Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Fungus Residue Amendment and Fermentation Enhance Black Soldier Fly Bioconversion of Fox and Raccoon Dog Manure into Quality Larval Meal and Frass Biofertilizer

Zi-Ru Li
Hebei Normal University of Science and Technology
Caili Kong
Hebei Normal University of Science and Technology
Zhixin Ji
Hebei Normal University of Science and Technology
Peng Sun
Hebei Normal University of Science and Technology
Jiahui Li
Hebei Normal University of Science and Technology
Guangxu Zheng
Hebei Normal University of Science and Technology
Chenyang Si
Hebei Normal University of Science and Technology
Yanying Zhang
Hebei Normal University of Science and Technology
Shuang Liu
Hebei Agriculture and Rural Affairs Department
Ju Wu
Zhaohe Agricultural Technology Co., Ltd
Chun-Liang Hou
Hebei Normal University of Science and Technology
Zhiwei Zhang
zhangzhiweia@163.com

Hebei Normal University of Science and Technology

Research Article

Keywords: Fox and raccoon dog manure, Fungus residue, Black soldier fly, Antibiotic residues, Frass biofertilizer, Waste valorization

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10647581/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Rearing black soldier fly (BSF) larvae on fox and raccoon dog manure (FRM) is an effective approach to both mitigate environmental pollution and produce high-quality feed and biofertilizer. However, FRM is rich in complex organic matter, has high ash content, an extremely high viscosity, and harbors pathogens, which lead to very low larval survival and pupation rates. To address these issues, this study introduced fungus residue (FR) amendment and fermentation. Compared with sole FRM (pupation rate 48.89%, survival rate 5.00%), the 1:1 FR : FRM mixture significantly improved pupation rate to 73.51% and survival rate to 61.35%, and fermentation of the 1:1 mixture further increased larval weight (0.0936 g) and survival rate (90.69%). BSF larval meal from the combined treatment contained 12.2% protein, 15.9% fat, and 31.95% total amino acids, with no detectable heavy metals. BSF frass had 68.7% organic matter and total NPK of 10.48%. When applied as a biofertilizer for Chinese cabbage, the 25% frass and 75% garden soil treatment gave the best results, with a germination rate of 98.33%, single-plant fresh weight of 515.11 g, and chlorophyll content of 2.52 mg/kg. In conclusion, FR-amended and fermented FRM is a suitable BSF rearing substrate, offering a sustainable solution for FRM management.

1. Introduction

The fur farming industry has experienced rapid growth globally(Wang et al. 2022), leading to the accumulation of large quantities of fox and raccoon dog manure (FRM)(Peng et al. 2019). FRM contains high levels of nutrients such as proteins, minerals, and organic matter, but improper disposal can cause soil and water pollution due to potential heavy metal accumulation and antibiotic residues from feed additives(Zalewska et al. 2021). Therefore, developing eco-friendly and efficient technologies for FRM valorization is crucial for sustainable fur industry development.

Black soldier fly (BSF, *Hermetia illucens* (Linnaeus, 1758)) larvae have emerged as efficient converters of organic wastes, including animal manure(Wang et al. 2021a), food waste(Ganesan et al. 2024), and agricultural by-products(Siddiqui et al. 2022a). BSF larvae can bioconvert organic wastes into high-value larval biomass (rich in protein and fat) for animal feed(Suryati et al. 2023), while the residual frass can be used as a biofertilizer(Siddiqui et al. 2022b; Lopes et al. 2024). However, the high electrical conductivity (EC)(Shen et al. 2026) and potential antibiotic residues of FRM may hinder its direct use as BSF substrate(van Dongen et al. 2024). Fungus residue (FR), a by-product of edible fungi cultivation, is rich in cellulose and hemicellulose, which can improve substrate structure as a bulking agent, while its beneficial microorganisms may promote nutrient release(Li et al. 2021; Yakti et al. 2023). Moreover, FR may help reduce antibiotic residues, as black soldier fly larvae have been shown to degrade various antibiotics during bioconversion(Liu et al. 2022; Wei et al. 2024).Thus, combining FR with FRM as a co-substrate may simultaneously alleviate the physicochemical constraints of FRM and mitigate its antibiotic risks, offering a promising strategy for FRM valorization.

Previous studies have investigated BSF rearing on various organic wastes, including co-conversion of different waste streams such as chicken manure with food waste or wet market waste(Jiang et al. 2024; Wang et al. 2025). Similarly, Kusumah et al. (2025) found that co-digesting oil palm empty fruit bunches with kernel meal substantially increased larval biomass(Kusumah et al. 2025).Beyond manure and agricultural waste, the co-digestion of mushroom root waste with soybean curd residues significantly improved larval survival and biomass production compared with pure mushroom root waste(Soomro et al. 2024).Mishra and Suthar (2025)

also demonstrated that bioconversion of spent mushroom substrate and fruit waste mixtures by BSFL yielded favorable larval growth and waste reduction, with the optimal mixture achieving 133.96% larval growth(Mishra and Suthar 2025).However, the co-conversion of FR and FRM—particularly the effects of FR inclusion rate and pre-fermentation—on larval growth has not been explored. Furthermore, the biofertilizer potential of frass derived from FR-FRM substrates remains to be systematically evaluated(Abd Manan et al. 2024).

This study aimed to: (1) characterize the basic nutritional and safety properties of FRM; (2) evaluate the effects of FR amendment ratio and fermentation on BSF growth and development; (3) analyze the nutrient composition of BSF larval meal and frass; (4) determine the optimal frass application rate for Chinese cabbage cultivation. The findings can provide a theoretical basis for the construction of a circular agriculture model of “fox and raccoon dog manure – black soldier fly bioconversion – biofertilizer – crop cultivation”.

2. Materials and Methods

2.1 Raw Materials

Fox and raccoon dog manure (FRM) was collected from a fur farm in Hebei Province, China. Fungus residue (FR) was obtained from a *Pleurotus ostreatus* cultivation base, dried at 60°C for 48 h, and crushed to pass through a 2 mm sieve. BSF eggs were provided by the Laboratory of Insect Resources Utilization, Hebei Agricultural University. Chinese cabbage *(Brassica rapa L. ssp. pekinensis)* seeds were purchased from a local seed company. Garden soil was collected from the experimental field of Hebei Agricultural University, with pH 6.78, organic matter 2.02%, available N 120.32 mg/kg, available P 51.86 mg/kg, and available K 72.26 mg/kg. Soil properties were analyzed by the following standard methods: pH by potentiometry, organic matter by potassium dichromate oxidation, available N by alkaline hydrolysis, available P by Olsen method, and available K by flame photometry.

2.2 Analysis of FRM Properties

FRM samples were air-dried, ground, and passed through a 0.25 mm sieve. Moisture content was determined by oven-drying at 105°C for 24 h. Crude protein, crude fat, crude fiber, and crude ash were analyzed using the Kjeldahl method, Soxhlet extraction method, acid-base digestion method, and muffle furnace combustion method, respectively**(**Jia et al. 2023). Total phosphorus (P), calcium (Ca), iron (Fe), magnesium (Mg), arsenic (As), Pb, and Cd were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x). pH was determined with a pH meter (soil : water = 1:5, w/v). Density was measured using the pycnometer method, and viscosity was determined with a rotational viscometer (NDJ-5S) at 25°C.

Table 1
Basic properties of fox and raccoon dog manure (FRM)

Determination Index	Test Result	Determination Index	Test Result
Moisture	76%	Magnesium (Mg)	14000 mg/kg
Crude Protein	29.30%	Total Arsenic (As)	0.88 mg/kg
Crude Fat	4.60%	Lead (Pb)	Not detected
Crude Fiber	7.60%	Cadmium (Cd)	Not detected
Crude Ash	41.60%	pH	7.8
Total Phosphorus (P)	2.80%	Density	1.35 g/ml
Calcium (Ca)	48000 mg/kg	Viscosity	73011 mPa·s
Iron (Fe)	4000 mg/kg	-	-

2.3 Black Soldier Fly Growth Performance Experiment

2.3.1 Substrate Formulation

Two sets of experiments were conducted: non-fermented and fermented substrates. For non-fermented substrates, three treatments were designed: NF-3 (FR:FRM = 1:1, w/w), NF-2 (sole FR), and NF-1 (sole FRM). For fermented substrates, three treatments were set: F-1 (FR:FRM = 1:1, fermented), F-2 (sole FR, fermented), and F-3 (sole fungus residue, fermented, as control). Fermentation was performed by adding 5% (w/w) *Bacillus subtilis* inoculant (1×10^8 CFU/g), adjusting moisture to 60%, and incubating at 30°C for 7 days with weekly turning.

2.3.2 BSF Rearing Protocol

BSF eggs (100 mg, approximately 2000 eggs) were inoculated into 500 g of each substrate in plastic containers (1 L), with three replicates per treatment. The containers were placed in an artificial climate chamber at 28 ± 1°C, 70 ± 5% relative humidity, and 12 :12 h light : dark cycle. Larval length and weight were measured every 3 days using a vernier caliper (accuracy 0.01 mm) and electronic balance (accuracy 0.0001 g), respectively. Survival rate was calculated as (number of surviving larvae / initial number of larvae) × 100%. Pupation rate was (number of pupae / number of surviving larvae) × 100%, and emergence rate was (number of adults / number of pupae) × 100%. Developmental durations (larval, pupal, adult stages) were recorded.

2.4 Nutrient Composition Analysis of BSF Larval Meal and Frass

Black soldier fly (BSF) larvae were harvested at the prepupal stage, blanched at 100°C for 5 min, freeze-dried, and ground into a fine meal. For the larval meal, proximate composition was determined using standard AOAC-based procedures(Egnew et al. 2021): moisture by oven-drying, crude protein by the Kjeldahl method, crude fat by Soxhlet extraction, crude ash by incineration in a muffle furnace, and crude fiber by sequential acid-base digestion. Mineral elements (Ca, Mg, P, and others) were quantified by inductively coupled plasma mass spectrometry (ICP-MS). Total sugar content was measured using the Fehling's titration method, and total

amino acids were analyzed with an automatic amino acid analyzer. The abundance of *Bacillus subtilis* was enumerated by the standard plate count technique.

BSF frass was collected from the residual substrate after larval separation, air-dried, and passed through a 2-mm sieve. The frass was analyzed for its fertilizing value and safety parameters, including moisture, total nitrogen, total phosphorus, total potassium, organic matter, and heavy metals (As, Cr, Pb, Cd, Hg). All these determinations followed the conventional methods prescribed for organic fertilizer quality assessment.

2.5 Chinese Cabbage Cultivation Experiment

BSF frass was mixed with garden soil at five ratios: 10% frass : 90% soil (C-10), 15% frass : 85% soil (C-15), 20% frass : 80% soil (C-20), 25% frass : 75% soil (C-25), 30% frass : 70% soil (C-30), and 0% frass : 100% soil (C-0). Each treatment was replicated three times in plastic pots (15 cm diameter × 12 cm height) with 1 kg of mixed substrate per pot. Ten Chinese cabbage seeds were sown per pot and thinned to 3 seedlings after germination. The pots were placed in a greenhouse with natural light, temperature 20–25°C, and regular watering to maintain soil moisture at 60%. Germination potential, germination rate, and emergence rate were recorded on the 7th day. After 45 days of growth, plant height, leaf number, leaf length, leaf width, fresh weight, and dry weight were measured. Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined using the ethanol extraction method(Liu et al. 2024b).

2.6 Statistical Analysis

All data were analyzed using SPSS 26.0 software. Normality and homogeneity of variances were tested prior to analysis. One-way analysis of variance (ANOVA) was performed to compare differences among treatments, followed by Duncan's multiple range test for post hoc comparisons. Significant differences were considered at $P < 0.05$. Data are presented as mean ± standard deviation (SD).

3. Results

3.1 Black Soldier Fly Growth Performance

As shown in Table 2, BSF larvae reared on sole FRM (NF-1) performed poorly, with a survival rate of only 5.00% and a pupation rate of 48.89%. In contrast, the addition of fungus residue (FR) at a 1:1 ratio (NF-3) dramatically improved survival to 61.35% and pupation to 73.51%. Sole FR (NF-2) gave the best results among the non-fermented treatments, with survival of 81.21%, pupation of 94.27%, and emergence of 80.68%. Larval and prepupal weights were similar between NF-2 (0.0778 g and 0.0715 g) and NF-3 (0.0739 g and 0.0659 g), both much higher than those of NF-1 (0.0551 g and 0.059 g). Developmental durations varied: NF-1 had the shortest larval period (29 d) but failed to produce adults (adult duration 0 d), whereas NF-3 and NF-2 had longer larval durations (31 d and 35.67 d, respectively) but successfully completed metamorphosis.

Table 2
Growth performance of BSF on non-fermented substrates

Index	NF-3 (FR:FRM = 1:1)	NF-2 (sole FR)	NF-1 (sole FRM)
Larval Length (mm)	15.4 ± 0.11 e	17.10 ± 0.01 cd	17.0 ± 0 d
Prepupal Length (mm)	15.4 ± 0.14 cde	16.0 ± 0.08 bcd	15.0 ± 0 de
Larval Weight (g)	0.0739 ± 0.0022 de	-	-
Prepupal Weight (g)	0.0659 ± 0.0044 cde	-	-
Survival Rate (%)	61.35 ± 1.79	81.21 ± 6.42 cde	5.00 ± 0 g
Pupation Rate (%)	73.51 ± 4.35 c	94.27 ± 13.32 b	48.89 ± 2.20 d
Emergence Rate (%)	80.69 ± 7.40 c	80.68 ± 7.40 c	70 ± 0 c
Larval Duration (d)	31 ± 3.61 d	35.67 ± 1.15 c	29 ± 0 de
Pupal Duration (d)	22.67 ± 2.08 a	19.67 ± 0.58 b	14 ± 0 c
Adult Duration (d)	6 ± 1 e	6.33 ± 1.15 de	0 ± 0 f
Note: Different lowercase letters indicate significant differences among treatments at P < 0.05;			

Fermentation with *Bacillus subtilis* further enhanced BSF growth (Table 3). The fermented 1:1 FR-FRM mixture (F-1) achieved the highest larval weight (0.0936 ± 0.0053 g), survival rate (90.69 ± 1.16%), and pupation rate (97.63 ± 15.34%), along with the shortest larval duration (28.33 d) and prepupal duration (5.67 d). In contrast, fermented sole FR (F-2) gave lower survival (69.36%) and longer larval duration (36.33 d). Unfermented sole FR (F-3) showed a high pupation rate (97.63%) but very low survival (37.96%), indicating that fermentation of FR alone did not improve overall performance. Emergence rates exceeded 70% in all fermented treatments, with no major differences. These results demonstrate that a combination of FR amendment and fermentation significantly improves BSF growth and development.

Table 3
Growth performance of BSF on fermented substrates

Index	F-1 (Fermented 1:1 mixture)	F-2 (Fermented sole FR)	F-3 (Fermented sole FRM)
Larval Length (mm)	14.0 ± 0 de	15.767 ± 0.0802 bc	16.667 ± 0.0557 ab
Prepupal Length (mm)	13.5 ± 0.0436 cd	14.567 ± 0.0153 bc	16.067 ± 0.0306 a
Larval Weight (g)	0.0936 ± 0.0053 c	0.0698 ± 0.0074 cd	0.0631 ± 0.0034 d
Prepupal Weight (g)	0.0537 ± 0.0041 cde	0.0657 ± 0.0035 cd	0.0475 ± 0.0158 cde
Survival Rate (%)	90.69 ± 1.16 bc	69.36 ± 1.89 d	37.96 ± 16.26 e
Pupation Rate (%)	97.63 ± 15.34 a	73.80 ± 7.31 b	97.63 ± 15.34 a
Emergence Rate (%)	86.05 ± 20.02 a	80.0 ± 0 a	70 ± 0 a
Larval Duration (d)	28.33 ± 2.89 c	36.33 ± 1.15 b	30 ± 2 c
Prepupal Duration (d)	5.67 ± 1.53 b	9.33 ± 2.08 a	7 ± 0 ab
Adult Duration (d)	5.33 ± 1.53 cd	7.0 ± 0 abc	7.33 ± 0.58 ab

Note: Different lowercase letters indicate significant differences among treatments at P < 0.05(The same below);

3.2 Nutrient Composition of BSF Larval Meal and Frass

The nutrient composition of BSF larval meal is shown in Table 4 and Fig. 1. The moisture content was 6.0%, crude protein 12.2%, crude fat 15.9%, crude ash 3.5%, crude fiber 2.7%, and total sugar 0.94 g/100g. It was rich in minerals, with Ca (10746 mg/kg), Mg (1108 mg/kg), and P (0.26%). Pb and Cd were not detected, meeting the feed safety standards. The total amino acid content was 31.95%, with glutamate (4.24%), aspartic acid (3.38%), and leucine (2.53%) being the major amino acids. The count of *Bacillus subtilis* was 6.5×10^4 CFU/g, indicating potential probiotic effects.

Table 4
Nutrient content of BSF larval meal

Determination Index	Test Result	Determination Index	Test Result
Moisture	6.0%	Phosphorus (P)	0.26%
Protein	12.2%	Calcium (Ca)	10746 mg/kg
Fat	15.9%	Magnesium (Mg)	1108 mg/kg
Ash	3.5%	Lead (Pb)	Not detected
Crude Fiber	2.7%	Cadmium (Cd)	Not detected
Total Sugar	0.94 g/100g	*Bacillus subtilis*	6.5×10^4 CFU/g
Determination Index	Test Result	Determination Index	Test Result
Moisture	6.0%	Phosphorus (P)	0.26%
Protein	12.2%	Calcium (Ca)	10746 mg/kg
Fat	15.9%	Magnesium (Mg)	1108 mg/kg

BSF frass properties are shown in Table 5. The graphs had a moisture content of 7.5%, total N 3.91%, total P 3.20%, total K 3.37%, and organic matter 68.7%. The total N, P, K content (N + P+K) was 10.48%, meeting the national standard for organic fertilizers (NY 525–2021). It was rich in trace elements, with Fe (0.24%), Mn (628 mg/kg), Zn (420 mg/kg), and B (31 mg/kg). Heavy metal contents were within the safe limits: As (1.6 mg/kg), Cr (5.5 mg/kg), Pb (1.6 mg/kg), Cd (0.084 mg/kg), and Hg (< 0.005 mg/kg).

Table 5
Nutrient content of BSF frass

Determination Index	Test Result	Determination Index	Test Result
Moisture	7.5%	Manganese (Mn)	628 mg/kg
Total Nitrogen (N)	3.91%	Boron (B)	31 mg/kg
Total Phosphorus (P)	3.20%	Zinc (Zn)	420 mg/kg
Total Potassium (K)	3.37%	Aluminum (Al)	1036 mg/kg
Organic Matter	68.7%	Arsenic (As)	1.6 mg/kg
Calcium (Ca)	6.22%	Chromium (Cr)	5.5 mg/kg
Sodium (Na)	0.21%	Lead (Pb)	1.6 mg/kg
Magnesium (Mg)	1.47%	Cadmium (Cd)	0.084 mg/kg
Iron (Fe)	0.24%	Mercury (Hg)	< 0.005mg/kg

3.3 Effects of BSF Frass on Chinese Cabbage Growth

The addition of BSF frass significantly affected Chinese cabbage seed germination and emergence (Table 6). The germination potential, germination rate, and emergence rate of C-25 (25% frass) were the highest: 98.89 ±

1.72%, 98.33 ± 2.79%, and 96.67 ± 5.16%, respectively, which were significantly higher than C-0 (88.89 ± 4.04%, 88.33 ± 3.50%, and 81.67 ± 14.72%, respectively) ($P < 0.05$). C-30 (30% frass) had the lowest germination potential (73.33 ± 4.71%), germination rate (71.11 ± 5.02%), and emergence rate (61.67 ± 9.83%) ($P < 0.05$).

Table 6
Effects of BSF frass on Chinese cabbage seed germination and emergence

Treatment	Germination Potential (%)	Germination Rate (%)	Emergence Rate (%)
0% Frass (C-0)	88.89 ± 4.04 c	88.33 ± 3.50 b	81.67 ± 14.72 b
10% Frass (C-10)	80.56 ± 2.51 d	77.78 ± 5.02 c	78.33 ± 9.83 b
15% Frass (C-15)	89.45 ± 3.90 c	89.45 ± 4.91 b	80.00 ± 12.65 b
20% Frass (C-20)	93.89 ± 2.51 b	95.00 ± 5.05 a	88.33 ± 7.53 ab
25% Frass (C-25)	98.89 ± 1.72 a	98.33 ± 2.79 a	96.67 ± 5.16 a
30% Frass (C-30)	73.33 ± 4.71 e	71.11 ± 5.02 d	61.67 ± 9.83 c

The 25% frass treatment (C-25) significantly improved Chinese cabbage growth parameters (Table 7). The leaf number (8.50 ± 1.05), leaf length (2.68 ± 0.13 cm), and leaf width (2.80 ± 0.09 cm) of C-25 were significantly higher than C-0 (6.33 ± 0.52, 1.79 ± 0.12 cm, and 1.88 ± 0.08 cm, respectively) ($P < 0.05$). C-30 (30% frass) had the lowest leaf number (5.17 ± 0.75), leaf length (1.94 ± 0.13 cm), and leaf width (2.15 ± 0.14 cm) ($P < 0.05$).

Table 7
Effects of BSF frass on Chinese cabbage growth parameters

Treatment	Leaf Number (pieces)	Leaf Length (cm)	Leaf Width (cm)
0% Frass (C-0)	6.33 ± 0.52 bc	1.79 ± 0.12 e	1.88 ± 0.08 d
10% Frass (C-10)	5.83 ± 1.17 cd	1.82 ± 0.08 de	1.87 ± 0.15 d
15% Frass (C-15)	6.17 ± 0.98 bc	2.15 ± 0.10 c	2.07 ± 0.16 c
20% Frass (C-20)	7.17 ± 0.75 b	2.41 ± 0.10 b	2.53 ± 0.08 b
25% Frass (C-25)	8.50 ± 1.05 a	2.68 ± 0.13 a	2.80 ± 0.09 a
30% Frass (C-30)	5.17 ± 0.75 d	1.94 ± 0.13 d	2.15 ± 0.14 c

The 25% frass treatment (C-25) achieved the highest single plant fresh weight (515.11 ± 13.08 g) and dry weight (60.46 ± 0.51 g), which were significantly higher than C-0 (212.07 ± 4.33 g and 29.28 ± 2.84 g, respectively) ($P < 0.05$) (Table 8). The chlorophyll a, chlorophyll b, and total chlorophyll contents of C-25 were 1.9783 ± 0.0175 mg/kg, 0.5383 ± 0.0006 mg/kg, and 2.5167 ± 0.0175 mg/kg, respectively, which were significantly higher than other treatments (Table 11). C-30 (30% frass) had the lowest chlorophyll content (total chlorophyll: 2.0657 ± 0.0334 mg/kg) ($P < 0.05$).

Table 8
Effects of BSF frass on Chinese cabbage weight

Treatment	Single Plant Fresh Weight (g)	Single Plant Dry Weight (g)
0% Frass (C-0)	212.07 ± 4.33 d	29.28 ± 2.84 d
10% Frass (C-10)	210.40 ± 1.20 d	24.64 ± 0.93 e
15% Frass (C-15)	338.15 ± 11.78 c	38.02 ± 0.76 c
20% Frass (C-20)	417.75 ± 7.97 b	47.70 ± 0.62 b
25% Frass (C-25)	515.11 ± 13.08 a	60.46 ± 0.51 a
30% Frass (C-30)	224.60 ± 3.71 d	30.22 ± 0.53 d

Table 9
Effects of BSF frass on Chinese cabbage chlorophyll content (mg/kg)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
0% Frass (C-0)	1.8793 ± 0.007 bc	0.4383 ± 0.0006 c	2.3177 ± 0.0075 c
10% Frass (C-10)	1.659 ± 0.03 d	0.2617 ± 0.0095 d	1.9207 ± 0.0214 e
15% Frass (C-15)	1.8657 ± 0.0092 c	0.4885 ± 0.0169 b	2.3542 ± 0.0158 c
20% Frass (C-20)	1.9332 ± 0.0722 ab	0.4885 ± 0.0169 b	2.4217 ± 0.0697 b
25% Frass (C-25)	1.9783 ± 0.0175 a	0.5383 ± 0.0006 a	2.5167 ± 0.0175 a
30% Frass (C-30)	1.64 ± 0.02 d	0.4257 ± 0.0172 c	2.0657 ± 0.0334 d

4. Discussion

4.1 Substrate Physical Properties and Their Constraints on BSF Performance

The inherent nutritional richness of fox and raccoon dog manure (FRM)—with 29.30% crude protein, abundant mineral elements (Ca 48,000 mg/kg, Mg 14,000 mg/kg, Fe 4,000 mg/kg), and undetectable Pb and Cd—initially positions it as a promising candidate substrate for black soldier fly (BSF) bioconversion(Wang et al. 2021b; Grassauer et al. 2023a). However, the present results revealed that sole FRM (NF-1) supported extremely poor larval survival (5.00%) and pupation (48.89%), which appears to be primarily attributable to its exceptionally high viscosity (73,011 mPa·s). This physical property likely restricts oxygen diffusion within the substrate matrix and mechanically impedes larval locomotion, thereby compromising feeding efficiency and overall development(Abdelmaksoud et al. 2026). This finding corroborates earlier observations by Yakti et al.(Yakti et al. 2023), who demonstrated that substrate physical characteristics—particularly porosity and viscosity—serve as critical determinants of BSF larval growth, often overriding the nutritional composition in importance. The addition of fungus residue (FR) as a structural bulking agent at a 1:1 ratio substantially alleviated these physical constraints, as evidenced by the marked improvements in survival (61.35%) and pupation (73.51%) in treatment NF-3 compared with the unamended FRM control. Nevertheless, a performance gap persisted

relative to the sole FR treatment (NF-2, 81.21% survival and 94.27% pupation), which may be explained by the high ash content (41.60%) of FRM. Elevated ash levels can dilute the organic fraction available for larval assimilation and potentially interfere with nutrient bioavailability(Grassauer et al. 2023b; Deng et al. 2024). This observation underscores the need for a more systematic optimization of the FR-to-FRM ratio, with future investigations exploring 2:1 or 3:1 formulation to identify the optimal balance between structural improvement and nutritional adequacy.

4.2 Effects of Fermentation on BSF Growth

Fermentation with *Bacillus subtilis* further improved BSF growth performance. The fermented FR-FRM mixture (1:1) (F-1) had the highest larval weight (0.0936 ± 0.0053 g) and survival rate (90.69 ± 1.16%), which may be due to the following reasons. First, *Bacillus subtilis* can degrade complex organic matter such as cellulose and protein into small molecules such as glucose and amino acids that are easily absorbed by BSF larvae(Quan et al. 2023; Memon et al. 2025). Second, fermentation reduces substrate viscosity and increases oxygen availability(Yakti et al. 2023). Third, *Bacillus subtilis* produces antimicrobial substances that inhibit pathogenic bacteria, improving larval health(Liu et al. 2024a). Compared with the non-fermented 1:1 mixture (NF-3), fermentation increased larval weight by 26.7% and survival rate by 47.8%, indicating that fermentation played a critical role beyond mere substrate mixing. Notably, fermentation of sole FR (F-2) did not improve BSF performance compared with the fermented sole FRM control (F-3). This may be attributed to the fact that FR itself already harbors a rich and functionally mature indigenous microbial community. The introduction of exogenous *Bacillus subtilis* into such a complex ecosystem could lead to intense competition for nutrients and ecological niches with native populations. Indeed, direct experimental evidence shows that exogenous B. *subtilis* remains only transiently in the larval gut and fails to establish stable colonization, thus being unable to alter the established microbial structure effectively(Carpentier et al. 2025). Moreover, excessive inoculation might disrupt the original micro-ecological balance, cause over-decomposition of the substrate and thereby reducing its nutritional value for larval feeding.

In contrast, fermentation of the 1:1 FR-FRM mixture (F-1) yielded substantial improvements in BSF growth. This differential response suggests that the efficacy of fermentation is highly substrate-dependent. While sole FR already possesses a robust microbial consortium that resists external invasion, the FRM component in the mixture—characterized by high viscosity and limited microbial diversity—benefits greatly from B. *subtilis* inoculation, which enhances substrate structure, releases nutrients, and suppresses potential pathogens. Similarly, Liu et al.(Liu et al. 2025)reported that probiotic-fermented chicken manure significantly improved BSF growth metrics, reinforcing the notion that fermentation efficacy is contingent upon the indigenous microbial ecology and physical properties of the substrate.

Collectively, these results position B. *subtilis*-mediated fermentation as a promising pre-treatment strategy for FRM-based BSF rearing, particularly for substrates with suboptimal physical properties or limited native microbial activity, although the economic cost of inoculant production and fermentation time should be considered in large-scale applications.

4.3 Integrated Valorization: BSF Larval Meal Quality and Frass Biofertilizer Potential

The nutritional profile of BSF larval meal derived from FR-FRM substrates—12.2% crude protein, 15.9% fat, and 31.95% total amino acids—presents both opportunities and constraints for feed applications. Although the protein content is lower than that of commercial fish meal (60–70% protein), the fat content exceeds the typical range (8–12%), suggesting that this larval meal may be better suited as an energy supplement rather than a primary protein source(Abd El-Hack et al. 2020; Matin et al. 2021). No detectable Pb or Cd were found, meeting feed safety standards. The presence of B. *subtilis* at 6.5×10^4 CFU/g is noteworthy, as this bacterium is recognized for its probiotic properties, which could potentially confer gut health benefits to animals(Long et al. 2026).

The BSF frass demonstrated promising fertilizer properties, with 68.7% organic matter and 10.48% total NPK, satisfying the Chinese organic fertilizer standard (NY/T 525–2021). Rich in secondary and micronutrients (Ca 6.22%, Mg 1.47%, Fe 0.24%, Zn 420 mg/kg, Mn 628 mg/kg), the frass represents a balanced soil amendment. In the Chinese cabbage trial, the 25% frass rate (C-25) optimized germination (98.33%), fresh weight (515.11 g/plant), and chlorophyll content (2.52 mg/kg). However, the 30% rate (C-30) caused growth suppression—germination (71.11%) and fresh weight (224.60 g/plant) fell below control levels—likely due to excessive soluble salts or nutrient imbalances(Zhang et al. 2026a). This biphasic response is consistent with recent reviews identifying 20–25% BSF frass as the optimal range for vegetable production(Borkent and Hodge 2021; Kagehi et al. 2025; Kaczor et al. 2026).

Collectively, these findings validate a dual valorization pathway: the harvested larvae serve as a functional feed ingredient, while the residual frass functions as an effective biofertilizer, offering a sustainable solution for fur-farming waste management.

5. Conclusions

This study demonstrates that fox and raccoon dog manure can be valorized through black soldier fly bioconversion with fungus residue amendment. The manure has high nutritional value and low heavy metal content, making it a suitable substrate for black soldier fly rearing. Fungus residue amendment at a 1 to 1 ratio and fermentation with *Bacillus subtilis* significantly improved black soldier fly growth performance, including larval weight, survival rate, and pupation rate. Fungus residue addition effectively reduced antibiotic residues in black soldier fly, with the 50% fungus residue treatment achieving relatively low residues. Black soldier fly larval meal contained no detectable heavy metals and could serve as a potential feed ingredient, while black soldier fly frass was a nutrient rich biofertilizer. The 25% frass plus 75% garden soil treatment was optimal for Chinese cabbage cultivation, significantly improving seed germination, growth, and yield. This study provides a sustainable solution for fox and raccoon dog manure management and promotes the development of circular agriculture.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Ziru Li: Writing – original draft, investigation.Caili Kong: Data curation, formal analysis.Zhixin Ji: Conceptualization, methodology.Peng Sun: Investigation.Jiahui Li: Investigation.Guangxu Zheng: Investigation.Chenyang Si: Investigation.Yanying Zhang: Funding acquisition.Shuang Liu: Supervision.Ju Wu: Resources.Chun-Liang Hou: Funding acquisition, writing – review & editing.Zhiwei Zhang: Project administration, supervision, writing – review & editing.All authors read and approved the final manuscript.

Acknowledgement

This work was supported by the earmarked fund for Hebei Agriculture Research System (HBCT2024280205) and the special project of basic scientific research business expenses of provincial universities of Hebei Normal University of Science and Technology (2025JK001). This work was funded by Science Research Project of Hebei Education Department (Project No. QN2026465). We also gratefully acknowledge the instrumental analysis center of Hebei Normal University of Science and Technology.

References

1. Abd El-Hack ME, Shafi ME, Alghamdi WY, Abdelnour SA, Shehata AM, Noreldin AE, Ashour EA, Swelum AA, Al-Sagan AA, Alkhateeb M, Taha AE, Abdel-Moneim A-ME, Tufarelli V, Ragni M (2020) Black Soldier Fly (Hermetia illucens) Meal as a Promising Feed Ingredient for Poultry: A Comprehensive Review. Agriculture 10(8):339. https://doi.org/10.3390/agriculture10080339
2. Abd Manan F, Yeoh Y-K, Chai T-T, Wong F-C (2024) Unlocking the potential of black soldier fly frass as a sustainable organic fertilizer: A review of recent studies. J Environ Manage 367:121997. https://doi.org/10.1016/j.jenvman.2024.121997
3. Abdelmaksoud EM, Hegazy SA, Abdelsalam SA (2026) Valorization of chicken manure with bread waste, yeast and cyanobacterium Spirulina platensis to enhance black soldier fly larval performance. Waste Manag 215:115423. https://doi.org/10.1016/j.wasman.2026.115423
4. Borkent S, Hodge S (2021) Glasshouse Evaluation of the Black Soldier Fly Waste Product HexaFrass™ as an Organic Fertilizer. Insects 12(11):977. https://doi.org/10.3390/insects12110977
5. Carpentier J, Noël G, Li B, Francis F, Caparros Megido R (2025) Black Soldier Fly Gut Microbiota Resists Invasion by *Bacillus subtilis* 168 and Pseudomonas putida KT2440. Appl Microbiol 5(3):82. https://doi.org/10.3390/applmicrobiol5030082
6. Deng B, Liu Z, Gong T, Xu C, Zhang X, Cao H, Yuan Q (2024) Addition of plantation waste to the bioconversion of pig manure by black soldier fly larvae: Effects on heavy metal content and bioavailability. Waste Manag 176:64–73. https://doi.org/10.1016/j.wasman.2024.01.018
7. Egnew N, Romano N, Fischer H, Sinha AK (2021) Purging black solider fly larvae (Hermetia illucens) compromises their nutritive value as a feedstuff. Int J Trop Insect Sci 41(4):3279–3286. https://doi.org/10.1007/s42690-021-00491-x
8. Ganesan AR, Mohan K, Kandasamy S, Surendran RP, Kumar R, Rajan DK, Rajarajeswaran J (2024) Food waste-derived black soldier fly (*Hermetia illucens*) larval resource recovery: A circular bioeconomy

approach. Process Saf Environ Prot 184:170–189. https://doi.org/10.1016/j.psep.2024.01.084

9. Grassauer F, Ferdous J, Pelletier N (2023a) Manure Valorization Using Black Soldier Fly Larvae: A Review of Current Systems, Production Characteristics, Utilized Feed Substrates, and Bioconversion and Nitrogen Conversion Efficiencies. Sustainability 15(16):12177. https://doi.org/10.3390/su151612177
10. Grassauer F, Ferdous J, Pelletier N (2023b) Manure Valorization Using Black Soldier Fly Larvae: A Review of Current Systems, Production Characteristics, Utilized Feed Substrates, and Bioconversion and Nitrogen Conversion Efficiencies. Sustainability 15(16):12177. https://doi.org/10.3390/su151612177
11. Jia Z, Zhou J, Yang M, Wang M, Li L, Fan X (2023) Preparation and evaluation of certified reference materials for crude protein, crude fat, and crude ash in feed. Microchem J 191:108854. https://doi.org/10.1016/j.microc.2023.108854
12. Jiang S, Sun J, Zhu X, Shen K, Zhang Z (2024) Co-treatment of agri-food waste streams using black soldier fly larvae (Hermetia illucens L.): A sustainable solution for rural waste management. J Environ Manage 370:122373. https://doi.org/10.1016/j.jenvman.2024.122373
13. Kaczor M, Bulak P, Waligórski P, Bieganowski A (2026) Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass. J Clean Prod 548:147855. https://doi.org/10.1016/j.jclepro.2026.147855
14. Kagehi N, Beesigamukama D, Tanga CMM, Ngugi MP, Subramanian S, Khamis FMM (2025) Contribution of different frass fertilizer products on enhanced growth, yield and nutrient quality of Broccoli [Brassica oleracea]. https://doi.org/10.3389/fpls.2025.1613814. Front Plant Sci 16
15. Kusumah MS, Panjaitan FR, Yudanto BG, Bajra BD (2025) Substrate degradation and black soldier fly larvae bioconversion performance profile on co-digested oil palm biomass-based feedstock. PLoS ONE 20(9):e0332046. https://doi.org/10.1371/journal.pone.0332046
16. Li TH, Zhang CR, Che PF, Ma Y, Zang LS (2021) Recycling of spent mushroom substrate and food waste: utilisation as feed materials for black soldier fly (Hermetia illucens (L.) Diptera: Stratiomyidae). https://chinesesites.library.ingentaconnect.com/content/wagac/jiff/2021/00000007/00000004/art00004. Accessed 18 June 2026
17. Liu C, Yao H, Cao Q, Wang T, Wang C (2022) The enhanced degradation behavior of oxytetracycline by black soldier fly larvae with tetracycline resistance genes in the larval gut: Kinetic process and mechanism. Environ Res 214:114211. https://doi.org/10.1016/j.envres.2022.114211
18. Liu H, Yang X, Yu X, Lin J, Peng S, Li Q, Yang Q, Wang D, Li Q (2024a) Untargeted metabolomics and PacBio analysis on bioactive components and microbial community in co-fermentation of black soldier fly larva. Food Res Int 197:115304. https://doi.org/10.1016/j.foodres.2024.115304
19. Liu K, Zhang G, Li Y, Jiao M, Guo J, Li J, Shi H, Wang X, Zhang W, Quan K, Xia W (2025) Effect of Rearing Substrate on Nutritional Composition, Growth Performance and Multi-Omics Characteristics of Black Soldier Fly. Insects 17(1):10. https://doi.org/10.3390/insects17010010
20. Liu Z, Liu Z, Wang P, Guo L, Sun L, Chen B, Li X (2024b) Physiological and biochemical responses of the waterlogging-tolerant pumpkin resources to waterlogging stress. Sci Hort 336:113347. https://doi.org/10.1016/j.scienta.2024.113347
21. Long L, Liu G, Gao G, Ding G (2026) Enhancing Immunity and Gut Microbiota Balance with Black Soldier Fly Larvae (BSFL) Meal: A Sustainable Feed Ingredient That Maintains Growth Performance in Pekin Ducks.

Insects 17(6):548. https://doi.org/10.3390/insects17060548

22. Lopes IG, Wiklicky V, Vinnerås B, Yong JWH, Lalander C (2024) Recirculating frass from food waste bioconversion using black soldier fly larvae: Impacts on process efficiency and product quality. J Environ Manage 366:121869. https://doi.org/10.1016/j.jenvman.2024.121869
23. Matin N, Utterback P, Parsons CM (2021) True metabolizable energy and amino acid digestibility in black soldier fly larvae meals, cricket meal, and mealworms using a precision-fed rooster assay. Poult Sci 100(7):101146. https://doi.org/10.1016/j.psj.2021.101146
24. Memon FU, Li C, Ahmad S, Cui Y, Feng X, Zeng P, Nabi F, Huang Z, Tettamanti G, Chen T, Tian L (2025) Efficiency of microbial fermentation on microbial shifts, enzymatic activity, and transcriptions in black soldier fly larvae during the sugarcane waste conversion. Environ Pollut 381:126588. https://doi.org/10.1016/j.envpol.2025.126588
25. Mishra A, Suthar S (2025) Cow dung biochar amendment affects the bioconversion of spent mushroom substrate and fruit waste by black soldier fly (Diptera: Stratiomyidae) larvae. J Environ Manage 392:126763. https://doi.org/10.1016/j.jenvman.2025.126763
26. Pei Y, Sun M, Wang M, Lei A, Liu X, Chen H, Yang S (2025) Characteristics of intestinal microbial communities and occurrence of antibiotic resistance genes during degradation of antibiotic mycelial residues by black soldier fly (*Hermetia illucens* L.) larvae. Environ Pollut 371:125940. https://doi.org/10.1016/j.envpol.2025.125940
27. Peng Y, Shi Q, Wang Y, Zhang F, Ji Z, Zhang J (2019) Dietary probiotics have different effects on the composition of fecal microbiota in farmed raccoon dog (Nyctereutes procyonoides) and silver fox (Vulpes vulpes fulva). BMC Microbiol 19(1):109. https://doi.org/10.1186/s12866-019-1491-x
28. Quan J, Wang Y, Cheng X, Li C, Yuan Z (2023) Revealing the effects of fermented food waste on the growth and intestinal microorganisms of black soldier fly (Hermetia illucens) larvae. Waste Manag 171:580–589. https://doi.org/10.1016/j.wasman.2023.10.002
29. Shen K, Liu L, Niu Y, Chen Y, Wang F, Chen Y, Lin L, Aye SL, Liu Z, Wu D, Li X (2026) Food waste treatment by black soldier fly larvae (BSFL) and pilot-scale aerobic composting of the BSFL frass using horizontal mechanical reactor. Process Saf Environ Prot 207:108413. https://doi.org/10.1016/j.psep.2026.108413
30. Siddiqui SA, Ristow B, Rahayu T, Putra NS, Widya Yuwono N, Nisa' K, Mategeko B, Smetana S, Saki M, Nawaz A, Nagdalian A (2022a) Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Manag 140:1–13. https://doi.org/10.1016/j.wasman.2021.12.044
31. Siddiqui SA, Ristow B, Rahayu T, Putra NS, Widya Yuwono N, Nisa' K, Mategeko B, Smetana S, Saki M, Nawaz A, Nagdalian A (2022b) Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Manag 140:1–13. https://doi.org/10.1016/j.wasman.2021.12.044
32. Soomro AA, Rehman K, ur, Cai M, Laghari ZA, Zheng L, Yu Z, Zhang J (2024) Larval biomass production from the co-digestion of mushroom root waste and soybean curd residues by black soldier fly larvae (Hermetia illucens L). Environ Sci Pollut Res 31(20):30112–30125. https://doi.org/10.1007/s11356-024-33173-5
33. Suryati T, Julaeha E, Farabi K, Ambarsari H, Hidayat AT (2023) Lauric Acid from the Black Soldier Fly (Hermetia illucens) and Its Potential Applications. Sustainability 15(13):10383. https://doi.org/10.3390/su151310383

34. van Dongen KCW, de Lange E, van Asseldonk LLM, Zoet L, van der Fels-Klerx HJ (2024) Safety and transfer of veterinary drugs from substrate to black soldier fly larvae. animal 18(7):101214. https://doi.org/10.1016/j.animal.2024.101214
35. Wang L, Zhang G, Dong Y, Tian S, Wu Z, Wang H (2025) Co-treating of chicken manure with wet market waste by black soldier fly larvae for synergetic bioconversion enhancement and gas reduction. Environ Technol Innov 39:104283. https://doi.org/10.1016/j.eti.2025.104283
36. Wang Q, Ren X, Sun Y, Zhao J, Awasthi MK, Liu T, Li R, Zhang Z (2021a) Improvement of the composition and humification of different animal manures by black soldier fly bioconversion. J Clean Prod 278:123397. https://doi.org/10.1016/j.jclepro.2020.123397
37. Wang Q, Ren X, Sun Y, Zhao J, Awasthi MK, Liu T, Li R, Zhang Z (2021b) Improvement of the composition and humification of different animal manures by black soldier fly bioconversion. J Clean Prod 278:123397. https://doi.org/10.1016/j.jclepro.2020.123397
38. Wang Y, Yan S, Ji Y, Yang Y, Rui P, Ma Z, Qiu H-J, Song T (2022) First Identification and Phylogenetic Analysis of Porcine Circovirus Type 4 in Fur Animals in Hebei, China. Animals 12(23):3325. https://doi.org/10.3390/ani12233325
39. Wei M, Li T, Khan S, Li H, Wen T, Yi T, Guo J (2024) Effects of black soldier fly larvae on biotransformation and residues of spent mushroom substrate and wet distiller's grains. Sci Rep 14(1):22392. https://doi.org/10.1038/s41598-024-72959-y
40. Xu X, Huang Y, Luo X (2025) Black soldier fly: a new model for bioremediation of antibiotic pollutants. Natl Sci Rev 12(3):nwaf043. https://doi.org/10.1093/nsr/nwaf043
41. Yakti W, Müller M, Klost M, Mewis I, Dannehl D, Ulrichs C (2023) Physical Properties of Substrates as a Driver for Hermetia illucens (L.) (Diptera: Stratiomyidae) Larvae Growth. Insects 14(3):266. https://doi.org/10.3390/insects14030266
42. Zalewska M, Błażejewska A, Czapko A, Popowska M (2021) Antibiotics and Antibiotic Resistance Genes in Animal Manure – Consequences of Its Application in Agriculture. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.610656
43. Zhang B, Xue Y, Pan C, Zeng G (2026a) Effects of Black Soldier Fly Frass on Plant Growth by Meta-Analysis. J Soil Sci Plant Nutr 26(2):7006–7016. https://doi.org/10.1007/s42729-026-03317-2
44. Zhang X, Gong Z, Chen X, Ludlow RA, Guo S (2024) Enhanced removal of antibiotics and heavy metals in aquatic systems using spent mushroom substrate-derived biochar integrated with Herbaspirillum huttiense. Environ Sci Pollut Res 31(35):47674–47689. https://doi.org/10.1007/s11356-024-34192-y
45. Zhang X, Zhao L, Yu G, Henawy AR, Zheng L, Huang F, Cai M, Yu Z, Zhang J (2026b) Black Soldier Fly Can Safely Co-Convert Antibiotic Fermentation Residue and Potato Peel Waste into a Valuable Feed Resource. Insects 17(6):550. https://doi.org/10.3390/insects17060550

Figures

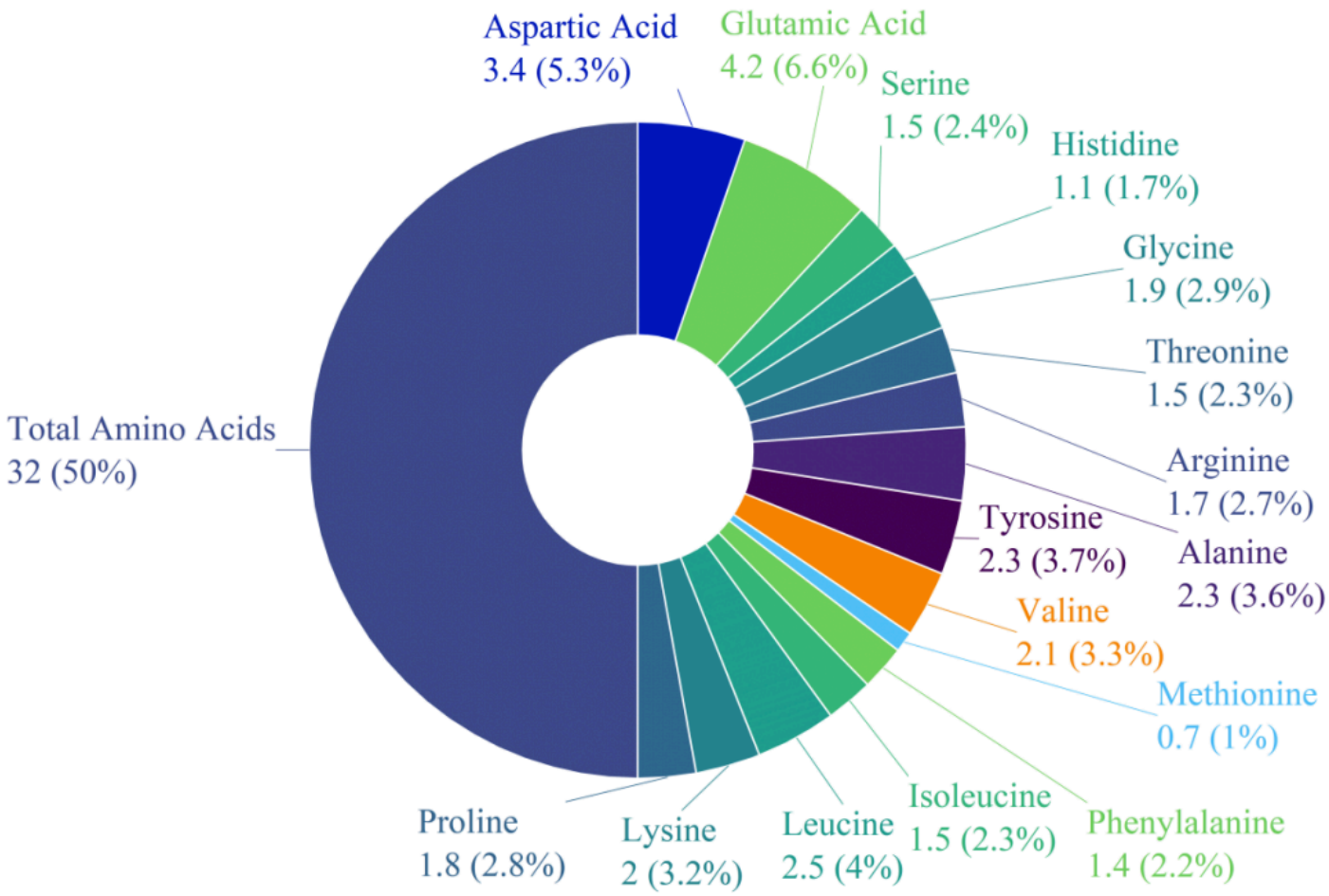


Figure 1

Amino acid composition of BSF larval meal