EFFECT OF DIFFERENT SUBSTRATE ON THE GROWTH AND WASTE UTILIZATION OF BLACK SOLDIER (*Hermetia illucens*) FLY LARVAE

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ABSTRACT

Black soldier fly (Hermetia illucens) larvae (BSFL) are a potentially sustainable source of protein for use as agriculture and aquaculture feeds, and a constructive means of disposing of organic wastes. Optimizing larval culture of these larvae depends on the refinement of husbandry methods. Towards this objective we investigated the use of different substrates on larval growth performance and utilization. The substrates were poultry waste (PW) T1, cow waste (CW) T2, swine waste (SW) T3 and mixture of PW, CW and SW (PCSW), T4. Efficiency of conversion of digested feed (ECD) were evaluated. The substrate of CW had the lowest assimilation rate while the mix PCSW was significantly (P<0.05) lower than PW and SW. The ECD levels of all treatments were significantly different (P<0.05) from each other with the PCSW substrate having the lowest ECD. Poultry waste (PW) and swine waste (SW) was more suitable for BSF hatching compared to CW and mixture of PCSW. This was evident after 11 g of BSF eggs hatched in 7 kg of PW which produced an average larva of 3.69 ± 0.10 kg. This was not significantly (P>0.05) different to swine waste (SW) which produced 3.65 ±0.10 kg of young larvae in five days. Cow waste produced a total of 0.80 ± 0.003 kg which was significantly (P<0.05) lower than the production in PCSW (2.83 ±0.033). It is therefore recommended that BSFL should be cultured in SW and PW to obtain a more optimal amount of larva for feeding livestock.

Keywords: Black soldier fly larvae, Poultry waste, Cow waste, Swine waste, Efficiency of digested feed

INTRODUCTION

The persistent increase in organic wastes generated worldwide is regarded as an emerging threat not only to human health but also to biodiversity and the ecosystem. Environmental concerns associated with this overwhelming level of waste, include contamination of water, air, and soil (Tanga *et al.,* 2021), and can also be a route of spreading pathogens (Kawasaki *et al*., 2020). In Nigeria, there is constant rise in the price of feed in the livestock sector. This results in large scale imports of animal feed, and consequently in surpluses of nitrogen (N) and phosphorus (P) in the form of manure and urine. Concomitant ammonia (NH_3) emissions lead to nitrification and acidification of soil, and eutrophication of water bodies, and more than half of the nutrients contained in feed are excreted as manure (Steinfeld *et al.,* 2006; Steinfeld, 2012). Black soldier fly (*Hermetia illucens*) larvae can utilize bio waste streams and convert them into high-quality larval biomass. In natural systems, manure and other decaying organic materials are broken down by a variety of organisms, among which the larvae of the BSF (Myers *et al.,* 2008). This species can digest SW, PW and CW (Myers *et al.,* 2008; Sheppard *et al.,* 1994; Bondari and Sheppard, 1981). Furthermore, BSF larvae have a high protein (32-64%), and fat content (20-40%) (Sealey *et al.,* 2011), and can be used as animal feed. As a diet ingredient, BSF larvae have been found to support growth in pigs and poultry (Elwert *et al.,* 2010). If BSF larvae are reared on manure from livestock production, and subsequently reused as animal feed, this could decrease the environmental impact of the livestock sector. Furthermore, this would decrease the need for feed imports, and thereby the pressure on unsustainable sources of dietary protein currently used, such as fishmeal or soybean meal. Several studies have reported on the provision of manure to BSF larvae. However, differences in experimental design prevent direct comparison of the suitability of manures as feed for BSFs. For these reasons, a study was conducted to determine the effect of different substrate on the growth and nutrient utilization of black soldier fly (*Hermetia illucens*) larvae

MATERIALS AND METHODS

Waste sourcing, processing and diet preparation

Prepupa was purchased from a reputable source and placed in an insectarium. After 7 days, most of the pupa developed into BSFs. The eggs were harvested from eggies placed in an insectarium and 11g each was measured in four places and put on 5g of chicken feed mixed with 2ml of water. After four days, the eggs hatch into neonates and fed with poultry waste (PW) T1, cow waste (CW) T2, swine waste (SW) T3 and mixture (PCSW) T4 which was collected at the animal experimental farm of Benson Idahosa University Edo State. In order to homogenize the waste, it was properly mixed with 4ml of water until a uniform consistency was realized. Homogenized waste (7 kg) was placed in four pens and mixed with 4 ml of water in order to create a diet for the neonates. The moistened manure was positioned at the center of the pen and wheat offal was placed in circles to serve as a wall to restrict movement of the larva. This is to allow the larvae to submerge in the substrate (Onincx *et al.,* 2015). The pen was highly ventilated, and the top was sealed with a lid.

Experimental design

Eggs from BSF were drawn, hatched and the resulting larvae were fed on the four experimental waste substrates to investigate growth performance and waste utilization. Four substrates were used in triplicates: PW (T1), CW (T2), SW (T3) and PCSW (T4). Each waste substrate measuring 7kg each. A total of 20kg of BSF pupa were used for the production of the eggs. The performance of these larvae was monitored for eleven days.

Substrate management, feeding and refeeding of larva

The colour and structure of the waste were evaluated daily. Before provision to the larvae, the colour o the PW was light grey, the CW was dark green, and SW contained both light and dark brown pieces whereas the mix contained shades of all the colours present in the different waste. For all four waste types, transformation to a homogeneous black slurry was considered an indicator that the waste had been consumed. If most waste had been consumed, more was added to allow continuous feeding. The amount of waste to be added per occasion was based on the amount consumed since the last feeding and the estimated larval mass present. For each gram of waste provided to the larvae, approximately 4ml of water was added. Feeding of the larvae was done following established protocols (Dortmans *et al.,* 2017) for a period of 11 days with re-feeding on day 5 and day 8 with 7 kg of waste until harvesting. Caution was taken such that the feed could not exceed a depth of 5 cm, in order to allow larvae to process the food completely and to avoid the attraction of unwanted flies to the remaining uneaten feed. All larva were harvested and weighed per replicate. Subsequently, residues of feed and faeces adhering to their integument were removed by using a standard kitchen sieve (Lardé, 1989). The difference between the two weights was considered to be residual material (faeces and leftover feed).

BSF larvae harvesting and sampling

Harvesting was done after 11 days of feeding and refeeding, at which time separation of larvae from the unutilized organic residues took place. Sampling of BSFL was done at day 5 and 8 to record growth. Weight measurements of every BSFL on different substrates was recorded using ACS Digital scale with readability of 0.1g.

Substrates utilization

The ability of the larvae to digest the different waste substrates (Conversion of Digested Feed) (ECD) was determined using the (Scriber and Slansky, 1981) method as:- B = (I-F)-M and ECD = B/I-F; where B = Assimilated food used for growth (measured as pre-pupal biomass); I = Total food offered during experiment; F = Residue in the experimental pen (Undigested food + excretory products) and M = Assimilated food metabolized (calculated by mass balance). All calculations were done in grams (g). The higher the ECD the better the Feed Conversion Efficiency (FCE). Mass balance was used to create a biomass production structure to aid in predicting the substrates utilization. The larvae feed intake was divided into three outputs; weight of the diet, weight of undigested feed and weight of harvested larvae

Data analysis

All the data were analyzed using one-way analysis of variance (ANOVA). Comparison of means between groups was done by Tukey's HSD test. Data were analyzed by SPSS) version 23 for Windows.

RESULTS AND DISCUSSION

Substrate utilization rate

Table 1 shows that PW and SW was more readily consumed and digested compared to CW and PCSW Substrate. The CW substrate had the lowest assimilation rate (14674) while the mix PCSW was significantly lower than PW and SW. The ECD levels of all treatments were significantly different (P<0.05) to each other with the PCSW substrate having the lowest ECD (9.68%).

Table 1. Efficiency of conversion of food digestion (ECD) of the different substrate

Waste substrates	I-F	B=(I-F)-M	ECD=B/I-F	ECD (%)
Poultry waste (PW)	26891^{c}	26833^{c}	997.9^{c}	9.98^{c}
Cow waste (CW)	14721^{a}	14676^{a}	996.9^{b}	9.97^{b}
Swine waste (SW)	26700^{c}	26691^{c}	999.7^{d}	9.99^{d}
Poultry, Cow, Swine waste (PCSW)	24171^{b}	23390^{b}	967.7^{a}	9.68^{a}

** Values expressed as Mean ±SE*

Biomass of larvae produced during hatching

Poultry waste (PW) and swine waste (SW) was more suitable for BSF hatching compared to CW and the PCSW. This was evident after 11 g of BSF eggs hatched in 7 kg of PW which produced an average of 3.69 ± 0.10 kg which was not significantly (P>0.05) different to SW which produced 3.65 ±0.10 kg of young larvae in five days. The CW produced a total of 0.80 ± 0.003kg which was significantly (P<0.05) lower than production in PCSW (2.83 ±0.033) (Table 2).

Table 2. Weight of five day old larvae (kg), extracted from 11 g of BSF eggs reared on poultry waste (PW), cow waste (CW), swine waste (SW) and PCSW substrates

Waste substrates	Mean larvae weight (kg)
Poultry waste (PW)	3.69 ± 0.10[c]
Cow waste (CW)	0.80 ± 0.003[a]
Swine waste (SW)	3.65 ±0.10[c]
Poultry, Cow, Swine waste (PCSW)	2.83 ±0.033[b]

** Values expressed as Mean ±SE*

Black Soldier Fly Larvae growth performance

When 5-day old larvae of 15 g were subjected to different waste substrates, the highest mean weight gain was recorded in the larvae reared on SW substrate (4.60 ±0.01 kg), which were significantly larger ($P < 0.05$) than the larvae reared on PW (4.34 ± 0.09 kg), CW (0.90 ±0.01 kg) and a mixture of the different substrates (3.83 ±0.04)-Table 3

Table 3. Growth performance of BSF larvae subjected in poultry waste (PW), cow waste (CW), swine waste (SW) and a mixture of PW, CW and SW (PCSW) substrates

Waste substrates	Initial average weight (kg)	Final average weight (kg)	Average weight gained (kg)
Poultry waste (PW)	0.02	4.34 ± 0.09[c]	4.33 ±0.09[c]
Cow waste (CW)	0.02	0.90 ±0.01[a]	0.89 ±0.01[a]
Swine waste (SW)	0.02	4.60 ±0.01[d]	4.58 ±0.01[d]
(PCSW)	0.02	3.83 ±0.04[b]	3.82±0.04 [b]

** Values expressed as Mean ±SE*

In this study, SW was more rapidly utilized by the BSF larvae compared to the other three substrates. The ECD levels were higher, this indicated that these larvae had a better FCE . These results are in agreement with previous studies by (Manurung *et al.,* 2016) found ECD levels of 5.69% to 10.85% while using rice straw and (Abduh *et al.,* 2017) obtained an ECD increment of 6.3% to 26.3% ECD using Screw pine (*Pandanus tectorius*) fruits. Contrary to the present study, (Supriyatna *et al.,* 2016), observed ECD level of 12 to 21% with cassava peel substrate, and Abduh et al. (2017) reported ECD levels of 12.5% to 25.9% while using rubber seed. Larvae fed on SW had a significantly better growth performance compared to PW and PCSW. The large sized larvae in the SW substrate could have resulted from the protein rich nature of SW substrate as compared to PW and PSCW substrate. However, nutritional content of the substrates may vary depending on the waste substrate materials. Larvae weight was observed to be proportional to the food nutritional composition (Tschriner and Simon, 2015). Research reported by (Nguyen *et al.,* 2013) recorded high growth rates on BSF fed on a diet high in protein, and higher growth rates. Certain bacteria, such as *Bacillus natto,* are present in poultry manure and promote larval growth and development (Yu *et al.,* 2011). Hence the significant increase in larva when compared to CW and PCSW substrate. The reduce growth of the larva reared on the CW and PCSW could be as a result of the age of the larvae; we used neonate larvae, whereas in other studies larvae were first reared on a housefly medium for four days (Myers *et al.,* 2008), before they received a manure diet. BSF larvae reared on CW from hatching, took approximately 120 days to develop to prepupae (Sealey *et al.,* 2011). These data indicate that larval age at transfer to manure, and dietary quality during early development could be a key factor for the development time of BSF larvae. The use of starter diets for optimal early development is common in BSF production systems (Delcroix *et al.,* 2015). Feeding regime also affects larva growth. However, Li et al. (2011) provided 48 grams of CW per 100 larvae in a single feeding, and Myers et al. (2008) provided 32.8-72.4 g in 26-30 days. In this study, feed was also restricted. Feed restriction leads to higher ECIs, while a surplus of feed leads to lower ECIs (Diener *et al.,* 2009). Similar to Diener et al. (2009) we assumed for our ECI calculation that all provided feed was ingested. Although this assumption has not been checked, the validity was supported by observed changes in colour and texture of the residual material, compared to the manure provided. These ECIs seem to reflect substrate nutritional quality, a trend generally observed in insect nutrition (Slansky and Rodriguez, 1987).

CONCLUSION

The results of this study indicate that nutritional composition of waste substrates affects the growth performance and substrate utilization and that growth trend of larvae slows with the increase in the larval size in all the different waste substrates. It is therefore recommended that Black Soldier Fly Larvae (BSFL) should be cultured in PW and SW to obtain a weighty larvae.

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