Effect of protein and carbohydrate feed concentrations on the growth and composition of black soldier fly (*Hermetia illucens*) larvae

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Abstract

Black soldier flies (*Hermetia illucens*) can be used for a wide range of applications, from screening their anti-microbial properties, entomophagy through to waste management. Although the use of black soldier flies for these purposes has been widely studied, mass-rearing black soldier flies is still in the preliminary phase. This study focussed on the nutritional composition of black soldier fly larvae over the course of their life history, and the impact of manipulating dietary protein and carbohydrate on the growth and composition (protein and fat) of the larvae. Larvae were collected every 24h over the course of this life-stage to test for differences in composition. To test the effect of dietary protein and carbohydrate differences, larvae were fed 25 diets with varying concentrations of protein and carbohydrates. Overall, the composition of larvae changed very little over their life history, with the higher concentration of protein mostly observed in the earlier instars of the larvae. The pre-pupal stage reduced the fresh and dry weight of the larvae, whereas the ash concentration was very stable throughout their life history. Both dietary protein and dietary carbohydrate had a significant effect on the fresh and dry weight of the larvae, but dietary protein was a stronger indicator of larval fresh and dry weight than dietary carbohydrate. Larval composition was also influenced by the feed-type, with heavier larvae producing significantly more fat than the lighter ones.

Keywords: dietary requirements, entomophagy, stratiomyidae

1. Introduction

Entomophagy might be able to solve some of the challenges facing our planet. One of these problems is the 805 million undernourished people in the world. Insects are rich in minerals and vitamins, and it has been proposed that they would be able to provide a healthy alternative food source (Gahukar, 2011; Nadeau *et al.*, 2015). Nadeau *et al.* (2015) suggest that if 15,586 – 92,976 ha^2 was devoted to insect rearing, it could reduce or eliminate this problem. Another problem to which entomophagy could offer a solution is overfishing, with populations of fish such as the Atlantic Cod (*Gadus morhua*) reportedly at 20% of their original population (Eriksson and Andersson, 2010). Most of these fish are either used for human consumption or to feed other fish/animals in the aquaculture/agriculture industries. Insects could replace between 25 and 33% of the fish meal in the diet of fish in the aquaculture industry (Burtle *et al.*, 2012; Cummins *et al.*, 2017; Kroeckel *et al.*, 2012). This percentage could be increased further by promoting chitinase activity or chitinolytic bacteria that would allow the fish to better digest the chitin, or the potential insect-feed had a higher concentration of omega-3 fatty acids (Barroso *et al.*, 2017; Kroeckel *et al.*, 2012; St-Hilaire *et al.*, 2007).

One of the insect species often used in the aquaculture industry is the black soldier fly (*Hermetia illucens*). This insect has been shown to have a wide variety of properties that can be beneficial. They have been used for forensic entomology, during dry post-decay stages in more tropical areas (Lord *et al.*, 1994; Pujol-Luz *et al.*, 2008). The black soldier fly also shows anti-microbial properties, producing hexanedioic acid, methanol and antimicrobial peptides that have been proven to effectively prohibit the growth/proliferation of both gram-positive and -negative bacteria and fungus (Choi *et al.*, 2012; Choi and Jiang, 2014; Elhag *et al.*, 2017; Park *et al.*, 2014). Lastly, the black soldier fly can also be very well applied as part of a waste management program. For example, studies have shown that black soldier fly larvae can reduce between 50 and 78% of the dry matter of manure (Lalander *et al.*, 2013; Sheppard *et al.*, 1994; Zhou *et al.*, 2013).

To date, the majority of studies on the black soldier fly have focused on the application of the larvae, rather than on finding an optimal rearing strategy. Previous studies have been executed on abiotic factors, such as temperature and humidity, and dietary requirements. Cammack and Tomberlin (2017) and Oonincx *et al.* (2015) have indicated that the black soldier fly larvae are a good candidate for converting lower value feed sources. However, these studies are often comparing different feed sources, such as 'Fish Renderings' with 'Swine Liver' (Cammack and Tomberlin, 2017). This different composition most likely has an influence on the growth of the larvae. For example, mealworms (*Tenebrio molitor*) have varying growth rates depending on the particular amino acids present in the feed(Davis, 1975).

In this study, the impact of various protein and carbohydrate concentrations on the growth of black soldier fly larvae was examined. To exclude the above-mentioned difference in feed compositions, a single base diet was enriched with the two macronutrients. It is hypothesized that the dietary protein concentration contributes to the growth of the larvae, and the carbohydrate contributes to the fat production of the larvae.

2. Material & Methods

Experiment 1: Composition over the course of the larval stage

This first experiment was executed to examine protein and fat accumulation in black soldier fly over the course of their larval stage. To examine this, 2,500 larvae, with an average weight of 15mg (commercially purchased from Swell Reptiles, UK), were placed on a chickenfeed-based substrate (70% moisture), which wet-weight is composed of 17.5% protein, 48.8%

carbohydrates, 5.9% fat, 3.93% calcium, 0.4% phosphorus and 0.14% sodium. The larvae were placed in a climate chamber (Sanyo MLR350; Sanyo, UK) at 28°C without any light. Each subsequent day, at least 5g of larvae were collected from the sample and fed additional chickenfeed, until 25% of the larvae had reached pre-pupal stage, upon which only pre-pupal stage larvae were collected and the remaining larvae were discarded. The collected larvae were washed to dispose of any remaining substrate or faecal matter. Afterwards, the larvae were euthanized in the freezer (-20°C) and stored until needed for chemical analysis.

Experiment 2: Rearing larvae at varying protein and carbohydrate dietary ratios

To determine the influence of dietary protein and carbohydrate on black soldier fly larvae, the following experiment was undertaken. A total of 12,500 insects were used in this experiment. Each cup had 11.8g of the chickenfeed diet, with 15±3 mL of tap water and the additional protein and carbohydrate depending on the treatment. Protein ratio was enhanced by Promax Maxinutrition Max Protein shake powder, which is composed of 71.4% protein, 5.9% carbohydrate and 5.7% fat on wet-weight basis. The carbohydrate ratio was enhanced by Polenta valsugana which was composed of 7.2% protein, 77.4% carbohydrate and 1.2% fat on wet-weight basis. Each treatment was replicated ten times. There were four and five concentrations of added protein or carbohydrates, 0g, 0.7g, 1.4g and 2.1g; 0g, 0.97g, 1.94g, 2.91g and 3.88g, respectively (Table 1). Each of these cups included 50 larvae and was placed in a Sanyo MLR350 climate chamber at 28°C without any light. Any cup that had been subjected to a drier environment will be discarded from the dataset. The larvae were harvested after eight days in the climate chamber and stored at -20°C until required for chemical analysis.

Table 1: The wet weight composition of the diets used for experiment 2, each additional level of protein adds 0.5g of protein and each level of carbohydrate adds 0.75g of carbohydrates. The first row represents the composition of the chickenfeed.

Protein Treatment	Carbohydrate Treatment	Protein (g)	Carbohydrate (g)	Fat (g)
1	1	2.06	5.75	0.70
1	2	2.13	6.50	0.71
1	3	2.20	7.25	0.72
1	4	2.27	8.00	0.73
1	5	2.34	8.75	0.74
2	1	2.56	5.79	0.74
2	2	2.63	6.54	0.75
2	3	2.70	7.29	0.76
2	4	2.77	8.05	0.77
2	5	2.84	8.80	0.78
3	1	3.06	5.83	0.78
3	2	3.13	6.58	0.79
3	3	3.20	7.34	0.80
3	4	3.27	8.09	0.81
3	5	3.34	8.84	0.82
4	1	3.56	5.88	0.82
4	2	3.63	6.63	0.83

4	3	3.70	7.38	0.84
4	4	3.77	8.13	0.85
4	5	3.84	8.88	0.86

Chemical analysis

All collected larval samples were subject to drying, by being placed at 80°C for 2-3 days, as similarly executed in protocol 950.46 (AOAC, 1990). Afterwards all the larvae of experiment 1 were ground, whereas only half of the repetitions from experiment 2. Three chemical analysis protocols were applied: the leco, soxhlet and ashing. The leco method (FP-528) allows for protein analysis by combusting the sample of approximately 0.15g and measuring the concentration of nitrogen (AOAC 976.05 as cited by (Surendra *et al.*, 2016), this can be converted to a percentage of protein by multiplying by 4.76, as suggested by Janssen *et al.* (2017). The soxhlet method was used to analyse the fat concentration in the sample, this methodology runs petroleum ether continuously through a sample to absorb and separate the fat. The samples used in this experiment weighed approximately 0.5g and were extracted for 1 hour and 30 minutes. Lastly, the ashing was only executed on Experiment 1; by burning all organic matter at a temperature of 400°C, the concentration of inorganic matter can be measured (920.153)(AOAC, 1990; Surendra *et al.*, 2016). Chemical analyses were carried out in either duplicate or triplicate.

Statistical analysis

A one-way ANOVA was executed to determine significant differences between the fresh weight, dry weight, survival, protein percentage and fat percentage (SPSS, ver. 24). Furthermore, a bivariate correlation matrix was applied to examine the effect of the variables. The shape of the response surface for each trait was then visualized using non-parametric thin-plate splines using the *fields* package in R-Studio. However, it should be noted that these are simply an aid to visualizing the surfaces and are not a direct output from the statistical models.

3. Results

Experiment 1: Larval macronutrient composition

Several observations can be made from the collected data (Table 2). Firstly, no obvious 'cut-off' point for the fat production was observed. However, the protein:fat ratio did rapidly increase during the first four days, after which, both protein and fat ratio appeared to stabilise. Secondly, the percentage dry-weight of the larvae increased from 18.1% to 38.7% over the course of 11 days (Figure 1). The protein and fat percentage appear to be negatively correlated as the increase of fat leads to a decrease of protein. Lastly, the ash percentage remained stable throughout the larval stage, until the pre-pupal stage. However, more data will be needed from the earlier time points the earlier days to validate this observation.

Table 2: An overview of the fresh weight (g), dry weight (g), protein (%), fat (%) and ash (%) concentrations over the lifetime of the black soldier fly larvae. The * indicates that for the chemical analysis has only been done once, as not enough sample was collected for a duplicate.

Time	Fresh weight (mg)	Dry weight (mg)	Percentage Dry weight	Protein (%)	Fat (%)	Ash (%)	Protein: Fat Ratio
0	15.4	2.8	18.1	47.0	12.4*		0.26
24	19.8	5.0	25.1	33.3	22.6*		0.68
48	54.1	13.8	25.6	36.6	28.5		0.78
72	96.5	25.3	26.2	35.0	27.4		0.78

96	123.7	38.3	31.0	31.8	35.2		1.11
117	151.0	49.6	32.8	31.4	34.7		1.10
141	141.0	43.3	30.7	32.0	35.1	9.3	1.10
166	180.2	61.2	33.9	30.2	38.0	9.1	1.25
192	199.6	67.6	33.8	29.6	39.4	9.1	1.33
216	220.6	76.2	34.5	29.8	38.8	9.0	1.30
240	225.3	80.5	35.7	29.0	38.9	9.1	1.34
264	162.7	63.0	38.7	32.7	36.9	11.0	1.13

Figure 1: This graph presents the growth of the black soldier fly over the course of 11 days, the left-axis indicates the weight of the larvae (mg) and the right-axis indicates the percentage of dry weight. The data used to create this graph is present in Table 2.

Experiment 2: Effect of protein and carbohydrates on the growth of black soldier fly larvae

The dataset is displayed in Figure 2 and Table 3. These data indicate that protein is more influential on the growth of the black soldier fly larvae than carbohydrate. This was further evidenced by the correlation matrix that indicated that the concentration of protein has a highly significant positive effect on the fresh and dry weight of the larvae (Pearson Correlation of: 0.638, 0.675; P-value: .000, .000, respectively). Whereas the effect of carbohydrates, although significant, was not as large of an influence on the fresh and dry weight of the larvae (Pearson Correlation of: 0.183, 0.194; P-value: .024, .017, respectively). The survival of the larvae was another good indicator of the weight, as the survival was significantly negatively-correlated with the fresh and dry weight (Pearson Correlation of: -0.673, -0.554; P-value: .000, .000, respectively). The protein concentration in the feed had a strong correlation with the percentage and concentration of protein (Pearson Correlation of: -0.470, 0.654; P-value: .000, .000, respectively) and fat in the larvae (Pearson Correlation of: 0.697 and 0.740; P-value: .000, .000, respectively). Whereas carbohydrates in the feed only had a small significant effect on the percentage of protein (Pearson Correlation of: -0.254; P-value: .022). Lastly, the birthweight of the larvae only had a significant effect on the dry weight (Pearson Correlation of: 0.204, P-value: .012).

Figure 2: This contour plot gives a visual representation of the effect of protein (x-axis) and carbohydrate (y-axis) on the fresh weight (left) and dry weight (right) of the black soldier fly larvae. The datapoints used to create this graph have been presented in Table 3. The colour regions represent a specific weight category as given in the legend of the figure.

Table 3: The effect of varying concentrations of protein and carbohydrate on the average survival, fresh and dry weight and the protein and fat content of the larvae of the black soldier fly. A different number in the column indicates a significant difference. The protein and carbohydrate concentration of the respective treatments are based on the information given in table 1.

Treatment	Fresh Weight (mg)				Dry Weight (mg)				Survival (%)				Protein (%)				Fat (%)			
P1C1	154.77	±	21.5	cd	47.35	±	5.8	fg	89.00%	±	16.4%	abc	36.36%	±	0.9%	bc	24.14%	±	1.7%	e
P1C2	150.30	±	21.3	de	49.01	±	5.1	ef	82.40%	±	20.3%	cd	36.35%	±	0.9%	bc	25.39%	±	3.4%	e
P1C3	150.98	±	15.7	de	47.44	±	6.8	f	94.80%	±	8.3%	ab	35.93%	±	1.0%	cde	26.53%	±	1.8%	cde
P1C4	154.98	±	16.5	cd	49.02	±	9.1	ef	95.00%	±	7.0%	ab	35.80%	±	1.0%	cde	26.14%	±	1.4%	de
P1C5	148.16	±	15.8	de	46.58	±	5.2	fg	99.60%	±	0.8%	a	34.90%	±	1.1%	def	24.54%	±	4.4%	e
P2C1	145.66	±	14.7	de	46.36	±	5.4	fg	87.80%	±	20.2%	bc	37.75%	±	0.9%	a	25.51%	±	1.9%	e
P2C2	133.89	±	14.1	e	41.99	±	6.3	g	99.00%	±	1.4%	ab	37.96%	±	1.3%	a	23.85%	±	2.4%	e
P2C3	154.69	±	18.5	dcd	33.53	±	4.7	h	90.80%	±	18.4%	abc	37.58%	±	0.5%	ab	23.93%	±	3.4%	e
P3C3	165.24	±	12.8	bcd	54.36	±	5.8	de	98.00%	±	3.1%	ab	36.00%	±	1.2%	cd	29.24%	±	2.4%	bcd
P3C4	169.06	±	14.8	bc	56.44	±	6.5	cd	96.80%	±	4.3%	ab	36.07%	±	2.1%	cd	30.47%	±	3.2%	ab
P3C5	171.37	±	17.7	bc	54.40	±	6.9	de	94.20%	±	11.7%	ab	36.33%	±	1.1%	bc	29.60%	±	1.7%	abc
P4C1	194.68	±	28.4	a	62.35	±	5.7	ab	55.60%	±	14.4%	f	34.15%	±	0.8%	g	32.47%	±	0.9%	a
P4C2	176.48	±	23.6	a	59.00	±	6.9	bcd	74.60%	±	18.1%	de	34.37%	±	0.8%	g	30.57%	±	1.6%	ab
P4C3	199.60	±	22.8	a	65.61	±	6.9	a	70.40%	±	13.1%	e	34.62%	±	0.3%	efg	31.13%	±	3.5%	ab
P4C4	184.80	±	11.6	ab	61.32	±	2.3	abc	80.20%	±	12.1%	cde	34.69%	±	0.8%	efg	30.13%	±	1.8%	ab
P4C5	195.46	±	25.9	a	63.89	±	4.0	ab	78.60%	±	14.1%	cde	34.16%	±	0.4%	fg	31.70%	±	1.1%	ab

4. Discussion

Experiment 1 showed that larvae have proportionally more protein in their earlier instars, and accumulate fat until a point where they grow similarly in protein and fat. These finding are like a study performed by Liu *et al.* (2017) who also found a lower percentage of fat in the early instars. Liu *et al.*, (2017) had a similar concentration of protein as this study. However, the fat percentage is generally 10% lower, this could perhaps be caused by the different strain of black soldier flies. The results for late-instar larvae are supported by the literature. These previous studies found protein concentrations such as 30.46-33.32% (original numbers were converted with protein factor 4.76 instead of 6.25)., and fat percentages of 30- 35% (Liu *et al.*, 2017; Newton *et al.*, 2005; Nyakeri *et al.*, 2017; Sheppard *et al.*, 1994; St-Hilaire *et al.*, 2007; Tschirner and Simon, 2015). Interestingly, the ash concentration throughout the sampling period was very stable, at around 9.2% and increased to 11% at the pre-pupal stage. These numbers are comparable to the study executed by Liu *et al.* (2017), but lower than in other studies, which found 15-16.6% of ash (Newton *et al.* (2005); Nyakeri *et al.* (2017); St-Hilaire *et al.* (2007). Again, this might be due to the difference in feeding substrate of the black soldier fly larvae: both Newton *et al.* (2005) and St-Hilaire *et al.* (2007) used manure to rear black soldier fly larvae, and Nyakeri *et al.* (2017) used fruit and vegetable waste, whereas in our experiment larvae were fed chickenfeed. The control substrate from Tschirner and Simon (2015), containing a wide spectrum of cereal leftovers such as broken pellets, spilled grains and grinding dust, would be most comparable to the chickenfeed diet that was used. Tschirner and Simon (2015) found larvae to be around 37.2% dry-weight, which is comparable to the larval dry weight in this study.

The results from the second experiment indicate that carbohydrate only have a small contribution to growth of the larvae, especially when compared to the importance of dietary protein. A previous study by Cammack and Tomberlin (2017) suggests that larvae should be reared on a balanced diet of 21% protein and 21% carbohydrates, in that treatment each larvae had approximately 0.24g of protein and carbohydrate and weighed around 80mg as a prepupae. For comparison the larvae in this study were fed between 0.041g and 0.076g of protein and between 0.115 and 0.178g of carbohydrate depending on the treatment and weighed between 133.9mg and 199.6mg. Although, this is not a good comparison due to the difference in instar that was weighed, it does further highlight the importance of understanding the impact of different amino acid profiles as larval growth in insects is affected by the variation in amino acids (Davis, 1975). A number of studies suggest that black soldier fly larvae do not grow as well on animal-based protein as they would on plant-based protein (Cammack and Tomberlin, 2017; Gobbi *et al.*, 2013; Nguyen *et al.*, 2015; Nguyen *et al.*, 2013; Nyakeri *et al.*, 2017). However, this is still an under-studied area as the whey protein used in this study would qualify as an animal protein and it is thought that not all plant-based feeds provide enough protein. For example, a study by Mohd-Noor *et al.* (2017) used coconut endosperm as a feed, and the low protein concentration (3-4%) in the coconut endosperm most likely prohibited the growth of the larvae. Another example would be the dried sugar beet pulp from the study of Tschirner and Simon (2015), this feeding substrate only had 8.5% protein and produced the lightest larvae of their study.

According to the correlation matrix the percentage of dietary protein was negatively correlated with the percentage of protein in the larvae, as the heavier larvae would produce more fat than the lighter ones and decrease the percentage of protein. The negative correlation of the fresh and dry weight with the survival of the larvae is understandable as well: the fewer larvae present in the substrate, the more nutrition is available to the remaining larvae.

5. Conclusion

To conclude, the macro nutritional composition of black soldier fly larvae does not greatly change over their life history, with the exception of freshly hatched larvae and the pre-pupal stage. The younger larvae showed higher percentage of protein compared to the older larvae. The pre-pupal stage larvae lost weight compared to late instar larvae, as well as exhibiting a higher percentage of ash and dry-weight.

The hypothesis regarding the influence of dietary protein on larval composition can be accepted, as the dietary protein concentration increased the fresh- and dry weight. The hypothesis with regard to the influence of dietary carbohydrate can be rejected, as the dietary carbohydrate did not have a significant effect on either the fat percentage or fat concentration in the black soldier fly larvae.

For future studies, it can be recommended to examine higher concentrations of protein to find the maximum concentration of protein, as well as examining the effect of other nutrients (e.g. micronutrients) and minerals on the growth and composition of black soldier fly larvae. Lastly, a similar experiment to Davis (1975) would allow for more accurate understanding of the protein requirement of the black soldier fly.

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