements are shown in Figure 9. This graph shows the flowthrough data, the estimated polynomial on this data, the measurements of the same crates in the accumulation chamber and the accumulation model. The comparison of the flowthrough and the accumulation data shows a similar trend with a gradual increase which ultimately leads to a peak CO_2 production rate at day 64 and a steep decline afterwards. The majority of the accumulation data points are below the curve of the flowthrough model which already suggests a systematic difference between both measuring techniques. This difference is even more obvious between both models. The flowthrough model gives an overall higher measured CO_2 production compared to the accumulation model. If we calculate the total amount of CO_2 produced for 1 kg of mealworms, the flowthrough model gives 1129 g of CO_2 produced while the accumulation model estimates it between 769 and 854 g (95% confidence interval).

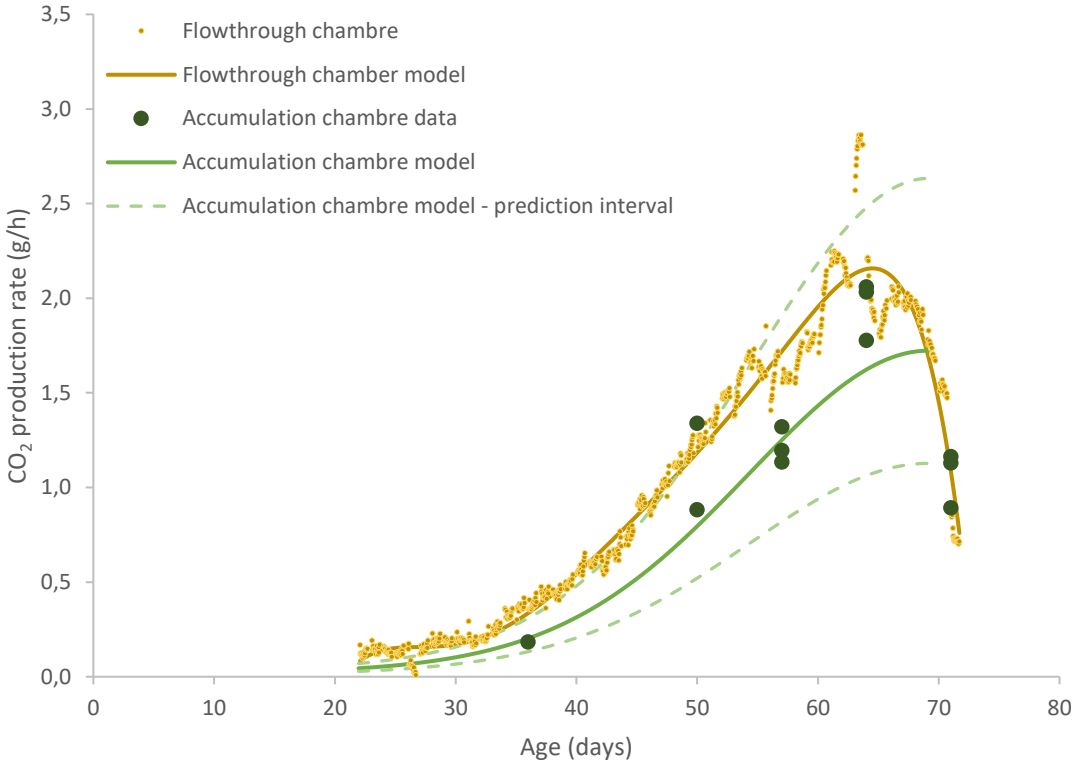


Figure 9: CO_2 production rate per hour for growing mealworms that will yield 1 kg of live mealworms at harvest measured with the flowthrough chamber and the accumulation chamber.

It is not possible to determine which technique is more accurate. The accumulation model has at least one issue as it assumes a 100% survival rate. Meaning that CO_2 emissions from all the mealworms that have died are not taken into account although these emissions have been produced. The real mortality rate is unknown at this stage. However, no significant difference between estimated amount of mealworms between day 22 and at day 69 was observed. This leads to the conclusion that the accumulation model is likely to be favored over the flowthrough model as it is the result of numerous independent measurements on different types of dry and wet feed. While the flowthrough model is the result of only one measurement period of three rearing crates simultaneously. Moreover, the accuracy of the flowthrough chamber is heavily dependent on how accurate the air flow rate can be measured. A recalibration of the system was already done but due to the complexity of fluid dynamics and how the air flows through the system it is hard to exclude that the current estimated air flow rate is not off by a certain factor. For future experiments the flowthrough chamber should undergo a new calibration. A suitable method could be adding a known amount of a marker gas. This could be done by adding a known amount of an ammonia solution with a known concentration which is left to evaporate inside the chamber. Ammonia could then be detected in the outlet air. The total amount of detected amount could then be corrected as the original amount dissolved in the solution is known.

Particulate matter

In none of the measurements an increase in particulate matter was observed as shown in Figure 10 which combines all timeseries data. In general the particulate matter concentration in de accumulation chamber gradually decreased despite the fact that ventilators in the accumulation chamber continuously mixed the air. This leads to the conclusions that

mealworms that live in a crate produce no net amount of particulate matter. The ambient particulate matter can settle as soon as the accumulation is sealed.

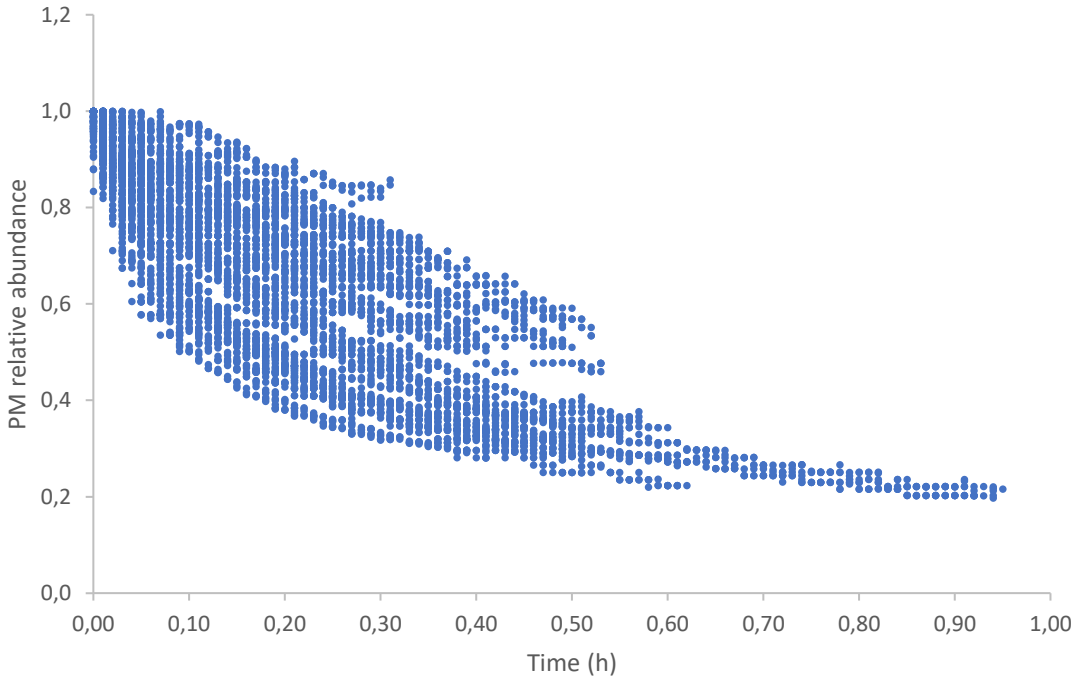


Figure 10: Relative evolution of the particulate matter (PM2.5) in the air over time.

Other emission

The most reliable data was collected on the CO_2 production of the insects. During none of the trials ammonia was detected and it was not practically feasible to test for other gasses such as methane and nitrous oxide due to the need for an analytical method which would require an external laboratory and the associated practical issues such as required air volumes, frequency of sampling and transporting conditions.

2.2.2 Cricket (*Acheta domesticus*) emissions

Carbon dioxide

The CO_2 production rate was determined for *Acheta domesticus* in a similar way as was done for mealworms. However, as the type of diet did not seem to affect the emission rate in *T. molitor*, measurements focused on one type of feed being a chicken starter feed and agar gel as a source of water. The results of these measurements are shown in Figure 11 and as reference the mealworm data was added to the graph. As shown both species seem to have a similar CO_2 emission rate pattern, at least on a log transformed scale.

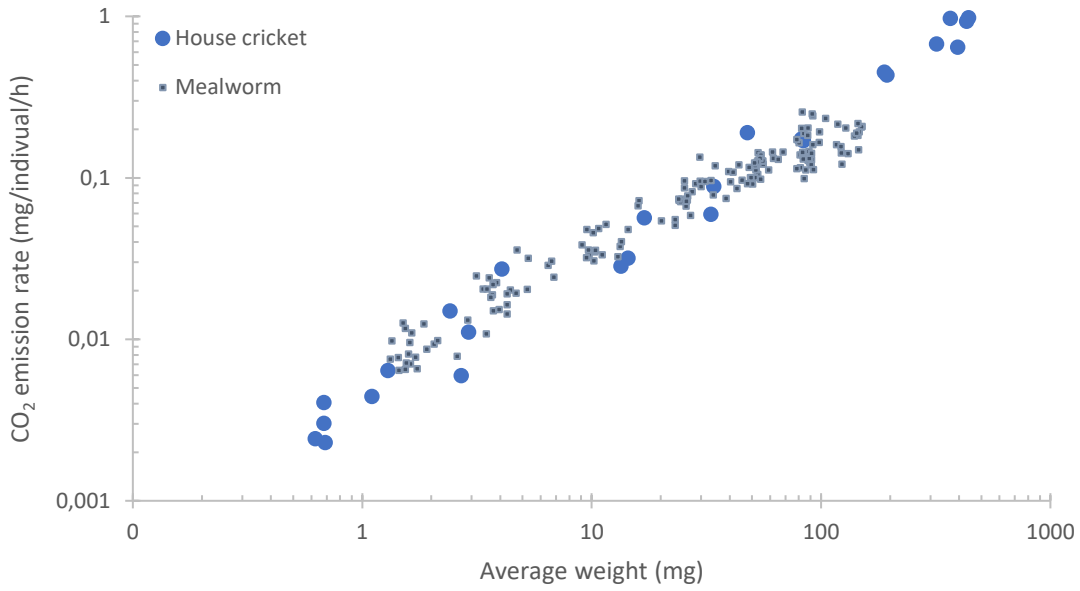


Figure 11: CO_2 emissions rate per hour for house crickets in function of its average individual weight (Avg (mg))..

The equation that we can fit on this data is shown in Equation 5 and differs of the CO_2 production rate curve of that of mealworms as the second order term is absent. The estimated coefficients are shown in Table 4 and is visualised in Figure 12. As crickets are a hemimetabolous species, measurements are not limited to their nymphal stage (as with the mealworms) and continued well into their adulthood. This resulted in a higher weight range that was tested for their emission rate. The model explains 98% of the observed variance.

Equation 5: The CO_2 production rate (mg/h) by a house cricket, "Avg" is the average weight of an individual cricket (mg).

$$\log_{10}(CO_2) = Intercept + \log_{10}(Avg)$$

Table 4: Coefficient estimates of the linear regression, the Standard Error (SE), the t-value and its according probability (R^2 : 0.98, p-value: <2.2E-16).

	Estimate	**SE**	**t-value**	**p-value**
Intercept	-2.37	0.040	-59.4	<2E-16
Log(Avg)	0.88	0.025	35.1	<2E-16

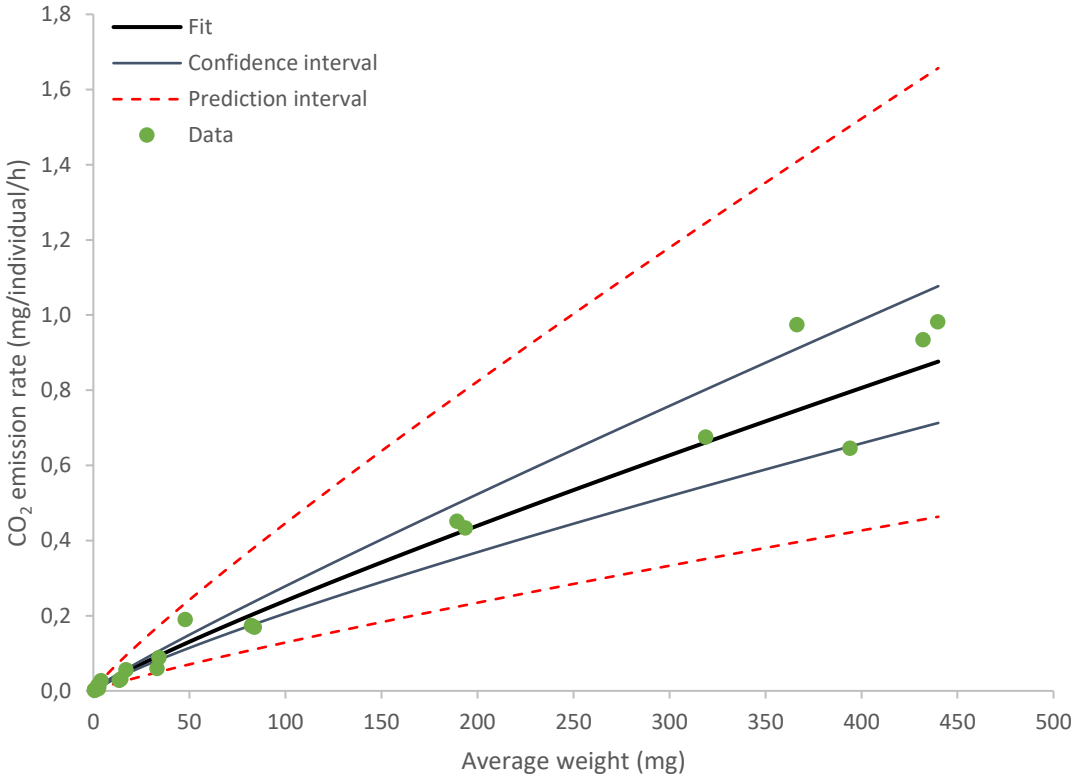


Figure 12: The final model based on including confidence interval and 95% prediction interval.

Now it is possible to perform similar calculations as done for the mealworms where we combined different growth curves with the CO_2 emission rate model. For the growth curves three different dry and wet feeds (Figure 13) were compared. The CO_2 production rate curves for each diet are shown in Figure 14, note that not all curves go all the way to day 0 as young crickets were nursed on control feed (of chicken feed and agar gel) for their first 14 days. It is clear that the diet that yielded the slowest growth (crickets grown on breadcrumbs and agar gel) also has a much higher emission rate curve as 4.5 times more crickets are needed to produce 1 kg in a same time interval. The other diets are all overlapping and do not show obvious differences. Similar conclusions can be drawn when looking at the total estimated CO_2 production Figure 15 with crickets grown on standard feed producing a total of 453 g of CO_2. One important remark should still be considered, this model assumes a perfect survival and mortality rates encountered in cricket rearing can be considerable (up to 50%).

According to results found in literature house crickets produce 1468 ± 971 g of CO_2 per kg of weight gain (Oonincx et al., 2010). The results reported in this study are quite different although the error bars are quite large and there is still some overlap with the results reported in this study. One probable explanation is that the current study does not take mortality into account, which means that the reported values in this study would increase. It seems unlikely that this would be with a factor of three, which implies only 33% survival and that all of the deceased crickets would have died right before harvest so that they could have contributed to the CO_2 emissions but could not be harvested. This seems unlikely and suggests other unidentified reasons for systematic differences between both studies.

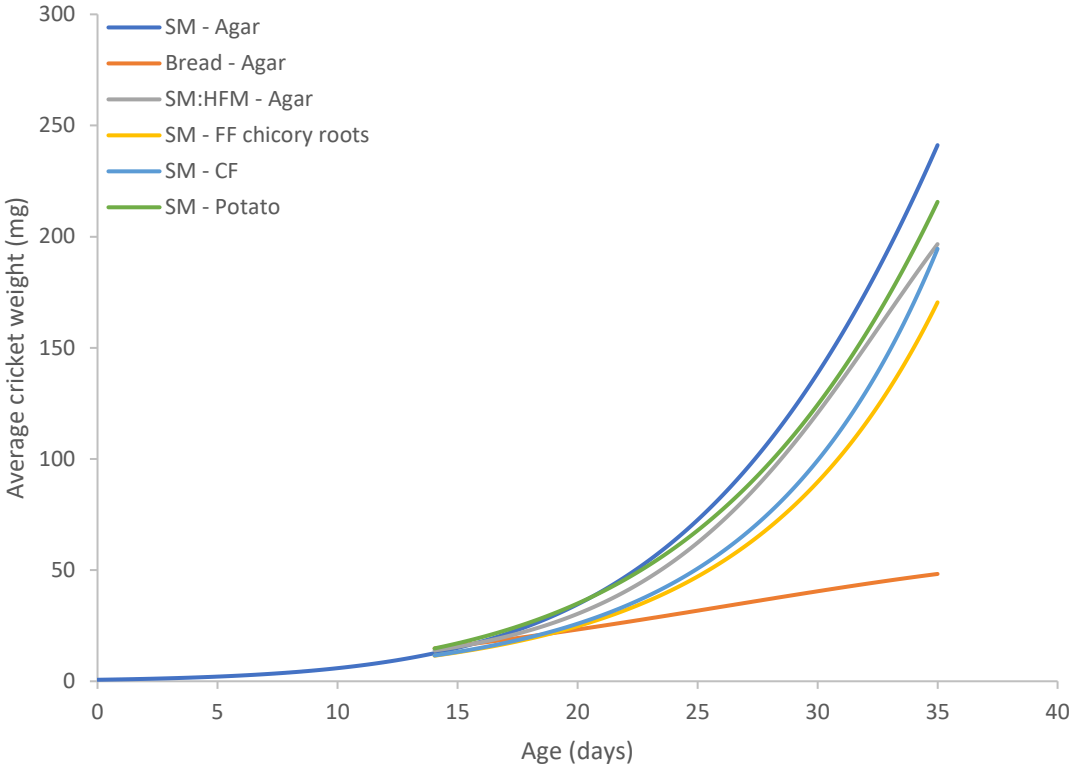


Figure 13: Growth curves for house crickets grown on different diets. "SM" is chicken feed starter mash, "HFM" is hydrolysed feather meal, "CF" is cucumber foliage, "FF" is fermented forced.

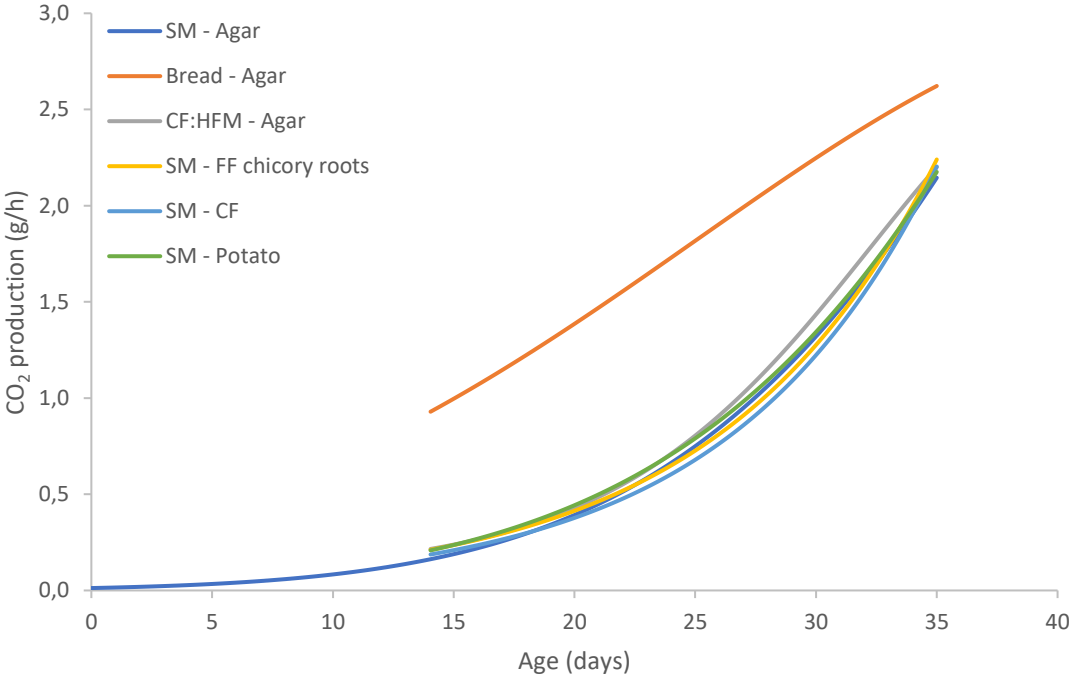


Figure 14: The CO_2 production rate per hour for growing crickets on different diets that will yield 1 kg of live crickets at harvest. "SM" is chicken feed starter mash, "HFM" is hydrolysed feather meal, "CF" is cucumber foliage, "FF" is fermented forced.

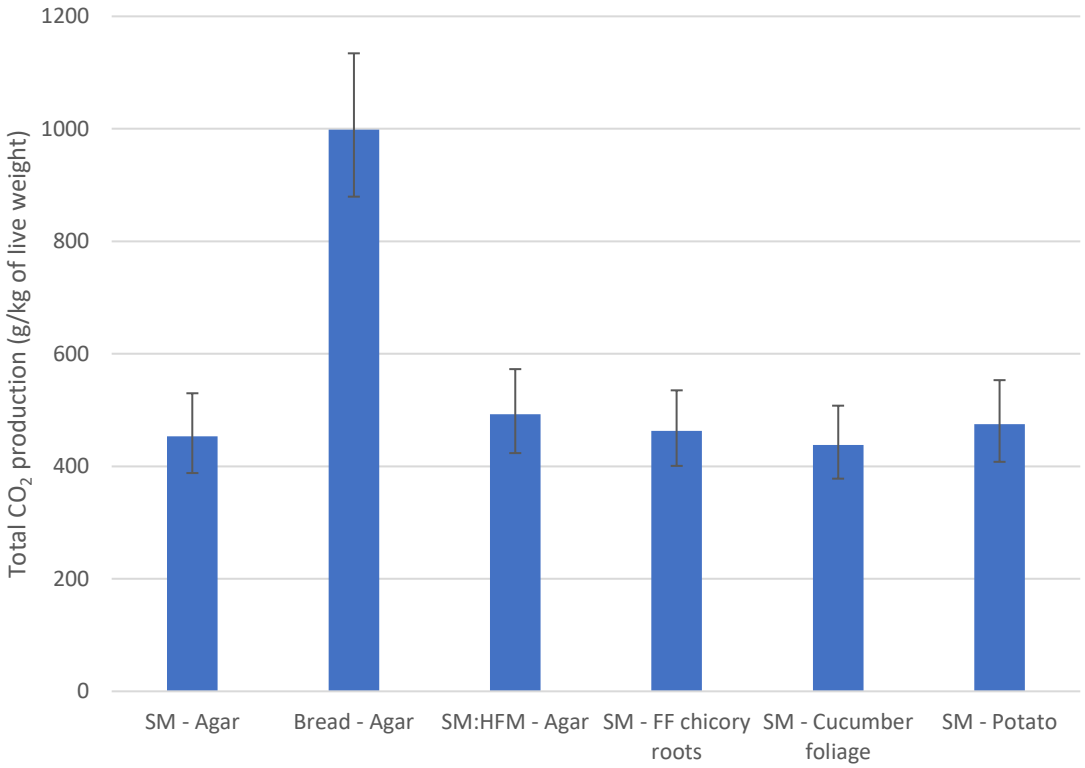


Figure 15: cumulative CO_2 production of house crickets from hatching until harvest for crickets grown on different diets represented as "dry feed component"-"wet feed component". "FF" is fermented forced. The error bars shown on the graphs are the 95% confidence interval.

Particulate matter

Particulate matter was below the detection limit of the sensors during the trials and never increased above the detection limit inside the accumulation chamber. This leads to the conclusion that no net amount of particulate matter is produced during cricket rearing.

Other emissions

The most reliable data was collected on the CO2 production of the insects. During none of the trials ammonia was detected and it was not practically feasible to test for other gasses such as methane and nitrous oxide due to the need for an analytical method which would require an external laboratory and the associated practical issues such as required air volumes, frequency of sampling and transporting conditions.

2.3 Conclusions

Emissions (CO_2, ammonia and particulate matter) were determined for mealworms and house crickets. Net ammonia and particulate matter emissions were not observed within the measuring range. However, two CO_2 emission rate models were successfully constructed for both species. Up to 40 mg mealworms and house crickets have very similar CO_2 emission rates. From then on the rate at which CO_2 is emitted increases almost linearly with an increasing average weight of the crickets but slows down for mealworms (Figure 16). These models estimate the total amount of respiratory CO_2 to produce one kg of live insects on control feed between 769 and 854 g (95% CI) for mealworms and between 388 and 530 g for

house crickets. More efficient feeds (with high growth rates and high end weights) lead to a reduction in overall CO_2 production. This study also provides information on CO_2 emission rates throughout the life cycle of 1 kg of potential harvest which is maximal for mealworms at around 1.8 g/h and 2.2 g/h for house crickets. This information can be used to further optimize ventilation systems and adapt air refreshment rates in accordance with what the insects really need. As such heat loss due to unnecessary ventilation can be prevented.

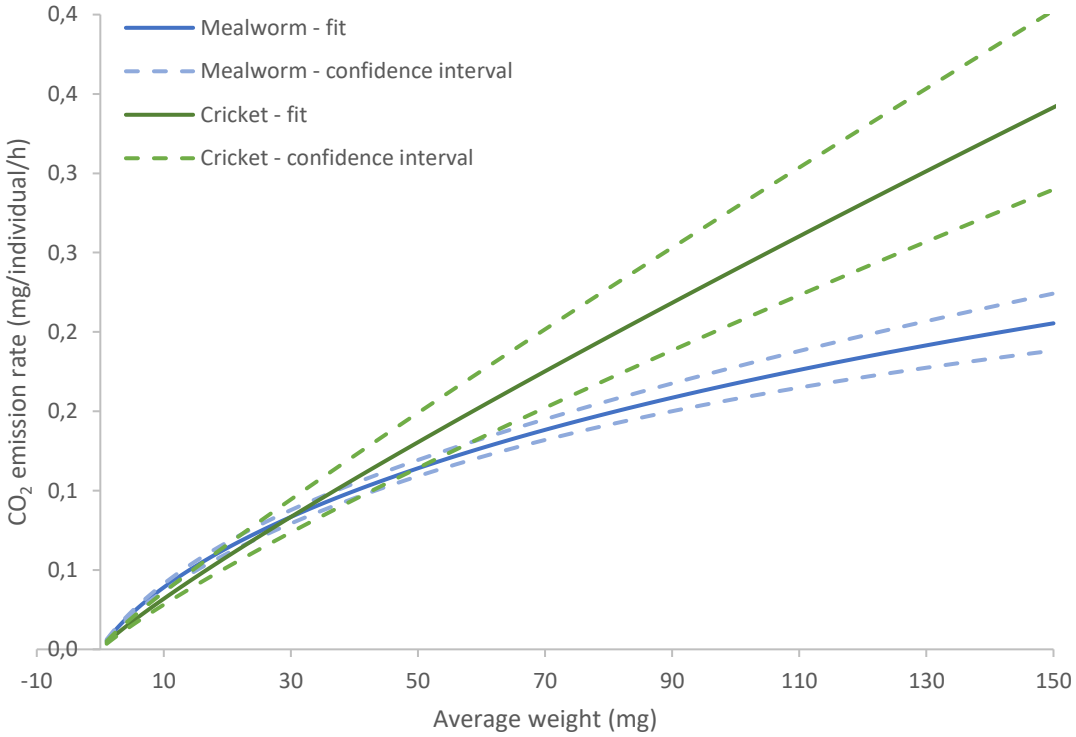


Figure 16: The emission rate models for mealworms and house crickets including confidence intervals.

3 Measuring techniques

To measure the emission of greenhouse gasses by the insect larvae a few concepts were designed by the GreenTechLab. We proposed four concepts, these are mainly different in the size of the group of insects that is placed in the measurement setup. During the meeting that was held with the partners, the concepts were presented and, based on feedback given during the meeting, a further elaboration was made of the concept that received the most positive response.

3.1 Concept generation

3.1.1 Petri concept

In this concept there is chosen for the following solutions for the challenges within this project:

- Measuring method: laboratorial gas sensing.
- Climate control: Yes.

In this concept the insects will be stored in a petri dish. Because the petri dish will not be ventilated there will only be one mealworm in the petri dish to not exceed the critical oxygen level to quick. A small hole will be made in the petri dish to extract the air by means of a syringe. This subtraction of gas will take place after a period of time after which the oxygen percentage of air will reach a critical point in which the insects might not survive. Next, the sample will be sent to one of the partners who will find out the concentration of gases. This partner is the Aberystwyth university in Scotland. It is possible to place this petri dish into a climate chamber to have the exact same climate for the one insect as for the whole group of insects which are going to be reared. A drawing of this concept is shown in figure 1.1.

Figure 1.1 Drawing of the petri dish with the syringe.

3.1.2 Lunchbox concept

In this concept there is chosen for the following solutions for the challenges within this project:

- Measuring method: laboratorial gas sensing.
- Climate control: Yes.

In this concept the optimal amount of insects will be around 7 mealworms. The subtraction of gas will take place after around 1/ 1.5 hours. Next, the sample will be sent to one of the

partners who will find out the concentration of gases. This partner is the Aberystwyth university in Scotland. Again it is possible in this concept to place the lunchbox in the climate chamber to mimic the exact conditions in which the insects are going to be reared in the industry. A drawing of this concept is shown in figure 1.2.

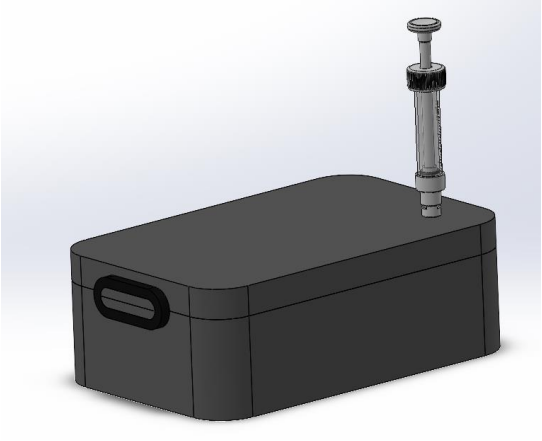


Figure 1.2 Lunchbox with the syringe

3.1.3 Chest concept

In this concept there is chosen for the following solutions for the challenges within this project:

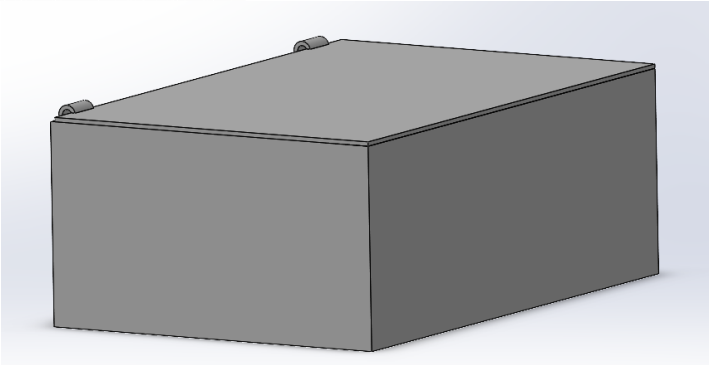
- Measuring method: laboratorial sensing, only oxygen, temperature, carbon dioxide and humidity will be sensed in house.
- Climate control: Yes.

In this concept the mealworms are stored in a chest with the following dimensions: length 60 cm, width 40 cm and height 23,5 cm this chest has a volume of 40 liters. During the measurements the chest will be placed into a measuring crate equipped with CO_2, O_2, temperature and humidity sensors. The desired amount of insects inside the 60 x 40 crate is around 15000 – 20000 mealworms.

During the process a couple steps need to be performed these are:

1. Take the crate with insects out of the climate chamber.
2. Place the crate with insects in the measure chest.
3. Push the green button to start the accumulation process of the gases.
4. When the oxygen level drops to a established value the accumulation is ready.
5. The measurement chest draws a test sample into a sample bag.
6. When this is done the crate with insects can be taken out of the measurement chest and put back in the climate chamber.

Five concepts have been devised of what can be implemented in the measurement chest to let the operator know that the process is finished, and a few automation proposals are made to make the process user friendlier. These are shown in table 1.1



Material

It is important that the measuring crate is not made of plastic because plastic can influence the concentrations of gases in the sample bag. The measuring crate can be made out of a number of different materials. For example plastic, steel, stainless steel or aluminum the different options are displayed in the figures 1.6 up to figure 1.9.

3.1.4 Climate chamber concept

In this concept there is chosen for the following solutions for the challenges within this project:

- Measuring method: Laboratorial gas sensing, oxygen will be measured in house.
- Climate control: Yes.

This concept foresees the use of the climate chamber available in the GreenTechLab. The benefits of this concept is that the climate can be regulated al the time and there are also holes in the climate chamber what means that air exchange is possible. The desired amount of insects in the chamber without air circulation is around 3500 mealworms. The chamber is able to ventilate with a speed up to 12 liter per hour, and at the same time maintain the climate conditions in the chamber. Again the best way would be to place an oxygen sensor in the chamber and when the concentration of oxygen would start to reach a critical value the sample should have to be taken and shortly after the sample is taken and the sample bag is closed the ventilator could blow fresh air into the chamber. Figure 1.5 shows a picture of how the climate chamber looks like.

Figure 1.5 Picture of the climate chamber

3.1.5 The chosen concept: (From minute 9-10-2020)

There are four concepts presented two on a small scale and two on a larger number of insects. There is no positive response to the measurements on a small scale because there are some dynamics in a 60 x 40 crate which you cannot mimic in a petri dish. The concept in which the 60 x 40 crate is placed in the box gives a great way to validate or to get an idea for a certain sensor what the range is in which the sensor need to measure. The time the insects spend in the crate is not very long so it is not a problem that there is no light coming into the box. A remark has been made that the crate should not be made of plastic because it could contaminate the gases and influence the results of the measurements. The climate chamber concept is a great real world breeding validation and also on a full time basis but the small closed chamber can give a nice proof of which sensor can be used during this project during a shorter period of time.

3.2 Development of chest concept

- Photo 1: The control chamber; it contains the PC, the controls for the sensors and the pump, the power supply and all electrical connections.
- Photo 2: The set-up seen from the top with the stainless steel chamber open. In the chamber the sensors are mounted: the particulate matter (PM2.5 and PM10), temperature and humidity, oxygen (O2) and carbon dioxide sensor. On the right side, the PC screen is mounted. The user interface is shown on this screen where the sensor values and the status of the system can be read. On the bottom right are two control buttons. These can be used to operate the set-up without the need for a keyboard or mouse. These are a white and a green button with a white and a green LED.

- We have currently programmed the following signals for the status of the setup:
- Flashing green: Ready to start. When the green button is pressed, the setup moves to the next step.
- Green on: Measurement started and accumulation runs until a certain CO2 value is reached. Then the system moves on to the next step.
- White and green on: Sampling in progress. After a few seconds (filling the sample bag) the setup will proceed to the next step.
- White flashing: The set-up is ready, the sample bag can be replaced and the crate with insects can be taken out of the set-up. By pressing the white button the set-up goes back to step A.

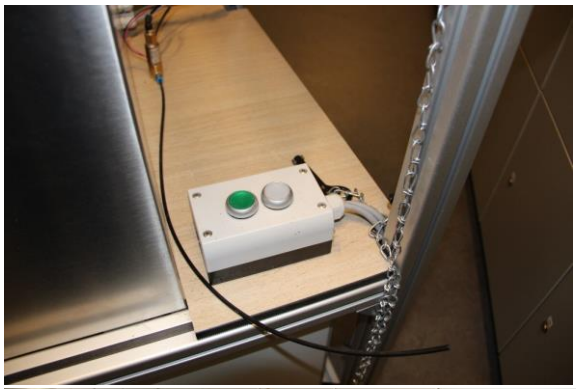
- Photo 3: Rear side of the set-up. In the screen a USB stick can be connected on which the measured sensor data are stored during the accumulation (in an excel sheet format).
- Photo 4: Photo of the complete set-up with the valve closed.




3.3 Feedback after the first tests by Inagro

The accumulation chamber was at Inagro from March 2021 till September of 2021. In this time the accumulation chamber is used to measure the gas emission of mealworms. This report shows the hardware decay during that time.

Outsides:



As can be seen from the pictures the chamber is covered in dust and fly droppings but still functional. Although it can use a thorough cleaning.

Insides:





Some leftovers from the tests are still inside the accumulation chamber but not more than expected. The sensors are still in good shape and seem to measure correct values within their accuracy's. Inside of the control chamber are some dead fly's. One of the cable glands was not fully tightened so they have probably crawled in. Or the door of the chamber could not have been closed properly.

3.4 Improvement areas accumulation chamber

Priority 1: Cameras so the activity can be seen. Lighting: Towards the light red colours or just further in the spectrum NIR. The insects can crawl together in a corner when feeding. BSF also has this normally. Mealworms are more difficult because they are not always on top. As they get older you should be able to see the substrate move.

Priority 2: More forced airflow in the room. This should be frequency controlled so they can experiment with it.

Priority 3: Moisture + temperature control. This would be a nice nice-to-have. Chamber temperature between 25 degrees and 32 degrees.

Extra: CO2 sensor replaced by 0-1% sensor. Supplier questions: can this also go above 1% and then come back up with the values when they go down again?

3.4.1 Adding the camera

The purpose of adding the camera is to see activity in the culture tank (does activity decrease as the CO_2 increases)?

For this purpose, several possibilities have been worked out.

3.4.1.1 *Concept 1: Fisheye lens*

Using a Fisheye lens, almost the entire tank can be brought into focus. The disadvantage is that the outside of the tank can become distorted and out of focus.

3.4.1.2 *Concept 2: Normal lens*

With a normal lens only a small part of the tank is visible but see more details.

With each concept you still have the choice between colour or monochrome (black and white) images. Lighting must also be added. There is a choice between visible light and infrared light.

3.4.1.3 *Concept 3: 4 cameras*

Using four normal cameras, the entire tank can be overseen without the disadvantages of concept 1.

3.4.1.4 *Hardware modifications:*

Drill a hole in the middle of the lid and let the camera lens stick through. That way the camera stays outside the 'dirty' part.

- Camera with infrared lighting 740 nm (does this affect the insects?)
- Small part 'zoomed in' or the whole tank but then you see them individually smaller.

Making a glass plate in the lid is an option for long delivery time cameras.

3.4.1.5 *Selecting the camera + Lens*

The camera and lens must meet various requirements. The requirements are listed below.

- The minimum working height of the camera is 270 mm.
- The maximum working height of the camera is 370 mm.

- The body is divided into 4 quadrants with a camera in each. Each camera has a FOV of 350 by 300 mm (overlap due to the fact that the photos are mounted together).
- The lens has a focus distance of 4 mm.
- The camera has the gigE vision interface to connect to the computer.
- The camera is compatible with Labview from National Instruments.
- The camera is at least 50% sensitive to light in the NIR spectrum (740 nm).
- The maximum price including accessories may not exceed €5000.

Based on these requirements and a discussion with an expert in the field of machine vision. The following camera was selected to be used together with concept 3. Prices are also listed below.

Part:	**Name:**	**Price for concept 3:**	**Characteristics:**
Camera	Basler a2A2590-22gmPRO	€1396	Monochrome, Ethernet connection, PoE possible, 60% efficiency at 740 nm, 5 mp camera
Lens	Basler Lens C125-0418-5M-P	€516	4.0 mm focus distance, suitable for 5 mp camera, suitable for NIR
Network cable	Basler Cable GigE, Cat6a, RJ45 sl hor/RJ45, S, 5 m - Data cable	€32.40	Cat6A cable, can be screwed to the camera.
Attachment	Tripod Mount ace2 - Camera Mount Adapter	€48	For fixing the camera
Confirmation 2	Camera Bracket: 360 / 90 - Mounting	€18	For fixing the camera
Enclosures	Housings to protect the cameras from impact and climate conditions	€80	(Glass, plexiglass, plate) €500
Housing	Housing for electronics	€100	
PoE Switch	NETGEAR GS108LP-100EUS Network switch RJ45 8 ports PoE function Gigabit Ethernet Unmanaged PoE/PoE+ switches	€86.78	
LED	LED Strip 5050 60 LED/m Infrared 740nm - per 50cm	€13.98	Lighting for the accumulation room. https://www.led-tech.de/de/lineare-led-module
PWM controller	NI-9472 783907-01	€128.00	PWM controller for LED lighting (also for the FAN).
Total:		€2419.16 ex	€2927.18 inc VAT

Cameras can be requested from DVC. Contact: Pascal Schoenmakers <Pascal@machinevision.nl>

3.5 Cooling and dehumidifying

Currently the temperature and humidity rises when the accumulation chamber is used. To prevent this, cooling can be applied. This cooling can be done by means of Peltier cooling. This peltier cooling is controlled by the TLK33 PID controller. This controller can be set manually or by means of a 0 to 10 v control signal. This control signal is sent by the compact rio controller.

The removal of the excess moisture from the accumulation chamber is also done by means of a Peltier cooling system. The control for this is implemented on the controller itself. Humidification is not possible with this setup.

Part:	**Name:**	**Price:**	**Remark:**
Peltier cooler	CA-040-AA-12	€246.00 (+€50 for IP65)	Peltier cooling to limit the temperature in the chamber. The capacity needs to be calculated.
PID controller	TLK33	€166.50	Can control peltier element for cooling and heating.
Peltier cooler (dehumidification)	CA-040-AA-12		Only a fan on the outside
Temperature sensor	VE122800 PT100	€20.00	
24v 200 watt power supply			
Total:		€432 ex	€522.72 inc

The calculation of the required cooling capacity is made in consultation with the supplier. In the first instance, the 40 watt cooling capacity is more than sufficient for 250g mealworms. In consultation with Carl of Inagro we will see if this is also sufficient for the other insects in the ValuSect project. Looking for a PID controller with 0 to 10 volt input to determine the setpoint. With this the temperature in the accumulation chamber can be controlled by the Labview program and the general controller.

3.6 Adding ventilation

At the moment there are doubts whether the gas composition in the accumulation chamber is a homogeneous mixture or whether certain gases are rising / falling and are therefore not properly picked up by the sensors or the sample bag. Because of this it was decided to add extra internal ventilators to increase the air flow. There is chosen for multiple water resistant fans that can be switched on / off in the program but the speed is set by means of a setpoint in the control box. See the table below for the detailed concept. https://www.elektroshop.nl/din-rail-led-dimmer-driver-0-10v-12-24v-25w-constant-voltage-dimbaar.html

Part:	**Name:**	**Price:**	**Remark:**
Fan	978-9WPA0624P4G001 (mouser)	€129.64	Fan for air distribution. 60 x 60

			mm, 53 m3/h, IP68, PWM
Setting the setpoint for the fan	TLK339PC8666X-S001	€230	PWM controller for the fans
Total:		€435.16	€526.55

3.7 Total

The total comes out to:

Part:	**Price inc:**	**Remark:**
Cameras	€2927.18	
Temperature control	€522.72	
Ventilation	€526.55	
Small parts	€400.00	Small parts such as, control box, terminals, connection cable, power supplies etc..
10% unexpected	€440	Unforeseen costs such as shipping costs, order costs, extra service
Total:	€4815	VAT inc.

4 References

Oonincx, D. G., Van Itterbeeck, J., Heetkamp, M. J., Van Den Brand, H., Van Loon, J. J., & Van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PloS one, 5(12), e14445.

Park, J. B., Choi, W. H., Kim, S. H., Jin, H. J., Han, Y. S., Lee, Y. S., & Kim, N. J. (2014). Developmental characteristics of Tenebrio molitor larvae (Coleoptera: Tenebrionidae) in different instars. International journal of industrial entomology, 28(1), 5-9.