Half-year report: 'Insect-based ingredients in aquafeed'

Manon Eggink, DTU Aqua
Covering August 2020 – January 2021

August 2020

Enorm has mechanically separated larvae meal into five different fractions by size (0-200, 200-400, >400 µm, of which the latter has been further separated into 0-200 and 200-400 µm as adhering smaller fractions were observed in the largest fraction). The purpose of this fractionation was to investigate whether it is possible to make fractions with different chitin levels, assuming that the smallest size particles have the lowest chitin content. In August, we analyzed these different fractions on chitin content (spectrophotometry) and amino acid profile (HPLC). We found that the separation method did fraction chitin (Figure 1), and that larger fractions had higher chitin contents than smaller fractions, finding 1.9% chitin of dry matter (DM) in the smallest fraction of the first separation (0-200 µm), and 18.7% DM in the largest fraction (>400µm) of the second separation.

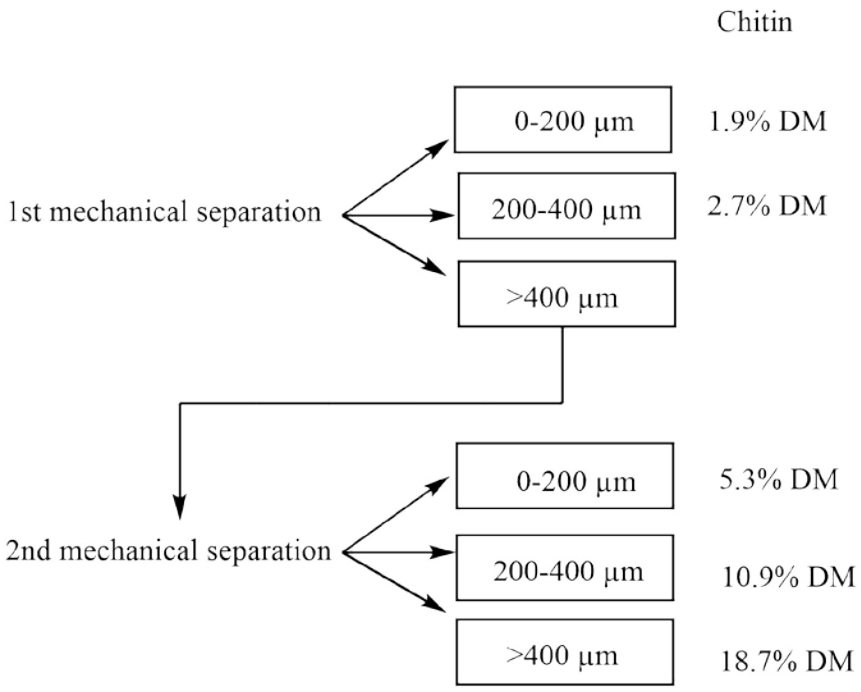

Figure 1: Chitin content on dry matter (DM) basis after the first and second mechanical separation.

Mechanical separation altered not only chitin content, but also amino acid content. In Table 1, the amino acid profile of the different fractions has been determined on DM basis, with the essential amino acids being in cursive. It also shows the amino acid content as % of crude protein. Crude protein was determined by Kjeldahl analysis, which measures not only protein nitrogen, but can also measure non-protein nitrogen, such as nitrogen found in chitin.

Separation seemed to decrease the content of arginine, aspartate, glutamate, threonine, cysteine, lysine, isoleucine, and phenylalanine whilst increasing the content of serine, glycine, alanine, proline, tyrosine, and valine (Table 1).

Table 1: Amino acid profile of the different fractions after the first (1) and second (2) mechanical separation. Amino acid levels are expressed as percentage on dry matter basis.

Amino acid % dry matter	**0-200 µm (1)**	**200-400 µm (1)**	**0-200 µm (2)**	**200-400 µm (2)**	**>400 µm (2)**
Hydroxyproline (Hypro)	ND	ND	ND	ND	ND
Histidine (His)	1,4	1,4	1,4	1,2	1,0
Taurine (Tau)	0,0	0,0	0,0	0,0	0,0
Serine (Ser)	2,2	2,2	2,3	2,4	2,6
Arginine (Arg)	2,9	2,9	2,7	2,0	1,8
Glycine (Gly)	2,3	2,4	2,6	3,1	3,5
Aspartate (+ asparagine) (Asp + Asn)	5,5	5,0	4,8	3,6	2,8
Glutamate (+ glutamine) (Glu + Gln)	6,2	5,9	5,5	4,2	3,4
Threonine (Thr)	2,3	2,3	2,2	1,8	1,7
Alanine (Ala)	2,6	3,1	3,3	4,5	5,0
Cysteine (Cys)	0,0	0,0	0,0	0,0	0,0
Proline (Pro)	2,4	2,8	3,0	4,0	4,6
Cystine (Csn)	0,2	0,2	0,1	0,1	0,0
Lysine (Lys)	3,5	3,3	2,9	2,1	1,5
Tyrosine (Tyr)	3,0	3,2	3,3	3,7	4,1
Methionine (Met)	0,8	1,0	0,8	0,4	0,3
Valine (Val)	2,5	2,7	2,8	3,1	3,4
Isoleucine (Ile)	2,4	2,4	2,3	2,0	1,9
Leucine (Leu)	3,8	3,8	3,8	3,7	3,8
Phenylalanine (Phe)	2,5	2,5	2,3	1,5	1,2
Tryptophan (Trp)	ND	ND	ND	ND	ND
Sum of amino acids	**46,4**	**46,7**	**45,8**	**43,2**	**42,3**
Amino acid content as % of crude protein	**87,6**	**87,0**	**86,7**	**79,6**	**77,0**

ND = not determined

All in all, the separation method seems to be a useful tool to make different grades of insect meal when looking at the chitin content, however, separation also seemed to change the amino acid profile.

September 2020

In September, a trial was designed to determine the effects of rearing substrate on larval growth and body composition. Different rearing substrates were selected: chicken feed, Enorm mix, rapeseed cake, brewer's spent grain, shrimp waste, and mitigation mussels. Chicken feed was used as an external control, and Enorm mix as an internal control. The other rearing substrates were selected due to their large availability and low cost, whilst not competing for

human nutrition. Chicken feed, Enorm mix, and rapeseed cake were already available at Enorm, whereas brewer's spent grain, shrimp waste, and mitigation mussels were sourced externally. Brewer's spent grain was obtained from Carlsberg (Fredericia, Denmark), shrimp waste (i.e. heads and tails) was obtained from Launis (Skagen, Denmark), whereas mitigation mussels were from the Shellfish center (Nykobing Mors, Denmark). Shrimp waste and mitigation mussels were individually grinded upon arrival (<5 mm) using a meat mincer (TS12E, OMAS,Oggiona S. Stefano, Italy), and mixed using a laboratory homogenizer (1094, Perstorp Analytical, Höganäs, Sweden). Brewer's spent grain, shrimp waste, and mitigation mussels were all stored in the freezer at -20°C for approximately two weeks until the start of the trial.

October 2020

Two trials were performed at Enorm, starting with one 6-day pilot scale trial (250g substrate, 357 larvae of 8 day old at the start of the trial) followed by a 7-day industrial scale sized trial with similar dimensions as used by Enorm (7000g substrate, 10000 larvae of 7 days old at the start of the trial). The pilot scale trial lasted 6 days so that the industrial trial could have a trial time of 7 days, using newly hatched larvae. The purpose of the pilot scale trial was to test whether or not the larvae would be able to grow on the six different substrates, whereas the large scale was designed to determine if growth and nutritional composition of the larvae would change over time, depending on the different rearing substrates.

Before the start of the trials, substrates were thawed at room temperature for 24h. Then, water content in the substrates was measured using a VWR moisture determination tool, and moisture content of substrates was adjusted to approximately 70% as recommended for BSF rearing (Cammack and Tomberlin, 2017). To each of the substrates, 2% sugar beet pellets was added to prevent accumulation of freestanding water in the boxes that can drown the larvae, except for the Enorm mix that already contained 2% sugar beet pellets. Subsequently, the correct amount of larvae and substrate were added to the containers, testing the six diets in triplicate, resulting in 18 boxes randomly allocated over the room.

During the pilot scale trial, larval weight was determined daily by taking 100 larvae per box (thus 300 larvae in total per treatment). These larvae were washed with distilled water to remove adherent substrate particles. Afterwards, larvae were dried with tissue paper to be ready for weighing. Larval weight was determined by weighing 10x10 larvae per box, after which they were returned to their subsequent box.

During the industrial scale-sized trial, in addition to daily larval weight determination, also substrate pH and core temperature were measured daily. For this trial, 500 larvae were daily taken per box (so 1500 larvae in total per treatment), washed and dried in a similar way as the pilot scale trial. For body weight, 10x10 larvae were weighed per box. The weighed 100 larvae were pooled together with the other 400 clean larvae were not returned to their box but frozen at -20°C to have sufficient quantities for body composition analysis. Furthermore, a 10 g substrate sample was taken daily and stored at -20°C until further analysis. Temperature was measured every morning 09.00 AM as core temperature of the substrate, whereas pH was determined using the protocol by Lalander *et al.* (2013), diluting the 10 g daily substrate sample

with 50 mL distilled water, leaving the sample stand for 1h at room temperature and determining pH with a pH meter (Lab 845, SI Analytics, Mainz, Germany).

November 2020

Black soldier fly trial

November was used to analyze data of larval growth, substrate temperature, and substrate pH of trial 1 and 2. In trial 1 and 2 similar trends were observed for larval growth, with the highest growth observed for larvae grown using/on chicken feed and the lowest growth observed for those grown on brewer's spent grain, mitigation mussels, and shrimp waste (Figure 2, 3, Appendix 1). In the latter three diets, it might be that the substrates physical and/or nutritional characteristics were not optimal. Physical characteristics that could affect performance may include viscosity, particle size, and water holding capacity. However, these could not be tested in this trial set-up due to the lack of appropriate materials required for these analyses. However, nutritional composition will be analyzed in the coming months to potentially explain, at least in part, the lower performance of larvae reared on brewer's spent grain, mitigation mussels, and shrimp waste.

Results of trial 2 showed that pH seemed to generally increase over time in most substrates (Appendix 2). An increase in substrate pH when rearing black soldier fly has been previously observed by other researchers (Green and Popa, 2012; Rehman *et al.*, 2017). As black soldier fly excrete microbial products and compounds (e.g. short chain fatty acids, ammonia, uric acid) into the rearing substrate, the pH will over time increase. Meneguz et al. (2018) showed that black soldier fly have a similar final body weight, when reared on pH ranging between 4.0-9.5. However, it is unknown whether the increase in ammonium and ammonia in the substrate will negatively impact black soldier fly rearing.

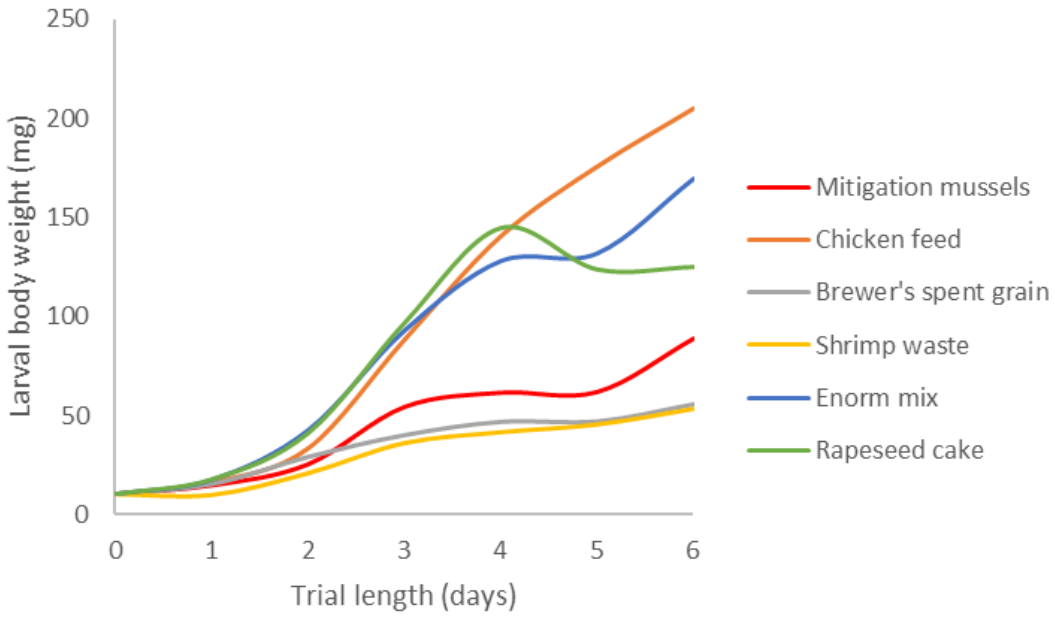

Figure 2: Body weight of 8-day old black soldier fly larvae (mg) over time of the pilot scale trial for six different rearing substrates.

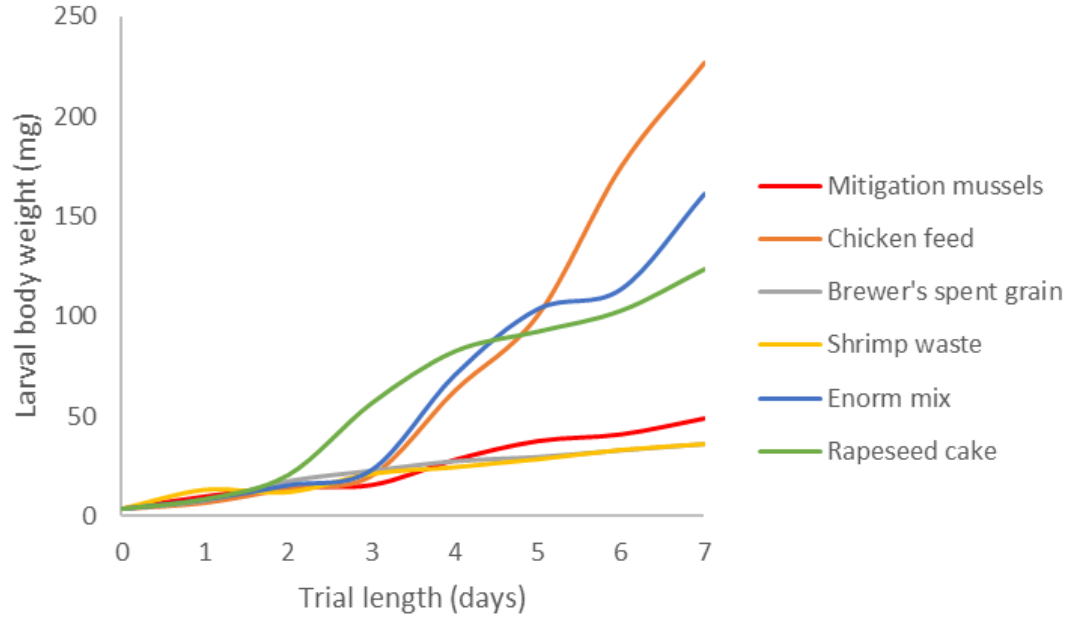

Figure 3: Body weight of 7-day old black soldier fly larvae (mg) over time of the industrial scale trial for six different rearing substrates.

Fish trials

To determine the possible uses of the previously fractionated black soldier fly meals in aquaculture, two digestibility trials will be performed – one on rainbow trout and one on Nile tilapia. Three fractions have been selected in collaboration with Aller Aqua, being the three different insect meals obtained after the first mechanical separation, i.e. 0-200 µm, 200-400 µm and > 400 µm. The composition of the three different insect fractions can be seen in Table 2.

Table 2: Composition of three different size fractions of black soldier fly meal

Parameter (%)	**Insect meal (0-200 µm)**	**Insect meal (200-400 µm)**	**Insect meal (>400 µm)**
Dry matter	96.5	96.4	97.6
Ash	13.1	13.0	11.0
Crude protein	53.0	53.7	54.6
Crude lipid	20.7	19.5	14.3
Chitin	1.8	2.6	15.0

Four diets were formulated for both aquaculture target species, rainbow trout and Nile tilapia (Table 3 and 4). The inclusion of black soldier fly meal was 25% for all diets except for the control diets. All diets were designed to be isoenergetic, isonitrogenous, and isolipidic. Titanium oxide was added as inert marker to be used for digestibility calculations.

Table 3: Raw material inclusion as total of the four test diets for rainbow trout

Raw material (%)	**Control**	**Diet 1**	**Diet 2**	**Diet 3**
Fishmeal	35.00	26.25	26.25	26.25
Insect meal (0-200 µm)		25.00		
Insect meal (200-400 µm)			25.00	
Insect meal (>400 µm)				25.00
Poultry meal	8.00	6.00	6.00	6.00
Feather meal	6.00	4.50	4.50	4.50
Hemoglobin	8.00	6.00	6.00	6.00
Rapeseed oil	12.00	9.00	9.00	9.00
Salmon oil	8.00	6.00	6.00	6.00
Wheat flour	20.60	15.45	15.45	15.45
Diamol	1.00	0.75	0.75	0.75
Vitamin premix	0.25	0.19	0.19	0.19
Mineral premix	0.15	0.11	0.11	0.11
Titanium oxide	1.00	0.75	0.75	0.75
Sum	**100**	**100**	**100**	**100**

Table 4: Raw material inclusion as total of the four test diets for Nile tilapia

Raw material (%)	Control	Diet 1	Diet 2	Diet 3
Soybean protein concentrate	25.00	18.75	18.75	18.75
Fishmeal	5.00	3.75	3.75	3.75
Insect meal (0-200 µm)		25.00		
Insect meal (200-400 µm)			25.00	
Insect meal (>400 µm)				25.00
Poultry meal	8.00	6.00	6.00	6.00
Feather meal	8.00	6.00	6.00	6.00
Hemoglobin	8.00	6.00	6.00	6.00
Rapeseed oil	3.00	2.25	2.25	2.25
Salmon oil	2.00	1.50	1.50	1.50
Wheat flour	37.58	28.19	28.19	28.19
Monoammonium phosphate	2.02	1.52	1.52	1.52
Vitamin premix	0.25	0.19	0.19	0.19
Mineral premix	0.15	0.11	0.11	0.11
Titanium oxide	1.00	0.75	0.75	0.75
Sum	**100**	**100**	**100**	**100**

December 2020

In December, composition of black soldier fly and rearing substrate of trial 2 was analyzed. In Table 5 the composition of black soldier fly larvae is described at initial (t=0 days) and final (t=7 days) time of the trial, when reared on different substrates. Values having large standard deviation still need to be replicated. The empty cell indicates that samples still need to be analyzed for that specific parameter.

Table 5: Composition of black soldier fly larvae 7-day old at initial sampling (t=0 days) and final sampling of 14 day old larvae (t=7 days) when reared on one of the six different rearing substrates.

	Initial	Final					
Parameter	**7-day old larvae**	**Chicken feed**	**Enorm mix**	**Brewer's spent grain**	**Mitigation mussels**	**Rapeseed cake**	**Shrimp waste**
Dry matter (%)	30.5±0.0	48.5±1.8	40.6±1.0	26.0±0.4	32.9±1.7	36.6±2.9	28.7±4.1
Crude protein (%DM)	55.4±0.0	43.6±0.8	44.0±2.9	59.8±0.7	46.9±2.5	51.5±0.5	49.4±1.1
Crude fat (%DM)	8.5±0.0	32.9±1.2	24.5±1.4	33.1±0.6	21.4±0.6	27.0±0.2	22.9±0.8
Ash (%DM)	13.1±0.1	7.5±0.2	7.0±0.3	10.0±0.6	22.0±0.4	12.5±0.1	16.9±1.2
Chitin (%DM)	10.5±0.6	13.0±0.5	9.3±2.3	13.1±0.3	7.0±0.2		8.0±0.8

January 2021

Black soldier fly trial

In January, the composition of rearing substrates was investigated before and after the 7-day exposure to the black soldier fly larvae (Table 6). Empty cells indicate the samples still need to be analyzed. Similar to the samples of December 2020, values with large standard deviation still need to be replicated.

Furthermore, insect and substrate samples are being prepared for fatty acid profile determination with GC-MS, as well as amino acid profile determination.

Fish trials

Additionally, Nile tilapias for the digestibility trial were moved into the digestibility system for acclimation, and the trial will be performed during February 2021. Afterwards, rainbow trout will be moved into the digestibility system for the next digestibility trial, by a one month trial period, so the second trial is estimated to run in March-April 2021.

Table 6: Composition of six different rearing substrates initial (t=0 days) and final (t=7 days) for the industrial scale trial

Parameter	Chicken feed		Enorm mix		Brewer's spent grain		Mitigation mussels		Rapeseed cake		Shrimp waste	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Dry matter (%)	31.2±0.2	31.2±1.2	28.7±0.2	30.8±0.8	31.2±0.2		26.9±0.7	32.9±1.7	31.7±0.4	39.2±0.7	20.4±0.3	21.6±0.6
Crude protein (%DM)	19.5±0.2	20.2±0.7	20.6±0.0		25.9±0.3	27.2±0.9	19.7±0.3		30.9±0.2	34.1±0.8	38.7±1.4	
Crude fat (%DM)	4.5±0.2	2.6±0.0	3.4±0.1	3.1±0.2	9.9±0.3	6.6±0.8	3.4±0.0	0.9±0.2	12.6±0.1	7.2±0.2	7.5±0.2	5.3±0.0
Ash (%DM)	5.4±0.2	7.8±0.4	4.5±0.3	5.9±0.6	4.3±0.0	6.3±0.1	61.0±0.7	72.0±1.1	7.0±0.0	13.7±0.1		

Appendix 1 Larval growth data

Table A1.1 - Body weight of 8-day old black soldier fly larvae (mg) over time of the pilot scale trial for six different rearing substrates.

	Day of the trial						
Substrate	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Brewer's spent grain	10.1	15.1	28.9	39.9	46.6	46.9	55.5
Chicken feed	10.1	16.0	33.1	88.0	140.2	175.4	204.7
Enorm mix	10.1	17.3	42.4	93.0	128.2	132.1	169.9
Mitigation mussels	10.1	14.5	25.3	54.5	61.7	62.0	89.2
Rapeseed cake	10.1	17.5	40.9	96.7	144.8	123.8	125.1
Shrimp waste	10.1	9.8	20.8	36.1	41.5	45.5	53.4

Table A1.2 - Body weight of 7-day old black soldier fly larvae (mg) over time of the industrial scale trial for six different rearing substrates.

	Day of trial							
Substrate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Brewer's spent grain	3.5	7.7	17.4	22.6	27.4	29.5	32.9	36.0
Chicken feed	3.5	6.6	13.8	20.1	62.7	100.6	175.1	227.1
Enorm mix	3.5	8.2	15.4	23.0	70.7	103.8	113.8	161.5
Mitigation mussels	3.5	9.8	14.6	15.5	28.2	37.6	40.9	48.9
Rapeseed cake	3.5	8.4	20.5	56.5	82.6	92.5	103.1	123.9
Shrimp waste	3.5	13.1	11.9	21.1	24.4	28.4	33.1	36.0

Appendix 2 Substrate pH

Substrate	Day of trial							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Brewer's spent grain	4.8	5.1	5.9	7.0	8.6	8.6	8.7	9.0
Chicken feed	6.2	5.4	4.8	4.2	4.8	4.5	5.5	5.8
Enorm mix	4.0	4.1	4.0	4.1	4.2	4.5	5.0	5.0
Mitigation mussels	6.9	6.3	6.7	6.7	6.9	7.3	7.3	7.6
Rapeseed cake	5.8	5.6	6.3	7.4	8.4	8.8	8.8	8.7
Shrimp waste	8.4	8.4	7.9	7.7	7.5	7.3	7.3	7.4

Table A2.1 – pH of rearing substrates for black soldier fly rearing over time at industrial scale trial

Appendix 3 Substrate temperature

Substrate	Day of trial							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Brewer's spent grain	25.0	37.0	41.5	51.9	47.2	45.0	38.2	37.3
Chicken feed	25.6	28.3	27.9	28.0	31.7	30.5	36.4	39.9
Enorm mix	26.5	27.6	27.7	26.2	30.6	30.6	36.2	36.9
Mitigation mussels	26.5	26.6	25.4	27.5	27.2	28.2	27.3	27.6
Rapeseed cake	25.5	37.1	44.7	43.9	45.2	46.5	45.2	36.7
Shrimp waste	30.3	28.7	30.0	30.2	18.7	27.5	27.9	28.6

Table A3.1 – Core temperature of rearing substrates for black soldier fly rearing over time at industrial scale trial