POULTRYNSECT

Compound diets formulation and *Hermetia illucens* larvae performance

Deliverables 1.1 and 1.2

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Abbreviations	
BCE	Bio Conversion Efficiency (dry larvae/ dry feed)
BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
DM	Dry Matter
DNF	Did Not Finish
FCR	Feed Conversion Ratio (dry feed/ live larvae)
FM	Fresh Matter
WP	Work Package

Content

Introduction

Introduction

The POULTRYNSECT Work Package 1 "Optimization of *Hermetia illucens* rearing protocols" aims to rear black soldier fly larvae (BSFL) for the poultry feeding trials in WP2. The WP is divided into four different tasks. The first two tasks concern to adjust the nutritional composition of the BSFL by testing different diet formulations for BSFL with by-products from the agri-food chain. This report joins the **preliminary results** obtained from the *in vivo* trials performed with *Hermetia illucens* (INAGRO) for deliverable 1.1 and 1.2.

Raising BSFL from their neonate stage to larvae of a harvestable size is not rocket science. However, to do this consistently on a feed composed of by-products from the agri-food chain with varying composition and properties, and meanwhile keeping feed conversion, larvae size, nutritional composition and harvestability of the final product consistent can be rather challenging.

Potential feedstocks of different origin were collected and analysed for their macro nutritional content in order to make mixtures that are more or less in line with the requirements of BSFL as they are currently presumed optimal (Table 1). A first category, agricultural by-products, involve by-products that arise on the farm. Most of these are plant parts (roots, stalks or foliage). However, there is one exception, being cheese whey, which is a by-product from cheese production. A second category are by-products that arise later on in the agri-food chain, these are former foodstuffs, unsold products from the shelves in retail. Animal proteins such as meat are legally not allowed in the diet of BSFL and this fraction should thus be sorted out of the retailer waste. Moreover, a lot of products in retail are packaged. All former foodstuffs should be unpackaged so no plastic wood or metal is present in feed for the larvae. A third category are feeds composed by companies or commercially available ingredients.

Table 1: Proximate composition of by-products from the agri-food chain.

FM: fresh matter, DM: dry matter.

		Dry matter content (% FM)	Crude protein (% DM)	Crude fat (% DM)
Agricultural by-products	Aubergine foliage	8.0	13.3	
	Brussels sprout stalks	18.0	12.5	1.1
	Cauliflower foliage	9.9	22.8	
	Cheese whey	6.2	12.1	0.8
	Chicory roots	9.6	4.5	1.7
	Cucumber foliage	6.8	13.0	1.2
	Green bean foliage	14.6	16.0	3.3
	Leek foliage	19.5	9.1	2.7
	Strawberry foliage	23.5	10.3	
Retailer waste	Bread	75.0	13.0	5.3
	Dairy and confectionery	31.0	26.0	34.0
	Fruit and vegetable waste	13.0	9.0	2.0
Commercial feed	DV 4614	92.0	16.7	3.4
	DV 4650	92.1	41.1	10.1
	Farm 1 Crumble	92.0	20.4	4.7
	Gainesville diet	91.1	16.0	4.1
	IBG	92.6	24.5	6.5
BSF requirements		**25 – 45**	**12 – 25**	**1 – 5**

A first thing that should be noted is that except for the commercial feeds, none of the by-products in itself is suitable for BSFL. In general the by-products are unbalanced in moisture, protein or fat.

The commercial feeds and mixtures of by-products were assessed as feed for BSFL in different feeding trials. The trials can be categorized in three subparts. First a general screening trial to select diets with the biggest potential, next a trial with the commercially available feeds and lastly an in depth trial with the most promising substrates.

1. Material and Methods

All feeding trials were performed in accordance with the "Standard protocol for performing insect feeding trials" that was developed in the "Susinchain" project (European Union's Horizon 2020 research and innovation programme under grant agreement No. 861976). Feeding experiments start with five day old larvae of around 3 mg and results are compared to the larval performance on the Gainesville diet which consists of 67% water, 17% wheat bran, 6.6% maize flour and 9.9% alfalfa.

Screening trial

A broad screening trial was set up with a variety of retailer wastes and agricultural by-products whether or not supplemented with feedstocks such as wheat bran or chicken feed to make for a more balanced BSFL feed. The proximate composition of the ingredients was already mentioned in Table 1, the specifics of the compound feeds tested are shown in Table 2. The number of replicates tested was dependent on the amount of by-products available.

Table 2: The specific composition (as given on a fresh matter basis) of the diets tested in the screening trial. No.: number of replicates tested.

Compound feed	No.	Feed description	BSFL density (No. larvae/g feed)
Bread – Fruit & vegetables	1	42.9% bread, 57.1% of a fruit and vegetables mixture. Both are retailer wastes.	1.5
Bread – Water	1	46.7% bread, 53.3% water. Bread is retailer waste.	1.5
Bread – Whey	2	46.7% bread and 53.3% cheese whey.	1.5
Brussels sprout stems – Wheat bran	10	90% brussels sprout stems, 10% wheat bran.	1.5
Chicken feed – Bran – Water d1	3	6.8% Farm 1 Crumble, 23.2% wheat bran, 70.0 % water.	0.9
Chicken feed – Bran – Water d2	3	6.8% Farm 1 Crumble, 23.2% wheat bran, 70.0 % water.	1.8
Gainesville diet	3	33.0% of dry Gainesville diet, 67.0% water	1.5
Gainesville diet – Whey	3	33.0% of dry Gainesville diet, 67.0% cheese whey	1.5
Leek – Wheat bran	8	83.3% leek foliage, 16.7% wheat bran	1.5
Wheat bran - Water	2	75.0% wheat bran, 25.0% water	1.5
Cauliflower foliage – wheat bran d1	3	74.6% cauliflower foliage, 25.4% wheat bran.	0.9
Cauliflower foliage – wheat bran d2	3	74.6% cauliflower foliage, 25.4% wheat bran.	1.8

Commercial feed trial

Three companies were contacted with the question to construct a feed for black soldier fly larvae production that was organic. Resulting in 4 tested commercially available feeds (DV 4614, DV 4650, Farm 1 Crumble and IBG, see Table 1).

Retailer waste trial

Different mixtures were made of former foodstuffs (unsold products from retail). Details can be found in Table 3.

Table 3: Feed mixtures (column titles) and their ingredients (as given).

Ingredients	Gainesville	Retail mix	Retail vegan	Retail dairy
Water	67.0%			
Wheat bran	17.0%			
Maize flour	6.5%			
Alfalfa	9.5%			
Bread		43.0%	49.0%	23.0%
Dairy & confectionary		20.0%		77.0%
Fruit & vegetables		37.0%	51.0%	

2. Results & discussion

Screening trial

The screening trial focussed on the overall performance of the BSFL on an array of different tested mixtures as described in Table 2. Feed conversion ratio (FCR) is used as a way of comparing the performance as it assesses the production potential of live larvae given the amount of substrate (on a dry basis) that was given to the larvae. This parameter is less dependent on the amount of larvae used per amount of feed opposed to the average weight of the larvae at harvest.

The results of the screening trial are shown in Figure 1. The lower the FCR is, the more efficient the larvae converted their feed into larval biomass. The lowest that is practically feasible for the larvae is an FCR 1.1 on chicken feed. None of the tested feeds is as efficient. The best performing diet that contained agricultural by-products was a mixture of leek foliage and wheat bran with an FCR of 1.8. Bread seemed to be a viable alternative as a dry ingredient compared to wheat bran. However, when mixed with a fruit and vegetable mixture from retail, the performance of bread drastically dropped. Probably because the efficient bread is partially replaced by inefficient vegetable fibres.

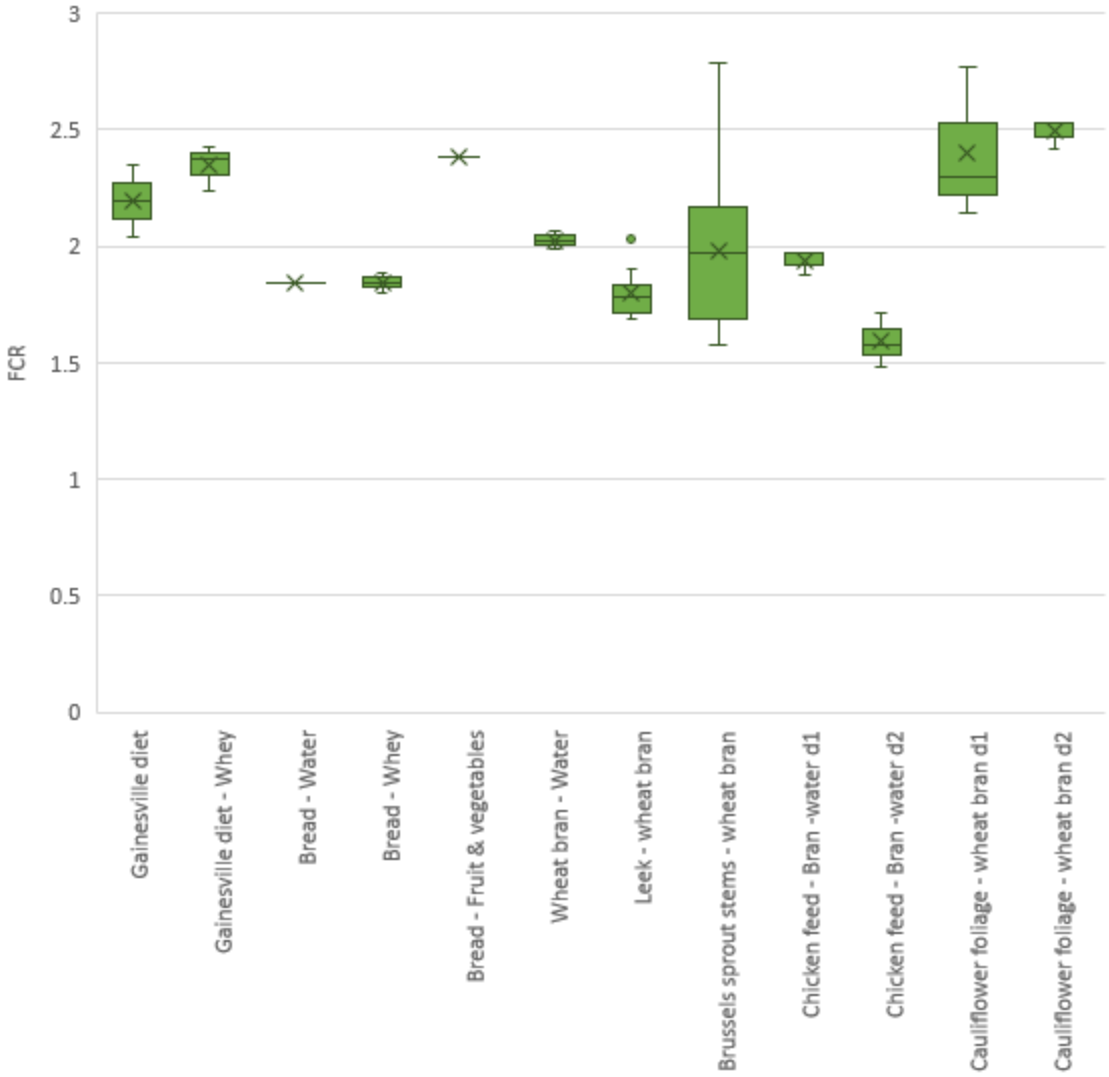

Figure 1: Feed conversion ratio (FCR) of larvae grown on different compound diets (amount of dry feed needed to produce an amount of live larvae).

Commercial feed and retailer waste

Following the screening trial, it was decided to proceed on trying to optimise a compound diet based on retailer waste. The advantage of retailer waste is that no external ingredients (such as wheat bran) are needed to compose such a diet, as the mixture in itself is already near the desired ranges for the BSFL. This means that there should be less competition over potential feedstock ingredients as currently most of the retailer waste is processed in a biogasplant, only bread is valorised to some extend in the agri-food chain as pig feed.

The results presented in Table 4, clearly show that a too high fat content can cause problems for the BSFL. The diet '"Retail dairy" was the most rich in fat (at around 21% on DM basis) and this diet was never processed in a way that would end up as a sievable frass at time of harvest. Moreover, the fat encouraged the larvae to escape on mass and as a consequence it was decided to discard this treatment. A similar problem was observed in the "Retail mix" diet however, to a far lesser extend (only 9% crude fat on a DM basis). The diet finally evolved to a

diet with some fairly large frass granules of similar size of the larvae. This complicated the separation of larvae and frass at harvest but is was still manageble. The other diets gave no such troubles and developed without many problems. In general the retail mixtures that could be harvested, performed very similar and managed to outperform the control diet and two commercial diets based on average weight of the larvae, BCE and nitrogen efficiency (the amount of nitrogen in the feed that was valorised in larval nitrogen). Farm 1 Crumble, a chick feed, was far superior in all assessed categories except for, although not formally tested, sustainability. It makes little sense to feed the larvae chicken feed and than feeding the larvae to chickens. All in all the retail diets performed similar to the commercial IBG diet (which contained, i.a., soy).

Table 4: Larval performance parameters for different feeds. BCE: Bio Conversion Efficiency.

Diet	Development time (days)	Average larvae weight [mg]	BCE (dry larvae/dry feed)	Nitrogen efficiency
Gainesville diet	7	87	11.7%	36.0%
Retail mix	9	152	18.9%	43.8%
Retail vegan	8	118	17.3%	42.7%
Retail dairy	DNF	DNF	DNF	DNF
Farm 1 Crumble	8	168	25.3%	61.4%
DV 4614	7	109	16.2%	54.7%
DV 4650	7	94	14.6%	22.3%
IBG	8	125	18.7%	40.8%

One of the more remarkable properties of the larvae grown on diets containing retailer waste, is their high fat content as shown in Table 5. This higher fat content seems to be specifically linked to the bread as it is observed in both the fat poor retailer feed (Retail vegan) and the fat rich one (Retail mix). Bread is especially rich in carbohydrates which are apparently quite efficiently converted into larval fat. This could be of major importance for the poultry feeding trials as the fat consumption could drastically differ depending on the origin of the larvae. This higher fat content has another trade-off as less crude protein can be consumed in the same feeding time.

Table 5: Nutritional composition of larvae Grown on different diets. DM: dry matter. Nitrogen conversion factor = 4.43 (Smets et al., 2021).

Diet	Crude protein content (% DM)	Crude fat (% DM)
Gainesville diet	35.0%	16.4%
Retail mix	24.2%	53.3%
Retail vegan	22.2%	52.9%
Farm 1 Crumble	29.4%	33.1%
DV 4614	33.5%	23.0%
DV 4650	37.2%	24.8%
IBG	31.9%	20.8%

When we look at the composition of this fat (Figure 2), the most striking observations are that the samples fed a Retail diet contained significantly more lauric acid C12:0, namely 55% vs.

40% in the other samples. As a result, the Retail samples contained less palmitic acid C16:0, but especially a lower concentration of linoleic acid C18:2.

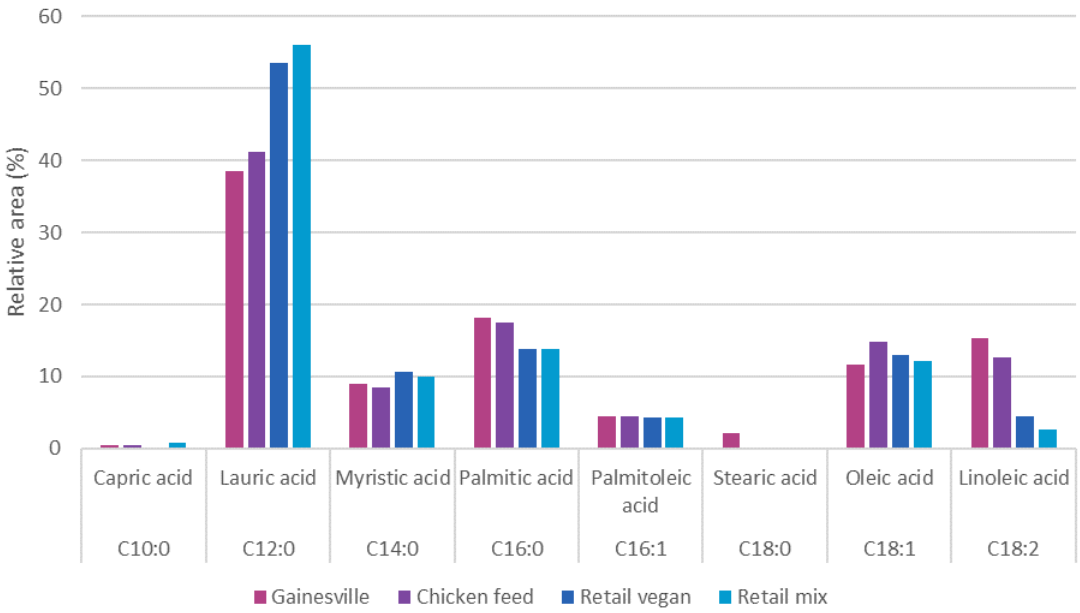

Figure 2: Fatty acid compostion of BSFL fed with different diets.

3. Conclusion

Black soldier fly larvae can be reared on an array of different by-products from the agri-food chain as long as their nutritional needs are met to some extend and the physical properties of the feed are hospitable for the larvae. However, using a different diet might result in larvae with a different composition. Especially fat content seems to vary strongly between diets. Especially when the diet is rich in non-fibre carbohydrates the larvae seem to incorporate it into fat.

For the poultry trials larvae of consistent quality will need to be reared over prolonged periods. Due to the inherent nature of agricultural by-products they are only available seasonally and often only for short periods. As for retailer waste, it is very variable in composition and also seasonally dependant. To have controlled conditions for the poultry feeding trials its seems therefore necessary to use a control feed for the larvae, preferably a feed that can be reproduced in other BSFL rearing facilities. It was therefore decided to do the poultry feeding trials with Gainesville diet.

References

Smets, R., Claes, J., & Van Der Borght, M. (2021). On the nitrogen content and a robust nitrogen-to-protein conversion factor of black soldier fly larvae (*Hermetia illucens*). Analytical and Bioanalytical Chemistry, 413(25), 6365-6377.

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