Interreg
2 Seas Mers Zeeën
European Regional Development Fund

BioBoost
Accelerating biobased horticulture

Insect Breeding

Lab scale and pilot scale experiments with mealworm and black soldier fly

(WP4)

December 2019

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

NIAB

vives hogeschool

AgriGrub

BioBoost – Insect Breeding

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Disclaimer

This report is a summary of the experiments carried out in the framework of the BioBoost project. This project has received funding from the Interreg 2 Seas programme 2014-2020 co-funded by the European Regional Development Fund under subsidy contract No 2S01-038, the province of West Flanders and the province of South Holland.

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1. Summary

BioBoost addresses the need for an environmentally friendly and resource efficient economy focusing on horticulture, using plant residues in a more sustainable, efficient and integrated manner. There is much potential for these residues in different sectors, however in this report we will be focussing on insects as a promising way of converting these horticultural residues into valuable insect protein, oil and derived products.

Insects are a class of arthropods and comprise millions of insect species. Two insect species were selected with the presumed highest economic potential and ability to process residues, the yellow mealworm (*Tenebrio molitor*) and the black soldier fly (*Hermetia illucens*). At the start of the project little (public) knowledge was available on how to rear one of these species on an industrial scale, their nutritional needs or how to process residues with them. To fill this knowledge gap, three project partners established dedicated insect facilities to conduct research about the topics mentioned.

In Belgium, Vives developed a protocol to test plant residues in the lab, paying not only attention to the performance and composition of the larvae, but also to the nutritional composition of the residues. Using this protocol multiple residues were assessed, however, the diversity of these plant residues is very big and a vast number of combinations are possible. Therefore, in order to select interesting residues and mixtures, experiments assessing the nutritional requirements of the larvae were conducted. Artificial diets were used in order to facilitate a baseline for diet formulations based on horticultural residues. The research showed that a diet with 30 % dry matter of which 15 % protein, 40 % non-fibre carbohydrates and 2 % fats, should be sufficient.

Meanwhile Inagro developed a way to rear mealworm and black soldier fly on a pilot scale. Their experiments show the potential of residues as a source of moisture for mealworm. In addition, the nutritional composition of various horticultural residues was determined at Vives. Based on Vives' recommendations for black soldier fly larvae, Inagro formulated multiple diets combining horticultural residues with by-products common in pig production, as demonstrated in two case studies.

The duplication of these activities at the two sites was vital to consider the potential for identifying different waste or co-product utilisation at different sites and under the environmental and legislative background in different states within the EU Interreg2Seas region.

The NIAB site chose the option of working directly with new start-up companies that were keen to try this system of waste valorisation. Initially, Entomics Ltd was working closely with NIAB to design and build a working facility and to identify waste feedstock's. An early decision was to source a range of waste fresh produce from the local supermarket Sainsbury. This enabled the group to trial a very broad range of feedstock's and the material was provided free (which was otherwise mainly landfilled). By mid-2018, Entomics ltd had grown and matured and split into two companies; the original parent decided to concentrate on high-end products. A new company was born; AGriGrub, that focused on the original plan to use waste food and co-products. AgriGrub was connected to companies and farmers until a good fit was found in terms of waste material direct from producers (rather than retail).

Apart from feedstocks some initial trails were performed on the use of insect manure (frass). Inagro analysed the nitrogen and phosphorous content of different frass samples, while AgriGrub performed bio repellent trials with frass of black soldier fly larvae as a bio control agent for aphids in cabbage.

All the research within BioBoost confirms the feasibility of (partial) implementation of horticultural residues as feedstock for the insect industry. Thereby contributing to a circular economy.

2. General introduction

2.1. The Problem

2.1.1. Animal Feed Supply Chains

The FAO estimates that food production needs to increase by 70% by 2050 to feed the world's growing population, with a big focus on higher quality protein diets in developing parts of the world. This is putting pressure on the sustainability credentials of the future animal feed supply chain, given that up to 7% of all greenhouse gases can be attributed to the growing of crops for animal feed.

The animal feed markets for aquaculture and poultry represent two of the most important growth sectors:

1. For aquaculture, there is increasing concern around the sustainability of farmed salmon feed supply chains, with a broad industry trend of moving away from fishmeal as a source of protein and Omega-3 oils. Fishmeal is currently produced using wild-caught forage fish such as anchovies in Chile and Peru, but due to increased demand and strict catch quotas designed to address overfishing, fishmeal prices have risen to £1,500 per tonne – four times as expensive as cheaper plant-based proteins. However, plant proteins such as soy are deficient in key amino acids needed for carnivorous fish diets, and contain anti-nutritional factors that limit digestibility and increase disease risk. The industry needs a sustainable, high quality feed alternative.
2. The poultry feed market is also looking for sustainable, high quality feed alternatives for two main reasons:
    a. Soy – the primary protein in poultry diets – is a resource-intensive crop that has been closely linked with deforestation, and there is ever increasing pressure from customers and supermarkets to move away from Soy. As a concrete example, Waitrose has already banned soy from its dairy supply chain in recent years for similar reasons.
    b. Health & Welfare – there is a growing recognition that bird health & welfare isn't being addressed through current soy-dominated diets, and there is an opportunity for natural feeds to target gut microbiome balance, immune system health and feather pecking mitigation

2.1.2. Food Waste

Simultaneously, 1/3 of all food produced globally is wasted. This represents £500 billion in lost value each year, and leads to 3.3 billion tonnes of carbon emissions. This is forcing a fundamental rethink around the 'food waste loop' and ways we might better utilise the unavoidable fraction of food waste.

This problem extends from all parts of the supply chain, from pre-farm gate to food processing factories, to retailers, to household food waste.

2.1.3. Opportunities

Insects, specifically the Black Soldier Fly (BSF), represent a natural 'food waste conversion engine', and can cheaply and efficiently transform organic waste into complex proteins and fats in their bodies. Black Soldier Fly larvae can reduce food waste volume by up to 95% over a rapid two week growing cycle, and they're found throughout the world, so a potentially global solution.

As the insect larvae eat the food waste, they efficiently synthesise and concentrate the low- value biomass into much more chemically complex and valuable compounds such as proteins and fat – an ideal feed source for farmed salmon and poultry.

Therefore, insects that can be fed on food waste, and with a resulting tiny carbon footprint, represent a massive opportunity for an animal feed industry that is desperate for new sources of high-quality, sustainable feed alternatives.

2.2. Legal aspects

2.2.1. Application in animal feed

Theoretically insects can be used as feed for various animals. At the moment in Europe, however, insects are considered livestock and can therefore not be used in feed for other livestock. As for December 2019 insects can be used in pet food (e.g. reptiles) and fish feed. A recently adopted Regulation (No 2017/893) authorises the use insect proteins originating from seven insect species for the production of fish feed:

- Black Soldier Fly (*Hermetia illucens*)
- Common Housefly (*Musca domestica*)
- Yellow Mealworm (*Tenebrio molitor*)
- Lesser Mealworm (*Alphitobius diaperinus*)
- House Cricket (*Acheta domesticus*)
- Banded Cricket (*Gryllodes sigillatus*)
- Field Cricket (*Gryllus assimilis*)

The fact that insects are livestock has more implications than just the ban on their use in livestock feed, because in that case the proteins are considered processed animal proteins. Livestock can't be fed with processed animal proteins, animal matter, faeces, waste (e.g. restaurant and household waste) etc.. Therefore insects may not be reared on products that are of animal origin.

There are some exceptions:

- Fishmeal
- Gelatine and collagen from non-ruminants
- Eggs and derived products
- Milk and derived products
- Honey

These regulations provide for the above restrictions, but also offer opportunities. At this moment we are not allowed to use 'processed animal proteins' in animal feed. Consequently, unprocessed insects (alive) and other fractions (e.g. fats) can be used as animal feed if we respect the restrictions on feed for the insects themselves. So for example the purified fats of insects grown on plant substrates may be used as feed and the larvae may be fed alive to non-ruminants.

Regulations may change in time, based on scientific research. It is to be expected that processed insects will be allowed in chicken feed in the near future.

2.2.2. Human food products

In addition to the 'general food hygiene requirements', the production and marketing of insects as food in Europe is governed by the so-called 'Novel Foods' legislation – i.e. Regulation (EU) No 2015/2283: this legislation applies to all categories of foods that 'were not used for human consumption to a significant degree' within the European Union before 15 May 1997, which is the case of insects, since January 2018. The use of insects in human food is therefore not allowed until approval of suchlike novel food dossier about that insect within the European Union. Several Novel Food dossiers about several insect species were provided to the EU. Approval of a dossier will allow the use of that certain insects species, reared and processed according to the described banner within the dossier, for human consumption in the EU. Until approval or disapproval of those several dossiers most of the EU nations don't allow human food with insects as an ingredient. Belgium is an exception: Prior to January 2018 the commercialisation of 10 insect species was allowed in Belgium. Pending a decision on admission, the species for which a novel dossier was submitted are currently still tolerated. There are other EU countries who tolerate products with insects until clarity on the dossiers, which in spring 2020. Switzerland is not a member of the EU and already allows the use of insects for human consumption.

2.2.3. Insect frass

Insects are considered livestock and therefore they produce manure. But as many insects species live in there feed substrate (e.g. yellow mealworm), the substrate becomes a mixture of feed, 'manure', exuviae and dead insects. The composition, the proportions of these fractions, depends on the used rearing techniques, such as amount and frequency of feeding, used separation techniques, etc.

In Belgium this substrate, the frass, is considered waste and not manure. This means on the one hand that no account has to be taken of regulations concerning emissions, storage, transport etc. On the other hand this means that the substrate is to be treated as waste. For safety reasons (e.g. bacteria populations) a high temperature (1h at 70°C) treatment is necessary. Incinerators, composting plants and biogas reactors with the right permits can also use the frass.

In the UK frass is classed as a manure, not waste, and therefore can be treated differently. Frass need not necessarily be pasteurised, although generally is pasteurised due to concerns about plant pathogen transmission, and to create a shelf stable product. While in Belgium previous companies applying frass to fields have been shut down, in the UK DEFRA takes a more open minded approach. It is up to producers (rather than regulators) to show that any disease risks have been adequately considered and appropriate action has been taken.

The frass of some insects has already been analysed and could be very useful for the production of fertilizers and growth enhancers. But the necessary heat treatment makes it economically less interesting because of the costs. On top of that the temperature will change the composition of the frass. More research on this topic is needed.

3. Selected insect species

Several insect species were taken into consideration for implementation in the BioBoost project (Table 1).

Table 1: Insects considered to be implemented in the BioBoost project.

Name	Latin name	Petfood	Feed	Novel food submitted	Growth rate	Processing residues	Separation of the rearing substrate	Low plague hazard	Remarks
Black soldier fly	*Hermetia illucens*	✓	✓	✓	✓	✓	✓	✓	
Common housefly	*Musca domesticus*	✓	✓	×	✓	✓	×	×	Flies are hard to control
Yellow mealworm	*Tenebrio molitor*	✓	✓	✓	×	✓	✓	✓	
Lesser mealworm	*Alphitobius diaperinus*	✓	✓	✓	✓	✓	✓	×	Adult beetle can fly
House cricket	*Acheta domesticus*	✓	✓	✓	✓	×	✓	✓	Highly susceptible to cricket paralysis virus
Banded cricket	*Gryllodes sigillatus*	✓	✓	✓	✓	×	✓	✓	
Field cricket	*Gryllus assimilis*	✓	✓	×	✓	×	✓	✓	
Migratory locust	*Locusta migratoria*	✓	×	✓	✓	×	✓	✓	Demands fresh grass
Dubia roach	*Blaptica dubia*	✓	×	×	×	×	✓	✓	

Based on their economical potential (applications in pet food, feed and food), black soldier fly, yellow mealworm, lesser mealworm, house cricket and banded cricket were still in the running. The BioBoost project specifically required the insects to be able to process horticultural residues, which limits the options to black soldier fly and the two mealworm species.

Overall, black soldier fly is a clear go. Strong arguments can be made for both yellow and lesser mealworms. Raising both species from egg to grown larvae is very similar. Growth rate favours the lesser mealworm, which takes only half the time to reach harvestable weight. However even fully grown they are a lot smaller than the yellow mealworm, which might cause more trouble during harvest. Eventually the deciding factor was the risk of pest formation. Lesser mealworm adults (the beetles) are able to fly and are therefore harder to contain, they can also cause damage to infrastructure. Taking these factors into consideration, makes the common mealworm the preferred option of the two for this project.

3.1. Mealworm (*Tenebrio molitor*)

3.1.1. General description

Mealworms are the larvae of the lesser known mealworm beetle. This species belongs to the family of darkling beetles, also containing other commercially reared species such as the lesser mealworm (*Alphitobius diaperinus*). Originally, the habitat of the mealworm was probably limited to southern Europe. Global trade has allowed the species to spread.

The beetle is a smooth, black beetle that measures 1.2 to 2 cm. The insect has active mouthparts and, just like the larvae, feeds on cereals (and derived products), vegetables and fruit. The beetles have wings, but will rarely fly under favourable conditions.

Mealworms are yellowish with a brown stripe at the end of each segment. They feed on the same food sources as the beetles. In addition, mealworms (just like the beetle) exhibit cannibalistic behaviour, often towards the less resilient life stages such as eggs and pupae.

3.1.2. Life cycle

The entire life cycle from egg to egg takes three to four months, but is highly dependent on the environment (temperature and humidity) and the quality of their feed.

Under favourable conditions, the beetles mate a few days after pupation. The females are fertile for a few months and lay a maximum of ten eggs per day. However, the frequency decreases as the beetles get older. Due to cannibalism, the productivity per beetle will decrease with higher numbers of beetles in a crate. The fertilized eggs are laid in a dry substrate or on a hard surface near a potential source of food for the larvae. After a few days the eggs hatch and the larval stage begins. This stage lasts eight weeks under ideal conditions. During this phase they undergo several moulds. At the end of the larval stage the mealworms pupate. The pupal phase lasts six to eighteen days, during which they do not eat or move. A newly pupated mealworm beetle is pale and soft, after a few days they harden and get the typical black colour.

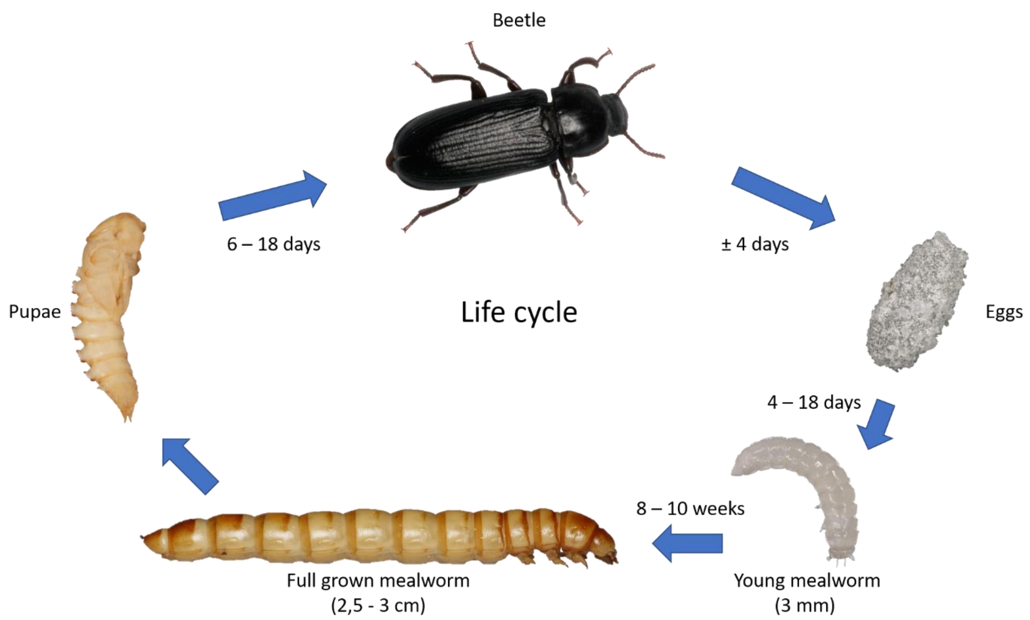

Figure 1: Lifecycle of the yellow mealworm (Tenebrio molitor).

3.1.3. Production process

The short life cycle and the possibility to grow a lot of mealworms in small boxes, makes this insect suitable for industrial production. The full grown larvae are the desired end product.

Both the larvae and the beetles can be kept in open plastic boxes. Using stackable boxes makes it possible to work vertically, resulting in a more efficient use of your production area. An opening between the stacked boxes is necessary to ensure sufficient air movement above the feeding substrate. To make sure the box can contain enough feeding substrate, a box should have a minimum depth of 8 cm.

Mealworms avoid direct light and will abide mostly underneath the surface of their substrate. The beetles lay eggs both in the substrate and on the bottom of the box. For optimal cultivation, it is advisable to move the beetles regularly (e.g. weekly) to a fresh container with fresh substrate so that the eggs and larvae can develop undisturbed by the beetles.

We recommend between 10 and 20,000 mealworm per box (which will result in a harvest of 1 to 2 kg), this can be achieved with 250 g of beetles for 7 days in a box.

Climate

Mealworms are typically reared at a constant temperature between 25 and 28 °C, the relative humidity is kept between 60 and 75 %. They develop the fastest at an ambient temperature of 31 °C. However, this is not recommended because active mealworms produce heat themselves and temperatures above 31 °C are detrimental for growth. The larvae will grow starting at 12 °C, although they will be strongly delayed. Mealworms can also survive for a long time at lower temperatures (5° C), such as when kept in the refrigerator.

A high relative humidity is beneficial for the rapid development of the mealworm. However, at a relative humidity higher than 80 %, problems can arise with the substrate in which the larvae live. The dry substrate can become damp, which increases the chance of mould formation. High humidity can also lead to the development of flour mites, a mite that occurs naturally on grain products. It is therefore often decided to set the relative humidity to a maximum of 75% as a compromise between optimal growth and maintaining hygienic conditions. The mealworms can also be cultivated in less humid conditions, but this has an impact on the development speed.

In densely populated production halls, it is also recommended to monitor the CO_2 concentration. It can quickly increase and therefore it is advisable to ventilate according to the CO_2 concentration.

Feed

The feed for mealworms can be divided into two groups, a dry feed source, such as wheat bran, and a wet feed source (also called a moisture source), such as (rejected) vegetables or fruit. Provide enough dry feed for them to eat for a few weeks.

The moisture source is preferably given on a daily basis. The portion should be consumed entirely by the next feeding in order to avoid mould in the boxes, but should be sufficiently present so that it is always available for the larvae. If there is a shortage of moisture source, the larvae will grow noticeably slower. To harvest 1 kg of mealworms, they will need to have consumed at least 2 kg of moisture source throughout their life.

If you choose wheat bran as a dry source of nutrition, this can be supplemented with other dry (grain) products with a higher nutritional value. This will accelerate the development of the larvae. Currently there are already dry feeds on the market that have been developed specifically for the cultivation of mealworms. The production of 1 kg of fresh mealworm requires 2.1 kg of wheat bran, while they only need 1.6 kg of the commercial mealworm feed.

3.1.4. Applications

The larvae are currently used in various food products for human consumption. They are also very popular as feed for pets and have been allowed to be processed in feed for aquaculture (the farming of fish) since 2017. Dried mealworms contain 48% protein and between 29 and 46% fat.

3.2. Black soldier fly (*Hermetia illucens*)

3.2.1. General description

The black soldier fly or BSF originates in the south of the United States. Since 1920, the species has spread to all (sub)tropical regions including Central and South America, Asia, Australia and Southern Europe.

The adult is a black fly with white legs. The body is narrow and about 2 cm long. The adult stage can only feed to a very limited extent. The insect survives mainly on the fat reserves that build up during the larval phase. The flies have no stinging, chewing or sucking mouthparts. Because of this they don't transmit diseases, contrary to other fly species. They are therefore not considered a vector or plague species in the countries where they occur naturally.

The larvae however are always hungry. They can feed on organic materials such as plant residues, manure and even carcasses. The fast growth of the larvae suppresses the development of other insects that enjoy the same substrate.

3.2.2. Life cycle

It takes 40 days for the life cycle to be completed (egg to egg), but this strongly depends on external variables such as the environment (temperature and humidity) and the feed. Under favourable conditions, flies mate two days after eclosion from the pupa. Two days after mating, the females lay their eggs in small slits or cavities above a suitable feed. An egg cluster contains several hundred eggs.

After 4 days the eggs will hatch and the larval stage begins. The larvae will feed and can double or even triple their weight each day in this stage. After 18 days the first prepupae will appear. In this stage their colour darkens, they stop feeding and they look for a quiet dark place tot pupate. The pupae are immobile and stay this way for approximately two weeks. The pupae enclose and the cycle starts again. Under unfavourable conditions it can take several months to complete the cycle.

3.2.3. Production process

Egg production

The flies are kept in cages. They don't really require feed, but it can be beneficial to supply them with some water as it can have a positive effect on the lifespan of the flies.

Light is required to trigger flies into mating. In greenhouses daylight should be sufficient, in closed rooms that are deprived of daylight, artificial lights are necessary (high kelvin LED's > 6000K). Light intensity and composition will determine the number of successful mating events and therefore the number of eggs.

The oviposition site should be centralised and random egg laying must be prevented. Flies can be lured to the oviposition site with an odour. A good recipe for odourful organic matter is a mixture of dead flies, frass from the larvae and water (other options are possible as well). Proper ventilation is recommended as well. If odour accumulates in the fly room, random egg laying will occur. Eggs can be collected in ribbed cardboard or between stacked wooden or plastic slats.

A tropical climate should be simulated. In general a high relative humidity (80 %) at 30 °C is recommended for fly reproductivity and hatching the eggs.

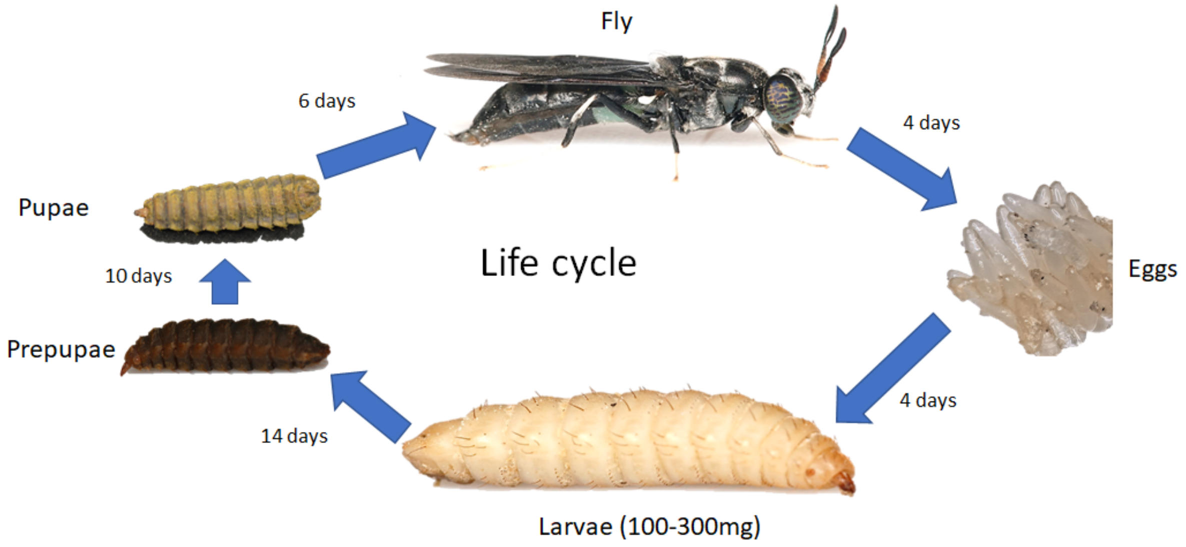

Figure 2: Life cycle of the black soldier fly (Hermetia illucens).

Larval growth

Raising BSF-larvae generally happens in two stages. First there is the nursery of 1 day old larvae for approximately 5 days. Nursery is always done on a high quality feed (e.g. chick starter crumble). Per gram of one day old larvae, 0.5 kg of wet feed is given (70 % water, 30 % starter crumble). All the feed is given at once, but make sure that the layer thickness does not exceed 3 cm. In one box of 60 x 40 cm 300,000 larvae can be nursed.

After these initial five days, the starter feed will be consumed. The larvae now can be raised on their final feed (consisting of residues from horticulture). A way that this can be done is the following. The 5 day old larvae are divided into groups with 20,000 individuals. All the feed is given at once, 10 kg of wet feed (70 % moisture). Other insect breeders might opt for a different feeding regime.

The moisture content of the substrate is very important and will decline throughout the raising process. The combination of larval and microbial activity will make the temperature in the box rise (50 °C is not uncommon) which makes the moisture evaporate. When the substrate has dried, growth will stop and the larvae can be harvested.

3.2.4. Composition

The larvae contain a lot of protein (42 % on dry matter basis), fats (15-49 % on dry matter basis) and calcium (5-8 % on dry matter basis). The exact composition strongly depends on the composition of the diet.

4. Insect lab (NIAB)

4.1. Entomics

In 2016 a new NIAB site at Hasse Fen was developed using local growth funding - and called the Eastern Agri-Tech Innovation Hub - providing an incubator for SME's with innovative ideas for reducing food waste. Entomics, 4 postgraduates from Cambridge discussed their ideas with NIAB for Black Soldier Fly breeding (Figure 3). With support from BioBoost, NIAB built an insect pilot facility which was initially for Entomics. This started off in the tractor shed (the largest building in Figure 4) as a small pilot using a glasshouse as a breeding chamber for the BSF's.

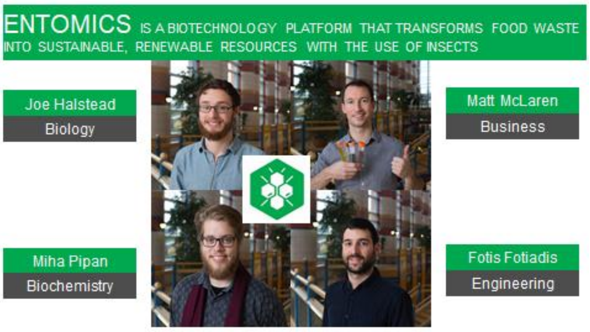

Figure 3: Four founders of Entomics.

Figure 4: Location of the facility.

Then NIAB provided Entomics with the whole of the barn next to the tractor shed to use as a designated insect pilot facility for breeding insects (Figure 5). It shows the barn as it was being done up. This larger area was equipped with a waste food shredding and preparing area, a heated feeding area and a large general area for output processing. NIAB also supplied Entomics with a well-equipped biochemical laboratory for studying BSF breeding outputs as more than 10 different inputs were being evaluated.

Figure 5: Inside Entomics.

4.2. The Birth of AgriGrub

In 2017 Entomics gained Innovate UK funding for the Metamorphosis project targeting aquaculture. In 2018 Entomics splits into two projects Metamorphosis and Genesis (targeting poultry). Also in 2018, three of the original founders of Entomics wanted to proceed in new directions and move to Laboratories at Cambridge University. The remaining founder, Joe Halstead stayed on at the Eastern Agri-Tech Innovation Hub and formed a new company called AgriGrub which is targeting pet food, fish food and Frass for horticultural use. Joe continued to establish assessment of inputs to outputs for the optimum results. Where Entomics have aligned themselves as technology developers for the burgeoning insect production industry, AgriGrub has a focus on commercialisation and increasing production. Over the course of its first year AgriGrub performed significant research into the suitability of available local feedstocks for BSFL as well as researching the properties of BSFL frass.

Figure 6: Inside AgriGrub.

4.3. Feasibility study for novel fruit and vegetable feedstocks for BSFL production

4.3.1. Key points

a) Diet trials were performed to assess the suitability of fruit and vegetable waste from AMT Fruit and G’s, two local waste producers, for processing by Black Soldier Fly Larvae (BSFL) into inputs for pet food and agriculture.
b) Fruit waste performed better than any previous feedstock, yielding 31% more BSFL.
c) Combining fruit with other vegetable feedstocks made these previously unsuitable feedstocks viable.
d) Frass production and nutritional profile did not differ between diets, meaning it is a consistent fertiliser input for organic growing regardless of waste feedstock.
e) Using fruit waste reduced retention times, resulting in 20% higher production of frass and BSFL.
f) BSFL grown on fruit have a higher fat content, are larger and contain more lauric acid and carotenoid pigmentation; nutritionally superior qualities compared to other larvae on the live pet feed market.

The three metrics of interest when performing BSFL diet trials are:

a) Average BSFL yield – wet weight live BSFL as a % of wet waste input.
b) Average frass yield – weight of frass (BSFL droppings and skin casings) as a % of wet waste input.
c) Retention time – number of days BSFL are kept in climate controlled conditions before reaching harvestable weight. This with yield determines the productivity of a plant of given capacity.

This diet project assessed a number of different waste types from AMT Fruit and G’s, against our control feedstock of 20% spent malt (a brewing by-product which soaks up excess water content) and 80% shredded waste potatoes by volume.

The first round of trials looked at combining each feedstock with 20% spent malt by volume to see if the nutritional profile was right to bring BSFL to harvestable size within 28 days. For all new feedstocks except fruit waste this proved not to be successful so through small scale trials a further eight diets were developed for testing at a larger scale.

All were compared to the control feedstock. Diet trial samples of each feedstock, BSFL and frass were sent to Rothamsted Research for nutritional analysis.

4.3.2. Results

All results are shown in Figure 7. Overall Diet 1 (fruit waste) performed best by far, with an average BSFL yield of 16.3% compared to 12.4% for control.

Diet 5 (a fruit and vegetable blend) yielded BSFL at 14.1%, and had a similarly low retention time (24 days) to diet 1 (23 days) while all other diets took between 27 and 32 days. This makes diet 5 a strong candidate for combining fruit and vegetable feedstocks. For frass yields, the picture is less clear, but where diet 5 had a high yield for BSFL and a low yield for frass (10.2%), diet 7 yielded low on BSFL and high on frass, suggesting that in the former more resources are going to producing the higher value output.

Using fruit instead of potato therefore resulted in an overall increase in absolute yield of 31%. When this is combined with a 20% average decrease in retention time, the productivity of a system using fruit is 56% greater than that of one using potato.

4.3.3. Nutritional Analysis

Micronutrient analysis showed little difference between BSFL and frass samples, especially in key nutrients for pet food (Ca, P) and fertiliser (N, P, K). The same analysis of the feedstocks showed that fruit waste is lower in nitrogen, despite giving higher BSFL yields, so these yields are likely due to the high sugar content.

4.3.4. Lipid Analysis

Live BSFL lipid contents for a range of diets were analysed by the University of Sheffield. Preliminary results show that, compared to the control, BSFL grown on fruit diets not only have a higher fat content thanks to the sugar rich diet, but also have much higher levels of Lauric acid, which is antimicrobial and linked to improved health in pets. Furthermore the lipids in fruit fed BSFL are rich in carotenoid pigmentation, important for reptile and bird health.

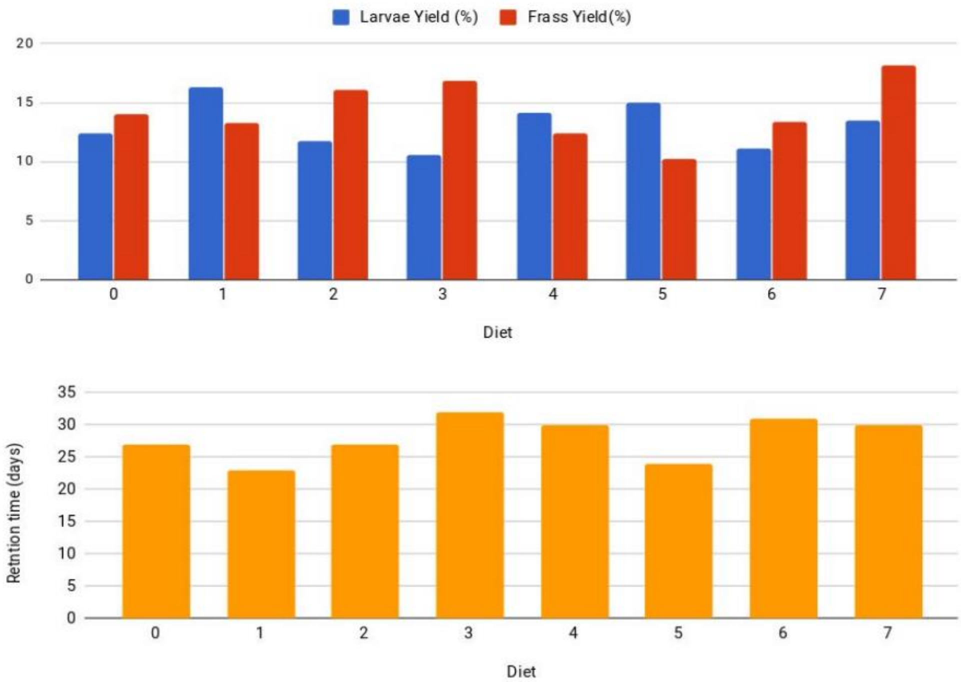

Figure 7: BSF-larvae performance on 7 different diets.

4.4. The impact of frass treatment on aphid populations in cabbage plants

Insect frass has been shown in previous trials to be an effective fertiliser, however, its unique nutritional breakdown means it has also been hypothesised to have pesticidal qualities (Kagata and Ohgushi, 2011). Frass is partially composed of insect skin casings, which is rich in chitin. By placing the chitin-rich frass around a plant, the plant is essentially tricked into responding to an insect attack. Much like a vaccination in humans, this raises the plant's systematic induced resistance system, making respond faster and more effectively when a genuine pest attacks. In this way, it is thought that insect frass can deter pests without the need for chemical intervention, by harnessing the natural immune systems found in plants (Kaku et al., 2006). Bio-repellents are often seen as preferable of conventional chemical pesticides, as they circumvent many common issues such as harm to key pollinators.

This trial was run on Black Soldier Fly (*Hermetia illucens*) larvae frass which was shown in internal trials to contain 14.52% chitin as a percentage of total dry mass. This is higher than other insect frass products commercially available, such as mealworm (*Tenebrio molitor*) frass which is marketed as containing 10%, though this figure could not be sufficiently confirmed (Farming, 2019). The higher chitin content found in Black Soldier Fly frass would suggest it would make an effective bio-repellent.

Aphids are among the most destructive pests on cultivated plants in temperate regions. They can cause yellowing, mottled leaves, stunted growth, curled leaves, browning, low yields and even death in plants, and therefore are a prime target of many pesticides (Jusoh and Norton, 1987).

4.4.1. Methodology

Aphid trials were conducted on a total of 96 cabbage plants over a span of 13 successive weeks. Four treatments were tested, control with no frass or aphid inoculation, Frass treatment and then again with inoculation of aphids. For frass treatments, 5% of soil on weight was replaced with Black Soldier Fly frass. To inoculate plants with aphids, approximately 200 aphids were introduced per aphid treatment set. Plant height was in duplicate each week to reduce variability, along with plant width - these measurements were carried out with a ruler. Leaf length, taken as the distance from leaf tip to petiole of the third apical leaf was measured on 6 randomly chosen plants from each treatment group until week 10. The number of leaves was recorded by visual inspection. Destructive sampling was taken at the end of the trial to estimate aphid population levels. This took place with the standard alcohol leaf washing technique. Plant area was calculated from the plant height and width data. Statistical analysis was carried out in R. 6.4.1 using Wilcoxon Signed Rank test, Unpaired ANOVA, variance test and Pearson product-moment correlation test.

4.4.2. Results

The number of aphids present in control pest inoculation was significantly higher than frass treatment counterparts ($p=0.012$). In inoculation trials over 9.4 times fewer aphids were found in Frass compared to control inoculation. Treatments without pest inoculation did not show significant differences, however, this data supports the hypothesis that Black Soldier Fly Frass functions as a bio-repellent, as illustrated in Figure 8.

Frass treatments produced significantly higher growth rate than controls. Statistically significant variation was seen after week 4, with a p-value of and a percentage difference of 14%. After 12 weeks the difference in growth rate was increased to 22%. However, no significant differences were found between the aphid inoculation treatments and controls, suggesting cabbage plant area growth is not affected by this specific pest.

4.4.3. Discussion

These results reaffirm the hypothesis that Black Soldier Fly frass is an effective fertiliser and deterrent to aphids. The inoculation trials showed over 9.4 times fewer aphids were found in Frass treatment compared to control inoculations, this strongly supports the deterrent action of frass. Although this was not uniformly seen across all the trends found, that is an indictment not on the impact of the product, but on the suitability of parameters measured as markers of pesticide activity. It is also possible that these measurements did not garner significant trends because of the specific morphology of cabbage plants. Regardless, these results strongly support further tests on different crops and using alternate pests to investigate how widely this bio-repellent acts.

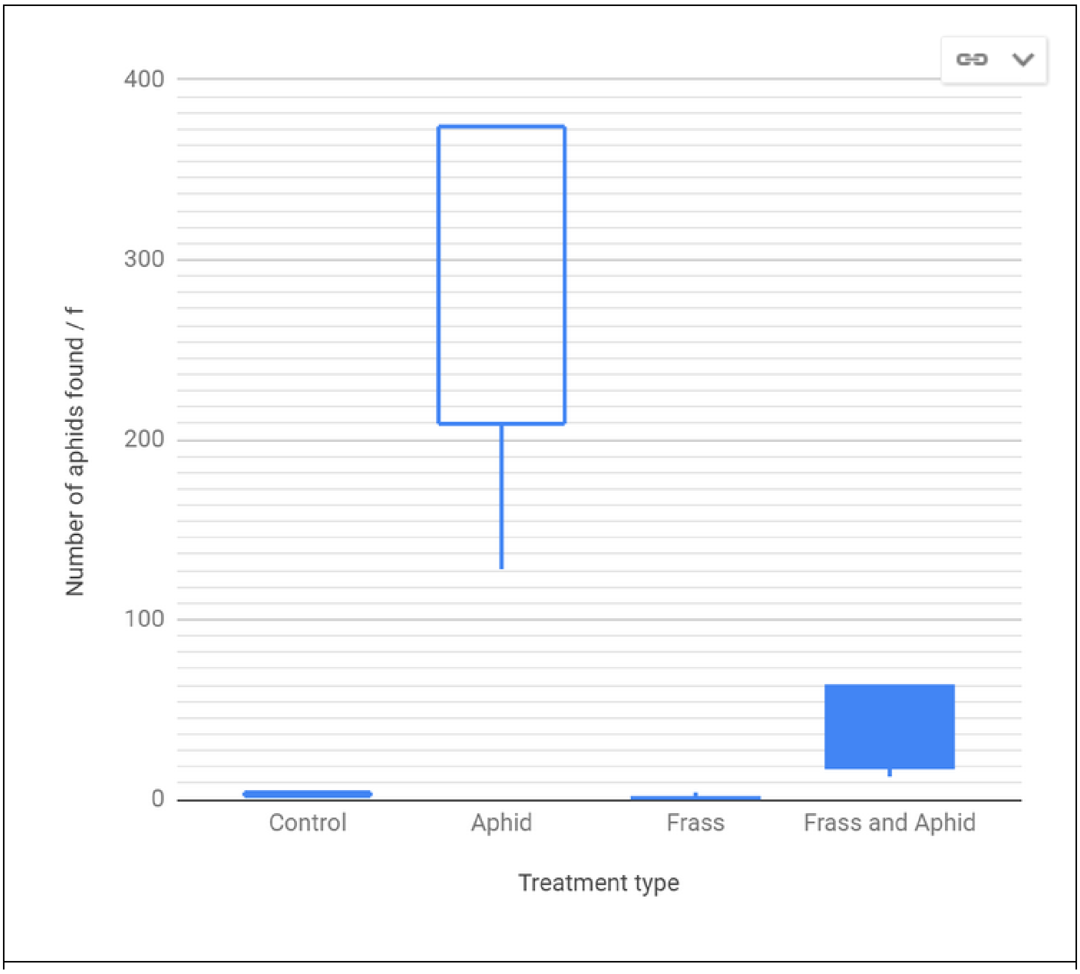

Figure 8: A plot to show the impact of Black Soldier fly frass on aphid populations found on cabbage plants. Pest inoculation control treatment (Aphid), shown here as an unfilled box, had significantly higher aphid populations than inoculation treatments with frass, p=0.012. There was no significant difference found between treatments without pest inoculation.

4.4.4. References

Jusoh, M. M., & Norton, G. A. (1987). Cabbage aphid control on commercial farms in the Thames Valley, UK. Crop Protection, 6(6), 379-387.

Kagata, H., & Ohgushi, T. (2012). Positive and negative impacts of insect frass quality on soil nitrogen availability and plant growth. Population Ecology, 54(1), 75-82.

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5. Insectlab (Vives)

5.1. Overview/ buildup

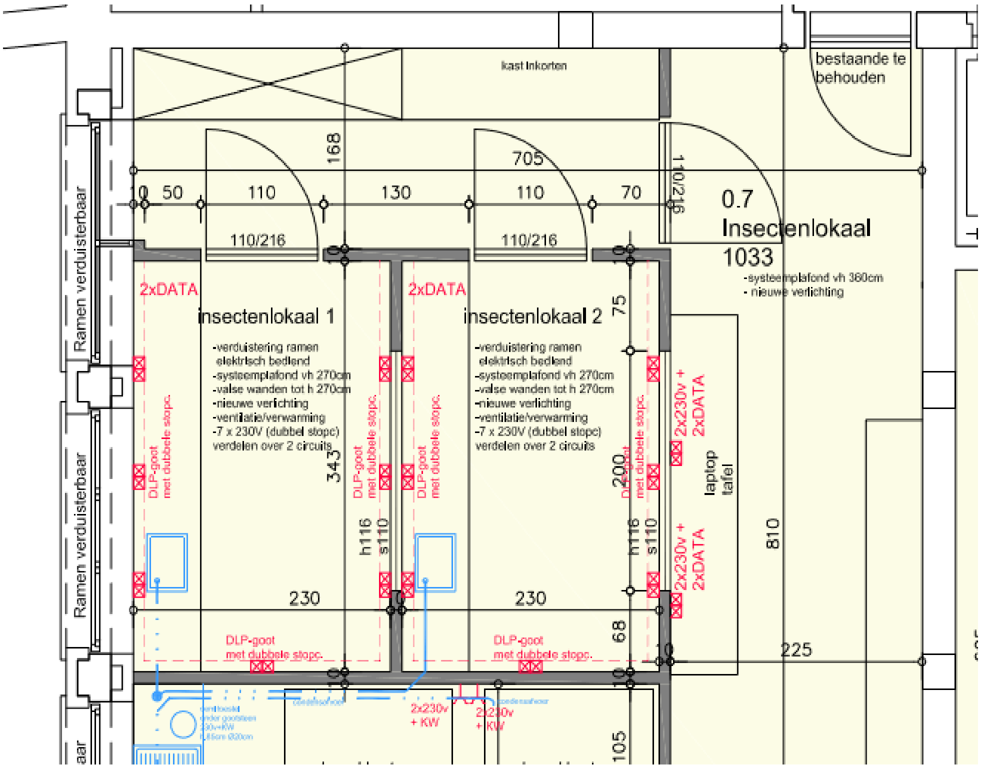

Figure 9: floor plan of the Insectlab

The Insectlab at VIVES is made up by 2 rearing rooms of 230 cm by 343 cm. One room is used to rear mealworms whereas the other contains BSF larvae and flies (Figure 9). The BSF room is climatized with a system provided by CTS Benelux (Type CW+15/21). In front of the rearing rooms there is a room with closets, containing materials, and a bench containing balances, microscope and drying furnace. The BSF standard feed (Farm 1 Crumble, Hobby First) is kept in a corridor separated from the rearing room. This corridor also contains 2 freezers and a fridge to store substrates and samples.

Figure 10: BSF rearing chamber

5.2. Standard rearing protocols

Both larvae and adults are grown in a climate cell at 27 ± 1 ° C and 75 ± 5% RH.

5.2.1. Larvae

Eggs are harvested 3 times a week and placed per 0.3 g (± 20 clutches; ± 90% hatching) in a container (17 x 12 x 6 cm) with cover (23 x 14 x 6 cm) (Figure 11). They hatch after 3.5-4 days and 1 day before hatching the cubes are placed on apple slices. The eggs hatch on the apple slices for 2 days and then 20 g of chicken feed / water is added. 3 days later they receive another 100 g of chicken feed / water and the cover is removed. 2-3 days later they are moved to a larger container (40 x 30 x 11 cm) (Figure 11) and receive 1 kg of chicken feed / water. 3 days later they receive another 2 kg of chicken feed / water.

Figure 11: rearing larvae

5.2.2. Flies

From bins that are not harvested, 10 days after the appearance of the first prepupae, trays (23 x 14 x 6 cm) of 150 g pupae/prepupae mixed with dry substrate are removed.

One tray is placed per cage (35 x 60 x 35 cm) and one day after the first flies appear (± 570 flies, 50/50 males / females), a tray (17 x 12 x 6 cm) with dead larvae and substrate, diluted with water, is placed to attract the flies. Plastic cubes (3 x 3 x 3 cm) with crevices are hanging over these trays and the flies lay their eggs in these crevices. For every cage the center of the widest side surface is illuminated during eight hours/day (13 Watts, Exo-Terra Natural Light Full Spectrum).

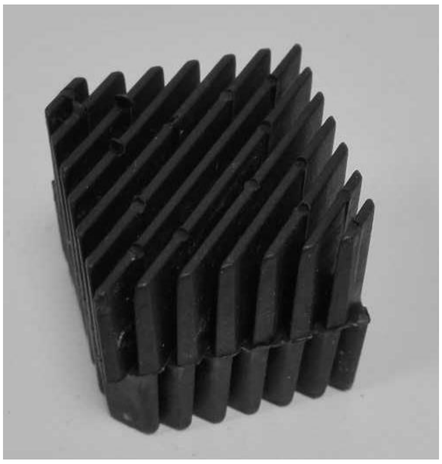

Figure 12: eggy

5.2.3. Optimization

Larvae

0.3 g of eggs yielded an average of 875 g of harvested larvae yield (= 3.39 kg yield / g of eggs), the feed conversion being 1.63. If 0.45 g of eggs were used, this yielded an average of 908 g (= 2.04 kg yield / g of eggs) and the conversion remained practically the same (1.69). When 0.6 g of eggs were raised, the harvest weight increased to 1219 g with a feed conversion of 1.22. Since the individual larvae weight in this case was only 150 mg, and the larvae in the Insectlab were mainly intended to grow into flies, it was decided to continue with the raising of 0.3 g of eggs (in addition, the kg yield / g of eggs was only 2.03 with 0.6 g of eggs).

Flies

The optimum density in the cages was determined and it was found that 100 g of pure pupae (without substrate) provided a maximum egg yield (at 200 g this no longer increased).

Initially, this yielded an average of 1.34 g of eggs per cage (29.49% of the females were laying).

By changing the position of the cages (and thus optimizing the illuminated area, the yield was increased to 2.10 g of eggs per cage (46.25% of the females were laying).

5.3. Chemical analyses

During this project the Insectlab of VIVES carried out multiple analyses on substrates and insects. In order to make mixtures with different horticultural side streams, that comply with the nutritional requirements of the larvae, many potential substrates (i.e. side streams) were analysed (8.3.2.: Table 6).

5.3.1. Dry matter and ash

Dry matter content of larvae and substrates is determined by drying in a furnace at 105°C until constant weight. Ash content is determined in a muffle furnace at 500 °C until constant weight.

5.3.2. Water activity

The Aw-value stands for water activity. It is the measure of the amount of free water that is present in a product. These values are measured with Aqua lab water point water activity meter. The Aw-value refers to the shelf life of the product. Because how lower the Aw-value, the more difficult it is for micro-organisms to grow. The value should be not higher than 0.6 in order to ensure a sufficient shelf life.

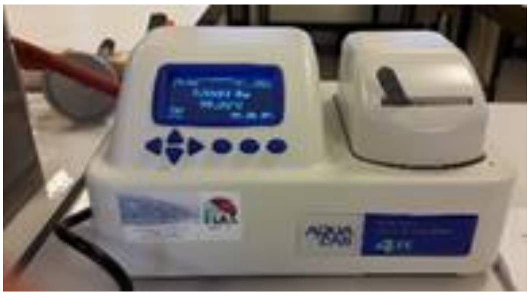

Figure 13: water activity meter

5.3.3. Protein

The Kjeldahl Method determines the nitrogen content which can be used to calculate the protein concentration. This is possible due to the conversion factor, which indicates the fixed ratio between the nitrogen content and the protein content in a specific product. The Kjeldahl analysis contains three different steps:

a) Destruction: Here, the nitrogen is converted to ammonium sulphate in presence of concentrated sulfuric acid and a catalysator.
b) Distillation: The formed ammonium sulphate will volatilize to ammonia when placed in the steam distiller by a surplus of NaOH. The volatilized ammonia is captured in boric acid, in which an ammonium borate complex is formed
c) Titration: Through a titration with HCl or sulphuric acid, the used amount of boric acid can be measured. From this, the nitrogen content can be calculated

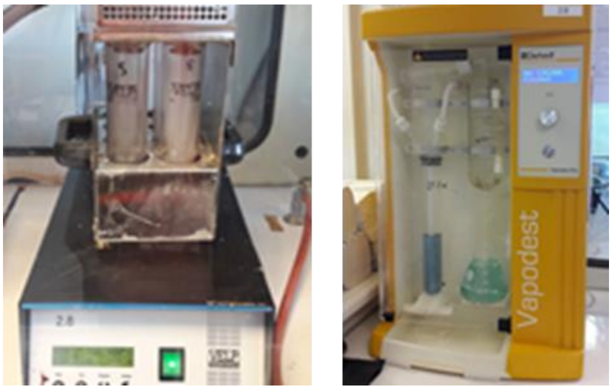

Figure 14: Kjeldahl analysis

5.3.4. Lipids (Ether extract)

The Soxhlet method is performed. Lipid is soluble in organic solvents and insoluble in water, because of this, organic solvents like petroleum ether have the ability to solubilize fat. Later the fat is collected by evaporating the solvent by placing it in the oven at 103 °C (± 2 °C) for 1 hour. The flasks are then allowed to cool and the mass can be determined.

Figure 15: Soxhlet fat extraction

5.3.5. Fibre

Crude fibre is determined gravimetrically after chemical degradation and solubilisation of other materials present. Two steps are involved in chemical degradation, deproteinization and demineralization. Deproteinization is the basic step with KOH and demineralization is the acid step with H2SO4. The weight of the fibre residue is then corrected for the ash content after ignition.

Figure 16: Non-fibre carbohydrates were estimated subtracting the sum of protein, fat, ash and fibre from 100.

6. Insect pilot (Inagro)

6.1. Location

The insect pilot of Inagro is located at Inagro vzw (Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium), integrated in the building of the aquaculture department, near the biogas installation (Figure 17). The biogas installation is fed with pig slurry and horticultural residues, and provides hot water and electricity for the greenhouses, the aquaculture department and now recently for the insect pilot as well. The use of renewable energy fits well within the general goal of insects in a circular economy.

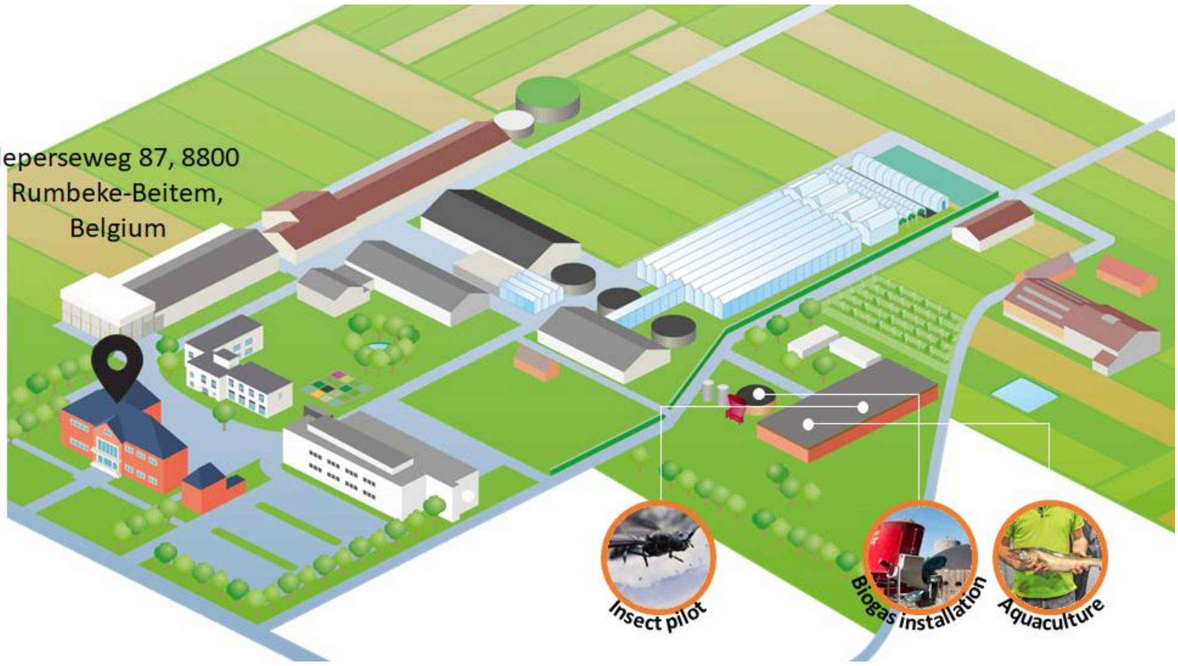

Figure 17: Location of Inagro's insect pilot.

The aquaculture department occupied a part of an old pigsty, but a part of the building was still unused. The BioBoost project gave us the opportunity to rebuild this desolated part of the building into a brand new insect pilot (Figure 18).

Figure 18: Construction of the insect pilot at Inagro.

6.2. Overview of the pilot plant

The insect pilot at Inagro consists of 6 climate controlled rooms, 4 larger rooms (6 x 4 m) and 2 smaller ones (4 x 4 m). The combined rearing area totals at approximately 130 m². Temperature and relative humidity can be controlled at desired levels, CO_2-concentration is measured continuously. Temperature and relative humidity can be controlled at a desired level.

The insect pilot is schematically represented in Figure 19:

- 6 rearing rooms (in blue):
  - 2 rooms for black soldier flies (production and reproduction separated)
  - 2 rooms for mealworms (production and reproduction separated)
  - 1 room for an alternative insect species, in the past Argentinian cockroach (*Blaptica dubia*), currently banded crickets (*Gryllodes sigillatus*).
  - 1 room is currently exhibiting an automation project, a feeding robot for mealworm.
- Sieving and harvesting (in orange): All activities that produce a significant amount of dust and odour take place in a separate room that is thoroughly ventilated. The mechanical sieve has a fixed place in this room.
- The washing station (in dark green): an outdoor spot where the rearing boxes are cleaned, equipped with a water drain.
- Horticultural residue preparation (dark blue): an outdoor spot where the crude residues are shredded.
- Fermented residue stock (in light green): all fermented residues are stored near the feed preparation spot where the mixer is located.
- Dry feedstock storage (in yellow)
- Box storage (in gray): cleaned unused boxes are stored on this spot.

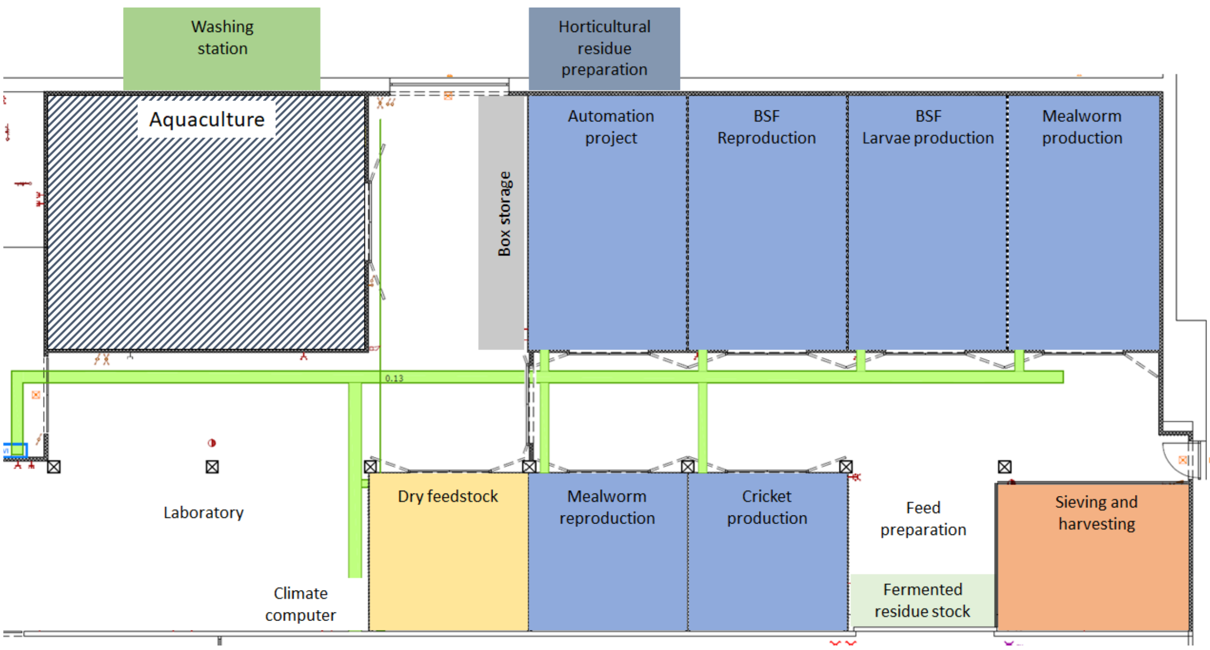

Figure 19: Schematic overview of Inagro's insect pilot.

6.3. Equipment

6.3.1. Breeding material

Boxes

Larvae and beetles are kept in boxes. The optimal dimensions of a production box is a topic for discussion. At the insect pilot of Inagro we opted for boxes of euro standard format of 60 by 40 cm (Figure 20). These dimensions are ideal for manual handling, bigger is unmanageable by a single person, smaller is not efficient enough. For sufficient air refreshment in each box, it is important to have cut-outs in the sides.

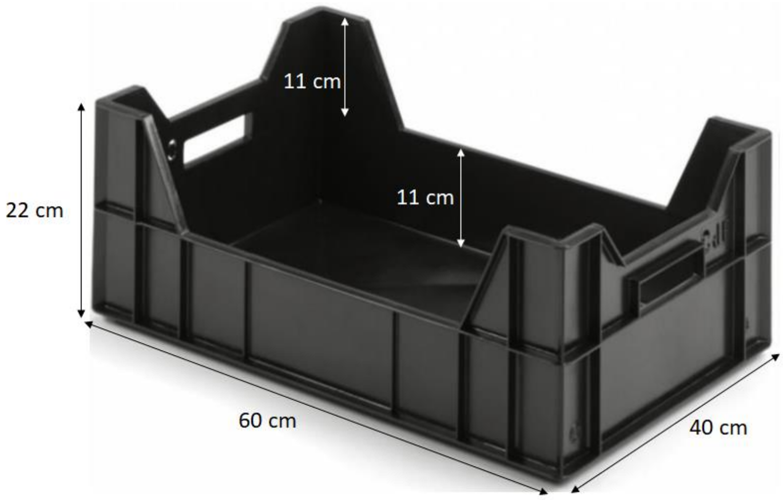

Figure 20: Insect rearing box.

We chose a box-design that works fine for both mealworm and black soldier fly, despite the fact there are more optimal designs for each specie. The current box is interchangeable. 1000 boxes that were already present, were included in the insect pilot.

Dead beetle separator

Some devices that we require are of our own making. One of the problems we encountered for mealworm rearing is that we needed to find a way to efficiently separate living beetles from dead ones. This can be done by exploiting the reflex of the beetles to grip on surfaces. A conveyor belt made of a fine mesh placed under an angle is the solution we came up with. The principle is demonstrated in Figure 21. The homemade device has its permanent spot in the sieving and harvesting room.

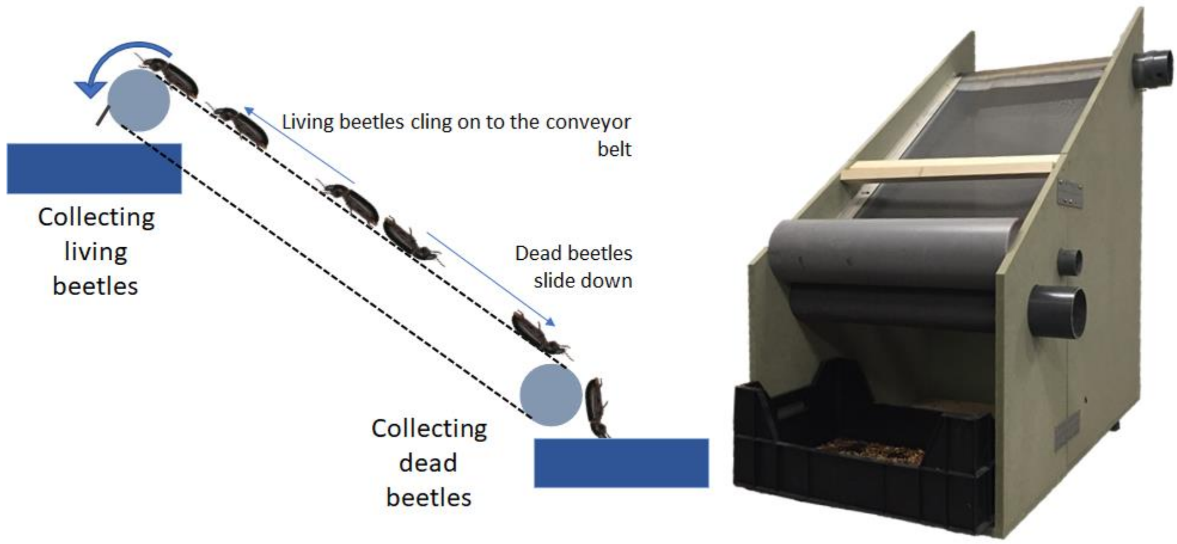

Figure 21: Dead beetle separator.

Black soldier fly cage

The adult phase of black soldier fly, the fly itself, needs to be kept in cages (Figure 22). 9 cages were given a permanent place in the insect pilot. The current average egg production per cage is around 40 grams over a two week period, but can be as high as 80 grams under optimal conditions. One gram of black soldier fly eggs can yield up to 30,000 larvae. In the end we want 20,000 larvae per box, giving the numbers, this means we have the potential of harvesting 38 boxes on a daily basis. Which is equivalent to around 80 kg.

The cages are mobile and are made of aluminium (light and easy to clean), with a height of 170 cm (excluding the light carrier, which can be adjusted and is up to 50 cm high). The bottom plate is removable and underneath the bottom plate, two boxes can be inserted. One box contains fresh pupae, the new fly generation, the other one the odourful attractant.

There are 8 holes (2 cm) in the bottom plate above the pupae drawer (for the pupae to enter the cage). Above the attractant, the bottom plate is perforated. The odour from the attractant is trapped in the shady house, inside this house there are eggies (stacked wooden/plastic slats).

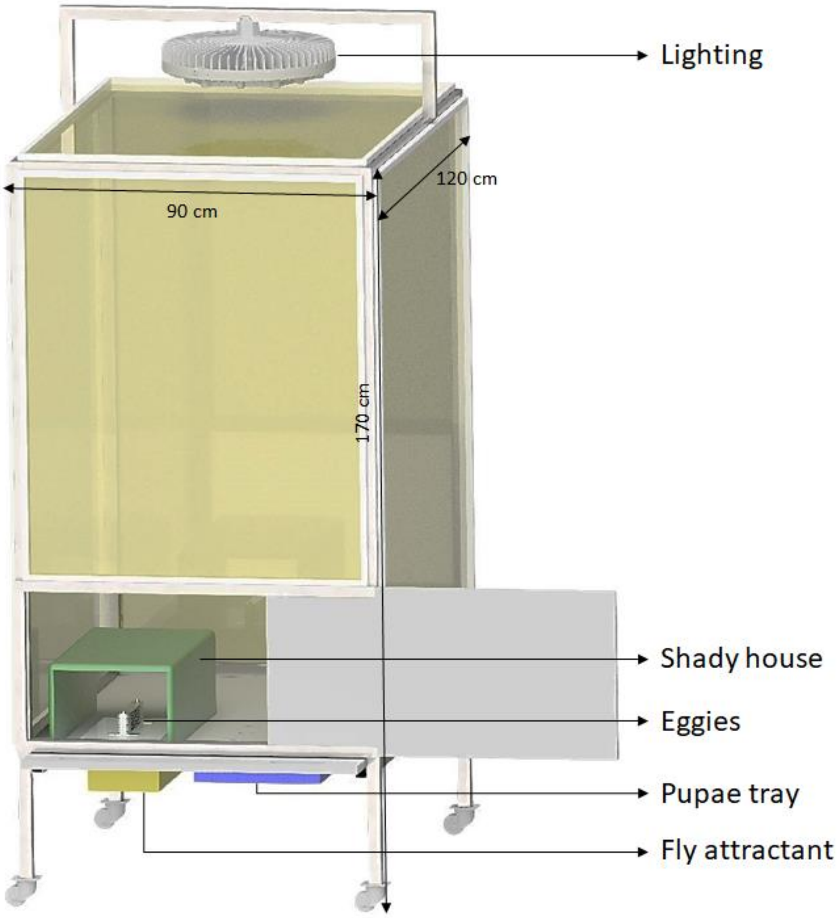

Figure 22: Black soldier fly cage. The 'shady house' is a small enclosure around the egg-laying-site that traps the odour of the attractant. 'Eggies' are the place where the black soldier fly lays its eggs and we collect them.

Dust mask

Rearing insects, and especially mealworms, is accompanied by a lot of dust production. Those dust particles carry allergens. In the long term employees might start to develop allergies and this cannot be undone. Allergies makes it impossible to keep on working near any mealworm production hall, prevention is therefore of utmost importance.

For mealworm breeding, we therefore recommended to protect yourself against the dust. Prevent inhalation and contact with the skin. Because exposure is highest while sieving, it is recommended to carry out all sieving activities in a therefore designated room. Entering this room is only allowed while fully protected by a full mask with air filtration (Versaflo TR-315E + motor unit + M-106 helmet) in combination with a disposable coverall and gloves. This offers sufficient protection against most allergens (Figure 23).

Figure 23: Employee of Inagro in protective clothing.

Fermentation barrels

Horticultural residues are often only present during several weeks each year. Prolonging the availability is important to keep your insect pilot running all year. Preserving the residues in a fridge is one option. A second, more sustainable option is fermenting the residue. Under anaerobic conditions, organic acids will be produced by microorganisms, the pH will drop and growth of other bacteria will be suppressed.

Several fermentation barrels (Figure 24) were made in order to preserve residues at the insect pilot of Inagro. The barrels have a volume of 60 litres and are equipped with a water trap, sealing of the inside from oxygen and allowing fermentation gasses to escape.

Figure 24: Fermentation barrel used at Inagro.

6.3.2. Machinery

Shredding

Processing horticultural residues is one of the major topics of this project. These residues are typically rich in fibre, which cannot be broken down by the insects. The fibres will still be present in the insect frass. Proper equipment is necessary to shred the residues to particles that are manageable by the larvae and that are sufficiently small to be separated from the larvae during sieving.

Different companies suggested a solution for our specific case. Their solutions were tested with actual residues and compared. Eventually our choice was made and a Caravaggi TPF15 E (Figure 25), with a modified engine of 15 hp and modified blades to cope with wet materials, was purchased.

Figure 25: Caravaggi TPF15 E.

Feeding

A diet for black soldier fly rarely consists of only one residue. Different ingredients need to be mixed in order to have a balanced diet. The process of mixing different ingredients needs to go fast and homogeneous. A blixer 30 (Figure 26) is used to fulfil this task. It can mix batches of 25 litres in one turn, this is too little for industrial insect breeding, but is sufficient for pilot scale research.

Figure 26: Blixer 30.

Sieving

Separating different life stages is an important part of insect rearing. Professional sieving equipment is therefore required for an insect farm. The different moments throughout the life cycle of both mealworm (Figure 27) and black soldier flies (Figure 28) where sieving is required are represented schematically.

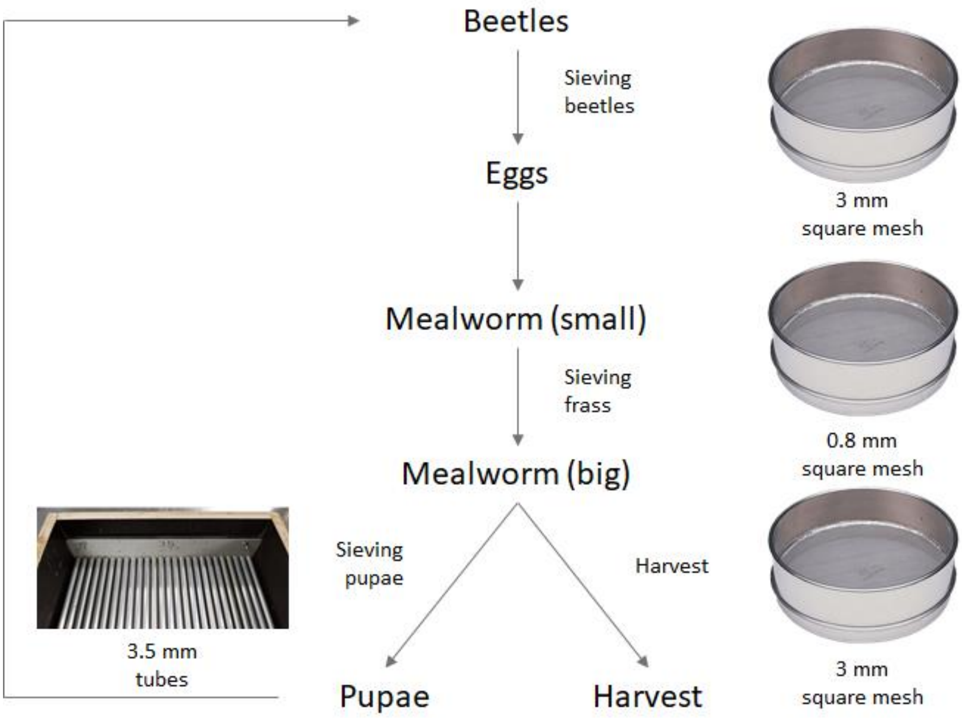

Figure 27: Recommended sieving steps and sieve types required for mealworm rearing.

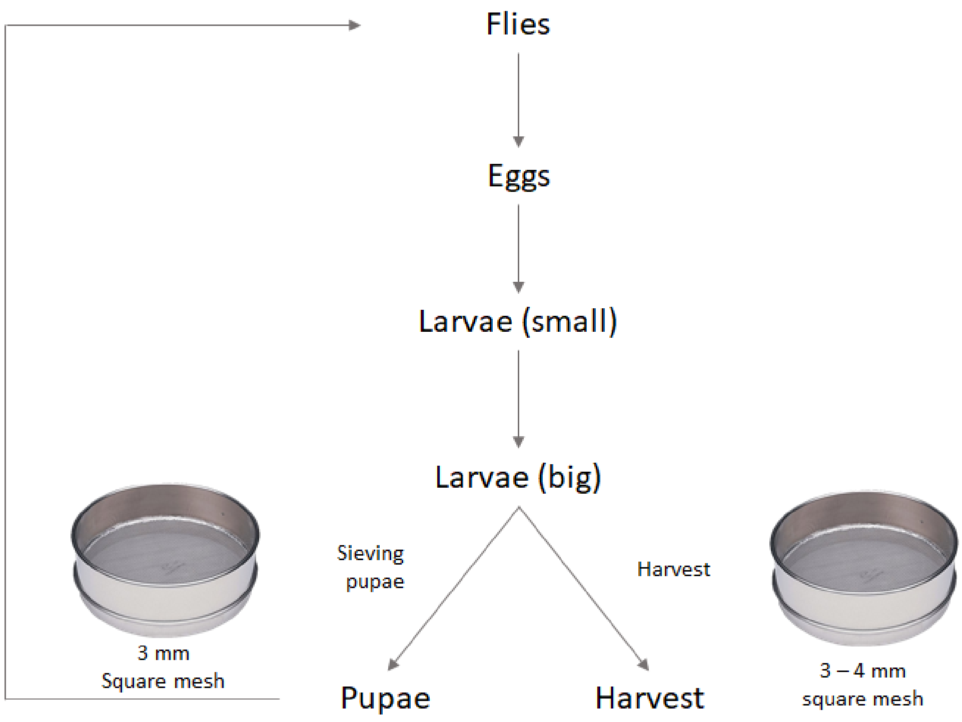

Figure 28: Recommended sieving steps and sieve types required for black soldier fly rearing.

A 30" FINEX SEPARATOR (Figure 29) is used to mechanize the sieving process. This machine can combine different sieve layers (with different mesh sizes). In addition, there is also a lot of freedom to adjust the horizontal and vertical movement of the engine. Making it the best option for the dual purpose, as it needs to be applicable for mealworm and black soldier fly breeding.

Figure 29: 30" FINEX SEPARATOR.

Washing/cleaning

An industrial crate washer is recommended for bigger insect production facilities. However due to budgetary reasons we were obliged to fall back on a high pressure cleaner that was already present (MH 4M - 200/960 FA (AT3715), Figure 30). The machine is capable of producing steam and spraying hot water at 90 °C, it also has the option to spray product (ketosan). All these options make it better equipped for cleaning rearing boxes than an ordinary high pressure cleaner.

Figure 30: MH 4M - 200/960 FA (AT3715).

7. Horticultural residues as feeding substrate

7.1. Insect lab research (Vives)

7.1.1. Experimental protocols

- Containers: polypropylene cups with mesh in the lid; diameter at the top 8.5 cm, bottom 7 cm; height 10 cm
- Temperature: 27 ± 1 ° C
- Relative humidity: 75 ± 5%
- Light regime: constantly dark
- 3 repetitions / treatment
- 100 larvae / replicate
- 6 ± 1 or 7 ± 1 days old at the start
- First week grown on ad libitum chicken feed (Farm 1 Crumble, Hobby First)
- Counted by hand, weighed per 100 and maximum 5% weight difference between groups
- First half of 2018: Initially 10 g is fed, then 20 g every 2-3 days for 2 weeks
- From the second half of 2018:
- Every 2-3 days 10 random larvae are individually weighed (and then placed back) to prepare a growth curve
- Harvest time per repetition when the first prepupae are determined (<10%) or when the growth stops. So not for all treatments at the same time!
- During harvest, a further 10 larvae are weighed individually, larvae and residual substrate are separated, weighed and frozen at –20 ° C for later analyses
- The parameters that can be determined are listed here. Important definitions: Substrate = total weight of fed material

Residual substrate = total weight of remaining material after harvesting the larvae, this is a combination of undigested substrate and the manure of the larvae:

- growth (g/larva/day)
- harvest weight (g / larva and g / 100 larvae): determine both the average individual weight and the total final weight of the 100 larvae, this can be expressed on both dry matter and wet material
- substrate processing (%) = ((substrate-remaining substrate) / substrate) * 100; can be expressed on both dry matter and wet material
- (waste reduction index) = (((substrate-remaining substrate) / substrate) / larval development time) * 100; can be expressed on both dry matter and wet material
- Feed conversion is best expressed according to the following formula, making it best comparable to other farm animals: feed conversion = (dry substrate (g)) / (wet harvest weight of larvae (g))
- dry matter content (g / 100 g)
- protein, fat, crude fibre and ash content (g / 100 g dry matter) of both the larvae, substrates and residual substrates. Protein and fat analyses used are Kjeldahl (correction factor of 4.76 for the larvae and 6.25 for the substrate) and Soxhlet, respectively.
- nutrient utilization of the substrate by the larvae (%)
- protein conversion = (protein in dry substrate (g)) / (protein in dry larvae (g))

- ECD (Efficiency or conversion or digested food) = (dry harvest weight) / ((dry substrate-dry residual substrate))

7.1.2. Side stream assessments

- Option 1: Add the residual flow to the already fed chicken feed at different ages. A start is made with larvae 6-7 days old. One treatment receives pure residual flow from the start, others receive chicken feed. With the 2nd feeding (2 days later), a 2nd treatment is also given residual flow and, if necessary, additional treatments can be started later at a later age depending on the nutritional value of the residual flows.
- Option 2: Mixing in residual flow with basic feed, starting with larvae 6-7 days old.
  - Depending on the quality of the residual flow, different ratios are tested (residual flow / chicken feed (%): 100/0; 80/20; 70/30; 60/40; 50/50; 40/60; 30/70; 20/80; 10/90)
  - Ultimately tested regimes based on literature, chemical analyses and preliminary tests
  - Control treatment with 100% chicken feed (0/100)
- Option 3: for substrates whose composition meets nutritional needs according to analyses or literature, the pure residual flow is given from day 6-7. Or different complementary residual flows are mixed, whether or not in different proportions.

The determined parameters are the same as those mentioned above

7.1.3. Report: Fruit mix (mango/avocado) as feed for BSF larvae

In the context of the BioBoost project, a fruit mix was investigated at the Insectlab at the request of the then partner Van Vliet Contrans (meanwhile taken over by Renewi). Not much was known about the composition of this fruit mix, only that it contained mango and mainly avocado.

The standard protocol for testing residual flows with BSF larvae was used. In addition to the fruit mix, a positive control was included in which a mixture of chicken flour with water (30/70) was tested. 100 larvae of 6 days old were placed on 10 g of substrate and this was repeated 3 times. Every 2-3 days, 20 g of substrate was fed and 10 larvae were randomly selected and weighed individually until the maximum weight was reached. The growth, yield and nutritional composition of the larvae was investigated together with the composition of the feeds and the residual substrates.

Growth and development

The performance of the larvae is shown in the growth curve below (Figure 31). These results show that the larvae grown on the fruit mix were only half as heavy as those on the chicken feed. However, it was also noted that the fruit mix was very oil-rich and that the young larvae had difficulty processing it. Therefore, in a 2nd experiment, larvae of 8 and 11 days old were fed with fruit mix. A new start was made with 6-day-old larvae that were divided per 100 into 9 pots. The first time 10 g of chicken feed was given to all jars. 2 days after (day 8) fruit mix was started at 3 jars while the remaining 6 jars were fed with chicken flour. From 3 days later (day 11), another 3 jars were fed with fruit mix. Larvae from the remaining 3 pots were further grown on the chicken feed. Figure 32 shows the resulting growth curves. The larvae from the 2nd experiment that received fruit mix from their 8th birthday were still significantly less heavy than those on chicken feed. But if we wait until the age of 11 days, this difference was much less. Figure 33 shows the total yields of the treatments. This shows that larvae that received fruit mix from day 8 reached only 64% of the weight of these on chicken feed. If you wait until day 11, the yield goes up to 82%.

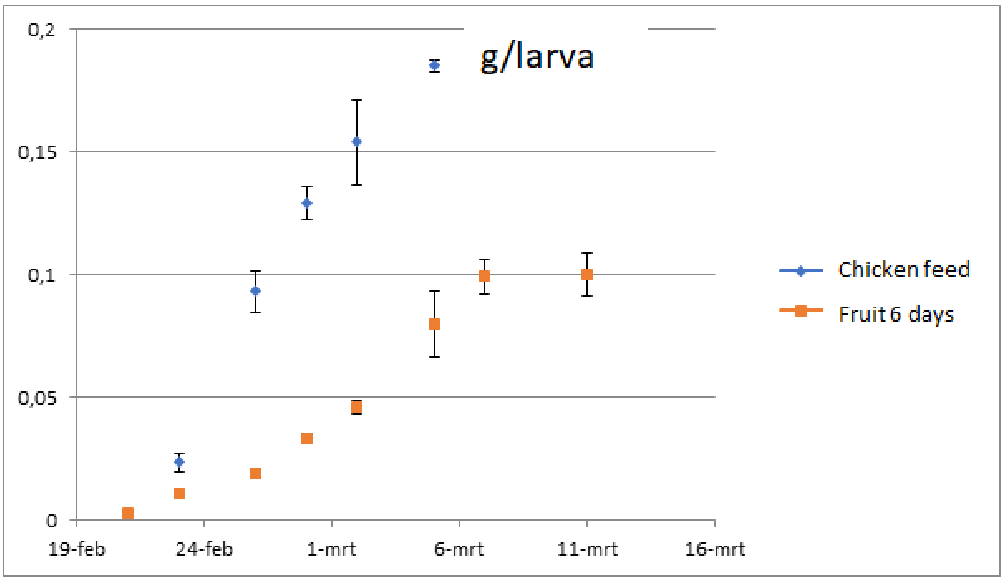

Figure 31: Growth curves showing the evolution of the individual weight of BSF larvae, grown on fruit mix and chicken feed mixed with water (30/70), over time

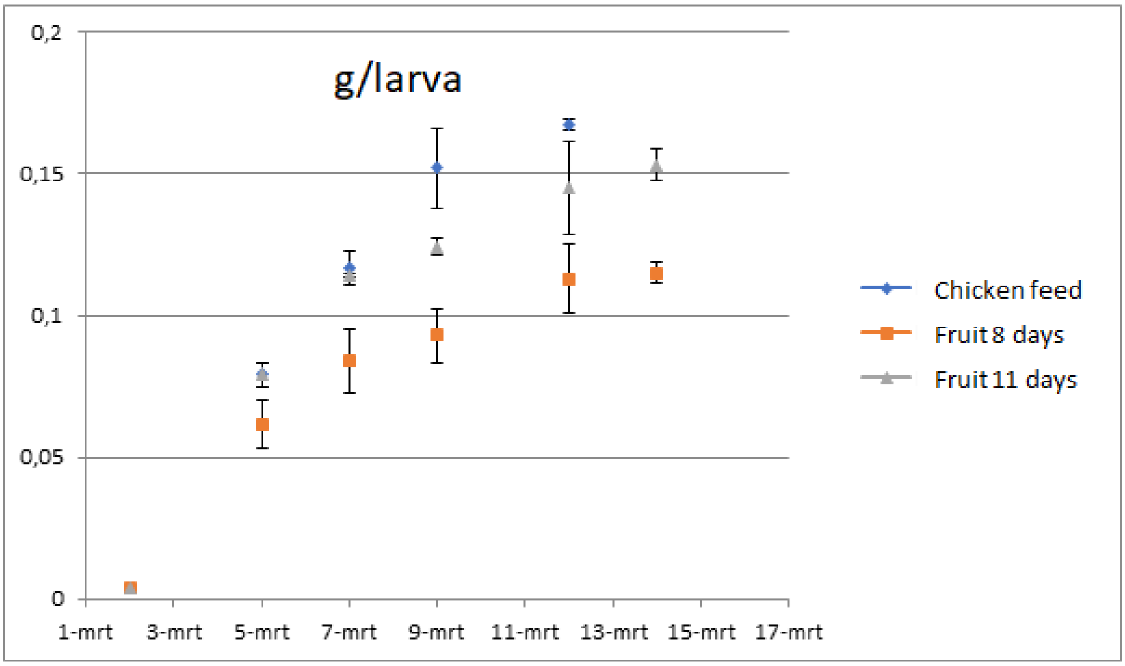

Figure 32: Growth curves showing the evolution of the individual weight of BSF larvae, grown on fruit mix from 4 March (= 8 days) and 7 March (= 11 days) and chicken feed mixed with water (30/70), over time.

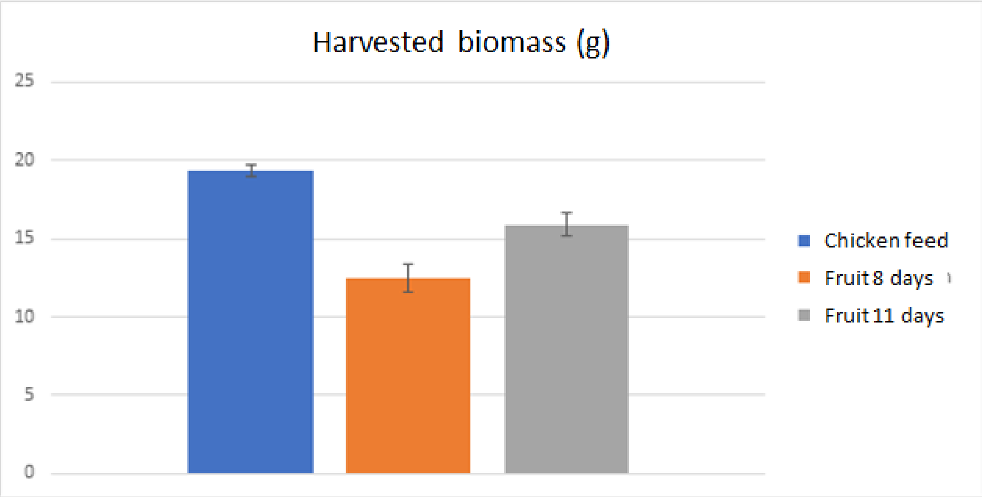

Figure 33: weight of 100 BSF larvae, grown on fruit mix from the age of 8 days and 11 days and chicken feed mixed with water (30/70).

Composition of larvae and substrates

The composition of the larvae, the feeds and the processed residual substrates were determined by Weende analyses: the larvae were dried at 70 ° C to a constant weight, then fat (Soxhlet; ISO 6492: 1999) ,protein content (Kjeldahl; ISO 5983-1: 2005) and chitin (Crude fibre: EG 152/2009) were determined.

The results are shown in Figures 34 through 36. The dry matter (DM) content of the chicken feed mixed with water (30/70) was almost twice as high as that of the fruit mix (Figure 34). This also translated into a lower DM content as the larvae grew longer on the fruit mix. However, it is especially noticeable that the larvae grown on the fruit mix were much fatter than those grown on chicken feed (Figure 35). Again, there appears to be a correlation between the level in the feed and that of the resulting larvae. Other studies at the Insectlab have shown that BSF larvae do not need many fats to reach a fat content of more than 30%. As long as there are enough easily digestible carbohydrates present. This was again established for the larvae grown on the low-fat chicken feed. However, it is the first time that such an oil-rich substrate, with 40% fat on DM, was tested as the fruit mix. The fat contents of the cultured larvae increased to over 50%. Such high levels have never been described and the fatty acid composition has not yet been investigated. Our hypothesis states that a significant proportion of those fats are still present in the intestinal tract. BSF larvae largely convert carbohydrates and fatty acids from their feed into lauric acid (C12:0). Accumulation of fatty acids from the feed, however, has already been described by St-Hilaire et al. (2007) where BSF was grown on fish-oil-rich substrates. Whether there was talk of incorporating these fatty acids into the body or just accumulation in the intestine was not traced. Dissections of the larvae and fatty acid analyses will provide an answer to this question but were not performed in our experiments.

The larvae that received fruit mix contained fewer proteins than those grown on chicken feed (Figure 36). However, the protein conversions (=% protein from the feed that ended up in the larvae) of the larvae grown on fruit mix were higher than of the larvae grown on chicken feed. Larvae reared on fruit mix contained more chitin (8%) than the control larvae (6%).

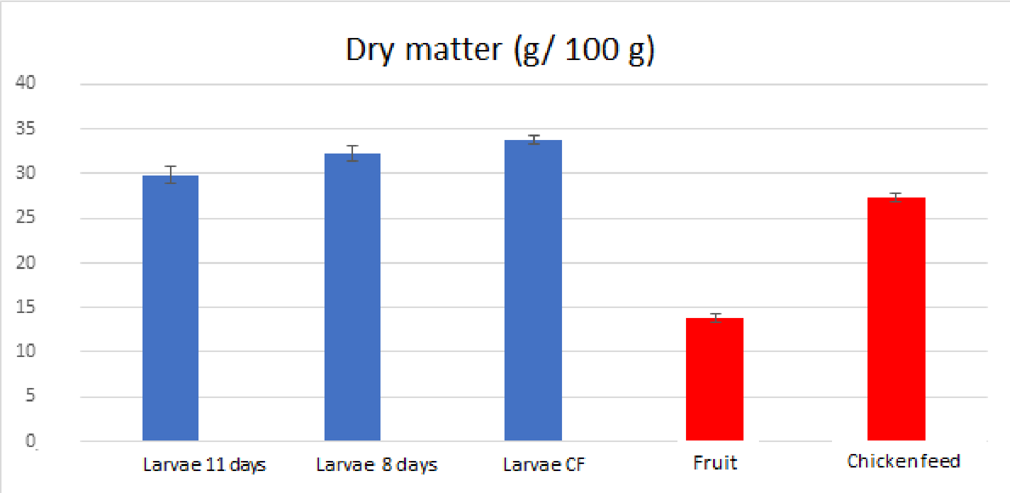

Figure 34: Dry matter contents of BSF larvae, grown on fruit mix from the age of 8 days and 11 days and chicken feed mixed with water (30/70) (= CF) and the feeds on which they were bred.

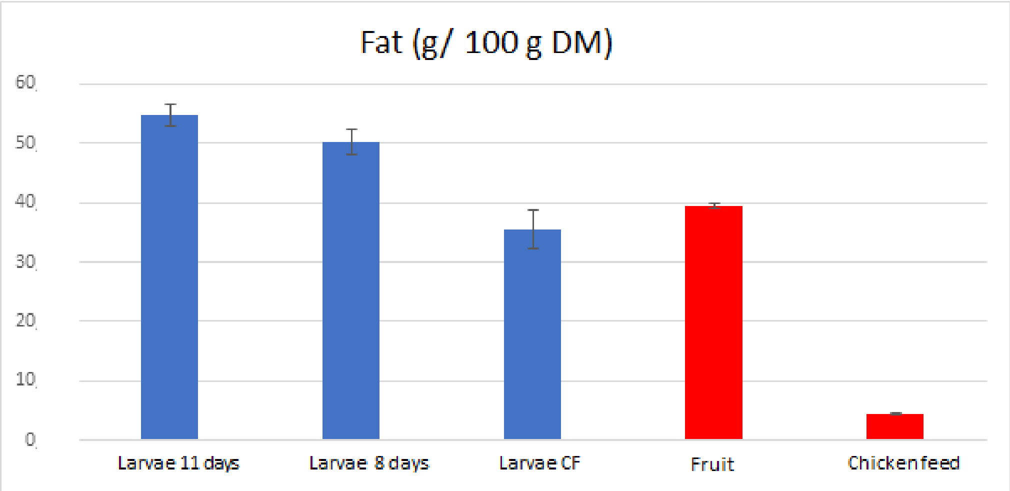

Figure 35: fat levels of BSF larvae, grown on fruit mix from the age of 8 days and 11 days and chicken feed mixed with water (30/70) (= CF) and the feeds on which they were bred.

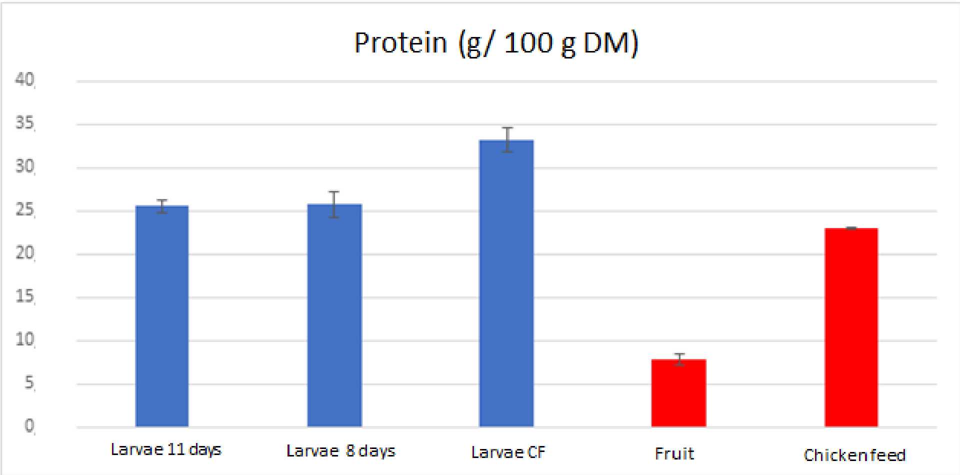

Figure 36: protein levels of BSF larvae, grown on fruit mix from the age of 8 days and 11 days and chicken feed mixed with water (30/70) (= CF) and the feeds on which they were bred.

Processing of the substrates

The processing of the feeds by the larvae is shown in Figure 37. From this we can deduce that the larvae that received fruit mix processed 40 – 50 % of the wet feed mass. If we express it in DM, we achieve a 59 % reduction in larvae that received fruit mix from day 8 while the larvae that received fruit mix from day 11 processed only 41 % of the DM. In Figs. 38 up to 40 show the DM, fat and protein contents of the residual substrates, respectively.

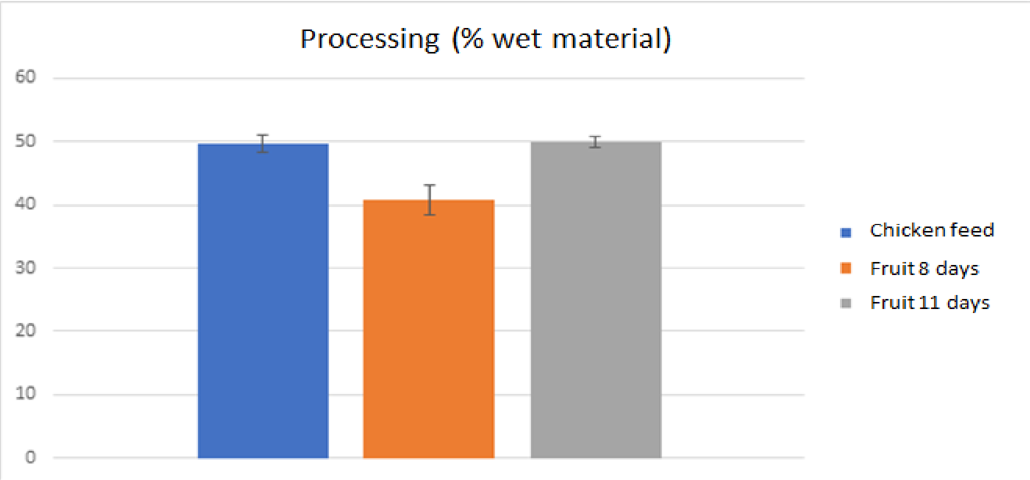

Figure 37: % of the feed processed by the larvae grown on fruit mix from the age of 8 days and 11 days and chicken feed mixed with water (30/70)

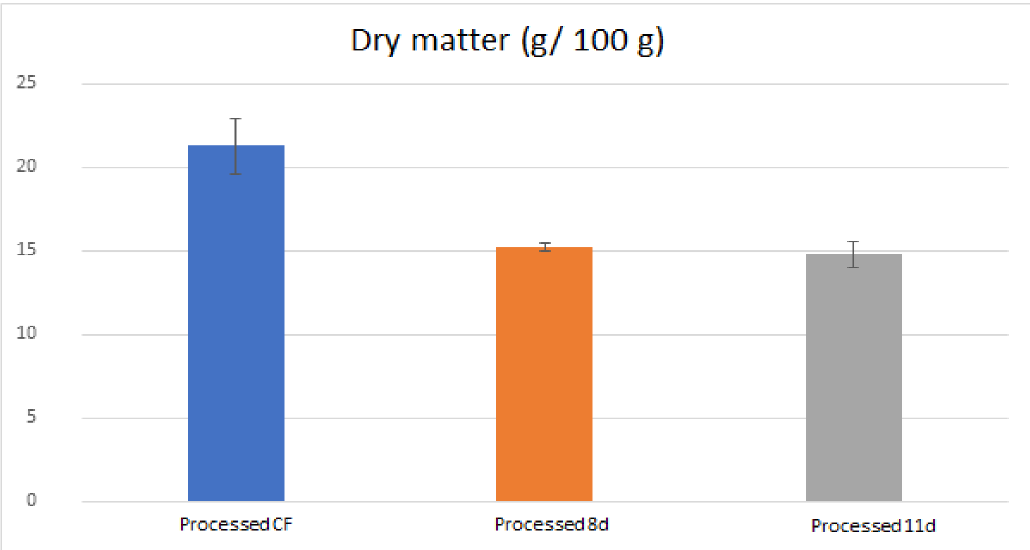

Figure 38: Dry matter content of residual substrates after processing by BSF larvae grown on fruit mix (= 8 d and 11 d) and chicken feed (30/70) (= CF).

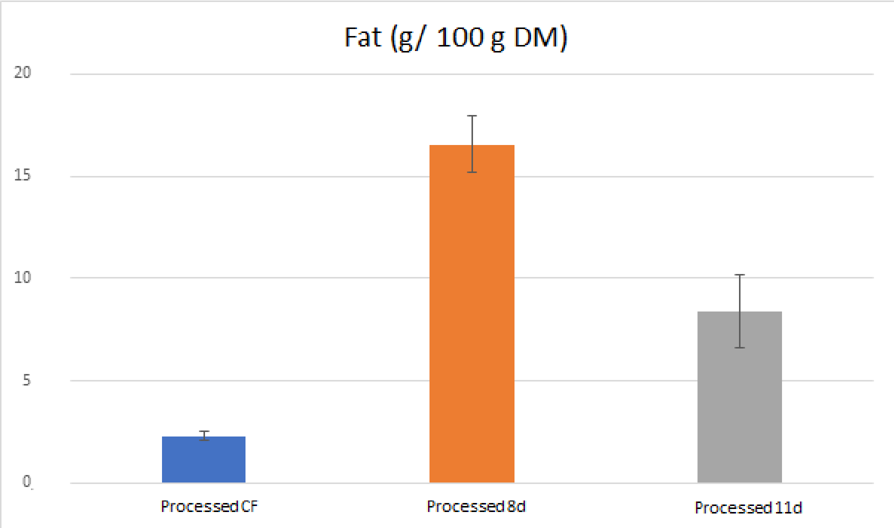

Figure 39: fat levels of residual substrates after processing by BSF larvae grown on fruit mix (= 8 d and 11 d) and chicken feed (30/70) (= CF).

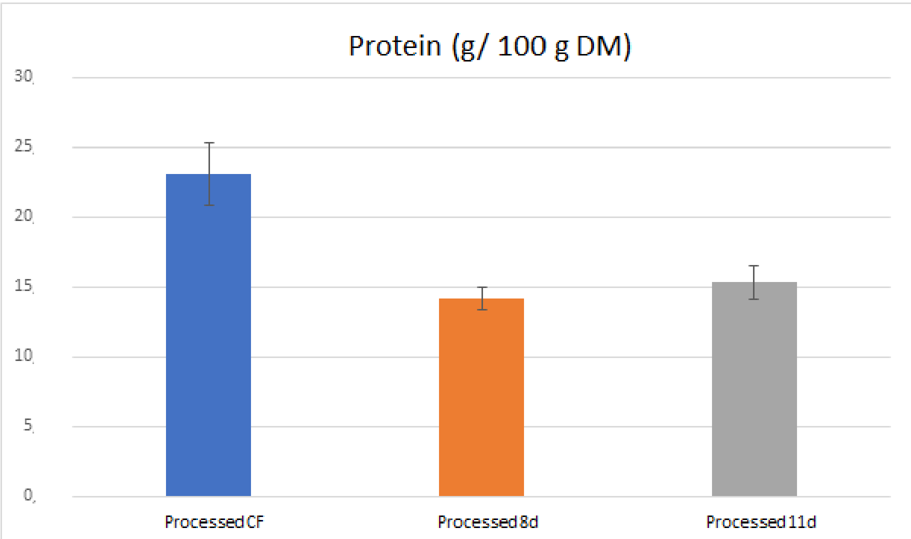

Figure 40: protein levels of residual substrates after processing by BSF larvae grown on fruit mix (= 8 d and 11 d) and chicken feed (30/70) (= CF).

Conclusions

From the results obtained in the Insect Lab we can state that it can certainly be interesting to use the fruit mix as a substrate for BSF larvae. It is best to work with larvae between 8 and 11 days old if you want to work with pure fruit mix. The reason for this is that the larvae can handle the oil-rich material better. Moreover, it is also best to provide a base layer (in our experiments this was chicken feed) that can absorb the oil. An alternative method may be to place the larvae on a mixture of chicken feed (or another oil-absorbing substrate) and fruit mix from the age of 6 days. These matters, however, need to be further investigated on a larger scale in order to arrive at reliable conclusions. The tests in the Insectlab are therefore a first screening in which interesting substrates are selected for further research. The fruit mix has passed this screening.

Finally, we can also provide the results of a cost-benefit analysis of the use of the fruit mix in relation to the chicken feed for larvae grown on a larger scale. This analysis was based on a price of € 0.27 / kg of chicken feed and € 0.00 / kg for the fruit mix. Based on the production results of the above tests, we can state that the feed cost / kg of larvae production for chicken feed was € 0.38, while that decreased to € 0.15 if fruit mix was started when the larvae were 11 days old. For larvae who already received fruit mix at the age of 8 days, the feed cost / kg production even dropped to € 0.07.

Furthermore, we assume that in practice a maximum of 3.88 kg of larvae can be produced per chicken feed (= upscaling from jar in the lab to production unit in practice: x 200). If we assume a selling price of € 3 / kg of larvae, this will bring us € 11.64. When we deduct the costs from this, we get a profit of € 10.17. If we produce 3.88 kg of larvae per unit by using fruit mix from day 8, the profit will increase by 12.0% to € 11.39. If we wait until day 11, there is only a 8.6% increase in profits (up to € 11.04) compared to a full cycle on chicken feed.

The above calculation example indicates that it can be economically advantageous to use the fruit mix as feed for the larvae. In addition, the profit margin can be increased if it should appear that the fruit mix is not only free but that potential processing costs can also be avoided. However, it should be stressed that this calculation example is purely hypothetical and that, in any event, additional testing on a larger scale is needed to draw 100% reliable conclusions. The next step in the assessment process of residual flows within the BioBoost project is carried out at the Insect Pilot of Inagro. The fruit mix has already been viewed, but due to the limited amount, thorough experiments could not be carried out. It has already been noted that due to the high oil and low DM content, the structure of the fruit mix is probably not optimal for pure use. This of course does not alter the fact that the fruit mix can be an important component in the diet of BSF larvae.

References

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., ... & Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society, 38(2), 309-313.

7.1.4. Nutritional requirements

Black soldier fly larvae are capable of converting many different residual streams into nutritional biomass. As part of the BioBoost project, a large number of interesting side streams from agriculture, horticulture and the food sector are being tested and optimum mixtures are being put together. However, the number of possibilities is very large and in order to have a better idea of which residual flows can be combined to guarantee optimum growth of the larvae, more knowledge is needed about the needs of the larvae. Practical situations have already shown that larvae grown on a mixture of 30% chicken feed and 70% water receive far too many proteins. Not using all these proteins translates into high ammonia emissions and, moreover, it is a waste of valuable nutrients. Interestingly, most side streams of vegetable origin have a much lower protein content.

To determine the minimum protein requirement to ensure good growth, experiments were set up with artificial diets. By working with components that are almost entirely composed of one specific ingredient, it is easier to vary only one component without also influencing the rest of the composition. The literature shows that proteins and non-fibre carbohydrates are the most important components and that is why pure starch / protein mixtures were initially started. The starch was pure potato starch while the protein consisted of soy protein isolate (93% pure protein).

The control diet was formulated to mimic the protein content of chicken feed (21% on dry matter and a similar amino acid profile). The rest of the dry matter was supplemented with potato starch. In addition, diets tested with 10.5% protein, 5% protein and 0% protein also work. In order to always obtain a comparable DM content (± 27%) and to avoid energy differences, the reduced protein was always compensated with an iso-energetic amount of starch (of course based on gross energy, since digestibility values are not known). 3 jars (polypropylene cups with mesh in the lid; diameter at the top 8.5 cm, bottom 7 cm; height 10 cm) of 100 larvae were set up per diet. The larvae were initially grown on wet chicken feed and were 6 days old at the start of the experiment (3-4 mg / larva). The larvae were fed every 2-3 days until their maximum weight was reached (a total of 130 g was fed per 100 larvae)

The results of this first experiment (Figure 41) showed no differences between the different protein levels. Only the 0% group was unable to develop into prepupa which was not surprising since this substrate contained only traces of protein. On the other substrates, the larvae grew to a final weight of 80 mg. This is a lot lower than on the chicken feed (more than 200 mg) but not surprising as there was a shortage of vitamins and minerals in the artificial diets.

That is why in a subsequent experiment a premix was added in which a broad spectrum of vitamins and minerals was present. The substrates tested now contained 21, 10.5, 5 and 2.5% protein and this time only the 2.5% treatment gave significantly reduced growth. The larvae grown on substrates with higher protein levels showed no mutual growth differences (Figure 42). But with 100 mg the final weight of these larvae was still a lot lower than the 200 mg we expect on a chicken feed.

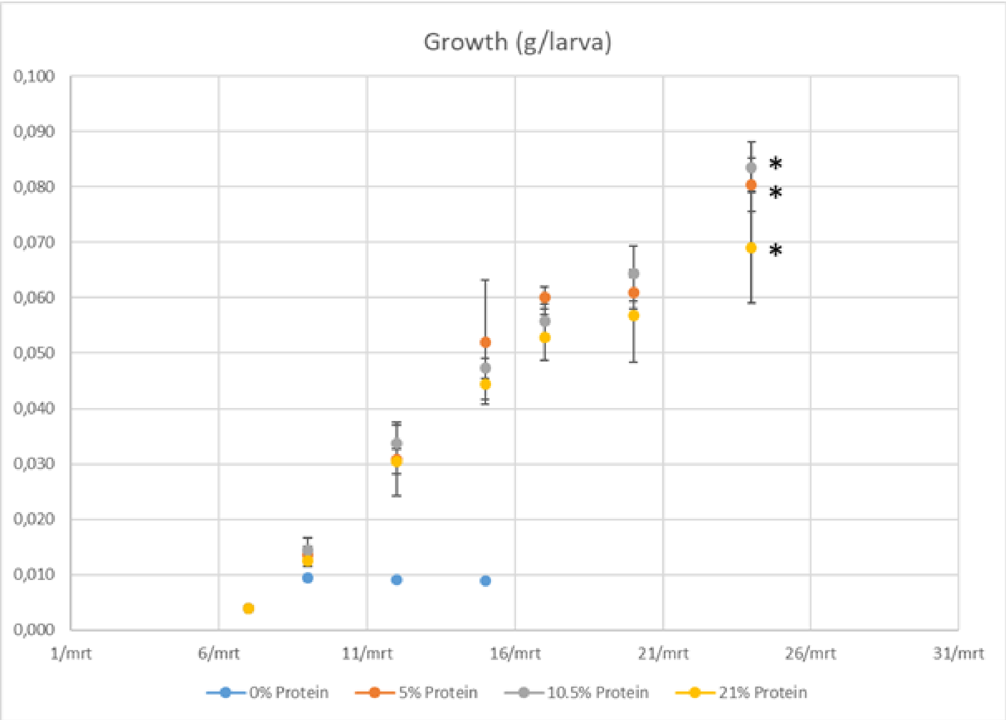

Figure 41: Evolution of the average individual weight of larvae grown on substrates (iso-energetic mixtures of soy protein and potato starch) with different protein levels. Different signs (or °) indicate statistically significant differences.*

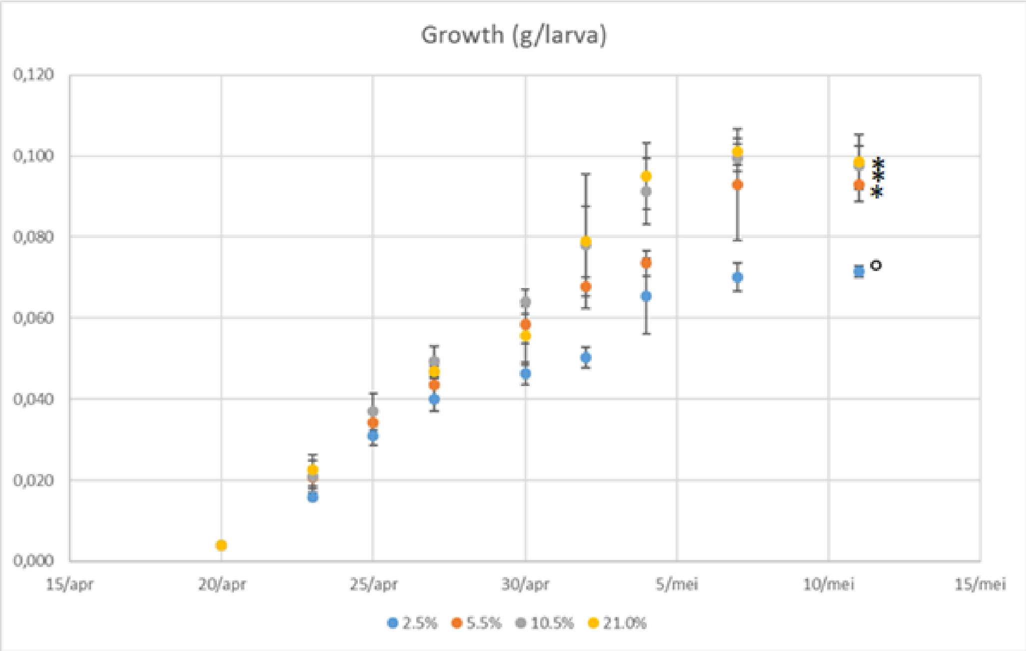

Figure 42: Evolution of the average individual weight of larvae grown on substrates (iso-energetic mixtures of soy protein, potato starch and premix) with different protein contents. Different signs (or °) indicate statistically significant differences.*

Therefore, another essential nutrient being fat was added in a following series of experiments. It was administered in the form of sunflower oil (rich in essential omega-6 and omega-3 fatty acids). Different fat levels were tested (8, 4 and 2%), at protein levels of 21% and 10.5%, but no significant differences were observed between treatments (Figure 43). Interestingly, the larvae from this experiment reached a final weight of 150 mg. The addition of premix and fat has therefore led to a growth improvement of 87.5%.

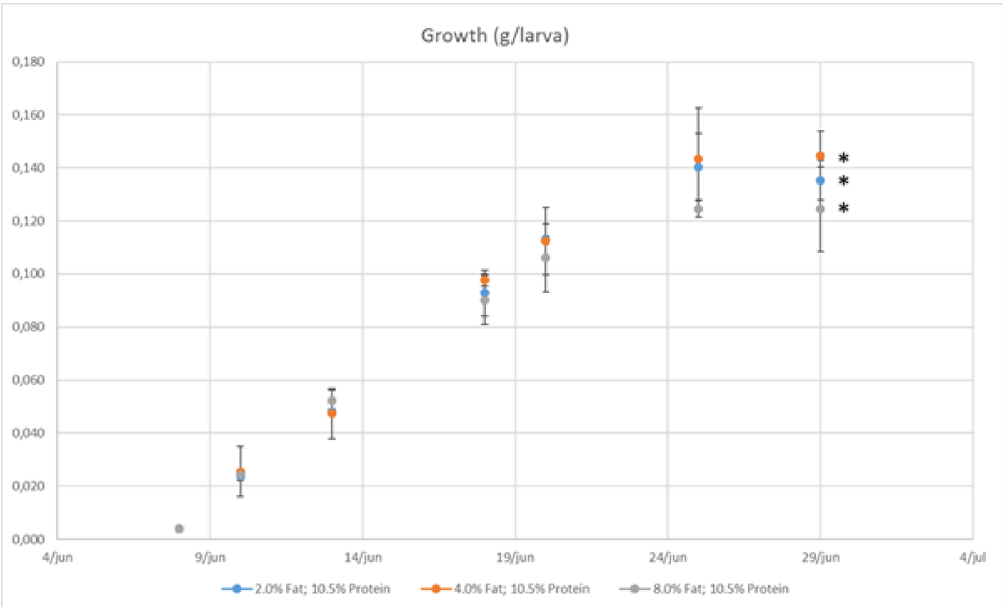

Figure 43: Evolution of the average individual weight of larvae grown on substrates (iso-energetic mixtures of soy protein, potato starch, premix and sunflower oil) with different protein contents. Different signs (or °) indicate statistically significant differences.*

From previous experiments we can deduce that a protein content of 10% (so half of what is in chicken feed) already leads to an optimal growth. The 2nd experiment (Figure 42) showed that there were not even differences between 10% and 5% protein. Since no fat was added at that time, this experiment was repeated with the addition of 4% fat. The 2 most interesting artificial diets, 10% and 5% protein, were compared. A diet with wet chicken feed was also included as a control (30% chicken feed and 70% water). In this experiment no differences were found between the artificial diets (Figure 44). This is very interesting as we can deduct from this that 5% protein, which is only 25% of what is in the chicken feed, is sufficient for an optimal growth of BSF larvae. Moreover, in this experiment everything was fed at the beginning (not every 2-3 days) and only 110 g of diet was given per repetition.

However, we must be careful when drawing conclusions as Figure 44 shows that the larvae on the artificial diets have grown 30% less than those on the control chicken feed. This is on the one hand surprising since the tested substrates contain all the necessary macronutrients and, moreover, they contain much more starch (and therefore more absorbable energy) than the chicken feed. On the other hand, many important micronutrients may be too short or completely missing.

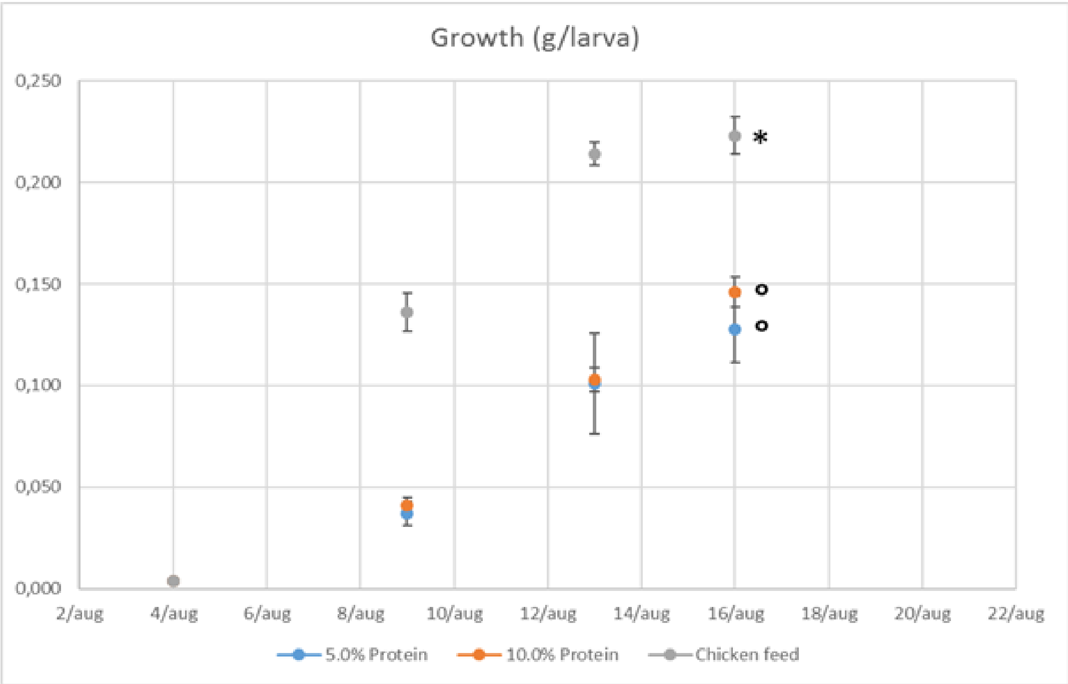

Figure 44: Evolution of the average individual weight of larvae grown on artificial substrates (iso-energetic mixtures of soy protein, potato starch, premix and sunflower oil) with different protein levels and larvae grown on a control substrate (chicken feed / water: 30/70) . Different signs (or °) indicate statistically significant differences.*

Sterols are essential nutrients for certain insect species and therefore a new experiment added an amount of 0.4% (Barragan-Fonseca, 2018). However, this proved to have no effect on our results. Furthermore, carotenoids such as lutein, which act as precursors of vitamin D, and other vitamins or precursors, may also be essential.

There may also be important differences in the digestibility of the macronutrients. Concerning the proteins, the levels of essential amino acids are particularly important. Soy is the most important protein source in chicken feed, but a significant proportion of synthetic amino acids are also added. The amino acid levels of the artificial diet are similar to the chicken feed, but the digestibility can vary considerably. The nature of the starch can also show important differences with regard to digestibility. The starch of chicken feed comes mainly from grains (corn, wheat and barley) and these (unprocessed) contain less resistant starch than potatoes. That is why different starch sources were compared with each other (wheat, corn and potato). No differences were found between larvae grown on potato starch and maize starch, while the larvae on wheat starch grew surprisingly less well. Replacing 25% starch with sucrose did yield an improved growth of 20%. This therefore indicates that there are problems with the digestion of the starch, but since starch also has a structural function, adding sucrose was not good for the structure of the artificial diet.

Figure 45: Larvae reared on artificial diets.

This brings us to another possible cause for the reduced performance on the artificial diet, namely the structure. On Figure 45 you can see that the artificial diet has a yoghurt-like structure that allows little air to pass through; the larvae are therefore inclined to leave this substrate. This is much less the case on chicken feed (Figure 46). Efforts have already been made to improve the structure of the substrate by means of cellulose (Barragan-Fonseca, 2018), but this did not result in any improvement. On the contrary, the cellulose absorbed a fair amount of water, as a result of which the substrates dried out too quickly and therefore the larvae could no longer absorb food.

Figure 46: Larvae reared on chicken feed/water (30/70).

Our first results provided a good basis for further development. In the future, the artificial diet will be further optimized and other nutrients will be considered in addition to protein. In addition, possibilities for improving the digestibility of nutrients for BSF larvae will also be looked into (e.g. by means of enzymes or symbiosis with micro-organisms).

7.1.5. Optimizing artificial diet

In a second series of experiments, the focus was still on protein. This time different protein sources were compared (i.e. soy and casein). Given the differences between these sources in amino acid profile, larval growth differences might reveal the essential amino acids for BSF larvae. However, the results didn't show any differences (Figure 47). Again the chicken feed control performed significantly better than the artificial diets. Interestingly, for the artificial diets, no differences were shown between 10% and 15% crude protein. These results confirm that 10% protein is sufficient for a BSF diet, however, the artificial diets still need to be improved in order to be sure.

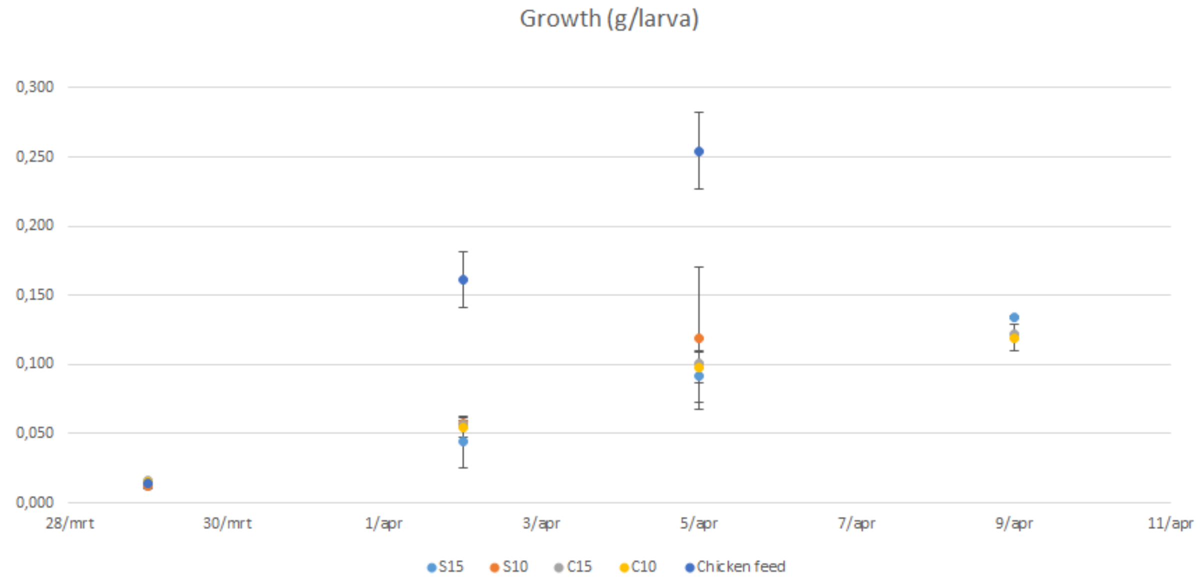

Figure 47: larval performance on different protein sources (S= Soy, C= Casein; 15% and 10% on DM basis)

Therefore, semi-artificial diets, with different protein contents, were developed. These diets had a Chicken feed basis and were added with soy protein and sugar (iso-energetic) (Table 2)

Table 2: semi artificial diets with different protein content

20% Protein	15% Protein	10% Protein	Chicken feed
50 g Chicken feed/water	50 g Chicken feed/water	50 g Chicken feed/water	100 g Chicken feed/water
6.85 g Sugar	8.30 g Sugar	9.75 g Sugar	
3.00 g Protein (Soy)	1.50 g Protein (Soy)		

The results showed that the Chicken feed control still yielded more biomass than the artificial diets, however, the differences were substantially smaller (Figure 48). In addition, in this set-up it was shown that larvae which received 15% protein in their diet performed significantly better than those who received only 10%.

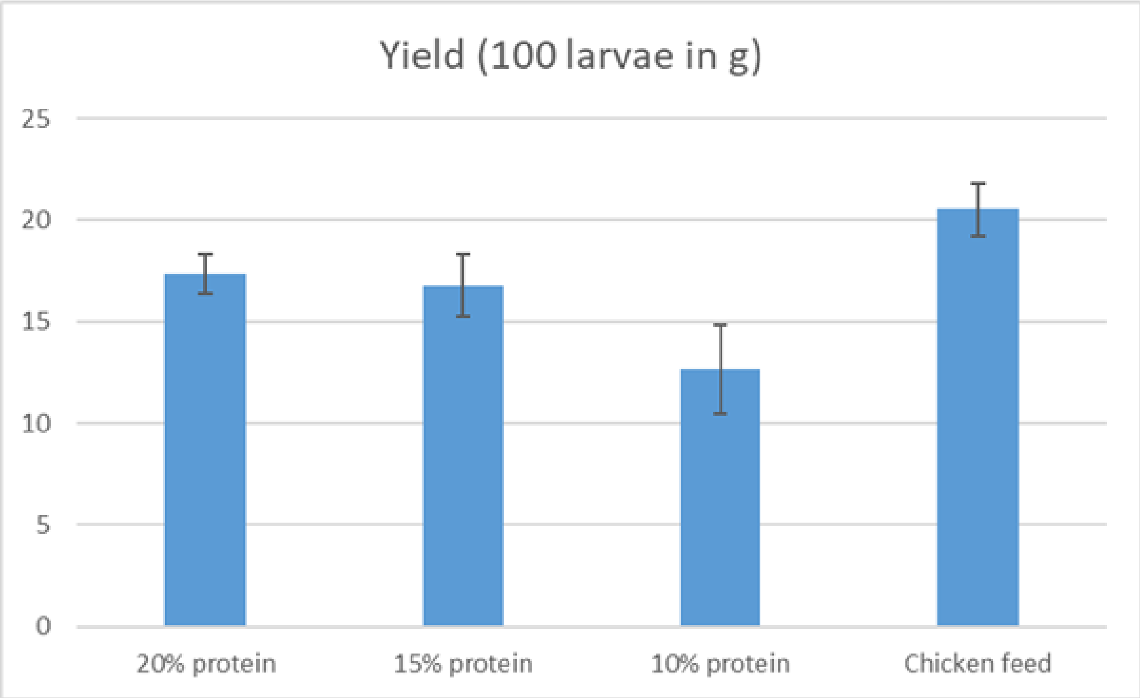

Figure 48: yield of 100 larvae reared on different semi artificial diets

Given that our artificial diets were substantially improved, the next step was to work with synthetic amino acids instead of soy protein powder. For most animals Lysine is the most important limiting amino acid. Another important essential amino acid for production animals is the sulphur containing Methionine. Therefore, the 10% protein diet from the previous experiment (Table 2) was modified with Lysine and Lysine + Methionine (Table 3). The amounts of synthetic amino acids were similar to what was present in the Chicken feed control diet.

Table 3: semi artificial diets with different amounts of essential amino acids

Lysine	Lysine + Methionine	Sugar	Chicken feed
50 g Chicken feed/water	50 g Chicken feed/water	50 g Chicken feed/water	100 g Chicken feed/water
9.57 g Sugar	9.52 g Sugar	9.75 g Sugar	
0.18 g Lysine-HCl	0.18 g Lysine		
	0.05 g Methionine		

The addition of amino acids didn't improve the performance of the larvae (Figure 49). However, unexpectedly, the "Sugar" diet (with 10% protein and no addition of synthetic amino acids) yielded more larval biomass than in the previous experiment (Figure 48). The produced larval biomass in the current experiment was similar as for the 15% protein diet in the previous experiment. Consequently, there was no space for improvement by adding the synthetic amino acids.

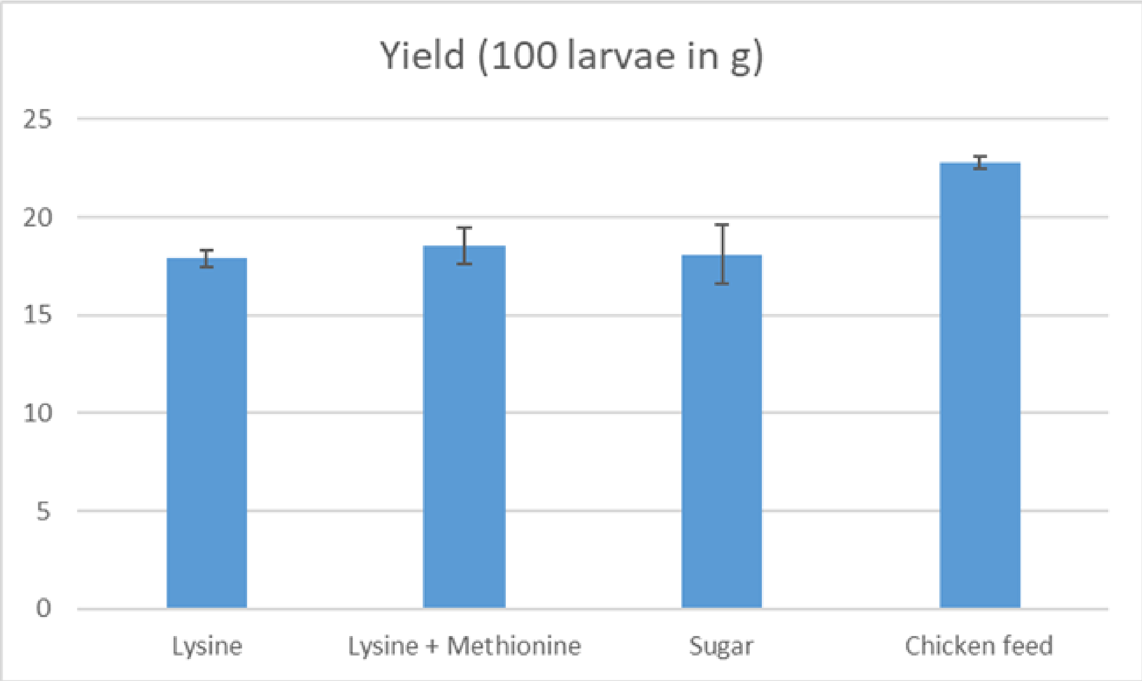

Figure 49: yield of 100 larvae reared on different semi artificial diets

Reference

Barragán-Fonseca, K. B. (2018). Flies are what they eat (Doctoral dissertation, Wageningen University).

7.1.6. Overview and advice

The results from the artificial diet experiments suggest that BSF substrates should contain 10-15% crude protein on a dry matter basis. This already has been confirmed by the pilot where multiple mixtures with different protein content were tested. Substrates with 10% protein could result in a good yield and favourable FCR, however, in general 15% protein substrates gave better results. Given that most horticultural side streams have lower quality protein (in terms of essential amino acids and digestibility), providing diets with 15% protein is advisable. Moreover, for most side stream mixtures it was no problem to reach 15% protein. Many horticultural side streams are surprisingly quite high in protein content.

From side streams tested in the lab (Table 4) (literature data could not be used because either data is lacking or larvae were over fed) a correlation could be found between the sum of proteins and non-fibre carbohydrates (NFC's) in the substrate and the larval biomass that could be harvested (yield) (Figure 50).

If we assume that 10% protein is sufficient, this substrate needs to contain at least 40 g NFC's/ 100 g dry matter in order to obtain a sufficient yield.

Table 4: overview of different side streams tested in the Insectlab. (CF: chicken feed, W: water)

	Chicken feed/water	Mango/Avocado	Mango/Avocado	Potato peels	Potato peels	Yacon/Green beans	Green beans	Brewer's spent grain	BasiQ/Spelt	BasiQ/Bran
Ratio	(30/70)	30% CF/W (30/70)	50% CF/W (30/70)	50% CF/W (30/70)	30% CF/W (30/70)	(50/50)	100%	100%	(90/10)	(90/10)
Protein (% DM)	20.45	13.49	16.15	15.72	13.09	10.85	19.61	24.7	11.93	14.86
Carbohydrates (% DM)	50	34.61	37.69	40.53	35.27	38.34	19.61	3	39.85	42.51
sum	70.45	48.1	53.84	56.25	48.36	49.19	39.22	27.7	51.78	57.37
Development time (days)	18	20	20	20	20	20	18	24	21	23
Harvest weight 100 larvae (g DM)	6.55	3.7	5.1	5.07	4.04	3.98	3.23	2.22	4.77	4.47
feed conversion (g dry feed/g larval growth in DM)	4.17	4.45	3.76	4.77	5.66	3.19	3.16	7.42	6.84	6.50

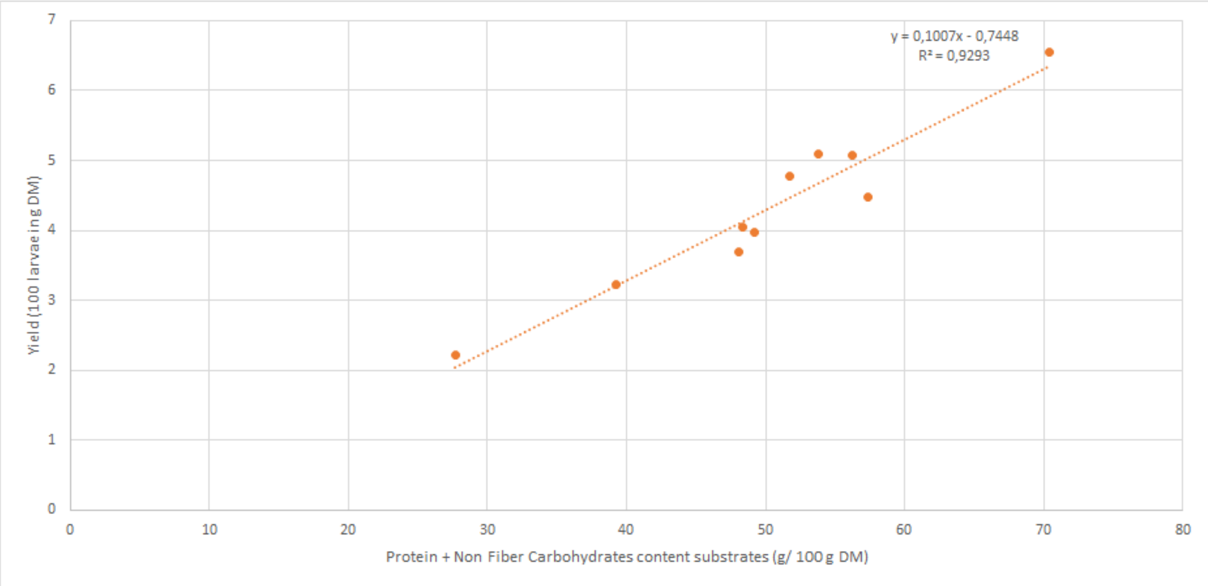

Figure 50: From side streams tested in the lab (literature data could not be used because either data is lacking or larvae were over fed) a correlation could be found between the sum of proteins and NFC's in the substrate and the larval biomass that could be harvested (yield).

7.2. Pilot plant research (Inagro)

Horticultural residues are diverse in availability, shape, structure and composition. For example, tomato stems are only released during a few weeks each year, are long-shaped, have a low feed value and contain a lot of fibre. While Belgian endive roots are almost year-round available, are low in fibre, high in non-fibre carbohydrates (starch and sugars) and may be contaminated with dirt. Even the same plant parts from different crops can differ strongly, the foliage of tomatoes contains almost no protein, while that of cabbage can be three times higher.

7.2.1. Processing and pre-treatment

Regardless of whether the residues are fed to mealworms or black soldier flies, the residues need to be shredded. Some insects can cope with larger pieces, but one has to be aware of the fact that unprocessed pieces that are too big to sieve out, might end up in your final larval product. We recommend to shred all plant residues in pieces smaller than 2 mm, or as small as practically feasible.

An insect pilot that is up and running year-round, will continuously need ingredients to feed to its larvae. Horticultural residues are not available year-round, most of the time a lot of biomass is released during a short period. Preserving this biomass with a minimal loss of quality is an important problem to tackle. Storing the residues in freezers or refrigerators is not cost-efficient on an industrial scale. An alternative is using natural anaerobic fermentation.

Table 5: availability of different crop residues. Peak availability in dark green, light green is a transition period with smaller amounts, grey is unavailable.

Crop	Residue	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Belgian endive	Forced roots	Dark green	Dark green	Dark green	Dark green	Dark green	Light green	Light green	Light green	Dark green	Dark green	Dark green	Dark green
Brussels sprouts	Stalks and foliage	Dark green	Dark green	Dark green	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Dark green
Cauliflower	Foliage	Grey	Grey	Grey	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Grey
Leek	Foliage	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Grey	Grey	Dark green	Dark green	Dark green	Dark green
Beans	Foliage	Grey	Grey	Grey	Grey	Grey	Grey	Light green	Dark green	Dark green	Dark green	Grey	Grey
Peas	Foliage	Grey	Grey	Grey	Grey	Grey	Grey	Light green	Dark green	Dark green	Dark green	Grey	Grey
Tomatoes	Foliage	Grey	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Dark green	Dark green

Anaerobic fermentation is a process where sugars are transformed by microbiota (such as lactic acid bacteria) in organic acids. This will make the pH drop, resulting in unfavourable conditions for bacteria that would otherwise spoil the residues.

Fermentation is already a common practice in agriculture, for example storing grass or maize in a silage or using fermented liquid feed for swine. Depending on the moisture content of the residue, different setups are possible. Moisture rich substrate can be fermented in fermentation barrels, dryer substrates are fermented in a silage. For our application, we chose for barrels, as most residues have a high moisture content. This moisture is initially still trapped inside the cells, but after shredding the moisture would cause leakage in a dry silage. Silage additives can be added to stimulate natural fermentation, we chose to add EM Silage™ as a standard practice.

7.2.2. Black soldier fly

Controlling the drying process of the rearing substrate during larval growth will determine the success rate of the harvest, a key factor of your production process. We have observed a delicate balance of different factors in order to achieve both fine dry frass and big larvae that can easily be separated from each other. These factors are:

- <u>The initial moisture content of the substrate:</u>
  Too much moisture (more than 75 %) will prevent sufficient drying, less moisture will make the larvae less efficient with the given nutrients. We aim for 30 % dry matter at the start.
- <u>Larval activity:</u>
  Higher larval activity, will create more movement in the substrate, making evaporation easier. We recommend at least 10,000 larvae. We aim for 20,000 larvae in a 60 x 40 cm box to create sufficient activity.
- <u>Layer thickness of the rearing substrate:</u>
  Obviously, the thicker the layer, the harder it is to dry out. We recommend not to exceed 10 cm, however a layer that is too thin (< 1 cm) will dry out within a few days, which is too fast for the larvae to process it. An optimal thickness is strongly dependant on the structure of the substrate. Fibres will make the substrate better aerated, allowing for a thicker layer (less chance for an anoxic region at the bottom of the box).
- <u>Microbial activity:</u>
  A lot of microbiota are active in the substrate and they will produce respiration heat (reaching up to 50 °C). More heat will make the water evaporate faster, but too much heat (> 40 °C) may

harm the larvae. The degree of heat production is of course also dependent on the composition of the substrate (e.g. sugar content).

- Ambient temperature, relative humidity:
  Dry, warm air is more efficient for drying, However if the air is too dry, there is a risk of crust formation (when the upper layer of the substrate dries faster than the larvae can process it). Which might cause trouble during harvest. We maintain 27 °C and 60 % relative humidity during larval growth.

- Air movement:
  Air replacement above the substrate is necessary to remove excess water vapour and CO_2. Therefor space between different boxes is necessary to guarantee air flow.

- Light:
  We recommend to rear the larvae in complete darkness. Larvae avoid light, making them crawl beneath the surface, which increases the risk of crust formation.

All horticultural residues share the same initial problem. As mentioned above we aim for 30 % dry matter in our initial rearing substrate, however all listed residues contain too much moisture (Table 6). As a result, rearing black soldier fly on pure residues in a production process is impossible. Apart from correcting the dry matter content, most residues will need a correction for either protein or sugars as well (in accordance to the advice given in 7.1.6 by Vives). Luckily in pig farming there is a wide range of feedstocks available that can be mixed with the residues in order to attain a well-balanced BSF diet.

Table 6: Nutritional composition of different crop residues.

Crop	Dry matter (% fresh matter)	Crude protein (% DM)	Ether extract (% DM)	Non-fibre carbohydrates (% DM)
Belgian endive				
Root (forced)	12.6	5.1	1.0	52.0
Foliage	4.3	25.4		
Brussels sprouts				
Foliage	17.1	20.7	3.1	20.0
Stalk	18.0	12.5	1.1	22.4
Green beans				
Foliage	14.2	16.0	3.3	25.6
Leek				
Foliage	12.0	14.4	2.5	31.3
Peas				
Foliage	20.4	18.4	3.1	
Tomato				
Foliage	12.6	8.8		
Stalk	24.2	5.4		
Tomato	6.8	16.6		
Whole plant	17.7	7.4		

The general work flow is shown schematically in Figure 51. Initially, the residue is collected fresh from the field and shredded. Depending on the availability of 5 day-old-larvae, it can be mixed in a balanced diet immediately, or preserved via natural anaerobic fermentation for later usage. The method is demonstrated on forced Belgian endive roots and Brussels sprouts. Other crop residues such as leek leaves, bean leaves and cauliflower leaves were also tested within BioBoost, but not within this standardized method. They were part of the necessary learning curve to establish this workflow, but this also means that these results are not representative for an industrial setting.

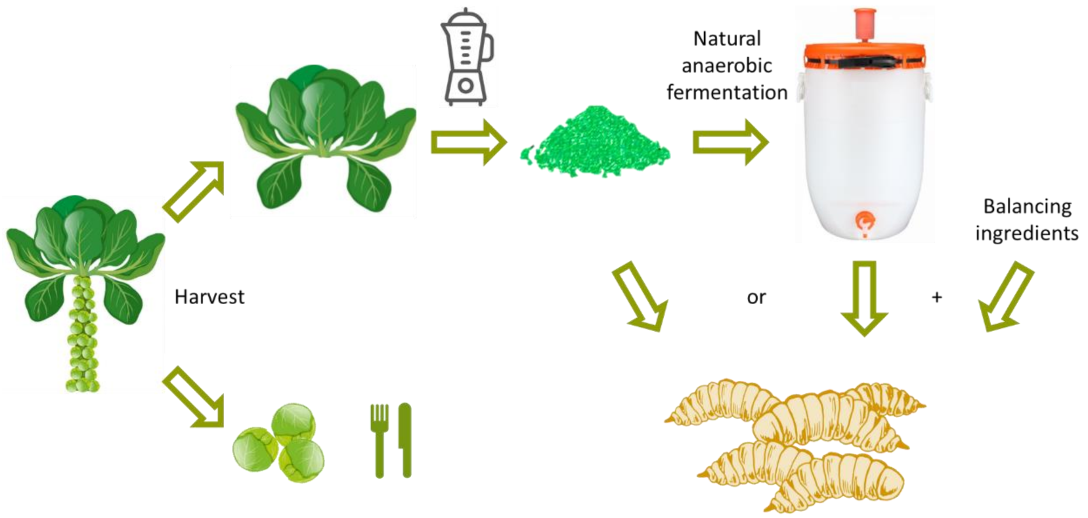

Figure 51: General work flow for testing residues with black soldier fly larvae.

Case study: forced Belgian endive roots

The white Belgian endive heads in the store are the result of a two-year crop. During the entire cultivation process, secondary streams are released at different times. First, green leaves are released during root harvest. Second, too large or too small roots are sorted out. Indeed, the aim is to obtain Belgian endive heads as uniform as possible after the forcing step. Only roots with an ideal size between 3 and 5 cm are used for the next step. After sorting the roots and the necessary vernalization- and/or storage period, the roots are planted again, in full soil or growing beds (hydroponics).

Third, to obtain the typical white colour of Belgian endive, forcing must be done in the absence of light. After 21 days in dark conditions, the heads are fully grown and ready for harvesting. The heads are separated from the root. Each root can be used only once to force Belgian endive as the growth point is also removed from the root together with the crop. For Belgium and the Netherlands, approximately 150.500 tons of roots are released as a third and perhaps largest side stream in this cultivation. It is this root that we will be focussing on in this case study.

Materials and methods

The composition of the forced roots is given in Table 6, generally it is high in moisture, low in proteins and high in sugars (compared to the desired levels determined under 7.1.6). Corrections will be necessary to formulate a balanced diet.

Correcting moisture can be achieved by adding dry feedstocks (spelt husks, wheat bran or FARM 1 crumble). Protein can be added in the form of wheat protein concentrate, soy paste, wheat bran or FARM 1 crumble. Potato starch can be added to balance the potential loss of sugars. The composition of all feedstocks is given in Table 7. No matter the diet, the same conditions have to be met: 30 % DM (as discussed earlier), at least 10 % protein (on DM basis), 40 % sugars (on DM basis) and maximizing inclusion of roots under these conditions

Table 7: Nutritional composition of all pure feedstocks in the Belgian endive case study.

		Belgian endive roots	Spelt husks	Wheat bran	Wheat protein concentrate	Potato starch	Soy paste	FARM 1 crumble
Dry matter	% fresh matter	12.7	94.0	87.0	26.3	30.0	8.3	91.7
Crude protein	% DM	5.1	7.4	17.3	29.4	4.4	21.8	19.2
Sugars (NFC's)	% DM	52	16.3	7.2	9.2	70.7	18	50

3 diets were formulated that met all conditions (diets 1-3). One additional diet, Diet 4, was formulated that was higher in protein (15 % on DM basis) and lower in sugar (35 % on DM basis). A last diet (diet 5), was constructed to be lower in protein (8 % on DM basis) and higher in sugar (51 % on DM basis). All formulations are visualised in Figure 52.

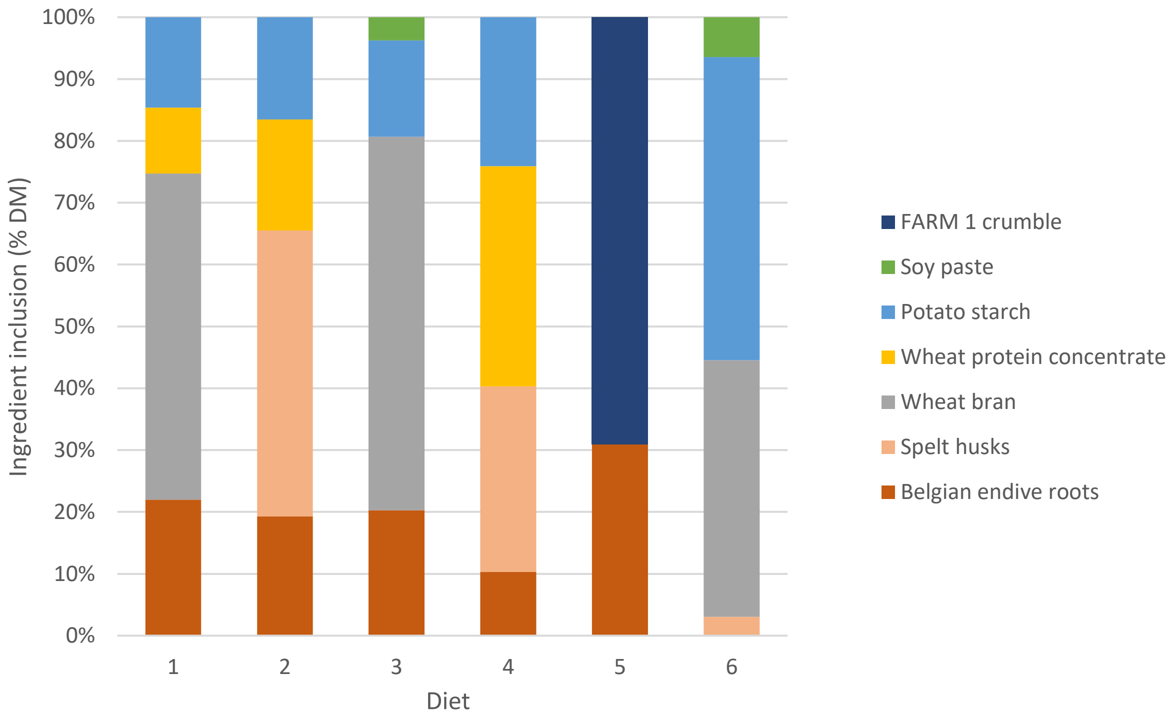

Figure 52: Composition of 6 different diets tested as black soldier fly larvae diet.

The experiment was carried out twice simultaneously for fresh forced roots and for fermented forced roots. Initially all roots were shredded, the fermented ones were shredded 7 days in advance and left to ferment in fermentation barrels.

All diets were mixed at the start of the experiment and tested in triplicate. The experimental conditions were the following;

- a box size of 60 x 40 cm,
- 10 kg of wet feed, all fed at the start,
- a density of 20,000 5 day old larvae (DOL) per box,
- a starting weight of 4.2 mg per larva (5 DOL),
- an ambient climate of 27 °C at 60 % RH.

After 7 days all boxes had finished eating (the substrate was dry and cooling down again). The larvae were separated from the substrate by mechanical sieving. The fresh weight of the larvae and the residual substrate was determined.

Results and discussion

There were no observable differences between fresh and fermented roots. It is perfectly fine to store the roots for at least one week after shredding them as long as the barrels stay closed.

Between the different diets, there were no observable differences in feed efficiency (Figure 53), except for the high protein diet (Diet 4) with an average FCR of 1.5, possibly due to wheat protein concentrate. The production of 2 kg fresh larvae requires 10 kg fresh feed. The larvae were still rather small (around 100 mg/larvae), but this was due to the fact that a limited amount of the diet was given at the start, ensuring a good processing of the substrate. Another notable result is that Diet 5 did not perform worse than diets 1, 2 or 3. Despite the high inclusion of roots (76 % on fresh basis), compared to only 50 % inclusion on fresh basis in the other diets.

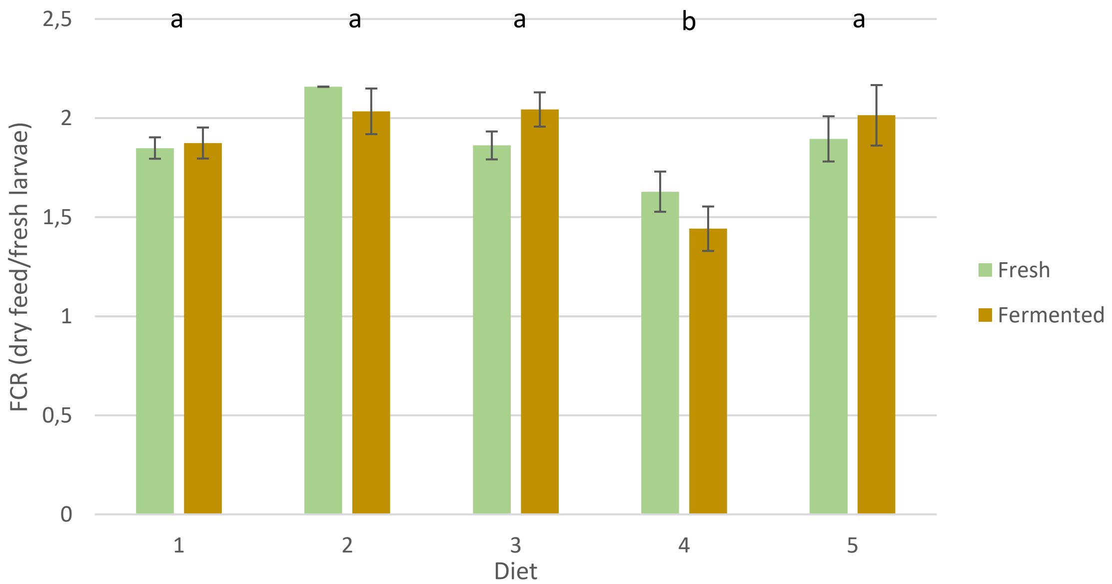

Figure 53: Feed conversion ratio of black soldier fly larvae on different diets as a mean ± standard deviation (n = 3) and their mutual significance.

Conclusion

Fermenting the roots does not have an adverse effect on larval performance, making this a cost-effective solution for storing this residue.

Mixing roots with the right ingredients can still result in an acceptable FCR (1.5 – 2), even for higher inclusion rates (50 – 75 % inclusion on fresh basis). In a 10 kg fresh diet, 7.6 kg roots and 2.4 kg chicken feed (FARM 1 crumble), will yield you 1.7 kg of fresh larvae and will leave you with 2 kg of residual frass.

Case study: Brussels sprouts

When harvesting the sprouts, stems (18 – 25 ton/ha) and foliage (11 – 18 ton/ha) remain on the field.

For Flanders, the Netherlands and the United Kingdom, a total average of 230 000 tons of stems and 190 000 tons of leaves is available. This high amount of available material makes this residue interesting for valorisation.

For this case study, the desired protein content will be raised from 10 % to 15 % on DM basis as a result of the findings of the previous case study.

Materials and methods

A mixture of stems and foliage was tested as a rearing substrate for black soldier fly larvae. The composition deviates from the ones in Table 6 as those are for pure stems and foliage. The composition of the mixture is given in Table 8. It has an overall balanced composition, but is too high in moisture (compared to the desired levels determined under 7.1.6). Corrections will be necessary to formulate a balanced diet. 6 different diets were formulated that needed to match the following conditions: 30 % DM (as discussed earlier), at least 15 % protein (on DM basis), 40 % sugars (on DM basis) and maximizing inclusion of residues (for diets 2 – 4) under these conditions. All formulations are visualised in Figure 54.

Table 8: Nutritional composition of all pure feedstocks in the Brussels sprouts case study.

		Brussels sprouts: stems and foliage	Forced Belgian endive roots	Wheat bran	Spelt husks	Dry potato starch	Industrial potato peel	Dry peas
Dry matter	% fresh matter	14.9	12.7	87.0	94.0	91.0	11.0	86.6
Crude protein	% DM	18.6	5.1	17.3	7.4	9.5	12.5	20.3
Sugars (NFC's)	% DM	45.7	52	7.2	16.3	62.5	53.9	41.6

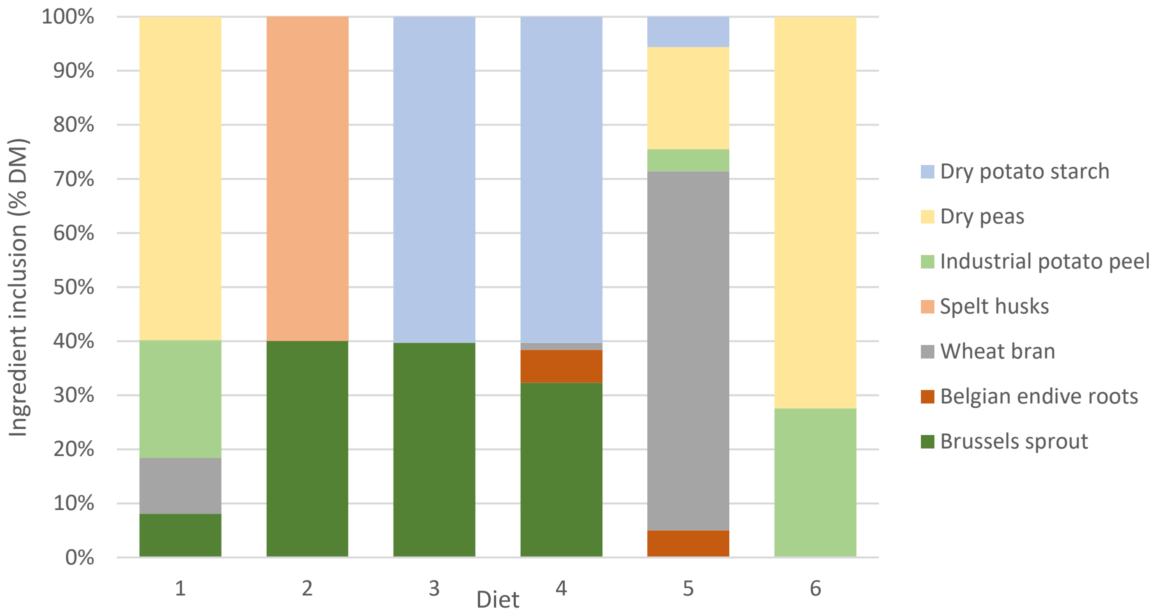

Figure 54: Composition of 6 different diets tested as black soldier fly larvae diet.

All diets were mixed at the start of the experiment and tested in triplicate. The experimental conditions were the following;

- a box size of 60 x 40 cm,
- 10 kg of wet feed, all fed at the start,
- a density of 20.000 5 DOL per box,
- a starting weight of 3 mg per larva (5 DOL),
- an ambient climate of 27 °C at 60 % RH.

After 7 days all boxes had finished eating (the substrate was dry and cooling down again). The larvae were separated from the substrate by mechanical sieving. The fresh weight of the larvae and the residual substrate was determined.

Results and discussion

Despite a comparable macro nutritional composition, there were significant differences between the different diets (Figure 55). A 10 kg diet containing 8 kg fresh Brussels sprout residues (40 % on DM basis), still yielded 1.6 kg of larvae. However the best (Diet 3) and worst diet (Diet 2), contained an equal amount of Brussels sprout residues.

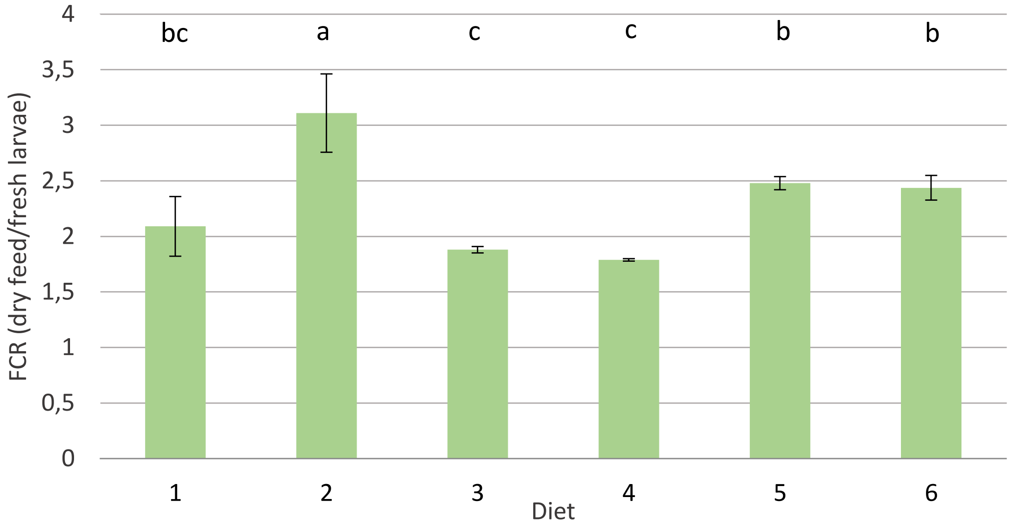

Figure 55: Feed conversion ratio of black soldier fly larvae on different diets as a mean ± standard deviation (n = 3) and their mutual significance.

Conclusion

Overall, growth was less efficient with Brussels sprouts than with forced Belgian endive roots. This could be due to the fact that the quality of the other feedstocks was worse, for example wheat protein concentrate is known for its high digestibility, but was not a part of the feedstocks in this case study. Despite that, a high inclusion rate (80 % on fresh basis) with a decent FCR (1.9) was achieved.

7.2.3. Mealworm

A big advantage of mealworm is that they live in a dry substrate. However they need moisture on a regular basis. As long as the dry feeding substrate is nutritionally balanced, the nutritional value of the residue is irrelevant, as long as there is sufficient moisture present and there are no adverse effects of certain components in the residues.

Dry feeding substrates are readily available on the market, ordinary wheat bran is commonly used, but is deficient in some nutrients. More specialised feeds are on the market as well (Table 9).

Table 9: Comparison of 2 dry feedstocks for mealworm based on FCR, price and development time.

Feed	Feed price	FCR	Feeding price	Development time
	EUR / kg feed	Dry feed / fresh mealworm	EUR / kg fresh mealworm	Weeks after starting oviposition
Wheat bran	0.35	2.14	0.74	10
INSECTUS Mealworm Grow ™	0.53	1.63	0.86	8

Importance of the moisture source

In an initial exploratory test, 9 different substrates (of which 5 were residues) were tested as a moisture source for mealworm. The dry feedstock was wheat bran and moisture source was daily supplied. At the start 17.5 g of mealworm (8.7 mg per mealworm) was placed per box. After 23 days the total fresh weight of the mealworms was determined (Figure 56).

Carrots are already commonly used as a moisture source. However some residues have similar potential, such as cauliflower leaves and the forced roots and white leaves from Belgian endive. Brussels sprout stems and leek foliage were outperformed by the others. Important to notice is that even carrots are not the best option, wet chicken feed outperforms all other substrates.

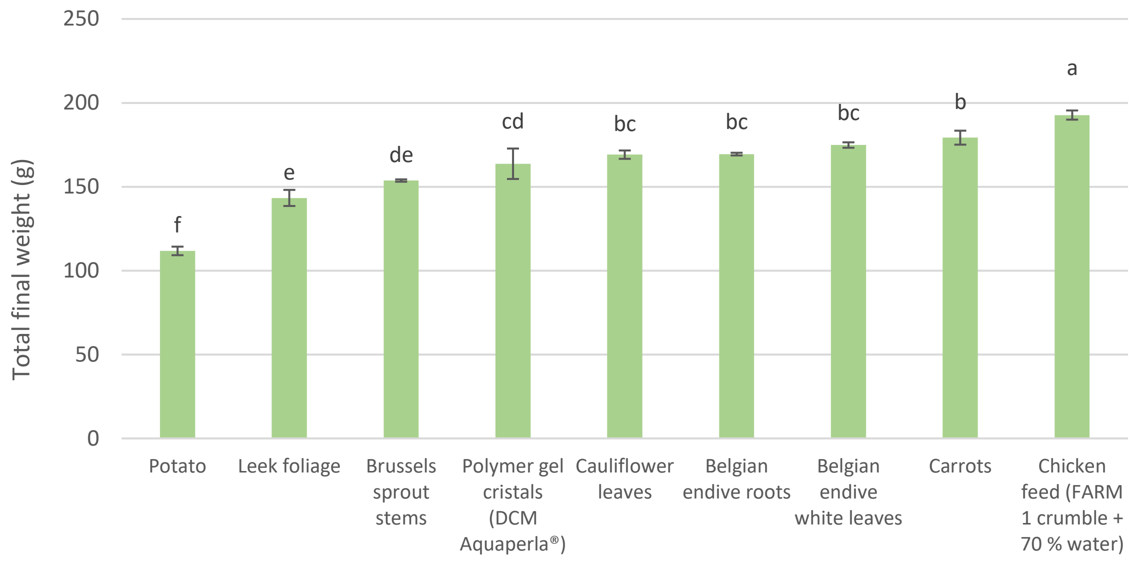

Figure 56: Total weight of mealworms harvested after 23 days on a diet of wheat bran and a daily supply of equal amounts of moisture source (pure uncut), the average value is given ± standard deviation (n = 3). The starting weight of the mealworm was equal for all treatments (17.5 g).

In a follow-up experiment, the influence of the original shape of the residue was excluded. For example, leek leaves will evaporate most of their water in no time, while a chunk of carrot will keep its moisture for a longer time. By mixing all residues into a similar wet pulp, only nutritional factors will play a role. The final weight of the mealworm is shown in *Figure 57*. A first notable difference is that mixed cauliflower leaves and mixed Belgian endive roots now outperform carrots. Secondly, leek foliage was one of the worst substrates in the previous test, now it differs no longer from the other substrates. This gives a strong indication that most residues could be used without much trouble, as long as they are properly mixed.

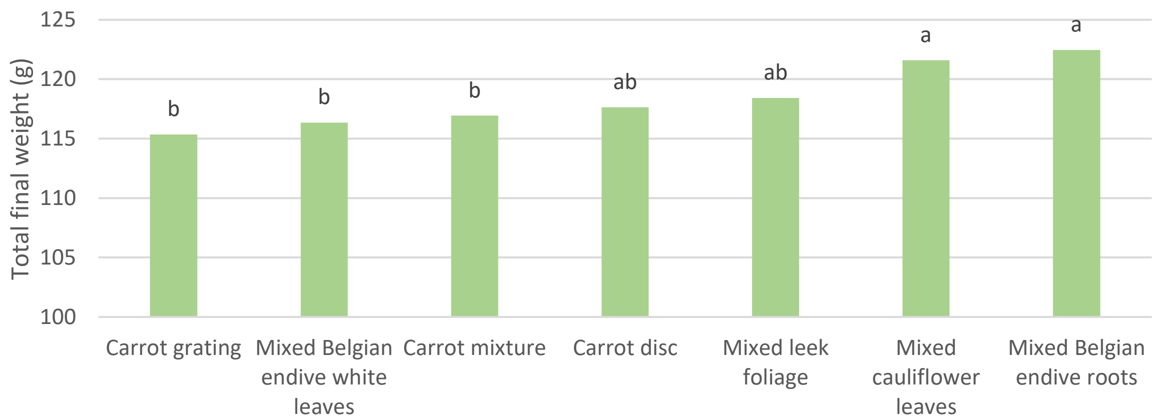

Figure 57: Total weight of mealworms harvested after 17 days on a diet of wheat bran and a daily supply of equal amounts of moisture source, the average value (n = 3). The starting weight of the mealworm was equal for all treatments (21.5 g).

Greenhouse foliage

Cucumber and tomato leaves were tested as a moisture source for mealworm. For this test wheat bran was replaced as a dry feedstock by INSECTUS Mealworm Grow (Table 9). Agar (a jelly-like substance, obtained from red algae) was used as a control moisture source. Agar was a significant better moisture source than uncut cucumber leaves (93 % yield compared to agar) and cucumber was in its turn significantly better than tomato leaves (83 % yield compared to agar) (Figure 58).

Unprocessed leaves are not very dense compared to agar. 200 g of agar are just some chunks spread over a box. A same amount of leaves will cover the entire surface of each box. The leaves evaporate water and create a nice humid micro-climate for the mealworm, this humid climate reduces mealworm mortality. There should be no difference between tomato and cucumber in number of mealworm per box after 7 days, however the boxes that got tomato leaves contained on average 13 % less mealworms (Figure 59). This difference can only be explained by toxins in the tomato leaves that effected mealworm survival. Cucumber did not differ from agar, the difference in yield has to do with a slower growth of the mealworm, possibly due to moisture that is hard to reach, as it is still trapped in plant cells. Mixing the cucumber leaves should solve this problem.

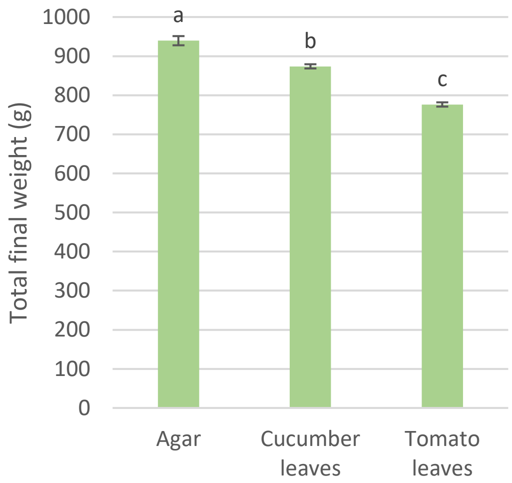

Figure 58: Total weight of mealworms harvested after 7 days on a diet of INSECTUS Mealworm Grow and a daily supply of equal amounts of moisture source (pure uncut), the average value is given ± standard deviation (n = 3). The starting weight of the mealworm was equal for all treatments (490 g).

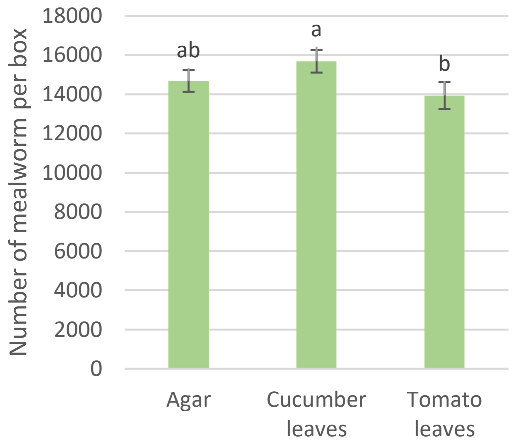

Figure 59: The estimated number of mealworms after 7 days on a diet of INSECTUS Mealworm Grow and a daily supply of equal amounts of moisture source (pure uncut), the average value is given ± standard deviation (n = 3).

A complete growth on Belgian endive roots

None of the tests mentioned above, were performed on a full rearing cycle of 8 weeks. Therefore, as a final test, mealworm were reared for 59 days on INSECTUS Mealworm Grow as a dry feedstock and forced Belgian endive roots (mixed) as a moisture source. The comparison was made between fermented and fresh forced roots (Figure 60). Fermenting the roots results in 6 % production loss on fresh basis (14 % on dry basis).

For the production of 2 kg of fresh mealworms from one box, you need 2.9 kg of INSECTUS and 4.3 kg of forced roots. In other words, per kg of fresh mealworm you need 1.45 kg INSECTUS and 2.15 kg forced roots. All dry feedstock can be given at once. Moisture source is given on a daily basis;

- 10 % during week 5,
- 10 % during week 6,
- 20 % during week 7,
- 40 % during week 8
- and 20 % during the last few days.

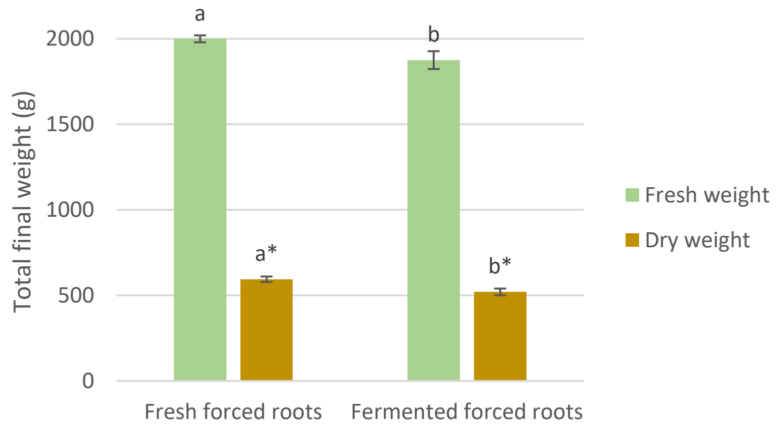

Figure 60: Total weight of mealworms harvested after 59 days on a diet of INSECTUS Mealworm Grow and a daily supply of equal amounts of moisture source (Belgian endive roots), the average value is given ± standard deviation (n = 4).

Conclusion

Mealworms are a reliable way to process a lot of pure residues as long as some conditions are met. First of all it is important to have a dry feedstock as a basis, this can be wheat bran, but better balanced alternatives are on the market. Secondly it is recommended to shred the residues, this way long plant fibres are cut and can be separated from the larvae in the end, it is also advantageous for the availability of moisture. A mealworm farm will need 2-3 kg of residues per kg of fresh mealworm product. A small farm that produces 100 kg mealworm each week will process at least 200 kg residues each week.

7.2.4. Residual substrate (frass)

In total 60 frass samples (20 from black soldier fly larvae, 20 from mealworm on wheat bran and 20 from mealworm on INSECTUS) were analysed to evaluate total nitrogen (Figure 61) and phosphorous content (Figure 62). The numbers are displayed next to the values of other livestock animals. It is clear that, especially mealworm frass, still contains a lot of nutrients and depending of the dry feedstock outcompetes all other sources of manure. This is partly due to the fact that mealworm frass is very dry (85 % DM), which concentrates the nutrients, for example broiler manure is only around 60 % DM.

The high nutrient content is positive news for applications as a fertilizer. However Flanders and the Netherlands have a history of a soil that is saturated with nitrogen and phosphorous, which might cause problems with emissions if fertilizing with insect frass is ill-considered.

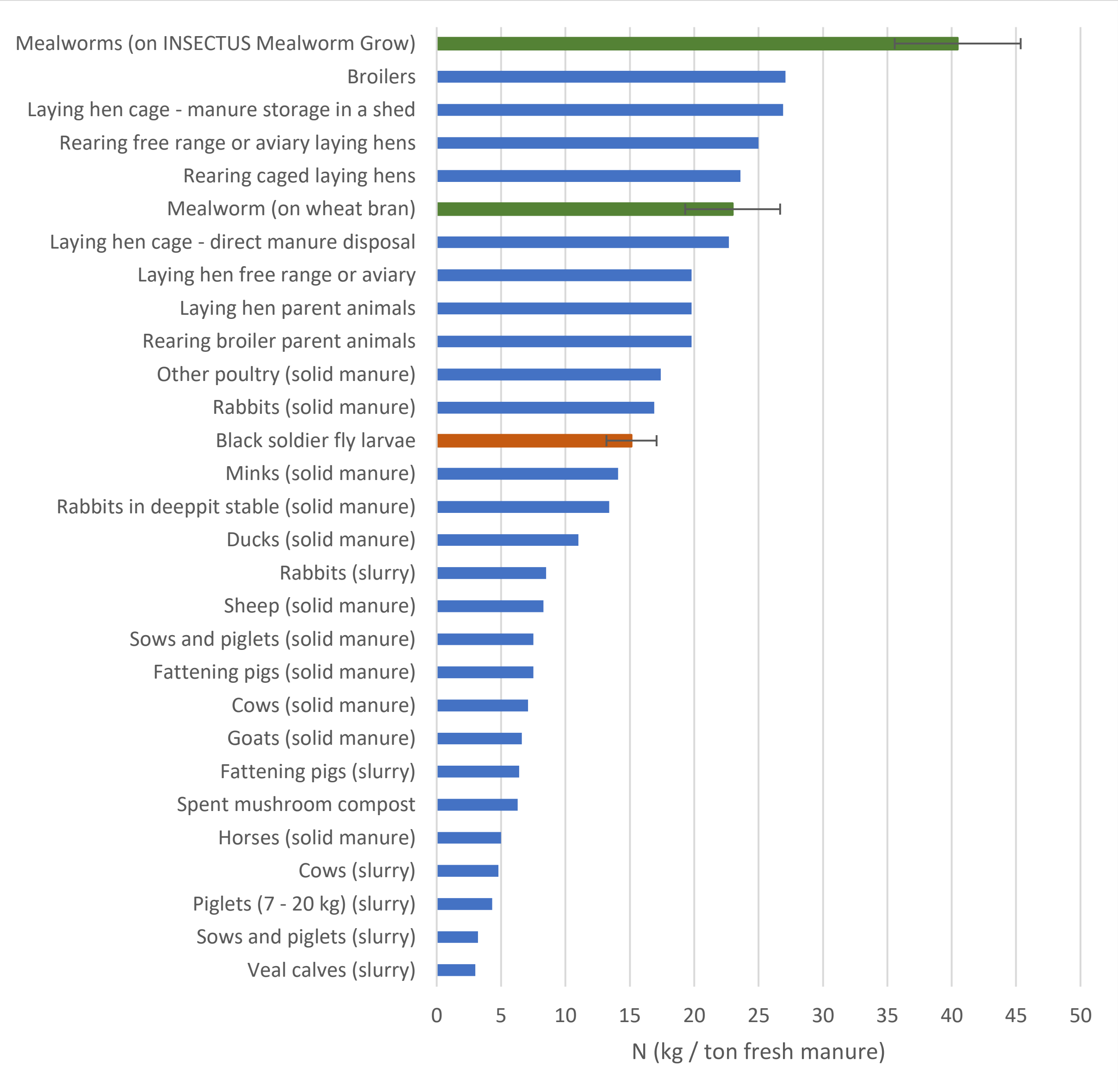

Figure 61: Total nitrogen content of different manure sources. The average of frass from mealworm and black soldier fly larvae are given ± standard deviation (n = 20, own laboratory analyses). The other figures in blue are from 'Normen en richtwaarden', VLM, versie juli 2019 – MAP 6.

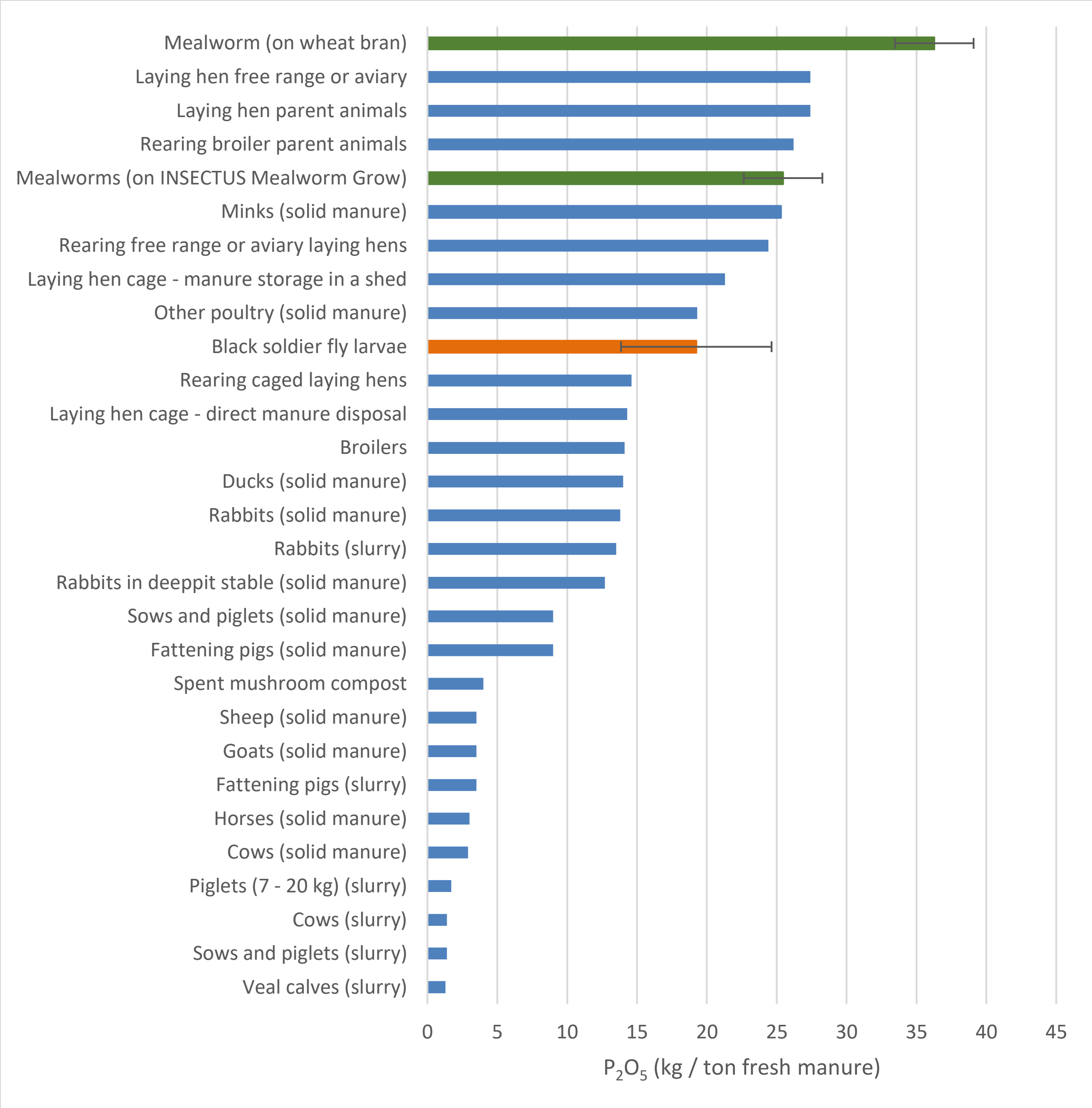

Figure 62: Total P_2O_5 of different manure sources. The average of frass from mealworm and black soldier fly larvae are given ± standard deviation (n = 20, own laboratory analyses). The other figures in blue are from 'Normen en richtwaarden', VLM, versie juli 2019 – MAP 6.

8. Future insect research (based on BioBoost)

Project name	Program	Involved partners	Start	End	Goal
Entobiota	The Strategic Basic Research (SBO) programme	Inagro (Partner)	1/01/2019	1/01/2023	Turning Black into 'Golden' Soldier Fly Larvae (Hermetia illucens): added value creation by exploring its microbiota and metabolism (e.g. amplifying the omega-3 fatty acid fraction of the insect fat).
Introsect	Vlaio-LA	Inagro (Leading partner) Hogeschool Vives (Partner)	1/09/2019	31/08/2023	The agricultural and horticultural sector in Flanders has been through a number of crises in recent years. As a result, farmers are looking for new, profitable activities. Professional insect rearing is an alternative. In Introsect the possibilities of transitioning farmers to insect breeding will be explored.
Valusect	Interreg NWE	Inagro (Partner)	26/09/2019	25/06/2023	The focus will be the development of a sustainable transnational Accelerator program including all partners with expertise on insects, food production, innovation and commercialisation to support and cooperate with enterprises. The knowledge will be transferred to enterprises by pilot demonstration and real life testing in close collaboration with end consumers via open/thematic calls for cases.
Susinchain	H2020	Inagro (WP leader)	1/10/2019	30/09/2023	Determining the specific requirements for the upscaling of insect rearing (Black soldier fly, yellow mealworm, cricket and house fly): 1. Validation of nutritional needs and preferred physical properties of feed substrates. 2. Optimisation of pre-treatment strategies for feed substrate ingredient and substrate mixtures. 3. Economic valuation of insect rearing by-products, other than chitin. 4. Actions to minimise effects of main pests and diseases in insect rearing systems. 5. Determination of transport and storage possibilities of insect eggs, small larvae and pupae. 6. Development of guidelines and organisation of courses focused on needs of insect rearing companies.

Project name	Program	Involved partners	Start	End	Goal
INSTEM	Provinciaal reglement inzake subsidiëring van projecten flankerend onderwijsbeleid	Hogeschool Vives (Leading partner) Inagro	1/09/2019	31/08/2020	Sustainability is a concept that is already extensively discussed in society and the media. Attention is also paid to this matter in the curricula of primary education and the first stage of secondary education. However, the concept of sustainability is very versatile and can sometimes seem rather hollow to young children. Young people in the 10-14 age group already have sufficient basis to give substance to this concept when it is made concrete from authentic and meaningful contexts. The intention is to make the abstract concept of sustainability tangible using a concrete case in which the role of insects is central. Within this project we want to integrate the case "insects as food for the future" into a STEM didactics. The children must be confronted with problems from this authentic context, which they can solve through "research", "design" and "optimize". The EC Agro and biotechnology, which already has a great deal of expertise on insects, and the EC Education Innovation of VIVES, which has built up a great deal of expertise around STEM, work together for this. By means of a design study, a process will be worked out in collaboration with a number of pilot schools and the process can then be further optimized based on try-outs. Hereby we have an eye for a link to the curriculum objectives regarding sustainability and STEM of the different networks.
Practical guide for insect growers and processors		Hogeschool Vives (Leading partner)	1/04/2019	31/12/2020	The preparation of a practical guide for insect growers and processors. This guide must provide clear guidelines for the breeding, harvesting and processing of insects. This manual must come about through closer cooperation between insect breeders (and / or processors), researchers and policy makers, so that the manual provides concrete answers to specific questions from practice. The manual should enable the entire sector to make better use of its time, quicker economic bridges will be formed and this will allow the sector to offer sufficiently sustainable, alternative proteins more quickly. At the same time, the manual protects the starting sector against malpractice arising from ignorance.