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Optimizing Black Soldier Fly (*Hermetia illucens*) Larval Growth on Spent Grain and Cow Dung

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ABSTRACT

Background: Growing global demand for sustainable animal protein has intensified interest in alternative feed resources. Black soldier fly (*Hermetia illucens*) larvae are increasingly recognized for their ability to convert organic wastes into nutrient-rich biomass suitable for animal feed. However, larval productivity is strongly influenced by substrate type and feeding strategy. This study investigated the growth performance, biomass yield, and population stability of *H. illucens* larvae reared on spent grain (SG) and cow dung (CD) under a progressively increasing feeding regime.

Methods: The study was conducted at the Biological Garden of the Department of Biological Sciences, University of Abuja, Nigeria. A completely randomized design was adopted using two substrates (SG and CD) with two replicates per treatment. Each rearing container was stocked with approximately 600 first-instar larvae. Feed quantity increased progressively from 1 kg on Day 1 to 5 kg on Day 12 to match larval nutritional demand. Larval biomass and population counts were measured on Days 1, 3, 6, 9, and 12. Data were expressed as mean ± standard deviation and analyzed using Student's T-test at $P < 0.05$.

Results: Larval biomass increased steadily with time in both substrates, but remained consistently higher in SG. By Day 3, mean biomass reached 53.44 ± 2.71 g in SG and 43.62 ± 8.66 g in CD. Growth continued by Day 6, with biomass values of 102.52 ± 17.02 g for SG and 75.47 ± 21.21 g for CD. A moderate increase was observed by Day 9 (116.64 ± 23.56 g in SG; 87.87 ± 33.73 g in CD). The most substantial biomass gain occurred between Days 9 and 12, when larvae reared on SG reached 1907.07 ± 242.45 g, significantly exceeding the 425.61 ± 11.12 g recorded for CD ($P = 0.012$). Maximum daily growth rates during this final phase were 596.81 g/day in SG and 112.58 g/day in CD. In contrast, larval population counts remained relatively stable throughout the experiment with no significant differences between substrates ($P > 0.05$).

Conclusion: The findings demonstrate that spent grain supports substantially greater biomass production than cow dung under progressive feeding conditions, highlighting its potential as an efficient substrate for large-scale *H. illucens* larval production and organic waste valorization.

Keywords: Black soldier fly larvae, *Hermetia illucens*, spent grain, Larval biomass, Organic waste bioconversion

1.0 INTRODUCTION

The increasing global demand for sustainable sources of animal protein has intensified the search for alternative feed ingredients that can reduce reliance on conventional resources such as fishmeal and soybean meal [1]. In recent years, insects have emerged as promising alternatives due to their high nutritional value, efficient feed conversion, and ability to recycle organic waste. Among these insects, the black soldier fly, *Hermetia illucens*, has attracted considerable attention because its larvae contain high levels of protein, lipids, essential amino acids, and minerals, making them suitable for incorporation into poultry, fish, and livestock diets [2]. Furthermore, the ability of *H. illucens* larvae to convert various organic residues into valuable biomass aligns with the principles of sustainable agriculture and circular bio-economy [2, 3].

Black soldier fly larvae (BSFL) can be reared on a wide range of organic substrates, including agricultural by-products, food waste, and animal manure. The type and quality of substrate used for larval feeding significantly influence larval growth, development rate, nutrient composition, and overall biomass yield [4]. Previous studies have shown that substrate composition, feeding rate, and environmental conditions play critical roles in determining larval productivity in mass-rearing systems [5]. Consequently, optimizing substrate selection and feeding strategies is essential for improving the efficiency and scalability of insect farming systems.

In addition to substrate quality, feeding regimes also influence larval growth dynamics and biomass accumulation. Progressive feeding strategies that gradually increase the quantity of feed supplied during larval development may enhance nutrient availability and reduce competition among larvae, thereby promoting optimal growth. However, excessive or poorly managed feed inputs can also lead to substrate deterioration, microbial competition, and reduced larval performance. Understanding how larvae respond to different feeding regimes and substrates is therefore important for designing efficient production systems that maximize biomass output while maintaining stable larval populations [5, 7].

Spent grain and cow dung are two widely available organic substrates that have potential applications in black soldier fly larval production. Spent grain, a by-product of brewing industries, is rich in carbohydrates, proteins, and residual nutrients. In contrast, cow dung is abundant in agricultural settings and represents a low-cost organic waste resource [8, 9]. Evaluating the growth performance and biomass yield of *H. illucens* larvae on these substrates under progressively increasing feeding regimes can provide valuable insights into the suitability of these substrates for large-scale insect farming. Therefore, this study aimed to compare the growth rate, biomass yield, and larval population stability of *H. illucens* larvae reared on spent grain and cow dung substrates across different developmental stages.

2.0 METHODOLOGY

2.1 Study Area

The experiment was conducted at the Biological Garden of the Department of Biological Sciences, University of Abuja, located in Gwagwalada Area Council, Federal Capital Territory, Nigeria. The garden serves as a semi-controlled outdoor research facility used for biological (including insect rearing) and ecological experiments. The area experiences a tropical savanna climate characterized by warm temperatures and moderate humidity, conditions suitable for the development of the black soldier fly, *Hermetia illucens* [10, 11]. During the experimental period, the rearing units were maintained under natural environmental conditions, with ambient temperature ranging from approximately 27–32 °C and relative humidity from 60–75%.

2.2 Experimental Design

The experiment was designed to evaluate the effect of progressively increasing feed quantity on the biomass production and growth rate of *H. illucens* larvae reared on two different organic substrates: spent grain (SG) and cow dung (CD). The study followed a completely randomized design with two substrate treatments and two replicates per treatment (N = 2) [12].

Plate 1. *Hermetia illucens* larvae

A total of four rearing containers were prepared, consisting of two containers for SG and two for CD. Each container was initially stocked with 600 first-instar larvae obtained from a laboratory colony maintained at the Ento'O'rganics fly farm, Iperu Remo, Ijebu, Ogun State, Nigeria (Plate 1) [12, 13]. The containers were randomly arranged within the rearing area to minimize positional bias and ensure uniform exposure to environmental conditions [13].

2.3 Substrate Preparation

Two organic substrates were used as larval feed:

Spent Grain (SG): Brewery spent grain was obtained from Kuje local farmers' market, Kuje Area Council, Abuja, Nigeria (Plate 2). The material was transported to the laboratory and allowed to stabilize for 24 hours before use. Large debris was removed, and the substrate was homogenized to ensure uniform nutrient distribution.

Plate 2. Fermented Spent Grain Substrate

Plate 3. Fermented cow dung Substrate

Cow Dung (CD): Dry cow dung was collected from the University of Abuja, Animal Farms, and fermented (Plate 3). The material was sieved to remove stones and large fibrous material, then thoroughly mixed to produce a uniform substrate.

Each substrate was weighed on a digital balance before being introduced into the rearing containers.

2.4 Larval Rearing Procedure

Plastic rearing containers (approximately 40 cm × 30 cm × 20 cm) were used for the experiment. Each container was perforated on the sides to allow adequate aeration and prevent moisture accumulation. A layer of the prepared substrate was placed into each container to serve as the feeding medium.

Larvae were introduced into the substrates and allowed to acclimatize. Feeding quantity was progressively increased throughout the experimental period in response to the rising nutritional demands of larval development. The feeding schedule was as follows:

Feeding schedule used during the rearing of *Hermetia illucens* larvae, showing a progressive increase in feed quantity over time. On the first day of rearing, the larvae were supplied with 1 kg of feed. The feed quantity was increased to 2 kg on Day 3 and to 3 kg by Day 6. By Day 9, the larvae received 4 kg of feed, and this was increased to 5 kg on Day 12. This gradual increment in feed quantity was designed to match the increasing nutritional demands of the growing larvae as their biomass and feeding capacity increased over the rearing period. Additional feed was added directly onto the existing substrate layer without disturbing the developing larvae. Moisture levels were maintained by occasional sprinkling of distilled water when necessary to prevent substrate desiccation.

2.5 Measurement of Larval Biomass

Sampling of larval biomass was conducted on Days 1, 3, 6, 9, and 12 of the rearing period. During each sampling event, larvae were gently separated from the substrate using a sieve. The recovered larvae from each replicate were rinsed lightly with distilled water to remove adhering substrate particles and then blotted dry with absorbent paper [14].

With an initial larval biomass of 10.2 kg per substrate before the experiment, the total larval biomass for each replicate was measured with a precision digital balance and recorded in grams [14]. Mean larval weight and standard deviation were subsequently calculated for each treatment. After measurement, larvae were returned to their respective containers to continue development.

2.6 Determination of Larval Population Count

Larval population counts were determined simultaneously with biomass measurements. A representative subsample of larvae was manually counted to estimate the population size per container. Counts were recorded as mean ± standard deviation for each treatment across the sampling days.

2.7 Growth Rate Analysis

The growth rate was calculated from changes in larval biomass between consecutive sampling intervals. The weight increase between sampling days was calculated as the difference between the subsequent and previous mean biomass. Daily growth rate (g/day) was calculated as the weight increase divided by the number of days between sampling intervals [15]. Percentage growth was calculated as the proportional increase relative to the preceding biomass value.

2.8 Statistical Analysis

All experimental data were expressed as mean ± standard deviation. Differences in larval biomass and larval counts between spent grain (SG) and cow dung (CD) substrates across the rearing days were analyzed using Student's t-test at a significance level of $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA).

3.0 RESULTS

The effect of progressively increasing feed quantity on larval biomass is summarized in Table 1. No measurable larval weight was observed on Day 1 at the initial feed level of 1 kg in either substrate (0.00 ± 0.00 g). However, by Day 3, when feed was increased to 2 kg, larval biomass rose considerably to 48.44 ± 2.71 g in the SG substrate and 38.62 ± 8.66 g in the CD substrate, demonstrating an early positive growth response to improved nutrient supply. On Day 6 (3 kg feed), larval weights were 49.08 ± 14.31 g in SG and 31.82 ± 12.52 g in CD. While SG maintained relatively higher biomass, the larger standard deviations, particularly in CD, indicate increased variability in growth among replicates. Interestingly, despite a further rise in feed quantity to 4 kg by Day 9, larval biomass declined markedly in both substrates, falling to 14.12 ± 6.58 g in SG and 12.40 ± 12.52 g in CD.

Table 1. Effect of Increasing Feed Quantity on Larval Weight (Mean ± SD) Across Developmental Days in SG and CD Substrates

Day	Feed Quantity (kg)	SG Substrate	CD Substrate
1	1	0.00 ± 0.00	0.00 ± 0.00
3	2	48.44 ± 2.71	38.62 ± 8.66
6	3	49.08 ± 14.31	31.82 ± 12.52
9	4	14.12 ± 6.58	12.40 ± 12.52
12	5	1790.42 ± 266.11	337.74 ± 22.61

Furthermore, Figure 1 below presents the progression of black fly larval growth on spent grain (SG) and cow dung (CD) substrates across the 12-day rearing period. Mean larval weights rose between days 3 and 6 in both treatments, with larvae maintained on SG consistently attaining higher values than those on CD. A slow increase in average weight was observed on day 9 for both substrates, followed by a pronounced increase by day 12. By the end of the experiment, larvae reared on SG exhibited markedly greater biomass than those reared on the CD substrate.

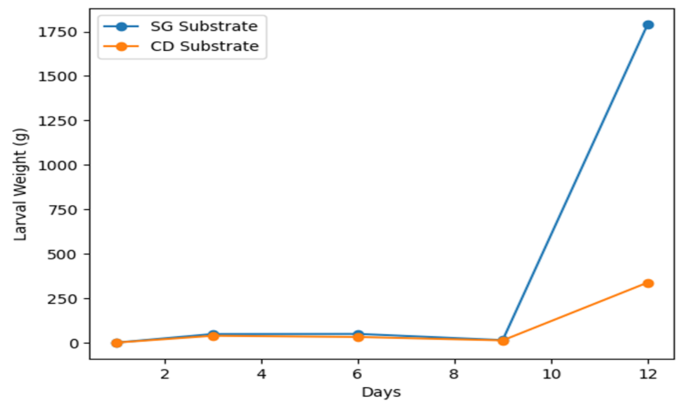

Figure 1. Growth Response of Black Fly Larvae on Spent Grain and Cow Dung Substrate

Table 2 presents the mean (± SD) larval weight and population count of *H. illucens* reared on spent grain (SG) and cow dung (CD) substrates across the experimental period. Larval weight increased progressively with time in both treatments; however, individuals reared on SG consistently attained significantly higher weights than those on CD from day 3 onward ($P < 0.05$). By day 12,

Table 2. Mean Larval Weight and Count of *H. illucens* Reared on Spent Grain and Cow Dung Substrates

Days	SG Weight (g) (Mean ± SD)	CD Weight (g) (Mean ± SD)	SG Larvae Count (Mean ± SD)	CD Larvae Count (Mean ± SD)
1	0.50 ± 0.00[a]	0.50 ± 0.00[a]	531.5 ± 123.04[a]	577 ± 230.31[a]
3	53.44 ± 2.71[a]	43.62 ± 8.66[b]	530.5 ± 125.17[a]	572 ± 226.27[a]
6	102.52 ± 17.02[a]	75.47 ± 21.21[b]	542.5 ± 140.01[a]	567 ± 228.64[a]
9	116.64 ± 23.56[a]	87.87 ± 33.73[b]	542 ± 140.01[a]	566.5 ± 226.73[a]
12	1907.07 ± 242.45[a]	425.61 ± 11.12[b]	542.5 ± 140.01[a]	566.5 ± 226.73[a]

N = 2; values carrying different subscripts along the rows are significantly different at *P* Å0.05

larvae cultured on SG exhibited a significantly ($p = 0.012$) greater mean weight (1907.07 ± 242.45 g) compared to those reared on CD (425.61 ± 11.12 g). In contrast, larval counts remained relatively stable throughout the rearing days in both treatments, with no significant differences observed between substrates ($P > 0.05$).

Growth rate analysis of H. illucens larvae reared on spent grain (SG) and cow dung (CD) substrates revealed distinct temporal differences in biomass accumulation (Table 3). Between days 1 and 3, larvae exhibited rapid early growth in both substrates, with biomass increasing by 52.94 g in SG and 43.12 g in CD, corresponding to daily growth rates of 26.47 g/day and 21.56 g/day, respectively. During the subsequent interval (days 3–6), growth remained higher in SG, where larvae gained 49.08 g (16.36 g/day), compared with 31.85 g (10.62 g/day) in CD.

Table 3: Growth rates of *H. illucens* larvae reared on spent grain (SG) and cow dung (CD) substrates

Growth Interval (Days)	SG Weight Increase (g)	SG Daily Growth (g/day)	SG % Growth	CD Weight Increase (g)	CD Daily Growth (g/day)	CD % Growth
1–3	52.94	26.47	10588.0	43.12	21.56	8624.0
4–6	49.08	16.36	91.82	31.85	10.62	73.02
7–9	14.12	4.71	13.78	12.40	4.13	16.43
10–12	1790.43	596.81	1534.60	337.74	112.58	384.30

A marked decline in growth rate was observed between days 6 and 9 in both treatments, with biomass increments of only 14.12 g (4.71 g/day) in SG and 12.40 g (4.13 g/day) in CD. However, a pronounced increase in biomass occurred during the final rearing phase (days 9–12), particularly in the SG substrate, where larval weight increased by 1790.43 g, representing a daily growth rate of 596.81 g/day. In contrast, larvae reared on CD recorded a comparatively smaller increase of 337.74 g (112.58 g/day) during the same period. Overall, larvae cultured on spent grain consistently demonstrated higher growth rates across all developmental intervals compared with those reared on cow dung.

4.0 DISCUSSION

The present study examined the growth performance of *Hermetia illucens* larvae under progressively increasing feed quantities using two organic substrates: spent grain (SG) and cow dung (CD). The absence of measurable larval biomass on day 1 at the initial feed level of 1 kg across both substrates likely reflects the larvae's early acclimatization phase. At this stage, larvae are newly introduced to the substrate and typically require time to adapt to the environmental conditions and initiate active feeding. During this period, most metabolic activity is directed toward physiological adjustment rather than measurable biomass accumulation. Similar observations have been reported in previous studies, in which early-instar *H. illucens* larvae exhibit minimal detectable growth during the first 24–48 hours after introduction to a new feeding substrate [16, 17]. This lag phase is considered a normal part of the larval developmental cycle and reflects the process of substrate exploration and the initiation of microbial digestion.

A marked increase in larval biomass was observed by day 3 when the feed quantity increased to 2 kg. At this stage, larval biomass reached 48.44 g in SG and 38.62 g in CD, indicating a strong growth response to increased nutrient availability. Early larval stages of black soldier flies are characterized by high feeding intensity and efficient nutrient conversion, which explains the rapid increase in biomass during this phase. Studies have consistently shown that the growth of *H. illucens* larvae is highly dependent on nutrient density and feed availability, particularly during the early instars when feeding activity is most pronounced [18]. The higher biomass recorded in the SG substrate compared with CD can likely be attributed to differences in nutrient composition. Brewery spent grain generally contains higher levels of protein, fermentable carbohydrates, and residual nutrients, making it a more suitable substrate for larval development than livestock manure [19].

By day 6, larvae maintained on SG continued to exhibit higher biomass (49.08 g) compared with those reared on CD (31.82 g). Although growth continued in both substrates, the larger standard deviations observed, especially in the CD treatment, suggest increased variability among replicates. Such variability is commonly reported when larvae are reared on heterogeneous substrates like animal manure. Cow dung often varies in nutrient distribution, moisture content, and microbial composition, which can influence larval feeding behavior and growth patterns. Similar variability in growth has been documented in earlier studies, in which manure-based substrates produced less consistent larval growth than agro-industrial residues [20]. Differences in microbial competition within the substrate may also contribute to these variations, as microorganisms can compete with larvae for available nutrients [21].

An interesting pattern emerged on day 9 when larval biomass declined substantially in both treatments despite a further increase in feed quantity to 4 kg. Rather than promoting additional growth, the larger substrate volume may have created less favorable environmental conditions within the rearing medium. Excessive substrate can reduce aeration, increase moisture retention, and promote anaerobic microbial activity. These conditions can negatively affect larval feeding efficiency and metabolic performance. Previous research has shown that overly thick substrate layers can limit oxygen diffusion and increase the accumulation of metabolic heat and ammonia, which may temporarily suppress larval growth [22]. Therefore, the decline observed around day 9 likely reflects suboptimal substrate conditions rather than a limitation in nutrient availability.

Despite this temporary slowdown, larval biomass increased dramatically by day 12, particularly in the SG treatment. At this stage, larvae reared on SG achieved a mean biomass of over 1900 g, while those reared on CD reached approximately 426 g. This large increase corresponds to the final active feeding stage of the larval life cycle, during which larvae rapidly accumulate nutrients in preparation for the prepupal stage. The considerably higher biomass obtained in the SG treatment suggests that nutrient-rich agro-industrial by-products can substantially enhance larval productivity. Similar patterns have been reported in other BSFL studies, in which substrates rich in organic nutrients supported significantly greater biomass production than manure-based substrates [23, 24].

The results of the present study further confirm the influence of substrate quality on larval growth. From day 3 onward, larvae reared on SG consistently attained significantly greater weights than those on CD, while larval counts remained relatively stable in both treatments. The stability in larval numbers indicates that survival rates were comparable across substrates and that the observed differences were primarily related to growth performance rather than mortality. This finding aligns with previous reports that substrate composition typically influences larval size and biomass accumulation more strongly than survival under adequately maintained environmental conditions [25].

The growth rate analysis in Table 3 provides additional insights into the temporal dynamics of larval development. During the first growth interval (days 1–3), larvae exhibited rapid biomass accumulation in both substrates, with SG showing slightly higher daily growth rates than CD. This phase corresponds to the period when larvae actively begin feeding and converting organic matter into body tissue. Rapid early growth has been widely reported in BSFL production systems and reflects the larvae's high feed conversion efficiency [26].

Between days 3 and 6, the growth rate remained higher in SG than in CD, reinforcing the nutritional advantage of spent grain as a substrate. However, the slowdown in growth observed between days 6 and 9 suggests that environmental or physiological constraints may have temporarily limited biomass accumulation. Similar mid-development growth reductions have been reported in BSFL systems and are often associated with substrate compaction, reduced oxygen availability, or nutrient redistribution within the substrate [27].

The most significant increase in biomass occurred during the final growth interval (days 9–12), particularly in the SG treatment. Larvae reared on SG showed a dramatic surge in growth rate during this period, indicating a phase of accelerated biomass accumulation before pupation. This stage is widely recognized as the peak feeding phase in BSFL development, during which larvae store large amounts of nutrients as fat and protein reserves for metamorphosis [28]. The comparatively lower growth rate observed in the CD treatment further highlights the limitations of manure-based substrates in supporting maximal larval growth.

The outcomes of this study provide important insights for the optimization of black soldier fly larval production systems. First, the superior growth performance observed in spent grain demonstrates the potential of agro-industrial by-products as highly effective substrates for large-scale BSFL production. Using such organic residues not only enhances larval biomass yield but also supports sustainable waste management by converting organic waste into valuable protein resources for animal feed [26].

Second, the results emphasize the need for careful management of feed quantity during larval rearing. While increasing feed availability initially promotes growth, excessive substrate accumulation may create unfavorable conditions such as reduced aeration and increased microbial competition. This suggests that optimal feeding strategies should consider both nutrient availability and physical substrate characteristics to maintain favorable rearing conditions.

Third, the significant differences observed between SG and CD substrates highlight the critical role of substrate nutritional composition in determining larval productivity. Although cow dung can support larval survival and growth, its lower nutrient density appears to limit biomass accumulation compared with nutrient-rich agro-industrial materials. Integrating manure with other organic wastes may therefore represent a practical strategy for improving nutrient balance in waste conversion systems.

Finally, the sharp increase in biomass observed during the final rearing phase underscores the importance of determining optimal harvesting time in BSFL production systems. Harvesting larvae at their peak biomass can maximize yield and improve production efficiency before they transition to the non-feeding prepupal stage.

In all, the findings reinforce the growing recognition of H. illucens larvae as an efficient biological agent for converting organic waste into valuable biomass. By optimizing substrate type and feeding regimes, insect farming systems can enhance both waste reduction and sustainable protein production.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

AD conceived and designed the study, contributed to data analysis tools, and manuscript writing. **AO** contributed to study design, data collection, analysis of data, and manuscript writing. **DL** contributed to study design, data collection, data analysis tools, analysis of data and manuscript writing. All authors approved the final copy of the manuscript.

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