Slutrapport för projekt

Ensiling as a method for storage and processing of black soldier fly larvae for use as animal feed

Projektperiod: from June 2017 to March 2018
Projektnummer: 44274-1

Med stöd från:
VINNOVA
Energimyndigheten
FORMAS
STRATEGISKA INNOVATIONS-PROGRAM

SLUTRAPPORT
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Datum
2018-05-20

Dnr
2017-001994

Projektnr
44274-1

Titel på projektet – svenska Ensilering som metod för lagring och bearbetning av soldatflugans larver för användning som djurfoder	
Titel på projektet – engelska Ensiling as a method for storage and processing of black soldier fly larvae for use as animal feed	
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Nyckelord: 5-7 st black soldier fly larvae, silage, fat extraction, animal feed	

Med stöd från:
STRATEGISKA INNOVATIONS-PROGRAM

Preface

The project was financed (75%) by the Innovation Program RE:Source as a Förprojkt, with the Department of Animal Nutrition and Management of the Swedish University of Agricultural Sciences co-financing (25%) the project.

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Sammanfattning

Soldatflugans larver (BSF) anses vara ett av alternativen för sojabönor och fiskmjöl i djurproduktion. I det här projektet syftade vi till att genomföra en livscykelanalys (LCA) och en kostnadsanalys (CBA) för olika lagrings- och behandlingsmetoder för larv-biomassa för att identifiera de bästa metoderna för energiförbrukning och produktutbyte. I synnerhet undersökte vi potentiella tillämpningar av ensilering som en alternativ lagringsmetod till torkning av insektsbiomassa. Vi var också intresserade av att ta reda på effekten av ensilering på utbytet av fett eller protein från insekterna. Olika tillsatsbehandlingar testades inklusive salt, socker och organiska syror. Det visade sig att ingen av dessa tillsatser var lämpliga på grund av höga biomassförluster (10-28 %) och ensilagen hade också höga pH-värden (5,3-6,8) och dålig lukt. Vi tror att en direkt försurning med svavelsyra skulle kunna leda till en bättre kvalitet på ensilaget. En intressant observation var att ensilagen utvecklade två faser - en vätskefas och en fast fas, med den flytande fasen huvudsakligen i form as vatten. Vi föreslår därför ett ensileringssystem utrustat med ett automatiserat vattenavlägsnande för att producera en insektsbiomassa med förhöjt torrsubstanshalt. Tyvärr kunde inte LCA- och CBA-analyserna utföras på grund av svårigheter att generera eller förvärva nödvändig data.

Summary

Black soldier fly (BSF) is considered as one of the alternatives for soybean and fishmeal in animal production. In this project, we intended to conduct a life cycle assessment (LCA) and a cost-benefit analysis (CBA) on different storage and processing methods of larval biomass to identify the best methods with regards to energy consumption and product yields. In particular, we investigated potential applications of ensiling as an alternative method to drying for the storage of insect biomass. We were also interested to find out the effect of ensiling on yields of insect fat or protein extracts. Different additive treatments were tested including addition of salt, sugar or organic acids. It appeared that neither of these approaches were suitable as there was a high biomass loss (10-28%) and silages had a high pH (5.3-6.8) and a bad smell. We suggest a direct acidification with sulphuric acid might result in a better quality silage. An interesting observation was that ensiled larvae developed two phases during ensiling: a liquid phase and a solid phase, with liquid phase comprising mainly water. We therefore suggest an ensiling system equipped with an automated water removal to produce partially dried insect biomass. Unfortunately we were not able to conduct LCA and CBA due to inability to generate or acquire data needed.

Background

According to the report of United Nations published in 2015, the human population will increase from 7.3 billion to 9.7 billion by 2050, indicating an urgent need to increase food production. Sustainability is a prerequisite for any production system, however, when it comes to the production of food, sustainability has unfortunately not always been one of the focal points.

Livestock products provide food of high nutritional value. Production occupies 30% of the world's ice-free surface or 75% of all agricultural land (including crop and pasture land) and consumes 8% of global human water use, mainly for irrigation of feed crops (Foley et al., 2011, Makkar et al., 2014). In addition, the livestock sector contributes between 7 and 18% of all anthropogenic greenhouse gas emissions (Hristov et al. 2013) and animal products generally have a much higher "water footprint" than plant-based foods (Mekonnen and Hoekstra, 2012, Makkar et al. 2014).

Considering that an indirect production of human food in form of meat and dairy products is less efficient than direct food production from agricultural lands (Foley et al., 2011) and that, it is unlikely that human consumption of animal-derived food will reduce in the future, it is necessary to find more sustainable solutions for feeding farm animals.

According to van Huis et al. (2013), rearing the insect could be one way to enhance food and feed security considering that insect larvae can feed on waste biomass including fruits and vegetable peels, food wastes, sewage, manure, slurry, etc. transforming it into high value food and feed resources (Diener et al., 2011, Sánchez-Muro et al., 2014, Čičková et al., 2015). Biodegradation of organic waste by insects is a fast process (between 4 and 27 days), with a feed:insect biomass conversion ratio of 2:1 (Collavo et al., 2005, Makkar et al, 2014, Čičková et al., 2015).

High demand for fish or soya meal, together with an increased poultry, pig and aquaculture production, has encouraged new research for using insect protein as an alternative protein source (De Marco et al., 2015). Insect-based feed products could have a similar market as fishmeal and soybeans, which are presently the major components used in feed formulae for aquaculture and livestock (Van Huis et al., 2013). Therefore, in the last decades there has been an increasing interest in feeding novel protein-rich ingredients (e.g. black soldier fly and housefly maggots) to fish, pigs, broilers and laying hens (Barroso et al., 2014, Makkar et al., 2014, De Marco et al., 2015, Henry et al., 2015, Vidaković, 2015, Li et al., 2017). In addition to turning organic wastes into high quality feeds, residues from rearing insect larvae can be used as plant fertilizers (Oonincx et al., 2015, Lalander et al., 2015). Excellent literature reviews on the potential use of insects as animal feed have been published in recent years (i.e., Barroso et al., 2014, Makkar et al., 2014, Sánchez-Muros et al., 2014, Henry et al., 2015).

Feeding insects to farm animals will not only contribute to animal feed security and a reduced competition between humans and animal farming for resources but will also have marked positive effects on environment, energy use and economy (Čičková et al., 2015).

Among >1 million known insects, Hermetia illucens (black soldier fly, BSF) is one of the most efficient species in disposal of organic wastes by converting them into protein- and fat-rich biomass suitable for animal feeding for all livestock species (van Huis et al., 2013). Black soldier fly larvae have been specifically pointed out as a candidate for replacing fish and soybean meals in animal feeds due to its favourable nutritional properties (St-Hillaire et al., 2007, Makkar et al., 2014). One major advantage of BSF over other insect species used for biomass production is that adult BSF does not feed and therefore require no particular care and are also not potential carriers of diseases (Sheppard et al., 2002, Makkar et al., 2014). Due to a large amount of fat in the prepupae, another application for BSF can be production of biodiesel (Zheng L. et al., 2012, Li et al., 2015, Cunwen Wang et al. 2017). In addition, BSF larvae are also a source of chitin (Wasko et al., 2016). Chitin and its derivatives have great economical values because of their numerous applications in food, cosmetics, pharmaceuticals, textile industries, etc. (Gortari & Hours, 2013).

The process of turning organic wastes into animal products by use of insects or microbes comprises: biomass production, harvesting, storage, biomass processing into quality feeds and animal production. Each step requires a complete evaluation and assessment from several viewpoints including production efficiency, energy use and food safety. Until now, most attempts have been mainly focused on optimizing insect biomass production and animal production, neglecting the steps in between.

In 2014, approximately 1.3 million metric tons of organic wastes were generated in Sweden, equivalent to 130 kg/person/year with household wastes accounting for 74% (http://www.naturvardsverket.se/). A successful evaluation and optimization of the whole process will not only benefit Swedish society but can also benefit other countries, particularly developing and under-developed countries.

At the Department of Energy and Technology of Swedish University of Agricultural Sciences (SLU), production of BSF on food wastes is currently under study. The production has until now been tested in batches of ~100 kg wastes, producing ~20 kg of larval biomass and ~20 kg of compost residue on a daily basis. In collaboration with this ongoing project at SLU, our long-term goal is to find suitable methods for storage and processing of BSF larvae for use as animal feed. Our specific goal in this project was to identify technical bottlenecks and tractable solutions for the storage and processing of BSF larvae.

This paper reports experiences with ensiling of BSF and extracting fat by means of pressure.

Materials and methods

We started our project by a literature survey, focusing on insect biomass processing, fat extraction and protein meal production. A summary of protocols utilized by other researchers is presented in Figure 1. With the exception of Kronckel et al. (2012) and Tschirner & Simon (2015), who reported working conditions (i.e., pressure, temperature and time) used for fat extraction, remaining authors did not describe procedures/protocols followed for defatting the insect biomass (Table 1).

Table 1. Working conditions for mechanical fat extraction from black soldier fly (Hermetia illucens) larvae reported in the literature

Authors	Press	Press trade mark	Pressing conditions
Kroeckel et al., 2012	Tincture press	Fischer Maschinenfabrik GmbH, HP-5MT-VA, Neuss, Germany	450 bar; 60˚C; 30 min
Tschirner & Simon, 2015	Tincture press	Fischer Maschinenfabrik, Neuss, Germany	250-450 bar; 50-60˚C; 10-30 min
Surendra et al., 2016	Lab-scale	Taby Press Type 20, Skeppsta Maskin AB, Sweden	Not reported
Schiavone et al., 2017	Not reported	Not reported	High pressure

Based on the available information and with the aim of doing a Life Cycle Assessment (LCA) as done by Smetana et al. (2016), Salomone et al. (2017) and Thevenot et al. (2017) and a Cost Benefit analysis (CBA), we set boundaries of the system under study and within the limits of our system boundary, we defined 4 scenarios to be compared (Figure 2). Our system boundary was set from harvested BSF larvae to the outcomes of storage/processing step, i.e. fat and defatted protein meals. Scenarios 1, 2, and 3 were based on published papers and Scenario 4 which included an ensiling step was our proposal.

Scenarios 1 and 2 are similar but differ in fat extraction procedures. In Scenario 1 (Fasakin et al., 2003), fat extraction was performed by solvent extraction (petroleum ether) resulting in a protein meal with a fat content of 6 to 7% on dry matter (DM) basis. In Scenario 2 (Schiavone et al., 2017), fat extraction was done by means of high pressure resulting in meals being highly or partially defatted (5 or 18% fat content, respectively). In Scenario 3 (Kroeckel et al., 2012), in contrast to Scenario 2 in which fat extraction was performed after drying and grinding the BSF larvae, frozen larvae were cut to enable leakage of intracellular fat during mechanical pressing resulting in a fat content of 11.8% (Kroeckel et al., 2012). In Scenario 4, our hypothesis was that ensiling is a more energy-efficient alternative to drying and has positive effects on yields of protein and fat from larval biomass.

To our knowledge, there is not much information in the literature about ensiling insect larvae beside the work of Rangacharyulu et al. (2003). These authors studied the inclusion of fermented silkworm pupae in diets for carps, as a substitute for fishmeal, and reported that under conditions of their study, ensiled pupae was nutritionally superior to untreated pupae or fishmeal (Rangacharyulu et al., 2003).

Ensiling

The prepupae larvae of BSF, reared on food waste, were provided by the Department of Energy and Technology of SLU and ensiling was done at the Department of Animal Nutrition and Management of SLU. The larvae were euthanized by freezing at -18˚C for 20 min before being gently crushed by a roller. Glass tubes (100-ml) equipped with water-locks were used as silo. Treatments in triplicate were: (i) addition of NaCl at 15% of fresh larval weight, (ii) addition of sugar (a mixture of glucose, fructose, and sucrose) at 3.75% of fresh larval weight, (iii) Promyr NT570 (30-40% formic acid, <25% propionic acid and <20% sodium formate) at 18 ml per kg of fresh larval weight and (iv) control (no additive). Silos were stored at room temperature (20-22˚C) for 98 days and were weighed at 0, 2, 7, 14, 21, 28, 50, and 98 d to determine weight losses. Weight losses were assumed to be a result of carbon dioxide production.

Chemical analyses

A representative sub-sample of euthanized larvae was dried at 60˚C until constant weight, ground in a coffee grinder and analysed for crude protein (CP; Kjelldahl method; Nordic Committee on Food Analysis, 1976), crude fibre (CF; Jennische & Larsson, 1990), fat (EE; according to O.J.E.C., 1998) and ash (by incineration at 550˚C for 3 h). Nitrogen free extract was calculated as NFE=100 - CP - CF - EE - ash. Chemical composition of ensiled larvae was measured in a similar manner. Upon silo opening, silage pH was measured in the liquid phase of the silage by a pH meter. Mean weight of fresh larvae, calculated after weighing 5 groups of 50 larvae with a precision scale, was 198 mg (± 11). Data on chemical composition of larvae before and after ensiling are presented in Table 2.

Microbial analyses

Fresh larval sample (30 g) was used for enumeration of lactic-acid bacteria (LAB), yeasts, moulds, clostridial spores and enterobacteria as described by Mogodiniyai Kasmaei et al. (2015).

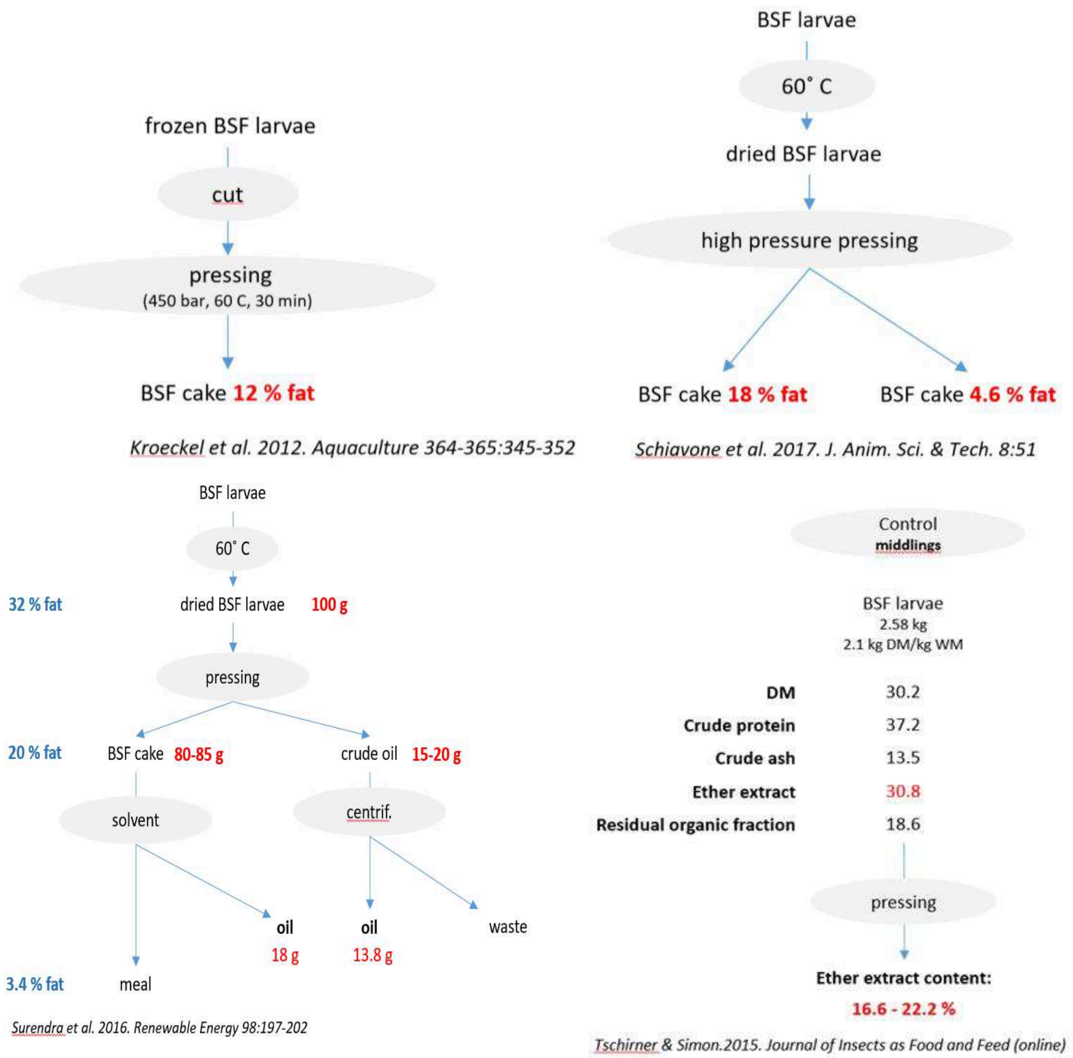

Figure 1: Yields of fat from black soldier fly larvae (BSF; Hermetia illucens) under different processing protocols.

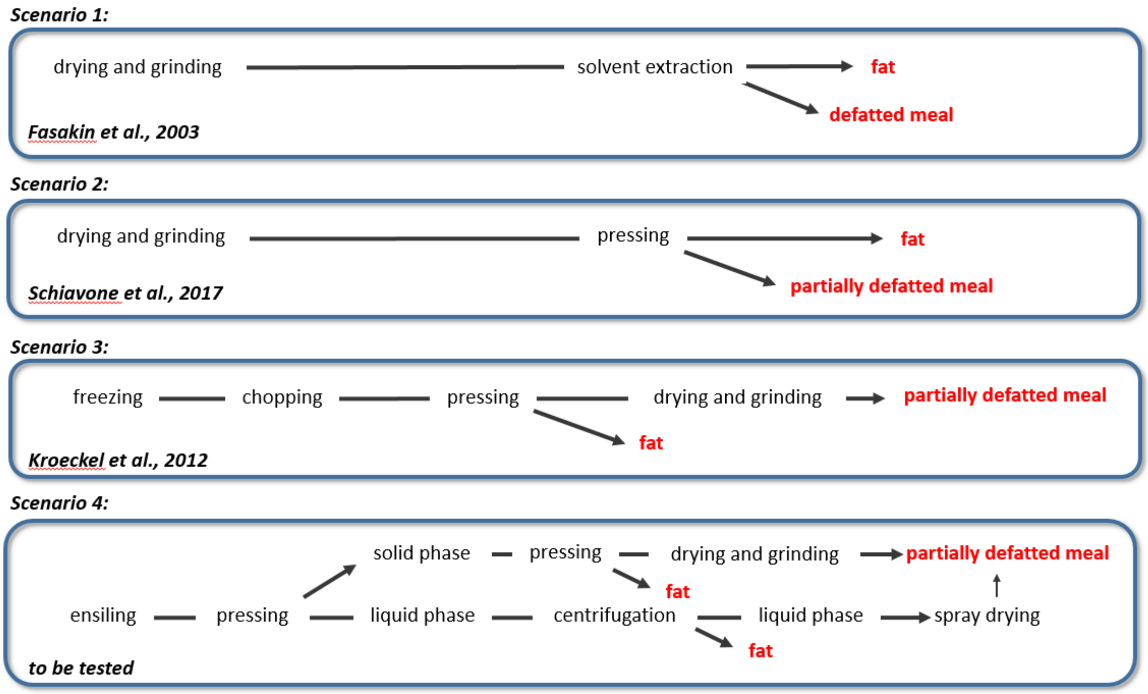

Figure 2: Different scenarios for the storage/processing of black soldier fly larvae intended to be used for Life Cycle Assessment and Cost Benefit Analysis.

Results and discussion

Chemical composition

Chemical composition of the fresh BSF larvae compares well to data published in the literature with protein and fat contents over 30% on DM basis (Table 2). The packing density of larval mass in silo was 271 kg DM m-3. Microbial analyses of fresh larvae revealed a high and diverse microbial composition. The fresh larvae were found to have high counts of enterobacteria (5.94 log cfu/g), LAB (5.49 log cfu/g), clostridia (5.24 log cfu/g) and yeasts (5.04 log cfu/g).

Chemical composition of the solid phase of the ensiled larvae resembled that of the pre-ensiled larvae with the exception that salt treated biomass had lower values for CP and EE due to the dilution effect from added salt (15% on fresh basis). The added salt also explains the high value of ash content in this treatment. The proportion of protein found in the liquid phase, again with the exception for the salt treatment, seems to be similar among treatments. No fat was detected in the liquid phase.

Table 2. Chemical composition of black soldier fly larvae (BSF, Hermetia illucens) before ensiling, and after 98 days of ensiling (n=3) with different additives (see Methods and Materials for treatment descriptions)

	DM	CP	CF	EE	NFE	Ash
				% of DM		
Before ensiling	35.8	39.4	5.2	35.7	10.3	9.4
Ensiled larvae						
Solid phase						
Control	n.a.	32.2	5.9	40.7	11.1	10.2
Salt	n.a.	28.7	4.1	25.8	10.9	30.4
Sugar	n.a.	30.9	6.1	41.4	12.2	9.4
Acid	n.a.	39.7	6.0	39.0	6.3	9.1
Liquid phase	**pH**					
Control	6.8	12.7	n.a.	-	n.a.	n.a.
Salt	5.3	6.7	n.a.	-	n.a.	n.a.
Sugar	5.7	13.8	n.a.	-	n.a.	n.a.
Acid	5.6	14.1	n.a.	-	n.a	n.a.

DM: dry matter % in fresh basis; CP: crude protein, CF: crude fibre, EE: ether extract-fat, NFE: nitrogen free extractives and ash. n.a.: not analysed.

Ensiling

Fermentation in all the treatments was very intensive resulting in excessive volume changes within the tubes causing liquid effluents to be forced upwards into the gas locks. These effluents were collected and weighed. The highest average formation of effluent was observed in acid treated larvae (12.4 g), followed by control (6.9 g) whereas salt and sugar treatments formed the least (3.1 g and 3.3 g, respectively). Quality of fermentation is often reflected in fermentation losses mainly in the form of CO_2 formation. A good fermentation process carried out by LAB normally results in low fermentation losses (3 to 4%) whereas fermentation losses in undesirable clostridia dominating process can be considerably higher (McDonald et al., 1991). The high losses in our study (Figure 3) suggests that fermentation was dominated by undesirable microflora even in additive treated samples. Such losses are unacceptable in a conventional forage silage and is a result of a mal-fermentation. The high pH (Table 2) and odorous smell of the silages provide further evidence of undesirable fermentation of the ensiled larval biomass The signs of undesirable fermentation were observed even in the salt treatment where DM losses were the lowest (10% DM loss). Further studies are required to investigate possibilities to obtain a silage with an acceptable fermentation quality.

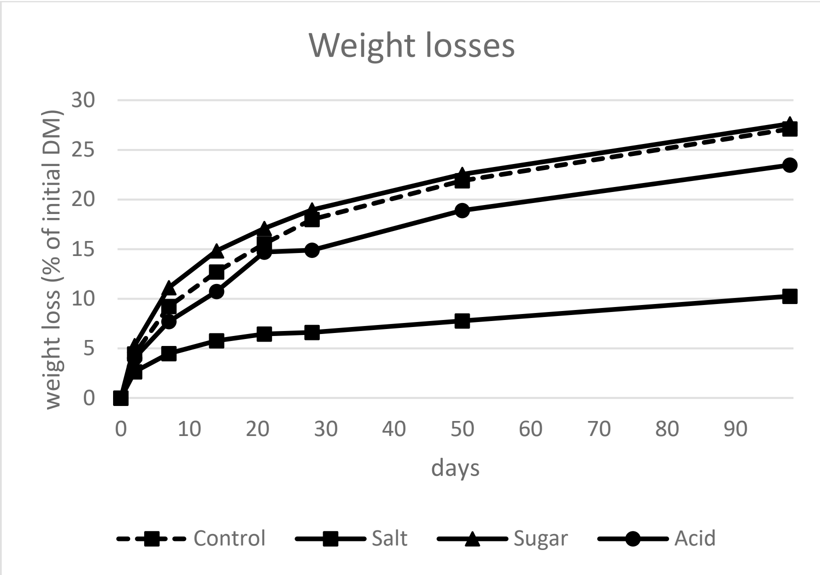

Figure 3. Dry matter (DM) losses in black soldier fly larval (Hermetia illucens) silages estimated from fresh weight losses of silage during 98 d conservation period. Salt=addition of NaCl at 15% of fresh larval weight, sugar=addition of sugar at 3.75% of fresh larval weight, acid=addition of organic acid (a mixture of formic acid, propionic acid and sodium formate) at 18 ml per kg of fresh larval weight.

Fat extraction

After ensiling, our major challenge was to extract the fat from the solid phase of silage to obtain yields of defatted meal and fat. This information was necessary for conducting the LCA and CBA.

We tried different alternatives:

1) In our first attempt, we used a hydraulic press (tincture type) specifically design for collecting liquid samples from ensiled forages. Samples of fresh and dry larvae were wrapped in a Dacron® fabric and pressed but our attempt failed.

2) In a second attempt, we tried a screw type press, normally used for small-scale oil extraction from oil seeds with an oil content >25%. In spite of the fact that BSF larvae we used had a fat content of 35.7% and that we tried different orifice diameters (open end) and working temperatures, no fat was extracted from the biomass.

3) Our third attempt was based on the work of Surendra et al. (2016) (see Table 1), in which, a screw type Taby Press Type 20, Skeppsta Maskin AB, Sweden was used. After contacting the authors to obtain more details about the protocol used, we visited the manufacturer of the press located near Örebro. We tested different preparations of larval sample (fresh, dry whole, dry milled, ensiled dry whole or milled). However, after trying the Surendra and co-workers' protocol as well as several other combinations of pressure and temperature, no fat was extracted.

A possible explanation for our failure in the mechanical extraction of oil from BSF larvae is that the fibre content of the larvae used were rather low. Surendra et al. (2016) noted that a low oil yield from mechanical extraction, when comparing insect meal to oil seeds, could be due to a low crude fibre content insufficient to create a sufficient backpressure required during the extraction process. While the crude fibre content of the BSF larvae used by Surendra et al. (2016) was approx. 10%, the BSF we used had a crude fibre content of 5.2% (Table 2).

Life Cycle Assessment and Cost Benefit Analysis

When we initiated the project, we believed that we are able to obtain information needed for LCA and CBA of storage/processing methods of BSF from literature and/or contacting the industry. However, we soon realized that information about yields and energy consumption of different storage/processing methods are not commonly reported in the literature. During the period of the project, we also contacted several insect based food/feed manufacturers and institutes, however, either they were not willing to share data with us or data provided were not relevant or sufficient. We therefore decided to produce our own data. Although the data we generated are in many aspects novel and interesting, we are unfortunately not able to conduct any LCA or CBA based on these data. We have contacted the Albrecht Daniel Thaer-Institute of Agriculture and Horticulture, Humboldt-Universität, Berlin, Germany to obtain more insights as to how successfully extract oil from insect biomass. They have had several successful experiences in processing insect biomass including fat extraction by a fully automated tincture press (Fischer Maschinenfabrik, Neuss, Germany; Tschirner & Simon, 2015).

Factory visits

We have not yet been able to visit any insect based food/feed manufacturers or institutes as promised in the application. This was mainly due to a time limitation. However, we are planning such visits in coming months and we can thereafter provide a separate report to Energimyndigheten upon request.

Conclusions

High counts of enterobacteria, LAB, clostridia and yeasts were found in the fresh larvae. Chemical composition of the solid silage phase was similar to that of the pre-ensiled larvae when salt dilution was taken into account. The fermentation weight losses at 98 d were 10 to 28 % and unacceptable. Hygienic quality was also poor as the silages smelled badly and pH was high, probably as a result of clostridial fermentation. Fat extraction by means of pressure was unsuccessful in spite of attempts with three different presses. No LCA or CBA were possible to perform due to lack of information from both manufacturers, institutes and this project. It seems that direct acidification, as opposed to fermentation, would be a better alternative for larval conservation and that more viable fat extraction methods must be investigated.

Implication and future research

An interesting observation during ensiling trial was that ensiled biomass developed two phases: 1) a solid phase with a chemical composition similar to the fresh larvae and 2) a liquid phase which comprised mainly water. The ensiling process can therefore be potentially looked upon as an alternative to drying for water removal from

insect biomass, with an obvious advantage of having considerably less carbon footprints. An automated system for liquid removal from silo together with a constant weight on top can perhaps be a novel idea to produce semi-dry insect biomass. In comparison with conventional energy demanding drying processes, such system would spare large quantities of energy, particularly in large scale applications.
Further studies are still needed for a successful ensiling of larval biomass. We did not measure buffering capacity of the larval biomass, but it is likely that the biomass has a high buffering capacity due to a high protein content. If so, a direct acidification with sulphuric acid (Jackson et al., 1984) would be more appropriate than promotion of lactic acid fermentation by inoculation with LAB or addition of sugars. We believe that although we were not able to fulfil all the promises made in the application, our results can pave the way for fruitful research and innovation in the near future in this line of research.

Publication

The results of this project will be presented at the 9th Nordic Feed Science Conference in Jun 12-13, 2018 hosted and organized by the Department of Animal Nutrition and Management of SLU.

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