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DEVELOPMENT AND EVALUATION OF A CRISPR-CAS13A-BASED PLATFORM FOR RAPID AND ULTRAPRECISE DETECTION OF RNA VIRUSES IN CLINICAL RESPIRATORY SAMPLES

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Abstract

Background: Respiratory RNA virus outbreaks have highlighted the urgent need for rapid, accurate, and affordable diagnostic tools. Traditional molecular diagnostics, while effective, often require complex infrastructure and have long turnaround times, limiting their use in resource-limited and point-of-care settings.

Objective: This study aimed to design and evaluate a CRISPR-Cas13a-based detection system for rapid identification of respiratory RNA viruses in clinical samples, focusing on sensitivity, specificity, and clinical utility.

Methodology: We developed a CRISPR-Cas13a diagnostic platform integrating reverse transcription isothermal amplification and Cas13a trans-cleavage activity. The assay targeted conserved genomic regions of SARS-CoV-2, influenza A/B, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV). Clinical validation was performed on 847 nasopharyngeal swab specimens from symptomatic patients, using RT-qPCR as the reference standard.

Results: The assay demonstrated high analytical sensitivity with detection limits of 10–20 copies/L for all target viruses. Clinical sensitivity was 96.7% (95% CI: 94.2–98.4) and specificity 99.1% (95% CI: 97.8–99.7). It showed 100% concordance with RT-qPCR in high viral load samples (Ct <25) and 92.3% in low viral load samples (Ct 30–35). Results were available within 45 minutes post-sampling.

Conclusion: This CRISPR-Cas13a platform offers rapid, accurate, and infrastructure-light detection of respiratory RNA viruses. Its performance and practicality support its use in point-of-care testing, outbreak response, and routine diagnostics, underscoring the clinical promise of CRISPR-based technologies.

Keywords: CRISPR-Cas13a, RNA virus detection, respiratory pathogens, molecular diagnostics, point-of-care testing, isothermal amplification.

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Introduction

Large quantities of agro-industrial residues, including broken rice, soybean meal, and various cakes, are often underutilised in animal feed [1]. The repurposing of these residues into animal feed is hindered by nutrient variability, anti-nutritional factors, and logistical challenges [2]. Simultaneously, reliance on unsustainable protein sources, such as fish meal, intensifies the pressure

on marine ecosystems, with the FAO reporting in 2025 that 35.5 per cent of global fish stocks are overfished [3,4]. Black Soldier Fly Larvae (BSFL) (*Hermetia illucens*) offer a promising circular solution for the efficient conversion of diverse organic waste into high-value biomass [5,6] study showed that BSFL achieved a 67 percent substrate reduction rate on fruit waste, producing larvae with 38.9 percent protein and 39.06 percent fat, which is rich in antimicrobial lauric acid. BSFL meal has proven effective

in animal diets, replacing up to 15 percent of fish meal in Atlantic salmon and 60 percent in Nile tilapia without compromising their growth [7,8]. In broilers, BSFL enhances performance and reduces feed costs, whereas in laying hens, 3 percent inclusion improves welfare and eggshell quality [9].

Despite the environmental advantages of a lower carbon footprint and water use, safety concerns persist. Contaminated substrates can cause heavy metal bioaccumulation, necessitating strict feedstock control [10]. Blanching reduces microbial risks, although heat-resistant spores and allergens require further study [11]. Additionally, the high production costs, which are more than twice those of dried fish meal in early 2024, limit large-scale adoption [12]. To address these gaps, our study conducted a comparative evaluation of BSFL reared on four regionally abundant by-products: broken rice, soybean meal, cottonseed cake, and rapeseed cake. We assessed larval growth, substrate conversion rate, proximate composition, and frass potential as a biofertilizer. This study also preliminary evaluated techno-economic analysis to advance sustainable feed alternatives and circular bioeconomy strategies.

Materials and Methods

Collection of Area-based industrial by-products

Broken rice, a byproduct of the rice milling process, was collected from a rice mill. Soybean meal from soybean processing units, cottonseed cake from the cottonseed oil industry, and rapeseed cake from the rapeseed oil industry were used.

Experimental Setup

This study was conducted at Arthro Biotech Private Limited, India. BSF eggs were incubated at 29 ± 2 °C with 70 percent humidity and a 12-hour light cycle, while airflow was monitored. After hatching, the larvae were fed poultry feed in a nursery for six days, achieving an average weight of 4.33 ± 0.92 mg. uniformly sized larvae were used in experiments. The experiments were conducted in plastic containers measuring 60.96 cm × 30.48 cm × 15.24 cm, ensuring an optimal environment and each container housed 23,000 larvae. Each treatment consisted of 15 replicates (n=15). Each treatment no combinations were mixed, and all four feed types were 100 percent of their own feed type. For each replicate containing 3000 g of feed and was administered in a single instance. Prior to distribution, the feed was soaked in water for two hours to ensure adequate hydration and facilitated to ease consumption [13,14].

Monitoring of BSF larvae growth

The larvae were fed uniformly on each replicate, and their developmental parameters were recorded each day before watering. The recorded parameters included the feed conversion ratio, survival rate (SR) and dry weight-based percentage of protein content, fat content, frass yield, fresh larval yield, crude protein and crude fat analyzed based on following standard methods [15].

Feed Conversion Rate (FCR)

Calculated by the formula: (Total yield of the larvae soon after harvest/ Total weight of the feed given)*100 [16]

Survival Rate (SR)

Calculated by the formula: (Total Number of larvae harvested / Total number of larvae deployed)*100

Table 1: Proximate analysis of the feed substrate and larvae by using the following standard methods.

Parameter	Method Used	Description	Reference
Moisture Content	Oven-drying method	5 g sample was dried at 75°C in a hot-air oven for 24 h until a constant weight was achieved. Moisture content was calculated as the percentage weight loss.	17
Crude Protein	Kjeldahl method	The sample was digested with H ₂ SO ₄ to convert nitrogen	18
Crude Fat	Soxhlet extraction	Dried larval fat was extracted using hexane in a Soxhlet	19
Ash Content	Incineration using muffle furnace	Five grams of dried larvae were incinerated at 550°C in a pre-weighed crucible for five hours, until the residue turned white or gray.	20
Crude Fiber	Acid-base digestion	The samples were digested with 1.25% H ₂ SO ₄ and 1.25% NaOH	21
Carbohydrates	Phenol-sulfuric acid method	The sample extract was reacted with phenol and concentrated H ₂ SO ₄ .	22

All proximate analysis were performed in triplicate, and the results are reported as mean values ± standard deviation. This ensured the accuracy and reproducibility of the data.

Statistical Analysis

The experimental data collected from various recorded parameters were analyzed using GraphPad Prism 8 Origin Pro statistical software. A two-way ANOVA was performed to assess the interaction effects between different types of feed and

larval growth, with statistical significance set at $p < 0.05$ (Gaddis, 1998). Growth curves were plotted to visualize the progression of larval weight over 12 days. Weight and time parameters were plotted using GraphPad Prism 8. Fresh larval yield and survival rates across feed types were compared using scatter plots, with FCR and SR reported for the four feeds [23].

Principal Component Analysis

PCA was performed to reduce the dimensionality of the dataset and identify the main factors driving the variation among feed types. The analysis focused on the following parameters: survival rate, frass percentage, feed conversion ratio (FCR), harvest weight, crude protein content, crude fat content, and dry FCR. The percentage of variance explained by each principal component was calculated to determine the most significant factors [24].

Results and discussions

Proximate analysis of feed substrate

Analysis of feed substrateresults showed distinct nutrient profiles (Table 2). Soybean meal, with its high protein ($48.5 \pm 1.2\%$) and moderate carbohydrate ($23.3 \pm 1.0\%$) content, is ideal for protein-intensive processes. The carbohydrate richness of broken rice ($75.4 \pm 1.5\%$) makes it energy-efficient, but it lacks protein ($7.8 \pm 0.5\%$), limiting its standalone use. Cottonseed cake balances protein ($45.0 \pm 1.1\%$) and carbohydrate ($34.0 \pm 1.3\%$) content, offering versatility, whereas rapeseed cake excels in mineral content (ash: $6.9 \pm 0.3\%$) but remains low in fat ($1.0 \pm 0.2\%$). These variations underline the importance of substrate-specific optimization for achieving targeted biological outcomes.

Table 2. Proximate analysis of Feed substrate (Agro-industrial by-products) in percentage.

Parameter s	Soybea n Meal	Broke n Rice	Cottonse ed Cake	Rapese ed cake
Moisture	6.02 ± 0.75	5.43 ± 0.5	4.71 ± 0.7	6.02 ± 0.5
Crude Protein	48.5 ± 1.2	7.8 ± 0.5	42.0 ± 1.1	38.6 ± 0.8
Carbohydrates	23.3 ± 1.0	75.4 ± 1.5	31.0 ± 1.3	35.4 ± 1.2
Fats	5.0 ± 0.4	1.1 ± 0.1	6.3 ± 0.5	1.0 ± 0.2
Ash	6.0 ± 0.5	0.8 ± 0.1	6.0 ± 0.4	7.9 ± 0.3

Growth performance of BSF larvae in different feed substrates

The BSF larvae fed on soybean meal exhibited a rapid initial growth phase, as shown in Figure 1, reaching a peak on day 8 (116.69 ± 4.02 mg). Growth stabilised afterwards, with a slight decline observed towards the end of the experiment (115.54 ± 4.11 mg) on day 12. This indicates that soybean meal is an effective feed for early larval development owing to its high protein content, but it may not sustain maximum growth rates over extended periods. Once the feed supply was depleted, the larvae exhibited

indications of weight loss, potentially owing to reduced fat reserves in their bodies. This suggests that the optimal harvesting time to achieve maximum protein and fat contents is during the peak growth period. Larvae fed broken rice showed a slower initial growth rate than those fed soybean meal, but demonstrated a consistent and substantial increase in biomass from day 4 onwards. The maximum larval weight (185.08 ± 6.45 mg) was recorded on day 10, with a minor decrease by day 12 (182.77 ± 6.48 mg).

Larvae fed with a cottonseed cake diet exhibited moderate growth in the initial days, with a notable acceleration after day 4. The growth curve peaked on day 12, with a larval weight of (140.53 ± 1.55 mg). These results indicate that cottonseed cake is a viable feed option that promotes steady growth, particularly during the later stages of development. The presence of gossypol, an anti-nutritional factor, in cottonseed cake may have influenced growth rates to some extent [25]. The growth of larvae fed rapeseed cake was characterised by a gradual but steady increase. The maximum weight was recorded on day 10 (122.34 ± 4.23 mg), followed by a stabilisation phase on day 12 (120.77 ± 4.32 mg), as shown in Figure 1. Rapeseed cake was less effective than the other feeds in promoting rapid growth; however, it sustained a stable growth rate throughout the study. The presence of glucosinolates, which are known to have anti-nutritional effects, in rapeseed cake may have contributed to the lower growth rates observed in this group [26]. The data from this study provide valuable insights into larval growth dynamics in response to different feed types. Although effective, cottonseed and rapeseed cakes resulted in comparatively lower growth rates. These findings can inform feed selection strategies for larval cultivation to optimize growth performance based on specific developmental goals.

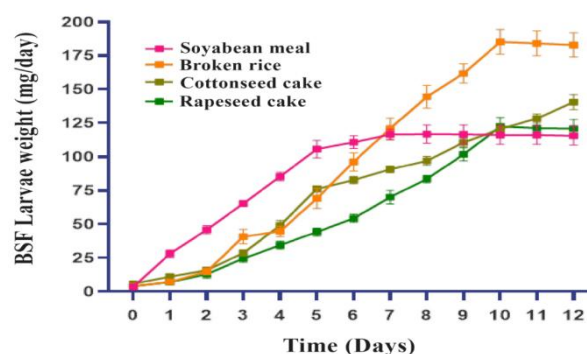


Figure 1: Line graph depicting the growth and development of larvae weight over specified time intervals (days).

BSFL feed conversion ratio and survival rate

Comparison between the feed conversion ratio (FCR) and survival rate of larvae fed four different feed types. The data suggest a complex interplay between FCR and survival rates across different feeds (Figure 2). A higher FCR indicates efficient nutrient utilization. However, this

relationship is influenced by the nutritional and anti-nutritional factors present in feed.

Soybean meal had a high FCR ($79.42 \pm 1.24\%$) and survival rate ($89.39 \pm 2.52\%$), suggesting that it effectively promoted growth and survival. Their high nutrient content and balanced profiles contribute to efficient nutrient conversion and larval vitality. Although broken rice had the highest FCR ($83.20 \pm 3.14\%$), its survival rate was moderate ($59.23 \pm 0.61\%$). This disparity is due to nutritional imbalances, deficiencies, and increased feed viability due to mortality. A high FCR indicates effective nutrient utilization, but a low survival rate suggests that additional factors may affect the population. The lowest FCR ($28.57 \pm 1.45\%$) and survival rate ($26.46 \pm 1.51\%$) indicated that cottonseed cake was less effective in supporting larval growth and survival. Gossypol likely hampers nutrient utilization and negatively affects larval health, resulting in poor growth efficiency and high mortality rates [27]. With an FCR of ($78.46 \pm 2.31\%$) and a survival rate of ($84.58 \pm 1.82\%$), rapeseed cake showed a balanced profile. The slightly lower FCR compared to that of broken rice suggests a somewhat less efficient nutrient utilization, possibly due to glucosinolates. However, the high survival rate indicates that the larvae were healthy and resilient.

The results revealed that although high FCR values with high survival rates exist, exceptions exist, as seen with broken rice. This suggests that factors beyond nutrient conversion efficiency, such as feed composition, anti-nutritional factors, and larval behavior, play crucial roles in determining the overall larval health and survival [28]. These insights highlight the need for a holistic approach to feed formulation, considering not only FCR but also the comprehensive nutritional profile and potential anti-nutritional components.

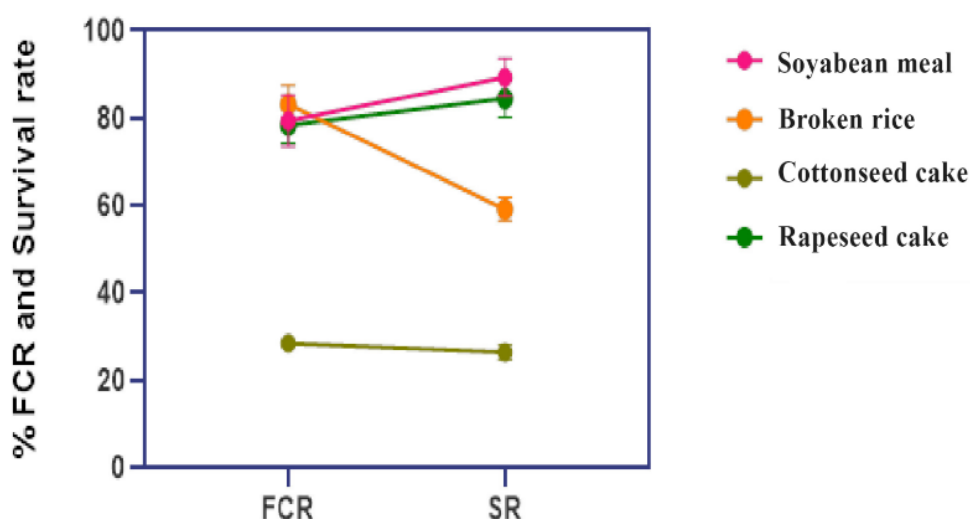


Figure 2: Stutter plot gives the relationship of FCR (feed conversion rate) and SR (survival rate)

Potential of BSFL for enrichment of crude protein and crude fat

Crude protein and crude fat content in four different feed types, the following data present the percentage of crude protein and crude fat on a dry basis for each feed type is shown in Figure 3. Soybean meal had the highest crude protein content ($47.12 \pm 4.63\%$), indicating its potential to support protein synthesis and growth in the larvae. The crude fat content was moderate at ($17.16 \pm 1.79\%$), suggesting that, while anti-nutritional fatty acids, its primary contribution is from protein [29]. The high protein-to-fat ratio makes it suitable for rapid growth stages, where protein demand is high. The broken rice had a protein content of ($31.35 \pm 1.48\%$) and the highest crude fat content of ($41.13 \pm 3.07\%$). The high fat content suggests that broken rice can be a source of energy, supplying lipids essential for metabolic processes [30]. Its balanced protein and high-fat composition make it advantageous for sustaining energy-intensive activities and overall growth, although its lower protein content compared to soybean meal may necessitate supplementation for optimal growth. Cottonseed cake had a crude protein content of $40.00 \pm 1.67\%$ and crude fat content of $27.21 \pm 1.44\%$. This feed type provides a good balance between protein and fat, thereby supporting both growth and energy requirements. However, the presence of gossypol, an anti-nutritional factor, may limit its effectiveness, as it can interfere with nutrient absorption and metabolism [31]. Processing methods that reduce gossypol levels could enhance the nutritional value of cottonseed cake. Rapeseed cake contained $31.05 \pm 0.55\%$ percent crude protein and the highest crude fat content ($41.57 \pm 1.63\%$), similar to that of broken rice. This high fat content supports energy production and storage, which is essential for larval growth. The moderate protein content complements the fat content, making rapeseed cake a balanced feed option. The presence of glucosinolates, which are anti-nutritional compounds, may affect nutrient bioavailability; however, with proper processing, rapeseed cake can be a valuable feed component.

The data revealed an inverse relationship between crude protein and crude fat content across feeds. Feeds with higher protein contents, such as soybean meal and cottonseed cake, have lower fat contents, whereas feeds with higher fat contents, such as broken rice and rapeseed cake, have lower protein contents. This correlation indicates that selecting feed types for specific growth stages requires balancing protein and fat to meet the nutritional demands of the larvae. The high protein content of soybean meal supports rapid tissue growth and development, which is essential during the early growth stages [31]. Conversely, the high fat content in broken rice and rapeseed cake provides sustained energy release, which is critical during the later growth stages when energy demand increases. Cottonseed cake, which contains balanced protein and fat, can serve as an intermediate feed to support both growth and energy needs, provided that its anti-nutritional factors are managed. These findings underscore the importance of understanding the nutritional composition and factors of feed types to optimize larval growth and development. By strategically combining feeds with complementary protein and fat contents, it is possible to enhance the growth efficiency and overall larval health.

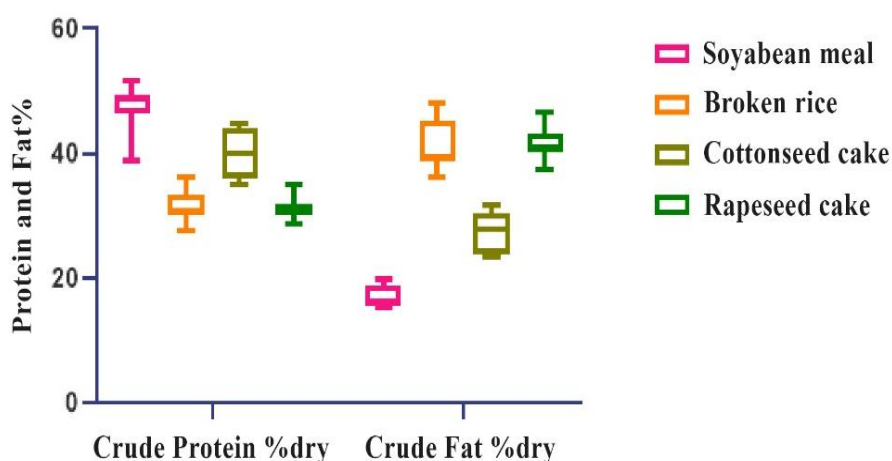


Figure 3: Correlation of conversions rate between crude protein and crude fat content in four different feed types.

Relationship of BSFL yield parameters

Heatmap describing the correlation between FCR, Crude protein, Crude Fat, frass, larvae weight at harvest, and larvae survival rate with four feeds. The color gradients (from negative to positive) indicate the strength and direction of the correlations between the parameters. Three asterisks (***) denote statistical significance levels, with being the most significant ($p \leq 0.001$) and (*) indicating a statistical significance level of $p \leq 0.05$. Positive correlations (red tones) indicate that as one parameter increases, the others also tend to increase. Negative correlations (blue tones) indicate that as one parameter increases, the other tends to decrease (Figure 4).

Soybean meal treatment showed significant correlations between the parameters. A positive correlation was found between the survival rate and FCR. This suggests that higher survival rates may lead to an increased FCR, possibly because of higher competition for feed among larvae. Similarly, a strong positive correlation observed between the larvae weight at harvest and FCR. This implies that larger larvae consume more feed, resulting in higher FCR values, which reflects the effectiveness of the feed in promoting growth, albeit at a higher expense. Conversely, a negative correlation was observed between larvae weight and crude fat percentage, indicating that as the larvae gained more weight, their crude fat percentage tended to decrease. This can be attributed to the metabolic allocation of nutrients; wherein heavier larvae prioritize growth over fat deposition. Additionally, a positive correlation with a significance level was observed between the crude fat percentage and dried larvae weight. This indicates that higher crude fat percentages are associated with less efficient feed utilization, leading to higher dried larvae weight. These findings highlight the complex interactions between different parameters and emphasize the need to optimize feed formulations to improve the overall efficiency of BSFL production using soybean meal feed.

Broken rice-fed BSFL treatment showed a positive correlation between weight at harvest and FCR ($p \leq 0.05$) and a stronger correlation with dried larvae weight ($p \leq 0.01$) indicated that increased larval weight required more feed, reflecting higher consumption. Additionally, the weight at harvest was positively correlated with the crude protein percentage ($p \leq 0.05$), indicating efficient protein utilization. However, higher crude protein percentages were linked to increased Dried larvae weight ($p \leq 0.01$), suggesting that more feed was necessary for protein conversion to biomass. A negative correlation between the crude fat and crude protein percentages indicates that a higher protein content reduces fat deposition, likely due to metabolic prioritization. Finally, the negative correlation between harvest weight and survival rate implies that larger larvae faced increased competition or stress, leading to lower survival rates. These findings underscore the importance of balanced feed formulations for optimizing growth and feed efficiency during BSFL production.

Correlation analysis of BSFL fed on cottonseed caketreatment revealed significant insights into nutrient dynamics and feed efficiency. A strong negative correlation between crude protein percentage and Dried larvae weight ($p \leq 0.001$) indicated that higher protein content leads to more efficient feed conversion, as evidenced by lower Dried larvae weight. This suggests that the high protein content of the cottonseed cake was effectively utilized by the larvae for growth. Additionally, the negative correlation between crude fat percentage and crude protein percentage ($p \leq 0.001$) highlights a metabolic trade-off in which increased protein synthesis occurs at the expense of fat deposition. The relationship of crude fat percentage and Dry Matter ($p \leq 0.01$) suggests that a higher fat content is associated with less efficient feed conversion, resulting in higher Dried larvae weight. These findings emphasize the importance of balancing the protein and fat content in feed to optimize growth and feed efficiency. By understanding these correlations, producers can make informed decisions to enhance the nutritional profile and overall efficiency of BSFL production using cotton-seed cake feed.

Analysis of the correlations for rapeseed cake feed in the BSFL treatment revealed interesting relationships between the parameters. A positive correlation between weight at harvest and FCR, marked by a single asterisk ($p \leq 0.05$), suggests that as the larvae's weight at harvest increases, the FCR also tends to increase. This implies that larger larvae consume more feed, resulting in higher FCR values, indicating the effectiveness of rapeseed cake in promoting growth. Conversely, a negative correlation between survival rate and harvest weight, also marked by a single asterisk ($p \leq 0.05$), indicates that as the larval weight at harvest increases, the survival rate tends to decrease. This could be due to increased competition for resources or the stress associated with rapid growth, leading to lower survival rates. These findings highlight the importance of balancing feed formulations to optimize growth and feed efficiency, while maintaining high survival rates in BSFL production using rapeseed cake feed.

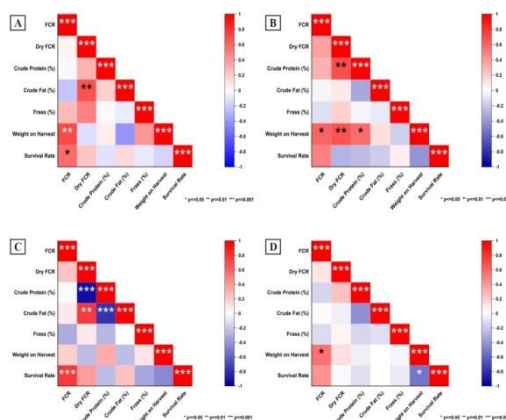


Figure 4: Correction Heatmap of Soybean meal (A), Broken Rice (B), Cotton Seed cake (C) and Rape seed cake (D).

Principal Component Analysis (PCA)

Principal component analysis was applied to simplify the complex dataset and identify the primary factors driving variation among the four feed types: Soybean Meal, Broken Rice, Cottonseed Cake, and Rapeseed Cake.

PC1 Analysis: The first principal component (PC1) captures the maximum variance in the data and highlights the most critical factors affecting the feed types. PC1 was primarily influenced by crude protein, survival rate, and feed conversion efficiency. Soybean Meal stands out in this regard due to its high protein content and exceptional survival rate, making it a significant contributor to PC1. This indicates that protein content and survival are key drivers of variation and effectiveness of livestock feed.

PC2 Analysis: The second principal component (PC2) captures the second-highest variance and focuses on different factors, such as crude fat and dry matter content. Broken Rice played a crucial role in PC2 because of its high fat content and dry matter percentage. This highlights the importance of energy-related variables and their impact on growth efficiency. Although Broken Rice demonstrates impressive weight gain, it shows different efficiencies in feed conversion compared to other feeds, positioning it uniquely in the PCA plot shown in Figure 5.

Interpreting Feeds in PCA Graphs

Soybean Meal: In the PCA for Soybean Meal, PC1 captured 30.92% of the variance, whereas PC2 accounted for 27.74%. The parameters are distributed across the first and second quadrants. The first quadrant included survival rate, frass percentage, FCR, and harvest weight, highlighting the importance of efficiency and growth metrics as Figure 5 represents. The second quadrant featured crude protein, crude fat, and dry FCR, emphasizing the role of nutritional content in driving variation. This distribution indicates that Soybean Meal performance is influenced by a combination of growth efficiency and nutritional quality, making it a robust feed option.

Broken Rice: For Broken Rice, PC1 explained 38.49% of the variance and PC2 accounted for 21.84%. The parameters were distributed as follows. The second quadrant contains the survival rate and frass percentage, indicating the significance of survival and waste production. The first quadrant includes FCR and crude protein, highlighting the importance of feed

conversion and protein content. The third quadrant contained crude fat and dry FCR, reflecting the role of energy content and efficiency. Broken Rice's high weight gain and energy contribution are evident, but its different conversion efficiencies and lower survival rate present challenges that need addressing.

Cottonseed cake: PC1 captured 43.67% of the variance in the PCA for cottonseed cake, while PC2 explained 26.81%. The first quadrant includes FCR and survival rates, highlighting the importance of feed conversion efficiency and survival. The second quadrant featured crude protein and harvest weight, emphasizing the roles of protein content and growth. The third quadrant includes crude fat, dry FCR, and frass percentage, indicating the contribution of fat content and waste production. The exceptional feed conversion efficiency of Cottonseed Cake is notable, but its lower survival rate suggests the need for further optimization.

Rapeseed Cake: For Rapeseed Cake, PC1 explained 23.40% of the variance and PC2 accounted for 22.66%. The parameters were distributed as follows. The first quadrant contained harvest weight, FCR, and dry FCR, thus underscoring the importance of growth and efficiency. The second quadrant featured crude protein and frass percentages, highlighting the roles of nutritional content and waste production. The third quadrant included the survival rate and crude fat, indicating the significance of survival and energy content. The balanced performance of rapeseed cake in terms of feed conversion and survival rates is evident, although its growth rates are slightly lower than those of the other feeds.

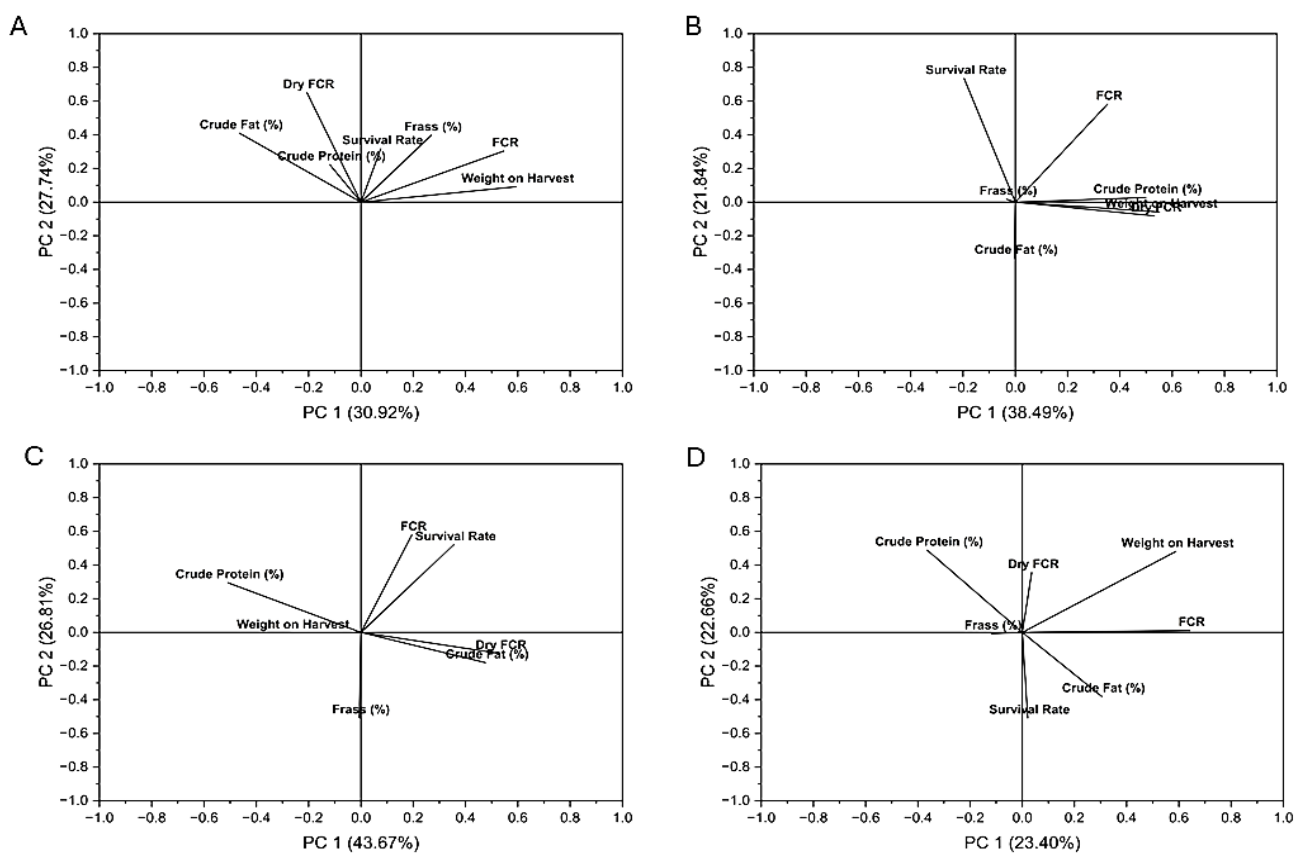


Figure 5: PCA analysis of BSFL four feeds: Soybean meal (A), Broken Rice (B), Cotton Seed cake (C) and Rapeseed cake (D).

In summary, PCA effectively reduced the complexity of the dataset, revealing the key drivers of variation among the feed types. Soybean Meal emerged as the most balanced feed with high protein content, efficient feed conversion, and high survival rates. Broken Rice demonstrated significant weight gain but faced challenges in conversion efficiency and survival. Cottonseed Cake showed excellent feed conversion efficiency but was limited by its lower survival rate. Rapeseed Cake displayed balanced performance, combining efficient conversion and high survival rates. This analysis provides valuable insights that can guide future research and practical applications for optimizing feed strategies for enhanced growth and sustainability.

Conclusion

This study investigated sustainable alternatives for Black Soldier Fly Larvae (BSFL) production by evaluating the nutritional suitability of selected agro-industrial byproducts as larval substrates. The results demonstrate that BSFL can efficiently convert diverse organic feed materials into valuable nutrient-rich biomass, reinforcing

their potential as a sustainable solution for livestock feed applications. Among the tested substrates, soybean meal emerged as the most promising option, exhibiting balanced nutritional support, superior feed conversion efficiency, and the highest survival rate, thereby establishing its reliability for consistent BSFL growth. Rapeseed cake also showed favourable performance, with

acceptable feed conversion and survival rates, although larval growth was comparatively moderate. Broken rice produced the greatest larval weight gain but was associated with reduced feed conversion efficiency and lower survival, indicating the need for further optimization. Cottonseed cake displayed excellent feed conversion efficiency; however, its very low survival rate highlights the impact of anti-nutritional factors and limits its immediate applicability. Overall, the study provides meaningful insights into the strengths and constraints of various substrates used for BSFL rearing. The findings support soybean meal and rapeseed cake as robust and practical protein sources for sustainable BSFL production. Adoption of optimized BSFL feeding strategies may reduce dependence on fishmeal, promote waste valorisation, and contribute to environmentally responsible, economically viable, and circular livestock production systems.

Future Research

Future investigations should focus on optimizing substrates that exhibited partial limitations in this study. Strategies such as nutrient balancing, blending of substrates, and pre-treatment techniques may improve feed conversion efficiency and survival rates, particularly for broken rice and cottonseed cake. Further research is warranted to explore detoxification methods for cottonseed cake to mitigate the effects of anti-nutritional factors such as gossypol. Long-term studies assessing the scalability, economic feasibility, and lifecycle environmental impacts of BSFL production using agro-industrial byproducts are recommended. Additionally, evaluating the effects of these optimized BSFL-derived feeds on animal growth performance, health, and product quality would provide practical validation for commercial adoption. Expanding investigations into microbial interactions, larval metabolism, and nutrient bioavailability may further enhance the efficiency and sustainability of BSFL-based feed systems.

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Author contribution statement

Balu Amaresh DJoragi: Conducted and performed the experimental analysis and played a key role in drafting and writing the manuscript. Veeresh SJ: Planned and supervised the research work, contributed to manuscript writing and revisions, coordinated with all collaborators involved in the research work, and final version of research article corresponded to the journal.

Disclosure Statement

The authors declare no competing interests.

Data Availability Statement

All data produced or analyzed in this study are included in the article.

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