Journal of Agriculture and Food Research 4 (2021) 100153

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.journals.elsevier.com/journal-of-agriculture-and-food-research/

Technical basis for the small-scale production of black soldier fly, *Hermetia illucens* (L. 1758), meal as fish feed in Benin

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ARTICLE INFO

Keywords:
Hermetia illucens
Insect breeding
Fish feeding
Polyunsaturated fatty acid
West Africa

ABSTRACT

Promoting black soldier fly (BSF) rearing in Benin to replace fishmeal in fish diet could reduce fish production costs. This study aims to set up a breeding technique of BSF as a protein source. BSF reproduction has been carried out in sunlight. Two densities (8500 individuals/m^3 and 10,500 individuals/m^3) associated with 2 attractants (soybean and pineapple waste) were tested on imago egg-laying. A larvarium with a prepupae self-harvesting system was designed and its efficiency was evaluated by testing 3 densities of larval rearing (1 larva/g, 2 larva/g, 3 larva/g). Two diets, VGD (soybean meal + euphorbia leaves and seeds + colza oil) and FOD (soybean meal + fish offal) were formulated to optimize polyunsaturated fatty acid profile of prepupae to meet nutritional requirements of tilapia. Best oviposition was obtained with 8500 individuals/m^3 with pineapple waste and optimal larval load density was 2 larva/g. Prepupae fed with VGD accumulated C18:2n-6 (10.1%) which is essential for tilapia. Prepupae fed with FOD contained C20:5n-3 (2.20%), a fatty acid beneficial to humans. The lowest production cost of BSF meal was estimated at $1.84/Kg and was obtained with the FOD diet. BSF can easily be reared under local conditions throughout the year. Its nutritional qualities are very interesting to meet Nile tilapia requirements.

1. Introduction

In Benin, fish farm installation throughout the country has increased simultaneously with various programs of support and promotion of aquaculture [1,2]. The two main species farmed are the Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) and the African catfish *Clarias gariepinus* (Burchell 1822) with a higher production of the former due to the preferences of Beninese consumers [3]. Among other factors, feed represents the production factors that strongly influences the cost of produced fish. The feed generally used in Benin for fish production is imported and expensive for the producers [4]. An economically and environmentally sustainable solution would be to directly produce fish feed in Benin with high-quality local ingredients. Currently, fishmeal, an important constituent of fish feed formulation, is mostly derived from small pelagic species (e.g. anchovies, sardines or herring) whose overfishing will have a long-term negative impact on the environment and on food security in the fishing areas [5]. One of the critical points in fish feed formulation and production is consequently to identify high-quality protein and lipid sources to replace fishmeal [6]. The substitute must meet the nutritional requirements of the targeted fishes, principally Nile tilapia in Benin. The protein requirements for optimal growth of *O. niloticus* are estimated at 29% [7] while the optimal level of total lipid intake is around 10% [8]. In farmed fish, lipid intake in the diet is essential to cover the essential fatty acids (EFAs) requirements of the fish (Guillaume et al., 1999) but have also beneficial effect on human health as lipids in fish are generally characterized by high levels of polyunsaturated fatty acids (PUFAs) [9]. Freshwater fish such as *O. niloticus* are able to biosynthesize long-chain polyunsaturated essential fatty acids of the C20 or C22 series from C18 PUFAs (C18:2-n3 and C18:3n-3) through enzymatic processes of elongation and desaturation [10]. However, recent study have shown that the presence of C18 in fish feed can influence this bioconversion [11]. In *O. niloticus*, only linoleic acid (C18:2n-6) is essential between 0.5 and 1% [12]. Among alternatives for the aquaculture development, insects are seen as serious candidates and among potential species, the black soldier fly (BSF, *Hermetia illucens* (L. 1758); Diptera, Stratyomyidae) are increasingly targeted as this

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https://doi.org/10.1016/j.jafr.2021.100153
Received 17 July 2020; Received in revised form 13 April 2021; Accepted 14 April 2021
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cosmopolitan species could be produced on a very broad scale of organic residues [6,13,14]. Moreover, its nutritional composition may be appropriate for use in fish nutrition. The protein content of *H. illucens* ranges from 37 to 63% of dry matter while lipid content also varied from 7 to 39% principally depending on the rearing substrates [13,15]. The BSF fatty acid profile has been characterized in several studies and was mainly composed of biosynthesized lauric acid (C12:0) [16]. In order to optimize the fatty acid composition of BSF larvae or prepupae (i.e. the developmental stage of a metamorphic insect prior to entering the pupal stage), several experiments enriched BSF diets with PUFAs and showed a substantial incorporation of linoleic acid (LA, C18:2n-6), alpha linolenic acid, eicosapentaenoic acid (EPA, C20:5n-3) or docosahexaenoic acid (DHA, C22: 6n-3) [14,17–19].

Generally, BSF production is carried out in two steps. The first one is carried out in an insectarium and consists in adult and egg production while the second step is carried out in a larvarium where the larvae are growing [20]. For incorporation into feed formulation, this species could be used at the larval stage following manual harvesting or sieving or it could be self-collected at the prepupal stage [21–24]. Several parameters have already been identified in order to successfully breed *H. illucens*. For adult reproduction, ambient temperature should be between 26 and 27 °C; relative humidity around 60–70% and light intensity for oviposition between 135 and 200 μmol/m^2/s [13]. Concerning adult densities in breeding cages, Hoc et al. [25] obtained maximum egg production at a density of 8500 individuals/m^3 inside a breeding unit with controlled environmental parameters. As attractant for female oviposition, several products can be recommended as decaying organic matter, poultry manure or carrots [25,26] but any other local co-products should be used with possible attractiveness differences. Current literature provides information on larval rearing devices in line with the development cycle of *H. illucens* [27,28]. Two systems could be used depending on the feeding substrates. A first system based on the self-harvesting of prepupae when using plant or animal co-products as in this study or a second system based on last instar larvae sieving when using high-quality products (e.g. flour or cereal bran) in a drier environment. In laboratory conditions the average larval growth time is 24.6 ± 6.2 days and the average weight of prepupa is 0.11 ± 0.01 g [13].

Promoting small-scale of BSF rearing in Benin suggests an optimization of the different rearing parameters under local conditions. The objectives of this work are to propose a device adapted to *H. illucens* larval rearing; to determine the optimal parameters for reproduction and growth of the species under local conditions and to optimize the lipid quality of the prepupae produced on local byproducts while maintaining low production cost of insect meal.

2. Materials and methods

2.1. Study site

The experiment was conducted in Benin (West Africa) within the Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi.

2.2. Reproduction of black soldier fly

The experiment was conducted in a fenced building (10 × 3.7 × 2.8 m) with windows. The experimental module consisted of 12 nylon breeding cages (75 × 75 × 115 cm, Bugdorm, Taichung, Taiwan). The cages were placed near the windows for maximum exposure to sunlight. A control density of 8500 individuals/m^3 (FD1) was used [25] while a density of 10,500 individuals/m^3 (FD2) was tested in triplicates for two egg-laying attractants: soybean dregs (i.e. waste from extraction of soy milk; SD) and pineapple waste (i.e. waste from the pineapple juice extraction; PW). 500 g of attractant were placed in a tray (37.5 × 28 × 7.5 cm) covered with a grid to prevent flies from laying eggs inside and were introduced into each cage. A 12 cm long wooden nesting box (model proposed by Hoc et al. [25]) was placed on the grid for the females to lay eggs. A water-soaked cloth was placed in each cage to allow the flies to hydrate. A datalogger DL-USB-171 (ATAL, Purmerend, Netherlands) was set up to record temperature data every 2 h throughout the experiment.

Every day, eggs were collected from the egg-laying structure with a cutter and weighed with an electronic scale (Sartorius, FB423, Germany) with an accuracy of 0.001 g. The egg collection was stopped when all the flies died. In addition to temperature, the sunlight duration was also recorded daily throughout the experiment. Each day, the time of the first sun rays was recorded and the time of the sunset as well. The collected eggs were incubated in trays (60 × 40 × 33.5 cm; Auer Packaging, Amerang, Germany) corresponding to each cage. The hatching substrate for the eggs was SD as its homogeneous and non-fibrous texture allows easy harvesting of the larvae after the egg hatching. After one week, the larvae were sorted with 2 mm and 1 mm mesh sieves. The 2 mm sieves allow the sorting of larvae with an average weight of 0.01 g. The number of larvae obtained per tray after hatching was estimated using an average weight in order to determine the yield per cage.

2.3. Larvarium design

A white-colored, rectangular and transparent plastic container (42 cm × 29 cm x 25 cm) closed with an opaque plastic lid was used (Fig. 1). This container was selected because of its high availability and accessibility on Beninese markets. In addition to the plastic container, a polyvinyl chloride pipe (PVC) of 50 cm in diameter was selected to serve as a collector for the prepupae. One of the side faces (42 cm × 29 cm) was cut along one length and the two widths to create an exit ramp for the prepupae (Fig. 1a). The opening was closed using a plastic from another container of the same model. A 45-cm PVC pipe was opened on the top to serve as a collector gutter. The pipe was fixed on the edge of the slope with its right end was closed with plastic. In order to aerate the system, the lid was cut in the middle and was covered with a 2.5-mm-mesh net (Fig. 1b).

2.4. Determination of optimal load density

One-week-old larvae with an average weight of 0.01 g were used for growth experiment. The experimental device consisted of 9 larvaria build according to the previously described model. All larvaria were placed on wooden shelves. Three larval densities were tested in triplicate: LD1 (1 larva/g of feed); LD2 (2 larvae/g of feed) and LD3 (3 larvae/g of feed). The feed used as a breeding substrate was a chicken feed (protein: 21% and lipid: 5.6%) from Veto Service Group (Cotonou, Benin). 2 kg of chicken feed (dry matter) with 70% moisture were distributed in each container. These 2 kg of chicken feed corresponds to 2 cm of substrate in the breeding containers [13]. Every two days, 60 larvae were sampled from each container of each treatment and weighed with an electronic scale (Sartorius, FB423, Germany). Larval growth was followed till the first prepupae emerged from the substrates. Finally, residual substrates were collected and dried in an oven at 105 °C for 1 h. The dried substrates were weighed to evaluate the substrate reduction effectiveness.

2.5. Harvesting system and feed production

For each larvarium, a 5-L bucket was placed at the left end of the PVC pipe. At the end of the experiment (On the 12th day when the prepupae no longer emerge from the substrate), the prepupae recovered from each bucket were counted, as well as those remaining in each container.

All the prepupae of each batch were transferred to labeled boxes and then kept in the freezer (Liebherr, France) at – 20 °C for 24 h. Frozen *H. illucens* specimens were dried in an oven at 50 °C for 6 h. They were then crushed/blended, batch by batch, in a kitchen blender. Because of its high fat content, some of the BSF flour gets stuck to the blender blades. This residual flour is removed each time and added to the different

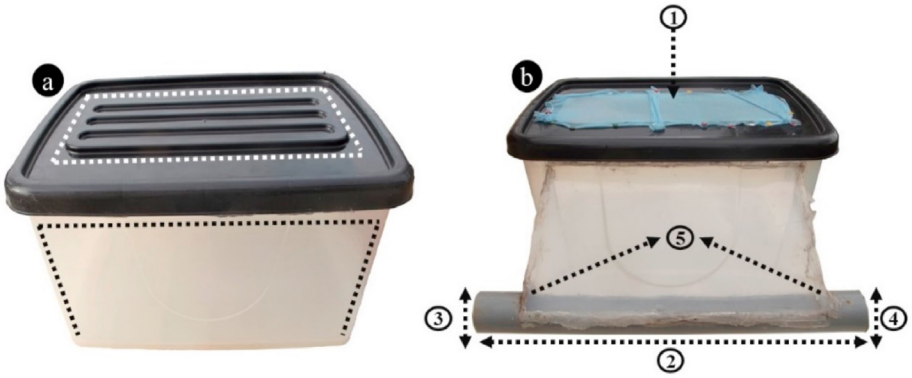

Fig. 1. (a) Model of the tank used for the design of the larvarium. The dots represent the cutting lines; (b) Larvarium for *Hermetia illucens*. 1: window opening covered by a mosquito net, 2: prepupae collection gutter, 3: exit for the prepuae, 4: closed end, 5: prepupae exit ramp.

samples. The meal obtained was weighed per batch.

2.6. Optimization of the lipid quality of black soldier fly prepupae

Five plant and animal ingredients were selected for this experiment: soybean meal (SM, Fludor-Benin, Cotonou, Benin), colza oil (CO, Super U Benin, Cotonou), leaves and seeds of *Euphorbia heterophylla* L. (EH) and fish offal (FO, Fishing port, Cotonou, Benin). The SM is a staple in all diets. The other ingredients are sources of contrasted fatty acids. CO and EH provide high levels of oleic (OA, C18:1n-9), LA, and ALA, acids. Other authors have already succeeded in enriching *Cavia porcellus* (L. 1758) [29], *Coturnix japonica* Temminck & Schlegel, 1849 [30] and *Oryctolagus cuniculus* (L. 1758) [31] with omega 3 from euphorbia. The EH plants, which are considered as weeds, have been harvested in Benin (Zinvie area) in cassava and maize plantations [32]. FO came from marine fishes and has been used to provide long-chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22: 6n-3) to BSF larvae. In order to obtain homogeneous substrates and to facilitate ingestion by the larvae, the various ingredients were reduced to powder. The leaves and seeds of EH were dried in shade for three days. FO was dried in an electric oven (Heraeus, Lille, France) at 70 °C for 24 h. All ingredients were ground in an electric mill (David 4 V, Italy).

Two experimental isolipidic diets VGD (vegetable diet, SM + EH + CO) and FOD (fish offal diet, SM + FO) were formulated from these ingredients and tested in triplicate on BSF larvae (Table 1). The diets were homogenized by mixing the ingredients and then moistened (70% water) in the larvarium designed. The control was chicken feed (CF) previously used to determine optimal load density. The larval load density was 2 larvae/g of feed. At the end of the experiment, prepupae were harvested and processed as described above. 100 g of prepupae meal was sampled from each diet for further nutritional analysis.

Table 1
Composition of formulated larval rearing diets.

Diet composition			
Diet	Ingredient	Inclusion (%)	Feed (g)
Control	CF	100	2000
VGD	SM	77.74	2000
	EH	15.00	
	CO	7.26	
FOD	SM	32.01	2000
	FO	67.99	
Nutritional composition			
Content	Control	VGD	FOD
CP	21.00	45.60	51.90
Lipid	5.60	10.0	10.00
C4:0	0.00	5.00	15.50
C14:0	0.00	0.00	3.50
C16:0	14.7	9.90	25.10
C16:1	0.00	0.20	7.30
C18:0	3.40	2.50	7.80
C18:1n-9	26.90	40.40	16.00
C18:2n-6	49.20	32.90	10.00
C18:3n-6	0.00	0.00	0.30
C18:3n-3	4.60	7.40	1.20
C20:0	0.40	0.40	0.40
C20:1n-9	0.30	0.80	0.90
C20:2	0.00	0.00	0.10
C20:3n-6	0.00	0.00	0.10
C20:4n-6	0.0	0.00	0.00
C20:3n-3	0.0	0.00	1.70
C20:5n-3	0.20	0.00	2.70
C22:6n-3	0.00	0.00	5.30
C24:0	0.20	0.00	1.40

Abbreviations: CF = chicken feed, SM = soybean meal, EH = *Euphorbia heterophylla*, CO = colza oil, FO = fish offal, CP = crude protein, VGD = vegetable diet, FOD = fish offal diet

2.7. Chemical analysis

Nutritional analyzes were done following the methods described by Caparros Megido et al. [33]. Dry matter content was determined by dehydrating samples in a drying oven at 105 °C overnight. Ash contents were determined by incinerating samples in a muffle furnace heated to 550 °C at a constant rate of 50 °C every 30 min for 4 h and then cooling in a desiccator. Crude protein content was determined by the Dumas method (Rapid N Cube Elementar, Hanau, Germany) with a conversion factor of 4.76 from nitrogen to protein [34]. Fat content was determined by Folch extraction method as described by Paul et al. [35]. Fatty acid profile was established by gas chromatography. Fatty acids of 10 mg lipids were transformed into fatty acid methyl esters with boron trifluoride (Sigma-Aldrich, Overijse, Belgium) and methanol (VWR, Oud-Heverlee, Belgium). Fatty acid methyl esters were diluted in 8 ml hexane (VWR) and analysed with a Trace GC Ultra gas chromatogram (Thermo Fisher Scientific, Asse, Belgium). The temperature program was as follows: hold at 50 °C for 1 min, increase to 150 °C at 30 °C/min, increase to 240 °C at 5 °C/min, and hold at 240 °C for 10 min. Fatty acid methyl esters were identified on the basis of their retention times compared to a reference mixture of 37 fatty acid methyl esters (Supelco 37 component FAME mix, Sigma-Aldrich, Overijse, Belgium). The relative percentage of each fatty acid was obtained by comparing the individual peak area with the sum of the peak areas of all identified compounds, using Chemstation software (Agilent Technologies, Palo Alto, CA, USA).

2.8. Financial evaluation of productions

The total cost of inputs used for the production of fly meal was evaluated. The inputs consisted of the equipment (cages, trays, nests) and

substrates used for the reproduction and larval rearing of the flies. These inputs have been categorized as fixed capital and circulating capital. The fixed capital represents the value of the tool used while the circulating capital indicates the raw materials entering the production. Labour was estimated based on the minimum wage for Beninese workers and the maximum amount of insect meal produced in one month. The maximum load-bearing capacity of the building was 200 larvaria. The evaluation of production cost was done by integrating the depreciation charges and made for 1 kg of BSF meal produced.

2.9. Data processing

The rate of degradation or substrate reduction (SR) was calculated with the formula:

SR=W−R/W, where W is the total amount of chicken feed applied during the experiment and R is the residue at the end of the breeding.

Prepupae harvesting rate (PHR) was also calculated.

PHR = 100 (HP/IP), where HP is the number of harvested prepupae and IP is the initial number prepupae.

One-way ANOVA test (F) was carried out to compare the breeding parameters (survival rate, harvesting rate, meal quantity) between the different densities tested and the nutritional parameters (dry matter, crude protein, lipid, ash, fatty acids) between the different diets. Two-way ANOVA test (F) was carried out to test the interaction of density and substrate type on egg production. Least Significant Difference (LSD) were used to compare paired samples. When the conditions for applying ANOVAs test (normality of populations and homoscedasticity) were not met, Kruskall-Wallis tests (H) were applied, followed by comparison of paired samples by the Mann-Whitney test (W). Normal distribution was tested by Shapiro-Wilk's test and homoscedasticity was tested by Leven's test.

3. Results

3.1. Reproductive parameters of Hermetia illucens

3.1.1. Ambient temperature and light duration

The atmospheric temperature during the experiment was 26.8 ± 0.48 °C and the average duration of sunlight exposure of was 12h10 min ± 2 min.

3.1.2. Evolution of oviposition over time

Oviposition began 2 days after imago emergence and was controlled every day. The total oviposition period was 21 days for flies bred with SD and 23 days for flies bred with PW. Regardless of the fly density (FD1 or FD2) or the attractant substrate used, egg laying reached maximum egg production on day 6 (Fig. 2). From day 8, egg-laying gradually decreased until the end of the experiment.

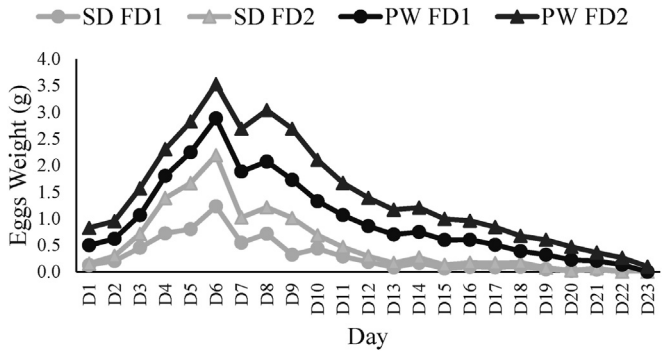

Fig. 2. Temporal evolution of oviposition of *Hermetia illucens*. SD FD1 = soybean dregs + fly density (8500 individuals/m^3), SD FD2 = soybean dregs + fly density (10,500 individuals/m^3), PW FD1 = pineapple waste + fly density (8500 individuals/m^3), PW FD2 = pineapple waste + fly density (10,500 individuals/m^3).

3.1.3. Variation of egg-laying and larval production according to densities and substrates

The highest egg and larval productions were recorded with pineapple waste under both density conditions (Table 2). The interaction between density and substrate has no significant impact on egg production ($F_{1,260} = 1.22$; $p = 0.27$).

3.2. Breeding parameters

At the end of the larval development, the average prepupal weight was significantly higher in LD1 and LD2 (0.24 ± 0.05 g) than for LD3 (0.14 ± 0.03, Table 3). In all density treatments, substrates reduction rate (SR) were equal or greater than to 50% and increased with density (Table 3). The maximum rate of prepupae harvested by the automated system was observed for LD1 (81.7 ± 5.75%) and LD2 (71 ± 8.53%) while containers at LD2 produced the maximum amounts of BSF meal (432.4 ± 54.36 g).

During the 10 days of larval growth, the larvae reached their maximum weight on the sixth day: 0.26 ± 0.04 g, 0.25 ± 0.04 g, and 0.21 ± 0.03 g respectively for LD1, LD2, and LD3 (Fig. 3). After day six, weight began to decline in all treatments till prepupal stage at day 10.

3.3. Prepupal weight and proximate composition of BSF reared on contrasted lipid diets

The prepupal weight of larvae fed on control diet was higher (0.23 ± 0.01 g) than for larvae fed on plant-based diet (VGD, 0.20 ± 0.00 g) and on fish offal diet (FOD, 0.17 ± 0.00 g) (Table 4). Dry matter and crude protein contents of prepupae did not vary between diets while lipid was significantly higher in VGD diet compared to Control and FOD diets. Ash contents were higher in the control diet and lower in VGD diet. (Table 4).

3.4. Fatty acids composition of BSF prepupae reared on contrasted lipid diets

Whatever the breeding substrate, the lauric acid (C12:0) content is higher (at least 32% of total lipids in FOD) than all other fatty acids (Table 5). Moreover C12:0 as well as linoleic acid (C18:2n-6) are found in higher proportions in the control diet (44.07 ± 0.14%) than in the two other diets. The highest concentrations of oleic acid (C18:1n-9) and α-linolenic acid (C18:3n-3) are found in VGD, while the highest concentrations of long-chain polyunsaturated fatty acids, eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) are found in FOD. (Table 5).

3.5. Evaluation of Hermetia illucens prepupal production costs

This production cost assessment was carried out for 1 kg of BSF meal. Only one breeding cage, 4.63 kg of feed and 2 larvaria are needed to achieve this production. The costs of the various inputs used in the production of BSF meal are shown in Table 6. The total cost was $333.32 for CF-based production, $334.12 for HDV-based production, and $331.6 for FOD-based production. The circulating capital made up of pineapple waste, diets and monthly labour represents 1.18%, 1.44% and 0.61% of the total capital for CF, VGD and FOD, respectively. All the material used in the manufacture of 1 larvarium and labour cost $14.94. Once the rearing equipment is acquired, it would only take the circulating capital in each rearing cycle to produce 1 kg of BSF meal. In this case, it amounts to $3.54, $4.33 and $1.84 per kg of BSF meal for CF, VGD and FOD respectively.

Table 2
Production of eggs and larvae according to densities and substrates at the end of reproduction cycle.

Factor	FD1-SD	FD1-PW	FD2-SD	FD2-PW	Statistical analyses	p
Eggs (g/23days)	6.74 ± 0.82^{a}	10.14 ± 0.43^{b}	5.62 ± 1.13^{a}	10.43 ± 0.31^{b}	$F_{1,260} = 20.1$	<0.001
Larvae (g/23days)	492.10 ± 17^{a}	701.13 ± 187.82^{b}	335.33 ± 30.66^{a}	726.4 ± 44.61^{b}	$F_{1,8} = 28.1$	<0.001
Numbers with the same letters are not significantly different						

Table 3
Hermetia illucens breeding parameters.

Parameters	LD1	LD2	LD3	Statistical analyses	p
Prepupae weight	0.24 ± 0.05^{a}	0.24 ± 0.04^{a}	0.14 ± 0.03^{b}	$H_2 = 319.41$	$p < 0.001$
SR (%)	50 ± 7.32^{b}	82.1 ± 0.2^{a}	85 ± 1.73^{a}	$F_{2,6} = 59.22$	$p < 0.001$
PHR (%)	82 ± 5.75^{a}	71 ± 8.53^{a}	38 ± 2.08^{b}	$F_{2,6} = 43.00$	$p < 0.001$
BSF meal (g)	255 ± 37.94^{b}	432.4 ± 54.36^{a}	289.3 ± 69.67^{b}	$F_{2,6} = 8.63$	$p = 0.02$
Numbers with the same letters are not significantly different. LD = Larval density					

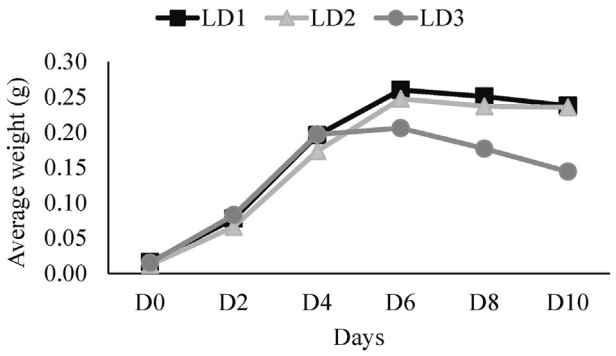

Fig. 3. Evolution of the average weight of the larvae of each treatment during the experiment. LD = Larval density.

4. Discussion

Mass production of black soldier fly for fish farming will only be achieved by controlling the different stages of the insect life cycle (e.g. adult reproduction and larval growth) while reducing the feeding and the rearing structure costs.

4.1. Black soldier fly artificial reproduction

The main factor known to influence mating of BSF is sunlight [36,37]. In this study, BSF were exposed to sunlight for about 12 h a day, which is within the recommended optimum range [38,39]. In Benin, a tropical country where the sun is omnipresent, the reproduction of BSF is possible throughout the year without artificial light and will consequently reduce the production cost compared to temperate countries. Environmental temperature is another factor influencing egg laying in *H. illucens*. During this experiment, temperature was around $26.8 \pm 0.48\ ^{\circ}C$ corresponding to the optimal temperature between 26 and $27\ ^{\circ}C$ [36]. According to Holmes et al. [40], a relative humidity of around 70% guarantees a successful egg-laying and increase the longevity of adult flies. In this study, relative humidity was not measured but without a moistening system, we recommend watering the cages periodically during the day with a sprayer. For optimized reproduction, the density of 8500 ind/m^3 associated with a fermented substrate such as pineapple waste is therefore the best combination. This device produced an average of 10.1 ± 0.43 g of eggs which corresponds to 187.8 g larvae g with an average weight of 0.01 g. However, these amounts obtained do not represent the entirety of eggs laid as losses are recorded. Females also lay eggs out of nests, especially on cage walls. The maximum quantity of eggs obtained by Hoc et al. [25] was 9 ± 0.2 g at the same density (8500 ind/m^3) with a female-dominant sex-ratio in a cage volume of 0.091 m^3. However, in term of productivity, it could be considered as lower in this study as the volume of the cages were around 0.22 m^3. In our study, the density of 10,500 ind/m^3 with unknown sex-ratio did not increase the quantity of eggs laid as recommended by previous authors. In addition to a potential sex ratio effect, this result could be explained by the nature of the attractant (i.e. fermented carrots in Hoc et al. [25]). In this study, pineapple waste induced higher egg-laying than soybean meal and the laying time was prolonged in adults reared with pineapple waste (23 days). A constant egg production requires the maintenance of a steady number of adults but our results show that the egg production falls under 0.5 g per day after 15 days due to adult mortality. In order to keep the egg production profitable, an adult renewal is recommended after 15 days. It is therefore evident that the type of attracting substrate significantly influences the amount of eggs produced. This difference would be due to the volatile compounds released during the decomposition process of these substrates. Indeed, aldehyde compounds, alcohol, organic acids are released during the degradation process to stimulate the smell of the adults flies [20]. The aromatic profile of pineapple has already shown the presence of acetate and butyrate esters [41]. Its decomposition would surely produce the attractive odors to the adult fly. The hatching substrate used here is soybean meal. This moist substrate was chosen because it is obtained at low cost ($ December 0, 1000 g) and its non-fibrous texture allows easy sorting of larvae after hatching. In order to find a possible correlation between substrate and hatching, future studies could test different substrates on egg hatching rates. In order to

Table 4
Larval weight and nutritional parameters of BSF prepupae reared on vegetable diet (VGD) and on fish offal diet (FOD).

	Control	VGD	FOD	Statistical analyses	p
Prepupal weight (g)	0.23 ± 0.01^{a}	0.20 ± 0.00^{b}	0.17 ± 0.00^{c}	$H_2 = 7.8$	$p = 0.02$
Dry matter (%)	35 ± 0.16^{a}	36.1 ± 0.56^{a}	36 ± 0.07^{a}	$F_{2,3} = 9.3$	$p = 0.05$
Crude protein (% dry matter)	43.4 ± 0.1^{a}	42 ± 1.54^{a}	45.1 ± 0.81^{a}	$F_{2,3} = 5.99$	$p = 0.09$
Lipid (% dry matter)	23 ± 0.17^{b}	33 ± 2.21^{a}	27 ± 2.03^{b}	$F_{2,3} = 17.7$	$p = 0.02$
Ash (% dry matter)	11 ± 0.07^{a}	7 ± 0.49^{c}	8 ± 1.41^{b}	$F_{2,3} = 1043$	$p < 0.001$
Numbers with the same letters are not significantly different					

Table 5
Fatty acids composition of black soldier fly prepupae reared on vegetable diet (VGD) and fish offal diet (FOD).

Fatty acid	Control	VGD	FOD	Statistical analyses	p
C10:0	0.83 ± 0.04^{a}	1.04 ± 0.10^{b}	0.84 ± 0.02^{ab}	$F_{2,3} = 6.82$	$p = 0.08$
C12:0	44.1 ± 0.14^{a}	39 ± 1.49^{b}	32 ± 0.50^{c}	$F_{2,3} = 87.5$	$p < 0.001$
C14:0	8.5 ± 0.07^{a}	6 ± 0.12^{c}	6.4 ± 0.01^{b}	$F_{2,3} = 630.7$	$p < 0.001$
C14:1	0.00 ± 0.00^{c}	0.2 ± 0.00^{b}	0.3 ± 0.01^{a}	$F_{2,3} = 543$	$p < 0.001$
C15:0	0.00 ± 0.00^{c}	0.2 ± 0.00^{b}	0.6 ± 0.02^{a}	$F_{2,3} = 2604$	$p < 0.001$
C15:1	0.00 ± 0.00^{c}	0.2 ± 0.01^{b}	0.31 ± 0.03^{a}	$F_{2,3} = 169.8$	$p < 0.001$
C16:0	14 ± 0.14^{b}	8 ± 0.45^{c}	17 ± 0.44^{a}	$F_{2,3} = 302.2$	$p < 0.001$
C16:1	2.3 ± 0.02^{b}	3 ± 0.03^{a}	0.6 ± 0.00^{c}	$F_{2,3} = 4811$	$p < 0.001$
C17:0	0.00 ± 0.00^{c}	0.2 ± 0.02^{b}	0.71 ± 0.04^{a}	$F_{2,3} = 482.1$	$p < 0.001$
C17:1	0.2 ± 0.01^{a}	0.3 ± 0.01^{b}	0.9 ± 0.01^{c}	$F_{2,3} = 2024$	$p < 0.001$
C18:0	2.8 ± 0.04^{b}	1.4 ± 0.36^{c}	3.5 ± 0.23^{a}	$F_{2,3} = 36.26$	$p = 0.01$
C18:1n-9	13 ± 0.20^{a}	22.13 ± 0.32^{b}	18 ± 0.88^{c}	$F_{2,3} = 136.4$	$p < 0.001$
C18:2n-6	13.4 ± 0.15^{a}	10.1 ± 0.28^{b}	6.2 ± 0.52^{c}	$F_{2,3} = 213.8$	$p < 0.001$
C18:3n-3	1.13 ± 0.03^{b}	2.3 ± 0.05^{a}	0.52 ± 0.04^{c}	$F_{2,3} = 978$	$p < 0.001$
C20:1n-9	0.00 ± 0.00^{b}	0.64 ± 0.00^{a}	0.00 ± 0.00^{b}	$F_{2,3} = 16{,}129$	$p < 0.001$
C20:3n-3	0.1 ± 0.01^{b}	0.00 ± 0.00^{b}	0.9 ± 0.03^{a}	$F_{2,3} = 1200$	$p < 0.001$
C20:4n-6	0.1 ± 0.02^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$F_{2,3} = 25$	$p = 0.01$
C20:5n-3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.2 ± 0.06^{a}	$F_{2,3} = 3025$	$p < 0.001$
C22:2	0.00 ± 0.00^{b}	0.26 ± 0.05^{a}	0.00 ± 0.00^{b}	$F_{2,3} = 57.33$	$p < 0.001$
C22:6n-3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.4 ± 0.05^{a}	$F_{2,3} = 121$	$p < 0.001$

Numbers with the same letters are not significantly different

Table 6
Total cost of inputs for the production of 1 kg of Hermetia illucens prepupae.

		Quantity	Unit Cost (USD)	Total (USD)	Service life (Year)	Depreciation (USD)	Costing (USD
Fixed Capital							
Breeding cages		1	16.50	16.50	1	16.50	
Plastic containers for attractant substrate		1	0.84	0.84	1	0.84	
Wooden nests		1	1.70	1.70	1	1.70	
Wooden shelf		1	16.90	16.90	3	5.62	
Plastic containers for larvarium		2	5.90	11.80	2	5.90	
PVC pipe (50 cm)		1	3	3	2	1.50	
Silicone + Silicone gun		1	7.69	7.69	2	3.85	
Plastic bucket for prepupae harvesting		2	1	2	2	1.00	
Mosquito net		1	4.22	4.22	1	4.22	
Larvarium labour		2	3.70	7.40	2	3.69	
Freezer		1	276.93	276.93	10	27.69	
Blender		1	64.62	64.62	2	32.31	
Oven		1	2250	2250	10	225.00	
Total fixed capital						**329.78**	
Circulating capital							
Pineapple waste (Kg)		0.50	0.30	0.10	0	0.10	
Chicken feed (Kg)		4.60	*0.6*	2.70	0	2.70	
VGD	SM	3.60	0.5	1.80	0	1.80	
	EH	0.70	0.10	0.10	0	0.10	
	CO	0.30	4.60	1.60	0	1.60	
	Total VGD					3.50	
FOD	SM	1.50	0.50	0.70	0	0.70	
	FO	3.10	0.10	0.30	0	0.30	
	Total FOD					1	
Labour force ($/Kg/month)		1	0.74	0.74	0	0.74	
Total circulating capital (Chicken feed)						**3.54**	**333.3**
Total circulating capital (VGD)						**4.34**	**334.1**
Total circulating capital (FOD)						**1.84**	**331.6**

keep the same size of larvae in the tanks at the beginning of the rearing process, it is recommended to use different sized sieves for sorting. Heterogeneous sizes within the same tank lead to differential development and bias feed ration calculations. Sieves with a mesh size of 2 mm are suitable for sorting larvae with an average weight of 0.01 g.

4.2. Larvarium prototype

The larvarium prototype that was designed seems very practical for insect producer because of its small size and its mobility. Moreover, all the material used in setting it up is available locally. The ramp planned for the exit of prepupae actually served this purpose. Automatic harvest rates varied from 37.7 ± 2.1 to $81.7 \pm 5.8\%$ depending on the larval densities. This function allows the producer to avoid as much as possible the fastidious sorting of prepupae from the substrates. However, substrate moisture is the primary factor that prompts prepupae to migrate through the exit ramp. Prepupae prefer dry areas to initiate their pupation [40]. Consequently, the ramp would only be effective for harvesting if moisture levels are high enough at the end of larval growth. However, this parameter is still poorly known. Some companies have already designed similar farming systems. In the United States, ProtaCulture has developed a composter called "BiopodPlus" preferentially used for breeding the BSF [42]. Being of larger dimensions ($67.31 \times 38.35 \times 40.64$ cm), it is also equipped with a self-harvesting system. The unit cost is relatively high (US $ 200) while the larvarium proposed here is around $14.94 including labour to build it. The proposed prototype has nevertheless some disadvantages. The transparency of the containers used could negatively influence larval development as

larvae are known to be photophobic [20]. In addition, the resistance of the containers is low; which could limit their lifetime. Opaque and more robust containers could reduce the impact of these factors. For purposes of our experiment, the prepupae were collected in buckets from each container. In semi-industrial systems, it will be recommended to use of elbow- and T-shaped PVC in order to connect the containers to each other on the same shelf. Only one collection container would be needed on each shelf. For example, for a row of 4 larvaria, 1 elbow-shaped PVC, 3 T-shaped PVC and 1 PVC pipe of the same diameter would be required to arrange them.

4.3. Larval rearing

Larval growth of *H. illucens* depends on several factors such as larval density, substrate moisture or feed quality (i.e. proteins or lipids) [13]. Of the three densities tested in this study, those of 1 larva/g and 2 larva/g allowed better larval growth. However, the density of 2 g/larva allows a higher rate of substrate decomposition (82.05 ± 0.2%). This density is within the optimum "min. 0.1 and max. 3.33 larvae/cm^2" recommended by Barragan-Fonseca et al. [13]. These authors' calculations consider the surface area of containers used in the various density works and the thickness of the substrates (1–2 cm). The 3.33 larvae/cm^2 corresponds in our case to 2 larvae/g, which is an easier expression of density. At this density, the control diet produced prepupae weighing 0.24 ± 0.03 g which is similar to results from other studies where prepupal weight range from 0.22 ± 0.01 to 0.25 ± 0.01 g [16,43]. BSF prepupae produced on local by-products were smaller (0.20 ± 0.00 g for VGD and 0.17 ± 0.00 for FOD) but higher than prepupae fed with vegetable waste (0.14 ± 0.04 g) or prepupae fed with cow manure and fish offal (0.15 ± 0.05 g) [16,17]. The nature of the different substrates characterized by their nutritional quality should explained these differences. Nevertheless, further studies are still needed to clearly identify the factors determining optimal growth of *H. illucens* larvae.

4.4. Nutritional composition of prepupae reared on contrasted lipid diet

In order to evaluate the ability of BSF to effectively substitute fish meal and fish oil in tilapia feed, a larval enrichment experiment was conducted. According to Caruso et al. [20], lipids will be the limiting factor for inclusion of BSF in fish feed. Consequently, particular attention was paid to the accumulation of valuable fatty acids in BSF in this study and feeds of animal and vegetable origin with interesting fatty acid profiles have been selected. For the plant-based diet VGD, a local species of Euphorbia (*Euphorbia heterophylla*) was used for its high level in LA (C18:2n-6) and ALA (C18:3n-3). Nevertheless, this species only grows in the rainy season in cassava or maize fields and its availability is still limited since it is considered a weed and not yet cultivated in Benin [32]. Consequently, it has been associated with rapeseed oil in order to reduce its incorporation rate. Concerning animal-based diet, fish offal (FOD) was used for their potential supply of EPA (C20:5n-3) and DHA (C22:6n-3). The total lipid contents of VGD (32.9 ± 2.21%) and FOD (26.7 ± 2.03%) prepupae clearly exceeds 10% requirement of Nile tilapia. The high lipid content could limit the incorporation rate of BSF meal in fish feed. This fat concentration may lead to problems in feed formulation or pellet storage and stability [6]. One solution would be a defattening of the BSF meal by physical or chemical extraction as used by Renna et al. [44] for *Oncorhynchus mykiss* (Walbaum, 1792) feed production. This process will strongly influence the cost of production. Therefore, this important parameter for profitability will have to be taken in consideration before any decision will be made to do so. This step increases the insect protein content and enriched oil can be used for other purposes. The fatty acid composition of the prepupae shows a high presence of lauric acid (C12:0) independent of diet while it does not appear in the substrates. This finding has already been made in other studies [16,17,19,45,46]. Oonincx et al. [45] speculated that this fatty acid is biosynthesized by the larvae. This tendency of the larvae to prioritize this fatty acid could limit the rate of valorization of diets enriched in essential fatty acids (EFAs). Some EFA from experimental diets were variably accumulated in BSF and their levels were positively correlated with those of the substrates. Linoleic (C18:2n-6; 10.08 ± 0.28%) and α-linolenic acid (C18:3n-3, 2.30 ± 0.05%) are mainly provided by the VGD as already shown by Oonincx et al. [45] who obtained a significant accumulation of these two fatty acids (respectively 17.1 ± 0.35% and 1.5 ± 0.02%) from vegetable diets (beet molasses, potato steam, spent grains, beer yeast, bread remains, cookie remains) with a lipid content of 9.5%. Although the C18:2n-6 content of prepupae fed on FOD is lower than those fed on VGD, its content is also very high (6.19 ± 0.52%). The prepupae from both diets largely meet the C18:2n-6 requirements of *O. niloticus* (0.5–1%). The essential long chain polyunsaturated fatty acids were only found in prepupae fed with marine fish offal (FOD). EPA and DHA contents are respectively around 2.20 ± 0.06 and 0.39 ± 0.05%. These results are close to those of St Hilaire et al. [17] who managed to have 1.66% EPA and 0.59% DHA in prepupae from a 50% inclusion of fish offal in their diet. Based on these results, an optimal diet could be formulated by including soybean meal, euphorbia and fish offal at a level of 10% of lipid. In order to eliminate the use of colza oil in an industrial production context, mass cultivation of *E. heterophylla* could be initiated in Benin. But some aspects should be taken into account. Studies have revealed toxic elements in this plant. Their chemical composition contains alkaloids, cyanides, tannins, flavonoids and saponins, which are toxic compounds [47,48]. This toxicity does not appear to affect BSF larval growth in the present study and may be related to the doses tolerated by the species. Moreover, the levels of toxicity are related to the nature of the plant samples used: fresh or dry [47]. On the other hand, these compounds could be bioaccumulated in the prepupae and thus constitute a potential risk for the fish for which they are intended. These points constitute research tracks prior to a safe use.

Concerning other nutritional parameters, the protein content of prepupae was around 43.4 ± 0.16% for CF, 41.6 ± 1.54% for VGD and 45.08 ± 0.81% for FOD. These results are in the range (37–63%) of those found in other studies [13]. These values show that in terms of protein, BSF prepupal meal can meet the 29% *O. niloticus* protein requirement. CF was better valued by BSF larvae in terms of protein, whereas there was a loss of protein in larvae from experimental diets. It is well known that CF is produced with high quality materials and optimized with nutritional supplements in order to ensure a better yield in chicken. It is used in this study for comparative purposes and cannot be recommended for BSF production. In addition to its high cost, its availability could be challenging for industrial production since its demand is already quite high for poultry. Dry matter contents of VGD (36.08 ± 0.56%) and FOD (35.81 ± 0.07%) prepupae are similar to those of Oonincx et al. [45] (33–36%) raised on agricultural by-products. Ash content (6.86 ± 0.49% VGD; 7.63 ± 1.41% FOD) is, however, below the values (10.8 ± 0.00%) of prepupae fed with vegetable waste by Ref. [16]).

4.5. Economic evaluation of production

In order to determine the production cost of BSF meal, the equipment lifespan has been included as a depreciation factor. This factor was evaluated based on the experience of using the various inputs of working capital. To reduce the production cost, the option of a home-made breeding cage was evaluated. This breeding cage would be built from local materials consisting of mosquito netting and wood. Its design would cost $16.5 based on market prices in 2020 instead of $98.6 for the current plastic cage. Finally, prepupae were killed in the freezer, dried in an oven and then ground to obtain insect meal. Oven drying is not very suitable for mass production as it consumes energy and would influence the cost of production. As Benin is a tropical country, it would be interesting to test a drying process using sun. The effects of this drying method will have to be evaluated on the nutritional composition of the prepupae. Similarly, an alternative to the freezing method used to kill prepupae must be developed like the use of hot water for example.

The cost of a first production of BSF meal is almost the same for the 3 tested diets ($333.3/Kg for CF, $331.62/Kg for VGD and $331.62/Kg for FOD). Considering only the circulating capital for future production, the VGD-based BSF meal is more expensive ($3.5/Kg) than the other two and the FOD-based BSF meal is cheaper ($1.84/Kg). However, these costs are only an approximation because the energy used by the oven, freezer and blender was not included in the calculation. In the experimental diet the SM and CO have a significant influence on the cost. The production cost can therefore be reduced by using other nutritionally valuable but low value-added substrates. Gougbedji et al. [49] have listed agricultural by-products of interest in Benin for BSF breeding. The costs and availability of the by-products listed could direct future research towards substitutions that integrate the economic aspect of production.

In order to assess the profitability of *O. niloticus* farming, financial analyses based on the production of tilapia from BSF meal-based feed will have to be carried out.

5. Conclusion

This study develops a breeding method for *Hermetia illucens* in Benin. The species can easily be reared under local conditions throughout the year. The proposed production technology can allow any farmer in animal sector to easily produce BSF larvae. It has also been shown that *H. illucens* breeding is a potential source of animal protein whose nutritional profile makes it possible to consider a substitution of fish meal in Nile tilapia, *Oreochromis niloticus* feed. However, any production must include the economical factor beyond the viable biological model. Possible ways of reducing BSF flour production costs have been listed for a good profitability of the livestock industry.

Declaration of competing interest

The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

Acknowledgments

Our sincere thanks go to the Académie de Recherche et d'Enseignement Supérieur (ARES) for financing the realization of this work within the framework of the institutional support program (PRD10, 2016) entitled “Optimization of the tilapia sector in Benin through genetic improvement and innovative food strategies”. We would also like to thank Lucien Mostade and Bertrand Hoc for their valuable assistance during the chemical analyses.

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