KLIPP

KLIMASMART PROTEINPRODUKSJON

Quality analysis of different biowaste streams (BWS) and their influence on mealworm (Tenebrio molitor) development

tebrito

Transforming the future of protein supply

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Introduction/Background

Protein production worldwide needs to increase in pace with the world's growing population. By 2050 a staggering 60% increase in protein production is demanded using the same land, causing less emissions of greenhouse gases[1].

The use of insects to convert waste to protein is an efficient way of making more with less. Insects have a protein quality comparable to beef but insect production has less environmental strain compared to beef production.

The Interreg project KLIPP - Climate Smart Protein Production, aimed to contribute to create the basis in Inner Scandinavia for a cross-border large-scale production of insects for food and feed for the European market.

Purpose

The objective for Tebritos participation in the KLIPP project was to analyse the quality of different biowaste streams (BWS) and their influence on mealworm (*Tenebrio molitor*) development in order to evaluate, verify and potentially adapt the method for large-scale production.

The purpose of the trials was to complement KLIPP project results by evaluating the technical suitability and effect on total yield by considering the dried or semi-dried feed types as an alternative to current dryfeed organic wheat bran. Within the scheme we were also able to evaluate the possibility to crush the materials to attain a better technical fit. Additionally, the possible increase in digestibility by soaking crushed material was briefly investigated for a possible future use as "instant wet feed".

[1] https://www.wri.org/

Materials and methods

Facilities

The experiments were performed at the facilities of Tebrito AB in Orsa. During the trials, Tebrito AB moved, changing location midway trials.

Site 1

Bornvägen 53, 794 32 Orsa, Sweden.
Pilot facility consisting of two rearing cells at approx 200 square m. Climate system consisting of manually handled mobile devices for heating and humidity control.

Site 2

Klockarvägen 3A, 794 32 Orsa, Sweden.
Testing facility for large scale automated rearing. Consisting of three rearing cells with a stationary climate control system, climate lock system and software to log climate and rearing data.

Biowaste streams

All feed types used in the trial were residual streams of Swedish or Norwegian food industry companies, identified by the KLIPP project management.

- Brewer's spent grain
  80kg sample, (20% DM) from Spendrups (Grängesberg, Sweden)

- Semi-dry spent brewer's grain
  80kg sample, (40% DM) from Spendrups (Grängesberg, Sweden)

- Milled dried spent brewer's grain
  80kg sample (100% DM) from Spendrups (Grängesberg, Sweden)

- Barley residuals
  100kg sample (100% DM) from Fiskå Mølle (Norway)

- Crispbread
  60kg sample from Leksands Knäckebröd (Leksand, Sweden)

- Gluten-free crispbread
  60kg sample from Semper (Falun, Sweden)

- Fermented carrot

- Brewer’s spent grain and gluten free crispbread mixed with water

Weight to volume comparisons, BWS

All feed types were added in equal amounts as the control at 0.98 kg/CU (carry unit). The weight to volume differs between the feed types compared to control feed. The humidity of samples is not known.

Trial materials/BWS	**Volume weight (L/0.98kg)**
Crisp bread LEKSAND	2.2
GLF Crisp bread SEMPER	2.7
Barley residue Fiskå MøLLE	6.1
Milled semi-dried spent grain SPENDRUPS	2.9
Ref Wheat bran	3.5

Fig 1

Equipment

To attain the same grain size as wheat bran, a mill was acquired for the trial. Dried grains from Fiskå Mølle, gluten free crisp bread from Semper and crisp bread from Leksands were all prepared in the mill. In order to test moisture content and ph-levels in the different bio waste streams a ph-meter, a water activity meter, a scale and a laboratory bench were acquired.

Fig 2

Fig 3

Method

The complete trial ended up consisting of three different parts. The different parts will, in this report, be referred to as Part 1, Part 2 and Part 3.

General trial design

The general trial design consisted of one trial batch per substrate and a control batch from the regular production. As the tests were conducted in an industrial setting alongside normal rearing activities and conditions the test batches consist of different sized batches in some cases. Trials were conducted according to regular rearing protocol of Tebrito under currently existing climate conditions (temperature target 29*C and humidity 65%rH). Cycle time from eggs to larvae mature for harvest at age 60-74 days.

- Beetles lay eggs in wheat bran (future feed of larvae)
- Week 0-1: Incubation of eggs in rearing cells
- Week 2-3: Watering small larvae
- Week 4-8: Wet feed period (potatoe, apple etc)
- Week 5: Rebran (refill of dryfeed wheat bran)
- Week 9: Harvest

The wheat bran in week 5 was in trials replaced with the dry KLIPP feed types. The same amount as when applying regular wheat bran (rebran) was used (0.98 kg). The feed materials in the trial had a particle size that could not pass through the sieve when separating adult insects and could therefore cannot be added from start. Trial substrates must therefore be used in combination with regular dryfeed.

Crushing and soaking

The Crispbread and the Gluten free crisp bread was successfully crushed into a harsh flour that could easily run through separation frames. The barley residue was not possible to alter through available crushing equipment.

The crushed material was used as a substrate for egg-laying to start new beetle batches as well as feed/bedding for the prepupae larvae stage (post-harvest).

Part 1

During Part 1 the usual rearing substrate (wheat bran) was replaced by the test substrates. No other changes to the rearing protocol were made. The trial design was flawed and tests should have been carried out differently. Dry feeding substrates were used as wet feeding substrates giving results not possible to evaluate or compare to the control. Feeding ad libitum was disregarded.

Fresh carrots were to be tested in one batch but had such poor shelf life that the test never was conducted.

Part 2

In Part 2, all dry feeding substrates including mill dry grain (Spendrups), dry grain (Fiskå Mølle), crisp bread (Leksands Knäckebröd) and gluten free crisp bread (Semper) were used to replace the usual rearing substrate (wheat bran) at the step in the rearing protocol where wheat bran usually would have been refilled (rebran).

Part 3

Part 3 was designed as a dry feed trial but was complemented with two wet feed tests including fresh, fermented carrots and a wet mix of Spendrups-Semper replacing the usual wet feed (potato peel). The main aim of the dry feed tests was to completely replace the usual dry substrate (wheat bran) with another dry substrate with the same grain size. In Part 3, substrate from Fiskå Mølle was not tested.

Potato peel residue was used as wet feed in all batches except the wet feed batches. The carrots in Part 1 had poor shelf life and to address this problem, fermented carrots were used in Part 3 and were compared to the usual wet feed substrate, potato peel.

Results

Harvest material fractions

Larvae

Live larvae are separated from the feeding substrate and residue at harvest based on attachment to a conveyor belt. Normally, some residual dry and/or wet feed ends up in the larvae fraction but is subsequently removed. Target yield is 1.2 kg/CU, but varies between 0.5-1.1 depending on feed type, temperature etc.

Frass

Insect excrements, small parts of feed residues, chitin and possible dead insects are separated based on particle size and is a second main product. Theoretical factor is 2.1x larval weight

Heavy residue

Larvae ejected by sorting machine (not attaching to conveyor belt, live or dead) along with dried wet feed residues. Proportion of larvae to feed residues differs a lot due to feed type and amount fed. Optimal is to aim for 0, but is seldom or never presented in regular harvest.

Light residue

Fractions separated based on weight, i e light enough to be sucked upwards from material layer. Possibly tiny larvae can end up in fraction but fraction mainly contains dry feed residue and is optimally close to zero.

Part 1

Results from Part 1, NA.

Part 2

With the current method for adult separation, none of the feed types are suitable to right off replace wheat bran without pretreatment like crushing. (Semi-dried spent grain was evaluated as mentioned within the KLIPP project and works out technically, but beetles were unhealthy.)

With available technique, residual feed from all trial materials appears in the final product, rejected material and in the frass, with one exception. Semi-dried spent grain rather ends up as light reject and leaves a clean final product. Results indicate that all trial feed types perform poorly compared to control.

Semi-dry spent grain is the top material presenting a yield of 92% of that of regular dryfeed. Around half of the added amount is harvested as residue. The final product is clean from contaminating fractions. The results from Part 2 clearly shows a superior performance from Spendrups dry spent grain, although none of the test feeds performed at the same level as the control feed substrate (wheat bran). The yield presented by Crispbread was 85%, by barley residue 67% and by Gluten free crisp bread 67% compared to reference performance on wheat bran.

Crisp bread - Leksand

Yield: 86 % of control.
Residues are present to an extent that impairs final product quality.

Gluten free crispbread - Semper

Yield: 53 % of control.
Residual amount of feed is larger than that of regular crisp bread, and also more visible due to bright colour. Along with the barley residue, this material was the most time consuming group to harvest. Larvae mortality was higher than ever seen before.

Barley residue - Fiskå Mölle

Yield: 67 % of control.
Larvae size possibly larger than control (size only estimated, not measured). Highest amount of feed residues in the final product. Highest amount of visible residual particles in frass. Along with the gluten free crisp bread, this material was the most time consuming group to harvest.

Semi-dry spent grain

Yield: 92,4 % of control.
Clean final product. Larvae possibly larger in size than control. Feed seems not to have been eaten to a noteworthy degree - large light residual fraction.

Part 3

The dry feed materials (Crisp bread - Leksand and Gluten free crisp bread - Semper) used for full substitution of the ordinary dry feed (wheat bran) were both observed to be less consumed during larvae growth phase than control. The materials appear too hard to digest rather than not accepted. Compared to the trials in Part 1, where the same test feed types were used but combined with regular dry feed, the results are poor.

Crisp bread - Leksand

Yield: 12-24% of control; 8-22% of same feed in trial set up Part 1.

Gluten free crispbread - Semper

Yield: 13-18% of control; 44-54% of same feed in trial set up Part 1.

The wet feed materials prepared for trial Part 3 both resulted in a clear increase of larvael growth compared to regular wet feed.

Wet blend Spendrups-Semper

Yield: 144% of control

Fermented carrot

Yield: 113% of control

Table - Complete results

Part 2

KLIPP feed type	Larvae yield kg/CU	Diff with control	Frass yield kg/CU	Diff with control	Residue HEAVY kg/CU	Residue weight (kg) LIGHT	Average individual weight (g)
Harvest date 2021-09							
Dry Leksand	0.43	85.11%	0.75	59.64%	0.694	0.108	0.035
Dry Semper	0.26	52.89%	0.754	59.99%	0.947	0.038	0.03
Fåske Mölle	0.33	66.89%	0.75	59.64%	0.721	0.194	0.058
Control	0.5	-	1.258	-	0.079	0.034	0.045
Harvest date 2021-09							
Semi dried spent grain Spendrups	0.57	92.42%	1.16	103.16%	0.076	0.464	0.061
Control	0.62	-	1.124	-	0.58	0.104	0.05

Fig 4

Part 3

KLIPP feed type	Larvae yield kg/CU	Diff with control	Frass yield kg/CU	Diff with control	Residue HEAVY kg/CU	Residue weight (kg) LIGHT	Average individual weight (g)
Harvest date 2022-08							
Wet blend Spendrups-Semper	1.4	144.33%	2.22	98.23%	0.17	0.126	0.086
Control	0.97	-	2.26	-	0.063	0.45	0.064
Fermented carrot	1.19	113.66%	2.35	94.00%	0.069	0.41	0.078
Control	1.047	-	2.5	-	0.077	0.6	0.071
Harvest date 2022-10							
Dry Semper, harvest 1	0.115	12.64%	NA	NA	1.617	2.25	NA
Dry Leksand, harvest 1	0.034	24.29%	NA	NA	2.3	6.25	NA

Control	0.91	-	2.09	-	0.118	0.3	0.073
Dry Semper, harvest 2	0.14	17.74%	NA	NA	1.95	4.04	NA
Dry Leksand, harvest 2	0.096	12.17%	NA	NA	1.886	9.62	NA
Control	0.789	-%	2.43	-	0.129	0.55	0.079

Fig 5

Discussion

Part 1

Feeding ad libitum was disregarded and it is therefore possible that the population experienced starvation giving flawed results when calculating individual weight, therefore Part 1 will not be reported.

Fresh carrots were to be tested in one batch but had such poor shelf life that the test never was conducted. The logistics and environmental stress that weekly deliveries of fresh carrot would entail were not applicable to this project.

Part 2

Choice of amount of dryfeed per CU could be optimized for the different feeds based on nutrition values, moisture content and weight volume to get a juster comparison. The bedding volume along the growth cycle seems to possibly affect the well-being of larvae. Materials with high density will in this case not offer so much environmental shelter within the box. Information on available amounts, price per kg could help in designing a logistical- and cost effective feeding protocol including the trial feed types.

The different harvest fractions (Larvae, frass, heavy residue and light residue) do not appear as pure fractions but more or less contaminated by other fractions and this implicates the need for further development of separation techniques.

The urge to solve contaminations in the end product, using for example air blow techniques utilised in the milling industry, shares a joint goal with the usage of wet spent grain as a wet feed.

Part 3

During the second part of the trials (Part 2) we learned a lot. The poor result left oss wondering what we could do differently to attain a better result. Hence we design a third part of the trials where we put the attained knowledge to good use.

As can be seen from the results, the results from dry Semper and dry Leksand were overall not great, there was an inability to compare the difference with the control as the larvae were so small that counting was not possible. We also see a high amount of light residue that does not correspond to the control. This is most likely due to the harvesting machine not being able separate the “dusty” material from the regular light residue.

Moult from the larvae fed fermented carrot was not digested at the same amount as the control (or the other test batches). The moult not being consumed by the larvae could stem from the low pH (approximately 3.5) of the ingested fermented carrots, however this requires further testing to know the exact reason, for example, the larvae and the moult would need to be analysed to see where the acidic material allocates.

As is seen in the results, the crisp bread and gluten-free crisp bread were not effective to promote growth in the larvae at an early stage. However there was a visual improvement in growth at later instars. Further studies would be needed to know its exact effect compared to regular wheat bran and also exactly what instar is the best to begin feeding with crisp bread.

Spent brewers grain untreated, especially in combination with thickening agent (glf) has great potential for industrial scale application. Carrots or other fresh, untreated agricultural residues need pre-treatment to prolong shelf life, for example fermentation. If shelflife is adequate, fermented carrots show great promise as a wet feed on its own or in combination with other substrates. Further studies are needed to better understand why moult is not digested at the same rate as with other substrates. Barley residue has potential but has substantial technical challenges if used in an industrial environment. Particle size and addition of water is key to make some substrate feasible. Our conclusion is that there are sufficient volumes of feeding substrats in the region for large scale industrial insect production.

Appendix 1 - Figures

Fig 1: Trial materials, Volume weight (L/0.98kg)

Fig 2: Mill used for milling substrates

Fig 3: Laboratory equipment

Fig 4: Results, complete table, Part 2

Fig 5: Results, complete table, Part 3