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Effect of Palm Kernel Cake as a Feeding Substrate on Post-Feeding Development and Reproductive Performance of Black Soldier Fly (*Hermetia illucens*)

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ABSTRACT

The Black Soldier Fly (BSF), *Hermetia illucens*, undergoes a complete life cycle consisting of egg, larval, prepupal, pupal, and adult stages. Feeding occurs only during the larval (BSFL) stage; therefore, the feeding substrate may influence subsequent BSF post-feeding performance. This study evaluated palm kernel cake (PKC), an abundant agro-industrial by-product, as a feeding substrate for BSFL by assessing its effects on adult emergence, egg production, and adult longevity. The feeding substrates comprised 100% PKC and commercial chicken feed (CF) as the control. BSFL were reared on the respective substrates, and their post-feeding development and reproductive performance were compared. Each replicate consisted of 50 randomly selected prepupae, with three replicate cages established for each treatment. Adult emergence, egg production, and adult mortality were monitored and recorded daily and analysed using two-way ANOVA followed by Tukey's multiple-comparison test, with statistical significance set at $p < 0.05$. From the results, feeding substrate influenced post-feeding development and reproductive performance. CF supported faster adult emergence and greater egg production, whereas PKC resulted in delayed emergence and lower egg production but prolonged adult survival. Complete adult emergence was achieved by Day 4 in CF compared with Day 9 in PKC. Cumulative egg production was higher in CF (234.27±12.89 mg) than in PKC (89.60±8.31 mg). Collectively, these findings indicate that although 100% PKC can support successful development and reproduction of BSF, its use

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is associated with slower post-feeding development and reduced reproductive output, suggesting that substrate optimisation is necessary to improve its suitability for continuous BSF production.

Keywords: Black Soldier Fly, palm kernel cake, post-feeding development

INTRODUCTION

The increasing generation of organic waste and its improper disposal present significant environmental and public health challenges, particularly in countries with limited waste management infrastructure and technology (Kanhai et al., 2021). Conventional disposal methods, such as landfilling and combustion, are widely used due to their low cost but can contribute to pollution and greenhouse gas emissions (Kim et al., 2021). In this context, insect-based bioconversion, specifically using Black Soldier Fly (*Hermetia illucens*) larvae (BSFL), has emerged as a sustainable alternative capable of transforming organic waste into high-value products within a short period (Siddiqui et al., 2022). Moreover, BSFL can feed on and convert a wide range of organic substrates, including agricultural and agro-industrial byproducts, highlighting their potential to reduce waste and environmental impacts (Hancz et al., 2024).

Palm Kernel Cake (PKC), a by-product of palm oil extraction from palm kernels, is abundantly generated in major palm oil-producing countries such as Malaysia and Indonesia (Nugroho et al., 2022; Shukor et al., 2013). As an agro-industrial by-product, PKC contains substantial levels of nutrients, particularly protein and residual fat, indicating its potential as a feeding substrate for BSFL. However, its suitability as a sole feeding substrate may be constrained by its relatively high fibre content, which can limit nutrient availability and larval utilisation (Liew et al., 2022). Previous studies have therefore explored substrate modification approaches, including supplementation (Nugroho et al., 2023), substrate mixing (Bajra et al., 2023), and microbial fermentation (Damanik et al., 2024; Liew et al., 2022), to improve the feeding value of PKC and support BSFL growth (Idzam et al., 2025).

Nevertheless, the suitability of feeding substrate should not be evaluated solely based on larval performance, as nutritional conditions during the feeding stage may also influence subsequent development (Zhang & Puniamoorthy, 2025). These effects are particularly relevant during post-feeding development, when BSFL progresses through the prepupal and pupal stages before adult emergence (Guillaume et al., 2024). During this period, larvae cease feeding, empty their digestive tract, and undergo physiological changes associated with metamorphosis (Rebora et al., 2023). Understanding these post-feeding stages is therefore important, as successful development into reproductively competent adults is essential for maintaining BSF rearing and production systems (Miranda et al., 2019). A recent study further demonstrated that carbohydrate-rich diets with lower protein and fat improved larval growth, female body weight, and egg production, whereas diets high in

both protein and carbohydrates reduced these outcomes, highlighting the carry-over effects of larval diet on adult reproductive performance (Zhang & Puniamoorthy, 2025).

Although previous studies have extensively investigated the effects of feeding substrates on BSFL growth and waste conversion efficiency, comparatively less attention has been given to their subsequent effects on post-feeding development and adult reproductive performance (Guillaume et al., 2024; Rebora et al., 2023). Particularly, information on whether PKC-fed larvae can successfully complete post-feeding development and sustain reproductive performance remains limited. Therefore, this study investigated the effects of PKC as a larval feeding substrate on subsequent adult emergence, egg production, and adult longevity, using commercial chicken feed as the control.

MATERIALS AND METHODS

Substrate Preparation

PKC and commercial CF were purchased locally from MJ FATHONAH Sdn. Bhd. and TVR TERNAKAN RESOURCES, respectively. The proximate composition of CF and PKC was 67.5% and 75.6% total carbohydrate, 19.9% and 15.4% crude protein, 3.8% and 1.9% crude fat, 5.3% and 4.5% ash, and 2.3% and 20.0% crude fibre, respectively. Prior to feeding, both substrates were separately mixed with dechlorinated water to achieve a final water content of 70% (Dzepe et al., 2020).

Prepupae Preparation

Two experimental groups were established in which larvae were fed separately on PKC and CF. The experimental feeding phase began at the neonate stage at 6 days of age and continued until the larvae reached the prepupal stage. Briefly, each container received approximately 485 g of the designated substrate to ensure sufficient substrate for larval growth. Once the larvae entered the prepupal stage, characterised by darkening of the cuticle and migration away from the food source, the actively prepupae were selected from each substrate group for post-feeding development observations (Giannetti et al., 2022).

Experimental Setup and Parameters Assessment

A total of three independent replicates were established for each treatment, with each replicate consisting of 50 randomly selected prepupae transferred into a separate 30 × 30 × 30 cm rearing cage. Adult BSF emergence from each replicate cage was monitored daily following the method described by Ferdousi and Sultana (2021). The number of resulting adults was counted, and the empty pupae shells were separated into small, covered containers to avoid counting the number of adult emergences repeatedly (Figure 1). The experiment was conducted at room temperature (30-32 °C) with an environmental humidity of 69-71% relative humidity (%RH).

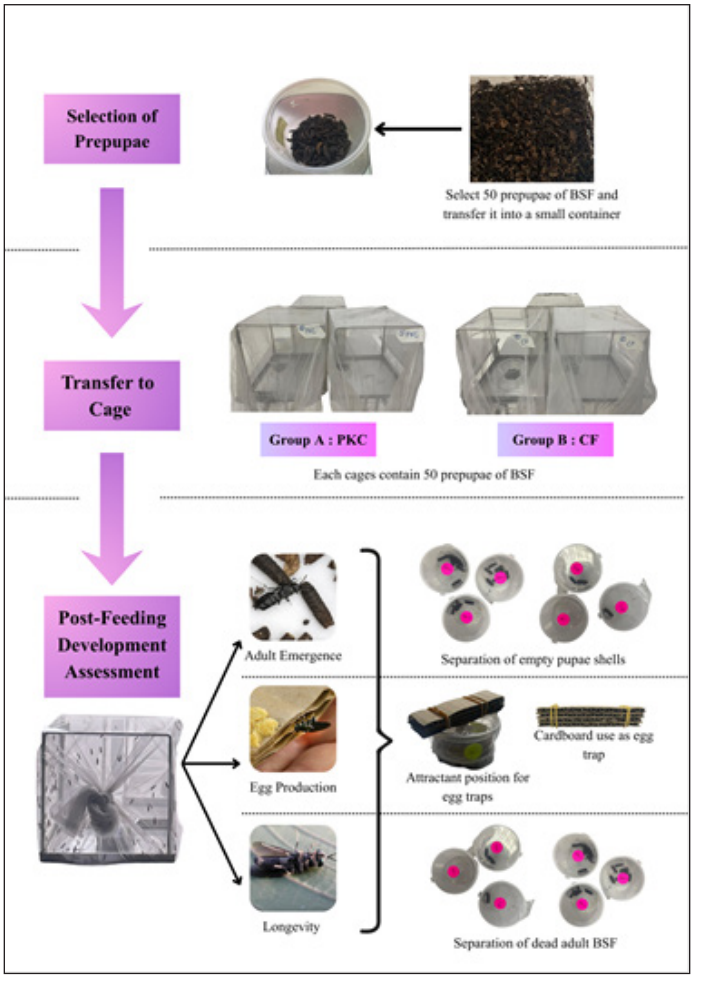


Figure 1. Experimental setup for the studies. Selection of 50 random prepupae from CF and PKC. 3 cages for each treatment represent 3 replications. Parameter evaluation included adult emergence, egg production and longevity

The success rate percentage of adult emergence was calculated using Equation 1:

$$\textit{Success rate of adult emergence, \%:}\ \frac{\textit{Number of Adult Flies Emerged}}{\textit{Number of Initial Pupae}} \times 100 \quad [1]$$

The egg production of the BSF was evaluated by mating a random 50 adults of BSF (unknown sex) in one cage. The oviposition substrates were placed in a container covered with a net filled with a moist attractant (fermented tofu mix with tempeh) and put underneath the cardboard to trigger the egg-laying response, as shown in Figure 1. The mixture of fermented tofu and tempeh can emit aromatic compounds that attract female flies to lay eggs in cardboard traps (Lestari et al., 2020). The traps were checked every day for 7 days,

and eggs were collected. The egg masses were measured, and the collected data were entered into the data tabulation.

The lifespan of the adult BSF was determined by observing mortality daily until all of them perished. Longevity was given in terms of the mean lifespan of adult BSF for each treatment in days (Resconi et al., 2025). Similar to the adult emergence, the dead flies were separated into a small, closed container to avoid counting the dead flies repeatedly. The percentage of dead flies is calculated using Equation 2:

$$\textit{Percentage of dead adult flies, \%: } \frac{\textit{Number of Dead Adult Flies}}{\textit{Number of Adult Flies Emerge}} \times 100 \qquad [2]$$

Statistical Analysis

All the data that have been collected were analysed through GraphPad Prism (version 10.6.0). Descriptive statistics were used to summarise the success rate of adult flies, egg production and longevity (mean and standard deviation). The comparison of different substates across days was analysed using two-way ANOVA, with $p < 0.05$ defined as significant. In cases where significant results were found, multiple comparisons were carried out to establish the differences between each possible pair, with Tukey's multiple comparison test as the post-hoc analysis being used.

RESULTS AND DISCUSSIONS

Successful Emergence of Adult BSF

Adult emergence differed between the substrate treatments (Figure 2). Based on cumulative emergence (Figure 2a), CF exhibited a faster emergence pattern, reaching 100% emergence by Day 4, whereas PKC showed a slower emergence pattern, requiring up to Day 9. The results supported by the daily emergence data (Figure 2b) showed that emergence in the CF group began on Day 1, peaked on Day 2 (54.67±9.24%), and declined rapidly, with no emergence observed after Day 4. Compared to PKC, it exhibited a more gradual emergence pattern, with emergence continuing until Day 9. Two-way ANOVA revealed a significant treatment × day interaction, $F_{(8, 36)}=6.932$, $p < 0.0001$, and a significant effect of day, $F_{(8, 36)}=10.04$, $p < 0.0001$. However, the overall effect of feeding substrate was not significant, $F_{(1, 36)}=0.001$, $p =0.9744$. Tukey's multiple-comparison test showed significant differences between CF and PKC on Days 1, 2, 4, and 6 ($p < 0.05$).

Egg Production of Adult BSF

Egg production of adult BSF differed between substrate treatments (Figure 3). Based on cumulative egg production (Figure 3a), CF exhibited a higher reproductive output, reaching a total of 234.27±12.89 mg by Day 5 with no further increase thereafter, whereas PKC

showed a gradual increase until Day 10, reaching 89.60±8.31 mg, corresponding to a 61.8% reduction in cumulative egg production relative to the CF control. Referring to daily egg production (Figure 3b), oviposition in the CF group began on Day 1 (64.37±27.24 mg), peaked on Day 2 (126.5±33.36 mg), and declined by Day 3 (18.23±21.20 mg), with no egg production observed after Day 6. In contrast, PKC resulted in lower daily egg production, with oviposition starting on Day 3 (28.13±20.25 mg) and continuing at low levels until

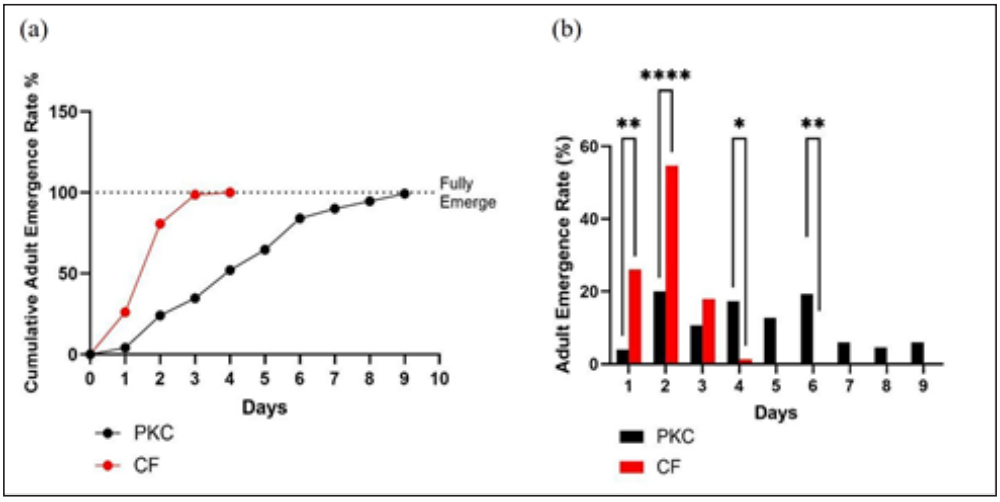


Figure 2. Adult emergence rate of BSF fed PKC and CF as substrate treatment: (a) Cumulative; (b) Daily. Data represent the mean (n = 3)
Note. *Asterisks indicate significant differences

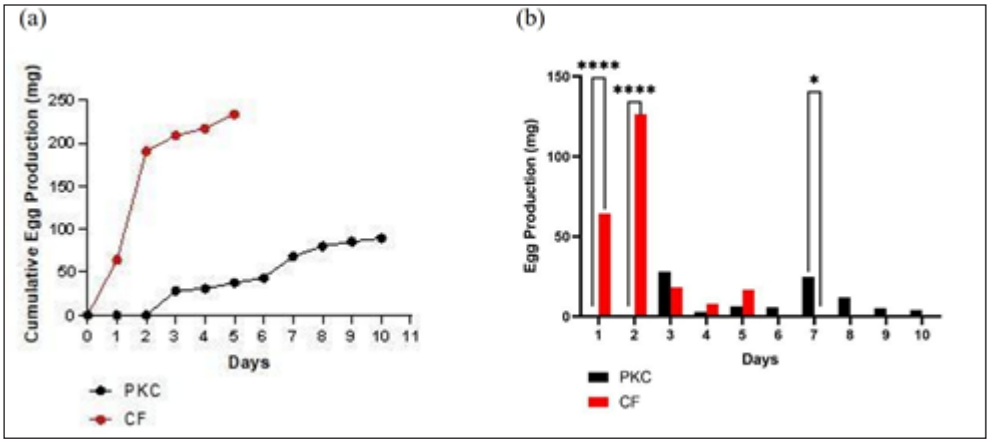


Figure 3. Egg production of BSF fed PKC and CF as substrate treatment: (a) Cumulative Egg Production of Adult BSF across 10 days; (b) Daily Egg Production of Adult BSF across 10 days. Data represent the mean (n = 3)
Note. *Asterisks indicate significant differences

Day 10 (4.00±6.93 mg). Two-way ANOVA revealed significant effects of feeding substrate, $F_{(1, 40)}$=15.20, p = 0.0004, and day, $F_{(9, 40)}$=10.74, p < 0.0001, together with a significant treatment × day interaction, $F_{(9, 40)}$=15.43, p < 0.0001. Tukey's multiple-comparison test showed significant differences between CF and PKC on Days 1, 2, and 7 (p < 0.05).

Longevity of Adult BSF

Adult longevity of BSF differed between substrate treatments (Figure 4). Based on cumulative mortality (Figure 4a), CF exhibited a faster increase in mortality, with most individuals dying earlier, whereas PKC showed a more gradual increase, reaching complete mortality by Day 14. Referring to daily mortality (Figure 4b), low mortality was observed in both CF and PKC groups during the initial days, followed by a marked increase from Day 3 onwards. In the CF group, mortality peaked on Day 5 (>33.33%) and declined thereafter. In contrast, PKC showed lower mortality on Day 5 (20%) but maintained relatively higher mortality on subsequent days, indicating a more extended mortality pattern. Two-way ANOVA revealed a significant effect of day, $F_{(13, 56)}$ =16.32, p < 0.0001, and a significant treatment × day interaction, $F_{(13, 56)}$=3.177, p= 0.0013. However, the overall effect of feeding substrate was not significant, $F_{(1, 56)}$ = 0.002, p = 0.9622. Tukey's multiple-comparison test identified significant differences between CF and PKC on Days 4 and 5 (p < 0.001).

The present study demonstrated that the larval feeding substrate produced distinct subsequent effects on post-feeding performance, with CF supporting earlier adult emergence and higher egg production, while PKC was associated with delayed emergence, reduced reproductive output, and prolonged adult survival. These differences may be associated with

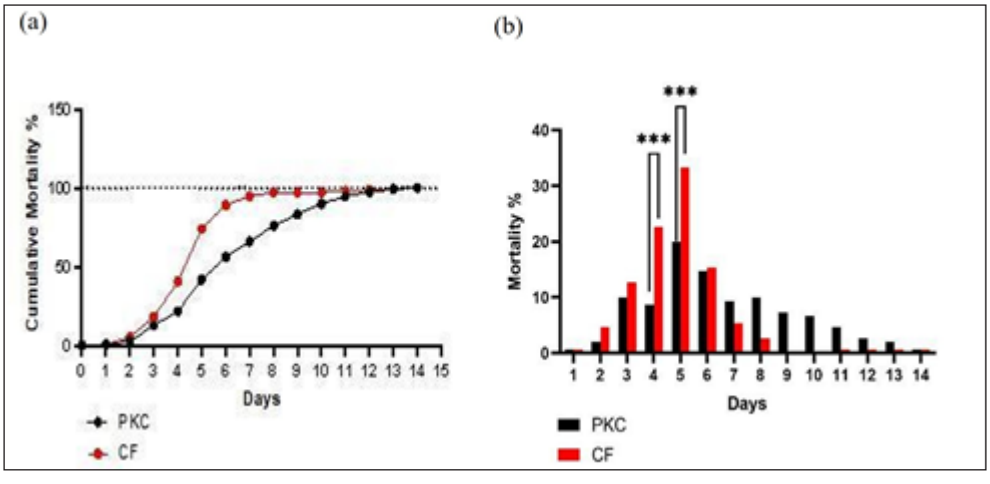


Figure 4. Longevity of BSF fed PKC and CF as substrate treatment: (a) Cumulative Mortality (%) of Adult BSF across 14 days; (b) Daily Mortality (%) of Adult BSF across 14 days. Data represent the mean (n = 3)
Note. *Asterisks indicate significant differences

the contrasting nutritional composition of the larval feeding substrates, as dietary nutrient concentration and macronutrient balance have been shown to influence BSF development time and subsequent adult life-history traits (Binsin et al., 2023; Laursen et al., 2024).

In the present study, CF contained higher crude protein (19.9%) and crude fat (3.8%) and substantially lower crude fibre (2.3%) than PKC, which contained 15.4% crude protein, 1.9% crude fat, and 20.0% crude fibre. The relatively high fibre content of PKC may restrict nutrient accessibility and utilisation by BSFL, as the lignocellulosic components of PKC are difficult for the larvae to digest (Damanik et al., 2024; Liew et al., 2022). Consequently, reduced nutrient availability during larval development may affect nutrient accumulation, as dietary nutrient quality has been shown to influence protein and lipid reserves in the BSF larval fat body, which are important for subsequent metamorphosis and adult reproductive functions (Barragan-Fonseca et al., 2018; Pimentel et al., 2017). This subsequent effect of larval nutrition is supported by Schneider et al. (2025), who demonstrated that dietary protein concentration influenced BSF development beyond the larval stage. The medium and high protein diets shortened development to the prepupal stage and resulted in larger prepupae and adults compared with a low protein diet (Schneider et al., 2025). Such nutritional differences during larval development may therefore contribute to the delayed adult emergence observed in PKC-fed BSFL.

The influence of larval nutrition was also reflected in reproductive performance. Adults emerging from CF-fed larvae produced 234.27 ± 12.89 mg of cumulative egg mass compared with 89.60 ± 8.31 mg from PKC-fed larvae, representing a 61.8% reduction relative to the CF control. This difference may reflect the subsequent effects of nutrient acquisition during larval development, as nutritional reserves accumulated during this stage contribute to adult development and reproductive investment (Laursen et al., 2024; Schneider et al., 2025). The lower egg production observed in the PKC treatment may therefore reflect reduced nutrient accumulation during larval development. Protein availability is particularly relevant to reproductive performance because protein supports vitellogenesis and egg formation in adult insects, while larval diet has been shown to influence adult body size, lifespan, and egg production in BSF (Laursen et al., 2024). Consistent with this, Zhang & Puniamoorthy (2025) demonstrated that larval substrate composition influenced larval growth, adult body weight, oviposition timing, egg production, and hatchability, with larger females generally producing more eggs. Their findings further showed that different compositions of protein, carbohydrate, and fat produced contrasting reproductive outcomes, highlighting the importance of overall dietary nutrient balance rather than individual nutrients alone.

Interestingly, despite the lower reproductive output, adults originating from PKC-fed larvae exhibited a more prolonged survival pattern, reaching complete mortality by Day 14 compared with Day 8 in the CF treatment. This contrasting response indicates that larval feeding substrate may influence adult life-history traits differently, with effects on

reproductive output not necessarily corresponding directly with adult survival. Similarly, Laursen et al. (2024) demonstrated that larval rearing diet influenced both adult lifespan and egg production, supporting the broader subsequent effects of larval nutrition on adult performance. However, prolonged adult survival in the PKC treatment should not necessarily be interpreted as superior reproductive performance, particularly given its substantially lower egg production.

Based on these findings, from a production perspective, the reduced reproductive output observed in the PKC treatment may limit breeder-colony productivity, as lower egg production can reduce the number of larvae available for subsequent production cycles and ultimately constrain biomass production potential. This is supported by Laursen et al. (2024), who highlighted that higher reproductive output can increase biomass production potential and breeding population size, while shorter development time can further improve production turnover in BSF mass-rearing systems. Therefore, although PKC supported successful adult development and reproduction, its delayed emergence and substantially lower egg production may constrain its use as a sole feeding substrate for breeder-colony production. Nevertheless, given its availability and potential for agro-industrial by-product valorisation, appropriate substrate optimisation could improve its suitability for continuous BSF production (Azizi et al., 2021; Idzam et al., 2025).

CONCLUSION

The findings of this study demonstrated that the feeding substrate used during the larval stage influenced the post-feeding development of BSF, including adult emergence, longevity, and egg production. Although BSFL reared on PKC showed delayed adult emergence and lower egg production compared to CF, PKC was still able to support successful adult development and reproduction. This study contributes to the understanding that the suitability of a BSFL feeding substrate should not be evaluated solely based on larval performance, as nutritional conditions during the larval stage may exert subsequent effects on developmental and reproductive traits**.** Given the high generation and abundance of PKC in palm oil-producing regions such as Malaysia, its utilisation in BSFL bioconversion represents a promising alternative that can support sustainable waste management and contribute to the advancement of a circular bioeconomy. Further research should focus on developing appropriate substrate optimisation strategies to enhance the overall suitability of PKC for continuous BSF production.

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