# Nutritional content and bioconversion efficiency of *Hermetia illucens* (Diptera: Stratiomyidae): harvest as larvae or prepupae?

**N. J. Parry and C. W. Weldon***

*Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0082, Pretoria, South Africa*

**cwweldon@zoology.up.ac.za*

**Running head:** Nutritional content of black soldier fly larvae

## Abstract

Most research on nutritional composition that relates to use of fly larvae as an alternative ingredient in animal feed has focused on the prepupal stage of *Hermetia illucens* L. However, crude protein and lipid content of *H. illucens* larvae vary dramatically during larval development and little evidence has been presented to support use of prepupae for animal feed. The aim of this study was to establish the nutritional value of larvae and prepupae of *H. illucens* reared on organic waste streams for use in animal feed. The effect of harvesting of either larvae or prepupae on waste reduction was also measured. Freshly hatched neonates (90 mg) were placed on 12 kg of mixed pre-consumer waste and held at 28˚C. Moisture content, crude protein, crude lipid, ash, fibre, survival, total biomass, individual larval biomass, waste reduction and bioconversion on a dry matter basis were determined and compared between larvae harvested on day 15 and prepupae harvested on day 20. Prepupae had higher fibre content than larvae, while moisture content, crude protein, crude lipid and ash did not differ between the two groups. Total biomass was higher in larvae than in prepupae due to mortality between the two stages. Size of individual larvae was very similar between the two groups. In terms of nutritional content or biomass production it is not detrimental to harvest larvae early, or to harvest a mixture of larvae and prepupae or prepupae only. However, harvesting later, when larvae have become prepupae, would lead to a reduction in potential harvest and total biomass output, especially as survival declines over time. Bioconversion and waste reduction did not differ between day 15 and day 20, indicating that nutrient recovery from waste was not improved by harvesting at the prepupal stage.

## Keywords

Proximate analysis, Biomass, Small industrial scale, Moisture content, Fibre, Waste reduction

## INTRODUCTION

Fly larvae can be used in a nutrient cycling process called bioconversion, whereby nutrients are recovered from organic waste streams to produce value-added products and reduce the total waste mass (van Huis, 2013; Čičková *et al.* 2015). The value-added products that can be produced from fly larvae include protein, lipids, chitin and fertilizer (Čičková *et al.* 2015; Caligiani *et al.* 2017).

The species most often studied for use in bioconversion is the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), due to its versatility in efficiently and effectively reducing numerous waste streams, such as vegetable waste and animal manure (Ĉiĉková *et al.* 2015). Dried *H. illucens* larvae contain approximately 44% protein and 33% lipids (St-Hilaire *et al.* 2007). The amino acid composition of *H. illucens* larvae compares favourably to soyabean meal and fish meal, so they can partially or completely replace traditional sources of protein in animal feed (Makkar *et al.* 2014; Renna *et al.* 2017; Schiavone *et al.* 2017). The high calcium content (7.56%) and high calcium to phosphorus ratio of larvae is also favourable for use in growing pigs and poultry (Makkar *et al.* 2014). In different trials using *H. illucens* larvae as a source of protein for farmed animals, growth rates and feed conversion efficiency were found to be similar to traditional feeds (Makkar *et al.* 2014). Between 20 and 25% of fish meal can be replaced by maggot meal for fish species such as Atlantic salmon (Lock *et al.* 2014), African catfish (Madu & Ufodike, 2003), yellow catfish (Zhang *et al.*, 2014), Nile tilapia (Ogunji *et al.* 2007), rainbow trout (St-Hillaire *et al.* 2007; Renna *et al.* 2017; Dumas *et al.* 2018) and sea bass (Lopez, 2016), with no adverse effects on growth, feed conversion or taste of these fish.

Research involving *H. illucens* has focused on rearing larvae on one waste type or on artificial diets, the safety and use of the species as an animal feed ingredient (Charlton *et al.*, 2015; Diener *et al.* 2009; Lalander *et al.* 2015; Nyakeri *et al.* 2019 Oonincx *et al.* 2015; Veldkamp & Bosch, 2015), or to ensure reproduction of the adult flies (Heussler *et al.* 2018). For use of *H. illucens* in animal feed, the majority of research on nutritional composition has focused on the prepupal stage (an intermediate stage between larvae and pupae), but little evidence has been put forward to indicate that prepupae are nutritionally the optimal life stage for use in animal feed (Liu *et al*. 2017). Changes in crude protein and lipid content of *H. illucens* larvae can be dramatic as larval development progresses (Liu *et al*. 2017). Amino acid content can also change based on larval stage as well as the substrate on which larvae feed (Diener *et al.* 2009). Once larvae reach the prepupal period, they stop feeding and migrate away from the food source to find a suitable place for pupation. This has the potential to change protein and lipid content, and affect the composition of minerals, vitamins, amino acids, fatty acids, and fibre (Liu *et al.* 2017; Aniebo & Owen, 2010). For this reason, the nutrient content of insect stages should be monitored so that harvesting occurs at a stage when their properties are tailored for final use (St-Hillaire *et al.* 2007).

In addition to considerations of nutrient composition and biomass of the insect product, commercial bioconversion facilities are required to reduce organic waste and ensure that their own waste production is limited. Rate and efficiency of reduction of waste on a dry matter basis changes over time for the larvae. These changes need to be investigated and understood as it can influence decisions that a bioconversion company needs to make. A bioconversion company needs to balance reduction of organic waste with fast throughput (the total amount of biomass produced over time), while also ensuring that the end products are of consistent quality suitable for their intended purpose. Therefore, greater understanding of waste reduction and the nutritional composition of *H. illucens* larvae and prepupae is needed to provide information that will help to improve mass breeding and product development. This will ensure cost-effective and optimized mass insect rearing to improve the use of *H. illucens* for waste management.

The aim of this study was to establish the nutritional value of larvae and prepupae of *H. illucens* reared on organic waste streams for use in animal feed. The effect of harvesting of either larvae or prepupae on waste reduction was also measured. By doing so the best stage for harvesting to balance feed quality, waste reduction and throughput was determined. It was anticipated that harvesting of younger larvae would be the optimal strategy due to reduced time in the system, maximised biomass production and more favourable nutritional content in terms of lower fibre and higher protein content. This work is novel due to the use of organic waste streams with variable nutrient content rather than artificial diets and the use of a small industrial scale rather than a lab scale study. As such, the results will be more applicable to large-scale, commercial bioconversion operations and will provide information required for optimum timing of harvest.

## MATERIALS AND METHODS

*Experimental insects*

Freshly hatched *H. illucens* larvae, called neonates, were collected from an established colony held at Aegis Environmental Bioconversion Facility, Centurion, South Africa. The adult flies were held under constant conditions in a climate controlled room (30 ± 0.5°C, 70% RH, 12:12 L:D h). The eggs were collected once a day in egg traps and were kept in an incubator (26 ± 0.5°C, 65% RH) until hatching began three to four days later. Egg traps are made from white, extruded twinwall fluted/corrugated polypropylene sheet (Correx, Duroplastic Technologies), cut into 3 cm x 8 cm sheets stacked in a group of eight and held together by an elastic band. The flutes of the sheet are open length ways in the trap and are orientated left and right for easy oviposition by the females. Neonates were collected less than three hours after hatching from containers placed underneath the egg traps. Neonates were transferred to weighing boats and weighed using a milligram scale (AE Adam ® HCB123 120g x 0.001g). A single neonate weighs approximately 0.015 mg (Cammack & Tomberlin, 2017, *personal observation*).

*Nutritional content and bioconversion efficiency of larvae vs prepupae*

Freshly hatched neonates (90 mg) were placed on 12 kg of mixed pre-consumer waste and held at 28 ± 0.5˚C. The trays used were plastic meat trays (60 × 33.5 × 21cm). There are approximately 6000 neonates per 90 mg and 12 kg of food provides approximately 2 g of food per larva for their lifetime. The preconsumer waste, collected from a local fresh produce market and comprising fruit and vegetables, was macerated into fine particles using an electric hammermill. Five different batches of waste were used, and for each batch, two trays were set up. On day 15, one tray was randomly selected, the larvae were sifted from the remaining residue, called frass, and the weight of all larvae, 20 individual larvae, and the frass were recorded. A 160 g sample of the larvae was collected, blanched at 80°C in hot water for between one and two minutes (until all larvae had stopped moving), and dried in a convection oven at 80°C to constant weight to determine the moisture content of larvae. This treatment was repeated on day 20 for the second tray, containing prepupae. The samples were sent to an accredited laboratory (ARC Irene Analytical Services) for proximate analysis, comprising of moisture content, crude protein, crude lipid, ash and fibre. Dry matter was determined using the Association of Official Analytical Collaboration (AOAC) Official Methods 934.01 and 930.15, described in Harris (1970). Fat was determined using ether extraction, AOAC Official Method 920.39. Ash was determined by combustion following the AOAC Official Method 942.05, described in Harris (1970). Total protein was determined using the Kjeldahl method, following the AOAC Official Method 954.01. The dry matter content of the initial waste and frass was determined by taking the average from three samples of five grams, dried in an oven for eight hours at 80°C.

**Figure 1.** (a) Larvae of *Hermetia illucens.* (b) Prepupae of *Hermetia illucens* with increased pigmentation in the integument. Photographer: Nina Parry

The final total biomass, final individual weight, total waste reduction, bioconversion and efficiency of conversion of digested feed (ECD) was compared and statistically analysed between the larvae and prepupae using paired t-tests for independent samples. Waste reduction, bioconversion and ECD were calculated based on equations in Diener *et al.*, (2009). Moisture content of larvae or waste mass was determined by subtracting final dry weight from the initial wet weight and dividing by the initial wet weight and converting it to a percentage of the original mass. This was used to determine total waste reduction, bioconversion, and ECD on a dry matter basis. Formulae below.

$$\text{Waste Reduction (\%)} = \frac{dry\ weight\ of\ waste\ mass(i) - dry\ weight\ of\ waste\ mass(f)}{dry\ weight\ waste\ mass(i)} \times 100$$

$$\text{Bioconversion (DM basis)} = \frac{larval\ biomass(i) - larval\ biomass(f)}{waste\ mass\ (i)} \times 100$$

$$\text{Efficiency of conversion of digested feed (ECD)} = \frac{larval\ biomass(f) - larval\ biomass(i)}{waste\ mass\ (i) - waste\ mass\ (f)} \times 100$$

Total biomass, individual larval mass, total waste reduction, bioconversion, and ECD at each harvesting stage were compared with paired t-tests. Survival was estimated by dividing the total weight of all larvae by the average weight of the individual larvae to calculate the number of larvae at the end of the experiment and expressing that number as a percentage of the original 6000 larvae added to the container at the beginning of the experiment. Survival was analysed using a logistic regression, with harvesting stage as the predictor variable. The proximate analysis results were compared between larvae and prepupae using a multivariate analysis of variance (MANOVA) as there were multiple response variables that may be correlated and not independent. This was followed by univariate analyses of variance (ANOVA) to investigate which of ash, protein, fat or fibre content contributed to the multivariate effect. All data analyses were run using R version 3.6.2 (R Core Team, 2020).

## RESULTS

Total biomass harvested per tray was higher when larvae were harvested at day 15 in comparison to prepupae harvested at day 20 ($t = 2.326$, $df = 14$, $p = 0.036$). The total biomass harvested as 15 days was on average 22.8% greater than at 20 days (Figure 2). Overall mean (± s.e.) individual larval weight was 205 mg ± 9.42 mg. There was no significant difference ($t = 1.743$, $df = 14$, $p = 0.103$) between the individual larval mass of larvae harvested at day 15 (220 mg ± 8.90 mg) in comparison to prepupae harvested at day 20 (190 mg ± 15.27 mg).

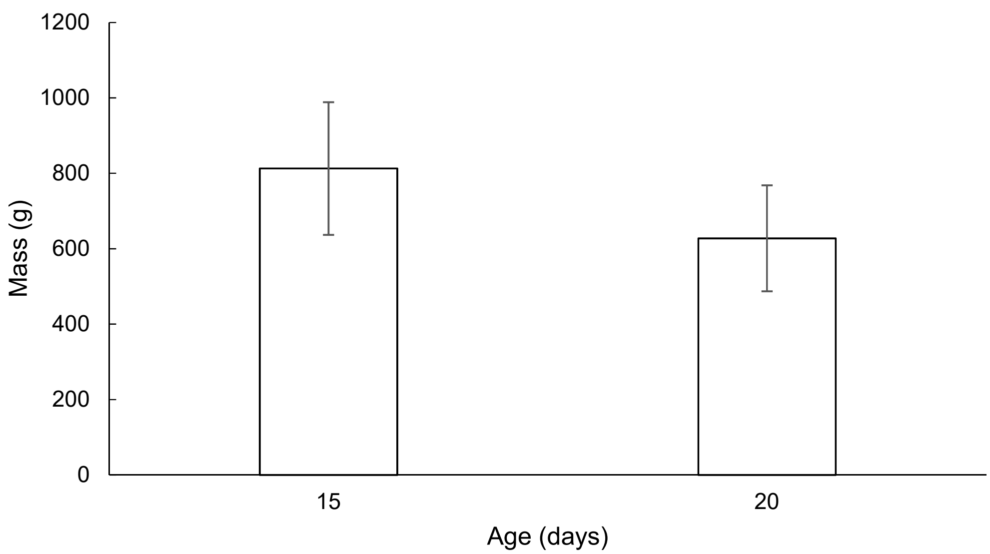


**Figure 2.** Mean total biomass of *H. illucens* harvested per tray on day 15 and day 20. Error bars indicate ± 1 S.D. Paired T-test: $t = 2.326$, $df = 14$, $p = 0.036$.

There was a significant difference in survival between larvae harvested at day 15 in comparison to prepupae harvested at day 20 (Logistic Regression: $\chi 2 = 0.208$, df = 2, $p < 0.001$). Survival was 8% higher for larvae collected at day 15 (Figure 3).

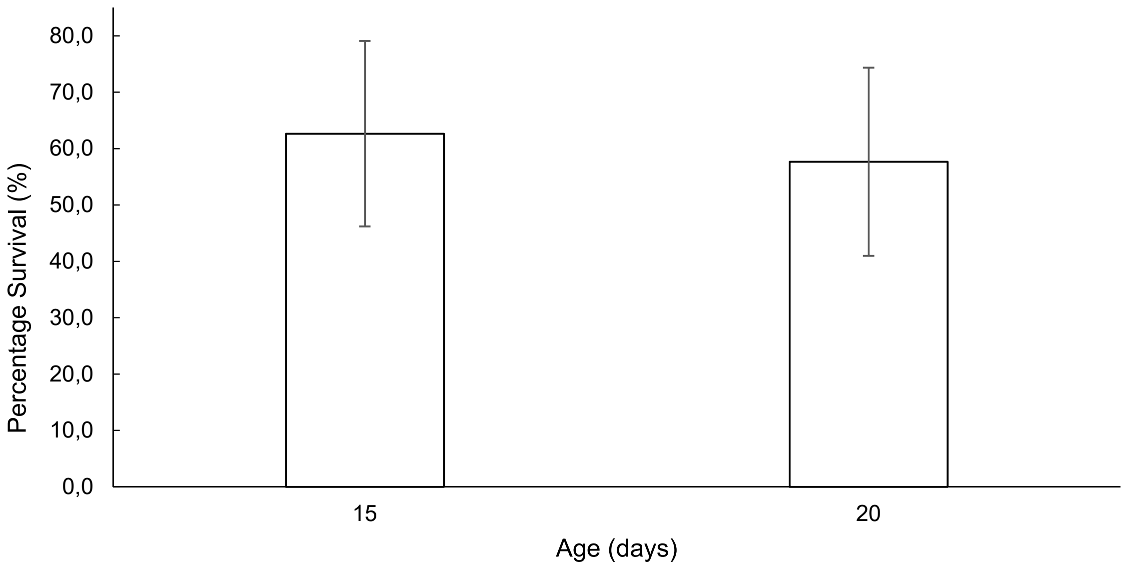


**Figure 3.** Mean survival, expressed as a percentage, of *H. illucens* larvae harvested on day 15 and day 20. Error bars indicate ± 1 S.D. Logistic Regression: $\chi 2 = 0.208$, df = 2, $p < 0.001$

Total waste reduction was measured and compared between the two groups of larvae (62% ± 1.56; t = -1.264, df = 14, p = 0.227) and, along with bioconversion (11.38 ± 0.65; t = 1.454, df = 14, p = 0.168) and ECD (18.58 ± 1.27; t = 1.899, df = 14, p = 0.078), no significant difference was found between the two groups.

The difference in dry matter content was analysed using a paired t-test between larvae harvested at day 15 vs day 20 (overall mean: 35.67% ± 0.88), with no significant difference (t = -1.361, df = 14, p = 0.195). Total protein, crude fat, crude fibre and ash were analysed and compared between the two different groups using a MANOVA (Wilk's $\lambda = 0.00$, $F_{4,11} = 3.778$, p = 0.036). The content of these nutrients was similar between the two groups of larvae, except for larvae harvested on day 20 having a significantly higher fibre content in comparison with those harvested on day 15 (Figure 4; Table 1).

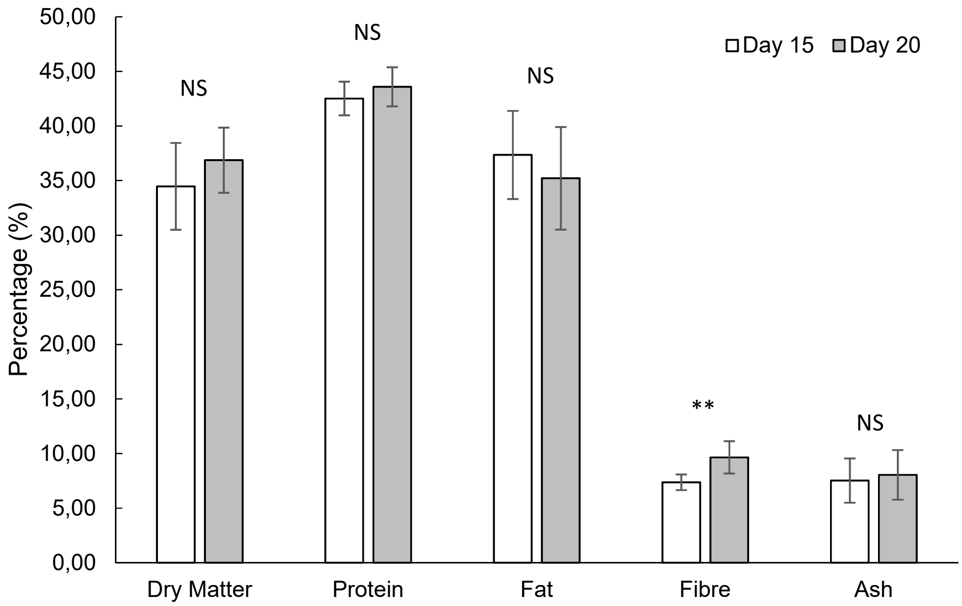


**Figure 4.** Average nutrient content of *H. illucens* larvae compared between trays harvested on day 15 and day 20. Error bars indicate ± 1 S.D. MANOVA: Wilk's λ = 0.00, $F_{4,11}$ = 3.778, p = 0.036. Annotations indicate comparisons between treatment groups (ANOVA: NS p>0.05; * p<0.05; ** p<0.01; *** p<0.001).

**Table 1**. Univariate ANOVA tests for the effect of harvesting stage on ash, protein, fat and fibre content of *H. illucens* larvae.

| Nutrient | Mean Square | *F* | df | *p* |
|---|---|---|---|---|
| Ash | 1.108 | 0.239 | 1 | 0.633 |
| Protein | 4.596 | 1.648 | 1 | 0.220 |
| Fat | 18.331 | 0.956 | 1 | 0.345 |
| **Fibre** | **20.802** | **15.317** | **1** | **0.002** |

## DISCUSSION

There was little difference between larvae and prepupae in terms of dry matter or nutritional content. With few differences between larvae and prepupae, it is not detrimental in terms of nutritional content or biomass production to harvest larvae early, or to harvest a mixture of larvae and prepupae or prepupae only. However, there is no additional mass gained from delaying harvest and it would be better for a bioconversion facility to harvest the larvae at an earlier stage than to delay and harvest when the larvae have become prepupae. Harvesting larvae later, with associated mortality experienced by the larvae, would lead to a reduction in potential harvest and total throughput.

Our current findings for crude protein and crude fat are consistent with other studies that have found crude protein to range from 37 to 45% and crude fat to range from 20 to 38% (Arango *et al.* 2004; Lui *et al.* 2017; Newton *et al.* 2005; Newton *et al.* 1977; Pastor *et al.* 2015; St-Hilaire *et al.* 2007b; Tschirner & Simon, 2015). The information provided in this study supports the use of black soldier fly larvae for both waste reduction and in animal feed. There was no difference found between larvae and prepupae in terms of crude protein and crude fat. However, it is possible that there were differences in amino acid and fatty acid composition that was not detected in this study (Liu *et al.* 2017). The higher fibre content may be an indication of the potential higher chitin content present in the exoskeleton of the larvae as they change from larvae to prepupae and the integument darkens and hardens slightly (Tomberlin *et al.* 2009; Jonas-Levi *et al.* 2017; Wang *et al.* 2020).

Other studies have found differences in nutritional content between stages of *H. illucens* larval development. While very few differences were found in this study between larvae and prepupae, it is possible the differences found in other studies are primarily between larvae and pupae, or late prepupae rather than larvae and early prepupae. Liu *et al.* (2017) found that protein increased steadily from young larvae to early prepupae before dropping between late prepupae and then increasing again during the pupation process. A similar pattern for crude fat was also found, where crude fat steadily accumulated in larvae from young larvae to 14-day old larvae, was similar in early prepupae and then dropped in late prepupae, until adult eclosion when fat was high again. During the prepupal stage the flies have likely not yet gone through the more intense changes that occur during metamorphosis from larva to reproductively active adult fly during the pupal phase. This is valuable information as larvae will occasionally begin developing from larvae to prepupae earlier if conditions such as temperature and nutrient availability are favourable, which can happen in a facility that uses a variable diet rather than a standard diet for the larvae. This can result in a mixture of larvae and prepupae being harvested at the same time.

Previous studies have focused on final larval or prepupal of *H. illucens* stages only for nutritional tests (Veldkamp & Bosch *et al.* 2015; Liu *et al.* 2017). Studying smaller life stages such as eggs or early larvae would not be conducive for the use of *H. illucens* larvae in bioconversion or as an animal feed ingredient due to the lack of biomass and difficulty in extracting larvae from feed during these earlier stages. However, the focus should not be limited to only prepupal or pupal phases either; larvae that have not yet become prepupae should be considered for nutritional testing and harvesting due to their potential for higher biomass and lower chitin content.

Bioconversion, an important measure of how efficiently the larvae breakdown and make use of the food available to them along with ECD, did not differ between larvae and prepupae, indicating that the extra time did not improve their digestion, nutrient recovery or processing of the food. This is also reflected in the lack of difference for waste reduction, indicating that the larvae do not break down any additional waste between day 15 and day 20, or in the process of changing from larvae to prepupae. This is further evidence that larvae should be harvested earlier for use in animal feed and for the purposes of waste reduction to maximise total larval mass and total turnover.

Insects benefit from having access to a more variable diet that more closely mimics their natural diet, such as a heterogenous diet sourced from multiple waste sources in the case of *H. illucens* larvae (Cammack & Tomberlin 2017; Barragán-Fonseca 2018). The effects of variable nutrients present in the diet on larval performance and nutrient content are not yet well understood; however the use of a heterogenous substrate may be more beneficial than making use of single waste source at a time, as the larvae are more likely to have access to the nutrients that they require (Gligorescu *et al.* 2018; Barragán-Fonseca 2018). More experiments should be conducted comparing larvae and prepupae on different waste types or diet mixes to determine the potential differences that nutrient content of the substrate may cause between larvae and prepupae (Oonincx *et al.* 2015).

Studies looking at the nutritional value of black soldier fly larvae have recently increased in number (van Huis *et al.* 2020). Understanding how different stages of larval development can lead to changes in crude protein and other nutrients over time is valuable. In this study there was very little difference found between larvae and prepupae in terms of nutritional content or in terms of bioconversion and waste reduction measures and therefore there are more benefits to harvesting larvae earlier. To further these results more experiments comparing larvae and prepupae on different waste types or diet mixes should be conducted.

**ACKNOWLEDGEMENTS**
This research was supported by the South African Department of Trade and Industry through a Technology and Human Resource for Industry Programme grant (THRIP/51/30/11/2017) to Aegis Environmental (Pty.) Ltd., the University of Pretoria (C.W.W.) and the University of Venda. N.J.P. received a PhD scholarship associated with the grant.

## REFERENCES

Aniebo AO & Owen OJ. 2010. Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal (HFLM). *Pakistan Journal of Nutrition* **9**, 485-487.

Arango Gutiérrez GP, Vergara Ruiz RA & Mejía Vélez H. 2004. Compositional, microbiological and protein digestibility analysis of larval meal of *Hermetia illucens* (Diptera: Stratiomyidae) at Angelopolis- Antioquia, Colombia. *Revista Facultad Nacional de Agronomía, Medellín* **57**, 2491–2499.

Barragán-Fonseca, K. B. 2018. *Flies are what they eat* (Doctoral dissertation, Wageningen University).

Caligiani A, Marseglia A, Leni G, Baldassarre S, Maistrello L, Dossena, A & Sforza S. 2018. Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. *Food Research International* **105**, 812-820.

Cammack, J, & Tomberlin, J. 2017. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). *Insects* **8**, 56.

Charlton AJ, Dickinson M, Wakefield ME, Fitches E, Kenis M, Han R, Zhu F, Kone N, Grant M, Devic E & Bruggeman G. 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as Food and Feed* **1**, 7-16.

Choi YC, Choi JY, Kim J. G., Kim MS, Kim WT, Park KH, Bae SW & Jeong, GS. 2009. Potential usage of food waste as a natural fertilizer after digestion by *Hermetia illucens* (Diptera: Stratiomyidae). *International Journal of Industrial Entomology* **19**, 171-174.

Čičková H, Newton GL, Lacy RC & Kozánek, M. 2015. The use of fly larvae for organic waste treatment. *Waste Management* **35**, 68-80.

Diener S, Zurbrügg C & Tockner K. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research* **27**, 603-610.

Dumas A, Raggi T, Barkhouse J, Lewis E, & Weltzien E. 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **492**, 24-34.

Einarsson S, Josefsson B & Lagerkvist S. 1983. Determination of Amino Acids with 9-Fluorenylmethyl Chloroformate and reversed-phase High-Performance Liquid Chromatography. *Journal of Chromatography* **282**, 609-618.

Gligorescu, A., Toft, S., Hauggaard-Nielsen, H., Axelsen, J. A., & Nielsen, S. A. 2018. Development, metabolism and nutrient composition of black soldier fly larvae (Hermetia *illucens*; Diptera: Stratiomyidae) in relation to temperature and diet. *Journal of Insects as Food and Feed* **4**, 123-133.

Harris LE. 1970. *Nutrition research techniques for domestic and wild animals.* Volume 1. Animal Science Department, Utah State University, USA.

Heussler CD, Walter A, Oberkofler H, Insam H, Arthofer W, Schlick-Steiner BC & Steiner FM. 2018. Influence of three artificial light sources on oviposition and half-life of the Black

Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. *PLoS One* **13**, e0197896.

Jonas-Levi A & Martinez JJI. 2017. The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis* **62**, 184-188.

Kaya M, Sargin I & Erdonmez D. 2016. Microbial biofilm activity and physicochemical characterization of biodegradable and edible cups obtained from abdominal exoskeleton of an insect. *Innovative Food Science & Emerging Technologies* **36**, 68-74.

Kumar S & Gupta SK. 2012. Applications of biodegradable pharmaceutical packaging materials: a review. *Middle-East Journal of Scientific Research* **12**, 699-706.

Lalander CH, Fidjeland J, Diener S, Eriksson S & Vinnerås B. 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development* **35**, 261-271.

Li Q, Zheng L, Cai H, Garza E, Yu Z, Zhou S. 2011a. From organic waste to biodiesel: black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel* **90**, 1545–1548.

Li Q, Zheng L, Qiu N, Cai H, Tomberlin JK, Yu Z. 2011b. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management* **31**, 1316–1320.

Liu X, Chen X, Wang H, Yang Q, ur Rehman K, Li W, Mazza L, Zhang J. & Yu Z. 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS One* **12**, e0182601.

Lock EJ, Arsiwalla T & Waagbø R. 2014. *Insect meal: a promising source of nutrients in the diet of Atlantic salmon (*Salmo salar*)*. In: Abstract Book Conference Insects to Feed The World, The Netherlands p. 74.

Lopez AS. 2016. *Potential of pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute: effects on growth performance and digestibility in European sea bass (*Dicentrarchus labrax*)*. MSc Thesis, The Technical University of Valencia, Valencia, Spain.

Madu CT & Ufodike EBC. 2003. Growth and survival of catfish (*Clarias anguillaris*) juveniles fed live tilapia and maggot as unconventional diets. *Journal of Aquatic Sciences* **18**, 47–51.

Makkar HP, Tran G, Heuzé V & Ankers P. 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* **197**, 1-33.

Mitrus M, Wojtowicz A & Moscicki L. 2009. Biodegradable polymers and their practical utility.*Thermoplastic Starch* 1-33.

Newton GL, Booram CV, Barker RW & Hale OM. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. *Journal of Animal Science* **44**, 395–400.

Newton L, Sheppard C, Watson DW, Burtle G, Dove R. 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. In: *Report for Mike Williams*, Director of the Animal and Poultry Waste Management Center. North Carolina State University.

Nyakeri EM, Ayieko MA, Amimo FA, Salum H & Ogola, HJO. 2019. An optimal feeding strategy for black soldier fly larvae biomass production and faecal sludge reduction. *Journal of Insects as Food and Feed* **5**, 201-213.

Ogunji JO, Nimptsch J, Wiegand C & Schulz C. 2007. Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of *Oreochromis niloticus* fingerling. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiolog* **147**, 942–947.

Oonincx DG, Van Broekhoven S, Van Huis A & van Loon JJ. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One* **10**, e0144601.

Pastor B, Cicková H, Kozánek M, Martínez-Sánchez A, Takác P & Rojo S. 2011. Effect of the size of the pupae, adult diet, oviposition substrate and adult population density on egg production in *Musca domestica* (Diptera: Muscidae). *European Journal of Entomology* **108**, 587.

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, Prearo M, Capucchio MT, Biasato I, Biasibetti E & De Marco M. 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology* **8**, 57.

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F. & Gasco L. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology* **8**, 51.

Sheppard CD, Tomberlin JK, Joyce JA, Kiser BC & Sumner SM. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology* **39**, 695-698.

St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, Sealey W, Sheppard C & Irving S. 2007. Fish Offal Recycling by the Black Soldier Fly Produces a

Foodstuff High in Omega-3 Fatty Acids. *Journal of the World Aquaculture Society* **38**, 309-313.

Tomberlin JK, Adler PH & Myers HM. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environmental Entomology* **38**, 930-934.

Tomberlin, JK, Sheppard DC & Joyce JA. 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America* **95**, 379-386.

Tschirner M & Simon A. 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed* **1**, 249-259.

van Huis, A. 2013. Potential of insects as food and feed in assuring food security. *Annual Review of Entomology* **58**, 563-583.

Veldkamp T & Bosch G. 2015. Insects: a protein-rich feed ingredient in pig and poultry diets. *Animal Frontiers* **5**, 45-50.

Wang H, ur Rehman K, Feng W, Yang D, ur Rehman R, Cai M, Zhang J, Yu Z & Zheng L. 2020. Physicochemical structure of chitin in the developing stages of black soldier fly. *International Journal of Biological Macromolecules* **149**, 901-907.

Zhang JB, Zheng LY, Liu XL & Yu ZN. 2014. *Kitchen waste converted by black soldier fly and partly substituting soymeal in chicken feed.* In: Abstract Book Conference Insects to Feed The World, The Netherlands, 14–17 May, p. 152.