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Research Article

Optimizing Black Soldier Fly (*Hermetia illucens* L.) Larval Production Using Artisanal Ferment-Based Substrates

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Abstract

Large-scale production of black soldier fly (BSF) larvae offers a promising approach to reducing competition for raw materials between humans and livestock. Efficient BSF production requires nutrient-rich, low-cost substrates capable of supporting rapid larval growth. Fermentation and microbial inoculation can modify substrate quality, but the use of a low-cost artisanal ferment (rice-washing water fermented with milk and sugar) in fruit-waste substrates has not been evaluated. This study assessed the growth performance of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae) reared on spoiled-fruit substrates formulated with increasing levels (0-50%) of artisanal ferment, in comparison with a chicken-feed control (T0). A total of 10,584 six-day-old larvae were allocated in a completely randomized design to seven treatment groups (substrates), with 1,512 larvae per treatment and three replicates per treatment. Data were analyzed using one-way ANOVA and generalized linear models in R software, version 3.6.2. Chemical analysis showed that dry matter, total nitrogen, and total ash contents were significantly higher ($p < 0.001$) in the control substrate (T0; chicken feed), whereas gross energy content was highest in the 100% spoiled-fruit substrate (T1). Larval performance parameters, including developmental duration, final larval weight, substrate reduction index, survival rate, relative growth rate, and bioconversion rate, differed significantly among treatments ($p < 0.001$), with the highest overall performance observed in the control substrate (T0; development time 12 days, survival 89.63%, bioconversion rate 14.23%). Among the experimental substrates, T1 (100% spoiled fruit) showed the best overall performance (34 days, 80.73%, and 7.04%, respectively), whereas increasing the ferment proportion progressively reduced performance, with survival falling to about 33% at 40-50% ferment. Under the conditions of this study, the artisanal ferment therefore did not improve larval performance; however, these findings indicate that T1 (100% spoiled fruit) may be a viable low-cost alternative substrate for black soldier fly larval production.

Keywords

Hermetia Illucens, Poultry Feed, Spoiled Fruit, Artisanal Ferment, Larval Production

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1. Introduction

The continuous growth of the global human population has increased demand for protein and energy sources [30], contributing to the scarcity and rising cost of feed raw materials [35]. In animal production, increasing raw-material prices may account for 60-70% of total production costs [6], partly because of competition between human and animal food systems. To address competition for protein resources, considerable research has focused on insect production [17, 30] and the use of insects in animal feed [4, 28]. Among edible insects, the black soldier fly (*Hermetia illucens*) has attracted considerable attention because of its tolerance of a wide range of environmental conditions and its cosmopolitan distribution [17, 30]. Importantly, black soldier flies can convert agro-industrial wastes and by-products into biomass rich in nutrients for animal feed [9, 30].

Black soldier fly larvae can efficiently feed and develop on diverse organic waste streams, including dairy cattle manure [22, 32, 40], chicken manure [40, 45], pig manure [47], human excreta [5], food waste [43, 46], soybean curd residue [40], and municipal organic waste [13], thereby contributing to waste recycling and environmental sustainability [42].

Chemical analyses indicate that BSF larvae contain approximately 37-63% crude protein, 7-39% crude fat, and a broad spectrum of amino acids, including lysine (2-8%) and methionine (0.7-2%) [6, 8]. Numerous studies have demonstrated the successful incorporation of BSF larvae into animal diets, including poultry [4], fish [24], and pigs [33], with satisfactory production outcomes.

Successful BSF larval production requires careful management of several critical factors, among which substrate quality is particularly important [17]. Substrate type can substantially affect both larval growth performance and the nutritional composition of BSF larvae [6, 14]. Furthermore, De Haas et al. [11] and Barragán et al. [6] reported that nutritionally rich substrates can shorten larval development time while increasing fat deposition and survival rates. Similarly, Meneguz et al. [29] reported that BSF larvae fed brewery by-products reached the prepupal stage more rapidly and had higher protein contents than larvae fed mixtures of vegetables and fruit or winery by-products. Protein-rich substrates can support mass production of BSF larvae over shorter periods [43], whereas protein-deficient substrates may prolong the insect life cycle [43]. However, protein-rich substrates may also increase ammonia emissions, raising environmental concerns [17]. For large-scale, cost-effective production of substantial larval biomass, it is therefore important to identify low-cost substrates that provide adequate protein and energy.

Microbial activity in the substrate is another factor that can influence BSF performance. Inoculating poultry manure with companion bacteria modified larval growth and development [45], microbe-assisted conversion of rice straw and restaurant waste has been reported [46], and pre-fermentation of fibrous substrates such as maize straw and coconut endosperm has been used to improve bioconversion and nutrient yield [15, 31]; the role of microbes in biowaste decomposition by BSF larvae has been reviewed by Gold et al. [17]. However, these studies relied on defined bacterial inoculants or on pre-fermentation of the substrate itself, which can be difficult to implement in smallholder settings. Artisanal ferments prepared from rice-washing water are inexpensive, easy to produce, and known to harbor lactic acid bacteria [1, 19], while spoiled fruit is available in large quantities at urban markets. To our knowledge, the effect of adding such an artisanal ferment to spoiled fruit at graded levels on BSF larval performance has not been evaluated, so it remains unknown whether the ferment improves or impairs larval growth, survival, and waste reduction. Addressing this gap is important for the development of low-cost, locally available substrates for BSF production.

In this context, the present study investigated the performance of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae) reared on spoiled-fruit substrates formulated with graded levels (0-50%) of an artisanal ferment, in comparison with a chicken-feed control. The study tested the hypothesis that adding the ferment would improve larval growth, survival, bioconversion, and substrate reduction relative to unfermented spoiled fruit, with the aim of identifying a low-cost substrate suitable for BSF larval production.

2. Materials and Methods

2.1. Acquisition of Black Soldier Fly Larvae

Black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae) were obtained from colonies established in 2016 in the Agro-Eco-Health sector of the International Institute of Tropical Agriculture (IITA), Tanguieta, Benin. Egg clusters were collected following the method described by Sheppard et al. [41].

2.2. Preparation of the Ferment

One kilogram of rice was soaked in three litres of water for 1 h. The resulting rice-washing water was transferred to a plastic container and incubated in the dark for five days. After incubation, several layers formed; all layers except the bottom layer were collected and filtered through cotton into a clean container. The filtered solution was mixed with milk at a ratio of 1 L of solution to 3 L of milk, with each litre of milk prepared from 300 g of powdered milk. The mixture was incubated in the dark for another five days. At the end of this period, a cheese-like layer formed at the surface, a yellow layer developed beneath it, and a sediment accumulated at the bottom. All layers except the bottom layer were collected again

using a cotton filter. The cheese-like layer was discarded, and the resulting yellow solution was used as the ferment. To prepare the final ferment solution, the collected ferment and 10 kg of sugar were added to a 1,000-L container. The container was filled to capacity with water, tightly closed, and left to ferment for three days in the dark before use in the experiment.

2.3. Characterization of the Artisanal Ferment

The artisanal ferment used in this study was produced according to the method described above, with minor adaptations based on previously published protocols. Its microbial characterization was based on Gram staining. A smear of the final ferment solution was Gram-stained and examined under a light microscope. Cell morphology and Gram reaction were interpreted by comparison with published descriptions of lactic acid bacteria isolated from fermented rice and rice-washing water [1, 2, 19] (Table 1); the identification of *Lactobacillus* spp. is therefore presumptive, because no culture-based or molecular identification was performed.

Table 1. *Gram staining test of the artisanal ferment.*

Ferment	Gram staining result	References
Fermented rice starch + sugar + milk	Predominance of Gram-positive bacilli (Lactobacillus spp.)	[1, 2, 19]
	Secondary flora: a few Gram-negative bacilli	[1, 19]

2.4. Collection of Spoiled Fruit and Preparation of Substrates

Spoiled fruits were collected from "Dantokpa" Market in 100-kg barrels and transported by vehicle to the IITA. Upon arrival, the fruits were sorted by type. The batch consisted primarily of oranges (60%), pineapples (25%), watermelons (12%), and lemons (3%). All fruits were then combined and mechanically crushed using a grinder to obtain a homogeneous mixture. The crushed fruit mixture was subsequently stored in baskets for three days to reduce its water content through passive drainage.

After 72 h, the fruit mixture (oranges, pineapples, watermelons, and lemons) was mixed with the prepared ferment at proportions of 0%, 10%, 20%, 30%, 40%, and 50%, except for the chicken-feed control, which was diluted with water to achieve 60% moisture [14]. Mixing was performed thoroughly with an aluminium spoon to ensure uniform distribution of the ferment throughout the substrate and to avoid localized differences in concentration. The resulting mixtures were tightly covered and allowed to ferment anaerobically for three days before use.

2.5. Experimental Design

Six-day-old larvae used in the experiment were obtained from a black soldier fly (Hermetia illucens) colony maintained in wooden cages measuring 2.83 m ×0.60 m ×2.20 m at IITA. During the larval growth phase, larvae were reared in containers measuring 10 cm × 5 cm × 2.3 cm, covered with netting secured with elastic bands and kept at ambient room temperature. The containers were placed on five-compartment shelving units, with each shelf compartment surrounded by boxes containing waste oil to prevent ant invasion. To ensure uniform access to nutrients, the substrate was mixed every two days.

Upon reaching the prepupal and pupal stages, as indicated by the development of a darkened cuticle, larvae were removed from the substrate using tweezers. They were transferred to plastic boxes containing 10 g of wood chips and covered with a layer of white mesh and an additional layer of black cloth, both secured with elastic bands. This setup provided darkness and facilitated adult emergence. Once flies began to emerge, the plastic boxes were transferred to adult breeding cages after a hole had been made in the center of the black cloth to allow the adults to escape.

The mating cages (45 cm ×44 cm ×58 cm) were equipped with mosquito netting on the sides and a glass top and were placed in a greenhouse to provide sunlight, which stimulates mating and oviposition. Each mating cage contained a container with 200 g of fermented palm-kernel meal mixed with water (60% moisture) [14] to attract gravid females. Females laid eggs on corrugated-cardboard oviposition trays positioned above and separated from the substrate container by a mosquito net. The trays consisted of strips of corrugated cardboard secured with elastic bands at both ends.

The study employed a completely randomized design with seven treatments and three replicates. The composition of each treatment was as follows:

- T0 (control): poultry feed
- T1: 100% crushed spoiled fruit (0% artisanal ferment)
- T2: 90% crushed spoiled fruit + 10% artisanal ferment
- T3: 80% crushed spoiled fruit + 20% artisanal ferment
- T4: 70% crushed spoiled fruit + 30% artisanal ferment
- T5: 60% crushed spoiled fruit + 40% artisanal ferment
- T6: 50% crushed spoiled fruit + 50% artisanal ferment

Each treatment was tested in white plastic containers measuring 14 cm × 9 cm × 7 cm (126 cm^2 surface area). Each container was covered with netting secured by elastic bands to prevent housefly infestation. A total of 10,584 six-day-old larvae were allocated to the containers at a density of 504 larvae per container (approximately 4 larvae/cm^2) [38]. Substrates were provided in three feedings throughout the experiment at a rate of 100 mg/larva/day over 15 days [12]. Finally, all containers were randomly arranged on a board.

2.6. Analysis of Nutritional Value of Experimental Substrates

For each substrate, dry matter (DM), gross energy (GE), total nitrogen (TN), and total ash (TA) were determined. All analyses were performed according to the official methods of the Association of Official Analytical Chemists [3]. Dry matter content was determined in accordance with ISO 1442 [18]. After DM determination, the sample crucibles were ashed in a muffle furnace at 550 ℃ for 6-7 h to determine total ash content. Total nitrogen was measured using the Kjeldahl method, and crude protein was calculated by multiplying the nitrogen content by a conversion factor of 6.25. Gross energy was determined using a bomb calorimeter.

2.7. Measurement of pH and Temperature

The initial pH and temperature of each substrate (before the introduction of larvae) were measured using an electronic pH meter-thermometer (model pH-B200EM, Infitek). The instrument was calibrated with a buffer solution at 20 ℃ before measurement. Each sample was placed in a 50-mL beaker, and the electrode was fully immersed in the substrate. The pH and temperature were then measured and recorded.

2.8. Data Collection and Parameter Calculation

During the experiment, the following data were collected: initial number of larvae, initial substrate quantity, initial and final larval weights, number of recovered larvae, remaining substrate quantity, larval development time, number of prepupae, number of emerged adults, pupal development time, temperature, pH, and adult longevity. These data were used to calculate performance parameters according to the methods described by Meneguz et al. [29], Diener et al. [12], and Parra et al. [38].

$$Survival\ rate\ (\%) = \frac{number\ of\ larvae\ harvested\ \times 100}{Initial\ number\ of\ larvae}$$

$$Substrate\ reduction\ rate\ (\%) = \frac{(substrate\ distributed - residual\ substrate)\times 100}{substrate\ distributed}$$

$$Bioconversion\ rate\ (\%) = \frac{Harvested\ larvae\ humid\ weight\times 100}{Initial\ weight\ of\ substrate}$$

$$Emergence\ rate\ (\%) = \frac{Number\ of\ emerged\ fly\times 100}{Number\ of\ pupae}$$

$$Relative\ growth\ rate\ (\%) = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight\times experimentation\ time}$$

$$Substrate\ reduction\ index = \frac{(substrate\ distributed - residual\ substrate)\times 100}{substrate\ distributed\times development\ time}$$

2.9. Data Analysis

Data were analyzed using R software, version 3.6.2 [39]. Prior to analysis, the normality of data distribution and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively. As the data satisfied the assumptions of normality and homoscedasticity, one-way analysis of variance (ANOVA) was performed to evaluate the effects of treatments on larval development time, initial weight, final larval weight, pupal weight, substrate reduction index, and adult longevity. For relative growth rate, bioconversion rate, emergence rate, survival rate, and substrate reduction rate, generalized linear models (GLMs) with a beta distribution were used. When significant differences were detected ($p < 0.05$), treatment means were separated using the Student-Newman-Keuls (SNK) multiple range test. The graphs were generated using GraphPad Prism software (version 8.0.0.288, released on 28 August 2021).

2.10. Ethical Considerations

This study was conducted in accordance with the institutional guidelines applicable to experimental work at the National Agricultural University (UNA), Benin. The protocol was approved by the relevant institutional ethics committee. All procedures involving black soldier fly larvae were conducted with appropriate care to minimize unnecessary stress or harm.

3. Results

Figure 1 illustrates the effects of pH and substrate temperature on BSF production (Figure 1a and 1b), as well as the effect of artisanal ferment-based substrates on BSF larval development time (Figure 1c). The inclusion of artisanal ferment had a highly significant effect ($p < 0.001$) on the initial pH of the experimental substrates. The highest pH, approaching neutrality, was observed in the chicken-feed substrate (6.18 ± 0.09). Similarly, initial substrate temperature differed significantly among treatments ($p < 0.005$), with the highest temperatures recorded in substrates T1 and T2 (32.6 ± 0.28 ℃ and 32.63 ± 0.32 ℃, respectively).

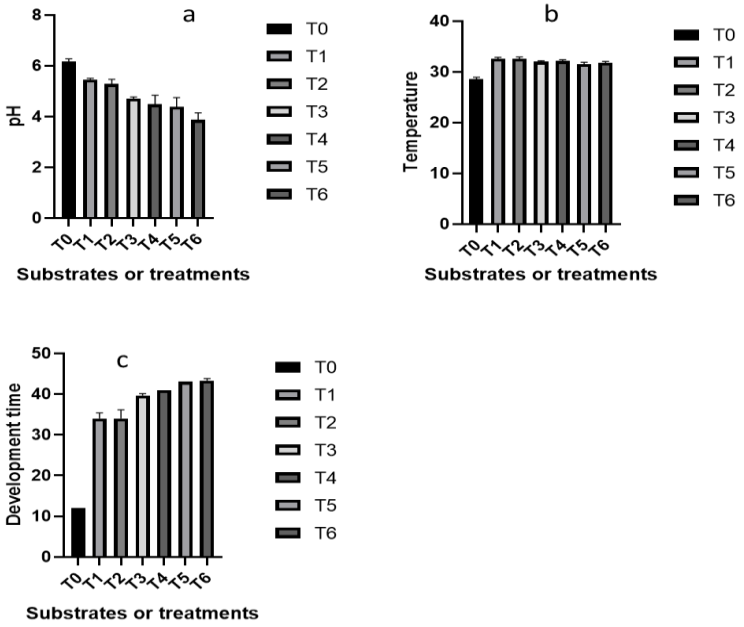


Figure 1. *Influence of pH and substrate temperature on BSF production (Figure 1a and 1b) and effect of artisanal ferment-based substrates on BSF larval development time (Figure 1c).*

Larval development time was significantly affected by substrate type ($p < 0.001$). Larvae fed chicken feed reached the prepupal stage in the shortest time (12 ± 0.00 days), whereas those reared on substrates T1 and T2 required significantly longer periods (34 ± 1 days).

Table 2 summarizes the effects of artisanal ferment-based substrates on substrate reduction rate, survival rate, bioconversion rate, emergence rate, and relative growth rate of BSF larvae. All five parameters differed significantly among substrates ($p < 0.001$). The substrate reduction rate was lowest in the chicken-feed substrate (77.39 ± 4.69%) and ranged from 90.82% to 95.87% in the fruit-based substrates (T1-T6). The survival rate was highest in larvae fed chicken feed (89.63 ± 1.09%), remained above 80% in T1-T3, decreased to 64.66 ± 0.65% in T4, and fell to about one-third in T5 and T6 (33.9 ± 1.65% and 33.4 ± 1.03%, respectively). The bioconversion rate was also highest in larvae receiving chicken feed (14.23 ±0.86%) and declined from 7.04 ±0.48% in T1 to 2.06 ±0.27% in T6. Relative growth rate and emergence rate showed the same overall pattern, with the highest values recorded for the chicken-feed substrate (55.6 ± 11.43 and 96.66 ± 1.52%, respectively); among the fruit-based substrates, the emergence rate was highest in T1 (83.33 ± 3.21%).

Table 2. *Effect of artisanal ferment-based substrates on substrate reduction rate, survival rate, bioconversion rate, emergence rate, and relative growth rate of BSF larvae.*

Parameters	Treatments/substrates							P-Value
	T0	T1	T2	T3	T4	T5	T6	
SRR	77.39 ±4.69^{b}	91.85 ±2.48a	92.81 ±2.8^{a}	94.1 ±1.27^{a}	95.87 ±1.23^{a}	95.17 ±0.85^{a}	90.82 ±3.1^{a}	<0.001
BC	14.23 ±0.86^{a}	7.04 ±0.48^{b}	6.83 ±0.7^{b}	6.07 ±1.09^{c}	4.42 ±0.68^{d}	2.71 ±0.16^{e}	2.06 ±0.27^{f}	<0.001
RGR	55.6 ±11.43^{a}	15.59 ±3.32^{b}	11.94 ±0.90^{c}	11.14 ±0.68^{c}	9.52 ±1.26^{d}	9.72 ±1.43^{d}	6.43 ±1.9^{e}	<0.001
ER	96.66 ±1.52^{a}	83.33 ±3.21^{b}	60.66 ±1.15^{c}	76.00 ±2.64^{d}	67.33 ±3.05^{e}	61.33 ±5.13^{f}	71.66 ±1.52^{g}	<0.001
SR	89.63 ±1.09^{a}	80.73 ±2^{c}	83.76 ±3.04^{b}	80.16 ±0.76^{c}	64.66 ±0.65^{d}	33.9 ±1.65^{e}	33.4 ±1.03^{e}	<0.001

SRR = substrate reduction rate; SR = survival rate; BC = bioconversion rate; ER = emergence rate; RGR = relative growth rate; T0 = control substrate (chicken feed); T1 = 100% fruit; T2 = 90% fruit and 10% ferment; T3 = 80% fruit and 20% ferment; T4 = 70% fruit and 30% ferment; T5 = 60% fruit and 40% ferment; T6 = 50% fruit and 50% ferment. Values are means ± SD (n = 3). Means in the same row followed by different letters differ significantly (SNK test, $p < 0.05$).

Table 3 presents the weights of larvae and pupae, the substrate reduction index, and adult longevity. Initial mean larval weight and adult longevity did not differ significantly among treatments ($p > 0.05$). However, final larval weight and pupal weight differed highly significantly among treatments ($p < 0.001$), with the highest values recorded in the chicken-feed group (T0; 0.24 ±0.018 g and 0.15 ±0.01 g, respectively). In the fruit-based substrates, final larval weight ranged from 0.05 to 0.10 g and pupal weight from 0.04 to 0.06 g. In addition, the substrate reduction index was strongly affected by substrate type ($p < 0.001$), with the highest value observed in larvae fed chicken feed (6.44 ±0.39), compared with 2.70 ±0.06 in T1 and 2.09 ±0.09 in T6.

Table 3. *Effect of artisanal ferment-based substrates on final larval weight, adult longevity, substrate reduction index, and pupal weight.*

Parameters	Treatments/substrates							P-Value
	T0	T1	T2	T3	T4	T5	T6	
Adult longevity (days)	7 ±1	7.33 ±0.57	7 ±0	7.33 ±0.57	6.66 ±1.52	9.33 ±2.08	8.66 ±3.21	0.409
Initial weight (g)	0.0003 ±4E-8	0.0002 ±1E-5	0.0003 ±4E-5	0.0002 ±1.9E-5	0.0002 ±3E-5	0.0003 ±3E-5	0.0003 ±1.5E-5	0.147
Final larval weight (g)	0.24 ±0.018^{a}	0.05 ±0.01	0.05 ±0.02	0.05 ±0.00	0.1 ±0.002^{bc}	0.06 ±0.00	0.09 ±0.015c	<0.001
Pupal weight (g)	0.15 ±0.01^{a}	0.05 ±0.01b	0.05 ±0.02b	0.05 ±0.00b	0.04 ±0.01^{b}	0.06 ±0.01^{b}	0.06 ±0.00b	<0.001
SRI	6.44 ±0.39^{a}	2.7 ±0.06^{b}	2.73 ±0.13^{b}	2.37 ±0.05^{c}	2.33 ±0.03^{c}	2.21 ±0.019^{c}	2.09 ±0.09c	<0.001

Adult longevity = longevity of adult flies; SRI = substrate reduction index. Values are means ± SD (n = 3). Means in the same row followed by different letters differ significantly (SNK test, $p < 0.05$).

Table 4 presents the chemical composition of the experimental substrates. Chemical analysis indicated that dry matter, total nitrogen, and total ash contents were significantly higher ($p < 0.001$) in the chicken-feed substrate (T0; 93.67%, 25.24%, and 6.79%, respectively) than in the other substrates (10.5-17.5%, 3.33-6.65%, and 1.64-4.40%, respectively). These parameters also differed significantly among the experimental substrates ($p < 0.001$) and decreased progressively with increasing ferment inclusion. Gross energy content was highest in the 100% spoiled-fruit substrate (T1; 4380.05 kcal/kg DM), whereas the lowest gross energy value was recorded in substrate T6 (2101.02 kcal/kg DM); gross energy also decreased with increasing ferment inclusion and was lower in T0 (2455.58 kcal/kg DM) than in T1-T5.

Table 4. *Chemical composition of the experimental substrates.*

Parameters	Treatments/substrates							P-Value
	T0	T1	T2	T3	T4	T5	T6	
DM (%)	93.67 ±0.76a	17.5 ±1.32b	17 ±1^{bc}	16.58 ±1.5bc	14.89 ±0.75c	12.5 ±0.5d	10.5 ±0.88e	<0.001
TN (%)	25.24 ±0.67a	6.65 ±1.88b	6.03 ±0.98b	5.96 ±0.05b	5.06 ±0.11bc	3.66 ±0.57c	3.33 ±0.57c	<0.001
GE (kcal/kg DM)	2455.58 ±1.4f	4380.05 ±1.95a	3888.04 ±0.98b	3439.04 ±0.97c	2966.03 ±0.95d	2530.03 ±0.95e	2101.02 ±0.97g	<0.001
TA (%)	6.79 ±1.11a	4.40 ±0.52b	3.06 ±0.95c	2.96 ±0.05c	2.30 ±0.51c	1.77 ±0.3d	1.64 ±0.76d	<0.001

DM = dry matter; TN = total nitrogen; GE = gross energy; TA = total ash. Values are means ± SD (n = 3). Means in the same row followed by different letters differ significantly (SNK test, $p < 0.05$).

4. Discussion

The results of this study showed that final larval weight and pupal weight were significantly higher in the control substrate (chicken feed). Among the fruit-based substrates, pupal weight did not differ significantly (0.04-0.06 g), and final larval weight (0.05-0.10 g) showed no consistent trend with the ferment level. The superior performance of the control may be attributable to the higher nitrogen and mineral contents of chicken feed (Table 4), as substrate quality is a key determinant of BSF larval growth performance [16, 33]. Protein-rich substrates, such as chicken feed, can promote rapid larval growth [43], which may explain the superior weight-related performance observed in the control group. In addition, insects must accumulate sufficient nutrient reserves to reach the critical weight required for successful metamorphosis [10, 20], and both the quality and quantity of dietary substrate can positively influence larval weight across developmental stages [20, 34]. Nutrient intake is a further likely explanation. Because all substrates were supplied at the same rate (100 mg/larva/day), and assuming that this rate refers to fresh substrate, larvae in T0 (moistened to 60% moisture, i.e, about 40% dry matter) received about 40 mg of dry matter per day, compared with approximately 10.5-17.5 mg in the fruit-based substrates (dry matter contents in Table 4), i.e., a 2.3- to 3.8-fold lower dry-matter supply. Moreover, the fruit-based substrates combined a relatively high gross energy content with low nitrogen contents (3.33-6.65% versus 25.24% in T0). Such a carbohydrate-rich but protein-poor composition probably limited tissue growth, because larval performance depends on the balance between protein and carbohydrate [7] and protein-deficient substrates prolong the life cycle [43]. Our findings differ from those reported by Jucker et al. [20], Nyakeri et al. [36], and Liu et al. [23], who reported different responses in larvae reared on fruit waste, vegetable waste, and chicken feed, respectively.

Larval development time and adult emergence rate were both significantly improved in the control substrate (chicken feed). Among the alternative substrates, the 100% spoiled-fruit treatment showed the best performance. These differences may be related to nutrient availability and initial substrate pH. Diets rich in protein or energy generally accelerate insect development [43]. The pH of the BSF midgut is buffered [17], and larvae reared on acidic substrates may increase substrate pH through digestive processes [27, 40], potentially facilitating development. Ma et al. [27] reported that substrates with an initial pH between 6.0 and 8.0 reduced development time, whereas pH values below 4.0 prolonged development. In the present study, the chicken-feed substrate had the highest initial pH (6.18; Figure 1a), whereas all fruit-based substrates were more acidic. In the latter, organic acids from the fruit and, probably, lactic acid and other metabolites produced during the three-day anaerobic fermentation of the substrates may have forced the larvae to spend time and energy buffering their environment before feeding efficiently, which may partly explain the longer development on fruit-based substrates. However, because the ferment and the fermented substrates were not characterized for organic acids, this interpretation remains to be tested. In the present study, larval development time was 12 days on chicken feed and 34 days on the 100% spoiled-fruit substrate. The development time on chicken feed was shorter than those reported by Gobbi et al. [16], Tariq et al. [43], and Oonincx et al. [37] for chicken-feed diets (15-24 days), as well as those reported by Jucker et al. [20], Mohd et al. [31], and Tinder et al. [44] for vegetable- and fruit-waste diets, whereas the 34 days observed on spoiled fruit exceeded the chicken-feed values, confirming that the fruit-based substrates strongly delayed development. Differences among studies may result from variation in substrate composition and texture [16], as well as feeding rate and larval density [7, 43]. The emergence rates observed in the present study (60.66-96.66%) were higher than that reported by Sheppard et al. [41] for the Gainesville diet (27.2 ± 6.7%), and the emergence rates in T0, T1, T3, T4, and T6 were also higher than that reported by Jucker et al. [20] for fruit and vegetable mixtures (63.55 ± 3.76%).

Survival rates were also significantly higher in the control substrate. In contrast, much lower survival rates were observed in substrates containing high proportions of artisanal ferment (40% and 50% in T5 and T6, respectively: 33.9 ± 1.65% and 33.4 ± 1.03%). These survival rates were lower than those reported by Gao et al. [15] for larvae fed fermented maize straw (93 ± 1%) and by Jucker et al. [20], whose survival rates ranged from 88.67 ± 6.12% to 96.44 ± 2.62%. The reduced survival observed in T5 and T6 may be related to excessive substrate moisture, which can negatively affect larval survival [6, 21]. The dry matter contents of T5 and T6 (12.5% and 10.5%; Table 4) correspond to moisture contents of about 87.5% and 89.5%, far above the 60% moisture used for the control [14]. Such wet substrates tend to become liquid and poorly aerated, which limits oxygen availability and may cause larvae to drown or to be stressed, and Lowet [26] reported that at substrate moisture levels as high as 97.5%, survival rates could decrease to 19.5%. In addition, higher ferment proportions probably increased the concentration of organic acids and other microbial metabolites in the tightly closed, anaerobically fermented substrates, which may also have impaired larval survival; this could not be verified because these compounds were not measured.

Finally, both the bioconversion rate and substrate reduction index were significantly higher in the control substrate. These parameters are important indicators of larval production efficiency, particularly when lower-quality substrates are used, and may contribute to environmental benefits through organic-waste reduction and the generation of nutrient-rich residues [15, 25, 28]. The higher substrate reduction index in T0 mainly reflects its shorter development time (12 days versus 34 days or more), because this index relates the reduction achieved to the duration of the trial; indeed, the substrate reduction rate itself was lower in T0 (77.39%) than in the fruit-based substrates (90.82-95.87%). The high reduction rates in

the fruit-based substrates probably include water loss by drainage and evaporation and microbial degradation of the highly moist, fermentable fruit, in addition to larval consumption, so reduction rates calculated on a fresh-weight basis should be interpreted with caution when substrates differ markedly in moisture. The decline in the bioconversion rate with increasing ferment inclusion (from 7.04% in T1 to 2.06% in T6) parallels the decline in dry matter, nitrogen, ash, and gross energy contents (Table 4). Notably, the bioconversion rates observed for T1, T2, and T3 (7.04%, 6.83%, and 6.07%, respectively) were higher than those reported by Miranda et al. [30] for BSF larvae reared on pig and dairy-cow manure substrates.

Overall, the graded inclusion of the artisanal ferment did not improve larval performance relative to unfermented spoiled fruit (T1): the bioconversion rate, relative growth rate, substrate reduction index, survival rate, and emergence rate all tended to decline as the ferment proportion increased. Three mechanisms may explain this pattern. First, dilution: the ferment is an aqueous solution that supplied sugar and microbial cells but few other nutrients, so each increment reduced the dry matter, nitrogen, ash, and gross energy contents per unit of fresh substrate (Table 4) and therefore the nutrients available to the larvae at a fixed feeding rate. Second, increased moisture and anaerobic conditions, as discussed above. Third, the microbial benefits reported for fermented substrates [15, 31] concern the pre-digestion of fibrous, poorly digestible materials such as maize straw or coconut endosperm; spoiled fruit is already rich in simple sugars, so pre-fermentation may offer little nutritional advantage, and the lactic acid bacteria may instead convert part of these sugars into organic acids, lowering the pH of the substrate. In addition, the citrus fruits that dominated the fruit mixture (oranges and lemons, 63%) may have contributed acidity and bioactive peel compounds that could affect larvae; this was not tested.

This study has limitations that should be considered when interpreting the results. The ferment was characterized by Gram staining only; its pH, titratable acidity, microbial counts, and organic-acid profile were not determined, and the microbial composition of the substrates was not monitored during the trial. Substrate moisture was derived from dry matter rather than measured at each feeding, the nutritional composition of the larvae produced was not analyzed, and the trial was conducted at a single site with three replicates per treatment. These aspects should be addressed in future work.

5. Conclusion

This study demonstrated that the control substrate (T0; chicken feed) produced the highest overall BSF larval growth and development performance (12-day development, 89.63% survival, 14.23% bioconversion rate, and 0.24 g final larval weight). Among the experimental substrates, 100% spoiled fruit (T1) gave the best results (34-day development, 80.73% survival, and 7.04% bioconversion rate), whereas increasing the proportion of artisanal ferment progressively reduced the bioconversion rate, relative growth rate, substrate reduction index, and survival rate, the latter falling to about 33% at 40-50% ferment. Under the conditions of this study, the artisanal ferment therefore did not improve larval performance. However, from an environmental-sustainability and waste-recycling perspective, substrate T1 (100% spoiled fruit) represents a potential low-cost alternative. The use of spoiled fruit as a rearing substrate can support BSF production while contributing to the recycling of organic waste and potentially reducing reliance on conventional feed substrates, provided that its slower larval development is taken into account.

6. Recommendations

Further research should (i) characterize the ferment (pH, titratable acidity, lactic acid bacteria counts, and organic acids) and test lower inclusion levels (below 10%) or drained substrates so that moisture is controlled; (ii) compare substrates at equal dry-matter and nutrient supply rather than at equal fresh-matter rates; (iii) evaluate spoiled fruit combined with protein-rich co-substrates to correct its nitrogen deficiency; (iv) analyze the proximate, amino acid, and fatty acid composition of the larvae produced and the economic cost of each substrate; and (v) validate the best substrates at larger scale while recording environmental conditions (temperature, relative humidity, and photoperiod).

Abbreviations

CERSA	Regional Center of Excellence on Poultry Sciences
UNA	National Agricultural University
FSA	Faculty of Agronomic Sciences
ISSMV	Higher Institute of Sciences and Veterinary Medicine
BSF	Black Soldier Fly
IITA	International Institute of Tropical Agriculture
DM	Dry Matter
GE	Gross Energy
TN	Total Nitrogen
TA	Total Ash
SRR	Substrate Reduction Rate
SRI	Substrate Reduction Index
BC	Bioconversion Rate
RGR	Relative Growth Rate
ER	Emergence Rate
SR	Survival Rate
ANOVA	Analysis of Variance
GLM	Generalized Linear Model
SNK	Student-Newman-Keuls
SD	Standard Deviation

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Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

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