Circular
Agricultural Systems

ARTICLE

Open Access

https://doi.org/10.48130/CAS-2023-0003
Circular Agricultural Systems **2023**, 3:3

Effects of different pretreatments on the physiochemical properties of pig manure and its potential in promoting the growth and nutrient accumulation of black soldier fly larvae (BSFL)

Xiaofei Wu[1], Wenyu Zhao[1], Xuan Wang[1,2], Zhaohai Bai[1,2] and Lin Ma[1*]

[1] *Key Laboratory of Agricultural Water Resources, Hebei Key Laboratory of Soil Ecology, Center for Agricultural Resources Research, Institute of Genetic and Developmental Biology, Chinese Academy of Science, 286 Huaizhong Road, Shijiazhuang 050021, Hebei, China*
[2] *Xiong'an Institute of Innovation, Chinese Academy of Sciences, Xiongan 071700, Hebei, China*
* Corresponding author, E-mail: malin1979@sjziam.ac.cn

Abstract

An economic and feasible way to use black soldier fly larvae consists of treating livestock manure to convert it into high value-added protein feed. This study was aimed to investigate the effects of different pretreatments on the physiochemical properties of pig manure and its potential in promoting the growth and nutrient accumulation of black soldier fly larvae (BSFL). Fresh pig manure was pretreated by aerobic fermentation (AE), anaerobic fermentation (AN) and high temperature anaerobic fermentation (HAN) for 2 or 5 d, and then inoculated with BSFL. Results indicate that EC value, DOC, DON, TN, NH_4^+-N and protein content were the highest in HAN pretreated manure. The individual body weight and protein content of BSFL fed with HAN pretreated manure increased by 20% and 30%, respectively, compared with those fed with AE pretreated manure. In terms of performance, BSFL fed with HAN pretreated manure showed the highest waste reduction, bioconversion rate, waste conversion efficiency and protein conversion efficiency. The conclusion of the present study may be helpful for further research on the conversion of organic waste by BSFL.

Citation: Wu X, Zhao W, Wang X, Bai Z, Ma L. 2023. Effects of different pretreatments on the physiochemical properties of pig manure and its potential in promoting the growth and nutrient accumulation of black soldier fly larvae (BSFL). *Circular Agricultural Systems* 3:3 https://doi.org/10.48130/CAS-2023-0003

INTRODUCTION

With the transformation of China's animal husbandry model to intensive industrial production, the decoupling of crops and livestock production has led to a shortage of land near livestock farms, and it has become increasingly difficult to return manure to the field[1]. Intensive livestock production causes a large amount of livestock manure being discharged directly into the environment[2], resulting in the loss of nutrients and environmental pollution. On the other hand, the transformation of the livestock industry has also increased China's dependence on forage imports. In 2017, China imported 170 Tg of crop products and 1.6 Tg of fishmeal, equivalent to 38 Tg of protein, 86% of which was used as intensive animal feed[3].

Due to resource shortages worldwide, circular economy is the way to sustainable development in the future. Muscat et al.[4] pointed out that by-products of food production, processing and consumption should be recycled and re-entered into the food system in a way that could add the highest value to the food system. Some edible insects have the ability to convert organic waste into high-quality proteins and fats. With insects as part of a closed loop of the food system, through their biotransformation, nutrients in organic waste are converted into proteins and fats for livestock consumption. Insects can solve two important problems that are plaguing the world: lack of sustainable feed and loss of nutrients from organic waste. As the nature's most powerful recyclers, black soldier fly larval (BSFL) have many outstanding advantages such as fast growth, strong reproduction, high output efficiency and suitable for industrial production[5]. BSFL are rich in protein, amino acids, iron, zinc, calcium and other minerals needed by animals[6]. They are an ideal source of feeding protein. Adult BSF do not eat and only need to replenish water, so that the risk of carrying and transmitting diseases is greatly reduced[7].

Currently, BSFL have been widely used in the treatment of chicken manure[8], cow manure[9] and pig manure[10]. Animal manure contains a lot of unused nutrients, which can be converted into body proteins through feeding and digestion by BSFL. While eliminating environmental pollution, it provides animal protein source feed additives for the market, so as to realize the resource utilization of agricultural waste. Despite the fact that BSFL can consume various types of substrates, high fiber content or unbalanced carbon to nitrogen ratio in the substrate may result in low treatment efficiency. How to improve the efficiency of BSFL converting waste into insect biomass has always been the focus and difficulty of research. Some pretreatments have been shown to increase the conversion rate of BSFL to their substrates. Mazza et al.[11] inoculated the associated bacteria into chicken manure, and found that almost all bacteria could promote the transformation of the manure and the accumulation of body weight and nutrients of the BSFL. Lindberg et al.[12] used enzymes to pretreat lettuce waste at different time before feeding BSFL, and found that compared with the controlled group, the bioconversion rate and waste reduction of direct enzyme addition increased by 22% and 14%, respectively. These pretreatments promote the decomposition of complex compounds such as cellulose in the substrate turning into carbohydrate forms that are more easily absorbed and utilized by larvae, thereby improving biomass conversion efficiency. However, addition of inoculants or enzymes will increase the treatment cost, and the treatment process needs to be carefully controlled to avoid food competition between microorganisms and BSFL. During the composting process, microorganisms can degrade the macromolecular organic matter in the manure into small molecule nutrients for their own metabolism. Reasonable control of composting conditions can increase the concentration of nutrients in manure that is easily absorbed and utilized by BSFL, thereby promoting the conversion efficiency of waste.

The purpose of this study was to investigate the effects of different pretreatment methods on the physicochemical properties of pig manure and its potential in promoting the growth and nutrient accumulation of BSFL. In addition, the mechanism of different pretreatments to improve the performance of BSFL by regulating the nutrients in pig manure was also discussed. The findings are expected to provide guidance for exploring simple and economical methods to improve the biotransformation efficiency of BSFL to pig manure.

MATERIALS AND METHODS

Materials

Fresh pig manure was collected from a pig farm in Niejiazhuang Village, Hebei Province, China. The eggs of BSFL used in the experiment were purchased from Jiangsu Anyou Biotechnology Co., Ltd., China. Firstly, eggs were laid in nylon mesh with a pore diameter of 20 mesh and placed 2−3 cm above wheat bran with moisture content of 70%. After two days, the eggs hatched, the nylon mesh was removed, and the eggs were cultured in wheat bran for 3 d, during which water was added continuously to keep the moisture content at 70%. Wheat bran used in the experiment was purchased from Chenxi organic feed (Huainan, Anhui Province, China). It was sterilized at 125 °C for 25 min before use. After sterilization, wheat bran was placed in an oven of 40 °C until dry. Sawdust was collected from Luancheng, Hebei, China.

Pretreatment of pig manure

The moisture content of fresh pig manure was adjusted to 65% with sawdust and then placed in 50 L composting reactors for different fermentation pretreatments. Six composting pretreatments were conducted in this study and each pretreatment was in triplication: (1) aerobic composting lasted for 2 d (AE-2); (2) aerobic composting lasted for 5 d (AE-5); (3) anaerobic composting lasted for 2 d (AN-2); (4) anaerobic composting lasted for 5 d (AN-5); (5) high temperature anaerobic composting lasted for 2 d (HAN-2); (6) high temperature anaerobic composting lasted for 5 d (HAN-5). Fresh pig manure was used as control (CK). In AE pretreatments, air was pumped at a constant flow rate with 0.2 $L·min^{-1}·kg^{-1}$ dry weight. In AN and HAN pretreatments, pig manure was naturally fermented in a closed composting reactor, in which AN was not provided with additional heat, while HAN was continuously supplied with heat to keep the compost temperature at 55 °C. After the pretreatment, part of the pig manure was stored at −20 °C for the analysis of physical and chemical properties.

Rearing of BSFL

The moisture content of fresh and pretreated pig manure was adjusted to 70% with deionized water, and then 300 g of pig manure was weighed and placed in a plastic box. Then 150 BSFL (5 days old) were put into pig manure and cultured at 30 °C for 10 d. Each pretreatment was set up in three parallels. After the test, the BSFL were separated from pig manure, washed with distilled water, weighed and freeze-dried for subsequent analysis.

Analysis of physiochemical properties of pig manure and BSFL

The pH value and electrical conductivity (EC) of manure (extracted with distilled water (1:5, w/v) at 150 $rmp·h^{-1}$ for 0.5 h and then stood for 30 min) were determined by a pH meter and a conductivity meter. The soluble carbon (DOC) and soluble nitrogen (DON) content of manure samples (extracted with distilled water (1:5, w/v) at 250 $rmp·h^{-1}$ for 1 h, centrifuged at 4,000 $rpm·min^{-1}$ for 10 min and the supernatant was passed through a 0.5 μm filter membrane) were measured by a carbon and nitrogen analyzer. An elemental analyzer (Elementar Analysensysteme GmbH, Germany) was used to determine the total nitrogen (TN) and total carbon (TC) content in pig manure. NH_4^+-N content was evaluated using a segmented flow analyzer (Skalar, Netherlands). The body weight of BSFL was obtained by randomly selecting five larvae after washing and draining and weighing them on a balance. The protein content in pig manure and BSFL was measured by Kieldahl Azotometer and then multiplied by 6.25. The amino acid content and fat content in BSFL were measured by an automatic amino acid analyzer (Hitachi L-8900) and Soxhlet extraction equipment (SOX 406), respectively.

The performance of BSFL

The survival rate, waste reduction, bioconversion rate and protein conversion efficiency were used to evaluate the performance of BSFL. The above indicators were calculated according to the formulas in the study of Gold et al.[13] .

Statistical analysis

Data were displayed as the mean ± standard deviation (n = 3). Significant differences were analyzed using a one-way analysis of variance (ANOVA) followed by post hoc Duncan multiple comparisons. Principal coordinate analysis (PCoA) and Redundancy analysis (RDA) were performed on the online tool of Majorbio Cloud Platform (https://cloud.majorbio.com/page/tools/). PCoA was carried out based on Bray–Curtis distance matrix, eigen values and eigen vectors were calculated to generate two-dimensional plots under different pretreatment conditions. Origin software (Origin 2021b, Origin Lab, Northampton, MA, USA) was used for graphical illustrations of the data.

RESULTS AND DISCUSSION

Changes in physicochemical properties of pig manure after different pretreatments

Figure 1 shows the changes in physicochemical properties of pig manure after different pretreatments. The pH values in AN and HAN pretreated manure were lower than those in fresh and

AE pretreated ones. This might be caused by the accumulation of organic and inorganic acids due to degradation of organic matter. In composting, the pH of pig manure is mainly affected by the decomposition and mineralization of organic matter[14]. The increase of pH values in AE pretreated manure might be due to the rapid volatilization of ammonium that counteracted the production of acids over time[15]. As shown in Fig. 1a & d, the EC and NH_4^+-N values in pig manure after AN, AE and HAN pretreatments were higher than those in fresh manure. EC is related to soluble salt content during composting, and it is generally believed that compost with an EC of less than 4,000 μs·cm^{-1} can be safely applied[14]. Higher EC values in HAN pretreated manure might be related to the formation of a large number of small molecular organic acids and inorganic salts. And with the prolongation of composting time, the accumulation of soluble acids and salts in the manure increased, resulting in the increase of EC value. Changes in the content of NH_4^+-N in pig manure are related to the activities of microorganisms. Degradation of nitrogenous compounds leads to an increase in NH_4^+-N, while microbial consumption leads to a decrease in its concentration[16]. In aerobic composting, the utilization of NH_4^+-N by microorganisms and the volatilization rate of ammonia were significantly higher than those in anaerobic composting, leading to lower NH_4^+-N content.

Figure 1b shows the TC and TN changes in different pretreated manure. It can be observed that different pretreatments all caused a decrease in TC content. This was mainly due to the degradation of macromolecular organic matter into smaller molecular substances by aerobic or anaerobic microorganisms, which were used for the development of their cell protoplasm[17]. Finally, the organic matter in the manure was released into the environment in the form of CO_2 under the decomposition and utilization of microorganisms. TC degradation in AE pretreated manure was the most prominent. The decomposition of organic substances is mediated by microorganisms, and in aerobic composting, an adequate oxygen supply can provide adequate electron acceptors for microbial respiration, resulting in stronger microbial activity[18]. Compared with fresh pig manure, TN values were higher in manure pretreated with AN and HAN. However, TN values of pig manure pretreated with AE were lower than that of fresh pig manure, and the decreasing trend became more obvious with the extension of time. The loss of TN in the AE pretreated manure might be attributed to the ammonia emissions. It has been reported that less than 1% of manure nitrogen is lost in the form of ammonia in anaerobic composting, whereas under aerobic conditions, ammonia volatilization reaches 9%–44%[19]. In pig manure pretreated with AN and HAN, the degradation of organic matter reduced the dry mass of manure, resulting in the increase of TN.

The change trends of DOC and DON in each group were similar (Fig. 1c), showing the highest value in HAN pretreatment and the lowest value in AE pretreatment. In aerobic composting, the utilization of small-molecule organic acids and

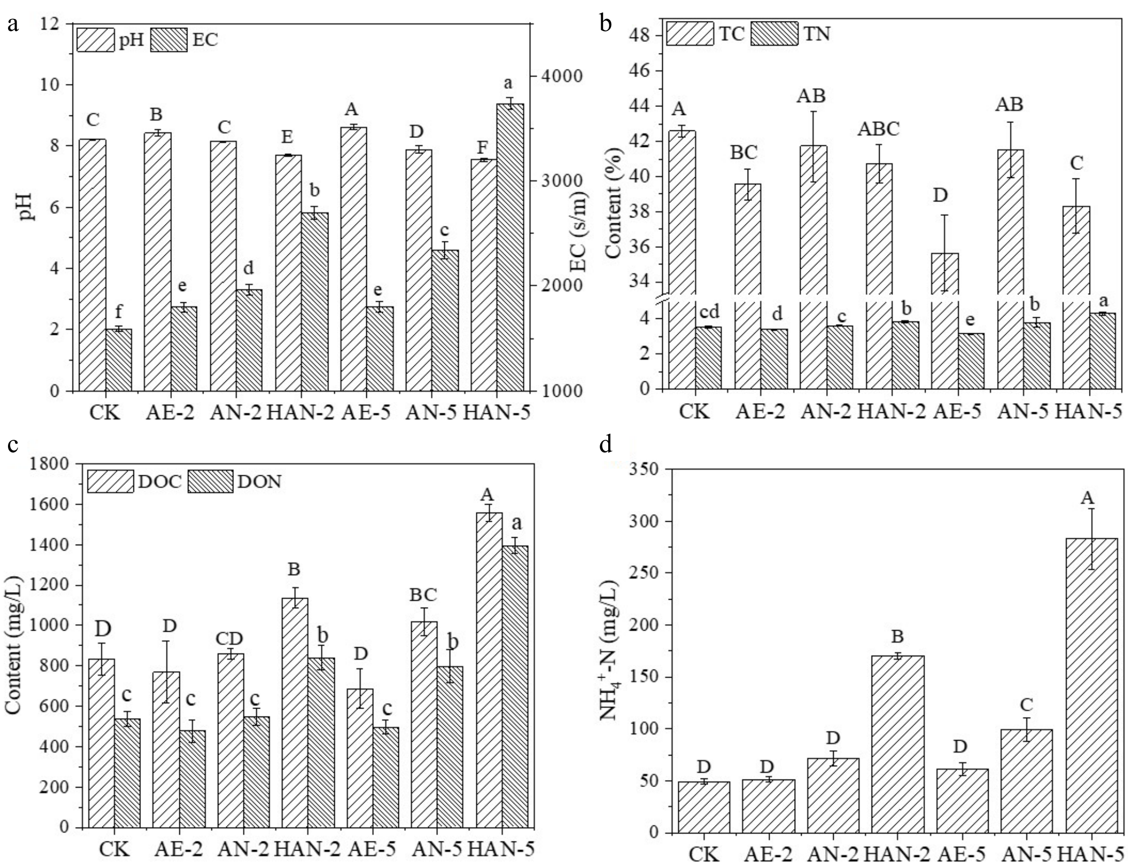

Fig. 1 Changes in physicochemical properties of pig manure after different pretreatments. (a) pH and EC; (b) TC and TN; (c) DOC and DON; (d) NH4+-N. CK, Control; AE-2, Aerobic fermentation for 2 d; AN-2, Anaerobic fermentation for 2 d; HAN-2, High temperature anaerobic fermentation for 2 d; AE-5, Aerobic fermentation for 5 d; AN-5, Anaerobic fermentation for 5 d; HAN-5, High temperature anaerobic fermentation for 5 d.

amino acids by aerobic microorganisms resulted in the reduction of DOC and DON[20]. Compared with macromolecular organic matter, these small molecular compounds were more easily absorbed and utilized by microorganisms. The DOC and DON increased in the AN and HAN pretreated manure because the utilization rate of anaerobic microorganisms was lower than the hydrolysis rate of macromolecular organic matter.

In anaerobic composting, temperature is the most limiting factor because the optimum growth temperature of hydrolytic bacteria is 55−70 °C. Under this condition, the solubility of refractory organic substances such as protein and cellulose in manure will be significantly improved. This can explain why HAN pretreatment has a more significant effect on the physicochemical properties of pig manure than AN pretreatment.

Protein and amino acids contents of pig manure after different pretreatments

Figure 2 shows the protein and amino acid content of pig manure with different pretreatments. Compared with fresh manure, ones pretreated with AE showed lower protein content. Aerobic microbes degrade proteins into amino acids that are more readily available to themselves, leading to the decrease of protein content. The protein content of AN and HAN pretreated manure was higher than that in fresh manure. Possible reasons were that the degradation of organic matter in manure reduced its dry weight, resulting in a relative increase in the concentration of protein[21]. Moreover, the nitrogen volatilization was inhibited in AN and HAN pretreatments, thus nitrogen loss was avoided.

In terms of amino acids, histidine accounted for the largest proportion in fresh pig manure, followed by glutamic acid and aspartic acid. As illustrated in Fig. 2b, with the concentrations of various amino acids in pig manure after different pretreatments decreased, the decreasing trend became more obvious with the extension of time. Among them, the decrease of histidine content was the most significant. Histidine is an amino acid necessary for the synthesis of hemoglobin in aquatic and terrestrial animals[22]. In each pretreatment, *Bacteroides* in manure degraded histidine to glutamic acid, which was then further degraded to propionic acid, acetic acid and butyric acid under the action of *Bacteroides* or *Anaerolineales*[23]. The decline of other amino acids was also related to the action of microorganisms. Compared with AN and HAN pretreatment, the content of amino acids in manure pretreated by AE was higher. This might be due to the transient accumulation of amino acids by aerobic microorganisms degrading proteins to produce amino acids faster than they consumed them.

Growth and nutritional characteristics of BSFL fed with pig manure with different pretreatments

BSFL reared on pig manure with HAN pretreatments showed the highest weight (Fig. 3a). AE pretreatments resulted in the lowest body weight in BSFL. The body weight of BSFL was highly dependent on macronutrients in the feeding medium. Usually, high dietary protein and digestible carbohydrates resulted in high larval weight[24]. After HAN pretreatments, the contents of DOC and DON in pig manure were markedly increased (Fig. 1c), leading to significantly higher body weight of BSFL fed with the manure than those in other groups. The protein content of BSFL was similar on all manure diets (ranging from 44.2 to 47.6 g/100 g DM), indicating that the protein accumulation in the larvae was not determined by the dietary protein content. Barragan-Fonseca et al.[25] also found that BSFL fed with 3.5% and 14% protein had similar protein content.

In living organisms, lipid deposition has physiological costs. It was estimated that the conversion of hex saccharides to fat can account for 20%−25% of the food energy supplied[26]. The fat content of BSFL in this study (Fig. 3c) was significantly lower than the values (30 g/100 g) reported in the literature[27]. This might be due to the fact that the addition of sawdust reduces the nutrient concentration of pig manure. The emergence of food limitation forced BSFL to reduce lipid deposition or mobilize fat reserves[28], resulting in lower body fat content. For animals, higher levels of dietary fat may be toxic to their gut microbes, leading to reduced feed intake and lower animal production[29]. For example, the fat content in beef cattle feed is generally between 2% and 5%, and when it exceeds 6%, diarrhea and digestive disturbance will occur[30]. Therefore, BSFL with low fat content are beneficial to increase their incorporation rate in the feed, thereby increasing their replacement rate of traditional feeds such as fishmeal and soybean meal.

The amino acid composition of BSFL fed with different pretreated manure is shown in Fig. 3d. Overall, glutamic acid,

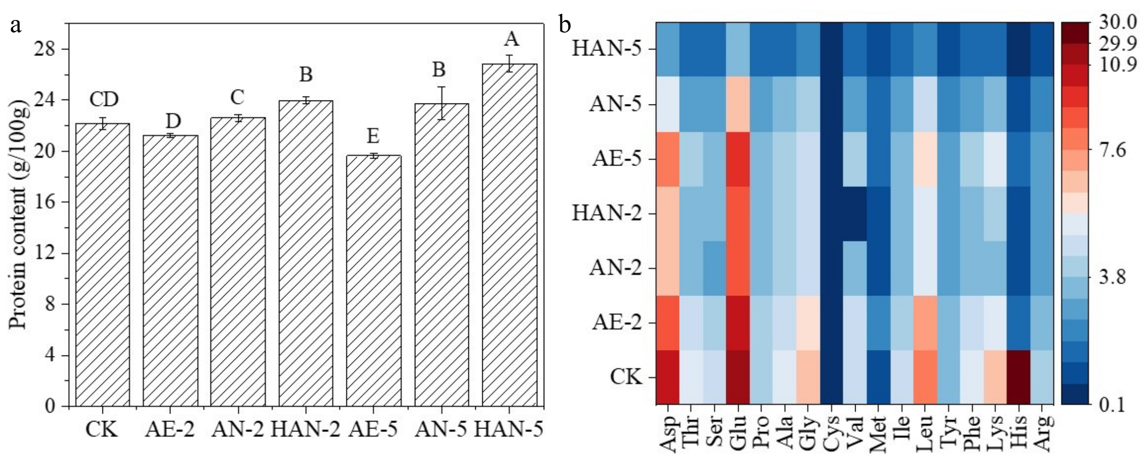

Fig. 2 Changes in protein and amino acids of pig manure after different pretreatments. (a) Protein content; (b) amino acid content. CK, Control; AE-2, Aerobic fermentation for 2 d; AN-2, Anaerobic fermentation for 2 d; HAN-2, High temperature anaerobic fermentation for 2 d; AE-5, Aerobic fermentation for 5 d; AN-5, Anaerobic fermentation for 5 d; HAN-5, High temperature anaerobic fermentation for 5 d.

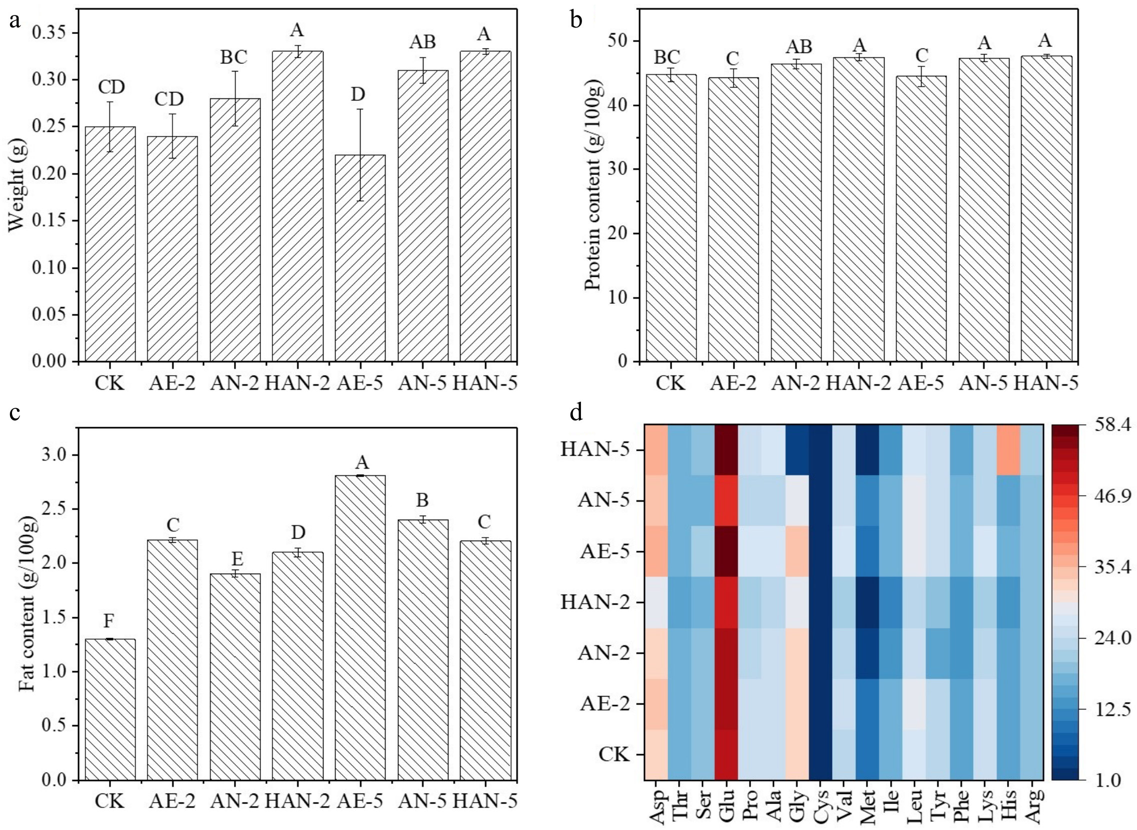

Fig. 3 Growth and nutritional characteristics of BSFL fed with pig manure with different pretreatments. (a) Weight, (b) protein content, (c) fat content, (d) amino acid content. CK, Control; AE-2, Aerobic fermentation for 2 d; AN-2, Anaerobic fermentation for 2 d; HAN-2, High temperature anaerobic fermentation for 2 d; AE-5, Aerobic fermentation for 5 d; AN-5, Anaerobic fermentation for 5 d; HAN-5, High temperature anaerobic fermentation for 5 d.

aspartic acid and glycine were the most abundant amino acids in BSFL. This is in accord with the study of Spranghers et al.[31] who found that the most dominant amino acid in BSFL reared on different organic substrates was glutamic acid, followed by aspartic acid. The BSFL in this study were also rich in a variety of essential amino acids such as leucine, lysine and valine, and the levels of these amino acids were sufficient to meet the requirements of poultry and pig[31]. It can be observed that BSFL of all groups are generally deficient in cysteine and methionine, so these amino acids need to be supplemented in a balanced diet. Makkar et al.[32] also reported the deficiency in cysteine and methionine. The variations in the amino acids of the larval reared on different pretreated manure were small, indicating that feeding substrates had small effect on the amino acid profile of BSFL.

Principal co-ordinates analysis (PCoA) of pig manure and redundancy analysis (RDA) of BSFL

Principal co-ordinates analysis (PCoA) was used to identify the effects of different pretreatments on the physicochemical properties of pig manure. Figure 4a shows the distribution of the first two principal components determined by PCoA, describing 92.75% and 2.64% of the cumulative variance contribution, respectively. Pig manure after different pretreatments is well differentiated on the distribution map, indicating that different pretreatments have a significant impact on the physical and chemical properties of pig manure. The pig manure with HAN and AN pretreatments can be well distinguished from those with AE pretreatments according to PC1.

The relationship between BSFL and physicochemical properties of pig manure was analyzed using redundancy analysis (RDA). As shown in Fig. 4b, the weight and protein content of BSFL were positively correlated with EC, NH_4-N, DON, DOC and TN of pig manure. Under the same conditions of density, temperature and humidity, the body weight and body composition of BSFL depend on the quality of the feeding substrates[24,33]. Fischer et al.[34] found that the BSFL fed with dough were significantly longer and heavier than those fed with spent coffee. The higher content of indigestible fiber, harder texture and lower nutrient concentration in spent coffee made it not suitable for BSFL growth. Nutrients in the feed profoundly influence the composition and quality of individuals[35,36]. Although insects can regulate growth through post-ingestive compensation for nutrients[37], feeding substrates with high nutrient concentration is still beneficial to their body weight and the accumulation of their own nutrients (such as protein and fat).

TC content in pig manure has little effect on the growth and nutrient accumulation of BSFL, mainly because lignin, cellulose and hemicellulose cannot be directly digested by the larvae. This is also the reason why the biotransformation rate of dairy manure by BSFL is higher than that of chicken manure even though dairy manure is higher in TC content[38]. The pH of the feeding substrates showed the least effect on the body weight and protein content of BSFL (Fig. 4b). It was reported that substrates with pH of 6–10 resulted in similar final weight of BSFL, but the growth time of BSFL was shortened by 3 d in the

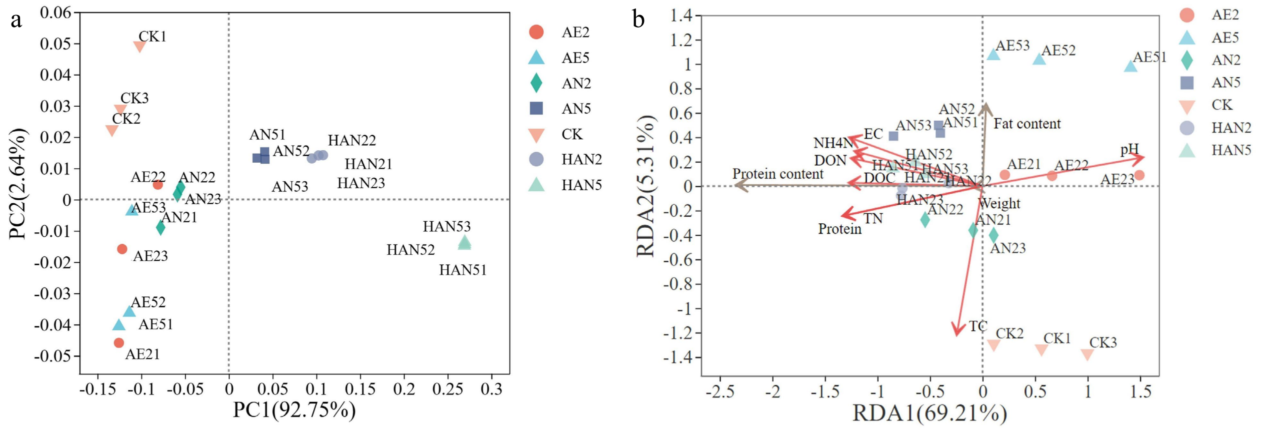

Fig. 4 (a) PCoA analysis of physicochemical properties of different pretreated pig manure and (b) RDA analysis of black soldier fly fed on different pretreated pig manure.

substrate at pH 8[39]. The pH values of fresh pig manure and those pretreated by different fermentation were in the range of 7.5-8.6, so they were suitable for the growth of BSFL. Within this range, lower pH values were beneficial to the growth and protein accumulation of BSFL. With regard to the body fat content of BSFL, EC, NH_4-N and DON seemed to have a distinct effect on it. In this study, the fat content of BSFL was significantly lower than the normal value because the addition of wood chips reduced the nutrient content of pig manure. However, low fat content of BSFL is desirable because it not only facilitates subsequent feed processing (especially crushing and pelleting) and storage, but also facilitates the admission of BSFL as feed. Tschirner & Simon[28] fed BSFL with sugar beet pulp, and the body fat content obtained was about 3%, which was close to the value of this study.

Performance of BSFL fed on different pretreated manure

All fresh and pretreated manure supported the growth of BSFL (Table 1). Survival rates of BSFL fed with different pretreatment pig manure were all above 94%, and there was no significant difference among the pretreatments. The BSFL have an extremely high tolerance to harsh dietary conditions, and their survival rate can reach 93% even fed with fermented maize straw[27]. In terms of waste reduction, bioconversion rate, waste conversion efficiency and protein conversion efficiency, BSFL fed with manure pretreated with AN and HAN showed better performance.

The waste reduction of AE pretreated manure by BSFL was 16.2%–25.9% lower than that of fresh manure. This might be because the addition of sawdust reduced the nutrient concentration of pig manure, and the microorganisms further consumed the nutrients during the pretreatment process, resulting in an increase in the relative content of lignin and other substances that cannot be used by BSFL. In addition, combining the results of waste reduction and larval weight, it could be found that there was a positive correlation between waste reduction and BSFL weight. The higher the waste reduction, the greater the weight of BSFL. The composition and microbial community of the feeding substrates had a major impact on the bioconversion rate[40]. The higher content of small organic molecules such as peptides, sugars and fatty acids in substrates was beneficial to the growth and feed consumption of BSFL, resulting in higher bioconversion rate. The waste reduction and bioconversion rate in this study were lower than previous studies, whose values were 29%–58% and 11%–14%, respectively[41,42]. This was due to the addition of sawdust, which was not easily degraded by BSFL. It has been reported that cow manure supported much lower waste reduction and bioconversion rate compared to the poultry feed, owing to its low protein and digestible carbohydrates contents[13].

As shown in Table 1, protein conversion efficiency by BSFL in AE pretreated manure was lower than that in AN and HAN pretreated samples. The protein conversion efficiency was related to body protein content of BSFL, which was largely dependent on the dietary protein concentration. High protein content diets usually resulted in higher body protein content of BSFL[43]. By increasing the protein concentration in the diet, BSFL can gradually convert a small portion of it into its body protein. However, Barragan-Fonseca et al[25]. reported that BSFL fed with a low-protein diet had higher body protein content than those fed with a high-protein diet. This might be due to

Table 1. Performance of BSFL fed on different pretreated manure.

Different treatments	Survival rate (%)	Waste reduction (%)	Bioconversion rate (%)	Protein conversion efficiency (%)
CK	95.67 ± 0.57^{bcd}	26.33 ± 3.01^{ab}	4.94 ± 1.84^{bc}	7.59 ± 0.011^{e}
AE-2	94.33 ± 1.52^{cd}	22.07 ± 6.80^{ab}	4.34 ± 1.07^{bc}	6.41 ± 0.036^{f}
AN-2	96.00 ± 1.00^{bc}	27.63 ± 8.25^{ab}	5.20 ± 1.64^{abc}	8.08 ± 0.061^{c}
HAN-2	98.67 ± 0.57^{a}	29.74 ± 7.95^{a}	7.32 ± 0.73^{a}	8.91 ± 0.023^{a}
AE-5	94.00 ± 1.00^{d}	19.52 ± 2.85^{b}	3.81 ± 1.63^{c}	5.09 ± 0.044^{g}
AN-5	95.67 ± 0.58^{bcd}	28.48 ± 4.54^{ab}	6.49 ± 1.64^{ab}	7.82 ± 0.017^{d}
HAN-5	97.33 ± 0.58^{ab}	28.97 ± 2.95^{a}	7.01 ± 1.08^{ab}	8.70 ± 0.032^{b}

The values before and after ± are mean values and standard deviation, respectively. Different lowercase letters indicate a significant difference ($p < 0.05$).

the requirement of dietary protein content for BSFL. When the protein content was lower than the threshold value, it was very important for the growth and protein accumulation of BSFL, but when the protein content was above the threshold value, it was not so important. It has been reported that on a low-protein diet, BSFL were able to convert more than 90% of the protein, and on the protein-rich diet, the conversion rate fell to 10%[44].

The relationship between performance of BSFL and physicochemical properties of pig manure

Figure 5 shows the relationship between performance of BSFL and physicochemical properties of pig manure. Since the protein content in the manure was calculated from TN, the correlation between them and the performance of BSFL was consistent. As can be seen from Fig. 5, the survival rate and waste reduction of BSFL were significantly positively correlated with TN and protein content of pig manure. The bioconversion rate and protein conversion efficiency were positively correlated with TN, DOC, DON, EC, protein content and NH_4^+-N of the pig manure. The survival rate of BSFL depends on the quality of the feeding substrate in which the protein level is the limiting factor[45]. Simon et al.[46] also reported that higher protein content in the diet helped improve the survival rate of some fly species. Waste reduction and bioconversion rate are related to the composition of feeding substrate, BSFL strain, feeding rate and larval density[40]. When these conditions are controlled, the addition of microorganisms will increase the bioconversion rate and waste reduction of BSFL. Xiao et al.[47] observed that addition of *B. subtilis* increased the biotransformation and substrate degradation rates in chicken manure by BSFL by 13%. This was attributed to the fact that the addition of microorganisms degraded some macromolecular nutrients in the feeding substrates into small molecular substances that were easily absorbed by BSFL, which promoted its biotransformation process. In this study, after 2 or 5 d of aerobic fermentation, the nutrients in pig manure were degraded and then consumed by aerobic microorganisms, resulting in the reduction of nutrient concentration. The activity of anaerobic microorganisms was significantly lower than that of aerobic microorganisms, so the nutrient content of pig manure pretreated with AN and HAN was higher, which was beneficial to the growth and biotransformation of BSFL. To a certain extent, EC reflected the concentration of soluble nutrients, which might explain the significant positive correlation between EC and the bioconversion rate of BSFL. Although it has been reported that BSF larvae fed a low-protein diet has higher utilization efficiency of dietary protein[24], the protein conversion rate of BSFL in this study was significantly positively correlated with the protein content in the feeding substrate at the 0.01 level. This might be due to the fact that the protein accumulation of BSFL was not only affected by the protein content, but also by other nutrients such as DOC and DON. The pH value was significantly negatively correlated with all performance indexes, indicating that the lower pH value in this study was beneficial to the treatment of waste and the transformation of its own substances by BSFL. There was no significant correlation between the TC content and the performance of BSFL, which was due to the fact that organic substances such as sawdust and cellulose in pig manure could not be utilized by BSFL. Figure 5b shows RDA analysis performance of BSFL and physicochemical properties of pig manure. Environmental factors in the RDA ranking reflected a total of 68.86% of the total variance in the handling performance of BSFL, with the first and second canonical axes showing 64.62% and 4.24% of the data variance, respectively. It can be clearly seen that the results of RDA further confirm the results of the correlation analysis.

CONCLUSIONS

High temperature anaerobic fermentation (HAN) pretreatments of pig manure significantly promoted the growth, protein accumulation and performance of BSFL compared with fresh manure. In HAN pretreatment, hydrolyzing bacteria were in the optimal growth temperature range, which accelerated the degradation of macromolecular substances such as cellulose in manure. At the same time, due to the limited consumption of nutrients in pig manure by microorganisms under anaerobic conditions, the TN, DOC and DON content in pig manure pretreated by HAN were significantly higher than that in other pretreatments. Correlation analysis and redundancy analysis (RDA) showed that body weight, protein accumulation and performance of BSFL were significantly

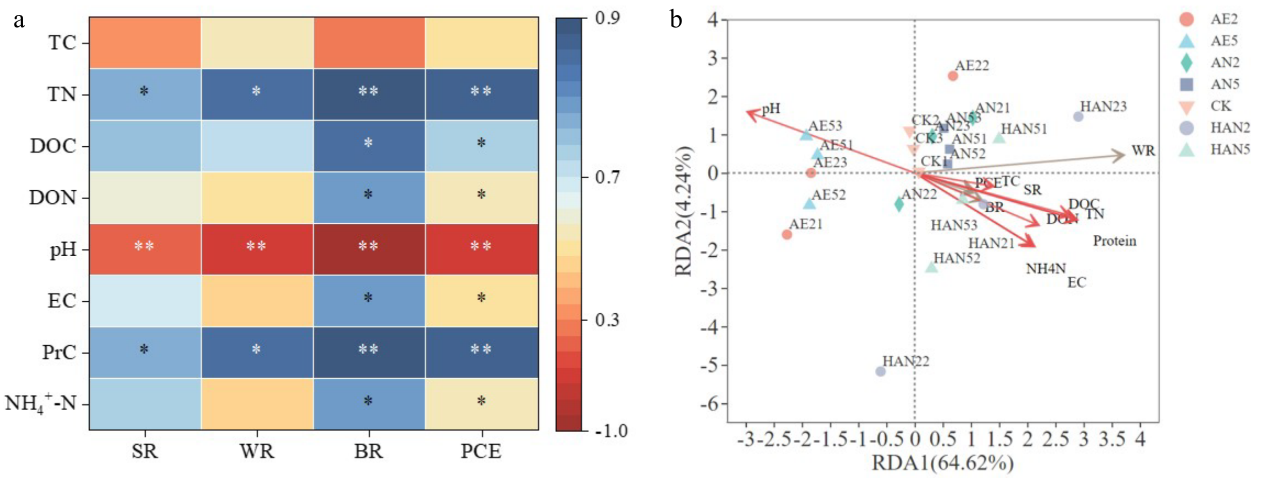

Fig. 5 Correlation analysis between performance of (a) BSFL and physicochemical properties of pig manure and (b) RDA analysis. SR, Survival rate; WR, Waste reduction rate; BR, Bioconversion rate; WCE, Waste conversion efficiency; PCE, Protein conversion efficiency.

positively correlated with TN, DOC, EC, DON and NH_4^+-N contents of pig manure, demonstrating that the growth characteristics and waste disposal performance of BSFL are related to the nutrients in their substrates. Based on the results of this study, HAN pretreatment can significantly improve the growth of BSFL and the conversion of pig manure, thus providing a potential strategy for the application of BSFL in organic waste management.

ACKNOWLEDGMENTS

This work was supported by Key R&D Program of Hebei (21327315D, 21373804D); the Special Research Assistant Funding Project of Chinese Academy of Sciences (E129ST15); the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28030302-4); Key Laboratory of Agricultural Water Resources-CAS (ZD201802); Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28030302); Youth Innovation Promotion Association CAS (2021095); Hebei Dairy Cattle Innovation Team of Modern Agro-industry Technology Research System (HBCT2018120206).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 28 September 2022; Accepted 8 February 2023; Published online 9 March 2023

REFERENCES

1. Bai Z, Ma W, Ma L, Velthof GL, Wei Z, et al. 2018. China's livestock transition: Driving forces, impacts, and consequences. *Science Advances* 4:eaar8534
2. Bai Z, Fan X, Jin X, Zhao Z, Wu Y, et al. 2022. Relocate 10 billion livestock to reduce harmful nitrogen pollution exposure for 90% of China's population. *Nature Food* 3:152−60
3. Bai Z, Schmidt-Traub G, Xu J, Liu L, Jin X, et al. 2020. A food system revolution for China in the post-pandemic world. *Resources, Environment and Sustainability* 2:100013
4. Muscat A, de Olde EM, Ripoll-Bosch R, van Zanten HHE, Metze TAP, et al. 2021. Principles, drivers and opportunities of a circular bioeconomy. *Nature Food* 2:561−66
5. Diclaro II JW, Kaufman PE. 2009. Black soldier fly *Hermetia illucens* Linnaeus (insecta: Diptera: Stratiomyidae). *EENY-461*. Entomology and Nematology Department, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida. https://edis.ifas.ufl.edu/in830
6. Parodi A, Leip A, de Boer IJM, Slegers PM, Ziegler F, et al. 2018. The potential of future foods for sustainable and healthy diets. *Nature Sustainability* 1:782−89
7. Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology* 39:695−98
8. Zhang X, Zhang J, Jiang L, Yu X, Zhu H, et al. 2021. Black soldier fly (*Hermetia illucens*) larvae significantly change the microbial community in chicken manure. *Current Microbiology* 78:303−15
9. Matos JS, de Aráujo LP, Allaman IB, Lôbo IP, de Oliva ST, et al. 2021. Evaluation of the reduction of methane emission in swine and bovine manure treated with black soldier fly larvae (*Hermetia illucens* L.). *Environmental Monitoring and Assessment* 193:480
10. Wu N, Wang X, Yan Z, Xu X, Xie S, et al. 2021. Transformation of pig manure by passage through the gut of black soldier fly larvae (*Hermetia illucens*): Metal speciation, potential pathogens and metal-related functional profiling. *Ecotoxicology and Environmental Safety* 211:111925
11. Mazza L, Xiao X, ur Rehman K, Cai M, Zhang D, et al. 2020. Management of chicken manure using black soldier fly (Diptera: Stratiomyidae) larvae assisted by companion bacteria. *Waste Management* 102:312−18
12. Lindberg L, Vinnerås B, Lalander C. 2022. Process efficiency in relation to enzyme pre-treatment duration in black soldier fly larvae composting. *Waste Manage* 137:121−27
13. Gold M, Cassar CM, Zurbrügg C, Kreuzer M, Boulos S, et al. 2020. Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. *Waste Management* 102:319−29
14. Mao H, Zhang H, Fu Q, Zhong M, Li R, et al. 2019. Effects of four additives in pig manure composting on greenhouse gas emission reduction and bacterial community change. *Bioresource Technology* 292:121896
15. Jiang J, Liu X, Huang Y, Huang H. 2015. Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting. *Waste Management* 39:78−85
16. Anjum R, Grohmann E, Krakat N. 2017. Anaerobic digestion of nitrogen rich poultry manure: impact of thermophilic biogas process on metal release and microbial resistances. *Chemosphere* 168:1637−47
17. Mehta C, Sirari K. 2018. Comparative study of aerobic and anaerobic composting for better understanding of organic waste management: A mini review. *Plant Archives* 18:44−48
18. Gong B, Zhong X, Chen X, Li S, Hong J, et al. 2021. Manipulation of composting oxygen supply to facilitate dissolved organic matter (DOM) accumulation which can enhance maize growth. *Chemosphere* 273:129729
19. Kirchmann H, Witter E. 1989. Ammonia volatilization during aerobic and anaerobic manure decomposition. *Plant and Soil* 115:35−41
20. Chen Z, Fu Q, Cao Y, Wen Q, Wu Y. 2021. Effects of lime amendment on the organic substances changes, antibiotics removal, and heavy metals speciation transformation during swine manure composting. *Chemosphere* 262:128342
21. Ren L, Schuchardt F, Shen Y, Li G, Li C. 2010. Impact of struvite crystallization on nitrogen losses during composting of pig manure and cornstalk. *Waste Management* 30:885−92
22. El-Dakar MA, Ramzy RR, Ji H. 2021. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). *The Science of the Total Environment* 772:145528
23. Mercado JV, Koyama M, Nakasaki K. 2022. Short-term changes in the anaerobic digestion microbiome and biochemical pathways with changes in organic load. *The Science of the Total Environment* 813:152585
24. Barragán-Fonsec KB. 2018. *Flies are what they eat: Tailoring nutrition of Black Soldier Fly (*Hermetia illucens *L.) for larval biomass production and fitness*. Thesis. Wageningen University and Research, Netherlands. https://doi.org/10.18174/449739
25. Barragan-Fonseca KB, Dicke M, van Loon JJA. 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomologia Experimentalis et Applicata* 166:761−70
26. Le Gall M, Behmer ST. 2014. Effects of protein and carbohydrate on an insect herbivore: the vista from a fitness landscape. *Integrative and Comparative Biology* 54:942−54
27. Gao Z, Wang W, Lu X, Zhu F, Liu W, et al. 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. *Journal of cleaner production* 230:974−80

28. Tschirner M, Simon A. 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of insects as food and feed* 1:249–59
29. Behan AA, Loh TC, Fakurazi S, Kaka U, Kaka A, et al. 2019. Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. *Animals* 9:400
30. Gautam DP, Rahman S, Borhan MS, Engel C. 2016. The effect of feeding high fat diet to beef cattle on manure composition and gaseous emission from a feedlot pen surface. *Journal of Animal Science and Technology* 58:1
31. Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, et al. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture* 97:2594–600
32. Makkar HPS, Tran G, Heuzé V, Ankers P. 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* 197:1–33
33. Oonincx DGAB, van Huis A, van Loon JJA. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *Journal of Insects as Food and Feed* 1:131–39
34. Fischer H, Romano N, Sinha AK. 2021. Conversion of spent coffee and donuts by black soldier fly (*Hermetia illucens*) larvae into potential resources for animal and plant farming. *Insects* 12:332
35. Konkol D, Wojnarowski K. 2018. The use of nanominerals in animal nutrition as a way to improve the composition and quality of animal products. *Journal of Chemistry* 2018:5927058
36. Ding X, Wu X, Zhang K, Bai S, Wang J, et al. 2020. Dietary supplement of essential oil from oregano affects growth performance, nutrient utilization, intestinal morphology and antioxidant ability in Pekin ducks. *Journal of Animal Physiology and Animal Nutrition* 104:1067–74
37. Zudaire E, Simpson SJ, Montuenga LM. 1998. Effects of food nutrient content, insect age and stage in the feeding cycle on the FMRFamide immunoreactivity of diffuse endocrine cells in the locust gut. *The Journal of experimental biology* 201:2971–9
38. Rehman KU, Cai M, Xiao X, Zheng L, Wang H, et al. 2017. 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* 196:458–65
39. Zhang J, Zhang J, Li J, Tomerlin JK, Xiao X, et al. 2021. Black soldier fly: A new vista for livestock and poultry manure management. *Journal of Integrative Agriculture* 20:1167–79
40. Surendra K, Tomberlin JK, van Huis A, Cammack JA, Heckmann L-HL, Khanal SK. 2020. Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (*Hermetia illucens* (L.))(Diptera: Stratiomyidae)(BSF). *Waste Management* 117:58–80
41. Lalander C, Diener S, Zurbrügg C, Vinnerås B. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *Journal of Cleaner Production* 208:211–19
42. Miranda CD, Cammack JA, Tomberlin JK. 2019. Life-history traits of the black soldier fly, *Hermetia illucens* (L.)(Diptera: Stratiomyidae), reared on three manure types. *Animals* 9:281
43. Nguyen TTX, Tomberlin JK, Vanlaerhoven S. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental Entomology* 44:406–10
44. Fuso A, Barbi S, Macavei LI, Luparelli AV, Maistrello L, et al. 2021. Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. *Foods* 10:1773
45. Gobbi P, Martínez-Sánchez A, Rojo S. 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *European Journal of Entomology* 110:461–68
46. Simon PP, Krüger RF, Ribeiro PB. 2011. Influence of diets on the rearing of predatory flies of housefly larvae. *Arquivo Brasileiro De Medicina Veterinária e Zootecnia* 63:1414–20
47. Xiao X, Mazza L, Yu Y, Cai M, Zheng L, et al. 2018. Efficient co-conversion process of chicken manure into protein feed and organic fertilizer by *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae and functional bacteria. *Journal of Environmental Management* 217:668–76