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Dynamic Effects of Initial pH of Substrate on Biological Growth and Metamorphosis of Black Soldier Fly (Diptera: Stratiomyidae)

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Abstract

Edible insects have become a recognized alternative and sustainable source of high-quality proteins and fats for livestock or human consumption. In the production process of black soldier fly (BSF), (*Hermetia illucens* L. [Diptera: Stratiomyidae]), initial substrate pH is a critical parameter to ensure the best value of insect biomass, life history traits, and quality bio-fertilizer. This study examined the impact of initial pH values on BSF larvae production, development time, and adult longevity. The BSF were reared on artificial diet with initial pH of 2.0, 4.0, 6.0, 8.0, and 10.0; the control was set at 7.0. Final BSF larval weight was significantly greater in substrates having initial pH 6.0 (0.21 g), control 7.0 (0.20 g), and 10.0 (0.20 g) with no significant difference among them, whereas larval weight reared with initial pH 2.0 and 4.0 were lowest at 0.16 g (–23%). Prepupal weight was greatest when larvae were reared on substrates with initial pH 6.0 (0.18 g), control 7.0 (0.19 g), 8.0 (0.18 g), and 10.0 (0.18 g). In contrast, the prepupal weight of larvae reared on diets with initial pH 2.0 was lowest at 0.15 g (–22%). Larval development time was 21.19 d at pH 8.0, about 3 d (12.5%) shorter than that of those reared on diets with initial pH 6.0, 7.0 control, and 10.0. In all treatments, pH shifted to 5.7 after 3–4 d and 8.5 after 16–17 d except for two groups (2.0 and 4.0) where the pH remained slightly acidic 5.0 and 6.5, respectively.

Key words: *Hermetia illucens*, development time, metamorphosis, pH value, adult longevity

Insects have received widespread attention over the last decade as a nutrient-rich and sustainable source of protein for human consumption or use as animal feed (Rumpold and Schlüter 2013, Sánchez-Muros et al. 2014, Liu et al. 2017). Considering the future world population will increase to more than 9 billion people by 2050, the demand for high-quality protein for human and livestock consumption will continue to grow (Tilman et al. 2011, Makkar et al. 2014). Thus, insects are being viewed more as conventional sources of protein (Biffi and Tuissi 2017). Because of the numerous benefits of insect farming, e.g., capable nutritional spectra of the insects, high productiveness and multivoltine life cycle, feed conversion rate, omnivorous diet, low substrate demand, and little space and water demand, this new sector of agriculture is expected to contribute to global food security (Rumpold and Schlüter 2013).

Among many insects considered for mass production, the black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), has great potential for large-scale production (EFSA 2015). Black soldier fly larvae are capable of transforming low-value organic wastes into

protein and fat (Myers et al. 2008, Barroso et al. 2014, Schiavone et al. 2016). In various feeding studies, larval or prepupal meal was found to be a suitable alternative to the partial and total replacement of fish meal, meat meal, and plant proteins meal in livestock (Makkar et al. 2014), poultry (Schiavone et al. 2016), and aquaculture (St-Hilaire et al. 2007, Cummins et al. 2017).

The black soldier fly life cycle consists of four life stages (eggs, larvae, pupae, and adult) (Li et al. 2011). The black soldier fly originated from humid and warm climate conditions with breeding conditions ranging between 24 and 40°C with optimal larval development at 27°C (Sheppard et al. 2002). Larval development takes about 2 wk, depending on the breeding temperature (Tomberlin et al. 2009), substrate composition, relative humidity (Holmes et al. 2012), and substrate availability (Harnden and Tomberlin 2016, Oonincx et al. 2015). Larvae were determined to be optimal regarding dry weight and developmental time when fed 200 mg/d per larvae on chicken feed (Diener et al. 2009). Black soldier fly larvae have no particular dietary restrictions. They can be reared on a variety of

substrates, including dairy manure (Myers et al. 2008, Li et al. 2011, Rehman et al. 2017b), chicken manure (Yu et al. 2011, Rehman et al. 2017a), swine manure (Zhou et al. 2013), human feces (Banks et al. 2014), vegetable wastes (Paz et al. 2015), food wastes (Zheng et al. 2012a, b, Nguyen et al. 2015), soybean curd residues (Rehman et al. 2017b), coffee pulp (Lardé 1990), and municipal organic waste (Diener et al. 2011).

In order to stabilize the black soldier fly conversion process and increase the biomass yield, co-digestion has been considered as an efficient method (Rehman et al. 2017a, b). Different waste materials have varying pH levels, such as food wastes having initially lower pH as compared to animal manures (Dennehy et al. 2016, Huang et al. 2016, Rehman et al. 2017b). Studies have shown mixing of different waste substrates rich in nutrients with another low nutritious substrate could increase alkalinity capacity, reduce volatile fatty acids (VFAs) concentrations, and increases production of larvae, because VFAs inhibition may be beneficial for the intestinal microbiota development (Wielen et al. 2000) and this insect gut microbiome play vital role in the larval growth (Lee et al. 2017). It is noteworthy that the gut microbiome of insects have a significant role in the degradation of nutrients and lignocellulose (Li et al. 2016, Abduh et al. 2017, Rehman et al. 2017b) and enhance larval growth (Lee et al. 2017, Rehman et al. 2017a, b). However, mixing only different waste substrates is not an optimal method for larval biomass production. With that said, pH of the culture medium greatly affects the activities of particular acid-producing microbial populations (Zhang et al. 2012) and other bacterial communities (Ghosh et al. 2000). Moreover, it was reported by Kim et al. (2017) that diet is one major determinant of intestinal bacterial community composition in insects. Thus, pH is one of the key factors affecting production efficiency of vermicomposting (Wang et al. 2017). Initial pH value in many waste management studies was controlled (Zhai et al. 2015). However, in digestion technology, the pH value varies with substrate, and an optimal initial pH value for black soldier fly digestion process needs to be evaluated to improve the biomass yield and life history traits. This study aims to investigate the effects of seven different pH values (2.0, 4.0, 6.0, 8.0, 10.0, and neutral control 7.0) on artificial feed in order to obtain the most stable digestion environment for black soldier fly and gut microbes, beneficial effects on various life history traits, and maximum larval meal production.

Materials and Methods

Experiment Site

The experiment was carried out in a greenhouse located at the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University (HZAU), Wuhan, Hubei, P. R. China. Black soldier flies used in this study were obtained from a colony housed in a cage (1.8 m³ and 1.5 mm mesh screen) maintained in a greenhouse at ~27°C with 14:10 (L:D) h.

Black Soldier Fly Larvae

A colony of black soldier flies (*H. illucens*), Wuhan strain, was established in 2008 at the State Key Laboratory of Agricultural Microbiology of HZAU, Wuhan, P. R. China. The Wuhan strain colony has been maintained in a greenhouse for 9 yr before it was used in this study (Zhou et al. 2013). Larvae were fed for 6 d with a standard colony diet before being utilized in this investigation.

Artificial Feed Preparation and Growth Conditions

The artificial feed ingredient used in the study was prepared by mixing 75 g bran, 30 g corn flour, and 350 g water at room temperature as described by Hogsette 1992, and Tomberlin et al. 2002 with slight modification. The bran and corn flour used in this study were dried at 60°C for 6 h before being used in this investigation. The mixture of bran and corn flour was sterilized at 121°C for 30 min before use. Each replicate consisted of 300 six-d-old larvae fed with 500 g of freshly prepared diet throughout the whole experiment, placed in a three-liter plastic container. Three replicates of each treatment were prepared. The experiment was conducted in the greenhouse at 27°C and 60% and 70% relative humidity.

Initial pH Adjustment and Measurement

The initial pH of the diet for a given treatment was 2.0, 4.0, 6.0, 7.0 (control), 8.0, or 10.0. Feed pH was adjusted by adding concentrated sodium hydroxide (NaOH; 2 M/liter) or hydrochloric acid (HCl; 2 M/liter) as previously described by Lay et al. (1997). The lowest initial pH of the feed was set at 2.0 because all larvae were killed when the pH value was less than 1.80. The pH values of the feed mixture were measured in a distilled water suspension at the ratio of 1:10 (W/V) using the Mettler Toledo FE 20 pH system.

Experimental Design and Operation

Two days after setting up the treatments, forceps were used to select 20 larvae from a given replicate of treatment randomly. The larvae were washed with distilled water, dried with cotton gauze, weighed, and then returned to their appropriate container. Larval development time was recorded daily. Prepupae were removed when observed in a replicate, individually weighed with electric balance (BS 210S, SARTORIUS AG, Germany), transferred to an individual 35 ml cups with breathable cover and identification information, and returned to the greenhouse. Percentage of prepupae that reached the adult stage was noted for each replicate of each treatment. Development time of larva, prepupa, and adult longevity along with sex was recorded.

Statistical Analysis

The statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Results of all experiments were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's HSD (honestly significant differences) for post-hoc testing to compare the significance (*P*) between the means of different groups. Whereas Fig. 1 and two only mean and standard error was calculated by SPSS 16.0. Alpha was established at *P* < 0.05.

Results

Percent Survival During Metamorphosis of BSF

Black soldier fly percent survival to prepupa 6.0, 7.0, 8.0 was significantly higher with 2.0, 4.0, and 10.0 treatments ($F = 15.92$; $df = 5, 12$; $P < 0.0001$). (Table 1). Survival rate of larvae to prepupa was significantly lower when reared on diets with high acidic and basic pH value such as 2.0 (91.62%), 4.0 (88.68%), and 10.0 (95.57%) ($F = 6.39$; $df = 2, 6$; $P = 0.063$) compared with 6.0 (99.11%), 7.0 (99.44%), and 8.0 (98.44%). It was noted that there was no significant difference in survival rate or prepupal rate among pH 6.0, 7.0, and 8.0 ($F = 6.54$; $df = 5, 8$; $P = 0.051$) (Table 1).

Percent survival to adult stage (Table 1) was higher in pH value 6.0 (95.30%), 7.0 (95.78%), and 8.0 (94.72%) with no significant

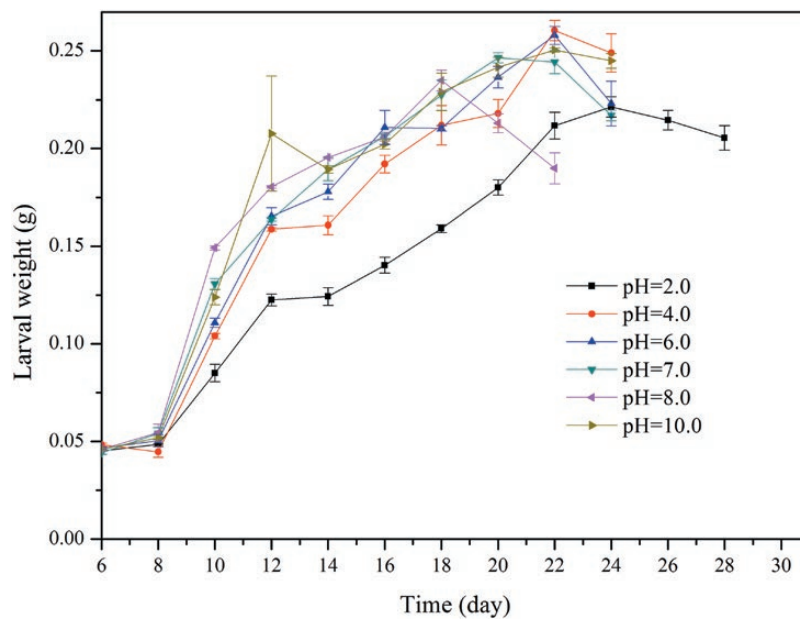


Fig. 1. Variations in initial adjusted pH of substrate during black soldier fly conversion process; bars indicate the standard error of the means ($n = 3$).

Table 1. Percent survival and female to male ratio of black soldier fly reared on identical substrate with various initial pH values

pH values	Percent survival to prepupal stage (%)	Percent survival to adult stage (%)	Female-to-male ratio
2.0	91.62 ± 0.89 ^{bc}	92.88 ± 0.88 ^c	0.86 ± 0.02 ^b
4.0	88.68 ± 2.18 ^c	93.59 ± 0.79 ^{bc}	0.89 ± 0.02 ^b
6.0	99.11 ± 0.89 ^a	95.30 ± 0.99 ^a	0.97 ± 0.01 ^b
7.0	99.44 ± 0.56 ^a	95.77 ± 0.79 ^a	1.33 ± 0.04 ^a
8.0	98.44 ± 0.87 ^a	94.72 ± 0.27 ^a	1.47 ± 0.07 ^a
10.0	95.56 ± 0.23 ^b	92.54 ± 0.47 ^c	0.86 ± 0.02 ^b

Data are expressed as mean ± SE; $n = 3$; values with different superscripts differ from each other at $P < 0.05$.

difference among these treatments ($F = 3.16$; $df = 5, 12$; $P = 0.074$). However, mean percent survival to adult stage for prepupae reared in high acidic (2.0–4.0) and extremely basic (10.0) media was lower compared with the slightly acidic pH (6.0), basic pH (8.0), and from control pH value (7.0).

Adult Female-to-Male Ratio of BSF

Sex ratio was determined for all treatments (Table 1). The female-to-male ratio differed significantly among the treatments ($F = 55.95$, $df = 5$, $P < 0.0001$). The sex ratio of pH 7.0 and 8.0 was significantly higher when compared with other tested values (2.0, 4.0, 6.0, and 10.0) but not significantly different between them ($F = 3.11$; $df = 1, 4$; $P = 0.153$). Moreover, it was recorded that there was no significant difference ($F = 12.44$; $df = 3, 8$; $P = 0.052$) in the female-to-male ratio with treatments having initial substrate pH values 2.0, 4.0, 6.0, and 10.0. The lowest female-to-male ratio (0.86) was observed in treatments with initial pH 2.0.

Biomass Generation, Development Time, Adult Longevity, and Dynamics of pH Value During BSFL Conversion

It was observed that the individual larval weight of was significantly different ($F = 6.58$; $df = 5, 12$; $P = 0.004$) across the treatments ranging from 0.16 to 0.21 g. The significantly greatest larval biomass was

obtained from treatment with initial substrate pH 6.0 but no significant difference with 7.0, and 10.0 ($F = 4.26$; $df = 3, 8$; $P = 0.45$). Moreover, the initial substrate pH 8.0 was significantly lower than 6.0 but no significant difference with 7.0, and 10.0 ($F = 34.44$; $df = 2, 6$; $P = 0.001$). The higher acidic pH (2.0, 4.0) has significantly lower biomass output during the conversion process with no significant difference between them ($F = 0.008$; $df = 1, 8$; $P = 0.933$). The mean prepupal biomass generation was significantly lower in 2.0, 4.0 than 6.0, 7.0, 8.0, 10.0 ($F = 32.64$; $df = 5, 12$; $P < 0.0001$) treatments and ranged from 0.15 to 0.19 g (Table 2). The treatments with initial substrate pH values 6.0, 7.0, 8.0, and 10.0 have no significant difference in prepupal weight, which is significantly higher than the 2.0 and 4.0 ($F = 9.65$; $df = 3, 83$; $P < 0.0001$).

Larval development time differed significantly ($F = 150.99$, $df = 5$, $P < 0.0001$) ranging from 28 to 21 d (Table 2). It was noted that larval development time delayed when fed with an acidic (pH 2.0) diet (28 d), whereas the development time reduced to 21 d at slight basic pH become basic (8.0). In fact, larval development was similar when diet initial pH ranged from 4.0, 6.0, 7.0 and 10.0 but higher when compared with 8.0 ($F = 3.64$, $df = 3$, $P = 0.013$). Prepupal development time showed significant difference ($F = 2.21$, $df = 5$, $P = 0.015$) across the pH treatments. The prepupa development time in initial substrate pH values 2.0, 4.0, 10.0 were significantly shorter ($F = 1.40$; $df = 4, 897$; $P = 0.031$) when compared with the pH 7.0, whereas pH 7.0 has no significant difference with

pH values 6.0, and 8.0 ($F = 1.56$; $df = 3, 729$; $P = 0.199$). However, the adult longevity ranged from 10.87 to 15.70 d and differed significantly ($F = 32.16$, $df = 5$, $P < 0.0001$). The shortest adult longevity (10.87 d) was observed in the group fed the diet with a neutral pH 7.0 and no significant difference ($F = 7.08$; $df = 3, 712$; $P = 0.051$) with 4.0, 6.0, and 8.0.

The overtime pH by treatment interaction is presented in Fig. 1. BSF was able to adjust the diet pH within 24 h after each feeding. However, this capability was not observed when diets were acidic (pH = 2.0). The final pH value at the termination of larval feeding (prepupa development) in 6.0, 7.0, 8.0, and 10.0 treatments ranged from 8.0 to 9.0; in contrast, strong acidic diets 2.0 and 4.0 had final pH values below 6.0.

Individual larval daily biomass gain during BSF conversion was greater when reared on diets with an initial pH of 6.0, or 10.0 as compared to neutral pH 7.0 or other tested pH values 2.0, 4.0, and 8.0 (Fig. 2). However, the lowest biomass production was noted for those reared on diets with an initial pH of 2.0 (Fig. 2).

Discussion

The survival rate of larvae, prepupa, prepupal weight, and adult longevity were significantly higher in substrates having initial pH of 6.0, control 7.0, and 8.0. Therefore, initial pH of 6.0 to 8.0 for bioconversion of organic waste using BSF is recommended. These findings

Table 2. Black soldier fly biomass production and development time on same substrate with various initial substrate pH values during metamorphosis

pH value	Sex	Final larval mass (g)	Prepupal mass (g)	Larval development (d)	Prepupal development (d)	Adult longevity (d)
2.0	F	–	0.16 ± 0.01	28.69 ± 0.32	22.52 ± 0.69	15.09 ± 0.38
	M	–	0.15 ± 0.01	27.29 ± 0.32	22.48 ± 0.50	15.70 ± 0.39
	Combined	0.16 ± 0.02 ^c	0.15 ± 0.01 ^c	28.08 ± 0.37 ^c	22.57 ± 0.38 ^a	15.39 ± 0.76 ^c
4.0	F	–	0.18 ± 0.01	24.36 ± 2.03	22.68 ± 0.50	11.90 ± 0.34
	M	–	0.15 ± 0.01	23.34 ± 0.23	22.03 ± 0.37	13.40 ± 0.39
	Combined	0.16 ± 0.01 ^c	0.17 ± 0.01 ^b	24.08 ± 0.12 ^b	22.40 ± 0.23 ^a	12.55 ± 0.46 ^{ab}
6.0	F	–	0.19 ± 0.01	24.28 ± 0.25	23.59 ± 0.59	11.73 ± 0.33
	M	–	0.17 ± 0.01	24.03 ± 0.23	23.90 ± 0.61	12.52 ± 0.36
	Combined	0.21 ± 0.02 ^a	0.18 ± 0.02 ^a	24.21 ± 0.14 ^b	23.72 ± 0.09 ^b	12.13 ± 0.31 ^{ab}
7.0	F	–	0.21 ± 0.01	24.26 ± 0.20	24.32 ± 0.53	10.78 ± 0.38
	M	–	0.18 ± 0.01	23.31 ± 0.21	23.78 ± 0.56	10.96 ± 0.39
	Combined	0.20 ± 0.01 ^{ab}	0.19 ± 0.01 ^a	24.00 ± 0.28 ^b	24.16 ± 0.05 ^b	10.87 ± 0.45 ^a
8.0	F	–	0.18 ± 0.01	21.33 ± 0.17	23.45 ± 0.47	11.79 ± 0.26
	M	–	0.16 ± 0.01	20.93 ± 0.22	24.06 ± 0.50	13.17 ± 0.42
	Combined	0.19 ± 0.01 ^b	0.18 ± 0.01 ^a	21.19 ± 0.03 ^a	23.83 ± 0.43 ^b	12.35 ± 0.29 ^{ab}
10.0	F	–	0.19 ± 0.01	24.90 ± 0.28	22.25 ± 0.45	13.01 ± 0.40
	M	–	0.18 ± 0.01	24.25 ± 0.28	23.48 ± 0.66	14.64 ± 0.35
	Combined	0.20 ± 0.01 ^{ab}	0.18 ± 0.01 ^a	24.63 ± 0.12 ^b	23.11 ± 0.52 ^a	13.93 ± 0.64 ^b

Data are expressed as mean ± SE; $n = 3$; values with different superscripts differ from each other at $P < 0.05$.

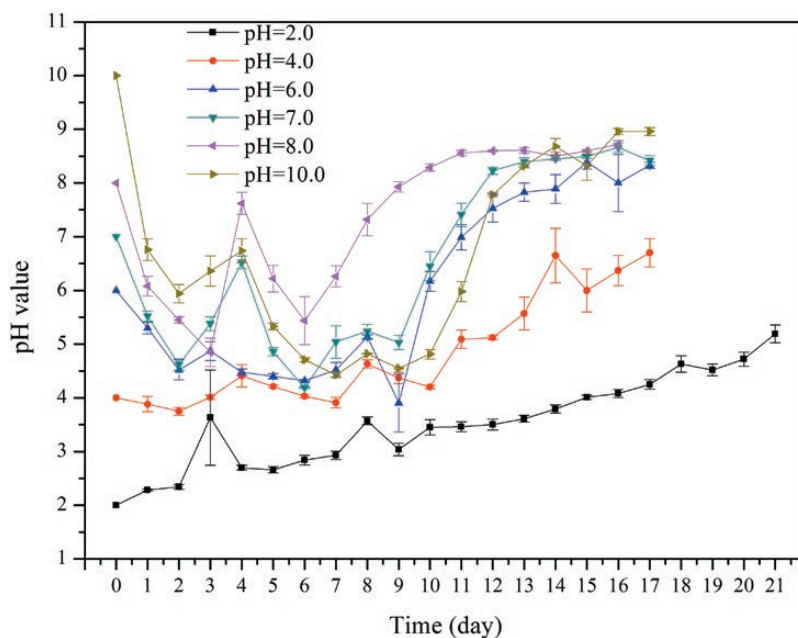


Fig. 2. Black soldier fly daily larval biomass gain during conversion process reared on different initial pH of feeding substrate; bars indicate the standard error of the means ($n = 3$).

indicated that initial pH significantly affects the life history parameters of BSF. Initial pH effects could favor beneficial bacteria (Sen and Chandra 2009, Castillo et al. 2013, Zhai et al. 2015, Hussain et al. 2016, Rehman et al. 2017b) and thus contribute to survival rate, larval growth, and development time; the insect gut microbiome promotes larval weight gain, growth, and egg production (Lee et al. 2017, Zheng et al. 2017). The present study is the first to describe the detailed effects of various initial pH of feed on the biological growth of BSF larvae, whereas a former investigation by Popa and Green (2012) only noted that BSFL neutralized acidity of compost leachate with no record of acidic and basic medium effects on life history characteristics of BSF.

Percent survival to the prepupal and adult stage with an adult female-to-male ratio interestingly fluctuated with the change in pH of substrate medium (Table 1). The significantly higher survival rate was noticed in a substrate, having pH value of 6.0 and 8.0 with similar values in neutral control pH (7.0). Moreover, the larval and prepupal weights were recorded highest in 6.0 and 10.0 pH value with no significant difference with control neutral pH substrate (7.0). Furthermore, the development time during metamorphosis of BSF varies with the fluctuation of pH (Table 2). The results were in agreement with previous investigations that slight acidic and basic initial pH value has significant effects on waste digestion and for better biomass production (Singh et al. 2006, Zhai et al. 2015, Zhang et al. 2015). The effects of initial pH adjustment on BSFL survival, development time, and biomass yield may be due to the establishment of gut and medium symbiotic bacterial communities as described previously during vermicomposting (Castillo et al. 2013; Huang et

al. 2013, 2014). The adjustment of initial pH value of BSF conversion technology is helpful for insect farmers as BSF could develop on the wide range of pH values such as animal manures and food wastes that have basic and acidic pH, respectively. Moreover, it is likely to be more basic or acidic with the passage of time, which may turn into an unhealthy medium for the development of BSFL and their gut microbes. Therefore, the mixing of different waste for BSFL development (Rehman et al. 2017a, b), with the adjustment of initial pH may produce an economical model of BSF farming. The findings of study is beneficial for the development of cost-effective BSF conversion system for the production of larval biomass that has potential to be used in human food chain by utilizing the BSFL meat directly for human consumption or used to replace the costly animal and plant protein and fat ingredients of livestock, poultry and aquaculture compound feed (Makkar et al. 2014).

Providing this information, i.e., initial pH adjustment of the substrate in BSF conversion system to industry could be beneficial for the development of cost-effective BSF conversion system to produce larval biomass. It was found that with adjustment of pH survival rate, development time, and BSF biomass production were significantly effected in different metamorphosis stages of BSF (Table 3). Moreover, current work noted the variation female-to-male ratio after adult development in response to pH change (Table 3).

Initially, substrates pH after the larval addition showed decreasing trends in pH during the larval development (Fig. 1). The treatments with initial pH of 6.0, 8.0, 10.0, and 7.0 (control), the final pH value were approximately 8.0 to 8.5 at the end of larval feeding period; however, strong acidic media (pH 2.0 and 4.0) did not exceed

Table 3. Comparison of selected parameters of black soldier fly conversion experiments

References	Feed source	Survival rate (%)		Development time (d)		Adult longevity	Mean fresh larval mass (mg)		Female-male ratio	Temperature (°C)	Humidity (%)
		Larvae	Prepupa	Larvae	Prepupa		Larvae	Prepupa			
Current investigation	Adjusted initial pH of artificial feed from 2.0 to 10.0	88.6–99.4	92.5–95.7	20.9–28.7	22.0–24.3	10.8–15.7	160–210	150–190	0.8–1.5	27	60–70
Manurung et al. (2016)	Rice straw	51.2–98.3	NA	38–54	NA	NA	NA	NA	NA	28	70
Ooninx et al. (2015a)	Cow manure	87.8	NA	214.5	NA	NA	70	NA	NA	28	70
	Chicken manure	82.2	NA	144	NA	NA	50	NA	NA	28	70
	Pig Manure	97	NA	144	NA	NA	60	NA	NA	28	70
Ooninx et al. (2015b)	Artificial feed	75–85	NA	21–37	NA	NA	NA	NA	NA	28	70
Gobbi et al. (2013)	Chicken feed	93	NA	15	NA	NA	NA	NA	NA	27	NA
Gobbi et al. (2013)	Meat meal	40	NA	33	NA	NA	NA	NA	NA	25	NA
Li et al. (2011)	Dairy manure	NA	NA	31	NA	NA	NA	NA	NA	27	NA
Sealey et al. (2011)	Dairy manure	NA	NA	~120	NA	NA	NA	NA	NA	25	NA
Diener et al. (2009)	Chicken feed	NA	NA	16–42	NA	NA	80–150	NA	NA	27	NA
Myers et al. (2008)	Dairy manure	71–85	NA	26–30	NA	10.2–14.9	140–170	NA	NA	NA	NA

NA, not available.

6.0 at the termination of feeding phase (Fig. 1). We noted earlier that vermicomposting resulted in the substrate becoming acidic due to the mineralization of nitrogen and phosphorus compounds and production of humic acid and fulvic acid (Bhat et al. 2015). The decrease in substrate pH by BSFL may be due to the gut microorganism responsible for the production of organic acids. However, increases in the substrate pH recorded in this study suggested the alkalization of the substrate caused by the release of ammonium ions (NH_4^+) and ammonia (Alidadi et al. 2016). The same phenomena had been described during composting with the earthworm (Majlessi et al. 2012). The trends in the individual larval biomass output during the conversion process showed that pH value 4.0 and 10.0 have higher daily larval biomass production (Fig. 2), the result was in agreement with the previous investigation (Bhat et al. 2015, Zhang et al. 2015). The final pH value of residues after conversion with BSFL was close to neutral or slightly basic, indicating that the final product could be useful for repairing acidic soils, basic compost was previously used for reconditioning of acidic soils (Huang et al. 2014).

Conclusions

This study found that initial pH of growth substrate has significant effects on the BSF larval biomass generation and life-history traits which is beneficial for the newborn insect farming industry for human and animal feed. The mixing of various organic waste for BSFL development with the adjustment of initial pH may produce economical model of BSF farming for larval biomass production that have potential to be used in human food chain by utilizing the BSFL meat directly for human consumption or used to replace the costly animal and plant protein and fat ingredients of livestock, poultry, and aquaculture compound feed. It was noted that the optimum pH value for the BSF production system (life history traits) was slight acidic (6.0) and slight basic (8.0 and 10.0), and larval biomass improved effectively by changing pH at the beginning of BSFL digestion. This approach provides insight into mixing of organic wastes materials having buffer capacity with easily acidifying materials.

Acknowledgments

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References Cited

- Abduh, M. Y., M. Jamilah, P. Istiandari, S. Manurung, and R. Manurung. 2017. Bioconversion of rubber seeds to produce protein and oil-rich biomass using black soldier fly larva assisted by microbes. *J. Entomol. Zool. Stud.* 5: 591–597.
- Alidadi, H., A. Hosseinzadeh, A. A. Najafpoor, H. Esmaili, J. Zanganeh, M. Dolatabadi Takabi, and F. G. Piranloo. 2016. Waste recycling by vermicomposting: maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators. *J. Environ. Manage.* 182: 134–140.
- Banks, I. J., W. T. Gibson, and M. M. Cameron. 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Trop. Med. Int. Heal.* 19: 14–22.
- Barroso, F. G., C. de Haro, M. J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez, and C. Pérez-Bañón. 2014. The potential of various insect species for use as food for fish. *Aquaculture.* 422–423: 193–201.
- Bhat, S. A., J. Singh, and A. P. Vig. 2015. Potential utilization of bagasse as feed material for earthworm *Eisenia fetida* and production of vermicompost. *Springerplus.* 4: 11.
- Biffi, C. A., and A. Tuissi. 2017. Stato dell'arte sulle tecniche di produzione additiva per metalli. *Metall. Ital.* 109: 5–10.
- Castillo, J. M., E. Romero, and R. Nogales. 2013. Dynamics of microbial communities related to biochemical parameters during vermicomposting and maturation of agroindustrial lignocellulose wastes. *Bioresour. Technol.* 146: 345–354.
- Cummins, V. C., S. D. Rawles, K. R. Thompson, A. Velasquez, Y. Kobayashi, J. Hager, and C. D. Webster. 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture.* 473: 337–344.
- Dennehy, C., P. G. Lawlor, T. Croize, Y. Jiang, L. Morrison, G. E. Gardiner, and X. Zhan. 2016. Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion. *Waste Manag.* 56: 173–180.
- Diener, S., C. Zurbrugg, and K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Manag. Res.* 27: 603–610.
- Diener, S., N. M. S. Solano, F. R. Gutiérrez, C. Zurbrugg, and K. Tockner. 2011. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization.* 2: 357–363.
- EFSA. 2015. Risk profile related to production and consumption of insects as food and feed. *EFSA J.* 13: 60.
- Ghosh, S., M. P. Henry, A. Sajjad, M. C. Mensinger, and J. L. Arora. 2000. Pilot-scale gasification of municipal solid wastes by high-rate and two-phase anaerobic digestion (TPAD). *Water Sci. Technol.* 41: 101–110.
- Gobbi, P., A. Martínez-Sánchez, and S. Rojo. 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Eur. J. Entomol.* 110: 461.
- Harnden, L. M., and J. K. Tomberlin. 2016. Effects of temperature and diet on black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), development. *Forensic Sci. Int.* 266: 109–116.
- Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. *J. Econ. Entomol.* 85: 2291–2294.
- Holmes, L. A., S. L. Vanlaerhoven, and J. K. Tomberlin. 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environ. Entomol.* 41: 971–978.
- Huang, K., F. Li, Y. Wei, X. Chen, and X. Fu. 2013. Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia foetida*. *Bioresour. Technol.* 150: 235–241.
- Huang, K., F. Li, Y. Wei, X. Fu, and X. Chen. 2014. Effects of earthworms on physicochemical properties and microbial profiles during vermicomposting of fresh fruit and vegetable wastes. *Bioresour. Technol.* 170: 45–52.
- Huang, X., S. Yun, J. Zhu, T. Du, C. Zhang, and X. Li. 2016. Mesophilic anaerobic co-digestion of aloe peel waste with dairy manure in the batch digester: Focusing on mixing ratios and digestate stability. *Bioresour. Technol.* 218: 62–68.
- Hussain, N., A. Singh, S. Saha, M. Venkata Satish Kumar, P. Bhattacharyya, and S. S. Bhattacharya. 2016. Excellent N-fixing and P-solubilizing traits in earthworm gut-isolated bacteria: a vermicompost based assessment with vegetable market waste and rice straw feed mixtures. *Bioresour. Technol.* 222: 165–174.
- Kim, J. M., M.-Y. Choi, J.-W. Kim, S. A. Lee, J.-H. Ahn, J. Song, S.-H. Kim, and H.-Y. Weon. 2017. Effects of diet type, developmental stage, and gut compartment in the gut bacterial communities of two Cerambycidae species (Coleoptera). *J. Microbiol.* 55: 21–30.
- Lardé, G. 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Biol. Wastes.* 33: 307–310.
- Lay, J.-J., Y.-Y. Li, and T. Noike. 1997. Influences of pH and moisture content on the methane production in high- solids sludge digestion. *Water Res.* 31: 1518–1524.
- Lee, J. B., K.-E. Park, S. A. Lee, S. H. Jang, H. J. Eo, H. A. Jang, C.-H. Kim, T. Ohbayashi, Y. Matsuura, Y. Kikuchi, R. Futahashi, T. Fukatsu, and B. L. Lee. 2017. Gut symbiotic bacteria stimulate insect growth and egg production by modulating hexamerin and vitellogenin gene expression. *Dev. Comp. Immunol.* 69: 12–22.
- Li, Q., L. Zheng, N. Qiu, H. Cai, J. K. Tomberlin, and Z. Yu. 2011. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Manag.* 31: 1316–1320.

- Li, L., M. Stasiak, L. Li, B. Xie, Y. Fu, D. Gidzinski, M. Dixon, and H. Liu. 2016. Rearing *Tenebrio molitor* in BLS: dietary fiber affects larval growth, development, and respiration characteristics. *Acta Astronaut.* 118: 130–136.
- Liu, X., X. Chen, H. Wang, Q. Yang, K. ur Rehman, W. Li, M. Cai, Q. Li, L. Mazza, J. Zhang, Z. Yu, and L. Zheng. 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS One.* 12: e0182601.
- Majlessi, M., A. Eslami, H. N. Saleh, S. Mirshafiean, and S. Babaii. 2012. Vermicomposting of food waste: assessing the stability and maturity. *Iranian J. Environ. Health Sci. Eng.* 9: 1.
- Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.* 197: 1–33.
- Manurung, R., A. Supriatna, and R. R. Esyantih. 2016. Bioconversion of rice straw waste by black soldier fly larvae (*Hermetia illucens* L.): optimal feed rate for biomass production. *J. Entomol. Zool. Stud.* 4: 1036–1041.
- Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environ. Entomol.* 37: 11–15.
- Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environ. Entomol.* 44: 406–410.
- Oonincx, D. G. A. B., S. van Broekhoven, A. van Huis, and J. J. A. van Loon. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One.* 10: e0144601.
- Paz, A. S. P., N. S. Carrejo, and C. H. G. Rodríguez. 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.), (Diptera: Stratiomyidae). *Waste Biomass Valorization.* 6: 1059–1065.
- Popa, R., and T. R. Green. 2012. Using black soldier fly larvae for processing organic leachates. *J. Econ. Entomol.* 105: 374–378.
- Rehman, K. U., M. Cai, X. Xiao, L. Zheng, H. Wang, A. A. Somroo, Y. Zhou, W. Li, Z. Yu, and J. Zhang. 2017a. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). *J. Environ. Manage.* 196: 458–465.
- Rehman, K. ur, A. Rehman, M. Cai, L. Zheng, X. Xiao, A. A. Somroo, H. Wang, W. Li, Z. Yu, and J. Zhang. 2017b. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). *J. Clean. Prod.* 154: 366–373.
- Rumpold, B. A., and O. K. Schlüter. 2013. Potential and challenges of insects as an innovative source for food and feed production. *Innov. Food Sci. Emerg. Technol.* 17: 1–11.
- Sánchez-Muros, M. J., F. G. Barroso, and F. Manzano-Agugliaro. 2014. Insect meal as renewable source of food for animal feeding: a review. *J. Clean. Prod.* 65: 16–27.
- Schiavone, A., M. Cullere, M. De Marco, M. Meneguz, I. Biasato, S. Bergagna, D. Dezzutto, F. Gai, S. Dabbou, L. Gasco, and A. Dalle Zotte. 2016. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Ital. J. Anim. Sci.* 16: 1–8.
- Sealey, W. M., T. G. Gaylord, F. T. Barrows, J. K. Tomberlin, M. A. McGuire, C. Ross, and S. St-Hilaire. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. *J. World Aquac. Soc.* 42: 34–45.
- Sen, B., and T. S. Chandra. 2009. Do earthworms affect dynamics of functional response and genetic structure of microbial community in a lab-scale composting system? *Bioresour. Technol.* 100: 804–811.
- Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *J. Med. Entomol.* 39: 695–698.
- Singh, N. B., A. K. Khare, D. S. Bhargava, and S. Bhattacharya. 2006. Effect of initial substrate pH on vermicomposting using *Perionyx excavatus* (Perrier, 1872). *Appl. Ecol. Environ. Res.* 4: 85–97.
- St-Hilaire, S., C. Sheppard, J. K. Tomberlin, S. Irving, L. Newton, M. A. McGuire, E. E. Mosley, R. W. Hardy, and W. Sealey. 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. *J. World Aquac. Soc.* 38: 59–67.
- Tilman, D., C. Balzer, J. Hill, and B. L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. *Proc. Natl Acad. Sci. USA.* 108: 20260.
- Tomberlin, J. K., D. C. Sheppard, and J. a. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Ann. Entomol. Soc. Am.* 95: 379–386.
- Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environ. Entomol.* 38: 930–934.
- Wang, D., J. Ai, F. Shen, G. Yang, Y. Zhang, S. Deng, J. Zhang, Y. Zeng, and C. Song. 2017. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. *Bioresour. Technol.* 227: 286–296.
- Wielen, P. W. J. J. Van Der, S. Biesterveld, H. Hofstra, B. a P. Urlings, and F. Van. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Appl. Environ. Microbiol.* 66: 6–11.
- Yu, G., P. Cheng, Y. Chen, Y. Li, Z. Yang, Y. Chen, and J. K. Tomberlin. 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. *Environ. Entomol.* 40: 30–35.
- Zhai, N., T. Zhang, D. Yin, G. Yang, X. Wang, G. Ren, and Y. Feng. 2015. Effect of initial pH on anaerobic co-digestion of kitchen waste and cow manure. *Waste Manag.* 38: 126–131.
- Zhang, X., W. Qiu, and H. Chen. 2012. Enhancing the hydrolysis and acidification of steam-exploded cornstalks by intermittent pH adjustment with an enriched microbial community. *Bioresour. Technol.* 123: 30–35.
- Zhang, T., C. Mao, N. Zhai, X. Wang, and G. Yang. 2015. Influence of initial pH on thermophilic anaerobic co-digestion of swine manure and maize stalk. *Waste Manag.* 35: 119–126.
- Zheng, L., Q. Li, J. Zhang, and Z. Yu. 2012a. Double the biodiesel yield: rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. *Renew. Energy.* 41: 75–79.
- Zheng, L., Y. Hou, W. Li, S. Yang, Q. Li, and Z. Yu. 2012b. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy.* 47: 225–229.
- Zheng, H., J. E. Powell, M. I. Steele, C. Dietrich, and N. A. Moran. 2017. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc. Natl. Acad. Sci. USA.* 114: 4775–4780.
- Zhou, F., J. K. Tomberlin, L. Zheng, Z. Yu, and J. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. *J. Med. Entomol.* 50: 1224–1230.