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Nutrition composition of black soldier Fly (*Hermetia illucens* L.) prepupae reared on different organic substrates

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Abstract
The rising population and enhanced living standards in developing nations have led to an increased demand for easily accessible animal derived proteins. Growing costs of animal feed, exacerbated by diminishing resources, intensify the competition between human food, fuel and animal feed. In order to address this problem, an experimental research was carried out in CRD design with four replications at the Division of Entomology, Dr. Sharadchandra Pawar College of Agriculture, Baramati during year 2023-24 with the purpose of assessing how various organic substrates affected the biochemical composition of black soldier fly reared on this substrate. The treatment included T_1-Poultry manure(excreta), T_2-Kitchen waste, T_3- fish waste, T_4-Poultry feed, T_5-Fruit waste and T_6-Combine waste. The data were analysed using descriptive statistics and the results showed that the fish waste also had high amounts of protein (39.20%) and fat (39.32%), but its utility as a feed item was limited due to its increased ash (13.84%) and moisture content (64.58%). In contrast, the kitchen waste was nutritionally better, with good levels of crude protein (36.14%) and fat (36.05%) improving larval performance. The best organic substrates for enhancing the development of larvae and pupae were found to be kitchen waste and chicken feed.

Keywords: Black soldier fly, waste, biochemical parameters, human food etc.

Introduction

Insects offer protein levels comparable to traditional sources like fish meal and soybean meal (SBM) when included in livestock feed (Khusro *et al.*, 2012) [24], and they support favorable growth rates (van Huis, 2013) [48]. Additionally, insect farming requires significantly less land and water than cattle production (van Huis *et al.*, 2013) [49]. Insects contribute to nutrient recycling and emit lower levels of greenhouse gases and ammonia compared to cattle or pigs, potentially reducing the environmental impact of food production (Oonincx *et al.*, 2016) [31]. However, despite these advantages, the consumption of insects as human food remains limited in Western cultures. For example, U.S. consumers tend to hold negative views about eating black soldier fly larvae (BSFL) directly, but are more accepting of food products from livestock fed on insects (Higa *et al.*, 2021) [21]. BSFL could serve as an alternative protein source for beef cattle (Fukuda *et al.*, 2022) [17], and identifying and adopting such alternative protein sources can enhance the sustainability of milk and meat production systems.

The black soldier fly, scientifically named **Hermetia illucens**, belongs to the phylum Arthropoda, class Insecta, order Diptera, and family Stratiomyidae. It is predominantly found in tropical and warm regions, within the latitudinal range of 45°N to 40°S (Doelle *et al.*, 2016) [15]. The BSF undergoes five life stages: egg, larvae, prepupae, pupae, and adult. The adult BSF typically lives between 8 to 20 days (Tomberlin *et al.*, 2009; De Smet *et al.*, 2018) [44, 13]. Flies generally mate two days after emergence, and females lay eggs two days after mating (Tomberlin and Sheppard, 2002) [46-47]. Mating can occur both in flight and on the ground (Tingle *et al.*, 1975) [43]. Male courtship, crucial for female acceptance, is thought to depend on acoustic signals produced by the male's wing fanning. BSF females are monogamous (Tomberlin and Sheppard, 2001; Giunti *et al.*, 2018) [45, 19], preferring dry sites for egg-laying, which takes around 20–30 minutes.

Each egg cluster contains about 324–998 eggs, with individual eggs weighing approximately 0.028 grams (Booth and Sheppard, 1984; Chia *et al.*, 2018) [5, 7-8]. Fertilized eggs typically hatch around four days after being laid (Booth and Sheppard, 1984) [5].

Adult black soldier flies lack chewing mouthparts and have a sucking mouthpart, feeding exclusively on liquids. Upon hatching, BSF larvae exhibit voracious feeding behavior, consuming various organic waste types and accumulating significant fat and protein for later life stages. The feeding phase of BSFL lasts about 2-4 weeks, depending on environmental factors like temperature and humidity, as well as food availability. Afterward, they enter the pre-pupae stage, stop feeding, empty their gut, and seek a dry location (Sheppard *et al.*, 1994; Diener *et al.*, 2009) [9, 14]. BSFL undergo six molts during their life cycle, progressing through six instar stages before pupation (Kim *et al.*, 2010) [25]. The final larval instar is non-feeding, and the pre-pupae stage usually lasts two weeks, but it can extend up to five months under unfavorable environmental conditions (Furman *et al.*, 1959) [15]. The protein-rich black soldier fly prepupae are a promising alternative for animal feed (Zhang *et al.*, 2010) [51]. BSF cultivation requires less space than traditional protein sources, and vertical stacking allows for higher production within the same area (Zurbrugg *et al.*, 2018) [52]. As BSF is grown on waste, its production costs are significantly lower than those of soybean and fish meal. The amino acid profile of defatted BSF prepupae is comparable to that of soybean meal (Caligiani *et al.*, 2018; Surendra *et al.*, 2016) [6, 42], though its composition can vary depending on the larvae's feed or substrate (Caligiani *et al.*, 2018; De Marco *et al.*, 2015; Onsongo *et al.*, 2018) [6, 12, 29]. Defatted BSF prepupae is a viable option for enhancing the protein content of animal feed (Cullere *et al.*, 2016) [10].

Incorporating BSF prepupae into animal feed has demonstrated substantial economic benefits. Replacing soybean and fish meal with 42% and 55.5% BSF prepupae in the diets of broiler chickens led to a 16% cost reduction and a 25% return on investment (Onsongo *et al.*, 2018) [29]. Eggs from Lohmann Brown Classic laying hens fed BSFL meal were comparable to those laid by hens fed soybean meal (Secci *et al.*, 2018) [34], though some studies have reported contradictory results (Ruhnke *et al.*, 2018) [32]. European seabass fed a diet with 45% BSF prepupae meal performed similarly to those fed a diet of 100% fish meal (Magalhaes *et al.*, 2017) [27]. Rainbow trout fed a diet in which 25% of fish feed was replaced by BSF prepupae also exhibited comparable growth patterns to those fed 100% fish feed (St-Hilaire *et al.*, 2007) [40-41].

Methodology

In this experiment, biochemical analyses of the prepupal stage of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), were conducted at Dr. Shardchandra Pawar College of Agriculture, Baramati.

The objective was to determine the biochemical composition of prepupae reared on six distinct organic substrates: poultry waste, kitchen waste, fish waste, poultry feed, fruit waste, and combined waste. Prepupae were selected, oven-dried at 60 °C for 24 hours to determine dry weight, and subsequently analyzed for various biochemical parameters.

Determination of crude protein content

The work was carried out at Department of Agricultural Chemistry and Soil Science, Dr. SPCOA, Baramati. Total nitrogen was estimated as per Kjeldahl modified method by Scales and Harrison (1920) [33] and converted to protein values by multiplying nitrogen percentage with the factor 6.25.

$$\text{Total nitrogen (g/100 g sample)} = \frac{\text{a}-\text{b x Normality x 14 x 100}}{g\ of\ sample \times 1000}$$

Where,
a = ml of standard acid for sample
b = ml of standard acid for blank
If total nitrogen value is X, protein content in 100 g = X x 6.25

Determination of crude fat content

The crude fat content was determined by ether extraction from oven dried prepupal sample using a Soxhlet apparatus (AOAC, 1975) [2].

$$\text{Crude fat (\%)} = \frac{\text{Weight of Soxhlet Flask with oil (g)} - \text{Weight of empty Soxhlet flask (g)}}{\text{Weight of the sample}} \times 100$$

Determination of moisture content

The moisture content was determined using the method outlined by AOAC (1975) [2]. For this, 10 grams of fresh powdered prepupal sample were precisely weighed into an aluminum moisture box. The sample was then dried in an oven at 100 °C (±2 °C) for 16 hours. After drying, the sample was cooled in a desiccator and re-weighed. This process was repeated until a constant weight was achieved. The percentage of moisture content was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight of sample (g)} - \text{Final weight dried sample (g)}}{\text{Weight of the sample (g)}} \times 100$$

Determination of ash content

The ash content was determined following the AOAC (1975) [3] method. For this, 5 grams of moisture-free powdered prepupal sample of black soldier fly were placed in a silica crucible. The sample was first charred over a low Bunsen flame and then ignited in a muffle furnace at 600°C for 6 hours. The ash content was calculated as a percentage using the following formula:

$$\text{Ash (\%)} = \frac{\text{Weight of the ash (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

Statistical Analysis

The data were statistically analyzed following the methods outlined by Panse and Sukhatme (1967), with further comparison conducted using Duncan's Multiple Range Test (DMRT). A Completely Randomized Design (CRD) was employed, with four replications used to analyze the data obtained from the laboratory experiments.

Results and Discussion

Crude proteins

The data presented in Table 1 and Fig. A show significant variations in the crude protein composition of black soldier fly prepupae reared on six different waste treatments. The highest crude protein content was recorded in prepupae from fish waste, with a concentration of 39.20%, which was significantly higher than all other treatments. This was statistically similar to prepupae from the combined waste substrates, which exhibited 38.06% crude protein content.

Kitchen waste reared prepupae showed 36.14% crude protein, which was on par with prepupae grown on poultry feed, reporting 35.09% crude protein. Prepupae from poultry waste showed 33.25% crude protein, while the lowest content was observed in fruit waste-reared prepupae at 28.00%. Black soldier fly larvae offer a sustainable protein source as they convert organic waste into high-quality protein, making prepupae valuable as feed for livestock, poultry, and fish.

These findings align with those of who recorded 36.1% crude protein in black soldier fly reared on kitchen waste, similar to the current results. The crude protein content for BSF reared on kitchen waste in this study is higher than the 33% reported by Shumo *et al.* (2019) [36] and lower than the 38.43% observed by Fitriana *et al*. (2022) [16]. Opoku *et al*. (2023) [30] reported a similar crude protein content of 37.6% for BSF reared on restaurant kitchen waste. The results of Belperio *et al*. (2024) [3], who recorded 28.1% crude protein in BSF reared on chicken feed, are slightly lower than the current findings. Seyedalmoosavi *et al*. (2022) [35] also reported similar crude protein content for BSF reared on poultry feed and poultry manure (33-39% and 32-45%, respectively). The protein content of BSF reared on poultry waste in this study is slightly lower than the 36.2% recorded by Boafo *et al*. (2023) [4].

Crude Fat

Table 1 and Fig. B reveal significant variations in crude fat content across treatments. The highest crude fat content was observed in prepupae reared on fish waste (39.32%), significantly higher than all other treatments. Prepupae from kitchen waste and combined waste showed 36.05% and 31.95% crude fat, respectively. Prepupae from poultry feed and fruit waste exhibited 28.80% and 22.71% crude fat, respectively, while those from poultry waste showed the lowest crude fat content at 17.66%.

These results align with Fitriana *et al*. (2022) [16], who reported 35.59% crude fat for BSF reared on kitchen waste. Belperio *et al*. (2024) [3] recorded 26.1% crude fat from BSF reared on chicken feed, which is slightly lower than the current findings. The crude fat content of BSF reared on poultry waste in this study is more or less consistent with Boafo *et al*. (2023) [4], who reported 25.6%, a value higher than our results. The crude fat content from fruit waste in this study is slightly lower than Singh *et al*. (2022) [37-38], who reported 19.89% crude fat. Deshmukh *et al*. (2024) [11] recorded 28.29% crude fat from fruit waste, slightly higher than the present findings. Li *et al*. (2011) [26] reported that the high fat content of BSF prepupae could limit their application as a feed ingredient.

Moisture Content

Table 1 and Fig. C demonstrate significant differences in moisture content among black soldier fly prepupae reared on various organic waste treatments. Lower moisture content reduces the risk of microbial attack and enhances preservation. Prepupae from poultry feed showed 58.51% moisture content, significantly lower than all other treatments and similar to prepupae from kitchen waste (58.53%). Combined waste yielded 60.20% moisture, while prepupae from fish waste and poultry waste showed 64.58% and 67.04% moisture, respectively. The highest moisture content was observed in prepupae from fruit waste (69.15%), posing a higher risk of microbial attack.

These results are consistent with Spranghers *et al*. (2016) [39], who reported slightly higher moisture content in BSF reared on chicken feed and kitchen waste (61.3% and 61.9%, respectively). Boafo *et al*. (2023) [4] recorded 60.6% and 61.8% moisture for BSF reared on fruit waste and poultry waste, values slightly lower than those observed in this study. Opoku *et al*. (2023) [30] reported lower moisture content for fruit waste and restaurant kitchen waste (62.2% and 57.8%, respectively). Insects with lower moisture content are less prone to microbial attack, as noted by Adeduntan (2005) [1].

Ash Content

Table 1 and Fig. D show that high ash content in black soldier fly prepupae may be undesirable for feed formulation. Prepupae from poultry feed had the lowest ash content (9.25%), significantly lower than all other treatments. Fruit waste reared prepupae showed 10.24% ash content, which was on par with kitchen waste (10.57%). Prepupae from combined waste and poultry waste exhibited 10.57% and 10.24% ash content, respectively. The highest ash content was recorded in prepupae from fish waste (13.84%).

These findings align with Spranghers *et al*. (2016) [39], who reported 10% ash content in prepupae reared on chicken feed, making them more suitable as a feed ingredient. Similar ash content was observed by Fitriana *et al*. (2022) [16] in food waste (10.40%). Shumo *et al*. (2019) [36] reported 9.6% ash content, slightly lower than our results (10.57%). Boafo *et al*. (2023) [4] recorded 8.8% ash content in BSF reared on fruit waste, lower than the present findings. Singh *et al*. (2022) [37-38] reported 12.87% ash content, slightly higher than our findings. Newton *et al*. (2005) [28] recorded 14.6% ash content for BSF reared on poultry manure, slightly higher than the current results.

Table 1: Biochemical parameters of black soldier fly reared on different organic substrates

Treatment	Crude Protein (%)	Crude Fat (%)	Moisture (%)	Ash (%)
T_1- Poultry waste	33.25^d	17.66^f	67.04^b	12.96^b
T_2- Kitchen waste	36.14^c	36.05^b	58.53^e	10.57^c
T_3- Fish waste	39.20^a	39.32^a	64.58^c	13.84^a
T_4- Poultry feed	35.09^c	28.80^d	58.51^e	9.25^d
T_5- Fruit waste	28.00^e	22.71^e	69.15^a	10.24^c
T_6- Combine waste	38.06^b	31.95^c	60.20^d	12.69^b
SEm (±)	0.42	0.23	0.39	0.26
CD (P=0.05)	1.29	0.70	1.20	0.80
CV %	2.07	1.33	1.07	3.87

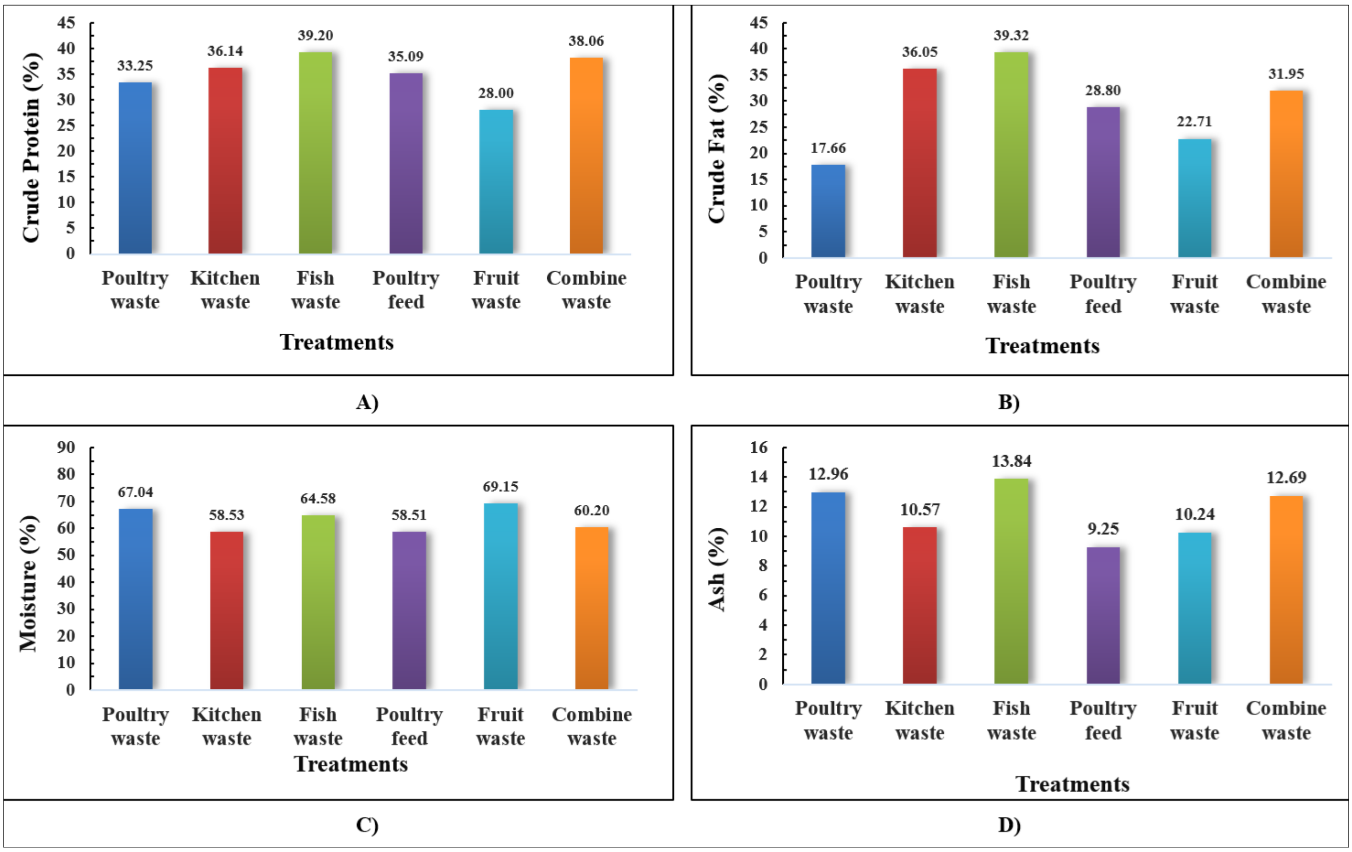

Fig 1: Biochemical parameters of black soldier fly reared on different organic substrates

Conclusion

The kitchen waste was nutritionally superior with the good levels of crude protein and fat enhancing larval performance, whereas fish waste also showed high protein and fat but increased ash and moisture led to limitations in its use as a feed ingredient. Overall, kitchen waste and poultry feed emerged as the most effective organic substrates for maximizing larval and pupal growth and nutrient composition of Black soldier fly.

Author Contributions

Shashianand U Kalbhor: Conducted research trial, Observations, Data collection, Collect reviews, Data analysis, Interpretation of results, Draft manuscript preparation, study conception and design.

Atul D Gonde: Critically reviewed the manuscript and served as scientific advisor.

Rajkumar V Bajolge: Reviewed manuscript and served as scientific advisor.

Sumedha J Shejuipatil: Reviewed manuscript

Sharad K Dalve: Reviewed manuscript
All authors reviewed the results and approved the final version of the manuscript

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