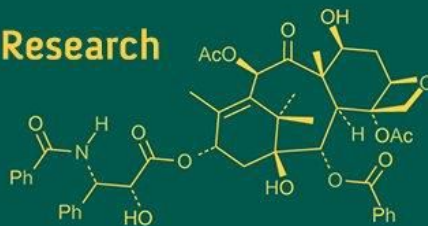


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## Nutritive pre-eminence and parsimony of Black Soldier Fly larval excreta over dried larval meal as feed supplement in Cattle and Buffalo ration

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### Abstract

Given that both human and animal population keep growing year on year leading to escalation of feed costs, animal nutritionists across academia and industry are in constant exploration of alternate feed protein sources to make livestock enterprise more productive and environmentally friendly. In this quest, insects have emerged as a more attractive alternates of traditional animal feed ingredients, especially for their protein nutritive value. This study was taken up to assess the nutritive value of black soldier fly larvae (BSFL) and its frass (BSFF). We reared black soldier fly following field established standards and elucidated the proximate composition, amino acid and fatty acid profile of BSFL meal and BSFF portions. Our results showed that protein nutritive value of BSFL meal and BSFF portion is on par with traditional animal feeds such as soya bean meal, fish meal and corn meal. Furthermore, the CP%, the fat% and the carbohydrate% of the BSFL are 36%, 37% and 11%, respectively, on dry matter (DM) basis. Same nutrients in BSFF clocked at 56%, 13% and 14% respectively, which suggest that frass may have better nutritive quality than BSFL. Furthermore, levels of almost all amino acids including the essential ones were found to be higher in BSFF portion when compared to BSFL meal whereas fatty acid profile showed lower levels of fatty acids in BSFF portion than BSFL meal. In conclusion, BSFF portion may offer a higher nutritive value at a highly economical price when compared to BSFL meal and its inclusion along with BSFL meal can significantly decrease the production cost in dairy sector.

**Keywords:** Black Soldier Fly larvae (BSFL), Black soldier fly larval Frass BSFF, crude protein (CP), dry matter (DM), dairy sector

### Introduction

Global human population has reached more than 8 billion, which has been projected to rise to almost 10 billion by 2050. According to a FAO report (2020), more than 92 billion land animals are raised and slaughtered every year in the global food system. With ever growing human population, the latter are to grow only more in numbers, which would put a lot of strain on animal feed supply chains. Besides, some of the feed ingredients used in animal feed directly competes with the human consumption adding more demand for them with apotential to drive their prices high. Given that feed is a major cost factor in any animal enterprise, the overall production cost of food animals is likely to soar. Additionally, limitations in the availability of food ingredients & lower nutritional value of vegetable protein for both human and animal consumption, compounds the issue further. Moreover, in some countries, usage of animal protein in food animal & aquaculture feeds is prohibited for ethical and philosophical reasons. Hence, academia and industry are always on a constant lookout for alternative protein feed sources that can supply cheap and nutritious protein to livestock, and of course, one that would not trigger ethical or philosophical concerns.

Insects are proteinaceous and have high feed conversion efficiencies and growth rates which make them an attractive source of animal feed <sup>[1, 2]</sup>. Among insects, Black soldier fly (BSF; *Hermetia illucens*) stood out as a choice of nutrient material, because it is not a pest and does not require precautionary measures for its rearing. BSF have high rate of oviposition, better survival rates of immature, and short generation interval, all leading to a high volume larvae production in a very short time <sup>[3]</sup>. Black soldier fly larval (BSFL) meal offers a sustainable protein for poultry <sup>[4]</sup>, pigs <sup>[5, 6]</sup>, pets and fish <sup>[7]</sup>. BSFL are available as live larvea, frozen,

or processed into a meal, for feeding the animals. Past studies indicate that BSFL may at least, partially replace traditional protein sources viz. soybean meal, fish meal, ground nut meal that supply high protein and essential micronutrients to the animals<sup>[8]</sup>.

Frass, which is a mixture of BSF excreta, moulted skins, undigested food, and other residual rearing substances, can be converted into nutrient-rich organic fertilizer that can boost soil fertility, promote plant growth, and help plants resist stress. Frass has high nutritive value for protein and be used as animal feed<sup>[9]</sup>. In most cases, the amount of frass can weigh more than the biomass of the fly itself, and converting frass into high-valued biomass for animal feeding would be a viable approach to reduce the animal feed costs.

Ruminants represent one of the major group of food animals. Of late, this sector has been under constant scrutiny for their high methane emission and its impact on climate change. Hence, a greater push has been going on to find alternate feed sources and feed supplements that may reduce methane emission by cattle industry. Cattle diets may get more economic upon inclusion of feed ingredients that have high incidental protein & low fats, and do not adversely affect digestibility, ruminal fermentation, supporting the feasibility as a feed ingredient. However, there are still critical research gaps including the need to assess efficiency of micronutrient and macronutrient extraction by the cattle as well as the gut microbes present in the rumen. Therefore, rearing and including BSF in livestock feeding enterprise may get rid of environmental detriments of food waste & ever increasing demand for cheap and nutritive protein, thereby creating a circular economy.

The present study was taken up to compare the nutritive value of BSFL meal and BSFL frass (BSFF) and its suitability as feed supplement.

## Materials and Methods

**Black soldier fly Larvae rearing:** Adult BSF are raised outdoors in a metal framed cage with screen mesh ( $1.8 \times 1.8 \times 1.8$  m with 1.5 mm mesh). The insects were provided with access to daylight and temperatures were maintained at  $30 \pm 5$  °C to encourage mating in BSF. The flies were supplied with ad libitum water to prolong their life. Corrugated cardboard were placed within the cage to attract adult females for laying their eggs.

**Substrate preparation for larvae feeding:** Kitchen waste containing a mixture of vegetable peels, fruit waste, rice and bread debris were supplied to the insects. Composition of kitchen waste was chosen purely based on their availability in the neighbourhood, considering for large scale future production of the larvae. Metallic trays were used to hold 1 kg of substrate. Five hundred neonatal BSFL were placed on the top of the substrate in each of the trays. The temperature was maintained at  $30 \pm 5$  °C and relative humidity at  $65 \pm 5\%$ . Edible water was sprinkled on the substrate to ensure 65-70% moisture content at all times. At prepupal stage, insect larvae were harvested, washed in tap water, dried in oven set at 60 °C for a duration of 48 hours. Dried worms were crushed using a laboratory blender and stored for later analyses in a refrigerator set to hold temperature at -20 °C. A pooled sample of 100 g of each of BSFL and BSFF was used for subsequent analyses.

**Proximate Analyses:** Proximate analysis of BSFL samples was conducted by employing standard methods of the Association of Official Analytical Chemist<sup>[10]</sup>. Dry matter was calculated by the weight differential of the sample before and after drying in an hot air oven set at 103°C for 6-8 hrs. Ash was determined by heating samples in a muffle furnace set at 600 °C for 2 hrs. The crude protein was determined using the Kjeldahl method as reported earlier<sup>[11]</sup>.

**Amino acids analyses:** Protein extraction was carried out following previously reported method<sup>[12, 13]</sup>. Briefly, insect samples were snap-frozen in liquid N<sub>2</sub> and crushed into fine powder using a mortar and pestle. Samples of ~2 g each were extracted for 1 hour in a beaker containing ice cold HEPES (pH 7.2), 2 mM dithiothreitol (DTT), 2.5% Polyvinylpyrrolidone (PVP), 0.5 mM EDTA, 1 mM benzamidine and 0.1 mM phenylmethanesulfonylfluoride (PMSF), with a magnetic stirrer. Solid debris was removed from the samples by filtering and centrifuged at 8000 rpm for 30 min at 4 °C.

Protein was precipitated between 45% and 80% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the pellet recovered by centrifugation at 21,000 rpm for 30 min at 4 °C. Protein pellets were then desalted in 20 mM HEPES-NaOH at pH 8 containing 2 mM DTT by utilizing a Sephadex G-25 gel filtration chromatography (PD-10 columns, GE Healthcare). 10 mg samples were transferred into a micro- reaction vial and 2ml of 6N HCl was added and capped after careful introduction of nitrogen gas. The samples were then hydrolyzed for 24 h at 110 °C. Tryptophan analysis was conducted separately using similarly processed samples.

After the hydrolysis, the mixtures were dried under vacuum, and the hydrolysates were reconstituted in 1 ml of solute made of water: acetonitrile (90:10) combination. Then the samples were briefly vortexed and sonicated for 30 min. Then the samples were centrifuged at 14,000 rpm, supernatant was then collected and analysed using, waters Acquity UPLC.

**Mineral analyses:** The proximate mineral composition of BSF larval meal and BSF frass portion was evaluated following the methods of the Association of Official Analytical Chemists (2002)<sup>[10]</sup>. Insect samples were snap-frozen in liquid N<sub>2</sub> and crushed into fine powder using a mortar and pestle. Then the samples were converted into ash and digested in 6N HCl and the content of the various minerals were analyzed on an Atomic Absorption Spectrophotometer (ECIL-AAS4141A).

**Fatty acid analyses:** Fatty acid profile was estimated by using a previously reported protocol<sup>[14]</sup>. First, total lipids were extracted from both the BSFL as well as BSFF using 100 mg of insect powder following the method described earlier, using chloroform/methanol (1:1), and the total lipid content was determined gravimetrically after the evaporation of solvent by a Laborota 4000 efficient rotavapor (Heidolph, Schwabach, Germany). Approximately 5 mg of sample was used for analyzing each lipid class. Methylation and analysis by gas chromatography with flame ionisation detection (GC/FID) were carried out as reported elsewhere<sup>[15]</sup>.

## Results

**BSFL & BSFF differed in proximate composition:** As reported in Table 1, crude protein (CP) and carbohydrate levels clocked in at 56% and 14% in BSFF compared to that of BSFL (36% and 11%), on a dry matter basis, respectively. However, a remarkably higher lipid levels were noted in BSFL (37%) as compared to that of BSFF (13%). These data indicate that frass, which comprised largely of the excrement, left over substrate and molted skin of the larvae has very high protein content compared to BSFL. However, the levels of CP seen with BSFL fraction are still comparable to that usually seen with other feed ingredients viz. Soya bean meal and fish meal [16, 17].

**Amino acid profile in BSFL and BSFF:** Except histidine, quantity of all the other amino acids were higher in BSFF as compared to that recorded in BSFL samples (Table 2). Frequently, values for amino acids in BSFF is nearly double than that seen in BSFL samples.

**Fatty acid profile in BSFL and BSFF:** As shown in Table 3, all fatty acids except for linoleic acid, clocked in conspicuously higher values, in BSFL as compared to that of BSFF. The higher the % of fatty acid in larvae, so is the value for that fatty acid higher in the frass, which is suggestive that excreta levels of fatty acids closely reflect their abundance in the larvae. Particularly, Lauric acid, Palmitic acid and Oleic acid levels are almost 3 fold higher in larval extracts when compared to that of Frass.

**Mineral composition of BSFL and BSFF:** As shown in Table 4, both macrominerals; Calcium, Magnesium, sodium, Potassium, Phosphorus and microminerals; Zinc, Copper, Iron, Manganese and Selenium, were present in measurable quantities. Values for most minerals were higher in BSFF than seen in BSFL. Except for  $\text{Ca}^{2+}$ , levels of all other minerals are higher in BSFF, and similar trend was seen with microminerals.

## Discussion

While the use of insect species for meeting the protein and nutrient requirement of animals has been exploited and practised for a long time, these alternate protein sources however have not yet come into fruition on a large scale, with tangible economic benefits, at the commercial scale. Overfishing and scarcity of land and water resources for growing traditional feed stuff has led to price escalation of protein rich feed ingredients [17]. Further, the nutritive value of BSF Frass vs. larvae has not been assessed in our market settings. Our findings in this paper would fill this knowledge gap. Our results suggest that frass is relatively high in calories and has a high nutritive value for CP, carbohydrates and minerals, while BSFL meal has a high nutritive value for fat when compared to BSFF.

A significant finding of this study has been the presence of higher protein (CP) and lower fat levels in BSFF, which were 56% and 13%, respectively (Table 1). Firstly, the CP content in BSFF is even higher than that found out in the BSFL meal (36%), suggesting frass has a higher protein content than the larval meal itself. Secondly, 56% CP in BSFF is at the higher end of CP values reported in the literature for BSFL meal (47.9%), Soyabean meal (46%), BSF prepupae meal (53.5%) and fish meal (56.7%) [17, 18, 19], suggesting that frass inclusion in these diets may enhance

the nutritive value of animal diets. Thirdly, presence of higher fat levels in BSFL meal has been suggested to be a limiting factor in its inclusion as an exclusive source of protein in animal diets, which had been primarily attributed to presence of high fat levels in BSFL meal (20 - 36%) (19). Finally, in addition to higher protein level in frass (56%), fat% was significantly lower (13%), and both these factors may support BSFF's better performance when included in animal diets.

A second remarkable finding of this study, but related to the first one, is that frass is highly proteinaceous and low in fat (Table 1), which may offer a right combination of nutrients that is suitable for significant replacement of traditional feed ingredients in animal diets. Furthermore, calorogenic value of BSFL meal is prominently higher than that of frass, which would be explained by the presence of higher fat levels in BSFF. Traditionally, fish meal, soya bean meal, meat meal and blood meal have been the choice of protein sources for use in animal diets, because of their nutrient value and commercial feasibility. However, limitations exist in procuring these ingredients due to their shortage, demand and cost factors. Sparophagous insects have the potential to help ease the demand for the traditional feed sources. Past studies reveal that the protein levels in BSFL meal ranges from 39% - 63% of DM [16]. The CP value and fat value in BSFL meal in our study is 36% and 37%, respectively, suggesting that the BSFL meal prepared in our study is on par with that reported earlier. Inclusion of BSFL meal in poultry and aquaculture diets showed that BSFL meal could replace traditional feedstuffs only partially. However, replacement by BSFL meal at a higher level or a total replacement resulted in reduced animal performance, which was ascribed to presence of higher fat (7 - 39% of DM) and ash (9 - 28% DM) in BSFL meal.

A third important finding of this study was that frass has higher level of carbohydrates (14%) compared to that noted in BSFL (11%) (Table 1). While these differences may appear marginal, it is relevant because removing carbohydrates from animal diets consistently showed to negatively affect growth and inclusion of carbohydrates reversed the growth deficits, which has been attributed to protein sparing affect of carbohydrates.

A fourth marked finding of our study was that, except for Histidine, a consistently higher levels of all amino acids were found in BSFF than in BSFL meal, often the differences are more than 2 fold (Table 2). For instance, levels of important amino acids in BSFL meal; lysine (1.9%), leucine (2.3%), valine (1.9%), methionine (0.7%), threonine (1.3%), arginine (1.7%) are comparable to those reported earlier in BSFL, soya bean meal, sunflower meal (20, 21). However, levels for these amino acids were ~ 2 fold higher in BSFF; lysine (3.8%), leucine (4.2%), valine (3.7%), methionine (1.2%), threonine (1.3%) and arginine (3%), than recorded in BSFL meal. Further, this trend continued even with non-essential amino acids and that BSFL and BSFF supplementation could be sufficient in most animal diets.

A fifth notable result of this study was that levels of macromineral and microminerals recorded were all comparable to the values reported in past studies for BSFL meal [22, 23]. Furthermore the mineral composition is very similar to that seen with fish meal for most minerals. Additionally, mineral levels in BSFF are even higher than

BSFL supporting its superiority over larval meal as feed supplement in terms of mineral nutrition.

Finally, our results showed that in addition to having higher fat% in BSFL meal, (Table 3), levels of Lauric acid, palmitic acid and linolenic acid are relatively higher than the levels of other fatty acids measured. Besides, the levels of these fatty acids are comparable to that reported in the literature (20). While lesser percent of fatty acids were recorded in BSFF when compared to BSFL, Lauric acid, palmitic acid and linolenic acids clocked in almost 3 fold lower values in BSFF. Collectively, these results suggest that the levels of fatty acids recorded closely reflect the total fat content measured in BSFF and BSFL.

In conclusion, black soldier fly signify a promising species for use as alternative feed source in animal diets, although the proportions (%) at which they can be included in animal diets and the stage of insect to use, has not been completely determined. However, our comparison of BSFL and BSFF, revealed comparative nutritive value of BSF larvae and BSF

frass and showed that BSFF has higher nutritive value with lower cost of production. Future studies will determine the performance of BSFL and BSFF inclusive diets on animal production. Further, trends seen in our data suggest that growth substrate used in rearing BSFL meal may be crucial to determining larval meal quality and frass nutritive value. An important caveat to our study was that we have not separated pupal and prepupal larval stages, and hence the nutrient analysis is reflective of meal made of both larval stages.

**Table 1:** Proximate Analyses of BSF Larvae & BSF Larval Frass

| S. No. | Proximate Analysis | Black Soldier Fly Larval meal (BSFL) | Black Soldier Fly larval Frass (BSFF) |
|--------|--------------------|--------------------------------------|---------------------------------------|
| 1      | Moisture (%)       | 8                                    | 8                                     |
| 2      | Crude Protein (%)  | 36                                   | 56                                    |
| 3      | Fat (%)            | 37                                   | 13                                    |
| 4      | Carbohydrates (%)  | 11                                   | 14                                    |
| 5      | Ash (%)            | 8                                    | 9                                     |
| 6      | Energy (Cal/100g)  | 493                                  | 400                                   |

**Table 2:** Amino acid profile of BSF Larval meal & BSF Larval Frass

| S. No. | Amino Acids        | Black Soldier Fly Larval meal (BSFL) | Black Soldier Fly larval Frass (BSFF) |
|--------|--------------------|--------------------------------------|---------------------------------------|
| 1      | Aspartic acid (%)  | 2.8                                  | 5.9                                   |
| 2      | Threonine (%)      | 1.3                                  | 2.4                                   |
| 3      | Serine (%)         | 1.5                                  | 2.4                                   |
| 4      | Glutamic acid (%)  | 3.8                                  | 6.4                                   |
| 5      | Proline (%)        | 2.2                                  | 3.4                                   |
| 6      | Glycine (%)        | 1.8                                  | 3.5                                   |
| 7      | Alanine (%)        | 2.3                                  | 3.7                                   |
| 8      | Cystine (%)        | 0.3                                  | 0.8                                   |
| 9      | Valine (%)         | 1.9                                  | 3.7                                   |
| 10     | Methionine (%)     | 0.7                                  | 1.2                                   |
| 11     | Isoleucine (%)     | 1.4                                  | 2.2                                   |
| 12     | Leucine (%)        | 2.3                                  | 4.2                                   |
| 13     | Tyrosine (%)       | 2.0                                  | 3.8                                   |
| 14     | Phenyl alanine (%) | 1.3                                  | 2.3                                   |
| 15     | Histidine (%)      | 2.9                                  | 1.9                                   |
| 16     | Lysine (%)         | 1.9                                  | 3.8                                   |
| 17     | Arginine (%)       | 1.7                                  | 3.0                                   |
| 18     | Tryptophan (%)     | 0.3                                  | 0.9                                   |

**Table 3:** Fatty acid profile of BSF Larvae & BSF Larval Frass

| S.No. | Fatty Acids                         | Black Soldier Fly Larval meal (BSFL) | Black Soldier Fly larval Frass (BSFF) |
|-------|-------------------------------------|--------------------------------------|---------------------------------------|
| 1     | Capric acid (C10:0) (%)             | 0.3                                  | 0.1                                   |
| 2     | Lauric acid (C12:0) (%)             | 15.3                                 | 5.0                                   |
| 3     | Myristic acid (C14:0) (%)           | 2.4                                  | 0.9                                   |
| 4     | Palmitic acid (C16:0) (%)           | 4.3                                  | 1.2                                   |
| 5     | Palmitoleic acid (C16:1) (%)        | 1.7                                  | 0.2                                   |
| 6     | Stearic acid (C18:0) (%)            | 0.5                                  | 0.3                                   |
| 7     | Oleic acid (C18: 1n-9 Cis) (%)      | 4.5                                  | 1.4                                   |
| 8     | Linoleic acid (C18: 2n-6 Cis) (%)   | 1.4                                  | 1.4                                   |
| 9     | Gama linolenic acid (C18: 3n-6) (%) | 0                                    | 0.2                                   |

**Table 4:** Mineral Acid profile of BSF Larval meal & BSF Larval Frass

| S. No. | Minerals         | Black Soldier Fly Larval meal (BSFL) | Black Soldier Fly larval Frass (BSFF) |
|--------|------------------|--------------------------------------|---------------------------------------|
| 1      | Calcium (ppm)    | 29933                                | 27077                                 |
| 2      | Zinc (ppm)       | 71                                   | 143                                   |
| 3      | Copper (ppm)     | 8                                    | 15                                    |
| 4      | Iron (ppm)       | 60                                   | 277                                   |
| 5      | Magnesium (ppm)  | 2960                                 | 4171                                  |
| 6      | Manganese (ppm)  | 87                                   | 349                                   |
| 7      | Phosphorus (ppm) | 7300                                 | 8812                                  |
| 8      | Sodium (ppm)     | 787                                  | 1245                                  |
| 9      | Potassium (ppm)  | 8467                                 | 14508                                 |
| 10     | Selenium (ppm)   | 0                                    | 1                                     |
| 11     | Molybdenum (ppm) | 0                                    | 1                                     |

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