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Original Research Article

Evaluation of Proximate Composition of Varying Black Soldier Fly (BSF) Larvae as an Alternative Protein Source

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ABSTRACT
The high cost of conventional protein sources, such as soybean and fish meal, has driven the search for alternative protein sources in fish feed. This study evaluates the proximate composition of least-cost feed incorporating black soldier fly (BSF) larvae as an alternative protein source. A total of 4 kg of BSF pupae were obtained from a farm in Benin and reared in controlled conditions for 30 days. Eggs were collected from attractant traps using pineapple, vegetable, and poultry waste, then incubated until they developed into larvae. Proximate analysis revealed that BSF larvae contain 45% crude protein, making them a high-quality protein source, while adult BSF contain 24% crude protein. Moisture content was higher in adults (15%) compared to larvae (7%). Ash content was similar, with adults at 10.5% and larvae at 9.8%. Ether extract (fat content) was higher in adult BSF (21.7%) than in larvae (16.4%), indicating differences in nutritional value at different life stages. These findings suggest BSF larvae as a viable, cost-effective alternative protein source for fish feed.

INTRODUCTION

Livestock production is a key sector in Nigeria's agriculture, contributing significantly to the economy and food security (Oni *et al*., 2017; Akpomughe *et al*., 2023; Awhefeada *et al*., 2022). Being the largest livestock population in Africa over 200 million cattle, sheep, goats, and pigs (FAO, 2021). This sector provides employment, maintains soil fertility, and supports industries such as leather and pharmaceuticals (Adegbola *et al*., 2019).

Despite its importance, the industry faces challenges, particularly high feed costs, which constitute 60–70% of total production expenses (Adegbola *et al*., 2019; Awhefeada *et al*., 2022). Researchers have explored alternative feed ingredients, including unconventional protein sources like insects and microbial proteins, to replace costly components such as soybean and fishmeal (Oluwatosin *et al*., 2020; Tohidi et al., 2020). Additionally, feed formulation models (González-Vega *et al*., 2019; Liu et al., 2021) and processing techniques like pelleting and extrusion enhance nutrient availability and reduce costs (Onwuka *et al*., 2019; Pfeiffer *et al*, 2020). Agricultural by-products such as cassava peels and palm kernel cake have also been evaluated as potential feed substitutes (Adejumo *et al*., 2020; Makinde *et al*., 2020).

A breakthrough in sustainable feed production is the use of black soldier fly (BSF) larvae (Hermetia illucens), which contain up to 63% crude protein and provide a cost-effective alternative protein source (Diener *et al*., 2009; Eze *et al*., 2020). Studies show that BSF larvae meal enhances growth performance in livestock, with optimal inclusion levels of 15% in rainbow trout and 10% in broiler chickens (Ebrahimi *et al*., 2021; Sun *et al*., 2020). Its inclusion in

layer diets has also been linked to improved egg production (Al-Qazzaz *et al*., 2016).
However, research on optimal inclusion levels and proximate composition of BSF-based feed remains limited. This study aims to determine the proximate composition of least-cost feed formulations incorporating BSF larvae as a primary protein source.

MATERIALS AND METHODS

The study took place at the demonstration farm of the Department of Fisheries and Aquaculture, Delta State University, Abraka. A total of 4 kg of Black Soldier Fly (BSF) pupae were obtained from a farm in Benin and kept in four 1 x 1 x 1 m cages for 30 days.

To rear BSF larvae, attractant traps containing pineapple, vegetable, and poultry waste were set in the cages to encourage egg-laying. The traps were covered with nets to prevent direct contact with the bait. The harvested eggs were incubated until they hatched into larvae, which were sorted using a 1.2 mm net and assigned to different rearing substrates.
Larvae were fed a specific regimen of 2 g of substrate per gram of larvae, with feeding occurring every two days. After 18 days, they developed into pupae and were reared for another 14 days until they became adults. The adults were fed for nine days before sex sorting. The study adhered to European Commission (EC) Regulation No. 1069/09 by avoiding animal products in the feeding substrates.

Figure 1: Harvested Black Soldier Fly

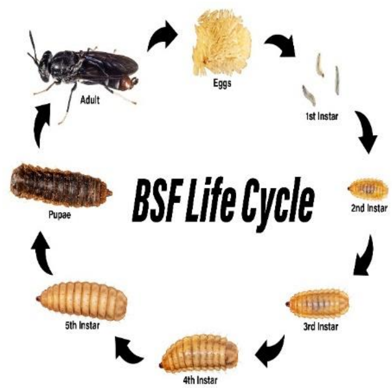

Figure 2: Life Cycle BSF

Figure 3: BSF Under Culture in 1m x1mx 1m Cage
Source: Field work 2024

Proximate Analysis

A proximate analysis of the BSF larvae was carried out. The proximate analyses in this study encompassed various analytical determinations, including moisture content, crude lipid content and crude fibre content, following the procedures outlined below.

Moisture Content Determination

2g of the sample was weighed out into the crucible with a known weight. The crucible was then dried overnight at temperature of 105^{0}C in an oven. This was removed and cooled in a desiccator and then reweighed. The following equation was used to calculate the moisture content

$$\% \, Mosture \, Content = \frac{Weight \, of \, Sample \, before \, Drying - Weight \, of \, Sample \, after \, drying}{Weight \, of \, Sample \, before \, drying} \times \frac{100}{1} \quad (1)$$

Ash Content Determination

2 grams of the sample was weighed with a known weight crucible and was placed in temperature-controlled furnace at 600^{0}C for about 1 -2 hours for proper ashing. The crucible contains the ashed sample was then cooled in a desiccator and immediately weighed. The percentage ash was then calculated using the following equation:

$$\% \, ash \, Content = \frac{(Weight \, of \, Crucible + ash) - Weight \, of \, Crucible \, alone}{Original \, Weight \, of \, Sample \, taken} \times \frac{100}{1} \quad (2)$$

Crude Fat Determination

The crude fat of the sample was measured using the Soxhlet apparatus. Some weight of the sample was weighed in a 250ml flask. The flask was filled with petroleum ether to three quarter full and sample into the thimble. The fat process was carried out for about 6 hours at temperature of 80^{0}C and the fat was collected into the flask. The solvent was expended by oven drying and then cooled in a desiccator and reweighed. The crude fat content was then calculated using the equation:

$$\% \, Crude \, fat \, Content = \frac{(Weight \, of \, flask + fat) - Weight \, of \, flask \, only}{Weight \, of \, Sample} \times \frac{100}{1} \quad (3)$$

Determination of Crude Fibre

1 gram of each sample was weighed into 1000ml conical flask and 100ml of crude fibre reagent (TCA digestion agents) was used. The mixture was boiled and allowed to reflux for about 40 minutes. It was then filter through Whatman filter paper of 15cm. The residue was rinsed with hot water and acetone. It was then transferred into a porcelain dish and then oven dried overnight at 105^{0}C. The sample was cooled in the desiccator and weighed as W1. It was then burnt at 500^{0}C in a muffle furnace for 1 hour, allowed to cool, then reweighed as W2. The percentage crude fibre was then calculated thus:

$$\% \, Crude \, fibre = \frac{W1 - W2}{Weight \, of \, Sample} \times \frac{100}{1} \quad (4)$$

Determination of Crude Protein

The sample was digested by adding 1 gram of copper sulphate, 2.5 gram of Potassium sulphate, and pinch of selenium powder. 20ml – 25ml of concentrated sulphuric acid was added to the mixture and digested for 40 – 50 minutes until the colour changes from black to light green. The digested solution was then cooled and transferred into 100ml volumetric flask which was made to mark with distilled water.

Micro kjeldahl distillation apparatus was used to distill 50ml of the prepared digest by adding 10m of 40% sodium hydroxide and 10ml of digest. The colour changes from green to dark brown. The released ammonia was condensed and collected into a receiver containing 10ml of boric acid with indicator solution.

The condensed ammonia was then back titrated with 0.1N HCL to pink colour end point.

$$\% \, Nitrogen \, by \, Weight = \frac{Titre \, value \times 14.007 \times volume \, made \times 100}{Atiquent \, taken \times weight \, of \, the \, sample} \quad (5)$$

Carbohydrates Content Determination

The carbohydrates content was determined by the difference in percentage:

Carbohydrate (%) = 100 - (Moisture + Protein + Fat + Ash + Crude Fibre)}

RESULTS AND DISCUSSION

The table and Figure are listed as follows Table 1 and Figure 1, to illustrates the proximate composition of Black Soldier Fly (BSF) at different life stages, specifically comparing the nutritional parameters between larvae and adults. BSF larvae have a significantly higher crude protein content compared to adult BSF. Nearly 45% of the larvae's composition is crude protein, making them an excellent source of protein. In contrast, adult BSF have about 24% crude protein. This dramatic difference suggests that larvae are particularly valuable for applications requiring high protein content, such as animal feed or human nutrition supplements.

Adult BSF have a higher moisture content than larvae, with about 15% compared to around 7% in larvae. This higher moisture level in adults could influence their preservation and storage, as higher moisture content typically requires more careful handling to prevent spoilage. The ash content, which indicates the total mineral content, is relatively similar between the two stages, with adults slightly higher at around 10.5% compared to 9.8% in larvae. This suggests that both larvae and adults provide a comparable mineral profile. Ether extract, which represents the fat content, is higher in adult BSF (approximately 21.7%) compared to larvae (around 16.4%). This increase in fat content in adults may make them more suitable for applications where higher energy content is desired, such as in high-energy animal feeds. Crude fiber is significantly higher in adult BSF, with a content of around 17%, whereas larvae have only about 2.5%. The higher fiber content in adults might affect their digestibility and use in certain feed formulations, potentially making larvae more suitable for easier digestion. Adult BSF also contain a much higher percentage of carbohydrates/nitrogen-free extract, at around 25.7%, compared to 4.4% in larvae. This substantial difference indicates that adults have a greater proportion of non-protein, non-fat components, which includes carbohydrates.

In general, BSF undergo significant compositional changes as they mature from larvae to adults. Larvae are highly protein-dense, making them valuable for protein-rich applications. Adults, on the other hand, have higher fat, fiber, and carbohydrate contents, which could be leveraged for different nutritional needs. These differences highlight the potential for BSF at different stages to be used in various industrial, agricultural, and nutritional contexts, depending on the specific nutrient requirements

Table 1: Proximate Composition of BSF adults and BSF larvae

Parameters (%)	Adult BSF	BSF Larvae
Crude Protein	24.318	44.976
Moisture Content	15.311	7.317
Ash Content	10.526	9.756
Ether extract	21.675	16.425
Crude fibre	2.475	17.164
Carbohydrates/Nitrogen free extract	25.695	4.362

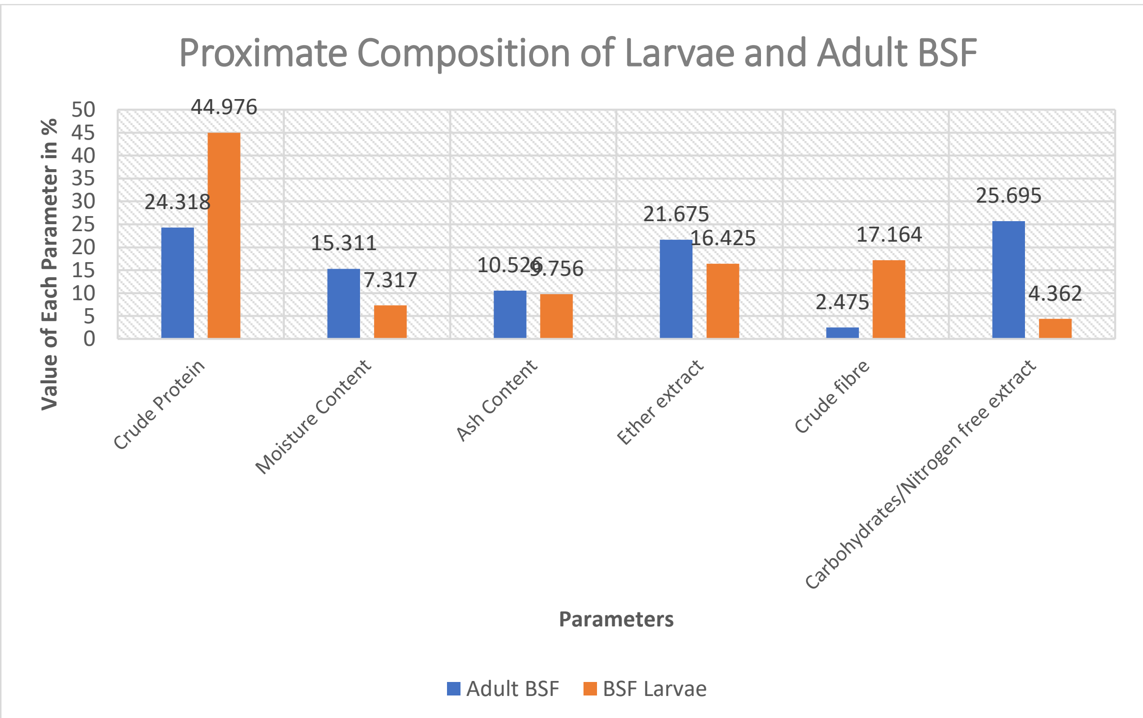

Figure 4: Bar Chart Showing the Proximate composition of insects and worms
Source: Field work 2024

In terms of moisture content, Adult BSF contains 15.311%, which is substantially higher than the 7.317% found in BSF Larvae. This indicates that BSF Larvae are comparatively drier, which could be advantageous for applications where lower moisture levels are desirable.

Ash content, however, does not differ significantly between the two groups, with Adult BSF containing 10.526% ash and BSF Larvae containing 9.756% ash. This similarity

Discussion

Crude Protein

Proximate Composition of Black Soldier Fly Larvae and Adults

This study found that Black Soldier Fly (BSF) larvae contain significantly higher crude protein levels than adult BSF, making them a superior protein source for animal feed. The crude protein content of BSF larvae consistently exceeds

40% on a dry matter basis, whereas adult BSF contain less than 30%. Barragán-Fonseca *et al*. (2017) reported BSF larvae crude protein levels between 40.8% and 55.6%, while adult BSF had only 24.4%. Similarly, Mertenat *et a*l. (2019) found larvae protein content ranging from 50.2% to 58.9%, with adult BSF t 26.8%. This difference is attributed to metabolic changes across life stages, as larvae accumulate protein and fat as energy reserves for pupation, while adults rely on stored energy for reproduction and flight without feeding on solid substrates. This reinforces the suitability of BSF larvae as an alternative protein source in animal diets, with a comparable amino acid profile and digestibility to conventional protein sources like soybean and fish meal.

Moisture Content

This study found that adult BSF have a moisture content of 15.31%, significantly higher than the 7.32% observed in BSF larvae. This variation is influenced by life stage and processing methods. Optimal BSF larvae rearing conditions maintain moisture content between 60% and 70%, with substrate moisture decreasing by 24–65% during larval development. Processing methods such as drying, freezing, oil extraction, and cooking impact BSF moisture content and nutritional quality. Cullere *et al*. (2019) reported fresh BSF larvae moisture at 61.8% and adult BSF at 72.5%, while Sogari et al. (2019) found fresh BSF larvae moisture at 63.2% and adult BSF at 75.4%. The higher moisture content in adults is likely due to their reliance on water and nectar for sustenance.

Ash Content

The ash content of BSF larvae (9.756%) and adult BSF (10.53%) does not differ significantly, indicating that their mineral composition remains stable across life stages. Literature suggests that ash content in BSF is influenced by factors such as substrate type, moisture content, processing methods, and storage time. Cullere *et al* (2019) and Sogari *et al*. (2019) reported similar ash content for BSF larvae (9.8–10.1%) and adults (10.2–10.4%). However, studies by Spranghers *et al*. (2017) and Biancarosa *et al* (2019) found a slight increase in ash content during metamorphosis, suggesting mineral accumulation in adult BSF. Variations in ash content may result from differences in experimental conditions, including diet and environmental factors.

Ether Extract (Fat Content)

This study revealed that adult BSF have a higher ether extract (21.675%) than BSF larvae (16.425%), suggesting lipid accumulation during metamorphosis. Cullere *et al*. (2019) and Sogari *et al*. (2019) reported ether extract percentages of 15.9–16.2% in larvae and 20.8–22.1% in adults. in adult BSF compared to larvae, indicating possible lipid mobilization for reproductive and metabolic activities. These discrepancies highlight the influence of factors such as diet, moisture content, and processing methods on BSF lipid composition.

Crude Fiber

This study found a significant difference in crude fiber content between BSF larvae and adults. Crude fiber, mainly composed of cellulose, hemicellulose, and lignin, plays a role in digestive health but can reduce nutrient digestibility if present in high amounts. The crude fiber content of BSF larvae ranges from 6.9% to 17.8%, while in adults, it is much lower (2.5% to 13.2%) due to their liquid-based diet. The study's findings align with those of Lu *et al*. (2022) and Rumpold *et al* (2013), who reported lower crude fiber in adults (2–2.5%) than in larvae (11–17.8%). However, Yusoff et al. (2022) found higher crude fiber in adults (13.2%) than in larvae (10.1%), likely due to differences in rearing conditions.

Carbohydrates/Nitrogen-Free Extract (NFE)

A notable difference was also observed in the carbohydrate (NFE) content between BSF larvae and adults. NFE, which includes sugars and starches, provides essential energy for animals. The NFE content of BSF larvae ranges from 5.1% to 16.9%, while in adults, it is lower (2.8% to 9.9%) due to their liquid diet. The study's results contrast with findings by Lu *et al* (2022) and Rumpold *et al* (2013), who reported lower NFE values in adults than in larvae. However, it aligns with Yusoff *et al* (2022), who found higher NFE in adults (25.7%) than in larvae (10.1%), suggesting that differences in rearing methods may influence NFE levels.

CONCLUSION

The findings indicate that BSF larvae have higher crude protein, crude fiber, and ash content than BSF adults, while BSF adults have higher moisture content, ether extract, and carbohydrates/NFE than BSF larvae. These differences suggest that BSF larvae and adults have distinct nutritional profiles and requirements, affecting their suitability and digestibility for different animal species. The proximate composition of BSF is influenced by factors such as substrate type, larval age, moisture content, and processing methods. Carbohydrate content in the feeding media and moisture levels impact their composition, while processing techniques like defatting or full-fatting affect ether extract and nitrogen-free extract (NFE) levels. Based on the results, the following recommendations are proposed: Future research should investigate the effects of different substrates, larval ages, moisture contents, and processing methods on the proximate composition of BSF larvae and adults, as these factors influence their nutritional value and quality for animal feed; future research should evaluate the digestibility, palatability, and performance of different animal species fed with BSF larvae and adults, as these parameters may vary depending on the proximate composition of the insect products; and future research should compare the environmental impacts and economic feasibility of using BSF larvae and adults as alternative protein sources for animal feed, as these aspects may affect their sustainability and scalability.

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