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ORIGINAL RESEARCH ARTICLE

# Cardboard supplementation on the growth and nutritional content of black solider fly (*Hermetia illucens*) larvae and resulting frass

**Nicholas Romano**[1]

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## Abstract

A 10-day trial was conducted to compare the production and fatty acid composition of black soldier fly (*Hermetia illucens*) larvae (BSFL) when grown without or with cardboard supplementation at 2.2% on a dry weight basis. The final biomass of BSFL or waste reduction was not significantly impacted by cardboard. The fatty acids of C10 and C22:6n-3 were significantly higher in BSFL in the cardboard treatment, but crude lipid significantly reduced. The leftover BSFL frass had significantly higher sulfur, zinc, manganese and boron at the expense of lower nitrogen (91.2% versus 8.73% in control versus cardboard, respectively). These preliminary results appear to indicate that the growth and nutritional value of BSFL were not adversely compromised while the frass can be enhanced by adding relatively small amounts (2.2%) of cardboard. Further studies could be conducted to investigate the implications of higher inclusion levels.

**Keywords** Capric acid · Organic fertilizer · Frass · Mineral elements · Cardboard · Black soldier fly larvae

## Introduction

Black soldier fly (*Hermetia illucens*) larvae (BSFL) are increasingly being investigated as sustainable protein and lipid sources in livestock feeds, either as a replacement to traditional ingredients or as a health promoting supplement (Lee et al. 2018; Harlystiarini et al. 2020; Kumar et al. 2021; Fischer et al. 2021a). The production of BSFL can be sustainable because BSLF can thrive on various waste streams, such as manure and spoiled foods that are unsuitable for human consumption (Rehman et al. 2017a, b; Fischer and Romano 2021; Van Huis 2021). The amino acid content of BSFL is not altered greatly by their diet, despite being fed diets with vastly different amino acid profiles. Moreover, the amino acid profile of BSFL is similar or even superior to soybean meal, which is often the dominant protein in aquafeeds (Fischer et al. 2021a, b). On the other hand, the proximate and fatty acid composition of BSFL tends to be more influenced by their food source. For example, vegetables lead to higher crude protein but lower lipid content in BSFL compared to fruits or starches (Jucker et al. 2007; Fischer and Romano 2021). High starch diets may increase the lipid content in BSFL by enhancing the synthesis of some saturated fatty acids, particularly capric acid (C10), lauric acid (C12), and myristic acid (C14) (Hoc et al. 2020). Indeed, bread/dough significantly increased C12 in BSFL compared to those fed fish or spent coffee grounds (Spranghers et al. 2017; Fisher et al. 2021a) that have a lower content of digestible carbohydrates.

Interestingly, there are indications, that BSFL have some ability to digest fibers (cellulose, hemicellulose and lignin) and can even enhance BSFL production when in the right combination (Rehman et al. 2017a, b; Beesigamukama et al. 2021a, b). For example, Beesigamukama et al. (2020) found that adding biochar at 15 or 20% to a control diet led to enhanced BSFL production, which was believed to be from increasing water absorption and thus facilitating larval feeding. Moreover, there are also indications that adding carbon rich materials, such as biochar, sawdust or maize straw can improve the nutritional composition and characteristics of the BSFL frass. This is believed to be from immobilizing ammonia and increasing nutrient retention, which can lead to improved crop production when used as a soil amendment (Beesigamukama et al. 2020; 2021a). One high carbon/low nitrogen waste material that has not yet been investigated on BSFL production or nutritional value to BSFL or resulting frass is cardboard.

✉ Nicholas Romano
romano.nicholas5@gmail.com; romanon@uapb.edu

[1] Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, USA

According to the Environmental Protection Agency, it was estimated that while the majority of carboard was recycled, approximately 25% ended up in landfills while none were composted (EPA, 2018). In 2018, China and the United States were the top two producing countries in the world at 110 and 72 million metric tones, respectively. Production has increased in recent years with more delivery services for products, especially during the COVID-19 pandemic (Dezember, 2021). Therefore, it is not often difficult to find used or discarded cardboard products and these could potentially be used as material during BSFL production. It is unclear, however, if this carbon rich material in the form of non-starch polysaccharides could influence the production and/or nutritional value of the BSFL as well as the leftover frass.

The aim of this preliminary study was to compare the production of BSFL and their subsequent fatty acid composition when grown with or without supplemental cardboard as well as the macro- and micro-nutrient composition of the resulting BSFL frass, which could have implications as a soil amendment for plants.

## Materials and methods

### Source of larvae

Black soldier fly eggs were purchased from Josh's Frogs, Owosso, Missouri, and hatched on a corn mash (ground up corn crumble; approx. 1 mm) that was periodically wetted with tap water. A plastic container was placed over the eggs to keep the substrate moist. After hatching the black soldier fly larvae (BSFL) were kept on the corn mash and after a week were provided with a combination of fruits, vegetables and spoiled fish feeds in an approximately 1:1:2 ratio (on an as is basis). After two weeks of culture, the BSFL (16 days old) were removed from the culture containers and thoroughly rinsed in tap water.

### Experimental design

A total of 2.3 kg of spoiled fish feeds were added into each of the six bins (45 cm length x 20 cm width × 20 cm deep), followed by adding 2 L of tap water and mixed. Only fish feeds (no pretreatments and added only once) were used to ensure nutrition was consistent. In the control treatments, nothing was added while in the other three bins a total of 50 g of corrugated carboard was added to each bin (to yield 2.2% dry weight). All cardboard had no wax, plastic or ink and were cut into approximately 2 cm × 5 cm pieces and gently sprayed with water. Therefore, this study yielded two treatments that were triplicated.

An equal biomass of 270 g of BSFL that were 16 days old was added into each of the plastic bins. A subpopulation of 20 BSFL were measured for their initial weight, which was 0.13 ± 0.1 g and were a mixture of instar III and IV larvae. Therefore, in each bin, approximately 2,076 BSFL individuals were added to each replicate, which all came from the same hatch and culture unit.

The BSFL were grown in a temperature controlled (30 °C) room (20 feet long × 25 feet width × 18 feet high) at a relative humidity of around 44%. A blue tarp was placed on top of all the containers to help maintain moisture.

### Final sampling

A day before ending the study the tarp was removed to help dry out the frass, which facilitates harvest and to help keep the BSFL cleaner. On the tenth day of the BSFL being cultured under these conditions, all the larvae were hand picked out of each container, placed in a sieve (500 μm) and thoroughly cleaned with running tap water. The larvae were blotted dry with dry tissue paper, batched weighed, and then stored in a freezer at -20 °C. The larvae were then dried in a forced air oven (Despatch; LBB Series 2–12-3) at 60 °C to determine the moisture content. The larvae were then measured for their proximate composition (crude protein, lipid and ash) as well as fatty acid composition according to AOAC (Association of Official Analytical Chemists) (2006) methods at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia.

The remaining frass after 10 days was weighed and then immediately dried in the forced-air oven at 100 °C to determine the dry weights. Various nutrients including nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, sodium, iron, manganese, zinc, copper and boron in the frass was then measured with an inductively coupled plasma spectrometry at the Fayetteville Agricultural Diagnostic Laboratory.

### Statistical analysis

All data are presented as the mean ± standard error. All data were subjected to an independent samples t-test after meeting the assumptions for equality of variance and normality. Analyses were conducted using Statistical Package for the Social Sciences (SPSS), Version 20.0.

Biomass gain and waste reduction (%) was calculated using the following formula,

$$\text{Biomass gain} = (\text{final wet weight} - \text{initial wet weight})$$

Waste reduction = ((final dry weight – initial dry weight) / final dry weight) x 100

## Results

### Production of BSFL and biochemical composition

There was no significant difference in the final larval biomass between the control and cardboard treatment (Table 1). The crude protein content was significantly higher in the BSFL in the cardboard treatment, while there were no significant differences in the moisture, crude lipid or crude ash content in BSFL fed the control diet or diet supplemented with cardboard (Table 1).

Among the detected fatty acids in BSFL, capric acid (C10) and docosahexaenoic acid (DHA; 22:6n-3) was significantly higher in the cardboard treatment, while the remaining fatty acids were not significantly different between treatments (Table 2).

### Waste reduction of substrate and nutrient composition of frass

Final wet weight, dry weight and substrate bioconversion was not significantly different between the control and cardboard treatment (Table 3). However, the nutritional composition was significantly altered by including cardboard. This included a significantly lower nitrogen content in the cardboard treatment compared to the control (Table 4). Sulfur, zinc, manganese, and boron in BSFL frass were significantly higher in the cardboard treatment (Table 4).

## Discussion

Results in this study show that within 10 days the final BSFL biomass was over 2.8-fold higher with no significant difference between BSFL grown with or without cardboard. Based on visual observations of numerous holes in the provided cardboard pieces, there are indications that the cardboard was at least being partially consumed. This appears to be further supported by the waste reduction values. Within 10 days, there was a substantial reduction to the initial substrate of 72.8 and 71.6%, with and without cardboard, respectively, and were not significantly different from each other. It could be anticipated that if the cardboard was not being consumed, there would be significantly lower waste reduction in the cardboard treatment. Nevertheless, there were some observable amounts of cardboard fragments still in the frass. Moreover, the frass appeared more fibrous in the cardboard treatment. These observations indicate that

**Table 1** Initial and final larval biomass (g) as well as proximate composition (% 'as is basis') of the black solider fly larvae fed a control diet without or with cardboard supplementations

| | Control | Cardboard | *P* value |
|---|---|---|---|
| Initial larval biomass (g) | $272.3 \pm 1.6^{a}$ | $270.9 \pm 1.3^{a}$ | 0.471 |
| Final larval biomass (g) | $798.9 \pm 11.8^{a}$ | $772.0 \pm 45.9^{a}$ | 0.134 |
| Larval biomass gain (g) | $526.6 \pm 11.83^{a}$ | $499.67 \pm 45.97^{a}$ | 0.257 |
| Moisture content | $35.23 \pm 0.21^{a}$ | $35.08 \pm 0.10^{a}$ | 0.419 |
| Crude protein | $42.88 \pm 0.05^{b}$ | $43.72 \pm 0.48^{a}$ | 0.155 |
| Crude fat | $37.72 \pm 0.18^{a}$ | $36.89 \pm 0.23^{a}$ | 0.045 |
| Crude ash | $6.52 \pm 0.12^{a}$ | $6.32 \pm 0.05^{a}$ | 0.198 |
| Crude fiber | $4.81 \pm 0.01^{a}$ | $5.56 \pm 0.71^{a}$ | 0.350 |

Different superscripted letters in each row indicate significant differences ($p < 0.05$)

**Table 2** Mean (±SE) fatty acid composition of black soldier fly larvae fed a control diet without or with cardboard supplementations

| Fatty acid composition | Control | Cardboard | *P* value |
|---|---|---|---|
| Saturated fatty acids | | | |
| C10 | $0.87 \pm 0.01^{b}$ | $0.96 \pm 0.02^{a}$ | 0.048 |
| C12 | $34.54 \pm 0.69^{a}$ | $34.10 \pm 0.58^{a}$ | 0.685 |
| C13 | $0.06 \pm 0.00^{a}$ | $0.06 \pm 0.00^{a}$ | 1.000 |
| C14 | $14.75 \pm 0.23^{a}$ | $14.06 \pm 0.17^{a}$ | 0.149 |
| C16 | $15.63 \pm 0.26^{a}$ | $15.84 \pm 0.12^{a}$ | 0.386 |
| C18 | $2.55 \pm 0.04^{a}$ | $2.65 \pm 0.04^{a}$ | 0.693 |
| C20 | $0.07 \pm 0.01^{a}$ | $0.07 \pm 0.01^{a}$ | 0.562 |
| C24 | $2.71 \pm 0.05^{a}$ | $2.64 \pm 0.01^{a}$ | 0.055 |
| Monounsaturated fatty acids | | | |
| C16:1 | $5.28 \pm 0.10^{a}$ | $5.39 \pm 0.03^{a}$ | 0.083 |
| C17:1 | $0.19 \pm 0.02^{a}$ | $0.18 \pm 0.01^{a}$ | 0.219 |
| C18:1n-9c | $14.71 \pm 0.12^{a}$ | $14.91 \pm 0.24^{a}$ | 0.172 |
| C18:1n-11c | $0.97 \pm 0.01^{a}$ | $1.00 \pm 0.02^{a}$ | 0.338 |
| C20:1n-9 | $0.41 \pm 0.00^{b}$ | $0.43 \pm 0.00^{a}$ | 0.016 |
| Polyunsaturated fatty acids | | | |
| C18:2n-6 | $5.68 \pm 0.11^{a}$ | $5.99 \pm 0.07^{a}$ | 0.276 |
| C18:3n-3 | $0.64 \pm 0.01^{a}$ | $0.67 \pm 0.01^{a}$ | 0.238 |
| C20:4n-6 | $0.29 \pm 0.01^{a}$ | $0.28 \pm 0.00^{a}$ | 0.065 |
| C20:5n-3 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | n/a |
| C22:6n-3 | $0.48 \pm 0.00^{b}$ | $0.52 \pm 0.01^{a}$ | 0.148 |

Different superscripted letters in each row indicate significant differences ($p < 0.05$)

**Table 3** Mean (±SE) initial weight of food, final weights of black soldier fly larval frass and waste reduction of a control diet without or with cardboard supplementations

| | Control | Cardboard | *P*-value |
|---|---|---|---|
| Initial dry weight (g) | $2300 \pm 0^{a}$ | $2530 \pm 0^{a}$ | n/a |
| Final wet weight (g) | $2881 \pm 83^{a}$ | $2946 \pm 124^{a}$ | 0.452 |
| Final dry weight (g) | $652 \pm 66^{a}$ | $686 \pm 31^{a}$ | 0.731 |
| Waste reduction (%) | $71.65 \pm 2.90^{a}$ | $72.87 \pm 1.20^{a}$ | 0.339 |

**Table 4** Mean (±SE) mineral composition (% 'as is basis') black soldier fly frass when fed a control diet without or with cardboard supplementations

| | Control | Cardboard | *P* value |
|---|---|---|---|
| Nitrogen | 9.12±0.10 [a] | 8.73±0.05 [b] | 0.024 |
| Phosphorus | 2.02±0.03 [a] | 2.13±0.06 [a] | 0.160 |
| Potassium | 1.15±0.04 [a] | 1.16±0.03 [a] | 0.816 |
| Calcium | 2.47±0.05 [a] | 2.66±0.07 [a] | 0.083 |
| Magnesium | 0.26±0.01 [a] | 0.25±0.02 [a] | 0.675 |
| Sulfur | 0.74±0.00 [b] | 0.77±0.00 [a] | 0.001 |
| Sodium | 0.5579±0.084 [a] | 0.5880±0.015 [a] | 0.154 |
| Iron | 0.0536±0.0015 [a] | 0.0597±23.90 [a] | 0.101 |
| Manganese | 0.0048±0.000 [b] | 0.0055±0.0001 [a] | 0.015 |
| Zinc | 0.2003±0.0006 [b] | 0.2173±0.0004 [a] | 0.019 |
| Copper | 0.0015±0.0000 [a] | 0.00016±0.0000 [a] | 0.101 |
| Boron | 0.0011±0.0000 [b] | 0.0012±0.0000 [a] | 0.047 |

Different superscripted letters in each row indicate significant differences ($p<0.05$)

the cardboard, was not fully utilized within the 10 days. The waste reduction values in this study are relatively high compared to other waste sources such as abattoir waste (46.3%), fruits/vegetables (46.7%), and general food waste (55.3%) but comparable to dog and poultry food at 60.5% and 84.8%, respectively (Lalander et al. 2019).

Studies have shown that accessible carbon with a high protein content generally support better BSFL growth (Lalander et al. 2019; Gold et al. 2020). Indeed, it is known that manure and general food waste are better substrates for BSFL production than fiber-rich material, such as grasses and various plant by-products (Liu et al. 2018; Palma et al. 2019; Van Huis 2021). However, their use at supplemental levels may provide benefits that appears to depend on the type and inclusion amount. For example, the addition of 20% biochar significantly increased BSFL dry weight (Beesigamukama et al. 2020). Bessigamukama et al. (2020) suggested this could be due to the biochar and gypsum holding onto more moisture that would enable the BSFL to more easily move and feed throughout the substrate. In the same study, however, adding 5–15% gypsum had no significant effect on BSFL wet or dry weight (Beesigamukama et al. 2020). In another study, Beesigamukama et al. (2021a, b) found that adding sawdust to Brewer's spent grain in a 8.5 / 91 ratio, led to similar BSFL for wet weights and bioconversion compared to only using Brewer's spent grain. This finding is consistent with the findings of this study. It should be pointed out that the cardboard in this study was cut into comparatively larger pieces and were placed on top while in other studies the carbon rich material was better homogenized in the substrates. However, Beesigamukama et al. (2021a, b) found that greater additions of cardboard beyond 8.5 / 91 led to a linear decrease in BSFL wet weights. Interestingly, however, the waste reduction and biomass conversion rate were similar regardless of the amount of cardboard added (Beesigamukama et al. 2021a, b).

There were two differences in the fatty acid composition of BSFL cultured with or without cardboard, which were statistically as well as biologically significant. Capric acid (C10) was higher in BSFL fed cardboard and this fatty acid has been demonstrated to be de novo synthesized by BSFL (Hoc et al. 2020). Nevertheless, the increase in C10 needs to be taken in the context of significantly lower crude lipid content of BSFL in the cardboard treatment. Based on these two findings it appears more likely that the increased C10 was a preferential conservation of this fatty acid. In contrast, it appears that BSFL have an inability or limited ability to synthesize long chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Hoc et al. 2020). The presence of DHA can be explained by the use of spoiled fish feeds that were then passed onto the BSFL. Interestingly, however, there are differences in the reported DHA content in BSFL even when this fatty acid was lacking in their diets. For example, Moula et al. (2018) found BSFL had a DHA content of 0.6% when fed on pig manure. On the other hand, DHA was not detected in BSFL fed vegetables or fruits (Jucker et al. 2017; Fischer and Romano 2021), bread/dough (Ewald et al. 2020; Fischer et al. 2021a, b), spent coffee grounds (Fischer et al. 2021a, b) or various cereals (Liland et al. 2017; Gao et al. 2019). Moreover, even when providing diets with a high EPA content, which is a precursor to DHA, this had no effect on the DHA content of BSFL (Erbland et al. 2020).

The increase in C10 and C22:6n-3 could have important implications on the nutritional value of BSFL to animals. In the case of C10, this can reportedly improve the health of chickens and reduce pathogenic bacteria in their eggs (Çenesiz and Çiftci 2020). Moreover, increasing research is showing the benefits of C10 alone, or in a blend with other medium-chain fatty acids, on the health and production of pigs (Hanczakowska et al. 2013; Gebhardt et al. 2020). The importance of C22:6n-3 in the diets of many marine fish/shellfish is well established (Mejri et al. 2021), as well as the health promoting properties for human consumers (Fard et al. 2019). Based on the importance of these fatty acids, this appears to warrant further research, such as using additional treatments to ascertain the best ratio of cardboard to add in BSFL substrates.

After BSFL are harvested, the remaining leftover material is 'frass', which consists of BSFL excrement as well as their chitin-rich exoskeletons. The type of food provided to BSFL greatly influences the nutritional profile, which is often gauged by the nitrogen-phosphorus-potassium (NPK) values. The NPK of BSFL frass when using a standard fly diet (50% wheat bran, 30% alfalfa meal and 20% corn meal) was 4.4–5.2–4.1 (Setti et al. 2019). However, feeding BSFL with spent coffee grounds or dough led to a more N heavy frass with a NPK of 4.2–0.31–0.63 and 2.84–0.25–0.65, respectively (Fischer et al. 2021a, b). In this study, the N was also substantially higher than either P or K and likely reflected the high protein content of the spoiled fish feeds. However, the N significantly decreased when adding cardboard. While this intuitively makes sense because cardboard is more carbon rich than N, it was reported that adding sawdust to BSFL food increased the N content (Beesigamukama et al. 2021a, b). Beesigamukama et al. (2021a, b) suggested that the added sawdust facilitated the immobilization of ammonia through microbial action and/or the ammonia became absorbed on the phenolic compounds of the undigested sawdust. This discrepancy could be related to the use of different materials (sawdust vs cardboard) and/or duration of culture (14 days versus 10 days) for bacteria to fully immobilize the ammonia. Adding sawdust had no significant effect on the P or K values in BSFL frass (Beesigamukama et al. 2021a, b), which is consistent with this study.

Currently, it is not common for researchers to report the other essential macro- and micro-nutrients within BSFL frass. In this study, using cardboard led to a significant increase in sulfur, zinc and boron, which are essential nutrients for various processes involved in plant growth and health. For example, sulfur is an essential macronutrient that is structurally necessary for two amino acids as well as chlorophyll synthesis (Li et al. 2020). Manganese, zinc and boron are also essential micronutrients for photosynthesis, regulation of growth, sugar transport/metabolism, and many other functions (Welch and Shuma 2011). In this case, it could therefore be argued that adding cardboard to BSFL diets improved the nutritional content of frass. It is likely that cardboard also increased the non-starch polysaccharide content of the frass, which may also provide benefits in terms of absorbing/retaining water and nutrients when used in the soil (Beesigamukama et al. 2021a, b). Indeed, there are increasing reports on the superiority of BSFL frass on crop production as well as reducing excessive nutrient runoff compared to synthetic fertilizers (Beesigamukama et al. 2020). Further research is required to examine any benefits of BSFL frass with added cardboard on plant growth.

## Conclusions

The added cardboard had no adverse effects on BSFL production and interestingly led to a significant increase in the fatty acids CA and DHA, although the values were numerically small. While this may indicate a preferential catabolism of lipids, which is supported by the reduction in crude lipid, this should be explored further. For the frass, it could be argued that cardboard improved the nutritional value of the resulting frass by significantly enhancing sulfur, zinc and boron, at the expense of N. Therefore, it could be recommended to include some cardboard for the production of BSFL and their frass, particularly considering the widespread availability of this resource. However, considering only one treatment was used in this preliminary study, further research with additional treatments should be conducted to determine the most appropriate amount of cardboard to include.

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**Author contributions** NR wrote the manuscript, cultured the larvae, conducted final sampling/measurements and acquired funding for this project.

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**Data availability** The data sets used during the current study are available upon reasonable request.

## Declarations

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** Author declares no conflict of interest.

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