Evaluation of Nutritional Value and Safety of Black Soldier Flies Fed on Dead Pig Meat and Bone Meal

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Abstract [**Objectives**] This study was conducted to evaluate the nutritional value and safety of black soldier flies fed with dead pig meat and bone meal (DPMBM) and to explore the resource utilization of DPMBM. [**Methods**] The general nutrient composition, amino acids, fatty acids and mineral elements of black soldier fly meal (BSLM) were detected and analyzed. [**Results**] The results showed that the contents of moisture, crude protein, crude fat and ash in BSLM were 3.42%, 42.31%, 34.04% and 5.40%, respectively. The contents of total amino acids (TAA), essential amino acids (∑EAA) and non-essential amino acids (∑NEAA) and umami amino acids (∑DAA), the EAA/TAA value and the EAA/NEAA value were 37.93%, 13.08%, 24.85%, 13.43%, 34.47%, 52.61%, respectively. A total of eight kinds of saturated fatty acids and seven kinds of unsaturated fatty acids were detected, accounting for 63.65% and 32.67% of the total fatty acids. Among the major mineral elements, the content of Ca was the highest, followed by K, Mg and Na. Among the trace mineral elements, the content of Mn was the highest, followed by Fe, Zn, Cu, Ni, Cr, Cd, As, Pb, Se, Sn, Ti, Sb and Hg. The contents of heavy metal mineral elements in BSLM were far lower than the limits specified in *Hygienical Standard for Feeds*. [**Conclusions**] In conclusion, BSLM has high nutritional value and good safety of heavy metals, and thus great potential for development and utilization as a high quality dietary protein, fat and mineral elements source.

Key words Dead pig carcass; Meat and bone meal; Black soldier fly; Nutritional components; Mineral elements

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The indiscriminate disposal or improper disposal of dead animal carcasses can not only cause air pollution and surface groundwater pollution, but also easily lead to the spread of major animal diseases and zoonotic diseases, and the dead animal carcasses may even enter human dining tables, posing a threat to human health. China is a big country in animal husbandry, with a high degree of intensive farming. In the process of farming, the number of dead animals caused by diseases is very large. It is estimated that there were about 21 million to 83.99 million dead pigs in China in 2022[1-2]. Exploring new ways of harmless treatment and resource utilization for sick and dead pigs has always been a hot concern in industry development. It not only affects the cost and enthusiasm of livestock breeding enterprises, but also concerns the sustainable and healthy development of animal husbandry and public health safety issues.

Black soldier flies (*Hermetia illucens* L.) are insects of Stratiomyidae in Diptera, which are distributed in most regions of the world and reproduce multiple generations a year. The Larvae are carnivorous and have a wide range of feeding habits. They feed on decaying organic matter and animal feces in nature. They have a short reproductive cycle, a large number, and a high biological conversion rate, and can achieve the resource utilization of organic waste[3-4]. Feeding black soldier flies with the residue (dead pig meat and bone meal, DPMBM for short) obtained after harmless treatment of dead pigs by the high-temperature drying method as a nutritional substrate converts waste organic matter into a non-grain raw material of insect feed, which might be an effective way to achieve the resource utilization of DPMBM.

The research on black soldier flies at home and abroad mainly focuses on their biological characteristics and artificial breeding[5-6], nutritional evaluation and antimicrobial peptide development[7-9], development of feed for aquatic animals, livestock and poultry[10-12], waste treatment and safety evaluation[13-15], and other aspects. The nutritional substrates used for raising black soldier flies mainly include organic waste such as kitchen waste and animal manure[15-16], traditional Chinese medicine residues[17-18], and shrimp waste[19-20]. However, there are few reports on the use of harmless treatment of dead animal residues as a nutritional substrate for feeding black soldier flies and the evaluation on their nutritional composition and safety[21].

In this study, using black soldier flies raised with DPMBM as the research object, their general nutritional composition, amino acids, fatty acids and mineral element composition were determined, and the nutritional value and safety of black soldier flies were comprehensively evaluated, aiming to explore the resource utilization of DPMBM waste.

Materials and Methods

Sample sources and pretreatment

Black soldier flies in this experiment were provided by a certain ecological and environmental protection technology Co., Ltd. in Hunan, and they were raised using the residue from harmless treatment of diseased and dead pigs as a nutritional substrate. The diseased and dead pigs were collected and transported from surrounding farms in Changsha County to a certain ecological and environmental protection technology Co., Ltd. in Hunan Province.

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The company adopted the high-temperature drying method for harmless treatment, and then separated the oil and residue (*i. e.* DPMBM), of which the latter was used for black soldier fly breeding. The black soldier fly larvae were dried into dry samples and stored in a cool and dry place. Before the detection of black soldier fly samples in this experiment, they were crushed by a small laboratory grinder and sieved through a 60 mesh sieve to produce black soldier fly powder, which was frozen and stored at −20 ℃ for the determination of general nutritional components, amino acids, fatty acids, and mineral elements.

Determination of nutritional composition and mineral elements

The determination methods of crude protein, crude fat, moisture, and ash in black soldier fly powder referred to the experimental methods in the paper of Cheng *et al.* [2]. The determination methods for amino acids, fatty acids and mineral element composition of black water fly powder referred to the experimental methods in the paper of Cheng *et al.* [22].

Data processing

The data were processed and analyzed using Excel 2007, and the results were expressed as mean ± *SD*.

Result and Analysis

General nutritional components of black soldier flies

The general nutritional components in black soldier flies were calculated based on dry weight, including moisture content (3.42 ± 0.06) %, crude protein (42.31 ± 0.35) %, crude fat (34.04 ± 0.23) %, and ash (5.40 ± 0.02) %.

Amino acid composition of black soldier flies

The amino acid composition of black soldier flies is shown in Table 1. In this experiment, the black soldier flies were detected to contain a total of 17 common amino acids (tryptophan was not detected due to acid hydrolysis), with a total amino acid content (TAA) of 37.93%, a total of 13.08% for seven essential amino acids (EAA), and a total of 24.85% for ten non-essential amino acids (NEAA). Among the 17 amino acids in black soldier flies, the content of Glu (4.65%) was the highest, followed by Asp (3.83%), His (3.35%), Ala (2.86%), Leu (2.75%), Tyr (2.65%), Val (2.32%), Lys (2.27%), Gly (2.10%), Arg (1.80%), Pro (1.76%), Phe (1.71%), Ile (1.69%), Ser (1.66%), Thr (1.61%) and Met (0.73%), and the content of Cys (0.21%) was the lowest. The total content of umami amino acids (DAA) in black soldier flies was 13.43%, and the EAA/TAA and EAA/NEAA values were 34.47% and 52.61%, respectively.

Fatty acid composition of black soldier flies

The fatty acid composition of black soldier flies is shown in Table 2. A total of 15 main fatty acids were detected in black soldier flies. Among them, eight were saturated fatty acids, accounting for 63.65% of the total fatty acid content; three were monounsaturated fatty acids, accounting for 32.67% of the total fatty acid content; four were polyunsaturated fatty acids, accounting for 3.69% of the total fatty acid content; two were highly unsaturated fatty acids, accounting for 0.12% of the total fatty acid content; n − 3 highly unsaturated fatty acids accounted for 0.12% of the total fatty acid content; and n − 6 highly unsaturated fatty acids accounted for 2.85% of the total fatty acid content; and n − 3/n − 6 was 0.11. The main saturated fatty acids in black soldier flies were lauric acid (C12:0), palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0), which accounted for 23.72%, 27.19%, 5.98% and 5.55% of the total fatty acids, respectively. Monounsaturated fatty acids were mainly composed of oleic acid (C18:1) and palmitoleic acid (C16:1), accounting for 28.88% and 3.36% of the total fatty acids, respectively. Linoleic acid (C18:2) was the main polyunsaturated fatty acid, accounting for 2.85% of the total fatty acids, while EPA (C20:5) was low, accounting for 0.07%.

Table 1 Amino acid composition of black soldier flies (by dry weight, %)

Amino acid	Black soldier flies	Amino acid	Black soldier flies
Aspartic acid (Asp)	3.83	Phenylalanine (Phe)	1.71
Threonine (Thr)	1.61	Histidine (His)	3.35
Serine (Ser)	1.66	Lysine (Lys)	2.27
Glutamic acid (Glu)	4.65	Arginine (Arg)	1.80
Glycine (Gly)	2.10	Proline (Pro)	1.76
Alanine (Ala)	2.86	TAA	37.93
Cysteine (Cys)	0.21	EAA	13.08
Valine (Val)	2.32	DAA	13.43
Methionine (Met)	0.73	NEAA	24.85
Isoleucine (Ile)	1.69	EAA/NEAA	52.61
Leucine (Leu)	2.75	EAA/TAA	34.47
Tyrosine (Tyr)	2.65		

TAA represents the sum of amino acids; EAA represents the sum of essential amino acids, including Thr, Val, Met, Ile, Leu, Phe and Lys; NEAA represents the sum of nonessential amino acids, including Asp, Ser, Glu, Pro, Gly, Ala, Cys, Tyr, Arg and His; and ∑ DAA represents the sum of umami amino acids, including Asp, Glu, Glu and Ala.

Table 2 Fatty acid composition of black soldier flies (by dry weight, in total fatty acids, %)

Fatty acid	Black soldier flies	Fatty acid	Black soldier flies
C8:0	0.07	C20:1	0.42
C10:0	0.55	MUFA[2]	32.67
C12:0	23.72	C18:2*n* − 6	2.85
C14:0	5.55	C20:2*n* − 6	0.72
C15:0	0.14	C20:3*n* − 3	0.05
C16:0	27.19	C20:5*n* − 3	0.07
C17:0	0.45	PUFA[3]	3.69
C18:0	5.98	HUFA[4]	0.12
SFA[1]	63.65	*n* − 3[5]	0.12
C16:1	3.36	*n* − 6[6]	2.85
C18:1*n* − 9	28.88	*n* − 3/*n* − 6	0.11

Saturated fatty acids ([1]SFA): C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0; monounsaturated fatty acids ([2]MUFA): C16:1, C18:1n − 9, C20:1; polyunsaturated fatty acids ([3]PUFA): C18:2*n* − 6, C20:2*n* − 6, C20:3*n* − 3, C20:5*n* − 3; highly unsaturated fatty acids ([4]HUFA): C20:3*n* − 3, C20:5*n* − 3; [5]n − 3: C20:3*n* − 3, 20:5*n* − 3; [6]*n* − 6: C18:2*n* − 6, C20:2*n* − 6.

Mineral element composition of black soldier flies

A total of 18 kinds of mineral elements were detected in black soldier flies, as shown in Table 3. Among the major elements, Ca had the highest content, followed by K, Mg and Na, and their contents were 17 854.45, 9 954.25, 2 233.70 and 1 190.70 mg/kg, respectively. Among trace elements, Mn had the highest content, followed by Fe, Zn, Cu, Ni, Cr, Cd, As, Pb, Se, Sn, Ti, Sb and Hg.

Table 3 Contents of 18 mineral elements in black soldier flies (by dry weight, mg/kg)

Element	Black soldier flies	Element	Black soldier flies
Ca	17 854.45 ± 31.18	Cr	0.955 0 ± 0.035 0
K	9 954.25 ± 24.25	Cd	0.368 5 ± 0.009 0
Mg	2 233.70 ± 47.24	As	0.196 5 ± 0.008 0
Na	1 190.70 ± 10.89	Pb	0.146 5 ± 0.001 0
Mn	395.20 ± 7.21	Se	0.077 0 ± 0.006 0
Fe	285.30 ± 13.01	Sn	0.032 5 ± 0.001 0
Zn	91.35 ± 1.62	Ti	0.027 5 ± 0.001 0
Cu	12.70 ± 0.28	Sb	0.017 5 ± 0.001 0
Ni	0.97 ± 0.03	Hg	0.003 2 ± 0.000 0

Conclusions and Discussion

Effects of DPMBM on general nutritional components of black soldier flies

In this study, the crude protein content of black soldier fly powder (42.31%) is close to soybean meal (41.8%), soybean meal (44.2%), peanut kernel meal (44.7%), and cottonseed meal (43.5%), but higher than shrimp shell powder (33.72%), full-fat soybean (35.5%), sesame cake (39.2%), rapeseed cake (35.7%), rapeseed meal (38.6%), cottonseed cake (36.3%), flaxseed meal (34.8%), sunflower meal (36.5%), DDGS (27.5%), and corn germ meal (20.8%), alfalfa powder (19.1%), wheat bran (15.7%), rice bran meal (15.1%), *etc.* [22-23], so it is a kind of high-quality animal protein feed. The crude fat content of black soldier flies (34.04%) is equivalent to that of yellow mealworms (34.10%), lower than that of sterile soldier maggots (58.50% – 68.20%), and higher than that of meat and bone meal (13.52%), shrimp shell meal (10.27%), earthworm meal (8.5%), and protein feed mentioned above[2,22-24]. The protein content in black soldier fly larvae and crushed insect powder fully meets or exceeds the standards of *High Quality Animal Protein* specified in *Catalogue of Feed Ingredients* issued by the Ministry of Agriculture, and can be used for livestock, poultry, fish, and culture of special species.

The nutritional components of black soldier flies are different due to different foods fed to its larvae, and a low protein content in the food is not conducive to the accumulation of nutritional components[16,25]. In this study, the crude protein content in powder of black soldier flies cultured with dead pig meat and bone meal is equivalent to those cultured with kitchen waste (42.05%) and mixed shrimp waste and bean curd residue (40.24% – 44.80%), higher than those cultured with pig manure (35.36%) and dregs (33.28% – 35.1%), and lower than those cultured with chicken manure (43.31% – 48.72%), tofu residue (52.3%), and peanut cake (50.76%)[5,8,16-18,20,26]. The crude fat content of black water fly powder in this study was 34.04%, which is equivalent to that in kitchen waste farming (31.2% – 33.70%), and higher than that in mixed farming of crayfish waste and tofu residue (18.63% – 22.63%), pig manure and chicken manure farming (4.30% – 21.6%), and herb residue farming (22.61%)[16-17,20,26-27]. The main reason for these differences may be different types and proportions of nutritional substrates for black soldier flies.

The nutritional components of the same type of nutritional substrate for breeding black soldier flies are also different. Huang *et al.*[6] reported that a dark environment was conducive to the growth and fat deposition of black soldier fly larvae, while a light environment was conducive to the protein deposition of black soldier fly larvae. As for chicken manure farming, He *et al.*[16] reported that the crude protein and crude fat contents of black soldier flies in chicken manure farming were 45.90% and 4.30%, respectively, while Yi *et al.*[27] found that the crude protein and crude fat contents of black soldier flies cultured in chicken manure were 38.0% and 21.60%, respectively. Similarly, in the case of kitchen waste aquaculture, Zhang *et al.*[26] reported that the crude protein and crude fat contents of black soldier flies in kitchen waste culture were 46.25% and 33.70%, respectively, while Cai *et al.*[9] reported that the crude protein and crude fat contents of black soldier flies in kitchen waste culture were 40.60% and 33.70%, respectively. The reason for this difference may be related to the breeding cycle and environmental factors of black soldier flies.

Effects of DPMBM on amino acid composition of black soldier flies

In this study, a total of 17 amino acids were detected in black soldier flies, with a total amino acid content (TAA) of 37.93%. Among the 17 amino acids, Glu showed the highest content (4.65%), and the umami amino acid content (DAA) was 13.43%, and the EAA/TAA and EAA/NEAA values were 34.47% and 52.61%, respectively, which is basically consistent with the report by Cai *et al.*[9] that the total amino acid content of black soldier flies cultured in kitchen waste was 36.16%, and the total essential amino acid content was 16.76%, accounting for 46.35% of the total amino acid content. According to the ideal model of FAO/WHO, high-quality proteins have an EAA/TAA of about 40% and an EAA/NEAA of over 60% of their amino acids. In this study, the amino acid composition of black soldier flies was slightly lower than the above requirements, but the values were still better than meat bone meal (EAA/TAA value of 32.90%, EAA/NEAA value of 49.14%)[2] and shrimp shell meal (EAA/TAA value of 31.23%, EAA/NEAA value of 45.42%)[22]. In order to improve the feeding value of black soldier flies, a certain amount of essential amino acids can be added to increase their balance.

Effects of DPMBM on fatty acid composition of black soldier flies

In this study, the fatty acid composition of black soldier flies fed with the residue after harmless treatment of dead pigs was mainly oleic acid (C18∶1), palmitic acid (C16∶0) and lauric acid (C12∶0), and saturated fatty acids accounted for more than

60% of the total fatty acid content, which is consistent with the research results of Li *et al.* [8] and Xu *et al.* [25]. However, He *et al.* [16] found that the content of unsaturated fatty acids in black soldier flies was higher than 50%, which might be related to the types and proportions of nutritional substrates, growth age and light conditions of black soldier flies[6]. Xu *et al.* [25] reported that the oil quality in black soldier flies was improved by adjusting the amount of algal residue added, and n-3PUFA in Schizochytrium residue in the diet could effectively enriched. Meanwhile, Li *et al.* [5] reported that adding 2% flaxseed oil to the diet for black soldier fly larvae could improve the growth performance and crude fat content of black soldier fly larvae, as well as improving the n-6/n-3 PUFAs ratio in the larvae, which to some extent enhanced the economic value of black soldier fly larvae. The types and contents of fatty acids in black soldier flies are different from the nutritional substrates used to feed larvae, so the value of fatty acids in black soldier flies can be improved by adjusting the ratio of nutritional substrates.

Effect of DPMBM on the safety of mineral elements and heavy metals in black soldier flies

In this study, the mineral elements of black soldier flies fed on the residue from harmless treatment of dead pigs were various and abundant especially of Ca at the highest content, followed by K, Mg, and Na. Among the trace mineral elements, Mn content was the highest, followed by Fe, Zn, and Cu. However, their contents of heavy metals Cr (0.955 0 mg/kg), Cd (0.368 5 mg/kg), As (0.196 5 mg/kg), Pb (0.146 5 mg/kg) and Hg (0.003 2 mg/kg) were far lower than the limit standards of Cr (5 mg/kg), Cd (2 mg/kg), As (2 mg/kg), Pb (10 mg/kg) and Hg (0.1 mg/kg) specified in GB 13078-2017 *Hygienical Standard for Feeds*. Therefore, black soldier flies had a lower risk of heavy metal residues and higher safety, which is consistent with the research results of raising black soldier flies from kitchen waste[9,15], traditional Chinese medicine residue[18] and diseased and dead pig feed[28].

The residue from harmless treatment of dead pigs used for feeding black soldier flies has high nutritional value and good safety, and can be used as a high-quality feed protein source, fat source, and mineral element supplement for livestock, poultry, aquatic animals, and special economic animal feed. The utilization of waste materials from the treatment of DPMBM by black soldier flies has great promotion and application value.

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(Continued on page 92)

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(Continued from page 38)

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