Original Research Article

EFFECT OF TYPES AND QUANTITIES OF SUBSTRATES ON GROWTH PERFORMANCE OF BLACK SOLDIER FLY LARVAE (Hermetia illucens)

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ABSTRACT

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| Despite the fact that black soldier fly larvae are being used as a supplement in livestock production. However, there is lack of information of the suitable substrates and their quantities that can be used in black soldier fly rearing. This study was conducted to determine the effect of different types and quantities of substrates on the growth characteristics of black soldier fly larvae.In this study, Black soldier fly (BSF) larvae were fed on five substrates, the experimental design used was a Randomized Complete Design with two factors namely: different types of waste and quantities of substrates fed, comprising of treatments; Pineapple Waste, Jack Fruit Waste, Rumen Content, Fish Offal, Mixed substrates and control replicated thrice per substrate and fed until the neonates larvae became fully grown larvae; length, weight and survival rate of the larvae fed on all five substrates at four feeding levels; 250g, 500g, 750g and 1000g were determined. **Pineapple waste (PW) and** Jack fruit waste (JFW) except for Mixed substrates (MS) recorded the highest length of 10.00mm, 11.85mm and 11.45mm, at 750g when compared at 1000g PW {9.60mm}, JFW {10.60mm}, MS {12.75mm} respectively (P<0.05), at 1000g of substrate. Mixed Substrate (MS) and Jack Fruit Waste (JFW) recorded the highest weight of 0.318g/FM and 0.212g/FM respectively (P<0.05), at 1000g of substrate. Control (Cont) and Mixed substrate MS achieved the highest Survival rate (SR) of 96.19% and 96.01% respectively (P<0.05). Results showed significant differences for all substrates influencing growth (p<0.05) but MS was the most influential.*Keywords: [Black Soldier Fly Larvae, Feeding Level, Growth Characteristics, Hermetia Illucens, Organic Substrates, Diptera Stratiomyidae*  |

1. INTRODUCTION

Animal production in general, and poultry in particular has several social-economic roles in many countries (Kleyn & Ciacciariello, 2021). Additional income and ensuring food security are the main reasons for poultry farming by many resource poor and local settings. However, due to faster growth in the sector, poultry farming is now considered a lucrative business to resource - constrained communities simply because they need slightly low initial capital, small rearing space, and reduced management costs (Omiti et al., 2009). This demand is driven by faster economic growth due to increasing population which has resulted into much desire to feed more animal protein (FAO, 2011). The demand in many developing countries for poultry products is predicted to hike by 70% in order to cater 9.2 billion people which are likely to occupy the globe by year 2050 (United Nation, 2019), hence putting much pressure on the need to provide enough animal proteins.

Globally silver fish and soya bean is widely used as protein ingredients in the mixing of poultry feeds. However, much of the silver fish produced is used by human beings for food and soya bean cultivation is also limited by land, water and poor yields (Veldkamp et al., 2015). Consequently, it is projected that the globe will have to produce more food by 70% by 2050 (FAO, 2011). However, with respect to animal protein production in particular, the International Feed Industry Federation (IFIF) believes that production of poultry meat, eggs, and pork likely to double. This poses many challenges to the world capacity to produce adequately enough livestock feeds.

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In sub -Saharan Africa, silver fish and soya bean are used as a cheap source of food mainly by poor households hence little and highly priced is available for use as ingredients in the manufacture of animal feeds. However, in Uganda, the prices for silver fish and soya are increasing every year because of competition for proteins between human beings and livestock, in this case affordability by poor livestock farmers is very low (Komi and Nakibugwe, 2017). The increasing scarcity of resources to produce silver fish, and soybean feed ingredients has doubled prices during the last 5 years and yet capital investment in livestock accounts for 60 to70% as feeding hence leaving marginal profits for livestock projects (Komi and Nakibugwe, 2017). Therefore, alternative livestock feeds are needed to reduce amount of the sources in a constituted feed (soya beans and silver fish)

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BSF Larvae have become common simply because of their potential to convert waste into a cheap protein ingredient for livestock feeds according to Banks et al., (2014). BSF Larvae bio-conversion technique have been successful in animal waste management particularly from confined animal facilities of livestock, and poultry with reduction values of 5. Additionally, BSF Larvae biomass is observed at 44.4% dry matter (DM) crude protein, 23% DM lipids, and a good balance of essential amino acid (Sheppard et al., 2008), associated with better feed conversion efficiency (Veldkamp et al., 2012).

Furthermore, studies on livestock feeding trials on BSF Larvae biomass have so far yielded good results predicting the ability to substitute fish meal and soy in livestock feeds (FAO, 2013). Fortunately, Use of BSF Larvae as a bio-conversion technique has been successful in animal waste management particularly from confined animal facilities of livestock, and poultry with reduction values of 76.5% (Rohmanna & Maharani, 2022) (Banks et al., 2014), for example jack fruit waste, pineapple waste, rumen content, fish offal among others. Therefore, successful development of BSF Larvae farming technology is key and entirely depend on the rearing substrates types and their feeding amounts which have so far not been studied widely. Available studies, have proposed feeding amounts for BSF Larvae as 100 mg larva−1 day−1 (Diener et al., 2009), 163 mg larva−1 day−1 (dry base) (Paz et al., 2015) and 200 mg larva−1 day−1 (Permana et al., 2018). Moo et al., (2022), these studies revealed that higher feeding amounts increased BSF larvae growth. Unfortunately, these studies are only limited to a few organic substrates while majority substrates that are readily available in our local communities are not being investigated in details. In this study however, different organic waste and their feeding levels were evaluated to determine their effect on growth which was documented as length, weight and survival rate of the larvae.

2. material and methods

**2.1.1 Black Soldier Fly Larvae**

The BSF larvae used in the experiment were collected from Ento organic farm of Makerere University Agricultural Research Institute Kabanyolo (MUARIK). The BSF larvae were picked from the same batch of five to six days old from the date of hatching which was reared under same conditions of room temperature between 21 – 24 degrees centigrade and uncontrolled relative humidity. Counting of 2500 larvae was done, and their initial weight was recorded before introduction to the substrate and substrate mixture. The experiment was carried out at MUARIK and Kyambogo University laboratories in a period of six months. The experiment was carried in the year of 2022 – 2023.

**2.2.2 Organic Substrates**

Two categories (two animal type; two plant type), four types of organic substrates were collected to assess the performance of the larvae in terms of length, weight and survival of the larvae on the substrates. The substrates comprised of rumen content from abattoirs of Wakiso and Kampala capital city, Fish offal from Kireka and Wakiso markets, Jack fruit and pineapple waste from fresh fruit markets around Wakiso and Kampala capital city. These substrates were selected on the basis of no or low purchase, non-use by human beings or livestock directly, agent need for disposal to improve sanitation, their protein content and palatability to black soldier fly larvae and sustained local availability in large quantities in Wakiso and Uganda with a plan for future use for large scale industrial BSF larvae production in Uganda.

**2.2.3 Collection of Substrates**

Pineapple and Jack fruit waste were collected daily in fruit markets at waste disposal points by placing a waste bin at Karlwe and Nakasero markets in Kampala capital, collecting in bucket reduces soil and fungal contamination. Additionally, Fish offal and Rumen content waste were collected from fresh fish markets and abattoirs in Kireka and Kampala capital city respectively. These were also collected in buckets which were later covered to stop other dipterans from ovipositing in the substrates and avoid soil contamination.

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**2.2.4 Processing and segregating substrates**

Unwanted wastes for example, grasses, plastics, polythene were separated and removed from Jack fruit and Pineapple waste. The pure Jack fruit and pineapple waste were then poured into the shredder and shredder for 5 – 10 minutes to break them into smaller particles before introducing to BSF larvae. This was done principally to increase surface area to be fed by BSF larvae. For the case of Rumen content and Fish offal, these were not processed since their particle size were already small and fine and thus easily consumable by BSF larvae therefore fed as they were.

**2.2.5 Experimental Design**

Pineapple waste, Jack fruit waste, Rumen content, Fish offal and Substrate Mixture were used as treatments. Four feeding levels were employed on each treatment. Feeding level conducted in this study constituted 250, 500, 750 and 1000g/2500larvae in 10 to 12 days with three replicates per feeding level. The selected quantities were different from amount suggested by Moo et al. (2022) and Permana et al. (2018). A total of 40 plastics (20cm x 15cm) without lids were used, four replicates four each feeding level in one single set of experiments. Each feeding container contained a designated substrate quantity and 2500 of five to six days old BSF larvae. Every container was covered with a mosquito net to avoid oviposition by other flies and also ensured uniform air circulation. All the experimental set ups were carried out at room temperature range of 21 – 24 degrees centigrade under uncontrolled relative humidity and light intensity. For each treatment, 2500 BSF larvae were weighed recorded before inoculating them into the substrate and its mixture and corresponding replicates. The weight of each designated substrate quantity was also measured and recorded before being fed to the BSF larvae. The feeding process was stopped after12days when the larvae is big, started to change color from white to creamy white. All the data recorded was in form of wet weight measurement. For weight determination, larvae from different petri dishes were picked and measured using a sensitive scale and recorded in grams. To determine length, larvae were picked and measured using a thread and the thread was later placed on a ruler (HACO school ruler, made in Kenya) the length was taken and recorded in millimeters. In addition, survival rate was determined by counting the number of the larvae that remained in the substrate after a rearing period of 12 days and divided by the original number of larvae introduced into the substrates multiplied by 100, Survival rate= Final number - initial number x100 . Initial number

**2.2.6 Statistical data analysis**

During data analysis first and foremost, the data were analyzed by Two-way Analysis of Variance (ANOVA) to determine the significant differences between substrates, and levels of substrates. Duncan’s multiple range test and least significant difference (LSD) was used to separate means between substrates and levels of substrates at 95% confidence interval (CI). Data were then presented in tables. Data that was tested for normality and found to be non-normal using the Shapiro wilk’s normality test (Ghasemi and Zahediasl, 2012), was transformed appropriately.

3. results

**Effects of types of substrates on growth characteristics of BSF Larvae**

BSF Larvae growth was interpreted as larval length, larval weight and larvae survival rate. There was a significant (*p*<.001) difference between length of the larvae; the highest larval length was recorded in Mixed substrate and the lowest was recorded in Fish offal. The Larval weight also showed a high significant difference (*p*<.001); the highest larval weight was recorded in Mixed substrate and the lowest was registered in Fish offal. There was a highly significant (p<.001) difference between Survival rate of the larvae; the highest survival rate and lowest was recorded on Mixed substrates and Fish offal respectively (Table 1).

There was significant (*P*=0.03) difference between levels of substrates which varied according to the substrate type. The larval survival rate showed several trends, whereby it generally increased from feeding levels 250g to 1000g in Control, Jack fruit waste, Rumen content and Mixed substrate. Fish offal decreased from 250g to 1000g (Table 2).

**Effects of levels of substrates on growth characteristics of BSF larva.**

**Larval Length**

There was a highly significant value (*P*<.001) between levels of substrates which varied according to the substrate type. The larval length showed several trends, whereby it increased from feeding levels 250 to 750g across levels of substrates;and decreased from 750 to 1000g with in the levels of substrates. The highest larval length was attained at feeding level 1000g, and the lowest was observed at level 250g across substrate levels (Table 3).

**Larval Weight**

There was a highly significant (p<.001) difference between levels of substrates which varies according to the substrate type. The larval weight showed several trends, whereby it generally increased from feeding level 250 to 1000g across levels of substrate; The highest larval weight was generally achieved at 750g, and the lowest was achieved at feeding level 250g (Tables 4).

**Table 1:** **Effects of different types of substrates on growth and survival of BSF Larvae**

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| **SUBSTRATES** |  |  |  |
| **Larval length (mm)** | **Larval weight (g/FM)** | **Larval survival rate (%)** |
| CONT | 10.06a | 0.205a | 96.01a |
| PW | 9.62a | 0.168a | 72.96b |
| JFW | 10.30a | 0.212a | 70.09a |
| RC | 10.01a | 0.171a | 85.16b |
| FO | 9.36a | 0.156a | 34.15a |
| MS | 11.3a | 0.318a | 96.01b |

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed, g/FM =gram Fresh Matter substrates. Duncan’s multiple range test was used to separate means using different letters to denote significant difference at 5% probability.

**Table 2: Effects of types and quantities of substrates on Survival rate of BSF Larvae.**

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| --- | --- |
| **Levels of substrates (g)**  | **Types of substrates****Survival rate of the Larvae (DM%)** |
| **CONT**  | **PW**  | **JFW**  | **RC**  | **FO**  | **MS** |
| 250 | 93.87a | 69.37a | 68.90a | 82.43a | 36.67a | 94.33a |
| 500 | 96.70b | 76.53b | 69.87ab | 85.73ab | 36.20ab | 96.37b |
| 750 | 96.47bc | 72.03c | 69.63bc | 86.33bc | 35.33abc | 96.53bc |
| 1000 | 97.73bcd | 72.83cd | 71.97d | 86.13bcd | 28.40d | 96.80bcd |

CONT= Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO= fish offal, MS= mixed substrates, DM= Dry Matter. For significant means at 5% probability letter superscripts were used to separate mean

**Table** **3: Effects of types and quantities of substrates on length of BSF Larvae.**

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| --- | --- |
| **Levels of substrates (g)**  |  **Types of substrates****Length of the Larvae (mm)** |
| **CONT**  | **PW**  | **JFW**  | **RC**  | **FO**  | **MS** |
| 250 | 8.00a | 9.20a | 8.75a | 9.50a | 8.75a | 10.05a |
| 500 | 8.62ab | 9.70ab | 11.00b | 9.60ab | 9.30b | 11.05b |
| 750 | 11.80c | 10.00bc | 11.85c | 10.70bc | 10.35c | 11.45bc |
| 1000 | 11.85cd | 9.60cd | 10.60d | 10.25cd | 9.05d | 12.75d |

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates, mm= millimeters. For significant means at 5% probability letter superscripts were used to separate means

**Table 4: Effects of types and quantities of substrates on weight of BSF Larvae.**

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| --- | --- |
| **Levels of substrates (g)**  | **Types of substrates****Weight of the Larvae (DM%)** |
| **CONT**  | **PW**  | **JFW**  | **RC**  | **FO**  | **MS** |
| 250 | 0.10a | 0.14a | 0.14a | 0.14a | 0.10a | 0.23a |
| 500 | 0.19ab | 0.17ab | 0.24ab | 0.18ab | 0.14ab | 0.36ab |
| 750 | 0.22bc | 0.21bc | 0.29bc | 0.17bc | 0.19bc | 0.36bc |
| 1000 | 0.29cd | 0.15cd | 0.15cd | 0.19cd | 0.19cd | 0.37cd |

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates, DM= Dry Matter. For significant means at 5% probability letter superscripts were used to separate means.

The control (Cont) comprised of brewers spent grains and mixed substrate (MS) was obtained by mixing plant and animal based substrates

**3.1 Discussions**

Effects of types and quantities of substrates on growth and Survival of BSF larvae, Survival rate (%) of the larvae was highly associated with protein content of larvae (prob<0.001). The highest survival rate of the larvae on Mixed substrate may have been attributed to the high mean crude protein (Carbohydrates (%) 45.00, crude protein (%) 15.08, crude fat (%) 9.60) of the substrate caused by substrate mixing. This finding is in line with Nguyen et al. (2013). Rumen content recorded higher value than Fish Offal among the animal type substrates. This is may have been attributed to the numerous bacteria that come from the rumen along with the substrate; these bacteria might have helped in decomposition of the substrate to generate more nutrients Li et al. (2011). However, Fish Offal substrate recorded the lowest survival rate, this is might have been caused by excess fats in the substrate which may have interfered with uptake and absorption of nutrients (Nguyen et al., 2013). Plant type substrates also had higher values in this study. However, Jack fruit waste recorded slightly lower survival rate of the larvae than Pineapple Waste. This could have been attributed to high fiber content of the Jack fruit that may have limited the larvae from feeding on the substrate, this study matches with the study of Tomberlin et al. (2009).

Contribution of substrate amount/levels to larval survival rate on the substrates

The high survival rate of the larvae obtained at high feeding level (1000g) across all substrates with exception of Fish Offal is possibly due to the highest crude protein content in the substrates that might have increased with additional substrate amounts from 250 to 1000g. However, the lowest survival rate recorded from the larvae fed on Fish Offal may have been caused by excess crude fat in the substrates that could have limited larvae aggregation, mobility, aeration, feeding, growth and hence survival. This finding is in agreement with Nguyen et al. (2013), the study reported high survival rate and growth, and decline in developmental time of larvae on higher protein diets. However, studies have shown that increased fat amounts, 20-36% Crude fat DM) can be harmful to the larvae and possibly adult survival, and reproduction. The decreasing survival rate was due to increasing fat levels in the substrate that might have increased with additional substrate amounts.

In comparison with other studies, the mean larval survival rate (96%) of this study is above the results of Oonincx et al. (2015), recorded the highest survival rate of 78.9±13.2 using vegetable waste as feeding substrate under controlled conditions.

There was a positive correlation between larval weight and larval length (prob<0.001, R=0.7774). The highest mean weight and length of the larvae fed on Mixed substrates may have been attributed to high nutritional quality of the diet generated by mixing the substrates; this finding is in line with Nguyen et al. (2013), who registered a greater weight and length on high protein diet substrates. Additionally, larvae fed on Jack fruit waste substrate still recorded higher weight and length. This may have been attributed to high fat in the substrates that could have attracted the larvae to feed faster and grow bigger and longer. Additionally, the lowest values recorded for Fish offal substrates is probably due to high production of ammonium compounds that might have been brought about by excess fats in the substrates that could have limited feeding and reduced larvae weight, and length. This finding is in line with Nguyen et al. (2015), extremely high fat amounts in diets (20 – 36% DM crude fat) contributed to slow growth (weight and length).

Contribution of substrate amount/levels to larval length

At 250g of substrate, the larvae were shortest; this is because 250g of substrates was not enough for the larvae. This finding agrees with Nguyen et al. (2013), reported an increase in larval length and weight, were proportional to the amount of feed given. The increasing length of the larvae fed on 250 and 750g of substrates may have been attributed to increasing nutrient amounts that increased with additional substrates. However, the decline of larval length fed on 1000g of substrates could have been caused by accumulation of waste in the feeding substrates; this finding is in agreement with Banks et al. (2014), observed that as more substrate was supplied to larvae, it positively affected development, length and weight in BSF, but negatively affected waste reduction efficiency.

Contribution of substrate amounts/levels to larval weight

Increase in mean larval weight from feeding levels 250g to 1000g in Mixed substrates is attributed to high nutritional superiority of the substrates that could have attracted the larvae to feed more and derives enough nutrients to grow, this finding matches with the findings of Leong et al. (2016). Weight gained by BSF larvae generally increased as the substrate quantities of palm oil waste increased (1g/day to 25g/day). In comparison, lower feeding levels used in this study; feeding at level 250g generally resulted in comparatively low average larvae weight compared to higher feeding levels. This might have been attributed to intraspecific competition that could have caused starvation, stunted growth, and development Mitchell- Foster et al. (2012). However, the lower larval mean weight of BSF larvae fed on Fish Offal at feeding level 1000g could be attributed to high production of ammonium compounds, caused by increasing fat amounts in the substrates that might have increased by adding substrates and thus compromising feeding and declining weight of the larvae. This finding is confirmed by Nguyen et al. (2015), increased fat mounts in diets; 20-36% crude fat contributed to reduced growth and low weight larvae.

Generally, the findings shows that the optimum weight that was obtained at level 750g/2500 larvae of 0.313g was highest in comparison with Oonincx et al. (2015) findings (0.158±0.02 and 0.13±13.2) of the experiment conducted using vegetable waste as feeding substrate conducted under laboratory-controlled conditions

**4 CONCLUSIONS**

Substrates used in this experiment generally increased growth and survival. However, excess fat in Fish offal substrate decreased growth and survival of the larvae. To effectively enhance utilization of the substrates and avoid wastage, substrates with extremely high fat should be integrated with low fat substrate to strike balance. On the other hand, increased feeding amounts increased growth but resulted into waste accumulation therefore high feeding rates are not recommended because they can cause inappropriate management of the substrates. An ideal feeding amounts should be designed for better utilization and save costs of feeding.

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