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# Abattoir sludge and food waste combination as a feed source for black soldier fly larvae: Optimisation of level of larval incorporation in the substrate#

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**Abstract**

*The study was conducted to optimize the level of Black Soldier Fly Larvae (BSFL) incorporation in the abattoir sludge and food waste combination. The substrate for the study included a combination of 70 per cent abattoir sludge as the principal substrate (PS) and 30 per cent hostel food waste as the co-substrate (Co- S) for BSFL rearing. Experiments were carried out to fix the level of larval incorporation into 320 g of the substrate and to assess the nutritional qualities of the harvested prepupae. The BSFL were incorporated in four different levels in 320 g of substrate ($T_1$: 150 mg larvae, $T_2$: 450 mg larvae, $T_3$:600 mg larvae and $T_4$: 750 mg larvae). The efficiency of BSFL to feed on the substrate and get converted into biomass was evaluated for the treatment combinations. Among the different treatments, $T_1$had significantly ($p<0.001$) higher mean prepupal weight and larval survivability. Hence the addition of 150 mg larvae to the 320 g of substrate was found to be optimum for BSFL biomass production.*

**Keywords**: *Abattoir sludge, food waste, black soldier fly larvae*

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Disposal of abattoir sludge is the most difficult part of its management. The current method of sludge utilisation includes composting, landfilling and its use as manure and soil conditioner. But these methods may cause some serious consequences like odour, pathogenic contamination and excessive nutrients in the soil.The challenge in establishing a sustainable waste management system is to find a method to dispose the sludge in such a manner that it does not permanently harm the ecosystem. There is a need to find new and improved waste recycling technologies to attain an efficient resource conservation and circular economy. One of the innovative methods is the conversion of organic waste by detritivorous insect larvae. The utilisation of high-impact waste streams for raising insects has been proven to be one of the best strategies for sustainable waste management. Among the various insect species, the larvae of Black soldier fly (*Hermetiaillucens*) are gaining significant attention.

Black soldier fly is a non-pest fly that can be found in tropical and warm climatic regions across the world. Among the different life stages, the larval stage is the only stage during which the fly feed and it is during this stage it stores nutrients for the further non-feeding stages. It consumes a wide range of organic wastes and get themselves converted into valuable biomass which is rich in protein and fat.Rekha *et al.* (2022) produced 0.567 kg black soldier fly larvae from 4 kg offood waste. Deepak *et al*. (2022) opined that the lechate produced after larval treatment of waste can be utilized for soil amendment after pretreatment. Thepurpose of the current study was to optimise the amount of larval incorporation in a combination of abattoir sludge and food waste to yield maximum biomass within the shortest development time.

## Materials and methods

### *Collection of abattoir sludge and food waste*

The sludge which remained after wastewater treatment (principal substrate-PS) was collected from Thrissur cooperation slaughterhouse, Kuriachira. Food waste (co-substrate- Co-S) was procured from PG ladies hostel, CVAS, Mannuthy.

### *Source of Larvae*

A BSF rearing unit pre-established at Meat Technology Unit, Mannuthy, provided the larvae required for the experiment. Larvae were reared in broilerchicken starter feed for about five days and five day- old larvae were used for experiments.

### *Optimisation of level of larval incorporation in the substrate*

The experiments were carried out in 7 cm deep earthen wares of 15.5 cm diameter andcovered with mosquito net to prevent from larvae predators.The BSF larvae were inoculated at four different levels in the substrate constituted of 70 per cent principal substrate and 30 per cent co-substrate,to identify the optimum quantity of larval incorporation for bioconversion of unit weight of substrate. The quantity of substrate was fixed as 0.32 kg for all treatments in this experiment. This diet was calculated for 16 days according to the feeding rate (200 mg per larva per day) recommended by Nyakeri*et al*. (2017).

Treatments were as follows
$\mathbf{T_1}$: 150 mg larvae in substrate
$\mathbf{T_2}$ 450 mg larvae in substrate
$\mathbf{T_3}$:600 mg larvae in substrate
$\mathbf{T_4}$: 750 mg larvae in substrate

The five-day-old larvae were inoculated in 320 g substrate.The substrate was introduced into the containers once at the start of the experiment.The substrate was thoroughly mixed before adding larvae to ensure uniform distribution of co-substrate in the principal substrate. The dry matter content of different substrates was determined gravimetrically. The five-day-old BSF larvae were picked using thumb forceps and inoculated in each treatment. Each larval cohort was evenlyspread on the allocated substrate surface. The feeding containers were randomly placed on the floor surface and covered with mosquito netting to keep off other fly species from ovipositing on the substrate. Water was added every day to the containers to compensate for the evaporation loss. The amount of water added was based on visual assessment of surface dryness.

The larvae were not handled until the appearance of the first prepupa, recognised by change in colour of integument from light/ cream to dark. As soon as the first prepupa was found in a particular container, that container was removed from the experiment and the date was noted (Ooninc*xet al*., 2015). The larvae from that container were collected manually using thumb forceps from the residual rearing substrate and counted. Cleaned BSF larvae and prepupae were put on tissue paper for a few seconds to absorb water adhering to their integument, weighed and that matter was noted as prepupal yield (g fresh matter). Dry matter content of the residual material was determined gravimetrically.

***Substrate consumption and waste reduction efficiency***

The efficiency of BSFL to consume and thereby reduce the organic matter content in the substrates was assessed by calculating the substrate reduction, bioconversion and feed conversion rates as previously described by Diener *et al*. (2009).

***Chemical and nutritional parameters of abattoir sludge and food waste***

The parameters of sludge such as pH (FCO, 1985), moisture content (FCO, 1985), crude protein content (Prasannakumar, 2016) and crude fat content (AOAC, 2012) were analyed. The food waste was analysed for moisture, crude protein and fat content as per the standard procedures (AOAC, 2012).

***Nutritional qualities of the prepupae***

The crude protein and fat content of the prepupae were determined as per AOAC, 2012. The nitrogen to protein conversion factor of 4.67 was used (Janssen *et al*., 2017).

**Results and discussion**

***Chemical and nutritional parameters of the sludge***

The average pH value of sludge was 7.4 ± 0.04. The pH value of the slaughter house sludge was within the range of initial substrate pH (6.0 to 8.0) recommended by Ma *et al*. (2018) for the effective conversion of organic wastes by BSFL. The moisture content of sludge collected was on an average 87.65 ± 0.02 per cent. Dortmans*et al*. (2017) reported that the larvae could perform well between 60 to 90 per cent substrate moisture. The sludge used for the experiments had a mean crude protein content of 18.86 ± 0.26. This value was lower than the protein content reported in the literature. Heddle (1979) reported that the recovered biomass from the activated sludge treatment of slaughterhouse effluent had a crude protein content of 50 to 55 per cent. The variation in the crude protein content of sludge from different slaughter houses may be attributed to the difference in the time between effluent collection, treatment and handling of the sludge. The bacteria present in the sludge might beresponsible for the breakdown of protein (Fransen*et al*., 1995). The crude fat content of the abattoir sludge was 1.09 ± 0.20. This value was comparable to the mean fat per cent reported by Fransen*et al*. (1995) for activated sludge (1.4 to 4.4 per cent) collected from poultry and pig slaughter houses. But the value was lower than the fat content found by Heddle (1979) (15 to 25 per cent). The difference in the fat content may be attributed to the difference in the fat content of the influent or might be due to the variation in the efficiencies of fat removal of different Effluent Treatment Plantunits.

***Nutritional parameters of the food waste***

The collected food waste had a mean moisture content of 78.61 ± 0.22 per cent. This value was within the range of optimum moisture content for larval growth recommended by Dortmans*et al*. (2017). The mean crude protein content of the food waste used was 9.03 ± 0.09 per cent and fat content was 11.16 ± 0.09 per cent. Food waste used by Ebeneezar*et al*. (2021) for rearing BSFL had a crude protein content of 16.89 ± 0.21 per cent and an average fat content of 12.34 ± 0.31 per cent. The authors had not mentioned about the composition of the food waste used. The lower crude protein and fat content obtained for the food waste used in the present study may be attributed to the variation in the composition of the food waste used.

**Parameters related to substrate reduction and prepupal growth**

Data on the average prepupal weight (milligrams), prepupal yield (grams), development time (days), substrate reduction (SRR) (per cent), feed conversion rate (FCR) and bioconversion rate (BCR) (per cent) of control and different treatments are presented in table 1.

***Average prepupal weight***

Statistical analysis showed that there was significant ($p<0.001$) difference in average prepupal weight (mg) between the treatments. Larvae reared on $T_1$ achieved a higher prepupal weight (38.44 ±6.34) which was significantly ($p<0.001$) different from average prepupal weights of those raised on T2 (14.52 ± 2.62), $T_3$ (12.15 ± 1.50) and $T_4$ (10.07 ± 0.80). Only a small increment in larval weight was observed in $T_2$, $T_3$ and $T_4$ which might be due to the insufficient amount of available feed for the increased larval numbers. The difference in the average weights of prepupae obtained from $T_2$, $T_3$ and $T_4$ was not significant.

In the present study, varied numbers of larvae were inoculated in a fixed amount of feed. As the number of larvae increased, the availability of feed per larvae decreased. This might have resulted in the decline in prepupal weight observed with increase in the larval incorporation. The larvae grown in treatments with lower feeding rates underwent food stress during their growth period and showed reduced prepupal weight in comparison with those reared at higher feeding rate. The results were in accordance with those reported by Diener *et al*. (2009) who observed increase in mean prepupal weight with increasing feeding rates in chicken layer feed. Nyakeri*et al*. (2019) also reported improved prepupal weight with increased feeding rate of faecal sludge. Larval growth was directly proportional to the quantity of feed supplied. Yakti*et al*. (2022) obtained higher individual larval weights at lower larval densities compared to higher densities. This was attributed to the higher availability of nutrients.

Barragan-Fonseca *et al*. (2019) opined that average prepupal weight was positively affected by higher larval densities. Higher temperatures resulting from larval aggregation enhanced the assimilation of feed by the larvae (Rivers and Dahlem, 2013). Green *et al*. (2003) suggested that the alkaline excretions of larval groups neutralized the acidity resulting from the bacterial growth and it pre-digested the substrate. Yakti*et al*. (2022) opined that even though substrate reached higher temperatures in high densities, earlier depletion of nutrients led to lower weight of harvested larvae.

***Prepupal yield***

There was no significant difference in the prepupal yield (g) among different treatments. The mean prepupal yield from $T_1$, $T_2$, $T_3$ and $T_4$ were1.51 ± 0.16, 1.47± 0.20, 1.46 ± 0.24 and 1.46 ± 0.16 g respectively. Nyakeri*et al*. (2019) observed an improvement in prepupal yield with increasing feeding rate of the faecal sludge. Diener *et al*. (2009) reported similar results in chicken feed. Prepupal yield was found increasing with higher feeding rates. Results of the present study was not in accordance with the above-mentioned researchers. Prepupal yield was not found to be varying with the different feeding rates in the current study. It can be concluded that the added amount of substrate was able to yield only 1.46 to 1.51 of larva irrespective of the amount of larva added initially.

***Larval survivability (per cent)***

Statistical results revealed that larval survivability varied significantly ($p<0.01$) between the treatments. The mean values for larval survivability rates were 82.67 ± 7.04, 69.67 ± 2.9, 59.75 ± 4.3 and 57.6 ±2.15 per cent for $T_1$, $T_2$, $T_3$and $T_4$ respectively. Larval survivability rate was significantly ($p< 0.01$) higher for $T_1$ compared to $T_3$and $T_4$. Lower survivability rates observed for treatments incorporated with higher larval numbers may be attributed to the lack of adequate feed to support the growth at higher densities. Competition over nutrients combined with the reduced quality of food might have resulted in lower survivability rates (Banks *et al*., 2014). The results suggest that the larvae were not capable of developing into prepupa at higher densities in the provided substrate.

### *Development time*

The days required by the larvae, in different treatments, to turn into prepupae did not vary significantly between the treatments. Among the different treatments, larval development appeared to be faster in $T_1$ with the emergence of the first prepupa in 36.00 ± 3.25 days. The mean development time observed for larvae in $T_2$and $T_4$ were 37.50 ± 2.51 and 38.00 ± 1.97 days respectively. Development of prepupae was slowest in $T_3$in which 40.33 ± 1.02 days were taken for the prepupa to emerge.

Diener *et al*. (2009) observed a prolonged development time of BSFL at a feeding rate of 12.5, 25 and 50 mg of chicken feed per larva per day (42, 32 and 20 days respectively). They concluded that larvae prolonged their development time in case of food shortage. Larvae fed at the rate of 100 and 200 mg per larva per day developed faster than those fed at lower rates. Nyakeri*et al*. (2019) also opined that higher feeding rates resulted in faster growth. Prepupae were observed after 14 days when BSFL was reared on feacal sludge at the rate of 200 and 250 mg per larvae per day. No prepupa was observed before 16 days with the feeding rates of 100 and 150 mg per larvae per day. Barragan-Fonseca *et al*. (2018) opined that development time was influenced by rearing density. Lower densities accelerated larval development. Parra-Paz *et al*. (2015) noted prolonged development time at higher larval densities as a consequence of the low fee availability per larva. Insufficient amount as well as unfavourable nutrient combination resulted in prolonged development time.

### *Substrate reduction rate*

Substrate reduction rates (%) were 37.62 ± 2.33, 37.61 ± 1.05, 36.60 ± 1.54 and 37.62 ± 1.13 per cent for $T_1$, $T_2$, $T_3$and $T_4$respectively. The mean values did not show significant variation between the treatments. The results were not in accordance with those reported by Diener *et al*. (2009) and Nyakeri*et al*. (2019) who observed differences in substrate reduction with varied feeding rates. Diener *et al.* (2009) fed larvae with chicken feed at different feeding rates *viz*., 12.5, 25, 50, 100 and 200 mg per larvae per day. An increase in substrate reduction was observed with supply of feed up to 100 mg per larvae per day. In the current study no such trend was observed. A substrate reduction in the range of 36.60 to 37.62 per cent observed in the present study suggests that only this much amount of substrate was reducible by the larvae. Increasing the larval amount did not result in difference in the substrate reduction.

**Table 1**. Parametersrelated to substrate reduction and prepupal growth

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | F-value | p-value |
|---|---|---|---|---|---|---|
| Average prepupal weight(milligram) | 38.44 ± 6.34$^a$ | 14.52 ± 2.62$^b$ | 12.15 ± 1.50$^b$ | 10.07 ±0.80$^b$ | 14.008 | 0.000$^{**}$ |
| Prepupal yield (gram) | 1.51 ± 0.16 | 1.47 ± 0.20 | 1.46 ± 0.24 | 1.46 ± 0.16 | 0.011 | 0.998$^{ns}$ |
| Larval survivability (%) | 82.67 ± 7.04$^a$ | 69.67 ± 2.9$^{a,b}$ | 59.75 ± 4.30$^b$ | 57.6 ± 2.15$^b$ | 6.429 | 0.003$^{**}$ |
| Development time(days) | 36.00 ± 3.25 | 37.50 ± 2.51 | 40.33 ± 1.02 | 38.00 ± 1.97 | 0.594 | 0.626$^{ns}$ |
| Substrate reduction (SRR) (%) | 37.62 ± 2.33 | 37.61 ± 1.05 | 36.60 ± 1.54 | 37.62 ± 1.13 | 0.101 | 0.959$^{ns}$ |
| Feed conversion rate (FCR) | 11.50 ± 0.75 | 12.43 ± 1.51 | 12.88 ± 2.34 | 12.17 ± 1.28 | 0.126 | 0.944$^{ns}$ |
| Bioconversion rate (BCR) (%) | 3.35 ± 0.36 | 3.28 ± 0.44 | 3.27 ± 0.54 | 3.26 ± 0.36 | 0.009 | 0.999$^{ns}$ |

### *Feed conversion rate (FCR)*

The mean values for FCR did not show significant variation between the treatments. Higher mean FCR value was observed for $T_3$ (12.88 ± 2.34) and lowest for $T_1$(11.50 ± 0.75). Feed conversion rates for $T_2$and $T_4$were found to be 12.43 ± 1.51 and 12.17 ± 1.28 respectively. Low FCR obtained for T1 suggested that the larvae reared on small densities (150 mg larvae per substrate) were more efficient in converting the provided substrate into biomass.

This result was supported by the findings of Nyakeri*et al*. (2019). They observed a decrease in FCR with increasing feeding rates of faecal sludge.

### *Bioconversion rate (BCR)*

The mean bioconversion rates of $T_1$, $T_2$, $T_3$ and $T_4$were 3.35 ± 0.36, 3.28 ± 0.44, 3.27 ± 0.54 and 3.26 ± 0.36 respectively. There was no significant difference in the BCR values between different treatments.Results of the present study suggested that the substrate used was not effective in producing larval biomass, though the waste reduction ranging from 36.6 to 37.62 per cent was observed. Lundgren (2019) observed higher substrate dry matter reduction and lower BCR on ventilating open boxes. He opined that increased ventilation enhanced the microbial respiration in the open containers which resulted in higher dry matter reduction rate.

The nutrients in the substrate might not be easily available to the larvae. The assimilated nutrients might have been utilised for maintenance purposes and thus unavailable for new biomass production. Even increasing the feeding rate also did not result in better bioconversion.

### *Parameters of the prepupae*

There was no significant difference in the crude protein (CP) content of prepupae raised on different treatments. These results were not in agreement with the findings of Diener *et al*. (2009) who observed high crude protein content of larvae at lower feeding rates of chicken feed. Oonincx*et al*. (2015) also observed increased larval nitrogen contents with feed restriction.

Contrary to the results obtained in the current study, Barragan-Fonseca *et al*. (2018) and Yakti*et al*. (2022)had reported that lower larval densities resulted in high crude protein content. Barragan-Fonseca *et al*. (2018) opined that the protein content of the larvae was regulated within narrow limits. There was no relation observed between the diet and that of larvae grown on it.

### *Crude fat (CF) (per cent)*

Crude fat content of prepupae harvested from $T_1$was 12.24 ± 0.80 and this was significantly ($p<0.001$) different from $T_2$(14.82 ± 0.42), $T_3$ (16.49 ± 0.71) and $T_4$ (18.83 ± 1.00). Difference in the fat contents between$T_2$ and $T_3$ was not significant. Fat content of $T_4$was significantly ($p< 0.001$) higher than $T_1$, $T_2$ and $T_3$and might be due to the higher larval density among the treatments. This finding was supported by the reports of Barragan-Fonseca *et al*. (2018)who obtained higher prepupal crude fat values at higher larval densities. Yakti*et al*. (2022) also observed similar results.

The different treatments compared in this experiment did not vary with respect to prepupal yield,development time, substrate reduction,bioconversion and feed conversion rates. Average weight and larval survivability rates were found to be highest for $T_1$. Mean individual weight is one of the main parameters to evaluate the productivity of a BSFL rearing system. In the waste management process utilizing BSF, achievement of higher larval survival rate is important. This will reduce the demand for eggs and enhance the larval production efficiency. In this perspective, $T_1$was selected as the best treatment in this experiment.

## Conclusion

The optimum amount of BSFL to be added to 320 g of substrate combination of 70 per cent abattoir sludge and 30 per cent food waste for the larvae to reach the maximum weight within the shortest development time was found to be 150 mg. That is, for the conversion of one gram of substrate 0.47 mg larvae were required.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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