Moving into a more sustainable future with the Black Soldier Fly - Black Soldier Fly Larvae as a substitute product to fish oil & meal in the aquaculture industry

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Field of Study: Environmental Sciences. After coming back from a job with Black soldier Fly Larvae (BSFL) in Malawi (Africa) the idea came up to compare the cultivation of BSFL in Africa with the tropical city Cairns in the tropical north of Australia. Using the knowledge for cultivating BSFL gained in Africa, this project was used to develop techniques to further process the cultivated larvae for the aquaculture industry as a substitute product to fish oil and meal derived pellets. Supervised by Dr. Sven Rohde.

Currently, fish pellets are constituted of fishmeal and -oil derived from small pelagic fish causing negative impacts on the environment due to overfishing. To counteract these problems a replacement with similar protein and lipid content needs to be found to meet the dietary requirements for farmed animals. Black Soldier Fly Larvae (BSFL) provide a promising alternative due to their adaptable nutritious composition depending on their diet. Researchers have already investigated optimal cultivation conditions to increase the protein and fat content in BSFL. However, cultivation BSFL and processing pellets with conventional techniques in a small scale needs further research to provide opportunities for low-income countries. Therefore, this project focused on the establishment of a wild BSFL culture, the cultivation on different locally collected organic waste in far north Queensland and the investigation of processing pellets in a small scale.

Black Soldier Fly Larvae (BSFL), Sustainable aquaculture, fish pellets

1 Introduction

1.1 Current Situation

Currently, fish pellets are constituted of fishmeal and -oil derived from small pelagic fish such as anchovies and sardines, whose overfishing already have negative impacts on the environment (FAO 2018). To prevent the need and processing of these species a replacement with similar protein and lipid content needs to be found to meets all dietary requirements for farmed animals.

Recent studies have examined the protein, fat, amino acid, and vitamin content of insects. It has been revealed that insects, especially the Black Soldier Fly, are a promising alternative and are therefore gaining more interest. Depending on the diet of the BSF larvae their nutritious compositions can vary enormously (Dortmans et al. 2021; Scieuzo et al. 2021).

Thus, the diet can be adapted to the final consumers need depending whether a high fat content or high protein content is to be achieved.

More advantages include their ability to break down a high diversity of organic substrates (faeces, manure, food scraps, etc. (Scieuzo et al., 2021)), and specific bacteria such as Escherichia.coli (Erickson et al., 2004; Liu et al., 2009) in organic matter due to the production of antimicrobial peptides (Moretta et al., 2020) and to create important by-products such as soil that can be used as fertilizer (Dortmans et al. 2021; Menino et al., 2021). However, there are regulations in place, particularly in Europe, stating that BSFL have to be cultivated on plant based feed if using the pellets for animal farming (Union 2021) .

These advantages do not only help the increasing food waste problem and the urban waste management in low income countries but moreover it helps with the reduction of diseases spread by e.g. House Fly (Musca domestica L.) (Miranda, Cammack & Tomberlin, 2019) due to the absence of a mouthpart in Black Soldier Flies to spread diseases.

1.2 General Background

The BSF Hermetia illucencs can nowadays be found all around the globe in tropical and subtropical regions between 45°N and 40°S (see figure 1a) (Dortmans et al., 2021) with almost constant temperatures. Optimal temperatures with fastest reproduction have been observed to be between 24 and 30°C (Dortmans et al., 2021) (Kim et al., 2021) even though temperatures between 15° and 47°C were tolerated (Kim et al., 2021) . Kim et al. (2021) have also observed that temperatures above 30°C decreased the life span of adult larvae.

The life cycle of a BSF is around 45 days (see figure 1b) (Dortmans et al., 2021), and it’s been observed that temperature, humidity and light influences their life cycle (Dortmans et al., 2021) leading to extended or shortened life cycle duration under unfavourable conditions (Dortmans et al., 2021) . A humidity of 70 - 80% has been concluded to be optimal. A higher humidity (around 70%) increases the survival and hatching rates but results in a slower development time (Kim et al., 2021) . The flies have no mouth part, meaning they neither eat nor drink during their short life as a fly. However, a moist surface or water is required for mating to stay hydrated. Furthermore, natural sunlight is essential as the wavelength stimulates the flies to mate. It’s been reported by Zhang et al. (2010) that a wavelength above 700nm cannot be seen by insects (Briscoe and Chittka, 2001) and wavelength between 450nm and 700nm are optimal condition to stimulate mating (Dortmans et al., 2021).

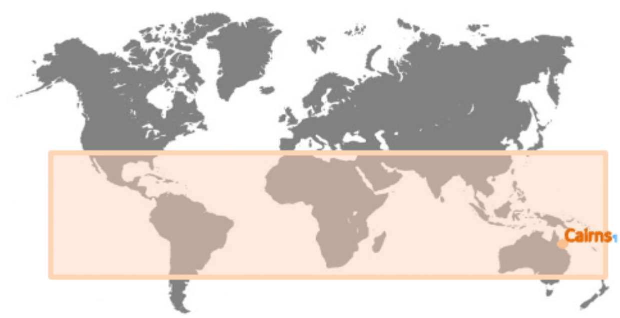

Figure 1a: World Map showing the tropical and subtropical regions where the Black Soldier Fly can be found, with Cairns as project location.

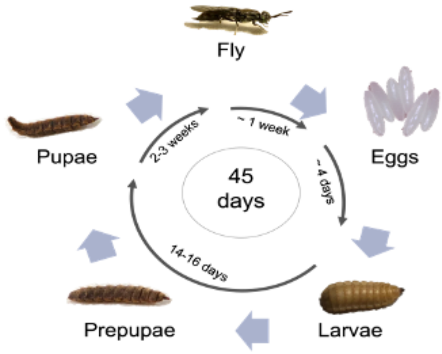

Figure 1b: Life Cycle of a Black Soldier Fly larva (Dortmans et al., 2021).

After mating, the female fly lays their egg packages of 400-800 eggs near organic material in dark, dry and sheltered cavities (Dortmans et al., 2021) such as corrugated cardboard. A sheltered environment protects the eggs from predators (Dortmans et al., 2021) but also prevents the egg membrane of dehydration by direct sunlight (Dortmans et al., 2021, Kim et al., 2021) .

The larvae hatch roughly after four days, depending on the environmental conditions. Barely visible with the naked eye they start feeding preferably on moist and mash-like material, going through different larvae stages until reaching the prepupae stage with a size of up to 2.5cm (Dortmans et al., 2021)

During the prepupae stage they crawl out of the moist substrate to find a dark, dry and protected environment (example can be seen in Figure A.1) and to turn into the pupae stage in which they remain for around two to three weeks (Dortmans et al., 2021). The emerging process only takes a few minutes, and their life expectation is short with a maximum of one week. During that time, they find a partner to mate with and the female fly lays their eggs in a protected environment and dies (Dortmans et al., 2021) .

Understanding the life cycle of BSF forms the basis for successful cultivation under controlled conditions. However, beside the cultivation, the processing of harvested larvae into storable pellets are from major interest to make the BSFL an attractive substitutional product to the currently used fish meal and oil derived pellets, especially to provide opportunities for low-income countries.

Thus, this project focused on (i) the cultivation with the aim to collect data about feasible cultivation condition on different locally sourced substrates in far northern Queensland and (ii) processing pellets in a small scale with simple techniques.

2 Material & Methods

2.1 Cultivation

Initial Stock and Cultivation

The BSFL were cultivated in the tropical north of Queensland, in the city of Cairns, Australia mainly during the winter months (dry season) between May and November 2022. This location was chosen due to the natural occurrence of BSFL in tropical and subtropical areas. An initial stock of larvae (100 larvae) was purchased locally from the company BioSupplies in Yagoona, NSW, Australia. The larvae were in different stages ranging from 2nd instar to pre-pupae stage. Another batch of larvae was ordered from PetWaves PTY lt.

The main cultivation was conducted in a cage (80cm x 110cm x 50cm) outdoors as seen in Figure A.1. to mimic natural conditions. The framing of the cage was built using small plastic irrigation pipes (Æ 5mm) which were purchased at a local hardware store (Bunnings). To prevent the larvae from escaping and from predators such as flies, geckos, ants etc. a mosquito net with a mesh size was glued onto all sides except the front door using hot glue. The front door was built with velcro tape to allow easy and fast access to the cultivation container. Additionally, to the flyscreen around the cage, the larvae were protected from intense sunlight during the day by covering the sides and the top with a shade tarp (ultramesh tarp, Anaconda). The side covers were attached with cable tie at the top of the cage to roll it up throughout the day. Hence, the cover was easy to remove to guarantee enough sunlight for the flies to mate during the day, but easy to roll down to prevent overheating in the afternoon sun.

The larvae were cultivated in small plastic containers with holes for aeration on the side and stacked on top of each other. Each container was separated into two compartments using the included divider. One compartment was used as cultivation chamber which was connected to the second compartment via a ramp (⌀ 5mm pipe) on a 40-50° angle. Since BSFL are light sensitive (Dortmans et al., 2021) the clear cultivation container (Montgomery Clear Medium White Storage Box, 452mm x 127mm x 243mm, W x H x L, Bunnings) were darkened using duct tape.

During the project the cultivation container were slightly modified. The ⌀ 5mm pipe ramps were replaced by a wider ramp which made it easier for the larvae to crawl out of the substrate through the pipes instead of on the container wall. The second compartment was kept dry using newspaper and cardboard and used as the harvesting chamber.

Temperature and humidity were monitored using the Govee Home Thermo-Hygrometer.

Additionally, to the initial stock, an insect farm (Tumbleweed Can-o-Farm, Bunnings) made of 100% recycled material with two working trays was purchased and set up to investigate whether native flies are present and a population can be obtained naturally.

It was kept outside covered with a waterproof plastic foil. The compost was initially started with shredded food scraps to attract wild flies and the lid was kept slightly open to give flies access for oviposition. The open lid also allowed the prepupae to escape the substrate for pupation. The larvae were fed with kitchen waste 2-4 times a week. During the day the insect farm was protected from excessive sunlight by the surrounding trees. Temperature and humidity were not specifically monitored inside the insect farm.

Substrate

The larvae were cultivated on three different substrates (A: fruits; B: vegetables; C; MIX). The MIX contained a mix of organic waste material that was obtained locally from several restaurants, coffee shops, juice shops and commercial operated boats. The substrate was shredded using the smallest grinder plate of a standard meat grinder (Ausbuy Stand Mixer, 5.5L 6-Speed Tilt-Head Food Mixer, Amazon*).*

The moisture content of the substrate was monitored every 2-3 days. If the substrate was too dry water was added with a spray bottle. In case of liquid excess the container was drained. The amount of substrate was adapted to the amount and the stage of the larvae in each container. A massive excess of food can result in mould (especially in the tropics) and might cause infections for the larvae. However, a lack in food deteriorates or effects the growth.

Pupae harvesting and storage

The prepupae were self-harvested using the pipes to crawl out of the substrate. After falling into the second compartment of the cultivation container they were collected and kept inside another small plastic container where the sidewalls were covered with duct tape. The lid was slightly opened to allow for aeration and the emerged flies to escape the container. The container was stored inside the cage in a covered corner.

Flies & Oviposition

The emerged flies escaped the dark container to seek sunlight in the cage, which was placed in the sun during morning hours, for mating. During the afternoon the shade cloths were rolled down to protect the flies from excessive sun. Corrugated cardboard was rolled up and hung inside the cage above the substrate. Some cardboard pieces were placed on the lid of the cultivation container. A bowl of water was placed inside the cage to allow for moisturization.

Eggs and larvae cultivation

Eggs found inside the cage within the cardboard pieces were kept in a small plastic container for 2-3 days and then transferred to the cultivation container filled with one tablespoon of soaked oats.

Eggs inside the insect farm were not transferred to observe the natural behaviour.

2.2 Processing

A thin layer of substrate with larvae was placed on top of the sieve with a black bucket placed underneath to separate the light sensitive larvae from the substrate. After separation they were rinsed in a smaller cooking sieve, killed in boiling water (100°C for 3min) to kill pathogenic bacteria, dried with a paper towel, and then further processed as shown in the figure below:

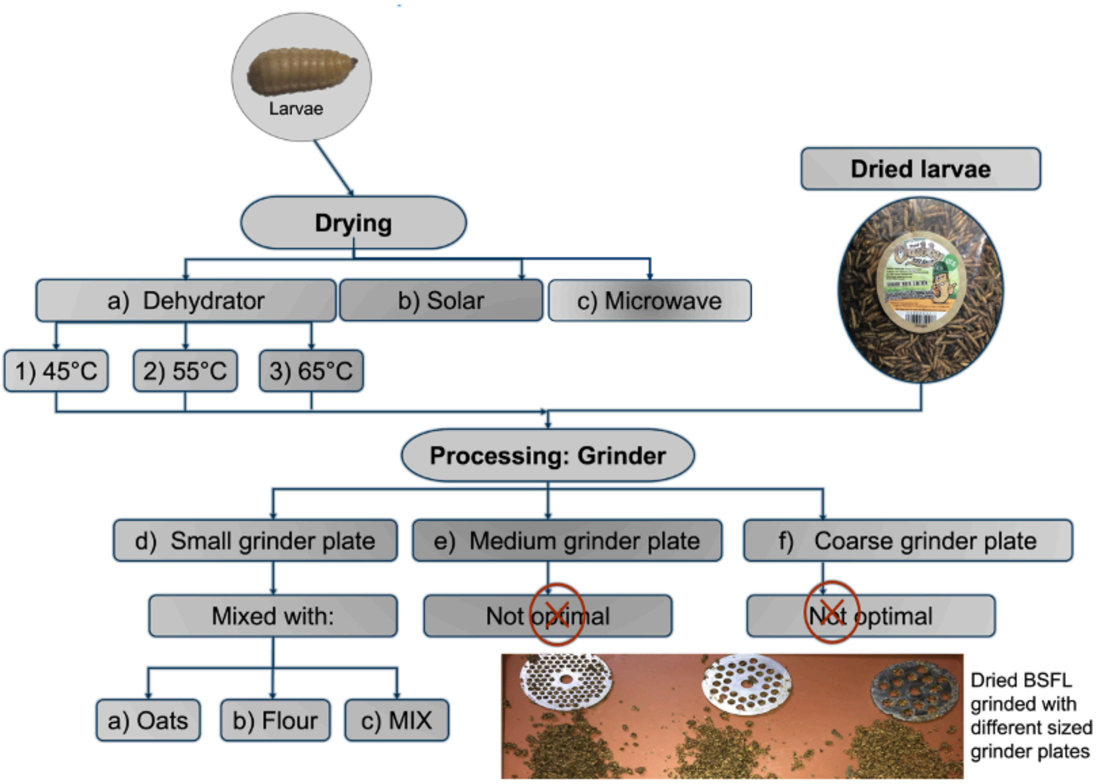

Figure 2: Process of BSFL. As the first step BSFL need to be dried before they are further processed and turned into pellets with a grinder.

Drying

Three different drying methods were tested to investigate the best drying technique for processing pellets. First, a dehydrator (Devanti Food Dehydrators, 10 Trays) was preheated to the aimed temperature of 45°C, 55°C or 65°C and 60g of boiled and dried BSFL were spread on each of the four drying trays. After the oven has reached the temperature, the larvae were dried for a 10h period until a constant weight was achieved. Second, a solar dryer was built of wood and plastic foil and left in the sun during sunny day. Roughly a batch of 1000 larvae were dried using the solar drier.

For the last method, microwave drying, 60g of larvae were weighted and evenly distributed on a ceramic plate and then placed in a conventional microwave. The microwave were microwave dried at 800W in three cycles, following the protocols of EAWAG aquatic research (2023) and Dortmans et al. (2021).

Producing pellets

The dried larvae were grinded using small, medium and coarse grinder plates. The meal produced by the small grinder plate was further mixed using the conventional kitchen machine with a) Australian Quick Oats b) Corn flour and c) LSA mix in different ratios. The final mix was processed with the small grinder plate to obtain pellets.

Ratio	$Mass_{Larvae}$	$Mass_{Flour}$
4:1	100 g	25 g
3:1	100 g	33.3 g
2:1	100 g	50 g

Table1: Mixing ratio for the production of BSFL pellets..

3 Results & Discussion

Cultivation

Wild BSF were easy to attract with the insect farm. The first egg packages from wild flies were found within the first day. On sunny days 5-7 egg clusters per week could be found on the inside of the insect farm whereas on overcast or rainy days no egg clusters were found at all. This is probably due to a lack of sunlight during the winter season with lower light intensities as a consequent result.

The semi- controlled cultivation in the cage, however, was less successful with only two egg clusters during the whole cultivation period. The maximum fly density was counted with 132 flies and the minimum was around 35 when the flies first started to emerge at the beginning of the experiment. The temperature ranged between 11.4°C (minimum at night) and 27.8°C (highest during the day) and the humidity was on average 80.9%. Both, temperature and humidity are within a normal range according to (Dortmans et al., 2021; Chia et al. 2018) and should not have affected the mating negatively. It is assumed that the unsuccessful mating might be due to either the insufficient light intensity or the low fly density. This is in line with (Tomberlin & Sheppard, 2002) who concluded that the intensity of the sunlight plays an important role in mating and oviposition behaviour and Zhang et al. (2010) who did not observe mating during winter months. This can be confirmed by the observations made with the insect farm. Regarding the fly density Nakamura et al. (2015) have achieved a reproduction in a smaller cage (27cm x 27cm x 27cm) with 100 flies, which doesn't underline the observation in this experiment. Furthermore, the water uptake during the fly stage might have affected the reproduction. According to Nakamura et al. (2015) the fly life span can be extended by providing sugar water.

Summarised, after 1-2 months a small colony of different stage larvae was established in the insect farm. One reason why the insect farm was more sufficient compared to the cage cultivation might be that the flies were mating in the surrounding area where they found the right mating conditions and were then attracted by the smell of the compost for oviposition. The results show that an initial stock can be naturally in the tropical study location and that the cultivation of BSFL with low effort and maintenance is feasible at low costs. Even though the cultivation with the insect farm under natural condition was highly productive, it takes several life cycles (months) to establish a suitable population size of larvae for further experiments.

However, a mass mortality was observed during the project, probably due to a lack of oxygen that was created by the high larvae density. An aggregation of larvae was noticed at the bottom of the substrate which might have caused higher temperatures (Barragan-Fonseca, Dicke & van Loon, 2018) and an insufficient aeration. Chia et al. (2018) have reported death and crippling malfunction if larvae are kept at temperatures above 40°C. Signs of deformed wings at newly emerged flies were observed before the mass mortality occurred and lead to the assumption that temperature as well as a lack of oxygen have caused the mass mortality. Due to the mass mortality, additional larvae were purchased locally for further processing experiments.

Processing – Drying

From all three drying methods the solar drying process was the least successful method due to the weather conditions as well as predators such as frogs, geckos, ants, birds, etc. feeding on the larvae. The rain throughout the day impeded the drying process and caused a longer drying period. Longer drying periods then again caused mould due to the high air humidity and resulted in an incompletely dried product. However, the solar dryer might work well on hot and sunny days with lower air humidity and is then the most energy efficient drying process.

The microwaved- dried larvae were crunchy and puffed and the dried product can be fed directly to fish and poultry. Since it was not defatted it can only be stored for a few weeks. Storing the larvae in vacuum bags might increase the storage time but was not tested during the experimental period.

Larvae dried with the dehydrator resulted in less voluminous and crunchy larvae and hence further processing is needed to increase its market value. Further processing also allows longer storage of up to 6 months if the water activity is lower than 0.4 (lower than 0.6 inhibits growth for bacteria and yeast) (Dortmans et al., 2021).

Processing – Pelleting

The medium and coarse grinder plate generated a larvae meal with visible larvae chunks which was unable to turn into pellets. The small grinder plate produced an evenly shredded meal that was further processed with the different ingredients.

Ratio	$\text{Mass}_{\text{Larvae}}$	$\text{Mass}_{\text{Flour}}$	Notes
a) Australian Quick Oats			
4:1	100 g	25 g	No homogeneous mass due to the oats; soaked oats or smaller grinded oats would have been better
3:1	100 g	33.3 g	No homogeneous mass; Pellets fall apart
2:1	100 g	50 g	No homogeneous mass; Pellets fall apart
b) Corn Flour			
4:1	100 g	25 g	Good consistency, but too soft; fall apart quiet easily
3:1	100 g	33.3 g	Better consistency; good pellet shape
2:1	100 g	50 g	Dry, firm and solid (almost powdery) consistency; good pellet shape
c) LSA Mix			
4:1	100 g	25 g	Greasy; soft consistency; fall apart easily
3:1	100 g	33.3 g	Greasy; Short pellets
2:1	100 g	50 g	Very greasy consistency; short pellets; pellets fall apart

Table 2: Results of the produced pellets.

The best obtained pellets were produced by mixing a 3:1 or even 2:1 ratio of larvae: Corn Flour as seen in Figure A3. However, the final product was not analysed for protein and fat content as it was beyond the scope of this study. A nutrient analysis would have been interesting to assess whether the larvae meet the protein & fat content requirements for the animal feed industry.

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5 Appendix

Figures

Cultivation Setup

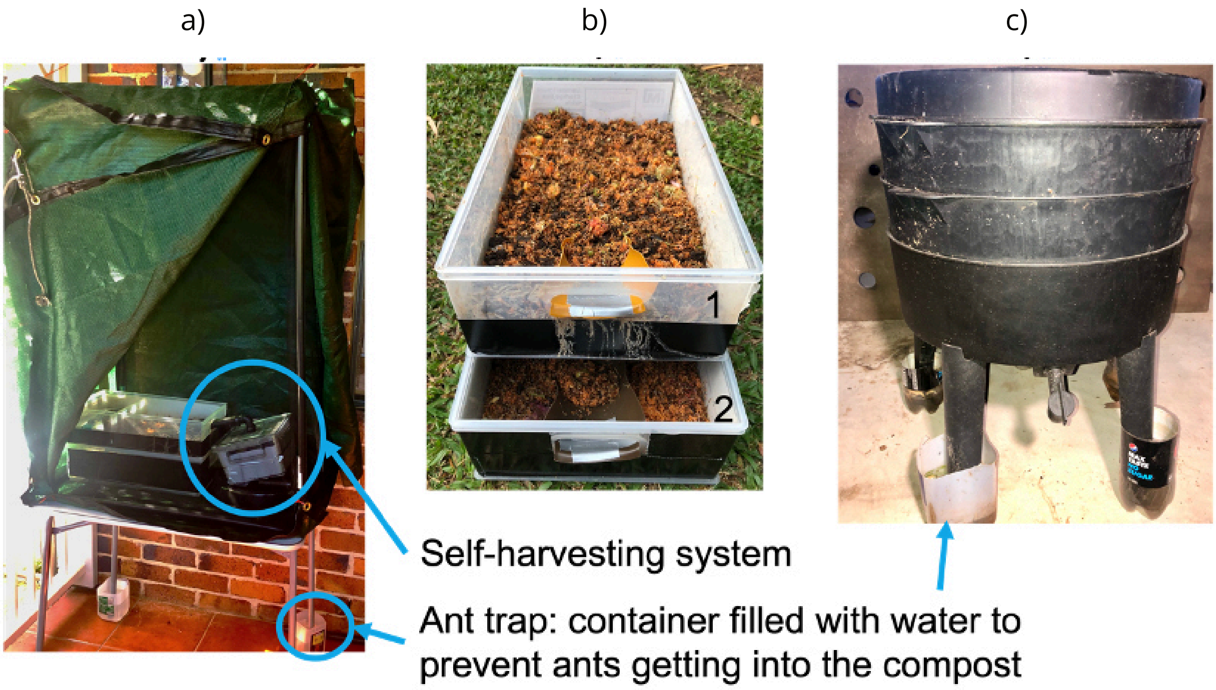

Figure A. 1: Cultivation cage (a) with two rearing containers (b) filled with fruits and mix of vegetables for rearing BSFL. The cage location provides sunlight during the morning for 3.5h hours and prevents overheating during the hottest time of the day. Figure (c) shows the insect farm with two working trays and a drainage system for excessive liquids.

Cultivation set up in a) cage with the cultivation container seen in b) and c) the insect farm.

Harvested Larvae in Pupae Stage

Figure A. 2: Larvae in pupae stage, in dry, dark, shaded and protected environment.

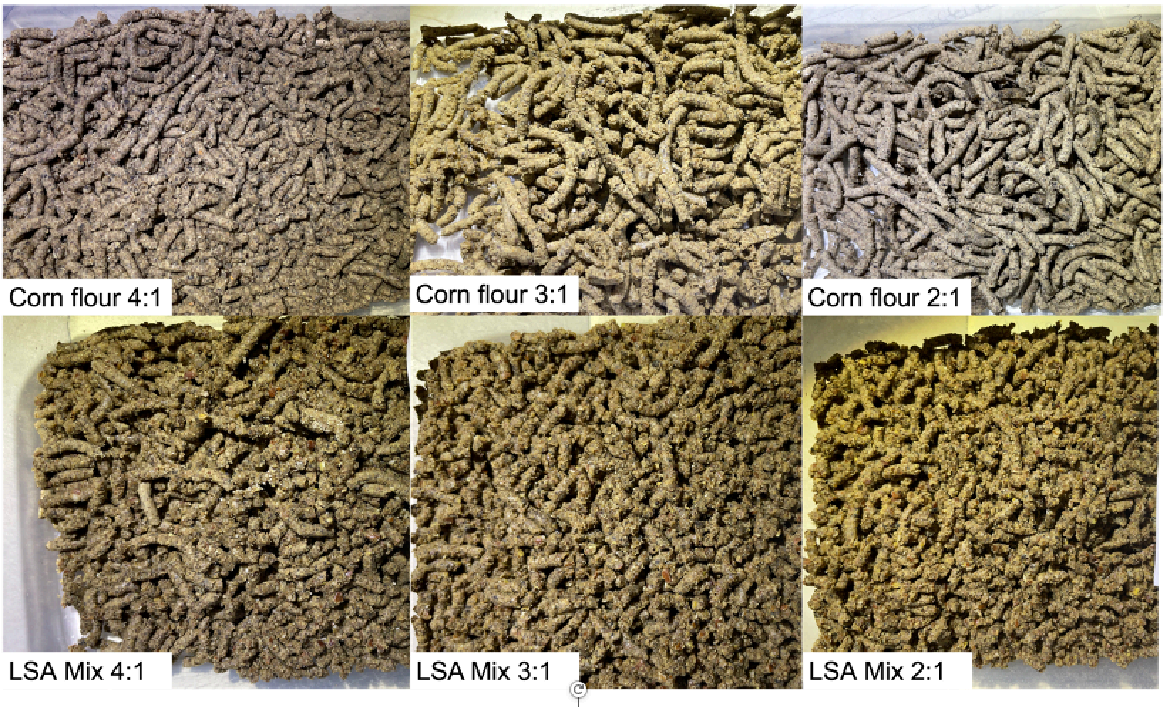

Figure A. 3: Overview of the produced pellets.