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Fatty Acid Bioconversion and Scaling-Up Effects of Swine Manure Treatment with Black Soldier Fly Larvae

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Abstract: Black soldier fly larvae (BSFL) treatment offers a promising avenue for manure valorization. However, there is a lack of larval density studies and ton-scale exploration in swine manure bioconversion. This study delves into the efficiency of larval fatty acid (FA) bioconversion, examining the impact of larval density on a kilogram scale and extending the analysis to a ton scale. Across a range of 50 to 600 larvae/kg, the larval FA content decreased from 15.3% to 7.85%. The peak larval FA yield, at 3.04% (based on manure dry matter), occurred at a density of 200 larvae/kg. Both low (50 larvae/kg) and high (600 larvae/kg) densities adversely affected BSFL bioconversion performance. Dominant larval FAs included C12:0 (39.7%), C16:1 (24.2%), C18:1 (17.5%), and C16:0 (8.3%). The scaling-up process maintained a consistent larval FA content and composition but resulted in decreased larvae FA yield due to increased larval mortality. Ultimately, each ton of swine manure yielded 12.4 kg of fresh larvae or 0.71 kg of larval FA components, corresponding to a 1.14% larval FA yield. This study underscores the feasibility of upscaling swine manure treatment using BSFL for FA bioconversion and emphasizes the necessity for large-scale studies to enhance larval survivorship and bioconversion efficiency.

Keywords: black soldier fly larvae; *Hermetia illucens*; swine manure; larval density; fatty acids; ton scale

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1. Introduction

Livestock farming plays a pivotal role in global food security and agricultural development. However, the growth of animal production results in greater volumes of animal manure, which poses an increasing environmental threat. Lessening these issues is a key concern for sustainable agriculture development [1,2]. Black solider fly, *Hermetia illucens*, is a tropical scavenging insect that has been introduced into manure treatment over the past decade [3]. Black soldier fly larvae (BSFL) treatment emerges as a promising approach for valorizing manure wastes, transforming them into larval biomass and frass residue [3–6]. These byproducts hold considerable potential for high-value applications, such as animal feeds and organic fertilizers for plants [7–10].

The larval biomass obtained through BSFL treatment is rich in crude protein, fat, fiber, and chitin [11,12]. The protein content serves as a valuable nutrient source for animal feed and antimicrobial compounds, while the fat component finds applications in feed oils, cosmetics, and biofuels [11–13]. The larval fat comprises fatty acids (FAs), with lauric acid (C12:0) dominating and other saturated (C14:0, C16:0, C18:0) and unsaturated (C16:1, C18:1, C18:2, C18:3) FAs [14,15]. Studies have demonstrated the positive effects of these oils in the nutrition of fish [16–19], chickens [20], and piglets [21]. The manure treatment process allows BSFL to be fed with manure, transforming macronutrients such as carbohydrates,

organic acids, proteins, and microbial flora into larval body FAs, which can be further used in agriculture or the energy industry [13].

Despite the increasing interest in manure treatment using BSFL [4,5], the efficiency of BSFL in the bioconversion of swine manure lags behind that of poultry manure [22,23]. Larvae yields from poultry manure treatments have been reported at 18–30% [24] and 10.2–11.5% [25], whereas swine manure treatments achieved lower yields of 1.9% [26] and 2.0% [22], respectively. The data above were calculated by dividing the dry matter of the larvae by the dry matter of the manure. The larvae derived from swine manure exhibit smaller body weights, lower yields, and longer development times compared to those from poultry manure [22,23]. This disparity is partly attributed to the lower energy and nutrient levels in swine manure [27,28] and also due to the limited research of the larval density effect on swine manure treatment. The high annual production of 4 billion tons of swine manure in China [4] necessitates improving the bioconversion efficiency of swine manure treatment and facilitating the application of BSFL technology. One critical yet understudied factor affecting waste bioconversion performance is larval density, particularly in the context of swine manure management. Previous research on poultry manure [29] and dairy manure [30] has emphasized the significant impact of larval density on waste reduction performance, necessitating a dedicated exploration of its effects in swine manure treatment.

A central challenge hindering the industrial adoption of BSFL treatment is the inconsistent performance observed during the scale-up process. While numerous studies are based on gram- or kilogram-scale experiments [26–32], ton-scale treatments, as conducted by Xiao et al. [25] on poultry manure, remain limited. Small-scale experiments may not accurately reflect performance on a larger industrial scale, as noted by Miranda et al. [22,33]. Therefore, scaling up swine manure treatment from a kilogram to a ton scale is crucial to understanding BSFL bioconversion performance on an industrial level.

This study aims to investigate the effects of larval density on BSFL performance in kilogram-scale treatment, with a specific focus on larval FA contents, profiles, and bioconversion rates. Furthermore, this research scales up the treatment from a kilogram to a ton scale, providing insights for the large-scale bioconversion of larval FA biomass based on swine manure management.

2. Materials and Methods

2.1. Larvae and Manure Source

Black soldier fly eggs were procured from a BSF farm located in Bijie, Guizhou, China. Upon arrival, the eggs were allowed to hatch in wheat bran with 70% moisture at 27 °C for two weeks, resulting in the development of small larvae with a body weight ranging from 0.01 to 0.02 g. Swine manure was sourced from a local swine farm situated in Hejiadian, Pingdu (Qingdao, Shandong, China), which houses 2000 growing pigs every five months. The pigpen underwent daily cleaning, with the collected swine manure stored in the septic tank within the facility. Swine manure used in the kg- and ton-scale treatment was obtained from the on-site septic tank.

2.2. Experimental Design

The kg-scale treatment was carried out at the Hejiadian pig farm in Pingdu, Qingdao, Shandong, China, during July–August 2022, with ambient temperatures ranging from 23 to 32 °C. The group design for the kg-scale treatment is illustrated in Figure 1a. Group A served as the control group with no larvae, while groups B, C, D, E, and F corresponded to larval densities of 50, 100, 200, 400, and 600 larvae per kilogram of manure, respectively. The upper limit of 600 larvae/kg was chosen based on the referenced upper limit of 1330 larvae/kg in a poultry manure study [29] and 370 larvae/kg in a dairy manure study [30]. Each group was accommodated in a blue plastic box (0.6 m × 0.4 m × 0.2 m) and conducted in duplicate. In each box, 10 kg of swine manure was weighed using a scale (Figure 1(b2)), and the corresponding number of larvae (0.0166 g per larva) were weighted and added. The boxes were covered with mosquito nets (Figure 1(b1)). The swine manure

in the box was considered as frass from Day 1 onwards. The frass was manually stirred on a daily basis, and duplicate frass samples (10 g) and larvae (10 individuals) were collected every three days. On the 27th day, most of the BSFL in groups B and C reached pupae stage. The treatment was stopped, and the frass and larvae were manually separated using steel sieves (6- and 8-mesh sieves, Figure 1(b3)), with larvae and frass (Figure 1(b4,5)) weighed separately and subsamples stored at −20 °C.

(a) Study design

Group	Larval density
kg	
A	0 larvae/kg
B	50 larvae/kg
C	100 larvae/kg
D	200 larvae/kg
E	400 larvae/kg
F	600 larvae/kg
ton	
FEC	100,000 larvae/ton

(b) kg-scale treatment

(c) ton-scale treatment

Figure 1. Experimental design and on-site perspectives of kg-scale and ton-scale treatments: (**a**) Experimental scales, groups, and larval densities. (**b**) Visual representation of on-site activities during the kg-scale treatment. (**1**), rearing boxes; (**2**), weighing swine manure; (**3**), manually sieving larvae; (**4**), final frass; (**5**), final larvae. (**c**) On-site depiction of activities during the ton-scale treatment. (**1**), rearing tanks equipped with mixing devices; (**2**), screening larvae using a vibrating sieve machine; (**3**), coarse chunk frass; (**4**), fine particle frass; (**5**), harvested larvae.

The ton-scale treatment was executed at the same location in September 2023, with an ambient temperature ranging from 19 to 32 °C (Figure 1c). The ton-scale treatment employed a larval density similar to that of group C (100 larvae/kg manure), being approximately 100,000 larvae/ton manure. The larvae rearing facilities consisted of duplicate tarpaulin tanks measuring 20 m in length, 2 m in width, and 0.5 m in height (Figure 1(c1)). These rearing tanks were positioned inside a plastic greenhouse equipped with a black shade net and a spray cooling system to prevent excessive exposure to sunlight and maintain an ambient temperature below 32 °C (Figure 1(c1)). Electric mixing devices were incorporated into the rearing tanks, operating at a speed of 0.2 m/s with a frequency of 30 min daily (Figure 1(c1)). Swine manure was pumped into each rearing tank using a slurry shear pump.

The pumping method used to transfer swine manure into the tarpaulin tanks resulted in more water being transferred into the initial manure in the ton-scale trial compared to the kg-scale trial. The initial moisture content increased from approximately 85% (kg-scale) to 90% (ton-scale), which led to the drowning of the BSFL. Therefore, 15 kg of wood chips and 15 kg of previously produced frass were added to each tank to reduce the manure's moisture to below 85%. The initial wet matter of swine manure after moisture adjustment was estimated based on the parameters of manure volume and kg/m^3 density. The initial heights of swine manure in the tank averaged at 7.3 cm, with manure densities of 1.05 kg/m^3. The initial wet matter of swine manure was estimated to be 3.07 tons.

A total of 300,000 black soldier fly larvae (0.0178 g per larva) were weighed and added to each tank, and the treatment was conducted for 30 days. Quadruplicate larvae samples were collected every three days for larval FA detection. The treatment was stopped on day 30, later than the 27 days of the kg-scale study, because the BSFL developed relatively

slowly in the ton-scale FEC group compared to the kg-scale C group. Larvae and frass were separated using a vibrating sieve machine (Figure 1(c2), 1.5 m in length, 0.5 m in width, Huzhou Xiaoxiang Abrasive Materials, Huzhou, Zhejiang, China). The sieve machine featured two layers of screens and three outlets. The upper layer used a steel bar screen with a spacing of 4 mm, while the lower layer used a 6-mesh mesh screen. Coarse chunk frass, larvae, and fine particle frass were obtained from the upper, middle, and lower outlets, respectively (Figure 1(c3–c5)). The larvae collected from the middle outlets were further manually processed to remove any frass impurities from the larvae collection. The larvae and frass were weighed separately post-screening, and subsamples were stored at −20 °C for subsequent analysis.

2.3. Chemical Property Analysis

BSF larvae and swine manure samples were detected for FA content and composition, following the methodologies outlined in Lu et al. [34] and Liu et al. [35]. Briefly, quadruplicate samples were oven-dried at 60 °C for 24 h and underwent repeated extraction with petroleum ether three times to obtain crude lipids. These lipids were subjected to a reaction with methanol, catalyzed by hydrochloric acid, and transformed to fatty acid methyl esters (FAMEs). The content of each FAME was then detected using gas chromatography and calculated accordingly with external standards. Gas chromatography was conducted using a Shimazu GC-2014C system (Shimazu Instruments Ltd., Suzhou, Zhejiang, China), equipped with a flame ionization detector (FID) and a FFAP capillary column (50 m × 0.25 mm × 0.33 μm, Zhongke Kaidi Ltd., Lanzhou, Gansu, China). The GC conditions were set as follows: an inlet temperature of 250 °C, detector temperature of 250 °C, injection volume of 1 μL, split ratio of 1:30, ultrapure nitrogen as the carrier gas, and a carrier gas flow rate of 1 mL/min. The GC heating program was initiated at 70 °C, increased from 70 °C to 240 °C at a rate of 1 °C/min, and maintained at 240 °C for 15 min. The GC profile was analyzed using GC Solution software Version 2.3 (Shimadzu, Suzhou, China).

BSF larvae, swine manure, and frass samples were also analyzed for moisture content, wet matter (WM), dry matter (DM), organic matter (OM), total carbon content (TC), and total nitrogen content (TN). Samples underwent drying in an oven at 60 °C for 24 h. The mass ratio reduced represented the moisture content, while the mass ratio remaining represented the DM ratio. Dried samples were ground using a ball grinding machine (SCIENTZ-48, Ningbo Xinzhi Bio-technology Co., Ltd., Ningbo, China) with the fine powder analyzed for TC and TN content using a Vario EL cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The OM content was estimated as twice the ratio of TC [36].

2.4. Evaluation of Bioconversion Efficiency

The reduction rates of wet, dry, and organic matter in manure were evaluated based on the following equations:

$$\text{Wet Matter Reduction Rate} = (1 - \frac{WM_F}{WM_M}) \times 100\% \quad (1)$$

$$\text{Dry Matter Reduction Rate} = (1 - \frac{DM_F}{DM_M}) \times 100\% \quad (2)$$

$$\text{Organic Matter Reduction Rate} = (1 - \frac{OM_F}{OM_M}) \times 100\% \quad (3)$$

where WM_M and WM_F are the WM of swine manure and final frass, respectively; DM_M and DM_F are the DM of swine manure and frass, respectively; and OM_M and OM_F are the OM of swine manure and frass, respectively; unit in kg or ton.

The larval FA composition and yields were calculated as follows:

$$\text{Individual FA ratio} = \frac{FA_i}{\sum_i FA_i} \times 100\% \quad (4)$$

$$\text{Larval FA ratio, body DM basis} = \frac{\sum_i FA_i}{DM_{body}} \times 100\% \quad (5)$$

$$\text{Larvae FA yield, manure DM basis} = \frac{DM_L \times FA_L}{DM_M} \times 100\% \quad (6)$$

$$\text{Larvae FA yield, manure WM basis} = \frac{DM_L \times FA_L}{WM_M} \times 100\% \quad (7)$$

where FA_i is the mass of individual FA species, $\sum_i FA_i$ is the sum mass of individual FA species, and DM_{body} is the DM of the BSF larvae used for FA extraction, unit in mg; FA_L is the larval FA ratio, unit in %; DM_L is the total DM of the larvae, DM_M is the total DM of the swine manure, and WM_M is the total WM of the swine manure, unit in kg or ton.

The bioconversion and survival rates of BSFL were evaluated as follows:

$$\begin{aligned} \text{Larvae yield, manure DM basis} &= \frac{DM_L}{DM_M} \times 100\% \\ \text{Larvae yield, manure WM basis} &= \frac{WM_L}{WM_M} \times 100\% \end{aligned} \quad (8)$$

$$\text{Survival rate} = \frac{WM_L \div WM_{body}}{Number_{initial}} \times 100\% \quad (9)$$

where DM_L is the total DM of larvae, DM_M is the total DM of swine manure, WM_L is the total WM of larvae, and the WM_M is the total WM of swine manure, all units in kg or ton; WM_{body} is the body weight of individual larva, and $Number_{initial}$ is the initial number of larvae added in the reared boxes or tanks.

2.5. Statistical Analyses

Figure plotting was performed using OriginPro 2024 version 10.1.0.40 (OriginLab Corp., Northampton, MA, USA). The comparison among groups at the kg scale employed the one-way analysis of variance (ANOVA) method, while the analysis between groups at the kg and ton scales utilized the Student's *t*-test. Quadruplicate data with duplicate conditions and sampling were used for the analysis. Statistical analysis was conducted using IBM SPSS Statistics (version 26.0.0), with a significance level set at $p < 0.05$.

3. Results

3.1. Manure Reduction Performance in Kg-Scale Treatment

The efficiency of manure reduction was evaluated based on the reduction rates of wet matter (WM), dry matter (DM), and organic matter (OM). The manure WM decreased from 10 kg to 1.33–1.75 kg across groups A to F, indicating a reduction rate ranging from 82.5% to 86.5% (Figure 2c). The reduction rates of DM and OM showed an increasing trend from group A to F, reaching peaks of 33.2% for DM and 41.8% for OM in group F (Figure 2f,i). Although the final DM of group F was not the lowest (Figure 2e), the DM reduction rate of group F was the highest. This is because the initial DM of groups A to F varied (1.11–1.55 kg) due to differences in moisture content (84.5–88.8%). Group F achieved a DM decrease from 1.55 kg to 1.03 kg (a 33.2% DM reduction), which was higher than the reset groups' reductions of 1.12–1.50 kg to 0.93–1.31 kg (12.2% to 20.1% DM reduction, Figure 2f). The higher larval density led to a higher DM reduction rate, which aligns with previous findings in poultry and dairy manure studies [29,30]. Notably, control group A, without larvae, demonstrated a higher WM reduction rate but lower DM and OM reduction rates compared to larval groups C-F, implying a slightly quicker drying process in the absence of larvae.

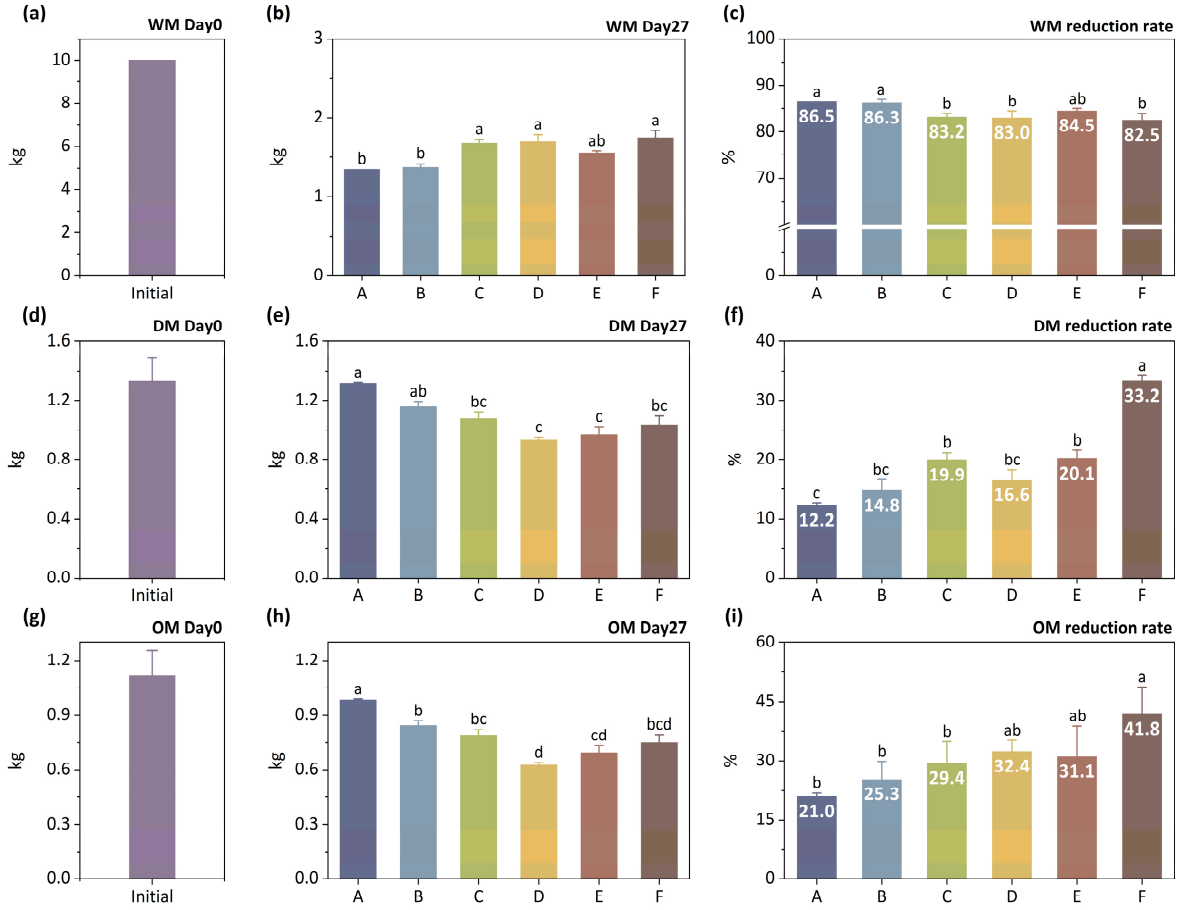

Figure 2. Manure reduction performance in the kg-scale treatment: (**a**,**b**,**d**,**e**,**g**,**h**), weights of manure wet matter (WM), dry matter (DM), and organic matter (OM), unit kg; (**c**,**f**,**i**), reduction rates, unit %, based on Equations (1)–(3). Data are presented as means ± standard deviation (n = 4, with duplicate conditions and sampling). Different letters within each graph signify significant differences among the groups.

3.2. Fatty Acid Profiling by Gas Chromatography Analysis

The FA components in both swine manure and BSFL were analyzed using gas chromatography and are presented in Figure 3. The FA content of swine manure was determined to be 4.32 ± 0.23% (on a dry matter basis). The BSFL in group C, harvested on day 27, were used as an example to illustrate the larval FA profiles in this section. The chronological changes in the FA profiles of groups C and FEC are further addressed in Section 3.5. Predominant FAs included palmitic acid (C16:0) at 28.4%, stearic acid (C18:0) at 32.6%, and oleic acid (C18:1) at 20.3%, alongside minor FA species such as lauric acid (C12:0, 6.3%), myristic acid (C14:0, 6.5%), and palmitoleic acid (C16:1, 5.9%) (Figure 3a,b). The FA content of larvae (group C) was determined to be 14.76 ± 0.95% (on a dry matter basis). The predominant FA species comprised lauric acid (C12:0) at 39.7%, palmitoleic acid (C16:1) at 24.2%, oleic acid (C18:1) at 17.5%, and palmitic acid (C16:0) at 8.3%. Minor FA species were also identified, including decanoic acid (C10:0, 1.8%), myristic acid (C14:0, 4.7%), stearic acid (C18:0, 2.3%), and linolenic acid (C18:3, 1.5%) (Figure 3c,d).

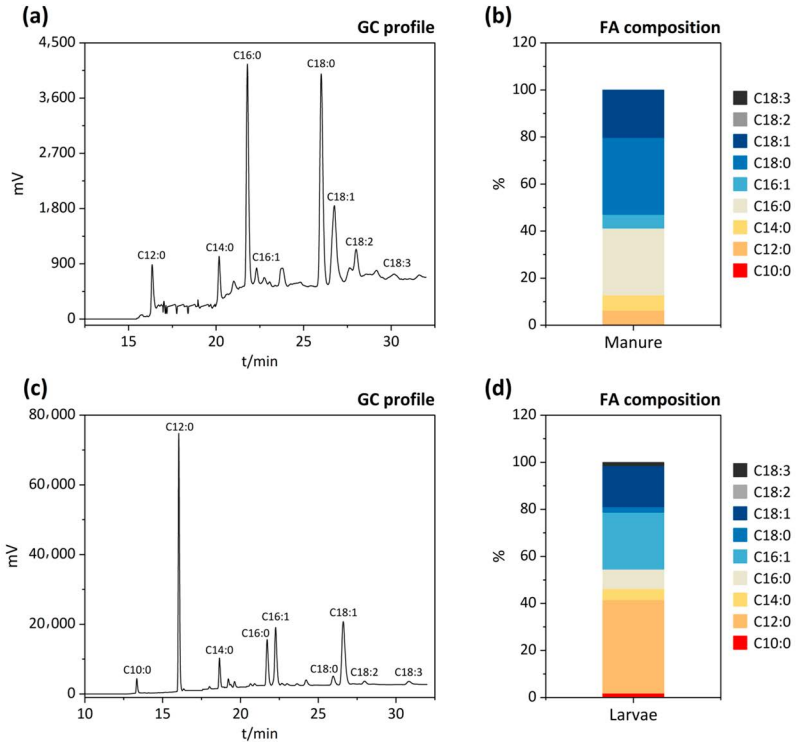

Figure 3. Fatty acid profiles of swine manure and black soldier fly larvae: (**a**,**c**) gas chromatography profile of swine manure and larvae; (**b**,**d**) fatty acid composition of swine manure and larvae; legend is FA species. Larvae presented in graphs (**c**,**d**) are samples derived from group C.

3.3. Larval Bioconversion Performance in Kg-Scale Treatment

The influence of larval density exhibited no discernible impact on larval FA composition (Figure 4a), with predominant FA species being lauric acid (C12:0, 26.7–43.7%), palmitoleic acid (C16:1, 21.6–28.3%), and oleic acid (C18:1, 18.6–27.0%) across groups B-F. However, the larval density factor significantly affected both larval FA content and yield (Figure 4b,c,i). The larval FA content displayed a decreasing trend from 15.3% to 7.85%, corresponding to an increase in larval density from 50 larvae/kg (group B) to 600 larvae/kg (group F) (Figure 4b, $p < 0.001$). Nonetheless, larvae FA yield reached its peak at 3.04% (manure DM basis) with a larval density of 200 larvae/kg (group D, Figure 4c,i, $p < 0.001$). Both low density (50 larvae/kg, group B) and high density (600 larvae/kg, group F) adversely affected FA bioconversion. The larval body weight exhibited a decrease from 0.196 g to 0.080 g with an increasing larval density from 50 larvae/kg (group B) to 600 larvae/kg (group F) (Figure 4d, $p < 0.001$). The larval total carbon (TC) ratio followed a declining trend from 49.3% to 43.2%, mirroring the patterns observed in larval body weight and FA ratio (Figure 4e, $p < 0.001$). In contrast, the larval total nitrogen (TN) ratio remained relatively stable, ranging from 6.30% to 6.74% (Figure 4f, $p = 0.022$), implying a consistent protein content within the larval body. Larvae survival rates were high in groups B, C, and D (97.2–98.8%) and lower in groups E and F (84.9–89.6%; Figure 4g, $p = 0.013$). The larvae yield (manure DM basis) peaked at 11.0% with a larval density of 400 larvae/kg (group E) (Figure 4h, $p < 0.001$). As both larval density and FA content contributed to the larval FA yield, the optimum density for larval FA conversion (200 larvae/kg, group D) differs from the optimal density for larvae yield (400 larvae/kg, group E) (Figure 4h,i).

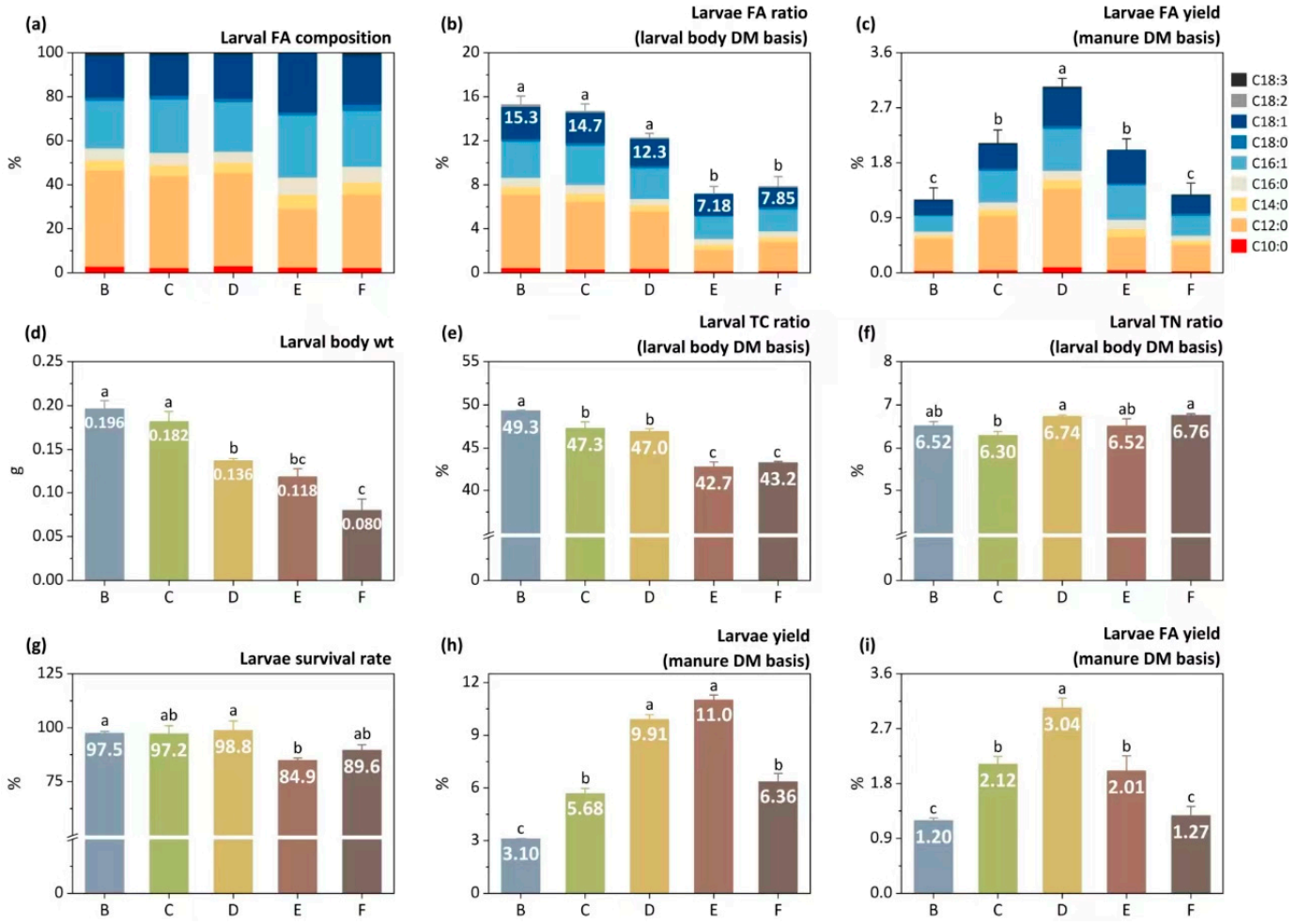

Figure 4. Bioconversion performance of black soldier fly larvae in the kg-scale treatment: FA, fatty acid; DM, dry matter; TC, total carbon; TN, total nitrogen; (**a**) larval FA composition; (**b**) larval FA ratio based on larval DM; (**c**) larvae FA yield based on manure DM; graphs a and b share the same legend as graph c, and the legend represents FA species; (**d**) larval body weight (wt); (**e**) larval TC ratio; (**f**) larval TN ratio; (**g**) larvae survival rate; (**h**) larvae yield based on manure DM; (**i**) larvae FA yield based on manure DM. Data are presented as means ± standard deviation (n = 4, with duplicate conditions and sampling). Different letters within each graph signify significant differences among the groups.

3.4. Comparison of Kg- and Ton-Scale Treatment

Following the separation of frass and larvae in the ton-scale treatment (Figure 1c), 576.3 kg and 622.7 kg of frass, along with 35.8 kg and 37.9 kg of larvae, were obtained from the duplicate 3-ton manure treatment. Despite the larvae rearing facilities, the ton-scale (FEC) and kg-scale (C) treatments also differed in larvae sieving methods (Figure 1). Small larvae, with a body weight of approximately less than 0.1 g, may not have been harvested in the ton-scale treatment but were collected in the kg-scale treatment.

In terms of waste reduction rates (Figure 5a–c), the ton-scale FEC group exhibited a lower wet matter (WM) reduction rate (79.8%) but similar dry matter (DM) (21.3%) and organic matter (OM) (33.9%) reduction rates compared to the kg-scale C group. This suggests that the ton-scale treatment achieved a reduction performance for swine manure that is comparable to the kg-scale treatment. Regarding larvae parameters, the ton-scale FEC group demonstrated similar body weight, total carbon (TC) ratio, total nitrogen (TN) ratio, and fatty acid (FA) ratio compared to the kg-scale C group (Figure 5e–h). However, the ton-scale FEC group had a lower larval survival rate (64.4%), along with

lower larvae yield (3.13%, manure DM basis) and larvae FA yield (1.14%, manure DM basis) (Figure 5d,i–l). Since the larval FA yield equals the larvae yield multiplied by the larval FA content (Equation (6)), the similar larval FA content between the C and FEC groups leads to the reduced larvae FA yield in the FEC group, derived from the declined larvae yield. Furthermore, the larvae yield reflects both larval survival rate and body weight (Equation (9)). The similar larval body weight (Figure 5e) suggests higher larval mortality in the FEC group. Therefore, the lower larvae FA yield in the FEC group compared to the C group is highly likely derived from the declined larvae survivorship.

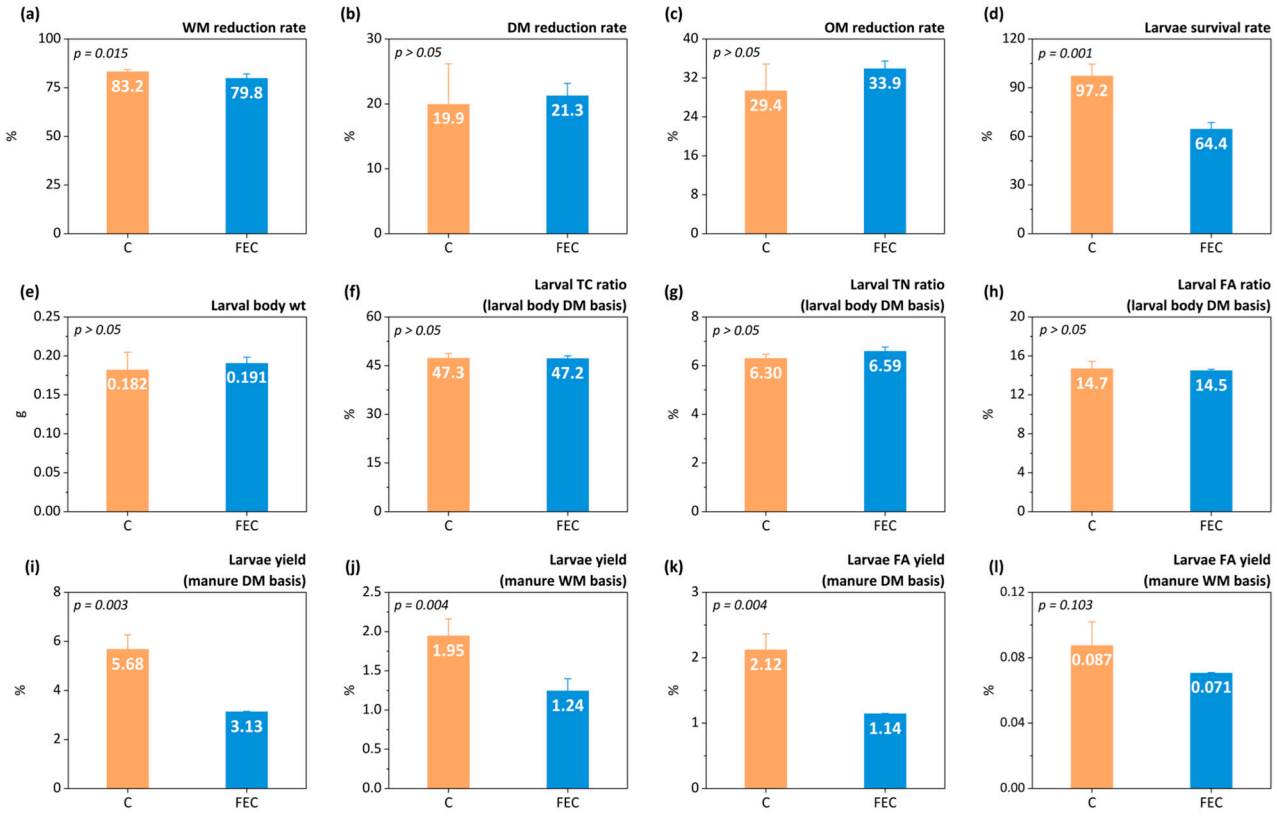

Figure 5. Comparison of kg- (C group) and ton-scale (FEC group) treatments: (**a**) wet matter (WM) reduction rate; (**b**) dry matter (DM) reduction rate; (**c**) organic matter (OM) reduction rate; (**d**) larvae survival rate; (**e**) larval body weight (wt); (**f**) larval total carbon (TC) ratio; (**g**) larval total nitrogen (TN) ratio; (**h**) larval fatty acid (FA) ratio; (**i**) larvae yield based on manure DM; (**j**) larvae yield based on manure WM; (**k**) larvae fatty acid yield based on manure DM; (**l**) larvae fatty acid yield based on manure WM. Data are presented as means ± standard deviation (n = 4, with duplicate conditions and sampling).

3.5. Chronological Changes in Fatty Acid Composition

Regardless of treatment time and scale, larval fatty acid (FA) components derived from swine manure treatment consistently exhibited dominance in lauric acid (C12:0), palmitoleic acid (C16:1), and oleic acid (C18:1) (Figure 6). In the kg-scale group C, the ratio of C12:0 ranged from 28.9% to 45.8%, C16:1 from 18.6% to 24.4%, and C18:1 from 17.9% to 26.6% (Figure 6a). Meanwhile, the ton-scale group FEC depicted an increased C12:0 ratio from 11.2% to 29.9% and a decreased C16:1 and C18:1 ratio from 35.0% to 26.9% and 31.1% to 22.0%, respectively, along with larval development (Figure 6d). A notable scale difference was observed, with larvae in group C exhibiting faster growth, reaching the peak body FA ratio on day 18 and maintaining body FA contents between 14.5% and 16.2% from day 18 to 27 (Figure 6b). In contrast, larvae in group FEC reached the peak body FA ratio on day 21 and maintained body FA contents between 14.3% and 16.4% from day 21 to 30 (Figure 6e). Before reaching the peak FA content, both groups C and FEC demonstrated

a gradual increase in the C12:0 ratio and a relative decrease in the C16:1 and C18:1 ratios (Figure 6a,d). The faster growth rate of group C compared to FEC could be attributed not only to scale differences but also to temperature variations, as the experiments were not conducted during the same time of the year due to the scale of the experiment. The daily mean temperature of group C was approximately 23–28 °C (July–August 2022), higher than that of group FEC, 20–26 °C (September 2023), which may have benefited the larval growth in group C. The final FA yields of larvae were 2.12% for group C and 1.14% for group FEC based on manure dry matter (Figure 6c,f). Since the body FA ratio of the harvested larvae was similar between the ton-scale FEC group and kg-scale C group (Figure 6b,e), the significant difference in larvae FA yield was likely derived from larvae survivorship.

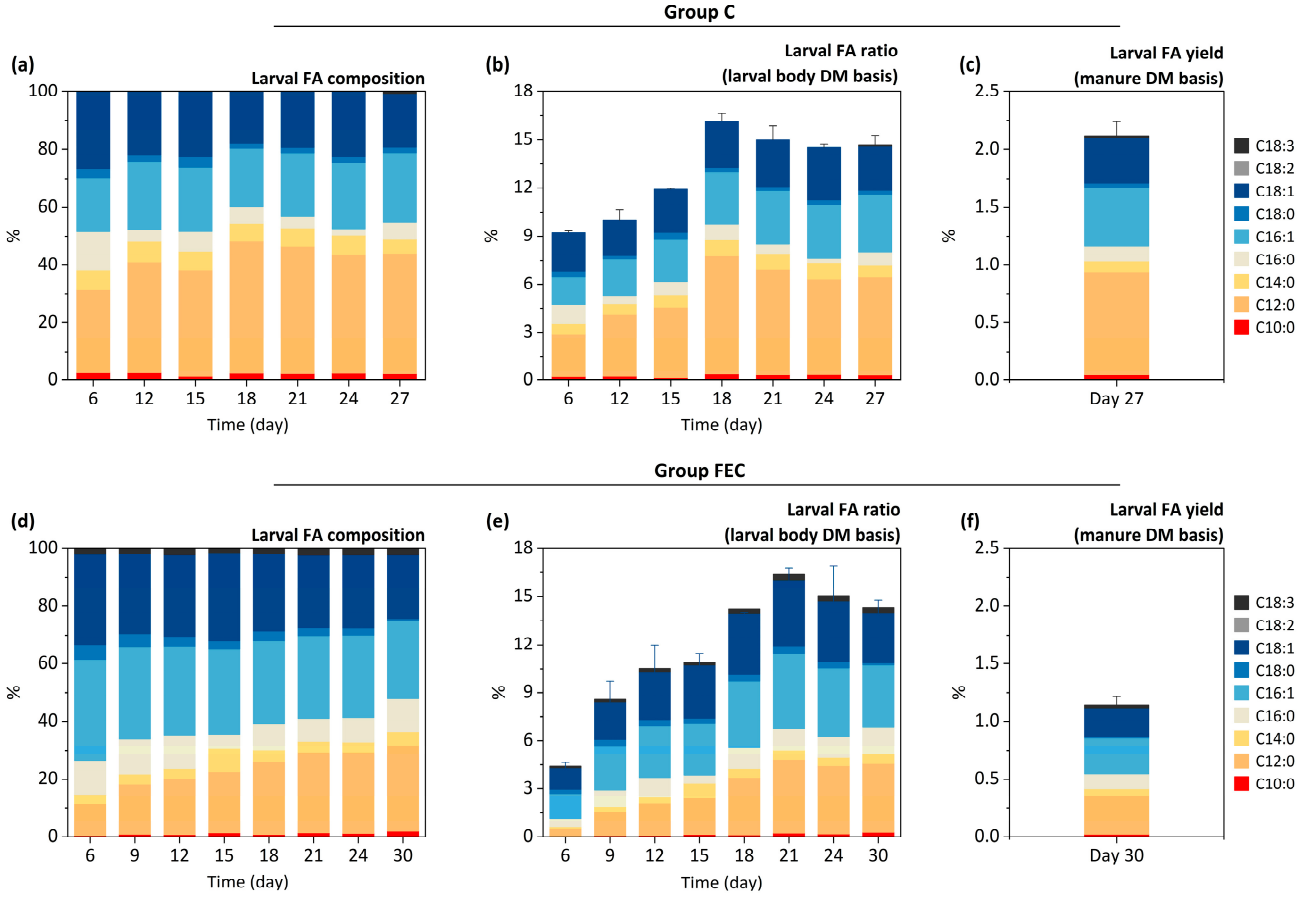

Figure 6. Chronological characterization of larval fatty acids derived from kg-scale (group C) and ton-scale (group FEC) treatments of swine manure: (**a**,**d**) larval fatty acid composition; (**b**,**e**) larval fatty acid ratio based on body dry matter (DM); (**c**,**f**) larvae fatty acid conversion yield based on manure DM. Legends represent fatty acid species. Data are presented as means ± standard deviation (n = 4, with duplicate conditions and sampling).

4. Discussion

4.1. Swine Manure Treatment and Larval Density Effect

Manure from swine, poultry, and dairy exhibits variations in chemical and physical compositions. Swine manure, characterized by lower nutrient content and higher fiber content compared to poultry manure, typically leads to lower bioconversion yields in BSFL [22,27,28]. For example, Dzepe et al. [29] and Xiao et al. [25] reported larvae yields of 18–30% and 10.2–11.5%, respectively, based on poultry manure treatment. Rehman et al. [37,38] documented a larvae yield range of 4.2–6.3% using dairy manure treatment, while Liu et al. [26] and Miranda et al. [22] found 1.9% and 2.0% larvae yields in swine manure treatment. Swine manure treatment typically results in lower larval weight, reduced

larvae yield, and prolonged development time compared to poultry and dairy manure treatment [22,23]. This discrepancy is primarily attributed to the lower nutrient content in swine manure [27,28], as well as the lack of studies on the effects of larval density.

The nitrogen content of swine manure (2.55%) is lower than that of poultry (4.78%) and dairy manure (2.74%), suggesting a lower protein-like component in swine manure [27]. Swine manure comprises nursery, growing, and finishing swine manure, with growing and finishing swine manure representing the majority but containing lower nutrients due to higher cellulose and hemicellulose content than nursery manure [28]. Despite these challenges, the present study, using growing swine manure, achieved larval yields of 3.1–11.1%, surpassing previous reports of 1.9–2.0% [22,26]. This improvement can be attributed to the optimization of the larval density factor, irrespective of potential composition differences in swine manure.

Larval density emerges as a pivotal factor influencing BSFL performance in manure management [29,30,39]. Low densities may result in poor bioconversion yield, as observed in group B with a density of 50 larvae/kg, while high larval densities may intensify competition, leading to reduced survivorship and development, as demonstrated in group F with a density of 600 larvae/kg. Group F achieved the highest manure dry matter (DM) and organic matter (OM) reduction performance (Figure 2c,f,i), likely due to increased oral secretions and gut microbiota aiding in manure digestion [33,40]. However, the lower larval growth and bioconversion yield in group F compared to groups B-E suggest that larvae in group F were less developed due to a lower individual nutrient level. Further, a larval density of 600 larvae/kg was found to be inefficient for larvae collection purposes in the current manure treatment. The optimum larvae yield of 11.0% is observed in group E, at 400 larvae/kg (Figure 4h), while the optimal larvae fatty acid (FA) yield of 3.04% is noted in group D, at 200 larvae/kg (Figure 4i). The non-overlapping optimal densities for larvae yield and larvae FA yield can be explained by the following reason: the increased larval density results in a decreased larval FA ratio. Group D obtained a significantly higher larval FA ratio (12.3%) than that of group E (7.18%, Figure 4b); however, the larve yield between group D (9.91%) and E (11.0%) did not differ (Figure 4h), leading to the higher larval FA yields in group D (3.04%) than in E (2.01%). In summary, optimizing the larval density factor contributes to improvements in larval FA yield, with 200 larvae/kg being the optimum for efficient larval FA bioconversion in swine manure treatment.

The larval density factor has been previously examined in poultry and dairy manure treatment. Dzepe et al. [29] investigated a feeding rate of 50–200 mg/larva/day in chicken manure management and found that 200 mg/larva/day, corresponding to approximately 1330 larvae/kg of manure, resulted in a high larval yield. Myers et al. [30] explored dairy manure management with 27–70 g of manure/300 larvae/day and discovered that 70 g of manure/300 larvae/day, corresponding to approximately 370 larvae/kg of manure, led to the maximum manure reduction performance. The current optimal larval density of 200 larvae/kg for swine manure is lower than that for poultry [29] and dairy manure [30], which is consistent with the nutrient differences in the manure [27].

4.2. Composition of Larvae Fatty Acids

This study identified a negative correlation between larval density and larval FA content (Figure 4b), aligning with prior research. Opare et al. [41] observed a similar trend, noting that an increase in larval density from 2150 to 21,500 larvae/kg (dog food) resulted in a decrease in larval fat ratio from 31.5% to 23.0%. Additionally, this study revealed that larval density did not interact with larval FA composition (Figure 4a). The dominant FA profiles were consistently characterized by lauric acid (C12:0) at 39.7%, palmitoleic acid (C16:1) at 24.2%, oleic acid (C18:1) at 17.5%, and palmitic acid (C16:0) at 8.3% (Figure 3c,d). While the growth of larvae led to increased ratios of C12:0 and decreased ratios of C16:1, C18:1, and C16:0 (Figure 6), the dominant FA species remained unchanged. These findings emphasize the positive impact of diet energy on larval FA content, while diet composition influences larval FA composition [41,42].

Various studies on lipid metabolism in BSFL have indicated their preference for converting dietary carbohydrates to C12:0 FA. They can also biosynthesize C16:0, C16:1, C18:0, and C18:1 FAs but cannot synthesize C18:2 and C18:3 FAs. Polyunsaturated FAs, such as C18:2 and C18:3, can only be accumulated from the diet [15,35,43,44]. Consequently, BSFL derived from food waste exhibit high ratios of C18:1 and C18:2 since these FAs are prevalent in the food waste [34,35]. Larvae reared on substrates like flaxseed waste or sea mussels may have a relatively high ratio of C18:3 FA, acquired from the flaxseed or mussel substrate [45,46]. In the current study, the swine manure used contained a minimal proportion of polyunsaturated FAs, such as C18:2 and C18:3 (Figure 2a,b). This resulted in BSFL dominated by saturated and monounsaturated FAs, namely C12:0, C16:0, C16:1, and C18:1 FAs, aligning with previous findings from animal manure treatment [47,48]. Given the positive nutritional effects of BSFL oil observed in feeding studies on fish [16–19], chicken [20], and piglets [21], the larval FA content derived from swine manure treatment could be a promising candidate for feed oil applications.

4.3. Scaling-Up Effect

The present study opted for group C, with a larval density of 100 larvae/kg, instead of group D with 200 larvae/kg for the scaling-up experiment, primarily for the following reasons: (1) to advance the application of BSFL technology in manure treatment, utilizing a lower larval density could reduce the financial cost of purchasing BSF eggs; (2) the larval FA yield of group C (2.12%) was lower than that of group D (3.04%) but comparable to group E (2.01%) and higher than groups B (1.20%) and F (1.27%), indicating promising potential for FA bioconversion performance of group C (Figure 2i); and (3) the larval body weight of group C (0.182 g) surpassed that of group D (0.136 g), which is advantageous for the mechanical sieving procedure.

While most previous research on BSFL for manure treatment has been conducted on a g-scale or a kg-scale [26–33,37], the application of BSFL treatment in industrial settings necessitates evaluation on a larger production scale, such as the ton-scale. Scaling up BSFL treatment is challenging not only because larger larvae rearing equipment is required but also because small-scale trials may not accurately represent what occurs on a larger scale. For instance, results from g-scale trials may not directly translate to kg-scale experiments due to variations in feeding regimes and larval densities, as noted by Miranda et al. [22,33]. However, in poultry manure treatment, studies by Mazza et al. [49] and Xiao et al. [25] achieved comparable larvae yields using kg- and ton-scales, respectively. These findings emphasize the importance of maintaining consistent larval density regimes in the scale-up process to reproduce and compare larvae performance.

The current ton-scale trial has undergone two unsuccessful attempts and one successful attempt prior to the formation of the FEC group (fourth enlarged C). Infrastructure requirements for ton-scale treatment included a disposal site with protection from rain and strong sun exposure, a temperature and humidity control system, horizontal ground, semi-automatic stirring systems, and vibrating sieving equipment. In comparing group FEC and group C, there were no differences in manure reduction rates (WM, DM, OM), larval weight, larval TC, TN, and FA ratio (Figure 5). However, the larval survival rate in group FEC was significantly lower than in group C, leading to similar trends in larvae yield and larval FA yield (Figure 5). These results suggest that the larvae harvested in group FEC are survivors of intense environmental and microbial competition, and the lower larval FA yield in group FEC is primarily due to the lower survival rate compared to group C.

The higher mortality rate in the ton-scale FEC treatment compared to the kg-scale C group could be attributed to various factors: (1) elevated environmental stress in FEC due to direct exposure to varying environments and uneven microenvironments [28,33,40], leading to a higher mortality rate among young larvae; (2) some larvae developing faster than the average, resulting in eclosion occurring earlier, with a few BSF adults flying at harvest time; (3) the vibrating sieving machine screened out larger larvae, while small larvae (approximately <0.1 g), due to growth competition or an uneven diet, could not be sieved

and were lost. In the kg-scale group C, steel sieves were used for manual sieving, resulting in a higher larvae–frass separation rate than in the ton-scale FEC group. In summary, each ton of swine manure in the ton-scale FEC treatment produced 12.4 kg of fresh larvae or 0.71 kg of larval FA component (Figure 5j,l). However, based on the performance of group C, each ton of swine manure should be capable of producing 19.5 kg of fresh larvae or 0.87 kg of larval FA component (Figure 5j,l). Further efforts are required to improve larval survivorship and bioconversion yield in ton-scale treatment. Potential strategies may include supplying microorganisms to aid food digestion and improve larval survivorship, optimizing the sieving device to enhance larvae–frass separation efficiency, and exploring treatments with higher larval density, such as group D with 200 larvae/kg, to increase oral secretions and gut microbiota aiding in microbial defense and manure digestion.

Altogether, this research represents the first ton-scale treatment of swine manure with BSFL technology, offering valuable insights into optimal larval densities for an efficient biomass conversion strategy and providing a ton-scale paradigm to optimize the mass-rearing conditions of BSFL-fed swine manure.

5. Conclusions

The current study elucidates the impact of larval density on the BSFL biomass conversion process and introduces a ton-scale paradigm for mass-rearing BSFL-fed swine manure. The larval density factor positively influenced manure reduction rates but had a negative impact on larval weight and FA content. Both low and high larval densities within the range of 50 to 600 larvae/kg swine manure proved disadvantageous to BSFL bioconversion performance. The larvae yield reached its zenith at 11.1% with a density of 400 larvae/kg manure, while the larval FA yield peaked at 3.04% with a density of 200 larvae/kg manure.

Although the larval density influenced the larval FA content, it did not impact the composition, which remained predominantly characterized by lauric acid C12:0 (39.7%), palmitoleic acid C16:1 (24.2%), oleic acid C18:1 (17.5%), and palmitic acid C16:0 (8.3%). The scale-up process demonstrated similar performance in manure reduction, larval weight, larval FA content, and composition. However, a reduction in both larvae yield and FA yield was observed, attributed to increased larval mortality at the ton scale. The decrease in larval survivorship could be associated with diverse environmental stresses and losses during mechanical screening. For every ton of swine manure, 12.4 kg of fresh larvae or 0.71 kg of larval FA components were produced, resulting in a 1.14% larval FA yield. This study highlights the feasibility of upscaling swine manure treatment using Black Soldier Fly larvae for larval FA bioconversion and emphasizes the necessity for future research to enhance larval survivorship and improve bioconversion efficiency in ton-scale treatment.

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