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Amino Acid Content in Black Soldier Fly (Hermetia illucens) Oil in the Larval, Prepupal and Pupal Phases as a Feed Additive in Broiler

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ABSTRACT: The global livestock sector is facing mounting pressure to adopt sustainable and efficient feed alternatives that can meet the growing demand for animal protein. Among the emerging options, Hermetia illucens (Black Soldier Fly, BSF) oil presents a promising candidate due to its bioactive compound richness and capacity to upcycle organic waste into nutrient-dense biomass. This study investigates the essential and non-essential amino acid composition of BSF oil extracted from larvae (8 days), prepupae (14 days), and pupae (28 days) stages and evaluates its potential as a feed additive in broiler production. High-Performance Liquid Chromatography (HPLC) revealed significant variations across developmental phases, with larval oil exhibiting the highest lysine (4,467.56 mg/100g) and glutamic acid (7,564.45 mg/100g), while pupal oil was enriched in leucine (5,670.76 mg/100g) and tryptophan (4,356.79 mg/100g). The dynamic amino acid profile supports the inclusion of BSF oil as a stage-specific supplement in poultry feed, offering both nutritional and economic advantages. Findings from this study contribute to the growing body of knowledge on insect-based feed ingredients and provide critical insight for formulating phase-targeted broiler diets with optimized functional performance. However, essential amino acids such as leucine, lysine, and tryptophan remained stable or even increased. Among all stages, larval oil exhibited the richest amino acid composition, suggesting its suitability for use in high-performance starter feeds for broilers.

KEYWORDS: Black Soldier Fly, oil, amino acid, feed additive, broiler.

INTRODUCTION

The intensification of poultry production, coupled with increasing global concern for environmental sustainability, has accelerated the search for alternative feed resources that are both nutritionally viable and ecologically sound. Traditionally, ingredients such as soybean meal and fishmeal have dominated broiler feed formulations due to their high protein content and favorable amino acid profiles. However, these sources are becoming increasingly unsustainable due to land-use pressure, deforestation, price volatility, and competition with human food systems (Barragan-Fonseca et al., 2021; Gadzama, 2025).

In this context, insects have emerged as a frontier solution, with the Black Soldier Fly (*Hermetia illucens*) gaining particular traction. Unlike plant-based alternatives, BSF can thrive on organic waste substrates, converting low-value biomass into high-protein and lipidrich material suitable for animal feed. While BSF larvae and meal have been well-documented for their protein content and positive effects on animal performance, less attention has been given to the nutritional potential of BSF oil, particularly regarding its amino acid composition—a critical determinant in the functionality of feed ingredients.

Amino acids are not only building blocks of proteins but also participate in various metabolic, immunological, and physiological functions in poultry. Essential amino acids (EAAs) like lysine, methionine, and threonine are often limiting in conventional diets, necessitating supplementation through synthetic or high-cost feed sources (Opoku et al., 2023). If BSF oil—widely regarded as a lipid component—also possesses functional concentrations of amino acids, its value as a dual-purpose feed additive dramatically increases. The present study aims to characterize the amino acid profile of BSF oil derived from three distinct developmental stages: larval, prepupal, and pupal. In addition, it explores the implications of each stage's profile for broiler nutrition, performance, and feed formulation strategies. This research not only fills a gap in insect oil utilization studies but also reinforces the potential of BSF as a strategic component in sustainable poultry production systems.

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MATERIAL AND METHODS

Insect Rearing

BSF were reared on a standardized mixture of fruit and vegetable waste (C/N ratio 25:1) under controlled conditions (28 ± 1 °C, 65% RH, 12L:12D photoperiod).

Oil Extraction

Samples were collected at larval (8 days), prepupal (14 days), and pupal (28 days) stages.

Black Soldier Fly Oil Extraction Method

Sample Preparation

1. Fresh maggots are soaked in hexane solution for 6 days.
2. After soaking, the samples are sun-dried for approximately 24 hours.
3. The dried maggots are ground into a fine powder.

Extraction Using the Soxhlet Method

1. The fat flask is dried in an oven at 105°C for 1 hour.
2. The fat flask is then cooled in a desiccator for 15 minutes and weighed.
3. Approximately ±5 grams of the powdered sample is weighed and wrapped in filter paper shaped into a thimble.
4. The extraction apparatus is assembled, consisting of a heating mantle, fat flask, Soxhlet extractor, and condenser.
5. The sample is placed in the Soxhlet and hexane is added to complete one cycle. Each cycle requires 150 grams of BSF larval meal and 1.5 liters of hexane solvent.
6. Extraction is carried out for approximately 4–6 hours until the solvent returns through the siphon into the fat flask, appearing clear.
7. The extract in the fat flask is separated from the hexane and oil using a rotary evaporator (50 rpm, 69°C).
8. The extracted oil is then heated in an oven at 105°C for 1 hour to remove residual solvent.
9. The fat flask is cooled in a desiccator for 15 minutes and then weighed.
10. The extracted oil is transferred into a modified aluminum pan equipped with an outlet for vapor.
11. A connecting hose is installed between the aluminum pan and a measuring glass.
12. A stove is ignited at a temperature of 1350–1500°C to generate vapor.
13. The process continues until the vapor condenses and oil is collected.
14. From 500 grams of BSF sample, approximately 50 ml of oil is produced.

Amino Acid Analysis

Amino acids were hydrolyzed using 6N HCl (110 °C, 24 h) under nitrogen, then derivatized with PITC. Samples were analyzed by HPLC with UV detection at 254 nm. Standard curves were generated using Sigma-Aldrich amino acid standards.

Statistical Analysis

Data analysis was conducted using a descriptive method.

RESULTS AND DISCUSSION Essential Amino Acids

Table 1. Essential Amino Acid Analysis Data

Essential Amino Acid	Larvae (8 days)	Prepupa (14 days)	Pupa (28 days)
Histidine	1.554,23	1.234,56	1.050,21
Isoleucine	3.456,76	2.987,28	3.210,86
Leucine	5.021,31	4.650,98	5.670,76
Lysine	4.467,56	4.210,45	4.532,18

Methionine	817,56	789,23	819,33
Phenylalanine	2.502,34	1.897,56	1.567,78
Threonine	3.210,45	3,010,32	2.810,16
Tryptophan	4.211,22	3.876,55	4.356,79
Valine	4.500,22	3.876,54	4.210,56

Source: Data obtained from Laboratory testing by Saraswanti.

Amino acid profiling across developmental stages of *Hermetia illucens* oil reveals significant nutritional potential, particularly in essential amino acids (EAAs) critical for broiler growth. In the larval stage, lysine and threonine concentrations peaked at 4,467.56 mg/100g and 3,210.45 mg/100g, respectively. Lysine is widely acknowledged as the first limiting amino acid in poultry diets, essential for muscle protein accretion and tissue repair (Opoku et al., 2023). Threonine contributes to immune response modulation and mucin production in the intestinal epithelium (Barragan-Fonseca et al., 2021).

The pupal phase was characterized by elevated levels of leucine (5,670.76 mg/100g) and tryptophan (4,356.79 mg/100g). Leucine, a branched-chain amino acid (BCAA), is central to mTOR pathway activation, stimulating protein synthesis and muscle hypertrophy (Lemme & Klüber, 2024). Tryptophan also functions as a serotonin precursor, influencing feed intake behavior and stress modulation (Huang et al., 2025).

Non Essential Amino Acids

Table 2. Non-Essential Amino Acid Analysis Data

Non Essential Amino Acids	Larvae (8 days)	Prepupa (14 days)	Pupa (28 days)
Alanine	4.213,44	3.910,21	3.100,17
Arginine	2.867,24	2.765,44	2.897,89
Aspartic Acid	6.765,83	5.876,55	5.976,41
Cysteine	160,22	87,65	85,22
Glutamic Acid	7.564,45	6.756,45	6.567,45
Glycine	4.211,67	4.310,22	4.210,22
Proline	3.554,12	3.224,56	3.445,27
Serine	3.245,33	3.564,34	3.210,33
Tyrosine	3.020,34	2.887,18	2.997,14

Source: Data obtained from Laboratory testing by Saraswanti.

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Glutamic acid was the most abundant across all stages, peaking at 7,564.45 mg/100g in the larval phase. Glutamate is essential for nitrogen metabolism, gut health, and enterocyte energy supply (Liu et al., 2019). Aspartic acid and alanine also supported energy metabolism and intermediary biosynthesis. Cysteine levels declined across development, reflecting its role in oxidative balance and chitin synthesis (Widiyastuti et al., 2024).

The profile illustrates that BSF oil contributes not only to energy supply through lipids but also to protein metabolic pathways via abundant NEAAs.

Functional Benefits in Broiler Diets

BSF oil offers dual value: it acts as a lipid source and a bioavailable amino acid contributor. Supplementation with BSF oil has improved feed conversion ratio (FCR), serum protein, and intestinal morphology in broilers (Azizah et al., 2024). Glutamate and threonine support epithelial barrier integrity, while leucine enhances muscle accretion in later growth phases (Maulana et al., 2025). Arginine and serine contribute to nitric oxide synthesis and antioxidative defense (Caligiani et al., 2018).

Sustainability and Economic Implications

BSF farming aligns with circular bioeconomy principles by converting organic waste into high-value feed ingredients (Gadzama, 2025). Unlike soy- or fish-based feeds, BSF production does not strain arable land or freshwater resources. Studies by Permana et al. (2024) and Kim et al. (2021) confirm that BSF oil can replace traditional lipid sources in poultry diets without adverse effects, potentially reducing dependency on imports and improving cost-efficiency in feed formulation.

CONCLUSION

BSF oil exhibits a dynamic amino acid profile that varies by developmental stage, with each phase offering unique nutritional advantages. Its high lysine, leucine, and glutamate content supports broiler growth, digestive function, and sustainability. These findings affirm the utility of BSF oil as a novel, eco-friendly additive in modern poultry nutrition.

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