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Bioconversion of Guldborgsund Municipality's residual sub-strates into insect meal

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1. Aim

The main aim of the research was to bio-convert different residual substrates from the municipality of Guldborgsund with Black Soldier Fly Larvae (BSFL) *Hermetia illucens* intended for fish-feed trials. The research is a part of the INTERREG North Sea region project BIOCAS which focuses on realizing concrete Biomass Cascading Alliances for a more sustainable conversion of residues, using the Circular Economy approach.

2. Methods

The research was divided in 4 phases: i) testing, ii) production, iii) processing and iv) perspectives. Temperature and relative humidity loggers were used during the testing and production phases, in order to monitor environmental conditions in the trays.

2.1 Testing

During the testing period, a series of experiments: dietary and silage experiments, were conducted. The experiments were conducted under similar and controlled temperature, density, humidity and light intensity conditions. All the substrates and diets were frozen before being used in the experiments in order to ensure no degradation and similar substrate quality during different experiments. The tests were conducted in small trays of 200 x 300 mm (se figure 1).

Figure 1: Production of BSFL reared on 4 different diets during the dietary experiment

Dietary experiment: A series of substrates (i.e. seaweed, apple pomas, spent grains, wheat, rapeseed cake, sugarbeet tops, malt and Danish cookies) from Guldborgsund Municipality were analyzed for their protein (P), fat (F), carbohydrate (C) and ash (A) concentrations (Table 1).

Table 1: Guldborgsund substrates and their characteristics, analyzed before being used in developing different diets

Promissing substrates	Seaweed	Spent grain	Apple pomas	Wheat	Rapeseed cake	Sugarbeet tops	Malt	Danish cookies
Ash %	4	1	1	1	7	3	7	0
Protein %	1	6	1	11	32	4	22	6
Fat %	0	3	1	2	9	0	3	21
Carbohydrates %	8	15	17	73	44	6	62	70
Dry Matter DM %	13	25	20	88	93	14	94	97
Ash % DM	31	3	4	1	8	24	7	1
Protein %DM	7	25	5	12	35	29	24	6
Fat %DM	1	11	4	3	10	3	3	22
Carbohydrates %DM	61	61	87	84	47	45	67	71
Seasonality	All year/sileage	All year	Sep-Dec /dried?	All year	All year	All year/sileage	All year	All year

Based on these substrates and the results from the analysis, 4 different diets were developed and used as feed for BSFL (table 2).

Table 2: Substrate fraction, dry mater, protein, fat, carbohydrate and ash concentration on both fresh weight (fw) and dry matter (dm) basis, of the 4 diets developed.

Substrates GBS	Fraction	Diet 1			Fraction	Diet 2			Fraction	Diet 3			Fraction	Diet 4		
Seaweed	0.10	DM%	20		0.05	DM%	19			DM%	18	17.59	-	DM%	20	
spent grain	0.15	A	1	% FW	0.20	Ash	1	% FW	0.19	Ash	1	% FW	0.25	Ash	1	% FW
apple pomas	-	Protein	5	% FW	0.10	Protein	5	% FW	-	Protein	3	% FW	0.10	Protein	6	% FW
	-	Fat	2	% FW	-	Fat	1	% FW	-	Fat	2	% FW	-	Fat	1	% FW
wheat	0.20	Carb.	25	% FW	0.10	Carb.	20	% FW	0.11	Carb.	15	% FW	0.10	Carb.	20	% FW
Rapeseed cake	-				0.02				-				0.02			
	-	Ash	6	%DM		Ash	7	%DM	-	Ash	3	%DM		Ash	6	%DM
sugarbeet tops	0.05	Protein	24	%DM	-	Protein	28	%DM	0.02	Protein	19	%DM		Protein	29	%DM
malt	0.05	Fat	10	%DM	0.10	Fat	7	%DM	0.03	Fat	9	%DM	0.1	Fat	7	%DM
danish cookies	0.05	Carb.	59	%DM		Carb.	59	%DM	0.03	Carb.	69	%DM		Carb.	58	%DM

The aim of this experiment was to develop efficient BSFL diets, based on residual substrates from Guldborgsund Municipality. The dietary experiment consisted of 3 replicates per dietary treatment and was conducted under stable temperature (27ºC), density (11 larvae/cm²) and light/dark ratio (14/10)[1]. The larvae were fed with a total of 375 g dry matter (dm) diet/replicate during 3 feeding episodes and were maintained under controlled conditions for a period of 12 days. The larval performance (larval biomass, substrate reduction and feed conversion rate (FCR)) and larval survival rate (%) were analyzed on both fresh weight (fw) and dm basis and used to identify the best diet required for the silage experiment as well as for the production phase.

Silage experiments: The aim of these experiments was to identify the impact of ensilaged sugar beet tops and catch crop on the performance of BSFL. Therefore, a series of diets were tested. In the first experiment, Diet 3, (the best diet identified during the previous experiment), was used under 2 different scenarios: i) the sugar beet tops were ensilaged before being used in the diet (Diet S) and ii) the sugar beet tops were not ensilaged before being used in the diet (Diets 3). These two dietary treatments were used together with a reference chicken feed (Paco Start) (Diet C) in the production of BSFL. The chicken feed was provided by Land & Fritid. In the second experiment, a cheap diet (Diet X) consisting of catch crop silage (8%), wheat (3%), spent grains (17%), malt (12%) and water (59%) was developed and used in the BSFL production. Both experiments consisted of 3 replicates per treatment and were conducted under stable temperature (25ºC), density (10 larvae/cm²) and light/dark ratio (14/10). The larvae were fed

[1] Carusco et al., 2013. Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae. Online source: https://uved-formation-aquaculture.cirad.fr/content/download/4328/32130/version/3/file/BLACK+SOLDIER+Technical+Handbook.pdf

with a total of 394 g (dm) diet/replicate during 2 feeding episodes and were maintained under controlled conditions for a period of 12 days. Similarly, as in the case of the dietary experiment, the larval performance and larval survival rate (%) were recorded.

2.2 Pilot production

During the pilot production the BSFL were fed on Diet 3 and the rearing was conducted in standard trays of 60x40, in 2 batches (total production trays: 38). The larvae were produced under controlled laboratory conditions: temperature 27ºC, density: 10 larvae/cm²; feeding episodes: 3; light/ dark ratio (14/10), production time: 12 days. The larval performance and larval survival were recorded. Moreover, the dm, protein, and ash content were analyzed through the production system (diet, BSFL and insect frass) and used to calculate the mass balance on the system. The fat content was determined in the diet and the larvae, while the nutrient profile (NPK) was analyzed in the insect frass.

2.3 Processing

The final pilot processing was carried out in the pilot scale biorefinery at DTI in Taastrup. Stirred and heated tanks in stainless steel with a connected wet mill was used to solubilize the biomass which was then fractionated stepwise with decanter centrifuge (GEA). The pH and temperature were adjusted during different steps of the process. Ventilated ovens and a pilot spray drier (DRY-TEC) was used for drying the products. All fractions were analyzed for content of water, ash, lipid and protein.

2.4 Business and LCA perspectives

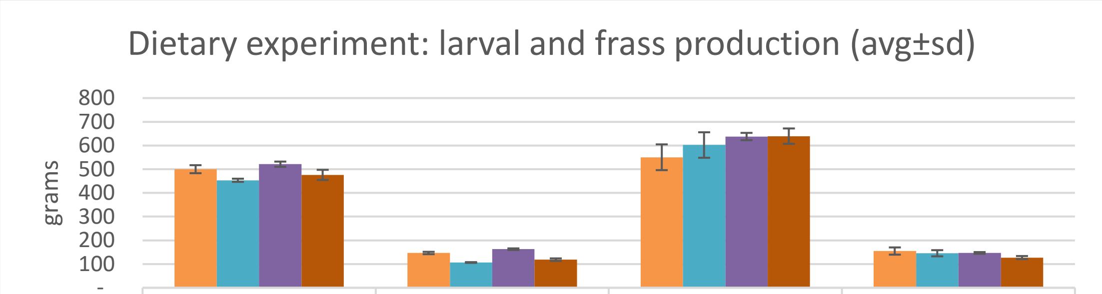
The data from the testing, production and processing phases were used together with input from the current scientific literature to describe the important steps of a BSFL production and processing systems and to develop a business plan of production of BSFL reared on residual substrates from Guldborgsund Municipality.

3. Results and Discussion

3.1 Dietary Experiment

The dietary mixtures were ground twice, using a kitchen blender, before being used in the experiment, making them very similar in terms of viscosity and structure. The tested diets were found to be suitable for BSFL production and showed overall promising results, as illustrated in the figures 2-5.

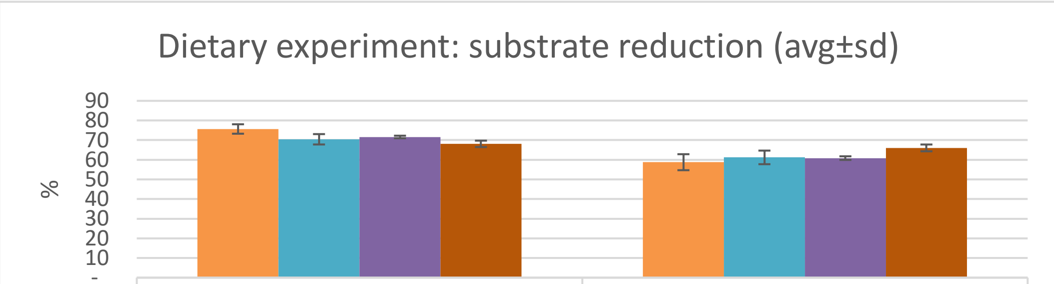
Overall, the larval biomass was found to be high for all dietary treatments (between 107-163 g (dm)). However, as illustrated below, the highest larval biomass production was when the larvae were fed on diet 3 (Mixture: 19% spent grain, 11% wheat, 2% sugarbeet tops, 3% malt, 3% Danish cookies and 62% water) (figure 2).



	Larval production (fw)	Larval production (dm)	Insect Frass (fw)	Insect Frass (dm)
Diet 1	500	147	550	155
Diet 2	453	107	602	146
Diet 3	521	163	638	147
Diet 4	476	119	640	127

Figure 2: Larval and insect frass production during the dietary experiment

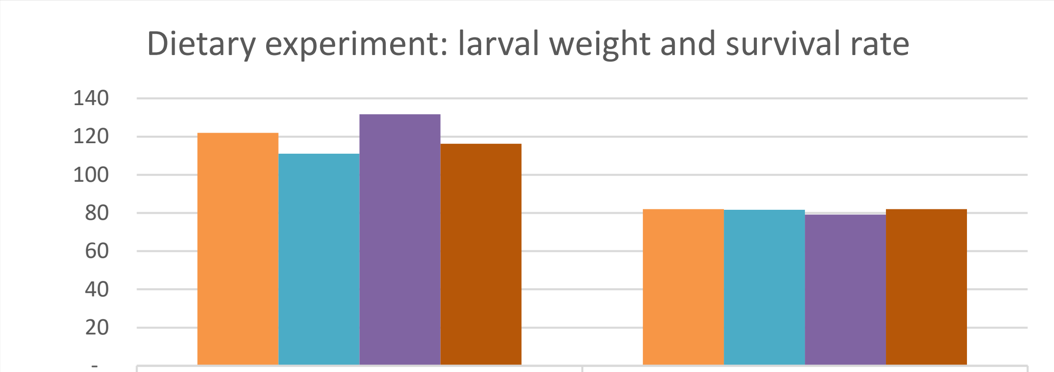
The larvae were successfully fed on all 4 diets, having an overall high substrate reduction rate (dm basis) ranging between 59-66%, being highest on diet 4 (figure 3).



	Residual reduction (fw)	Residual reduction (dm)
Diet 1	76	59
Diet 2	70	61
Diet 3	72	61
Diet 4	68	66

Figure 3: Substrate reduction during the dietary experiment

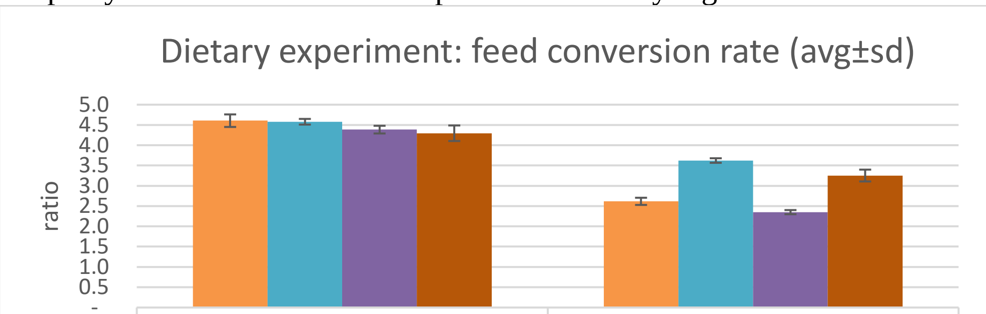
The larval survival rate was found to be high and similar between all dietary treatments, between 79% when fed on diet 3 and 82%, when fed on the other diets (figure 4), hereby indicating that the larvae encounter low mortality, when fed on substrates from Guldborgsund Municipality. Moreover, positive larval size was obtained, with the highest average weight (132 mg/larva) being obtained when larvae were feed on the diet 3.



	Avg weight (mg)	Survival (%)
Diet 1	122	82
Diet 2	111	82
Diet 3	132	79
Diet 4	116	82

Figure 4: Larval weight and survival rate from the dietary experiment

The overall feed conversion rate (FCR) obtained during this experiment was relatively high, ranging between 3.6, when larvae were fed on diet 2 and 2.4 when fed on diet 3 (figure 5). These FCR values indicate that the potential of using substrates from the Guldborgsund Municipality as feed source in BSFL production is very high.



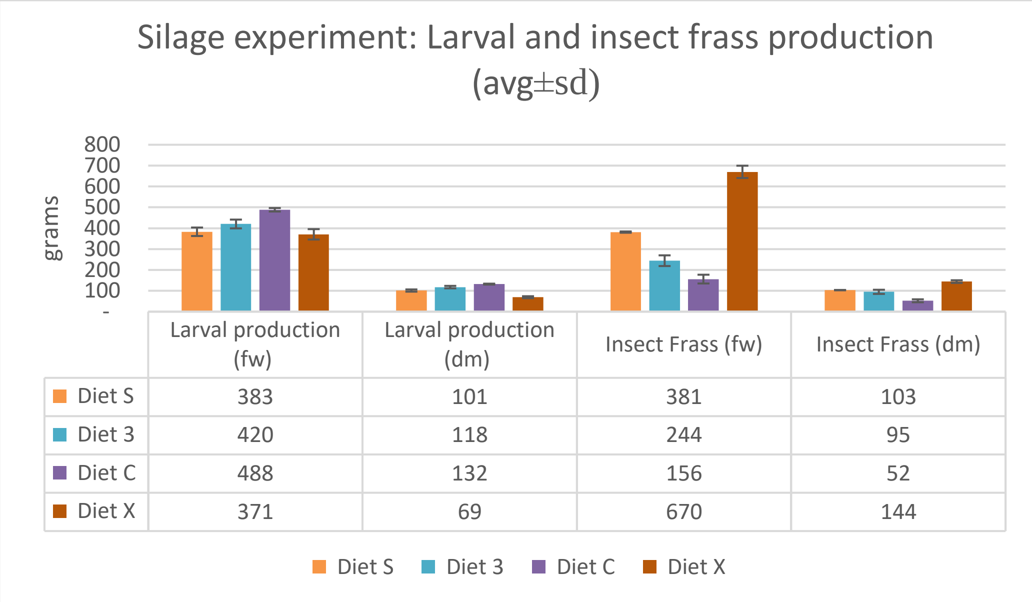
	FCR (fw)	FCR (dm)
Diet 1	4.6	2.6
Diet 2	4.6	3.6
Diet 3	4.4	2.4
Diet 4	4.3	3.3

Figure 5: Feed conversion ratio on both fresh and dry weights, from the dietary experiment

On the basis of the overall results from the dietary experiment (figures 2-5), it was indicated that the best diet for further use was diet 3. Therefore, the next activities (silage experiment and for the pilot production) were conducted on diet 3.

3.2 Silage experiment

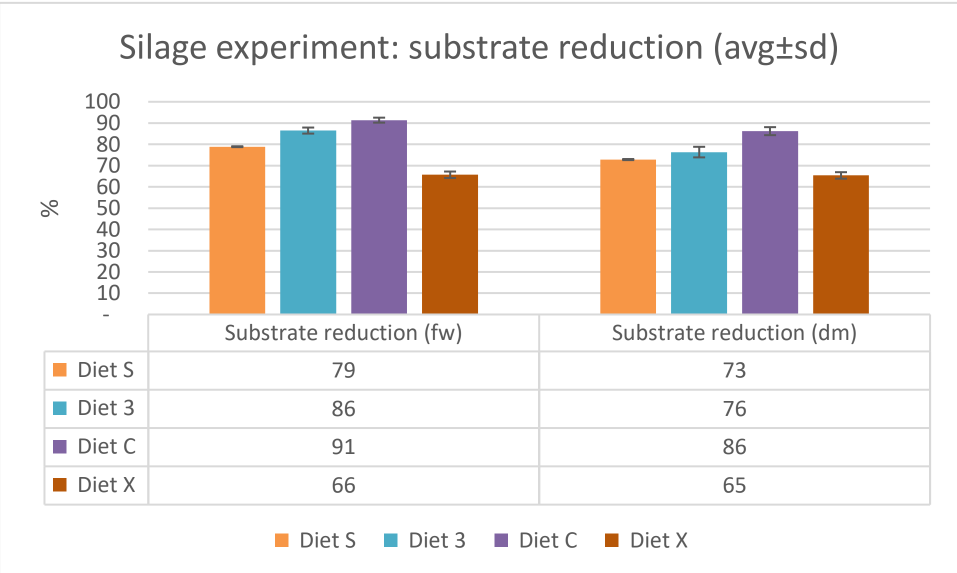
The diets used in this experiment were ground once in order to decrease handling and test the rigidity of the system. The grinding was performed using the same kitchen blender and the BSFL were found to grow on all the tested diets. However, as illustrated in figures 6-9, the BSFL were found to perform differently when reared on the different diets. As expected, the larvae production was highest when larvae were reared on the reference chicken feed diet (diet C). This was followed by the larvae reared in the diet 3 and by the larvae reared on the silage diets (diet S and X) (figure 6).



	Larval production (fw)	Larval production (dm)	Insect Frass (fw)	Insect Frass (dm)
Diet S	383	101	381	103
Diet 3	420	118	244	95
Diet C	488	132	156	52
Diet X	371	69	670	144

Figure 6:Larval and insect frass production during the silage experiment

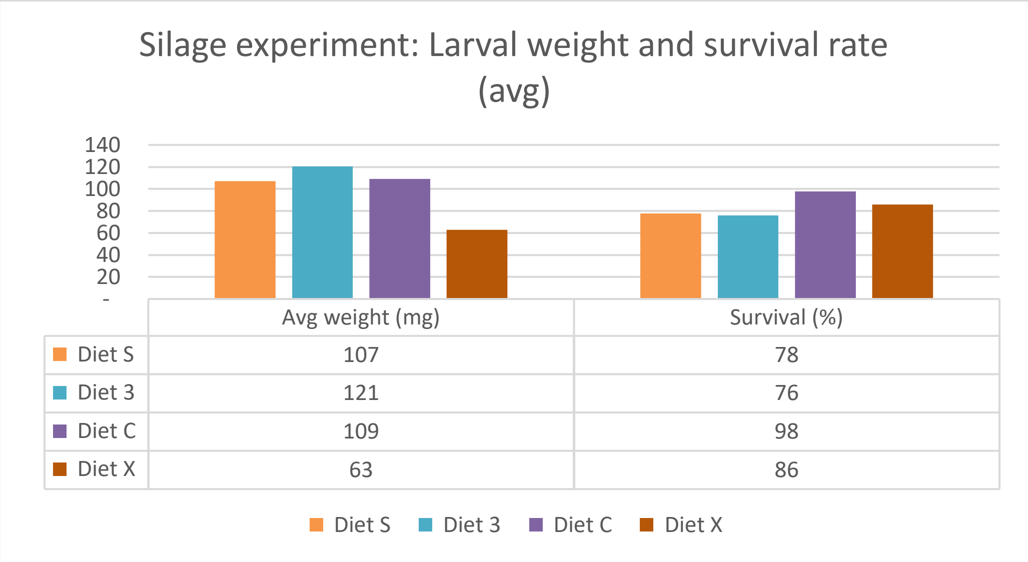
The substrate reduction rate was found to be different between the dietary treatments. The highest reduction rate (86%) was found when larvae were reared on the reference chicken feed (Diet C). The substrate reduction rate was found to be lower when larvae were reared on Diet S (78%), Diet 3 (76%) and lastly on Diet X (65%) (figure 7).



	Substrate reduction (fw)	Substrate reduction (dm)
Diet S	79	73
Diet 3	86	76
Diet C	91	86
Diet X	66	65

Figure 7: Substrate reduction during the silage experiment

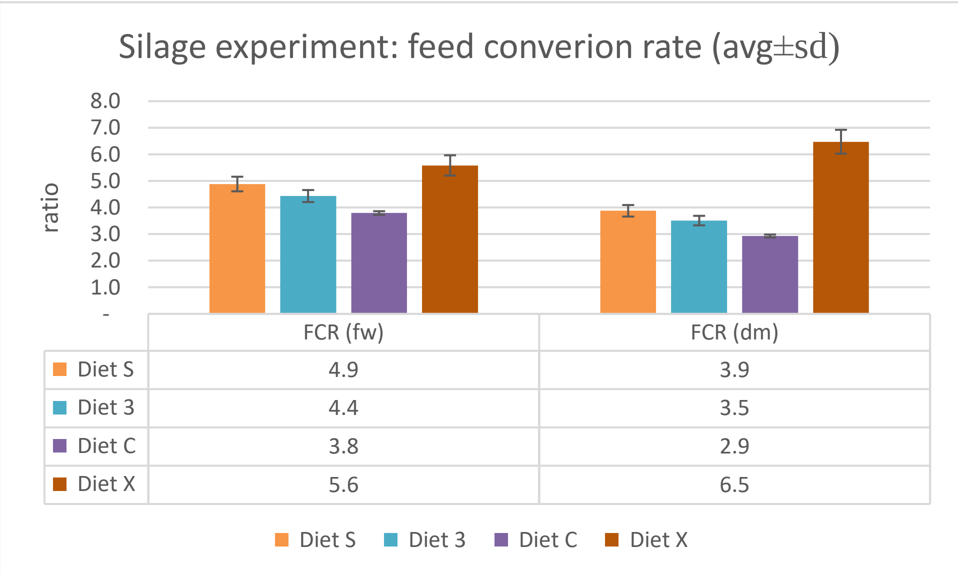
Overall the larval average weight was found to be high when reared on diet 3 (121 mg/larva), and lower when reared on the reference chicken feed (Diet C: 109 mg/larva) and Diet S (107 mg/larva). Moreover, a very low average larval weight was found when larvae were read on the cheap diet (Diet X: 66 mg/larval) (figure 8).



	Avg weight (mg)	Survival (%)
Diet S	107	78
Diet 3	121	76
Diet C	109	98
Diet X	63	86

Figure 8: Larval weight and survival rate from the silage experiment

The FCR (dry matter basis) was found to be low when larvae were reared on the reference chicken feed (2.9) and Diet 3 (3.5) and high when reared on the silage diets (Diet S: 3.9 and Diet X: 6.5) (figure 9).



	FCR (fw)	FCR (dm)
Diet S	4.9	3.9
Diet 3	4.4	3.5
Diet C	3.8	2.9
Diet X	5.6	6.5

Figure 9: Feed conversion ratio on both fresh and dry weights, from the silage experiment

These results indicate that the larvae do not perform well when the silage substrates were used in the diets of BSFL. However, these results are not surprising as the BSFL were not previously reared on silages and therefore, as in the case of other animals, will require a period of adaptation before being able to perform optimally on silage. In regard to this, DTI would recommend strongly that a study, where BSFL are reared on silages from Guldborgsund Municipality over a few generations should be made, in order to develop a highly specific BSFL strain for bio-converting this type of substrate. The recommendation is based on previous studies on BSFL adaptation conducted at DTI (figure 10)

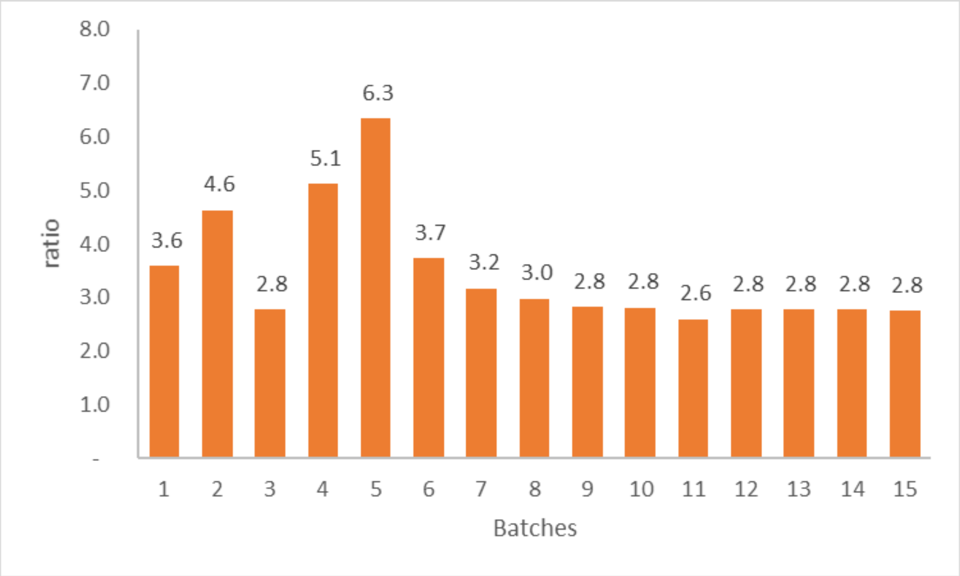


Figure 10: Adaptation of BSFL on chicken feed over 15 generations, illustrated as FCR

Overall the performance of BSFL during the silage experiments was found to be lower when compared with their performance during the initial dietary experiment, even though a similar diet 3 and a control (reference) feed were used. However, this cannot be considered surprising, as the parameters of the 2 experiments were not exactly the same. Firstly, the temperature was decreased from 27ºC during the dietary experiment to 25ºC during the silage experiments. Secondly, the density of larvae was decreased from 11 larvae/cm^2 during the dietary experiment to 10 larvae/cm^2 in the silage experiments. Thirdly, the total feed amount was slightly increased from 375 to 394 g, the feeding strategy was changed from 3 episodes to 2 episodes and the fractionization was decreased from two grindings to only one grinding, referring to the dietary experiment and the silage experiment respectively. These approaches were taken in order to assess the rigidity of the system and generate comparative data for the business assessment. DTI would recommend a further study where different cost-efficient grinding methods should be assessed.

3.3 Pilot production

Overall a good production of BSFL biomass (0.5kg/tray, dm basis) and insect frass (1.1 kg/tray fw. basis) was obtained during the pilot production (figure 11). Based on this result, it is estimated that a total of 2.5 kg of dried larvae and a total of 5.45 kg of insect frass (fresh weight basis) can be produced per m^2.

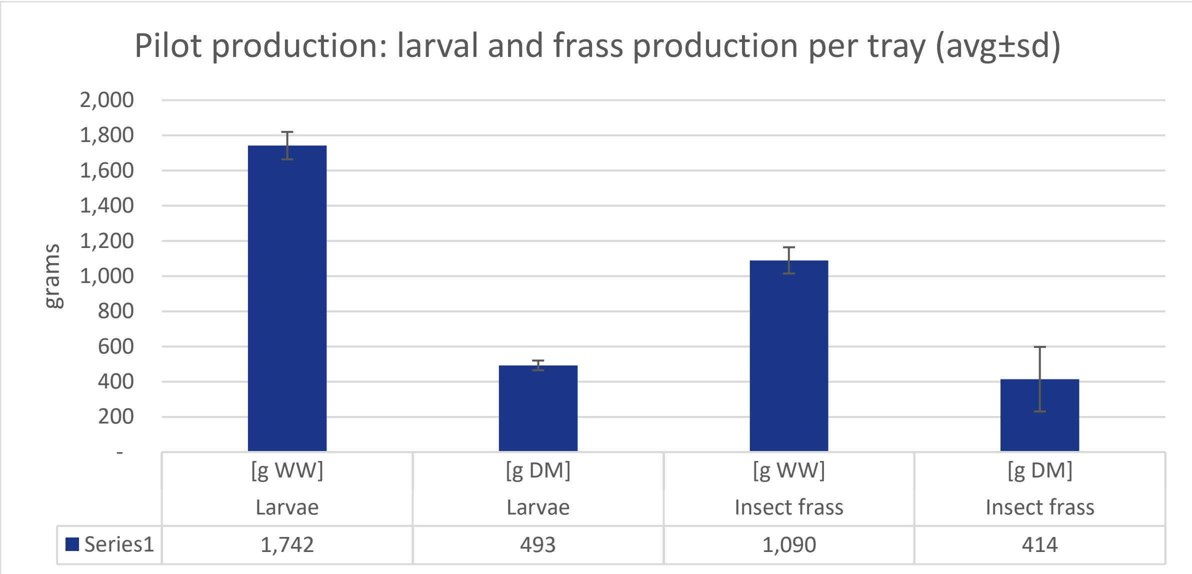


Figure 11: Example of larval and insect frass production per tray during pilot production

As illustrated in the figure 12, BSFL were able to consume 71% of the substrate when reared in large trays at pilot scale.

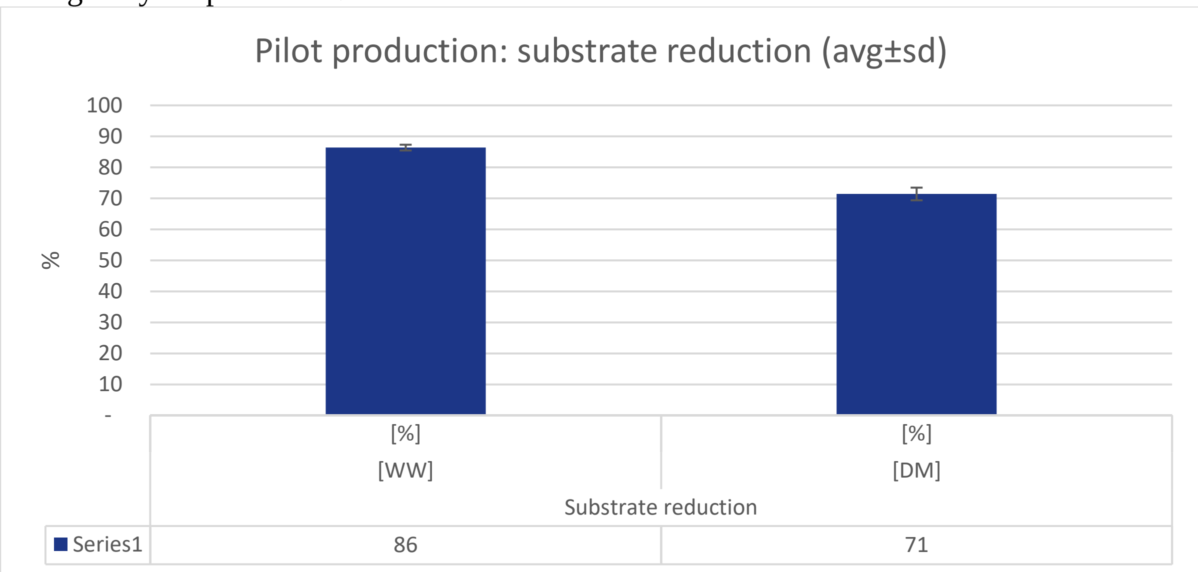


Figure 12: Example of substrate reduction on both fresh weight and dry matter basis, from the pilot production

The larvae were found to weigh, on average, around 104 mg and had a high survival rate during the pilot production (figure 13).

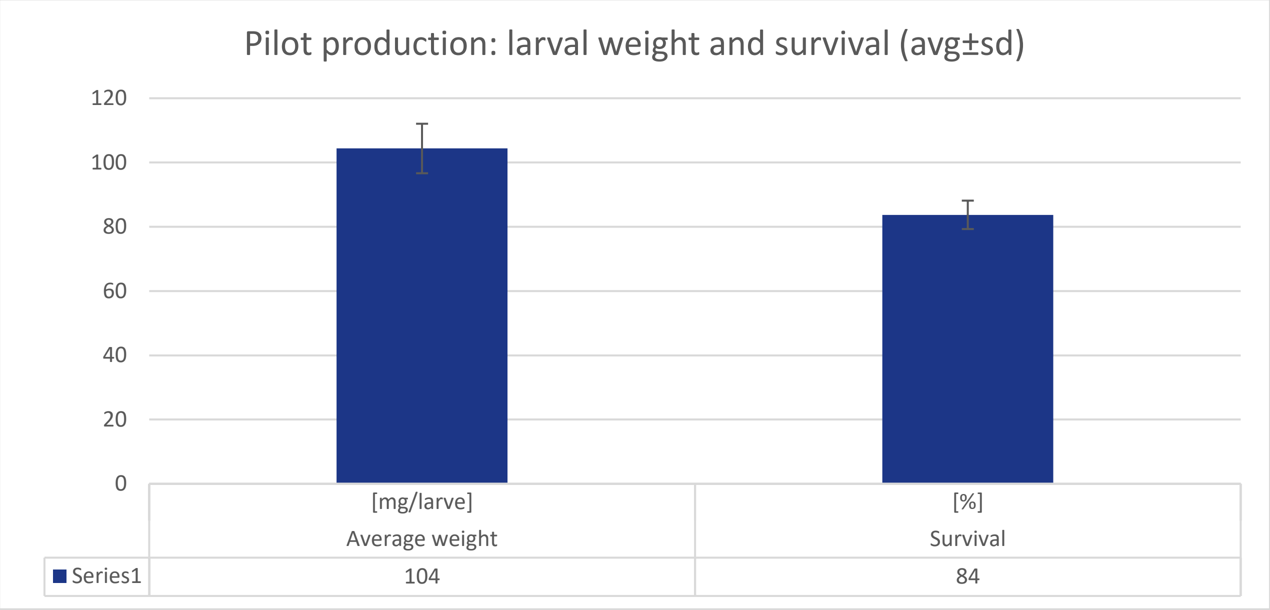


Figure 13: Example of average larval weight (mg) and survival rate (%) of BSFL from the pilot production

The FCR obtained during the pilot production was low (figure 14), indicating that high productivity can be obtained when larvae are reared on Guldborgsund Municipality's substrates.

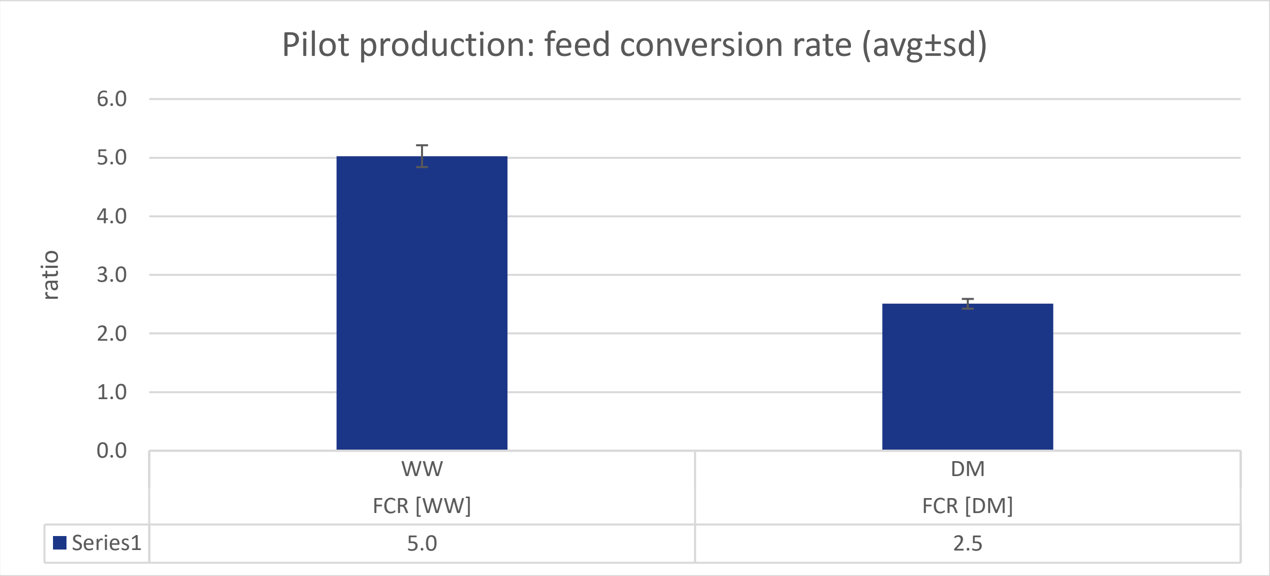
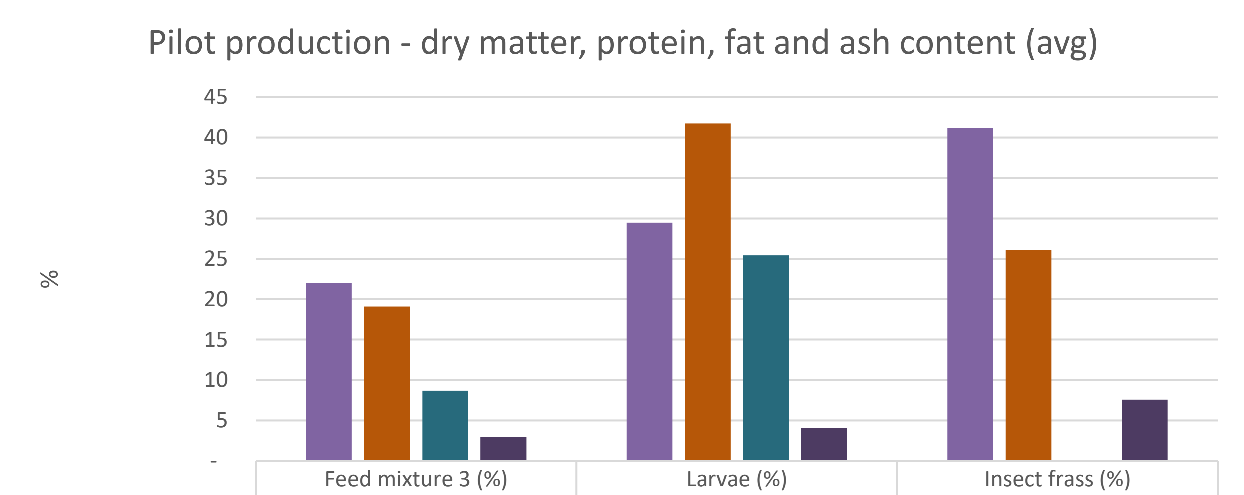


Figure 14: Example of feed conversion rate on both fresh and dry weights from the pilot production

Overall, the larval performance and survival rate during the pilot production were found to be similar or higher when compared with the larval performance and survival rate from the dietary and silage experiments. These results indicate that a transfer from the experimental scale to pilot scale and thus to more industrial-like conditions can be ensured. The only negative tendency seen when up-scaling from the experimental level to the pilot production was associated with average larval weight. This was found to be lower (104 mg/larva) in the pilot production compared to the larvae obtained during the dietary (132 mg/larva) and silage (121 mg/ larva) experiments. Such tendencies can be attributed to a faster loss of water observed during the pilot production. This tendency is further believed to decrease the overall biomass produced.

As presented in figure 15, the BSFL were found to have high protein and fat content and low ash when reared on substrates from Guldborgsund Municipality; confirming again that BSFL can be used for upcycling protein and fat from residual substrates into BSFL biomass suitable to be used as feed. Moreover, a by-product from the BSFL production, insect frass, is suitable to be used as fertilizer, in potting compost or in a biogas production as identified in e.g. the WICE project[2]. The conducted analysis reveals that the obtained insect frass from the pilot production has a balanced nutrient profile (figure 16) and therefore can be potentially used as a fertilizer ingredient.



	Feed mixture 3 (%)	Larvae (%)	Insect frass (%)
Dry matter	22	29	41
Protein (dm basis)	19	42	26
Fat (dm basis)	9	25	
Ash (dm basis)	3	4	8

Figure 15: Content of dry matter, protein, fat and ash in the feed mixture, larvae and insect frass from the pilot production.

[2] WICE Report 2017, online source: https://www2.mst.dk/Udgiv/publikationer/2018/05/978-87-93710-13-9.pdf

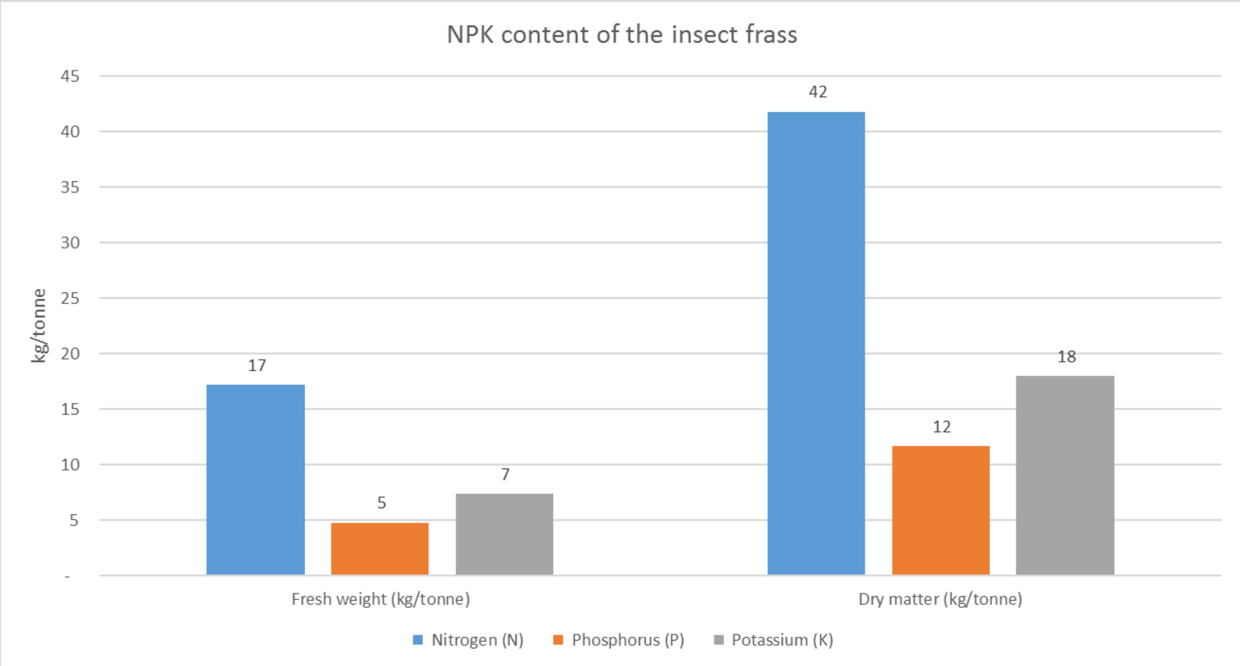
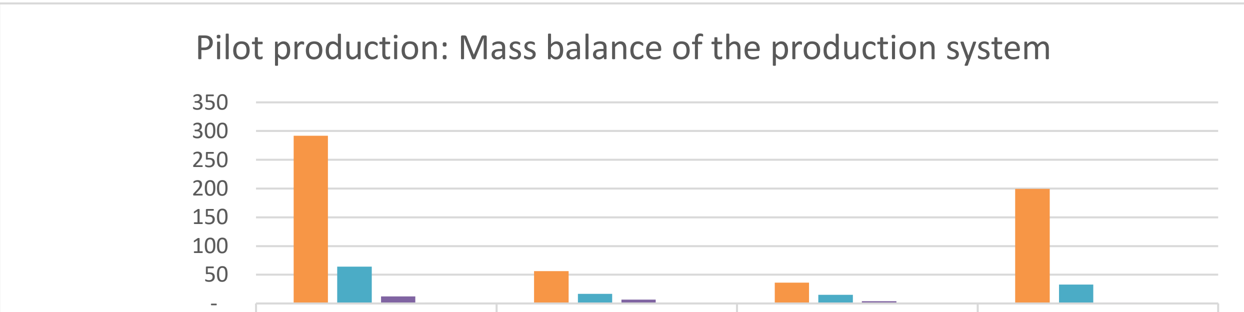


Figure 16: Nitrogen (N), Phosphorus (P) and Potassium (K) of the insect frass obtained after production of BSFL reared on diet 3 during the pilot production.

The mass balance of the system was made for fresh weight, dry matter, protein and ash and is presented below in figure 17. As shown by the ash balance, the pilot production is stable. However, half of the dry matter was lost from the system as a result of fast weight lost seen in the production, due to rapid water loss. Moreover, a small fraction of protein was found to be lost from the system due to the evaporation of nitrogen.



	Feed mixture 3 (kg)	Larvae (kg)	Insect frass (kg)	Balance (input-output)
Fresh weight	292	57	36	199
Dry matter	64	17	15	33
Protein (dm basis)	12	7	4	1
Ash (dm basis)	2	1	1	0

Figure 17: Mass balance of BSFL pilot production for fresh and, dry weights, protein and ash. The balance shows the difference between the inputs: feed mixture 3 and the outputs (larvae and insect frass) of the pilot production.

Overall, the production of BSFL at pilot scale was feasible on substrates from Guldborgsund Municipality with positive results such as a low FCR 2.5 (dm basis). Although these results confirm that the developed diet can be applied in the production of BSFL, further optimization of BSFL performance can be obtained. Therefore, DTI recommends further studies at pilot production level, where different feeding strategies, larval density and harvesting times are used to optimize the production and ensure no larval weight loss. Moreover, the production of BSFL on substrates from Guldborgsund Municipality during a few consecutive generations could ensure the high substrate adaptation of BSFL and further improve production output.

3.4 Processing

The larvae from the pilot production (approx. 60 kg) was dried at 65-70°C in a ventilated oven. The overall outcome of a review of existing literature and patents showed that oil extraction from the larvae biomass by screw pressing is challenging but can work under the right circumstances (oil and water content). The target is to produce an oil fraction and a press cake; the press cake representing a protein enriched and partially defatted larvae meal. As this process is simple and of relatively low-cost investment at industrial scale, it was intended to process the larvae in this way. Initial tests on a laboratory scale oil press indicated that the oil extraction was possible and an oil fraction with 94% lipids and a press cake with a lipid content of 21% was obtained. However, despite several attempts to reproduce or upscale the process, it was unfortunately not successful.

An alternative process was therefore designed at lab scale with the intention of fractioning the larvae in a more advanced aqueous system. Such a process requires more machinery, chemicals, water and staff and will naturally result in higher processing costs. The process was scaled up to pilot and was successful. The process flow chart is shown in figure 18.

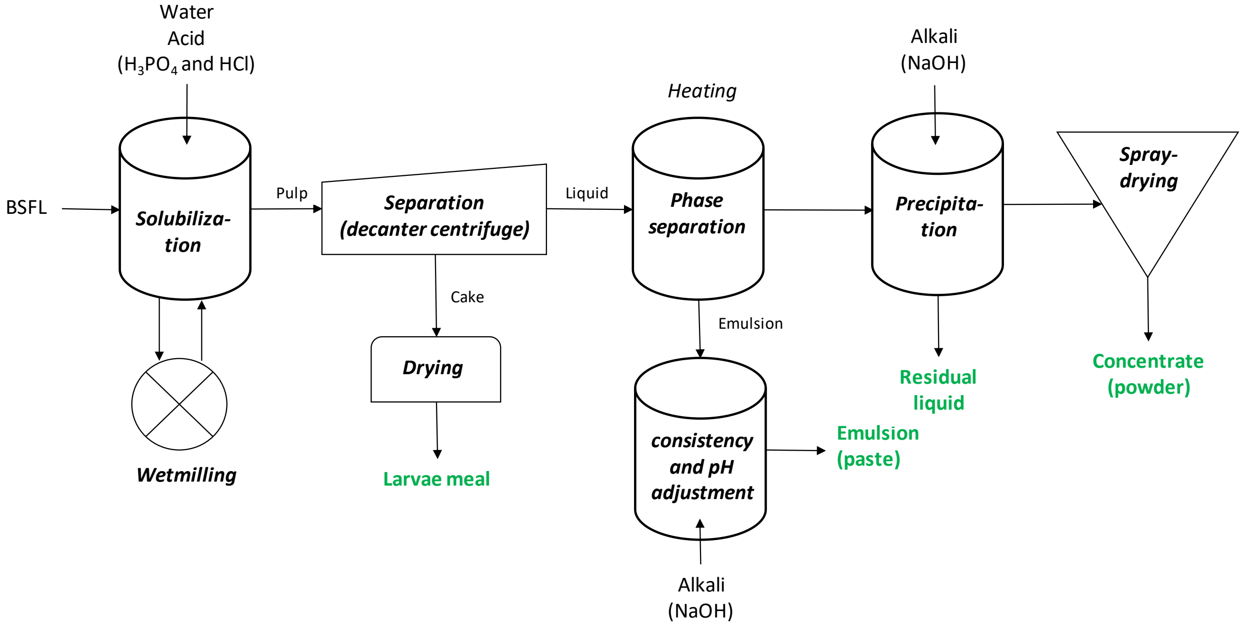


Figure 18: Process flow chart – pilot processing of BSFL

The dried larvae were solubilized in water at pH 2.7 and mechanically ground in a connected wet mill. The resulting pulp was separated into a liquid fraction and a ‘cake’ in a decanter centrifuge. The cake was oven dried and blended to achieve a larvae meal in powder form, see figure 19. The liquid fraction was heated to 85°C and left unstirred overnight to phase separate. The liquid was drained, and an emulsion was collected. The pH was elevated with NaOH to 8.0 where the consistency turned thick and slimy, see figure 18. This emulsion was stored at -20°C. The pH in the liquid from before was elevated to 8.7 and also left overnight for protein precipitation. The precipitate was drained, and spray dried to collect a fine powder.

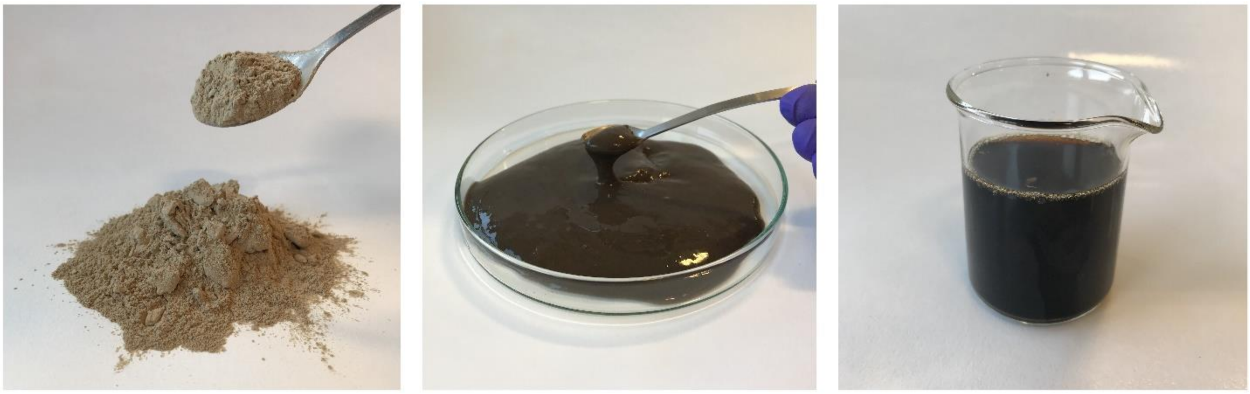


Figure 19: Pictures of the collected fractions. From left: insect meal, emulsion and residual liquid. Photo of concentrate not available.

The composition of the 4 produced fractions and the mass balance of protein and lipids can be seen in Table 3. Approximately 60 kg of fresh larvae were dried resulting in approx. 13 kg dried larvae for wet processing. The dried larvae contained 39.9% protein, 22.7% lipids and 6.3% ash. The mass balance is calculated based on the recovered material before the drying process, which is connected to a significant loss, especially for spray drying of relatively small quantities such as these.

Table 3: Composition and mass balance. Recovery values were corrected relatively to sum up to 100 %. No mass balance available for ash due to disturbance from addition of acid/alkali. Concentration of protein, lipid and ash is dm-based.

Fraction	Form	Dry matter		pH	Protein (%)		Lipid (%)		Ash (%)
		%	kg		Conc.	Rec.	Conc.	Rec.	
Insect meal	Powder	97.7	5.4	2.7	46.2	39.2	29.5	37.1	6.0
Emulsion	Paste	16.0	5.7	8.0	22.7	20.4	47.3	62.7	17.9
Concentrate	Powder	93.2	1.7	8.7	31.9	28.8	0.4	0.2	51.3
Residual liquid	Liquid	5.4	2.3	8.7	32.4	11.6	0.0	0.0	37.0

It proved to be challenging to separate the proteins from the lipids in the aqueous process as also experienced earlier in other process designs with similar biomass. The main part of the lipids (62.7%) was recovered in the emulsion fraction while the remaining part (37.1%) ended in the insect meal. Following the protein, 39.2% and 28.8% ended in the insect meal and concentrate fractions, respectively, while the remaining 20.4% was recovered in the emulsion. A more efficient fractionation of the protein and fat is desirable. Due to acid and alkali addition a high ash content is seen in the fractions. In the concentrate this could be reduced by centrifugation of the material before spray drying which was not possible at the actual quantities.
In conclusion of the pilot processing, the aqueous fractionation of larvae showed to be so challenging that a screw press process is to be preferred, as this is simpler and has the potential to be more efficient. However, the oil pressing technique (process) needs more work to be

successful. An alternative approach could be to directly oven dry and grind the larvae to produce a larvae meal with its natural composition; or to cold press oil from the fresh larval biomass before drying and grinding it to a meal.

3.5 Business consideration:

Based on the results from the pilot production, a business plan consisting of CAPEX, OPEX and Return on Investment was developed. The business model is divided in 6 different sections: 1. Experimental data; 2. Factory data and estimated yield; 3. CAPEX; 4. OPEX; 5. Revenues and 6. Return on investment and described below.

Table 4: Legend of different colors used in the business plan

Inputs
Calculation
Outputs

Experimental data

The experimental data from the pilot production: larval density, larval weight, development time, survival, FCR and insect frass were used to calculate the feed requirement, the larval biomass and insect frass production per m^2. Moreover, the rest of the data: feed and larval dry matter were used for further calculations in the next sections. The protein and fat profile of the larvae were not used to calculate the production of different BSFL derivates (BSFL protein and BSFL oil), as these were difficult to be separated in a cost-effective manner, during processing (for this particular pilot production). An overview of the experiments data and calculated parameters are presented in table 5 below.

Table 5: Experiment data and calculated parameters used in the business model

1. Experiments data		
Larval density	10	BSFL/cm2
BSFL weight	132	mg/BSFL [FW]
Development time	10	Days/batch
Survival	84%	%
FCR (DM)	2.5	
Insect frass	1.3	kg/batch [FW]
Feed - DM	19%	%
BSFL - DM	30%	%
Protein content	42%	% (of DM)
Fat content	25%	% (of DM)
Handeling	10	seconds/handeling
Feed	8.75	kg/ batch [FW]
Larval biomass	2.22	kg/batch [FW]
Larval density	100,000	BSFL/m2
BSFL weight	0.13	g/BSFL
Feed requiered	4.38	kg/day/m2 [FW]
Larval biomass	1.11	kg/day/m2 [FW]
Insect frass	0.65	kg/day/m2 [FW]

Factory data and estimated yield

In this section a series of assumptions, such as production areal (600 m²), production days (350), tray size (1m^2), number of handling per trays (2 handlings), rack height (10 m) and salary costs (25,000 DKK/person/month?) were made. These assumptions were used together with the experiment data to calculate the number of employees required (3.3), feed requirements (4,600 tons (fw) and neonates (300 million/day) required in the production. Furthermore, these were used to estimate the production of BSFL meal and insect frass. As presented in table 6, by utilizing about 873 tons (dm) of Guldborgsund based diet in a BSFL production of 6,000 m^3, an investor can annually produce 349 tons of BSFL meal and 683 tons of insect frass.

Table 6: Factory calculated data and estimated yield used in the business model

2. Factory data and estimated yield		
Production areal	600	m2
Production days	350	days/yr.
Tray size	1	m2
No. Handlings/tray	2	Handlings/tray/productions cycle
Tray stack height	10	m
Employees salary	25,000	dkk/month
Production trays	30,000	trays in production
Production trays	3,000	no. trays/day
No. Handlings/day	6,000	no tray handeled /day
Requiered time for handeling	16.67	hour/day
Requiered personel	3.33	personel/day
Feed required	4,596	tons/year [FW]
Feed required	873	tons/year [DM]
Neonates required	300	mil neonates/day
BSFL production	1,164	tons/year [FW]
BSFL meal	349	tons/year [DM]
Insect frass	683	tons/year [70% DM]

CAPEX

For a better estimation of a BSFL production facility in Denmark, a high degree of automatization was considered. Therefore, as presented in table 7, a total of 5.95 million DKK is expected to be required to establish the BSFL production. This will consist of storage, handling, conveyor belt and ventilation equipment. (These automatization requirements were identified during the WICE project[2]).

Table 7: CAPEX associated with implementing a BSFL in Guldborgsund

3. CAPEX	Price in dkk	
Automatic high storage	3,000,000	
Handling Robot	1,000,000	
Processing machinery	400,000	
Feeding Silo	200,000	
Conveyor belt	100,000	
Control panel	500,000	
Ventilation systems	750,000	
Total	5,950,000	dkk

OPEX

For the OPEX, a series of annual costs associated with neonates (small larvae) production, feed requirements, operational/maintenance and personnel were considered (table 8). The costs associated with the neonate requirements were calculated at a fixed price of 10 DKK/million neonates, based on internal data. The feed costs were calculated based on the price of different components of the diet, provided by biomass producers during a survey conducted by Guldborgsund Municipality (table 9). The operational costs were assumed to represent 5% of the actual investment and the personnel costs were calculated to be 1,000,000 DKK for 3.3 workers.

Table 8: OPEX associated with implementing a BSFL production in Guldborgsund

4. OPEX		
Production of neonates larvae	1,050,000	10 kr. pr. 1million neonates
Feed	1,723,382	@ 375 kr/tone
Operational and maintanace	297,500	5% of the CAPEX
Personel cost	1,000,000	salary cost/year
Total	**4,070,882**	dkk/year

Table 9: Price for different ingredients used in diet formulation and the overall price per ton of BSFL feed

Diet 3 components	**price/ton**	**ratio**	**price/ton diet 3**
Spent grain	400	0.19	76
wheat	1400	0.11	154
sugarbeet tops	215	0.02	4.3
malt	700	0.03	21
butter cookies	4000	0.03	120
total			**375**

Revenues and Return on Investment:

The overall revenues were considered on 2 streams: 1) BSFL meal, in form of dried and ground larvae and 2) insect frass, derivate from the production. The revenue associated with BSFL meal was set at 12,000 DKK/ton) and the revenue associated with insect frass was set to 1,000 DKK/ton. The prices were estimated based on inputs from industrial BSFL producers in Europe.

The overall annual revenues associated with BSFL meal (4.2 mill. DKK) and insect frass (0.7 mill. DKK) wre estimated to about 4,874,000 DKK. This calculates a net profit of 803,000 DKK/year and leads to a return on investment of 7.4 years. (table 10)

Table 10: Revenues and return on investment associated with implementing a BSFL production in Guldborgsund

5. Revenues		
BSFL meal	**4,191,264**	12000 dkk/ton
Insect frass	**682,500**	1000 dkk/ton
Total	**4,873,764**	dkk/year
6. Return on investment		
Revenues	4,873,764	dkk/year
OPEX costs	4,070,882	dkk/year
Diference	802,882	dkk/year
CAPEX	5,950,000	dkk/year
Return on investment	**7.4**	years

3.6 Life Cycle Assessment (LCA) perspectives

Since the production of BSFL on Guldborgsund Municipality side streams was found to be suitable from an economic perspective, a series of steps from the pilot production will be addressed together with existing literature to identify the energy consumption and environmental impacts.

The BSFL value chain follows the animal production conceptual frame: feed formulation, growing, processing, distribution and consumption[3]. In the current study, the feed formulation, growing and processing were considered and further used together with the existing literature to identify a series of production stages requiring high energy inputs.

The feed formulation at the pilot scale was found to be labour intensive, especially during grinding and to require low utilization of electricity for mechanical grinding and freezing. In a Life Cycle Assessment (LCA) conducted at pilot scale, where BSFL were fed on residual substrates (wheat starch slurry, wheat middlings and condensed distilled soluables) the overall electricity consumption associated with this stage represented 9.9% (0.8kWh) of the total electricity used (8.1 kWh)[3].

The highest consumption of energy, in the form of electricity, during the pilot production was associated with climate system used to ensure optimal temperature (approx. 27°C) and humidity levels (60%) for BSFL growing. Similarly, Smetana *et al*. (2019) found that the climate system had the highest electricity consumption 2.7 kWh (33%) and that the separation step will require 0.53 kWh (6.6%) of the total electricity used during the production of BSFL at pilot scale[3].

The electricity consumption during the processing of BSFL was found to be very high due to different processing steps used (see section 3.4 processing). However, this can be significantly reduced if a simpler separation procedure is implemented. Smetana *et al*. (2019) found that the total electricity consumption during processing was 0.61 kWh or 7.5% of total electricity consumption[3].

In additional to electricity, used primarily in our production system, natural gas and water were also considered in the LCA study conducted by Smetana *et al*. (2019). The utilization of these resources is presented in figure 20 below.

[3] Smetana *et al*., 2019, Sustainable use of *Hermetia illucens* insect biomass for feed and food: Attributional and consequential life cycle assessment, online source: https://www.sciencedirect.com/science/article/pii/S0921344919300515

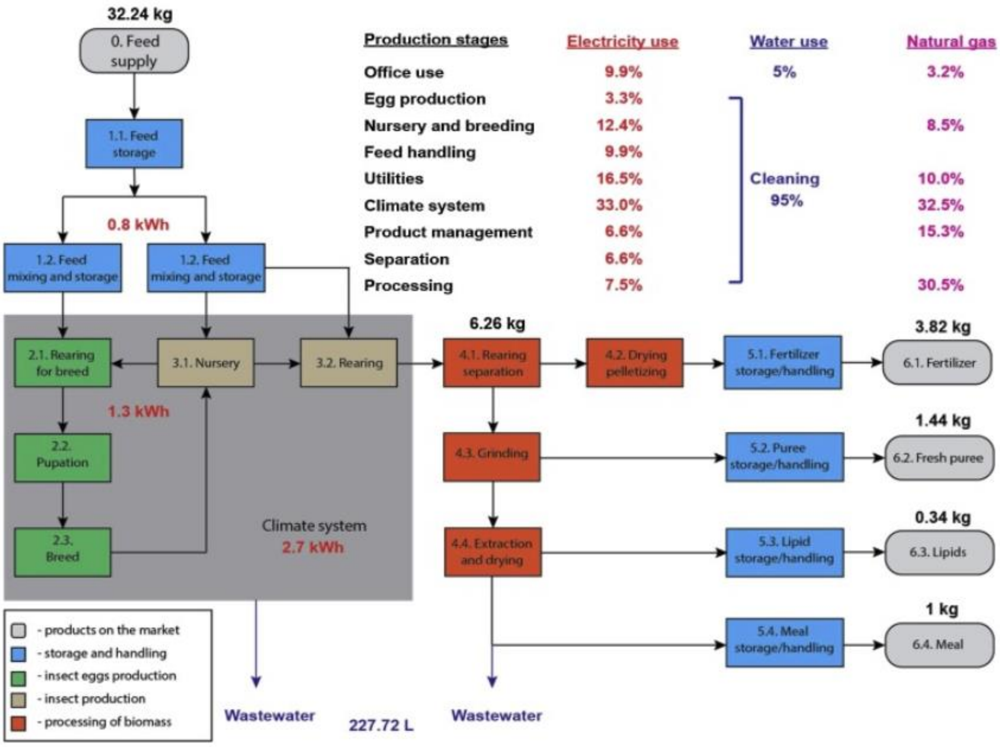


Figure 20: System boundaries of the study (attributional modelling) including input distribution and relative mass flow[3].

The production of BSFL is known to require a high degree of automatization, controlled environment, and therefore could be associated with high environmental impacts. However, the utilization of residual substrates in the production as well as their short life cycle, low FCR and their high-quality protein profile, are making the BSFL more sustainable than other sources of proteins. As presented in figure 21 below, the BSFL meal (HM) and BSFL pulp (HP) were found to have an overall lower environmental impact (GWP – global warming potential; OD – ozone depletion; AC – acidification; EU – eutrophication; ED – energy demand; FD – freshwater depletion; LU – land use) compared with fishmeal, egg protein concentrate or fresh chicken meat, although not lower than those of plant proteins, such as soymeal. An exception from this was seen in FD and LU, where BSFL meal or pulp were found to require less than soymeal and it is believed that further improvement of the BSFL production can lead to low environmental impacts similar to plant-based proteins.[3]

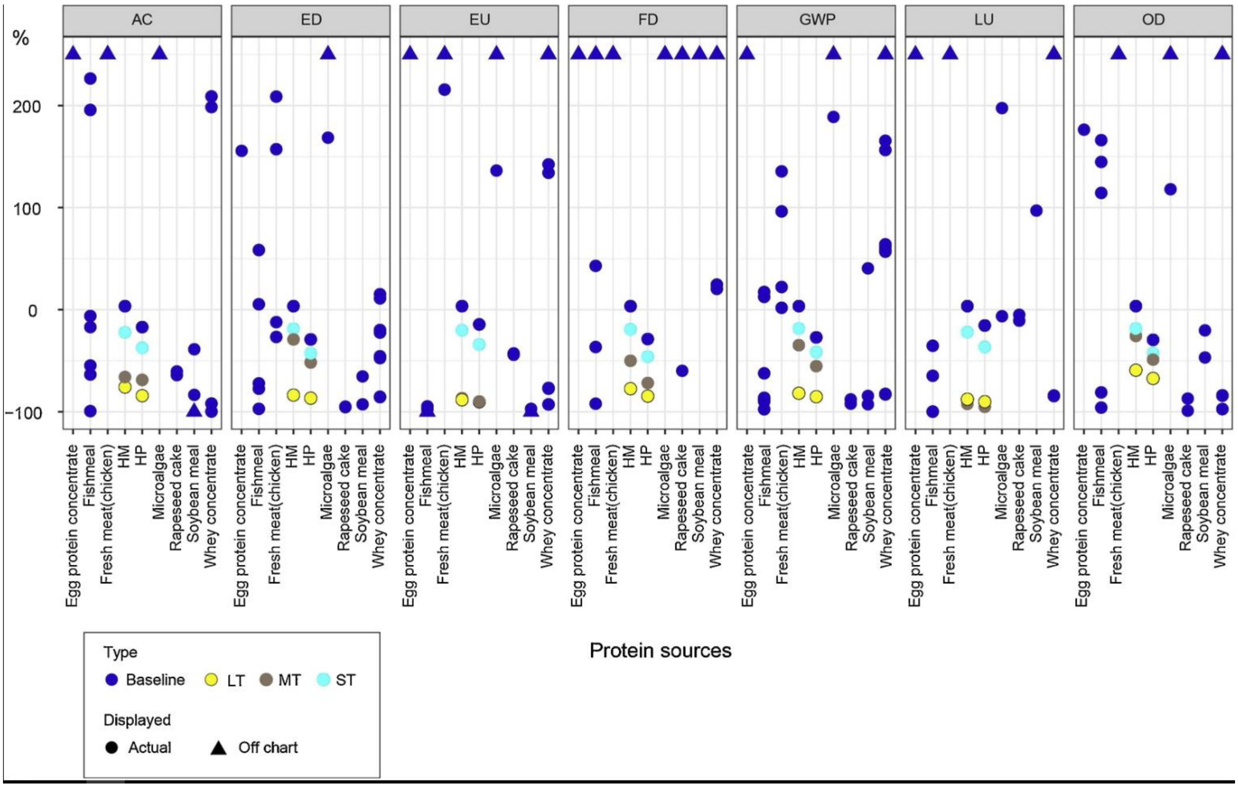


Figure 21: Environmental impacts of different sources of proteins (dry matter basis) weighted against HM (*H. illucens* meal (defatted protein concentrate)) and HP (H. illucens pure), GWP – global warming potential; OD – ozone depletion; AC – acidification; EU – eutrophication; ED – energy demand; FD – freshwater depletion; LU – land use, relative impacts are censored at -100% and 250% to maintain the readability of the plot and as triangles at these limiting values[3].

In addition to the BSFL meal, another product which is obtained is insect frass. A comparative study focusing on assessing the global warming potential between 2 waste management systems: i) BSFL feed with organic household waste (OHW) and ii) composting of OHW, found that CO_2 emission from the BSFL system is 47 times lower than the that from composting[4]. This indicates further potential of using BSFL for bio-converting other waste streams. Similarly, Smetana *et al*. (2016), reveal that the production of BSFL on low quality substrates (i.e. dried distillers' grains with solubles) can lead to lower environmental impacts when compared with other feed sources (i.e fishmeal, chicken feed and whey protein)[5].

Based on the available literature and on the experimental results conducted by DTI, the utilization of Guldborgsund Municipality by-products in the rearing of BSFL can lead to the production of a more sustainable insect meal than conventional fishmeal, with lower environmental impact. Moreover, by constructing a BSFL production facility in the proximity of the utilized by-products, we believe that the overall environmental impacts as well as resource and energy utilization associated with transportation of these by-products will be further decrease.

[4] Mertenat *et al*., 2019, Black Soldier Fly biowaste treatment – Assessment of global warming potential, online source: https://www.sciencedirect.com/science/article/pii/S0956053X18307293

[5] Smetana et al., 2016, Sustainability of insect use for feed and food: Life Cycle Assessment perspective, online source: https://www.sciencedirect.com/science/article/pii/S0959652616310447

4. Conclusion and Recommendations

The conducted experiments found that by-products from Guldborgsund Municipality can be used to produce BSFL meal suitable to be used as feed. Moreover, the FCR was low (2.5) indicating the high efficiency of the system. The business model indicates that high revenues can be obtained from BSFL meal and insect frass, leading to a return on investment in 7.4 years. Overall, the environmental impact associated with BSFL production on by-products is lower when compared with fishmeal and can be further decreased to a level comparable to plant-based proteins.

Based on the experimental work conducted by DTI, the LCA literature and the business perspectives highlighted several challenges identified in the production system and a series of recommendations for industrial setup are presented below (table 11).

Table 11: Identified stages with potential environmental effects from the pilot production and recommendations for industrial setup.

Conceptual frame	Stages with potential environmental effects	Challenges during pilot production	Recommendation for industrial set-up
1. Feed formulation	1.2 Feed grinding	Require high degree of manipulation and energy at lab scale	Wet fractionization post fermentation might decrease the energy consumption associated with mechanical grinding
	1.3 Feed storage	Require big freezing units and energy consumption	Stabilization of feed through fermentation can decrease energy consumption and environmental impacts associated with large scale freezing units
2. Growing	2.1 Production of BSFL	1) Utilization of temperature and humidity control production facility is energy intensive; 2) setting up production trays and feeding the larvae is labor intensive; 3) potential high emissions of different greenhouse gases (GHG).	1) Decrease the environmental impacts associated with energy consumption through utilization of green energy sources; 2) decrease labor by implementing automatization; 3) decrease GHG emission through diet optimization and implementation of efficient ventilation systems.
	2.2 Feeding	The feedings were performed manually and can be labor intensive	The utilization of automatized feeding systems can decrease the labor
	2.3 Larval separation	The separation was performed manually and can be labor intensive	Implementation of automatized separation systems, can decrease the use of labor, but increase the energy consumption
3. Processing	3.1 Slaughter and Storage	Require additional freezing units for slaughter and storage	Slaughter through drying and then milling into a BSFL meal will decrease the energy

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