

Article

Transforming Coffee and Meat By-Products into Protein-Rich Meal via Black Soldier Fly Larvae (*Hermetia illucens*)

Claudia L. Vargas-Serna ^{1,*} , Angie N. Pineda-Osorio ¹ , Heidy Lorena Gallego-Ocampo ^{2,3},
José L. Plaza-Dorado ¹  and Claudia I. Ochoa-Martínez ¹ 

¹ GIPAB Group (Agrifood and Biotechnological Processes Research Group), Escuela de Ingeniería de Alimentos, Universidad del Valle, Cali 760042, Colombia; angie.pineda@correounivalle.edu.co (A.N.P.-O.); jose.plaza@correounivalle.edu.co (J.L.P.-D.); claudia.ochoa@correounivalle.edu.co (C.I.O.-M.)

² Corporación Colombiana de Investigación Agropecuaria, Agrosavia, Rionegro 250074, Colombia; heilogao@hotmail.com

³ Escuela de Ciencia Básicas Tecnología e Ingeniería ECBTI, Universidad Nacional Abierta y a Distancia UNAD, Bogotá 110231, Colombia

* Correspondence: claudia.vargas@correounivalle.edu.co

Abstract: In response to increasing food waste and the necessity for sustainable resource utilization, this study evaluated the effectiveness of black soldier fly (*Hermetia illucens*) larvae in converting a mixture of coffee and meat residues into protein-rich meal suitable for animal feed. A two-component mixture design optimized the substrate composition, followed by model validation and a comprehensive nutritional characterization of the larvae-derived protein. The larval meal contained 30–39 g of protein per 100 g (dry basis). The results indicated that increasing the meat residue content to 35% in the substrate maximized the protein yield. The optimized larval meal contained 52.9 g of protein per 100 g (dry basis) and favorable parameters such as moisture and fat, demonstrating a nutrient profile suitable for aquaculture feed. These findings suggested that *Hermetia illucens* larvae could convert agro-industrial by-products into high-quality protein. Coffee and meat residues served as suitable substrates for larval growth, supporting proper metabolic development and yielding a high bioconversion rate. This work contributes to the constant efforts in food waste valorization by integrating nutrient recovery processes into circular economy principles.

Keywords: black soldier fly larvae; coffee residues; meat by-products; sustainable protein production; food waste valorization; circular economy; agro-industrial waste recycling; alternative animal feed; protein optimization



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1. Introduction

The black soldier fly (*Hermetia illucens*), an insect of the *Stratiomyidae* family native to tropical and subtropical regions, offers an innovative solution for managing agro-industrial waste. Its larvae convert a wide range of organic residues into high-quality proteins during their development, representing a sustainable, economical, and ecological approach [1,2]. Black soldier fly larval meal has been utilized in producing animal feed, biodiesel, biopolymers, and soil composting [3]. In a world where nutrient-rich food often ends up in landfills, repurposing such waste into value-added products can alleviate resource strain while advancing sustainability goals.

The need for effective food waste management is particularly urgent in the face of global challenges such as climate change, population growth, and food crises. Efficiently

converting food waste into valuable resources aligns with key environmental sustainability and food security goals. The diet of black soldier fly larvae significantly influences the quality and quantity of protein produced, affecting their growth, development, and amino acid profile [4,5]. Nutrient-rich dietary components are essential to enhancing protein yields in larvae, making food waste streams, especially those rich in proteins and carbohydrates, suitable candidates for sustainable feedstock [6]. Waste derived from plant and animal sources, such as poultry and coffee residues, has been identified as an optimal substrate due to its nutritional profile, which supports high protein and fat yields in larvae. Meat residues offer high protein and essential amino acids, while coffee residues (pulp) supply carbohydrates, minerals, and antioxidants, adding to the nutritional complexity of the substrate [7–9].

The growth of black soldier fly larvae and their ability to convert waste into protein have been studied by various authors [9–12]. It has been reported that the type of substrate influences the protein content of the larval meal. For instance, when larvae are fed exclusively on fruit and vegetable mixtures, the protein content of the meal ranges between 21.8% and 38% [13,14]. In contrast, when the substrate includes highly protein-rich components, such as meat residues, protein levels can reach up to 44.2% [4,6,15]. Each substrate offers advantages and disadvantages for larval growth; the nutritional content of the substrate can modify the metabolism of the larvae and enhance bioconversion. Since coffee residues are low in protein, mixing them with a portion of meat residues could promote the growth of black soldier fly larvae and increase the protein content of the larval meal.

The decision to include coffee residues in this study was based on their abundance as an agro-industrial by-product, their complementary nutritional profile, and their ability to support energy metabolism in larvae. Meat residues, with their high protein and amino acid content, provide a nutrient-dense component that, when combined with coffee residues, creates a balanced and cost-effective substrate. However, the combination of meat and coffee residues remains underexplored, and this study aimed to fill this gap by evaluating their combined potential for optimizing protein production in black soldier fly larvae. This approach not only enhances larval growth but also contributes to sustainable waste management practices by repurposing these residues into valuable resources.

This study examined the impacts of various organic residue mixtures, specifically raw meat and coffee residues (pulp), on the protein content produced by black soldier fly larvae. Aligned with the principles of a circular economy and sustainability, this research sought to maximize the protein content of larval meal and promote high-value utilization of agro-industrial waste.

The research was structured in three phases: (1) evaluating the nutritional properties of each substrate and their impacts on protein production in *Hermetia illucens* larvae, (2) optimizing the protein content of the larval meal using statistical methods to refine the mixture composition, and (3) validating the optimized mixture. These steps were designed to demonstrate how food waste, typically viewed as an end-product with limited use, can be transformed into a resource with significant applications, promoting a shift toward high-value waste utilization.

2. Materials and Methods

2.1. Residue Collection and Larval Meal Preparation

2.1.1. Obtaining Residues

Coffee and meat residues were used as feeding substrates for the larvae. Coffee residues (pulp) were collected after coffee pulping from a farm in Morales-Cauca, Colombia. The meat residues consisted of a 1:1 mixture of chicken and hen remnants post-deboning, including residues such as viscera, heads, fatty tissues, skins, and low-quality parts not

suitable for sale (e.g., wings and drumsticks) in their raw state, sourced from local markets in Cali, Colombia. Both coffee and meat residues were characterized following the official methods of the AOAC for moisture (934.01), protein (981.10), crude fat (920.39), and ash (942.05) [16].

2.1.2. Larval Rearing

Hermetia illucens larvae were obtained from hatched eggs under controlled conditions at 25 ± 2 °C and $87 \pm 2\%$ humidity. The eggs were sourced from a fly breeding facility in Tulua, Valle, Colombia. Before hatching, the eggs were placed on the coffee substrate with a plastic barrier to prevent them from getting wet. This barrier allowed the larvae, once hatched, to move and come into contact with the food. This stage lasted two to three days after the eggs were collected. After hatching, the larvae were maintained on a coffee pulp substrate for 5 days, with 1 kg of pulp per gram of eggs. The larvae were then distributed across nine harvesters and fed mixtures of 65–75% coffee residues and 25–35% meat residues, following the experimental design described in Section 2.3. The total larval growth period after egg hatching was 15 days. Substrate was added every two days, with the amount corresponding to 2 g of new substrate per gram of initial substrate. Larvae were reared under controlled conditions at 25 °C and 87% humidity, ensuring uniform growth. Growth conditions were monitored daily to maintain consistency across experiments.

On day 14 the larval length was measured using a calibrated digital caliper. Measurements were taken from the anterior to posterior extremities of the larvae. For each experiment, 10 random larvae were measured.

2.1.3. Larval Sacrifice and Processing

Larvae were sacrificed by immersion in boiling water at 100 °C for 3 min on day 15 of growth. Then were dried in a convection oven at 55 °C for 48 h to reduce moisture.

2.1.4. Production of Larval Meal

The dried larvae were ground to a fine powder using a blade mill and sieved for uniform particle size, producing black soldier fly larval meal.

2.2. Proximate and Microbiological Analysis in Larval Meal

The obtained meal was subjected to proximate analysis according to AOAC official methods [16] (1990). The parameters measured included protein content, hereafter referred to as protein content in larval-derived meal (PCLM), and determined using the Kjeldahl method, fat (Soxhlet extraction), moisture (oven drying), ash (incineration at 550 °C), and carbohydrates (by difference).

Larval meal was subjected to microbiological tests for *E. coli* (NTC 4458) [17], *Bacillus cereus* (NTC 4679), mold and yeast plate counts, and *Salmonella* (NTC 4574) [18].

2.3. Experimental Design, Statistical Analysis, Optimization, and Validation

Experimental Design and Statistical Analysis

The experimental setup utilized a mixture design with two components: coffee residues (65–75%) and meat residues (25–35%). Five replicates were included at the central point to estimate the experimental error, resulting in a total of nine experiments. The response variable, PCLM, was used to evaluate the nutritional quality of the larval meal.

To minimize the risk of systematic errors, the experimental runs were randomized. The sum of the proportions of the components is equal to 1, as shown in Equation (1):

$$\sum_{i=1}^k X_i = 1 \quad 0 \leq X_i \leq 1 \quad i = 1 \dots k \quad (1)$$

where X_i represents the proportion of the i th component of the mixture, and k is the total number of components in the mixture.

This study used the regression model presented in Equation (2):

$$\hat{y} = \sum_{i=1}^K \beta_i X_i + \sum_{j=1}^{k-1} \sum_{i=j+1}^k \beta_{ij} X_i X_j + \sum_{i=1}^k \frac{\delta_i}{X_i} \quad (2)$$

where $\hat{y}$ is the response variable, i.e., PCLM; β and δ are the coefficients of the equation; and X_i and X_j represent the proportions of the i th and j th components of the mixture, respectively.

An analysis of variance (ANOVA) determined the significance of each component. The statistical significance of the model and the terms of the equation were evaluated based on the p -value.

2.4. Model Quality

The R^2 (Equation (3)) and adjusted R^2 (Equation (4)) coefficients were calculated to determine the variation between the models. For both coefficients, values close to 1 indicated a good fit of the model.

$$R^2 = \frac{\sum(\hat{y} - \bar{y})^2}{\sum(y - \bar{y})^2} \quad (3)$$

$$Adjusted R^2 = 1 - \frac{(1 - R^2)(n - 1)}{n - P - 1} \quad (4)$$

where n is the number of observations and P represents the number of independent variables in the regression model, which in this study were the proportions of coffee residues (X_1) and meat residues (X_2).

2.5. Optimization

The optimal mixture that maximized PCLM was identified through the response optimizer tool in Minitab 19 software (Minitab, State College, PA, USA), applying composite desirability.

2.6. Experimental Validation

The optimal growth conditions were validated by applying the optimal conditions experimentally. The relative error was calculated using Equation (5):

$$RE = \frac{y - \hat{y}}{y} \times 100 \quad (5)$$

where y is the experimental value and $\hat{y}$ is the predicted value of PCML.

3. Results

The results are structured into four sections. Section 3.1 presents the nutritional characterization of the substrates used as larval feed, highlighting the key differences in moisture, protein, fat, and ash content between coffee and meat residues, as well as the

mixtures derived from them. Section 3.2 focuses on the statistical analysis and model evaluation for predicting protein content, providing an analysis of variance and describing the regression model used to represent the relationship between substrate composition and protein content. Section 3.3 addresses the optimization of substrate composition for maximizing protein content, utilizing the desirability compound and the selected model to achieve optimal results. Finally, Section 3.4 presents the experimental validation of the optimized larval meal, comparing the predicted and observed protein content and discussing the nutritional and microbiological quality of the final product.

3.1. Nutritional Characterization of Substrates

Table 1 presents the nutritional characterization of the residues used as larval feed and the composition of each mixture used. All substrates exhibited similar moisture levels; the main differences lay primarily in the protein and fat content. Substrates with a higher proportion of meat residue showed higher fat and protein content.

Table 1. Nutritional characterization of substrates.

CR:MR	Moisture (%)	Crude Protein (%)	Fat (%)	Ash (%)
100:0	81.36 ± 0.60	12.42 ± 0.01	1.04 ± 0.01	1.83 ± 0.18
75:25	77.63 ± 0.29	23.07 ± 0.19	7.84 ± 0.37	1.63 ± 0.14
72.5:27.5	77.26 ± 0.35	24.13 ± 0.21	8.52 ± 0.41	1.61 ± 0.13
70:30	76.89 ± 0.42	25.19 ± 0.23	9.20 ± 0.44	1.59 ± 0.13
67.5:32.5	76.52 ± 0.49	26.26 ± 0.25	9.88 ± 0.48	1.57 ± 0.12
65:35	76.14 ± 0.57	27.32 ± 0.27	10.56 ± 0.52	1.55 ± 0.12
0:100	66.45 ± 2.60	55.00 ± 0.73	28.24 ± 1.06	1.02 ± 0.03

CR, coffee residue; MR, meat residue.

The nutritional characterization of the substrates, as shown in Table 1, revealed key distinctions between coffee and meat residues, as well as the mixtures derived from them. The moisture content remained relatively stable across all substrates, ranging from 66.45% to 81.36%. Coffee residues exhibited the highest moisture level (81.36%), likely due to their fibrous and absorbent nature [19]. In contrast, meat residues, although still high in moisture (66.45%), displayed significantly lower water content, which could be attributed to the more compact structure of meat tissue compared with plant-based coffee residues [20].

The most notable differences arose in protein and fat content. Meat residues contained a notably high protein level (55.00%) compared with coffee residues (12.42%), reflecting the nutrient-dense nature of animal by-products. This high protein content in meat residues was a critical factor when considering the potential nutritional value of the resulting products. Similarly, fat content was substantially higher in meat residues (28.24%) than in coffee residues (1.04%), suggesting that meat residues could provide a more energy-dense feedstock, potentially influencing subsequent processing applications [4,5].

In the mixtures, the proportion of meat residues correlated directly with increased protein and fat content. For instance, the mixture containing 35% meat residue showed a protein content of 27.32% and a fat content of 10.56%, indicating a balanced nutritional profile from both sources. The ash content remained relatively low and consistent across all samples, suggesting that the mineral content did not vary substantially with changes in composition.

3.2. Statistical Analysis and Model Evaluation for Protein Content Prediction

Table 2 presents the analysis of variance for each component of the model. The *p*-value indicated significant effects (*p*-value < 0.05) for both the linear and the quadratic terms. However, a strictly linear relationship was not observed between the mixture components and PCLM, as the statistical model indicated significant non-linear interactions. While a general correlation existed between the proportion of meat residues and the protein content, the predictive model revealed additional complexities. Interactions between coffee and meat residues, including potential inhibitory or synergistic effects, point out the need for non-linear terms to accurately represent the influence of the mixture on PCLM.

Table 2. Analysis of variance for protein (component proportions).

Term	<i>p</i> -Value
Regression	0.074
Linear	0.029
Quadratic	0.03
Coffee residue X meat residue	0.03
Inverse	0.051
1/coffee residue	0.029
1/meat residue	0.034

The obtained model equation is presented below in Equation (6).

$$PCML = -310142 * CR - 1055203 * MR + 915456 * CR * MR + \frac{222995}{CR} + \frac{6866}{MR} \quad (6)$$

where CR stands for coffee residue, and MR stands for meat residue.

Table 3 presents the protein content results and the predicted values from the proposed mixtures.

Table 3. Observed and predicted protein content of black soldier fly larval meal based on the percentage composition of coffee and meat residues.

Coffee Residue (%)	Meat Residue (%)	Length (mm)	Protein Observed (g/100 g db)	Protein Predicted (g/100 g db)
75	25	18.75 ± 1.69	31.89	31.42
65	35	18.80 ± 1.54	39.81	39.26
72.5	27.5	17.35 ± 1.46	32.34	31.85
67.5	32.5	17.98 ± 0.91	30.98	30.45
70	30	18.00 ± 0.72	37.57	36.41
70	30	18.19 ± 1.10	35.26	36.41
70	30	18.84 ± 1.13	38.85	36.41
70	30	17.44 ± 1.44	36.01	36.41
<i>R</i> ²				90.14
<i>Adj R</i> ²				76.77
<i>RMSE</i>				1.2269

The results from the mixture design experiments, as presented in Table 3, provided key insights into the relationship between the substrate composition and protein content in the larval meal. Coffee and meat residue proportions were varied to evaluate their impacts on both the observed and the predicted protein content.

The data indicated a consistent trend where increasing the proportion of meat residue in the substrate correlated with higher protein content in the larval meal. However, slight discrepancies, such as the 32.34% protein content observed for 27.5% meat residue compared with 30.98% for 32.5% meat residue, could be attributed to substrate heterogeneity, natural variability in larval metabolism, and potential interaction effects between the residues. These factors, inherent to biological systems, may lead to variations in nutrient assimilation and protein synthesis despite consistent experimental conditions.

The protein content varied with the mixture composition; however, the length of the larvae at day 14 remained relatively consistent across all experiments, with no significant differences observed. This suggested that the larval length was not directly related to the protein content in the final meal, indicating that the substrate impacted nutritional quality more than physical growth. Length measurements, ranging from 17.35 mm to 18.84 mm, did not show any clear pattern relative to the substrate composition.

The comparison between the observed and predicted protein content showed a good fit for the regression model. The R^2 value indicated that 90.14% of the variability in the protein content was explained by the model, and the adjusted R^2 of 76.77% suggested a strong model performance, with some room for improvement. The RMSE of 1.2269 indicated a reasonable degree of error between the predicted and observed values, further supporting the accuracy of the model accuracy in estimating the protein content based on the proportions of coffee and meat residues. According to Gutiérrez and Salazar [21], a regression model was considered to have an acceptable fit when the adjusted R^2 value exceeded 0.75.

3.3. Optimization of Substrate Composition for Maximizing Protein Content

Figure 1 presents the optimization results. The optimization process utilized a desirability function to determine the substrate composition that maximized the protein content in the larval meal. The desirability score, which ranged from 0 to 1, indicated the quality of the prediction, with values closer to 1 representing higher alignment with the optimization target. The results indicated that a meat residue proportion of 35% achieved the highest desirability score of 90%, reflecting optimal protein content in the larval meal.

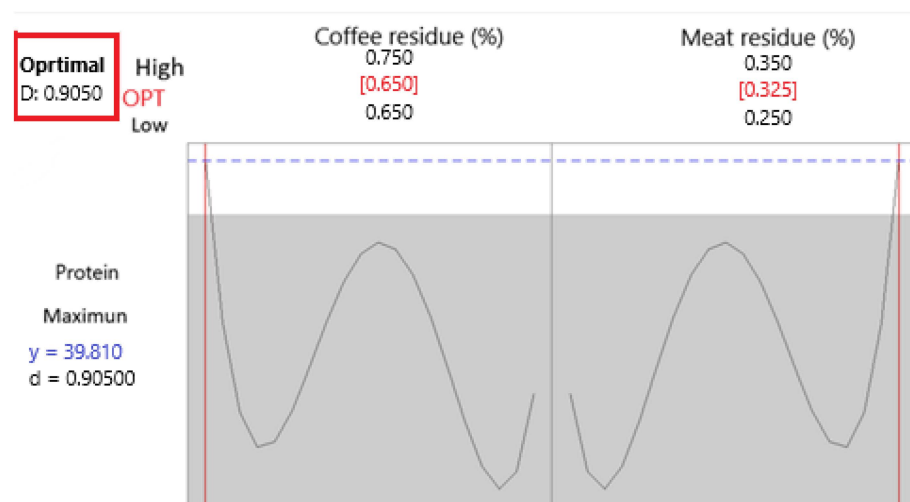


Figure 1. Optimization results for protein content.

3.4. Experimental Validation and Nutritional Quality of Optimized Larval Meal

An experimental run was conducted to verify the optimization results (Table 4). Test 1 and test 2 were replicates of the optimized conditions. The protein content of the optimized larval meal was found to be higher than that predicted by the model, with a

percentage error of 24% in the observation. This considerable error suggested that although key factors were used to develop the mathematical model, it may be necessary to include additional factors, such as climatic conditions (temperature and relative humidity) or the origin of the larvae. While these factors were controlled in the experiment, they were not incorporated as variables in the mathematical model.

Table 4. Optimization results.

Optimized	Protein (Observed)	Protein (Calculated)	RE
Test 1	52.97	39.81	24.8442515
Test 2	52.93	39.81	24.7874551

RE, relative error.

Table 5 presents the proximal and microbiological analysis of the larval meal obtained from larvae fed with the optimized substrate. The results indicated that the larval meal had low moisture content and water activity, suggesting that it could maintain stability over time. The fat content was consistent with the findings of Arena et al. [22]. The ash content was low compared with that reported by other authors [6]; however, the composition of the larval meal fell within the reference values of NTC 3688 [23] for aquaculture feed. These findings suggested that the larval meal could be used as feed for cultured fish.

Table 5. Nutritional composition of larval meal.

Optimized Larval Meal	
Moisture (%)	3.38 ± 0.12
Fat (%)	21.08 ± 1.19
Protein (%)	52.95 ± 0.03
Ash (%)	5.73 ± 0.33
Aw	0.41 ± 0.00
<i>E.coli</i> (CFU/g)	<10
<i>Bacillus cereus</i> (CFU/g)	200
Molds and yeasts (CFU/g)	7200
<i>Salmonella</i> (/25 g)	Absence

In terms of microbiological analysis, the larval meal was free from *E. coli* and *Salmonella*; however, the detectable levels of molds and yeasts were above the reference values established in NTC 3688. This indicated that additional thermal treatments were necessary to improve the microbiological quality of the larval meal.

4. Discussion

In general, black soldier fly larvae exhibited the ability to generate metabolic responses adapted to various substrates, which explained the lack of morphological differences in larvae fed with substrates of differing compositions [24]. Regarding the protein content in the larvae meal, differences were observed based on the supplied diet, with the highest protein content found in meals derived from larvae-fed substrates with higher protein levels. In all cases, the protein content of the larvae meal exceeded that of the substrate, demonstrating the larvae's capacity to convert nutrients from organic waste into high-quality protein. This conversion capacity increased when the substrate contained higher-quality protein [6,25].

The findings of this study highlight the potential of using black soldier fly larvae to valorize agro-industrial by-products, particularly coffee and meat residues, which are

commonly discarded as waste. This aligns with the broader sustainability objective of reducing food waste by converting it into valuable resources. By developing high-protein feed suitable for aquaculture, this study addresses the issue of organic waste disposal and provides a circular economy solution by reintegrating waste into the production chain as a nutrient source.

The larval meal obtained from larvae fed with the optimized mixture exhibited a high protein content, surpassing those reported by other studies. Research has shown that larvae fed organic waste can achieve protein content ranging from 30% to 46%, depending on the substrate [11]. Specifically, larvae fed animal waste reach a protein content between 21.71% and 41.90% [4,6,15], while those fed fruits and vegetables attain 36.20% and 24.36%, respectively [4,6,13]. Larvae fed with other type of residues present a protein content between 32.97% and 37.6% [13,14]. These findings indicate that using coffee waste and meat waste as feed sources for black soldier fly larvae is viable as they promote proper larval development and a high conversion rate of waste into protein.

The proximal analysis indicated that the larval meal had low moisture content and water activity, suggesting that it could maintain stability over time. The fat content was consistent with the findings of Arena et al. [22]. The ash content was low compared with that reported by other authors [6]; however, the composition of the larval meal fell within the reference values of NTC 3688 [23] for aquaculture feed. These findings suggested that the larval meal could be used as feed for cultured fish.

The optimization process employed in this study highlights the importance of fine-tuning substrate composition to enhance protein yields, demonstrating that increasing the proportion of meat residues up to 35% significantly boosts protein content. Protein-rich substrates support larval development, leading to a higher bioconversion rate and larval survival [25].

This targeted optimization approach reflects an efficient use of resources, enhancing the overall sustainability of the process by maximizing the output with minimal input changes. Such practices align closely with the United Nations' Sustainable Development Goals (SDGs) [26], particularly those focused on responsible consumption and production (SDG 12), as well as life below water (SDG 14), by reducing pressure on marine resources through alternative protein sources for aquaculture.

The results presented in this article demonstrate that black soldier fly larvae offer a viable alternative for the treatment of coffee and animal waste. However, while the current results are promising, further investigation into scaling up this approach would be beneficial, assessing the performance of larvae-derived protein in large-scale aquaculture environments, as well as investigating the impacts of larvae meal on fish growth, health, and overall feed conversion ratios.

The relative error observed under the optimization conditions indicates that although the mathematical model can predict the final protein content, other factors may influence the results. This is because the larvae are living organisms, and while their growth conditions can be controlled, external factors may accelerate their metabolism and the bioconversion rate. These factors include differences in enzymatic activity, variations in the substrate ingestion rate, or metabolic adaptative responses to minor environmental fluctuations.

5. Conclusions

The results of this study indicate that using coffee and meat residues is a viable approach for feeding black soldier fly larvae (*Hermetia illucens*). The optimized dietary composition, particularly with an increased proportion of meat residues up to 35%, resulted in significantly higher protein yields in the larval meal, demonstrating the critical role of balanced nutrient input in maximizing protein production. This finding features the

potential of black soldier fly larvae as effective bioconverters, transforming organic waste into high-quality, nutrient-dense protein that is suitable for use in animal feed, especially aquaculture. By utilizing agro-industrial by-products, this process not only addresses the pressing issue of food waste but also contributes to environmental sustainability by reducing the demand for conventional protein sources.

Although the statistical models used in this study showed a good fit, future studies are encouraged to include additional factors—such as climatic variables, the nutritional variability of feedstocks, and the geographic origin of the larvae—to further refine the predictive accuracy and adaptability across diverse environments.

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