

Feasibility Study on Lipid Extraction from *Hermetia Illucens* Larvae (Black Soldier Fly) for Potential Biodiesel Production

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The research evaluates the potential of producing biodiesel using *Hermetia Illucens* (black soldier fly) as a strategy for waste valorization at the Center for Research in Horticulture and native Plants (CIHPNA) of the UPAEP. The evaluated residues include feces from *Charolais*, *Beefmaster* and *European Swiss cattle and Dorper and East Friesian* sheep, with a combined daily generation of 390 kg/day. Additionally, tomato crop residues (*Solanum lycopersicum*) contribute 1.5 kg/day. These substrates were physicochemically characterized (pH, density, moisture, ash, total organic carbon-COT, Phosphorus-P, total Nitrogen-N and lignin content). The experimental design included three dependent variables: feeding rate (mg larva⁻¹ day⁻¹), larval density (larvae/m²), and type of larval diet. Larvae were fed at 444.4 and 666.6 mg larva⁻¹ day⁻¹, using 150 and 100 larvae, across five mixtures over 15 days. Lipid extraction was carried out using Soxtherm equipment. Prior to extraction, larvae were ground, and 0.5 g of dried biomass was mixed with 150 ml of ethyl ether. Fat content was determined gravimetrically. The highest fat yield (12.9%) was obtained in experiment 9 (460 g of sheep, 370 g of cattle and 170 g of tomato), followed by experiment 7 (400 g of sheep, 460 g of cattle and 140 g of tomato) with 11.3%, and experiments 8 (380g of sheep, 480g of cattle and 140g of tomato) and 10 (410 g of sheep, 430 g of cattle and 160 g of tomato) with 10.9%. All larvae selected for extraction were those fed at rate 666.666 mg larva⁻¹ day⁻¹.

1. Introduction

The Earth is currently facing one of the most severe ecological emergencies in history, driven by the overexploitation of natural resources to meet unsustainable social and economic demand. These pressures, characteristic of advanced industrial systems, have led to significant environmental degradation and negative impacts on human health (Rodríguez & López, 2020). Among the primary contributors to environmental damage are the burning of fossil fuels, excessive use of chemical, intensive agriculture, habitat destruction and unplanned urban growth.

Fossil fuels such as coal, crude oil, and natural gas have historically been the dominant sources of energy due to the high energy density and ease of transport and storage (Jung et al., 2022). However, their continued use contributes to climate change and environmental pollution, prompting the search for cleaner and more sustainable alternatives. In parallel, Mexico stands out as a major producer of vegetables, yet more than 50% of this production is discarded as an organic waste due to quality standard. In addition, livestock operations generate large quantities of untreated manure, which naturally decomposes anaerobically, releasing greenhouse gases and unpleasant odors (Cristerna-Sosa et al., 2022).

To address this challenges, biological treatments have gained attention as environmentally friendly waste management strategies. These methods employ living organisms such as bacteria, microalgae, earthworms, and insects to accelerate organic matter decomposition, recovering valuable elements like carbon and nitrogen in the form of biofuels and biomaterials (Liew et al. (2022); Gutiérrez et al., 2024). Among these, the black soldier fly (*Hermetia Illucens*) has emerged as a particularly effective solution. Its larvae can convert a wide range of

organic residues including food waste, animal manure, and agricultural by products into high fat biomass, suitable for biodiesel production (Su et al., 2019).

Hermetia Illucens offers several advantages: a short life cycle (10-31 days), rapid reproduction (females can lay up to 600 eggs per clutch), adaptability to diverse waste streams, and an optimal growth temperature of 27-33°C with 50-70% relative humidity (Oviedo and Gutiérrez, 2022). Moreover, larvae can reach fat levels of up to 40% of their dry weight, which can be extracted and converted into biodiesel. For instance, Singh & Kumari, (2019) demonstrated that oil extracted from 2,000 larvae raised on 1 kg of manure yielded 43.8 g of biodiesel, highlighting the potential of this species as a renewable energy source.

This study aims to evaluate the feasibility of producing biodiesel using *Hermetia Illucens* larvae as a biological conversion agent for agricultural and livestock waste generated at the Center for Research in Horticulture and native Plants (CIHPNA) of UPAEP. The research focuses on determining the optimal feeding conditions and substrate mixtures that maximize fat yield for potential biodiesel applications. The main objectives of this research are:

1. To evaluate the effect of feeding rate, larval density, and diet composition on biomass and fat accumulation in *Hermetia Illucens* larvae.
2. To extract and quantify the fat content of larvae fed with different organic waste combinations using gravimetric analysis.
3. To identify the most efficient waste mixture for maximizing lipid yield suitable for biodiesel conversion

2. Methodology

2.1 Design of experiment

To conduct the study, an initial diagnostic assessment was carried out using the quartic method to quantify the daily waste generated at the CIHPNA. The analyses revealed that the primary residues originated from *Charolais*, *Beefmaster* and *European Swiss cattle* and *Dorper* and *East Friesian* sheep, with a total output of approximately 390 kg/day. Additionally, tomato crops (*Solanum lycopersicum*) contributed around 1500 kg/day of organic waste.

Based on this findings, these material were selected as substrates for larval feeding. Subsequently, physicochemical characterization was performed on the three substrate types, evaluating parameter including Ph, moisture, ash, TOC, P, N and lignin content.

Following substrate characterization, a completely randomized design was established. The experiment considered three independent variables: feeding rate (mg per larva per day), larval density (#larvae/m²) and type of larval diet (specific mixtures of these selected residues). The experimental period lasted 15 days, after which results were recorded and are presented in Table 1.

Table 1: Randomized design of experiment

Number of experiments	Feed Rate mg.larva/day	Number of larvae	Sheep feces (g)	Bovine feces (g)	Tomato waste (g)
Ex 1	444.4	150	470	380	150
Ex 2	444.4	150	400	460	140
Ex 3	444.4	150	380	480	140
Ex 4	444.4	150	460	370	170
Ex 5	444.4	150	430	410	160
Ex 6	666.6	100	470	380	150
Ex 7	666.6	100	400	460	140
Ex 8	666.6	100	380	480	140
Ex 9	666.6	100	460	370	170
Ex 10	666.6	100	430	410	160

2.2 Fat removal

The fat extraction method used was the "Hydrotherm total fat in fish feed (for aquaculture), described in the Soxtherm equipment manual, since this was the equipment used to carry out the procedure.

As a first step, the larvae were dried in a convection oven at 100 °C for 4 h, in order to reduce their moisture content by 80% and reach a constant weight. Subsequently, the dried samples were ground in a mortar until a homogeneous powder was obtained and stored under refrigeration for conversion.

Then, 3 to 5 boiling stones are added to each extraction beaker. The beakers were then placed in the oven at 100 °C for 1 hour to remove moisture, then allowed to cool in the desiccator and weighed to an accuracy of ± 1 mg.

Next, 3 to 5 g of sample were weighed and placed on cartridge paper. The paper with the sample was inserted into the extraction thimble and covered with absorbent cotton. Then, 100 ml of ether was added as extraction solvent. Finally, the Soxhlet equipment was programmed to run the extraction process where it was programmed to perform 5 refluxes and waited until the completion of the process.

At the conclusion of the extraction program, the beakers were placed in the oven at 70 °C for 45 minutes. Then, they were moved to the desiccator and allowed to cool. Finally, the beakers were weighed to the nearest 1 mg. For the calculation of the total fat extracted was done by gravimetric method where the formula used for the total fat content is shown below.

$$w = \frac{(m^2 - m^1) * 100}{m_0} \quad \text{Eq (1)}$$

Where:

W: Total Fat Content

m¹ : mass of the empty extraction vessel with boiling stones (g)

m²: mass of the extraction vessel with grease after drying (g)

m₀: initial sample weight (g)

The result is rounded to one decimal place.

3. Results and Discussion

A The results of the fat percentages extracted in each of the experiments are shown in Table 2.

Table 2: Fat Removal Results

Number of experiments	Feed Rate mg.larva/day	Number of larvae	Sheep feces (g)	Bovine feces (g)	Tomato waste (g)	Total Fat Content (W %)
Ex 1	444.4	150	470	380	150	2.9
Ex 2	444.4	150	400	460	140	4.8
Ex 3	444.4	150	380	480	140	6.4
Ex 4*	444.4	150	460	370	170	45.2*
Ex 5	444.4	150	430	410	160	8.4
Ex 6	666.6	100	470	380	150	8.4
Ex 7	666.6	100	400	460	140	11.3
Ex 8	666.6	100	380	480	140	10.9
Ex 9	666.6	100	460	370	170	12.9
Ex 10	666.6	100	430	410	160	10.9

The fat extraction results obtained in this study demonstrated significant variability among the different larval diet mixtures, clearly indicating that the composition of the substrate plays a decisive role in lipid accumulation by *Hermetia illucens* larvae. The influence of the type of organic waste used as a larval diet on the percentage of lipids is consistent with the findings of Oviedo et al. (2022), who emphasize that the nutritional content of the substrate directly impacts the metabolic conversion of biomass into fats. According to their study, larvae fed with oil rich organic waste accumulated between 42% and 49% lipids, while those fed with bovine manure achieved up to 35%, and larvae fed with poultry litter showed lower fat percentages, ranging between 15% and 25%.

In the present research, although the overall fat percentages were lower than those reported by Oviedo et al., a distinct trend was observed: larval diets with higher proportions of sheep feces consistently resulted in greater fat accumulation. The highest percentage of extracted fat was obtained in experiment 9, with 12.9%, using a substrate composed of 460 g of sheep, 370 g of cattle manure and 170 g of tomato residues. This was followed

by experiment 7, with 11.3% fat, and experiment 8 and 10, both with 10.9% fat. These results suggest that sheep feces, due to their highest digestibility and possibly better nutrient profile, may enhance lipid biosynthesis in larvae when compared to cattle manure or tomato residues.

Table 3: ANOVA results for larval fat content

Origin of variations	Sum of squares	Degrees of freedom	Average of the squares	F	Probability	Critical value for F
Between groups	163.8244	8	20.4780	4.422768769	0.019758253	3.229582613
Inside the groups	41.625	9	4.625			
Total	205.4494	17				

A summary of the one-way ANOVA applied to assess differences in fat content across the treatments is presented in table 3.

The analysis yielded an F value of 4.43 and a P value of 0.0197, indicating that there are statistically significant differences in the fat content among at least two of the experimental treatments. Since the P value is below the standard significance threshold of 0.05, it can be concluded that the composition of the larval diet had a measurable and significant effect on lipid accumulation in *Hermetia illucens* larvae.

Furthermore, an analysis of the feeding rate variable revealed that all the experiments with the higher feeding rate of 666.6 milligrams per larva per day. This finding supports the notion that a greater availability of organic matter allows for higher metabolic activity and, consequently, greater lipid deposition. This is consistent with the work of Su et al. (2019), who report that the optimizing feeding rates is essential for maximizing larval growth and lipid conversion efficiency. In this context, the combination of substrate quality (high in sheep feces) and feeding rate (nutrient availability) appears to be critical for enhancing the bioconversion process.

It is important to note that while tomato crop residues are not particularly lipid rich, their inclusion may have contributed to maintaining the moisture balance and carbon to nitrogen ratio (C: N), which are important parameters for microbial activity and substrate palatability. Santiago et al. (2022) demonstrated that tomato residues with a C: N ratio of approximately 19:1 and high moisture content can enhance microbial activity and nitrogen mineralization in soil substrates.

A limitation of the study was observed in experiment 4, which was marked with an asterisk due to a procedural error during the fat extraction process. The sample was inadvertently exhausted during the analysis, and it was not possible to repeat the procedure under the same condition. While this limits the comparison across all experiments, the consistency of the data from the remaining treatments still allows for robust conclusion.

Figure 1 shows the graph of fat extraction.

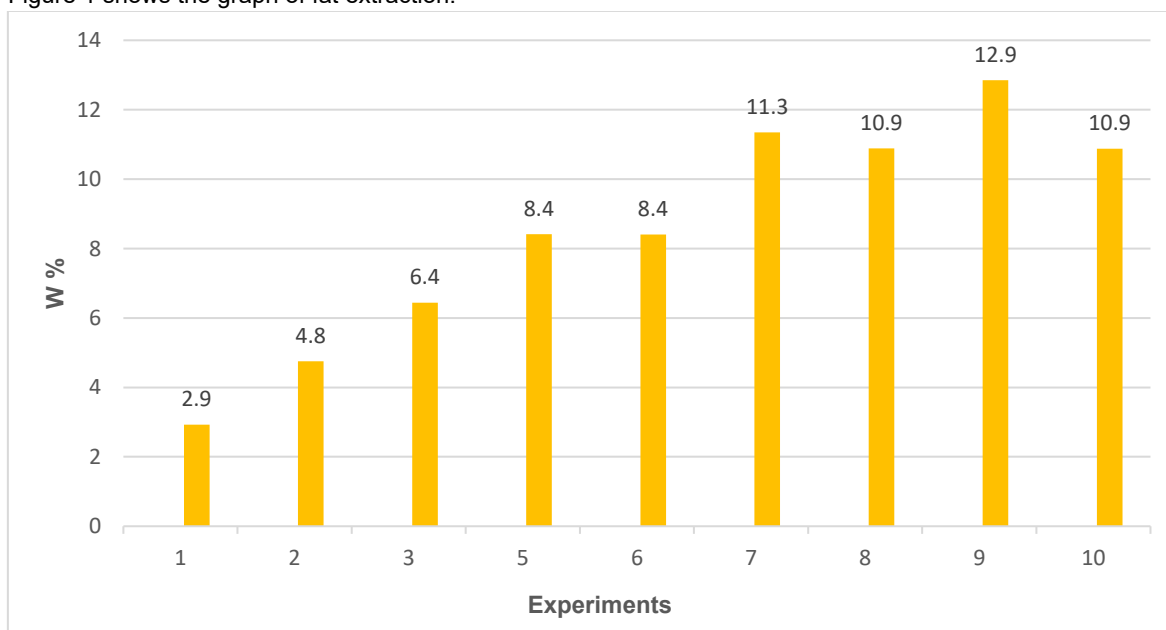


Figure 1: Fat extraction

It can be observed that experiment 9 presented the highest fat content among all trials, reaching 12.9% with a substrate composed of 460 g of sheep manure, 370 g of cattle manure and 170 g of tomato residues. This mixture, characterized by a relatively high proportion of sheep waste and a moderate inclusion of tomato residues, appears to provide a favorable nutrient profile for lipid biosynthesis in the larvae. Following closely by experiment 7, 8 and 10 which are shown in figure 1.

All these experiments shared a high feeding rate of 666.6 mg/larva-day, which allowed to standardize the quantity of ingested substrate, thus ensuring that observed differences in fat content were attributable primarily to substrate composition rather than feeding quantity. This observation supports the findings of Widjastuti et al. (2021), who emphasized that both the nutrient profile and digestibility of organic residues significantly influence the larval biochemical composition, particularly lipid accumulation.

The results from this study underscore the pivotal role of substrate formulation in optimizing fat yields in *Hermetia illucens*. In particular, sheep manure appears to offer a superior nutritional substrate, likely due to its favorable carbon to nitrogen ratio and fatty acid precursors, enhancing larval lipid biosynthesis, a phenomenon similarly reported by Mwaniki et al. (2021) in trials using pig and poultry manures.

When compared to other studies, the maximum lipid yield in this work (12.9%) is modest. For instance, Olivar Barrero et al. (2021) achieved 21% fat using pig manure, which is known for its higher fat and protein content compared to ruminant waste. Similarly, Cabrera & López (2021) reported 30% to 70% fat in larvae fed with a mixture of rice straw and restaurant organic waste, showing the potential of high energy substrates to maximize fat deposition. Moreover, Acosta & Guzman (2022), in a 23-day trial, reported 34.6% and 39.8% fat in larvae fed with fresh vegetable fruit waste and custom waste mixtures, respectively. These valuable notably exceed those obtained in the present study, reaffirming that substrate energy density and lip availability are key drivers in larval fat synthesis.

These discrepancies may be attributed to both the nature of organic residues and the duration of larval development, as longer rearing periods and energy rich feedstock enhance fat accumulation. Nonetheless, this study contributes valuable insights into the use of mixed ruminant and agricultural waste for larval biomass production, highlighting that even substrates considered less lipid rich such as tomato residues can play a supportive role in substrate structure, microbial activity, and moisture balance when properly proportioned.

4. Conclusions

The results obtained in this study underscore the importance of substrate composition in influencing the biochemical characteristics of *Hermetia illucens* larvae, particularly in terms of fat accumulation. Among the ten experimental treatments evaluated, the highest fat content (12.9%) was obtained in larvae fed with a substrate composed predominantly of sheep feces, moderate cattle manure, and a small proportion of tomato crop residues. This specific formulation demonstrated a favorable balance of nutrients, likely contributing to improved larval metabolism and lipid biosynthesis.

A key finding was the significant influence of sheep manure and larval fat content, which may be attributed to its higher organic matter content, favorable C: N ratio, and potentially higher digestibility compared to cattle manure. Although tomato residues are not lipid rich, their inclusion in moderate quantities appears to support substrate moisture, microbial activity, and palatability factors which indirectly promote nutrient assimilation by the larvae. However, the study also revealed that excessive tomato content may dilute the nutritional density mixture, resulting in reduced fat yield.

Furthermore, the feeding rate played a critical role in standardizing results across treatments. By maintaining a high feeding rate of 666.6 mg/larva-day, it was possible to isolate the effect of substrate composition on lipid accumulation, minimizing variability due to ingestion levels. This methodological consistency adds robustness to the conclusions and reinforces the correlation between waste formulation and larval biochemical output.

When compared to other studies in the field, such as those conducted by Oliver Barreto et al. (2021), Cabrera & López (2021), and Acosta & Guzman (2022), it is evident that substrates with higher energy or lipid content such as pig manure, restaurant waste or fruit vegetable mixtures tend to produce larvae with higher fat content, in some cases exceeding 30-70%. While the values reported in this study are comparatively lower, the use of readily available ruminant manures and agricultural residues offers a more sustainable and locally adaptable alternative, especially in rural or agricultural regions where such wastes are abundant.

In addition to their nutritional potential, the valorization of these residues through bioconversion processes involving *Hermetia illucens* aligns with circular economy principles, reducing environmental impacts associated with traditional waste disposal and offering promising pathways for producing insect based fat as potential feedstock for biofuel production or animal nutrition.

Nevertheless, further research is required to optimize substrate formulations for higher lipid output, investigate longer larval growth periods, and explore pretreatment methods. Moreover, biochemical profiling of the extracted fats would provide critical data to assess their industrial applications.

In conclusion, this study demonstrates that black soldier fly larvae can effectively transform a combination of livestock and agricultural waste into a value added biomass with notable fat content. The findings contribute to the growing body of knowledge on sustainable insect rearing and highlight the potential of locally sourced organic waste streams for enhancing lipid production in *Hermetia illucens* and finally, a lipid profile analysis is recommended to determine the composition of the fatty acids present in the extracted fat. This will allow to evaluate the feasibility for biodiesel production and explore its potential in industrial applications.

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