Article

In-Situ Yeast Fermentation Medium in Fortifying Protein and Lipid Accumulations in the Harvested Larval Biomass of Black Soldier Fly

Chung Yiin Wong [1], Yeek Chia Ho [2], Jun Wei Lim [1,*], Pau Loke Show [3], Siewhui Chong [3], Yi Jing Chan [3], Chii Dong Ho [4,*], Mardawani Mohamad [5], Ta Yeong Wu [6,7], Man Kee Lam [8] and Guan Ting Pan [9]

[1] Department of Fundamental and Applied Sciences, HICoE-Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, Seri Iskandar 32610, Malaysia; johnsonwcy@gmail.com

[2] Department of Civil and Environmental Engineering, Centre of Urban Resource Sustainability, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, Seri Iskandar 32610, Malaysia; yeekchia.ho@utp.edu.my

[3] Department of Chemical and Environmental Engineering, University of Nottingham Malaysia, Broga Road, Semenyih 43500, Malaysia; PauLoke.Show@nottingham.edu.my (P.L.S.); faye.chong@nottingham.edu.my (S.C.); Yi-jing.chan@nottingham.edu.my (Y.J.C.)

[4] Department of Chemical and Materials Engineering, Tamkang University, Tamsui, New Taipei City 251, Taiwan

[5] Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Malaysia; mardawani.m@umk.edu.my

[6] Chemical Engineering Discipline, School of Engineering, Monash University, Jalan Lagoon Selatan, Bandar Sunway 47500, Malaysia; wu.ta.yeong@monash.edu

[7] Monash-Industry Palm Oil Education and Research Platform (MIPO), School of Engineering, Monash University, Jalan Lagoon Selatan, Bandar Sunway 47500, Malaysia

[8] Department of Chemical Engineering, HICoE-Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, Seri Iskandar 32610, Malaysia; lam.mankee@utp.edu.my

[9] Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 106, Taiwan; gtpan@mail.ntut.edu.tw

* Correspondence: junwei.lim@utp.edu.my (J.W.L.); cdho@mail.tku.edu.tw (C.D.H.); Tel.: +60-5368-7664 (J.W.L.); +886-2-2621-5656 (C.D.H.)

check for updates

Abstract: Recently, worldwide researchers have been focusing on exploiting of black soldier fly larval (BSFL) biomass to serve as the feed mediums for farmed animals, including aquaculture farming, in order to assuage the rising demands for protein sources. In this study, yeast was introduced into coconut endosperm waste (CEW) whilst serving as the feeding medium to rear BSFL in simultaneously performed in situ fermentation. It was found that at a 2.5 wt% yeast concentration, the total biomass gained, growth rate and rearing time were improved to 1.145 g, 0.085 g/day and 13.5 days, respectively. In terms of solid waste reduction, the inoculation of yeast over 0.5 wt% in CEW was able to achieve more than 50% overall degradation, with the waste reduction indexes (WRIs) ranging from 0.038 to 0.040 g/day. Disregarding the concentration of yeast introduced, the protein productivity from 20 BSFL was enhanced from only 0.018 g/day (the control) to 0.025 g/day with the presence of yeast at arbitrary concentrations. On the other hand, the larval protein yield was fortified from the control (28%) to a highest value of 35% with the presence of a mere 0.02 wt% yeast concentration. To summarize, the inclusion of a minimal amount of yeast into CEW for in situ fermentation ultimately enhanced the growth of BSFL, as well as its protein yield and productivity.

1. Introduction

The black soldier fly (BSF) thrived in North America before it migrated to tropical other countries during WWII. It mimics the appearance of a wasp, confusing the public with its appearance. BSF larvae (BSFL) are intrinsically polyphagous as well as saprophagous, since the larvae only consume organic matter during this stage and can ingest different kinds of decaying organic matters such as animal manure, animal carcasses or sometimes even decaying wood matters. Unlike houseflies, the BSF does not carry any transmitted diseases, as the adult fly does not feed and only relies on body fat or the energy accumulated during the larval stage for metabolism. Upon maturing sexually, the female BSF will oviposit eggs at the cracks near to food sources to ensure the newly ecloded BSF larvae (neonates) have enough food to complete their life cycle [1]. Generally, after the copulation process, the female black soldier fly will oviposit the eggs after two to three days. The whole life cycle of a black soldier fly from egg to adult will take up to around 40 to 44 days [2].

Owing to its high protein content, the direct introduction of BSFL biomass into animal feed has been explored as an alternative fishmeal, which is growing in cost. From previous research studies, the inclusion of BSFL biomass at 17%, 33%, 49%, 64% and 75% into aquaculture feed was found to decrease feed consumption due to its low digestibility. In this case, the highest protein retention in fed fish was obtained when 33% of BSFL biomass was used, thereafter decreasing as BSFL biomass was incorporated. From the study, the inclusion of BSFL biomass into aquaculture feed was feasible at low percentages, and it has been suggested that the presence of chitin in BSFL biomass contributes certain benefits to the growth performance of the turbot from the feed intake, including the availability and digestibility of nutrients [3]. The BSFL protein was also introduced to rainbow trout as a replacement meal with the partial inclusion at 25% and 50%, and the outcome showed that the BSFL biomass degraded the lipid health indexes of the rainbow trout while negatively impacting the contents of polyunsaturated fatty acids with increases of BSFL biomass. In order to prevent the negative impacts of BSFL inclusion on trout, it was suggested that a 40% inclusion level of BSFL biomass could be used without impacting the survival, growth performance, condition factor and so on [4]. Apart from the aquaculture field, BSFL biomass can also be introduced as animal feed for broilers in either a partial or highly defatted form. From the past study, an inclusion of partially defatted BSFL biomass into broilers' feed showed higher digestibility by the chicken. [5]. According to Schiavone et al. [6], an inclusion of defatted BSFL in broiler chicken diets at 10% showed improvements in carcass and meat quality parameters as well as the heavy metal contents, and there were no negative consequences. Moreover, when the BSFL biomass was incorporated into quail feed to replace fishmeal, the outcome showed a similar result as with the fishmeal. When 25% to 50% BSFL biomass at 25% and 50% was included, no impact on the palatability of ration or quail appetites was detected. In short, the 50% replacement of fishmeal with BSFL biomass was generally recommended, as no negative impact was demonstrated on the growth performance of most of the farmed animals [7].

The study by Loponte et al. [8] showed that the corn-soybean meal diet used for *Barbary partridge* rearing could be replaced with *Tenebrio molitor* and *Hermetia illucens* biomass at 25% and 50%. Even though the control group had heavier weight of partridges fed and longer intestinal and caecal lengths, the live weights of the birds that were fed *T. molitor* and *H. illucens* meals were significantly higher than the control due to improved nutrient digestibility. Apart from these, several studies were carried out to determine the impacts of insect meal on the egg characteristics of laying hens. With the inclusion of *H. illucens* into laying hens' diets, lay percentage and egg mass were found to be affected only at 25% replacement, owning to higher methionine and lysine. A replacement by insect meal more than 50% negatively impacted dry matter, organic matter and crude protein digestibility due to the presence of chitin; hence, a 25% insect meal replacement was recommended for the diets of laying hens [9]. A 100% soybean meal replacement by

H. illucens was found feasible in Lohmann Brown Classic laying hens during 21 weeks of rearing. Eggs laid by the hens fed with the insect diet were found to possess higher quality of yolks than the control group, which was fed soybean meal. Also, the red index of the eggs laid was found to be higher in the insect treatment group (5.63) compared with the control (1.36). Moreover, the insect treatment group laid eggs with higher γ-tocopherol (4.0 against 2.4 mg/kg), lutein (8.6 against 4.9 mg/kg), β-carotene (0.33 against 0.19 mg/kg) and total carotenoids (15 against 10.5 mg/kg) than the control. Nonetheless, the insect treatment group eggs contained 11% less cholesterol than the control group, and no differences were found in fatty acid composition [10].

Recently, worldwide researchers have focused on exploiting BSFL biomass to serve as a feed medium for farmed animals, including aquaculture farming, in order to sustain the rising demands for a protein source. In this regard, various low-cost organic wastes had been employed to farm BSFL without truly optimizing its larval protein content. It has been hypothesized that increasing the protein content of BSFL would directly permit a higher inclusion of larval biomass in animal feeds whilst reducing the costs attributed mostly as a result of the unsustainable use of fishmeals. BSFL is currently proposed as the best protein source for animal farming and aquafarming, since the cost of animal feed and fishmeal continue increasing year after year due to marine overexploitation and a limited availability of lands. Animal feeds consist mainly of fishmeal and soybean, which serve as the protein alimentation, in addition to fish oils, seed cakes and other grains [11]. Thus, the main objective of this study was to enhance the protein content of BSFL by introducing yeast to execute fermentation on low-cost organic waste for larval feeding (i.e., coconut endosperm waste). The presence of yeast to ferment coconut endosperm waste would improve the nutritional content of larval feeding medium and eventually the larval protein content upon feeding. The degree of fermented coconut endosperm waste valorization by BSFL has also been reported to unveil organic waste treatment potentiality.

2. Materials and Methods

2.1. Acquisition of Coconut Endosperm Waste

The grated fresh coconut endosperm waste (CEW) was initially acquired from a local stall selling coconut milk and kept within 2 to 4 °C in a refrigerator. The moisture content of the CEW was determined through a gravimetric method and adjusted to 70% by homogenizing with sterile distilled water as calculated using Equation (1) prior to being used in the experiment.

$$V_{H_2O} = \frac{(\%_{H_2O})(M_S)}{1 - (\%_{H_2O})} - M_{H_2O} \tag{1}$$

where V_{H_2O} represents the total volume of sterile distilled water to be added (in g considering the density of water 1 g/mL), $\%_{H_2O}$ represents the percentage of desired moisture (which was 70% (0.7 was inserted into the equation) in this study), M_S represents the total dry weight of the CEW (in g) and M_{H_2O} represents the initial moisture content of the CEW (in g).

2.2. Attainment of Black Soldier Fly Larvae (BSFL)

We weighed 200 g of fresh CEW and transferred it into a plastic container with a size of 35 × 25 cm (height × diameter). We left the ventilated container in a sun-shaded area, serving as a bait to lure female BSFs. Several pieces of paper box cardboard with a size of 8 cm × 3 cm (length × width) were attached to the inner wall of the plastic container about 3 to 5 cm above the CEW medium, acting as a platform for the female BSF to oviposit her eggs. This cardboard was checked daily for BSF eggs. The attained eggs were then transferred into sterile Petri dishes and incubated until the larvae emerged. The new BSFL (neonate) were reared on CEW until 6 days old prior to being used in the experiments [12].

2.3. Rearing of BSFL Using CEW Inoculated with Yeast

Figure 1 presents the schematic flow of the reported works. Different quantities of dry yeast powder (commercial brand: Bunga Raya) with 0.02, 0.1, 0.5, 1.0 and 2.5 wt% were separately homogenized with CEW to serve as an initial inoculum for fermentation to take place. A 10 g, dry weight basis of each CEW that had been inoculated with yeast medium was then immediately administered to 20 six-day-old BSFLs. The larval rearing using each CEW medium inoculated with different percentages of yeast was stopped once the BSFL reached its fifth instar, as determined by head size and body color [1,13]. Each batch of harvested BSFL was deactivated at 105 °C for 5 min then dried at 60 °C until reaching a constant weight. This was followed by grinding the BSFL into powder and storing it at −20 °C prior to the chemical analyses [14]. All CEW residues were also separately collected and dried at 105 °C until reaching a constant weight. All setups were (at least) duplicated to verify the statistical reproducibility.
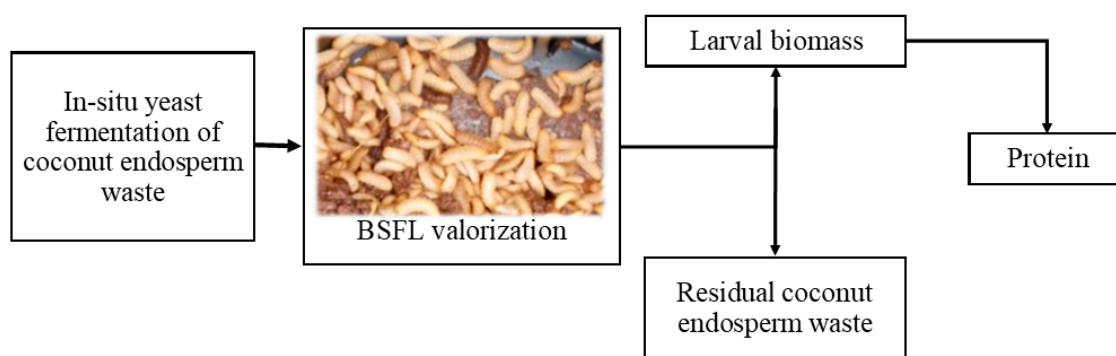

Figure 1. Schematic flow of the experimental procedures.

2.4. Growth Performance of the BSFL

Upon the completion of experiments, growth of the BSFL was evaluated using Equation (2) for the total biomass gained and Equation (3) for the BSFL growth rate [15], as shown below:

$$\text{Total biomass gained (g)} = \text{Final BSFL dried mass (g)} - \text{Initial BSFL dried mass (g)} \qquad (2)$$

$$\text{BSFL growth rate (g/day)} = \text{Total biomass gained (g)/Rearing time (day)} \qquad (3)$$

2.5. Treatment of CEW Via Valorization by BSFL

In order to determine the degree of CEW reduction, two parameters were measured including Equation (4) for overall degradation (OD) and Equation (5) for the waste reduction index (WRI) [16], as shown below:

$$\text{Overall degradation} = \text{Total feed consumed (g)/Total feed offered (g)} \qquad (4)$$

$$\text{WRI (g/day)} = \text{Total feed consumed (g)/Rearing time (day)} \qquad (5)$$

2.6. Nitrogen, Chitin and Protein Analyses

Nitrogen contents of dried BSFL biomass were determined through the Dumas combustion method (Perkin Elmer, CHNS/O 2400). The sample was weighed in the range of 1 to 1.5 mg then transferred into a tin capsule, wrapped and combusted at 925 °C. The nitrogen compounds were then converted into NO_x, further reduced to nitrogen gas at 640 °C and detected by a thermal conductivity detector (TCD) [17]. In this study, the larval protein contents were estimated with a multiplication factor of 6.25 [18]. However, the presence of chitin in BSFL biomass will influence the larval protein content and, hence, nitrogen from chitin has to be deducted from the total larval nitrogen content prior

to protein conversion in order to avoid over-estimation [19]. Chitin is a polysaccharide that can be found in yeast, fungi, crustaceans and insects [20], as well as being present in the exoskeleton of BSFL, where it accounts for 6.89% of the nitrogen content [16]. The formic acid method was applied for chitin determination in this study [19,21], with modification to suit a small sample size. We mixed 10 mL of 90% formic acid with 1 g of BSFL dried fat-free biomass (the initial mass prior to being defatted had been recorded) at room temperature for 24 h. Then, the mixture was centrifuged, and the supernatant was decanted. The residue was washed with 10 mL of 100% acetone, followed by 10 mL of 70% acetone before being recentrifuged to separate the acetone. The residue was refluxed with 5% of 10 mL sodium hydroxide for 90 min before being filtered and washed with distilled water on ashless filter paper (Whatman No. 1 with a 55 mm diameter). Next, the residue was dried in the oven to a constant weight at 105 °C, then later it was ashed at 550 °C for 24 h. The final weight of the sample was recorded and assumed to be intact chitin.

$$\text{Chitin content (\%)} = \text{Mass of residues after ashing (g)/Initial mass of BSFL (g)} \times 100\% \qquad (6)$$

$$\text{TN}_{\text{Chitin}} \text{ (\%)} = [\text{Chitin content (\%)} \times \text{Nitrogen content in chitin (\%) (which is 6.89\%)]/100\%} \qquad (7)$$

$$\text{Corrected protein yield for BSFL (\%)} = [\text{TN}_{\text{BSFL}} \text{ (\%)} - \text{TN}_{\text{Chitin}} \text{ (\%)}] \times 6.25 \qquad (8)$$

$$\text{Protein productivity (g/day)} = \text{Protein content (g)/Rearing time (day)} \qquad (9)$$

where TN_{BSFL} is the total nitrogen from the BSFL biomass and $\text{TN}_{\text{Chitin}}$ is the total nitrogen from the chitin.

3. Results and Discussion

3.1. Growth Performances of BSFL

Initially, 10 g of yeast-inoculated feed was introduced to 20 BSFL at different concentrations. The total biomasses gained for the BSFL were recorded once every setup had reached the fifth instar, as shown in Table 1. Under the control condition, the total biomass gained by the BSFL was attained at only 0.998 g from a total of 20 BSFL. This value increased with the increment of yeast concentrations rising from 0.02 to 2.5 wt%, and it attained its highest point at 1.145 g. As compared with a previous study by Zheng et al. [22], the performance of in situ yeast fermentation at the highest concentration in this study was comparable to the best RID-X dosage (w/w), which was equivalent to 1.228 g per 20 BSFL with a difference of merely 0.08 g per 20 BSFL. RID-X was the active bacterial product introduced into the larval feeding medium in the study by Zheng et al. [22]. On the other hand, besides changing the nutritional properties of larval feed by introducing microorganisms, the growth of the BSFL could also be altered by feeding with a protein-rich medium, as suggested by Rehman et al. [23]. At a 1:4 ratio of dairy manure to protein-rich soybean curd residue, the total dry larval mass that could be attained was 28.1 g, which is equivalent to 0.56 g from 20 BSFL. This showed that the performance of BSFL growth through the co-digestion treatment was still lower compared to the microorganism inoculation treatment (i.e., yeast in this study). Thus, the inoculation of microorganisms into larval feed is strongly recommended for better BSFL growth.

Table 1. Growth performances of BSFL fed with CEW having been inoculated with different yeast concentrations.

Yeast Concentration (wt %)	Total Biomass Gained (g)	Growth Rate (g/day)	Rearing Time (day)
0 (Control)	0.998 ± 0.125	0.065 ± 0.011	15.5 ± 0.7
0.02	1.013 ± 0.115	0.070 ± 0.011	14.5 ± 0.7
0.10	1.082 ± 0.019	0.077 ± 0.001	14.0 ± 0
0.50	1.088 ± 0.014	0.081 ± 0.005	13.5 ± 0.7
1.00	1.064 ± 0.030	0.079 ± 0.006	13.5 ± 0.7
2.50	1.145 ± 0.099	0.085 ± 0.012	13.5 ± 0.7

Moreover, the growth rate of the BSFL also increased in parallel to the increasing concentrations of yeast from an initial 0.065 g/day to a maximum of 0.085 g/day. This phenomenon can be explained by the shortening of the rearing time of the BSFL. The in situ yeast fermentation of feeding medium had a reduced rearing time from 15.5 days to 13.5 days. This occurrence could have been due to the introduction of yeast that favored the digestibility of carbohydrate compounds in CEW [24] and thus improved the assimilation of nutrients into the BSFL body mass in the form of lipids. Also, Yoon et al. [25] reported that the yeast was capable of breaking down carbohydrates through fermentation, especially common monosaccharides such as D-glucose, D-fructose, D-mannose and D-galactose. On the other hand, it has been proven that the BSFL was also able to convert additional glucose into lipids upon excess availability [26]. Indeed, the measured lipid content increased from about 40% for the control to 50% for a 1.0 wt% yeast concentration. The lipids could later serve as a potential source for biodiesel production, which is something that could be explored further.

3.2. CEW Valorization by BSFL

Due to its polyphagous nature, BSFL is able to reduce solid organic wastes during the rearing process. In this study, the overall degradation of CEW was 0.48 under the control, and this value was maintained for low yeast concentrations of 0.02 and 0.1 wt%. With the addition of yeast at more than 0.5 wt%, the overall degradation of CEW increased to a range of 0.51 to 0.53. Thus, it could be concluded that the 20 BSFL were able to degrade about half of the CEW upon completion of the rearing process, disregarding the concentrations of yeast inoculated. With the introduction of yeast at different concentrations in the feeding medium, it was shown that the WRI increased from 0.31 g/day under the control, to 0.33 g/day with a 0.02 wt% of yeast and 0.38 g/day with a 0.5 wt% yeast concentration. At last, the WRI reached its highest point of 0.40 g/day with a 2.5 wt% yeast concentration. The WRI increment was about 15% faster in 0.5 wt% compared to the 0.02 wt%. This could plausibly be because the addition of 0.5 wt% yeast reached the concentration threshold for maximizing the in situ fermentation to spur the ingestion of CEW by BSFL [27]. Also, it can be observed from Table 1 that the rearing duration for BSFL decreased from 15.5 days and reached a plateau at 13.5 days when the 0.5 wt% yeast concentration (and beyond) were employed for in situ fermentation. Above the 0.5 wt% yeast concentration, the effect on WRI was not significant, if not deteriorating, as reported by Palma et al. [28]. In their study of managing high fiber food waste using BSFL, incremental larval growth led to a decrease in almond hull consumption and vice-versa. The authors presumed that the occurrence was the result of a competition for resources between the BSFL and microbial communities, or because of enhanced synergy between the larvae and their associated microbiota.

3.3. Protein Contents in BSFL

The chitin content from the BSFL was determined to be around 8%, and the nitrogen from the chitin was deducted from the total nitrogen of the BSFL to prevent the over-estimation of BSFL protein content. Figure 2 shows that the corrected protein of the BSFL was only attained around 28% under the control system, and that this value increased to its peak at about 35% when the lowest yeast concentration was used for fermentation. The corrected protein value dropped to around 30% and remained at that level with yeast concentrations from 0.5 to 2.5 wt%. Looking into the protein productivity from 20 BSFL, the value was attained at around 0.02 g/day under the control system and increased to around 0.025 g/day with the introduction of yeast at 0.02 wt%. The value fluctuated within the range of 0.023 to 0.025 g/day with higher yeast concentrations from 0.5 to 2.5 wt%.

As reported by Diener et al. [19], a daily feeding rate of 100 mg of chicken feed per larva was proposed to produce better larval quality and higher waste reduction in the shortest period of time. At this rate, the corrected protein content of BSFL was 34.4%, which is comparable with the current study in which an average of 34.0 ± 3.4% was attained. This result shows that it is possible to attain an output with a similar larval protein content through the initial "one-off feeding method" by using microorganisms to execute fermentation. The introduction of microorganisms into larval feeding media

has been widely practiced as a means to improve the growth of BSFL. According to Gao et al. [29], the addition of *Aspergillus oryzae* into maize straw for fermentation ultimately improved the growth of BSFL and was able to obtain approximately 42% of larval crude protein. At the same time, the BSFL reared on fermented maize straw were found to contain higher amounts of monounsaturated fatty acids and polyunsaturated fatty acids, and were lower in saturated fatty acids as opposed to the control medium without exo-microorganisms. Concisely, it could be confidently deduced that the introduction of microorganisms into BSFL media through larval farming systems could promisingly enhance larval growth and, eventually, achieve more harvested larval biomasses.
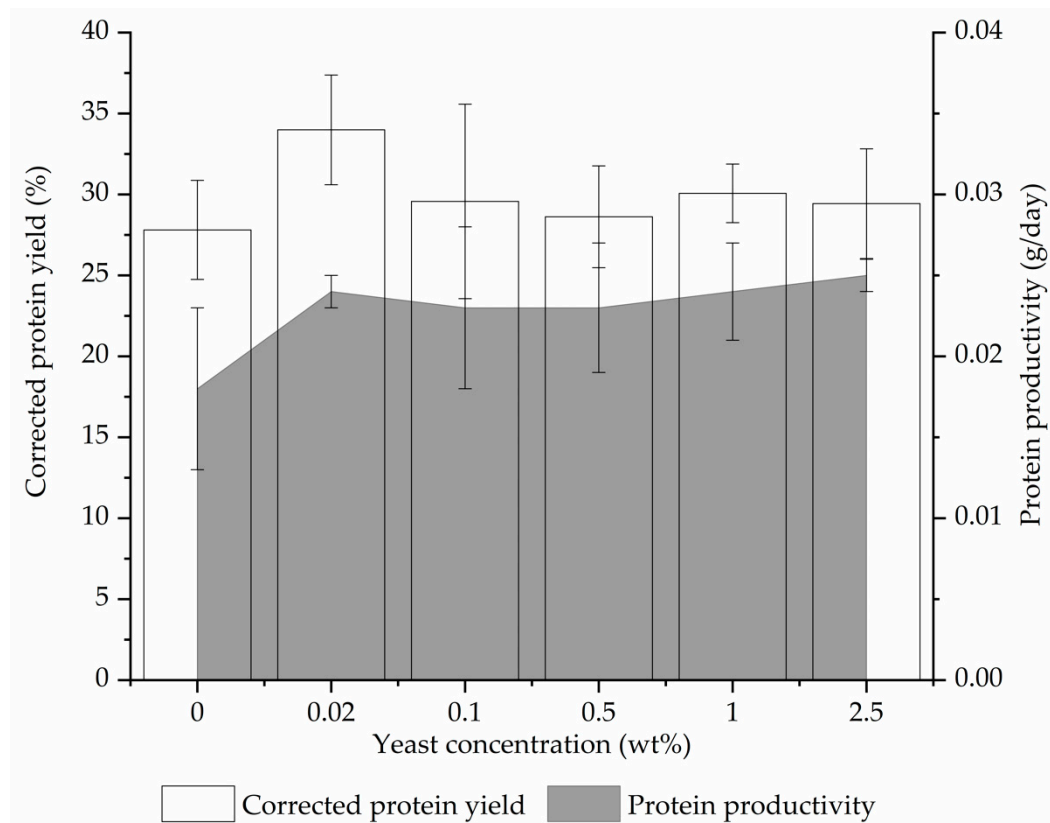

Figure 2. Impact of different yeast concentrations inoculating CEW on corrected protein yields and protein productivities from BSFL.

The introduction of BSFL biomass into animal feed could plausibly replace the exploitation of unsustainable soybean and fishmeal. Indeed, BSFL could serve as the sole protein source, since larval biomass is generally fortified with high protein as well as fat [30]. The inclusion of BSFL into animal feed for laying hens had been found to significantly increase the production of both day and house eggs. At the same time, it has also positively impacted the characteristics of eggs and the growth of laying hens [31]. In the case of aquaculture cultivation, a partial inclusion of BSFL into feed at 25%, serving as fishmeal protein has been shown to increase the growth performances of yellow catfish by 21.7%, while also improving their immune indexes [32]. Moreover, it was reported that the replacement of fishmeal by BSFL between 28.4% and 50% into the diets of juvenile barramundi could promote fish growth, fish whole body proximate and amino acid composition [33]. A 100% replacement of fishmeal by BSFL was also possible in Jian carp cultivation, as it had been reported that there was no unfavorable impact on the growth of Jian carp. BSFL meal could be an economic and sustainable replacement for current fish diets that could circumvent both feed shortages and the increasing price of fishmeal [34]. Thus, it is recommended that BSFL biomass meal be utilized as a substitution for protein alimentation in animal feed and fishmeal in the long-term, whilst also advocating for the green and sustainable farming of land and aquatic animals, respectively.

4. Conclusions

The inoculation of yeast at different concentrations into CEW to serve as the feeding medium for BSFL rearing enhanced larval growth. For a setup initially containing 20 neonates of BSFL, a final weight of 1.145 g, a growth rate at 0.085 g/day and a rearing period of 13.5 days were achieved when BSFL were fed with fermented CEW inoculated with 2.5 wt% yeast. With an increase in yeast concentrations, the overall degradation of CEW was found to improve from 0.48 to 0.53, with the waste reduction indexes fluctuating between 0.38 and 0.40 g/day. Likewise, the protein yield from BSFL was boosted from the control (28%) to its highest value of 35% in the presence of merely 0.02 wt% yeast concentration. On the other hand, protein productivity was increased from 0.018 g/day for the control to around 0.025 g/day across 0.02 to 2.5 wt% yeast concentrations. To conclude, the growth of BSFL was promoted with the inclusion of yeast as the fermentation precursor, and the harvested larval biomass can potentially be used as a replacement of protein sources in animal feeds and fishmeals.

References

1. Diclaro, J.; Kaufman, P.E. Black soldier fly hermetia illucens linnaeus (insecta: Diptera: Stratiomyidae). *EENY* **2009**, *461*, 1–3.
2. Win, S.S.; Ebner, J.H.; Brownell, S.A.; Pagano, S.S.; Cruz-Diloné, P.; Trabold, T.A. Anaerobic digestion of black solider fly larvae (bsfl) biomass as part of an integrated biorefinery. *Renew. Energy* **2018**, *127*, 705–712. [CrossRef]
3. Kroeckel, S.; Harjes, A.-G.; Roth, I.; Katz, H.; Wuertz, S.; Susenbeth, A.; Schulz, C. When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (hermetia illucens) as fish meal substitute—growth performance and chitin degradation in juvenile turbot (psetta maxima). *Aquaculture* **2012**, *364*, 345–352. [CrossRef]
4. Renna, M.; Schiavone, A.; Gai, F.; Dabbou, S.; Lussiana, C.; Malfatto, V.; Prearo, M.; Capucchio, M.T.; Biasato, I.; Biasibetti, E.; et al. Evaluation of the suitability of a partially defatted black soldier fly (hermetia illucens l.) larvae meal as ingredient for rainbow trout (oncorhynchus mykiss walbaum) diets. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 57. [CrossRef] [PubMed]
5. Schiavone, A.; De Marco, M.; Martínez, S.; Dabbou, S.; Renna, M.; Madrid, J.; Hernandez, F.; Rotolo, L.; Costa, P.; Gai, F.; et al. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (hermetia illucens l.) meal for broiler chickens: Apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 51. [CrossRef] [PubMed]
6. Schiavone, A.; Dabbou, S.; Petracci, M.; Zampiga, M.; Sirri, F.; Biasato, I.; Gai, F.; Gasco, L. Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on carcass traits, breast meat quality and safety. *Animal* **2019**, *13*, 2397–2405. [CrossRef] [PubMed]
7. Widjastuti, T.; Wiradimadja, R.; Rusmana, D. The effect of substitution of fish meal by black soldier fly (hermetia illucens) maggot meal in the diet on production performance of quail (coturnix coturnix japonica). *Anim. Sci.* **2014**, *57*, 125–129.
8. Loponte, R.; Nizza, S.; Bovera, F.; De Riu, N.; Fliegerova, K.; Lombardi, P.; Vassalotti, G.; Mastellone, V.; Nizza, A.; Moniello, G. Growth performance, blood profiles and carcass traits of barbary partridge (alectoris barbara) fed two different insect larvae meals (tenebrio molitor and hermetia illucens). *Res. Vet. Sci.* **2017**, *115*, 183–188. [CrossRef]

9.　Bovera, F.; Loponte, R.; Pero, M.E.; Cutrignelli, M.I.; Calabrò, S.; Musco, N.; Vassalotti, G.; Panettieri, V.; Lombardi, P.; Piccolo, G. Laying performance, blood profiles, nutrient digestibility and inner organs traits of hens fed an insect meal from hermetia illucens larvae. *Res. Vet. Sci.* **2018**, *120*, 86–93. [CrossRef]

10.　Secci, G.; Bovera, F.; Nizza, S.; Baronti, N.; Gasco, L.; Conte, G.; Serra, A.; Bonelli, A.; Parisi, G. Quality of eggs from lohmann brown classic laying hens fed black soldier fly meal as substitute for soya bean. *Animal* **2018**, *12*, 2191–2197. [CrossRef]

11.　Onsongo, V.; Osuga, I.; Gachuiri, C.; Wachira, A.; Miano, D.; Tanga, C.; Ekesi, S.; Nakimbugwe, D.; Fiaboe, K. Insects for income generation through animal feed: Effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *J. Econ. Entomol.* **2018**, *111*, 1966–1973. [CrossRef] [PubMed]

12.　Mohd-Noor, S.-N.; Wong, C.-Y.; Lim, J.-W.; Uemura, Y.; Lam, M.-K.; Ramli, A.; Bashir, M.J.; Tham, L. Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. *Renew. Energy* **2017**, *111*, 646–654. [CrossRef]

13.　Kim, W.-T.; Bae, S.-W.; Park, H.-C.; Park, K.-H.; Lee, S.-B.; Choi, Y.-C.; Han, S.-M.; Koh, Y.-H. The larval age and mouth morphology of the black soldier fly, hermetia illucens (diptera: Stratiomyidae). *Int. J. Indust. Entomol.* **2010**, *21*, 185–187.

14.　Li, Q.; Zheng, L.; Qiu, N.; Cai, H.; Tomberlin, J.K.; Yu, Z. Bioconversion of daiy manure by black soldier fly (*diptera: Stratiomyidae*) for biodiesel and sugar production. *Waste Manag.* **2011**, *31*, 1316–1320. [CrossRef] [PubMed]

15.　Lalander, C.; Diener, S.; Zurbrügg, C.; Vinnerås, B. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (hermetia illucens). *J. Clean. Prod.* **2019**, *208*, 211–219. [CrossRef]

16.　Parra, J.R.; Panizzi, A.R.; Haddad, M.L. Nutritional indices for measuring insect food intake and utilization. In *Insect Bioecology and Nutrition for Integrated Pest Management*; Panizzi, A.R., Parra, J.R., Eds.; CRC Press: London, UK; New York, NY, USA, 2012; p. 13.

17.　Culmo, R.F.; Shelton, C. *The Elemental Analysis of Various Classes of Chemical Compounds Using Chn Application Note*; Perkin Elmer: Waltham, MA, USA, 2013.

18.　Jones, D.B. *Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins*; US Department of Agriculture: Washington, DC, USA, 1941.

19.　Diener, S.; Zurbrügg, C.; Tockner, K. Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. *Waste Manag. Res.* **2009**, *27*, 603–610. [CrossRef] [PubMed]

20.　Rinaudo, M. Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* **2006**, *31*, 603–632. [CrossRef]

21.　Lovell, R.T.; Lafleur, J.R.; Hoskins, F.H. Nutritional value of freshwater crayfish waste meal. *J. Agric. Food Chem.* **1968**, *16*, 204–207. [CrossRef]

22.　Zheng, L.; Hou, Y.; Li, W.; Yang, S.; Li, Q.; Yu, Z. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy* **2012**, *47*, 225–229. [CrossRef]

23.　Rehman, K.U.; Rehman, A.; Cai, M.; Zheng, L.; Xiao, X.; Somroo, A.A.; Wang, H.; Li, W.; Yu, Z.; Zhang, J. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*hermetia illucens* l.). *J. Clean. Prod.* **2017**, *154*, 366–373. [CrossRef]

24.　Wiedmeier, R.D.; Arambel, M.J.; Walters, J.L. Effect of yeast culture and aspergillus oryzae fermentation extract on ruminal characteristics and nutrient digestibility1. *J. Dairy Sci.* **1987**, *70*, 2063–2068. [CrossRef]

25.　Yoon, S.-H.; Mukerjea, R.; Robyt, J.F. Specificity of yeast (saccharomyces cerevisiae) in removing carbohydrates by fermentation. *Carbohydr. Res.* **2003**, *338*, 1127–1132. [CrossRef]

26.　Li, W.; Li, M.; Zheng, L.; Liu, Y.; Zhang, Y.; Yu, Z.; Ma, Z.; Li, Q. Simultaneous utilization of glucose and xylose for lipid accumulation in black soldier fly. *Biotechnol. Biofuels* **2015**, *8*, 1–6. [CrossRef] [PubMed]

27.　Arcos-García, J.L.; Castrejón, F.A.; Mendoza, G.D.; Pérez-Gavilán, E.P. Effect of two commercial yeast cultures with saccharomyces cerevisiae on ruminal fermentation and digestion in sheep fed sugar cane tops. *Livest. Prod. Sci.* **2000**, *63*, 153–157. [CrossRef]

28.　Palma, L.; Fernandez-Bayo, J.; Niemeier, D.; Pitesky, M.; VanderGheynst, J.S. Managing high fiber food waste for the cultivation of black soldier fly larvae. *npj Sci. Food* **2019**, *3*, 1–7. [CrossRef] [PubMed]

29.　Gao, Z.; Wang, W.; Lu, X.; Zhu, F.; Liu, W.; Wang, X.; Lei, C. Bioconversion performance and life table of black soldier fly (hermetia illucens) on fermented maize straw. *J. Clean. Prod.* **2019**, *230*, 974–980. [CrossRef]

30. Wang, Y.-S.; Shelomi, M. Review of black soldier fly (hermetia illucens) as animal feed and human food. *Foods* **2017**, *6*, 91. [CrossRef]

31. Al-Qazzaz, M.F.A.; Ismail, D.; Akit, H.; Idris, L.H. Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. *Rev. Bras. Zootec.* **2016**, *45*, 518–523. [CrossRef]

32. Xiao, X.; Jin, P.; Zheng, L.; Cai, M.; Yu, Z.; Yu, J.; Zhang, J. Effects of black soldier fly (hermetia illucens) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (pelteobagrus fulvidraco). *Aquac. Res.* **2018**, *49*, 1569–1577. [CrossRef]

33. Katya, K.; Borsra, M.; Ganesan, D.; Kuppusamy, G.; Herriman, M.; Salter, A.; Ali, S.A. Efficacy of insect larval meal to replace fish meal in juvenile barramundi, lates calcarifer reared in freshwater. *Int. Aquat. Res.* **2017**, *9*, 303–312. [CrossRef]

34. Zhou, J.; Liu, S.; Ji, H.; Yu, H. Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of jian carp (cyprinus carpio var. Jian). *Aquac. Nutr.* **2018**, *24*, 424–433. [CrossRef]