ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Effects of meal processing of black soldier fly on standardized amino acids digestibility in pigs
Running Title (within 10 words)	Black soldier fly as a sustainable protein source
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through ((Companion Animal Life Cycle Industry Technology Development Program), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(322094-3)
Acknowledgements	
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Hosseindoust A, Ha S.H, Mun J.Y. Data curation: Hosseindoust A, Kim J.S. Formal analysis: Hosseindoust A, Mun J.Y. Validation: Hosseindoust A, Ha S.H. Investigation: Ha S.H, Mun J.Y. Writing - original draft: Hosseindoust A, Ha S.H, Mun J.Y, Kim J.S. Writing - review & editing: Hosseindoust A, Ha S.H, Mun J.Y.
Ethics approval and consent to participate	The animal care and experimental protocols used in this study received approval by the Institutional Animal Care and Use Committee of Kangwon National University (Ethical code: 210503-6).

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Abstract

The aim of this study was to investigate the effect of incorporating black soldier fly (BSF) larvae and its processed form as an alternative source of protein to fish meal on the digestibility of amino acids (AA) in weaned pigs. Four cannulated pigs with an initial bodyweight of 13.25 ± 0.25 kg and aged 30 days were subjected to a 4 × 4 Latin square design with three treatments, as well as a nitrogen-free treatment. The diets used for each treatment consisted of a fish meal diet (FM), a diet containing BSF larvae meal (BSFM), and a diet containing extruded BSF (BSFE). The study was conducted over four stages, with a total duration of 28 days. The apparent ileal digestibility (AID) of protein was higher in the FM treatment compared with the BSFM. Among essential AA, the AID of Arg, His, Leu, and Thr were higher in the FM compared with the BSFM and BSFE. A greater AID of Ile and Phe was observed in pigs in the FM treatment compared with the BSFM. The average AA digestibility did not show any difference between treatments. Among non-essential AA, the AID of Ala ($p = 0.054$) and Glu ($p = 0.064$) tended to be increased in the FM compared with the BSFM. Among essential AA, the standardized ileal digestibility (SID) of Arg, His, Ile, and Leu were higher in the FM compared with the BSFM. Among non-essential AA, the SID of Cys ($p = 0.074$) tended to be increased in the FM compared with the BSFM. In conclusion, the processing and thermal conditioning techniques utilized for BSF larvae meal showed a tendency for increased AA digestibility. Therefore, when formulating a diet, it is important to take into account the difference in AA digestibility between fish meal and BSF larvae meal.

Keywords: Fish meal, larva meal, standardized, apparent, extrusion, sustainable

INTRODUCTION

Black soldier fly (BSF) larvae have gained increasing attention as a potential contributor to sustainable agriculture [1,2]. The utilization of insect larvae in agriculture can have several positive impacts, including the provision of animal feed, waste management, and carbon sequestration [3–6]. The potential role of BSF larvae as a source of nutrition for livestock, particularly swine, has been a topic of growing interest in recent years [7,8]. BSF larvae are rich in protein, lipids, and other essential nutrients, making them suitable feed for livestock. This presents a sustainable alternative to conventional feed sources, such as soybeans and fish meals, which are frequently cultivated or harvested using environmentally damaging practices [1]. The high feed conversion efficiency of BSF larvae translates to a reduction in the amount of feed required to produce a given amount of meat, making BSF larvae a valuable component of swine diets. BSF larvae are capable of decomposing a diverse array of organic materials, including plant residues, food waste, and manure. This process sequesters carbon and other nutrients in the soil, helping to mitigate greenhouse gas emissions and enhance soil health.

Black Soldier Fly larvae meal and fish meal are both commonly employed as sources of protein in swine nutrition. However, there are certain disparities in their composition that are imperative to consider when formulating swine diets. In terms of crude protein (CP), BSF larvae meal generally exhibits higher levels compared to fish meal [4,9]. However, fish meal is often deemed to have a more balanced amino acid (AA) profile in comparison to BSF larvae meal [3]. Particularly, fish meal is rich in essential AA, such as lysine, that play a crucial role in growth and development [3]. Conversely, BSF larvae meal may be lower in some essential AA, particularly methionine, which could limit its use as a standalone protein source in swine diets [7,10,11]. Fish meal is frequently higher in unsaturated fat content compared to BSF larvae meal, which could have beneficial effects on swine health. Therefore, its essential to increase our knowledge about the digestibility of each individual AA in order to formulate pigs diet professionally and correctly with the highest efficiency. It is crucial to enhance our understanding of the degree to which each unique AA can be digested, in order to develop a swine diet that is accurately formulated, and capable of achieving the highest possible efficiency.

The quality of BSF larvae meal as a feed ingredient can be significantly influenced by various processing and thermal conditioning techniques. Thermal conditioning, such as roasting or extrusion, has the potential to improve the digestibility of BSF larvae meal by denaturing its proteins and breaking down the cell walls, thus improving the bioavailability of nutrients. However, high-temperature thermal conditioning may result in a loss of certain nutrients, including vitamins [12]. Thermal conditioning is a process that involves heating a feed ingredient, to increase its digestibility and enhance the release of nutrients, such as AA [12]. The process of thermal conditioning results in the denaturation of proteins, which makes the AA more accessible to digestive enzymes. This, in turn, leads to an increase in the overall digestibility of AA in the feed ingredient. There are several ways to perform thermal conditioning, including steam treatment, extrusion, and roasting. The choice of method depends on the desired end product, the equipment available, and the costs involved.

Extrusion, on the other hand, involves heating and compressing the feed ingredient through a die, resulting in a high-temperature, short-time exposure that results in an increase in digestibility [12,13]. In addition to increasing the digestibility of AA, thermal conditioning can also have other benefits, such as reducing anti-nutritional factors, improving palatability, and increasing the stability of the feed ingredient during storage. It is important to note that the degree of improvement in AA digestibility is dependent on various factors, such as the feed ingredient, the processing conditions, and the type of thermal conditioning method used.

MATERIALS AND METHODS

The experiments were conducted at the farm facility of Kangwon National University and were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, South Korea (ethical code: KW-210503–6).

Heat processing

For the BSFE diet, the mixture of BSF (50%) and corn flour (50%) was extruded by subjecting the mash diet to a 300 hp twin-screw expander (Brabender Mod. DSE 35/7D) with 316 rpm screw speed, and 47-atmosphere pressure in 2.8 mm die size.

Cannulation and sampling

A T-cannula was surgically implanted in the distal ileum of four weaned pigs. The pigs with an average of 13.25 ± 0.25 kg initial body weight (BW) were used in 4 periods to determine apparent ileal digestibility (AID) and standardized ileal digestibility (SID) contents of 3 feed sources (FM, BSFM, and BSFE; Table 1) in a 4 × 4 complete Latin square design. To determine basal endogenous ileal AA flow, one of the four pigs was fed a nitrogen-free diet. Each experimental period, which consisted of a 4-day adaptation period to the experimental diets followed by a 3-day total collection of samples, lasted for 7 days.

The SID calculation of AA was calculated based on the following equation described by Jeon et al. [14]:

$$AID\ (\%) = [1 - (AA_{digesta/AAdiet}) \times (Cr_{diet}/Cr_{digesta})] \times 100,$$

$$I_{AAend} = (AA_{digesta}) \times (Cr_{diet}/Cr_{digesta}),$$

$$SID\ (\%) = AID + (I_{AAend}/AA_{diet}) \times 100,$$

In the equations provided, AID represents the apparent ileal digestibility based on dry matter (DM); AA_{diet} and $AA_{digesta}$ represent the AA concentrations of the diet and ileal digesta, respectively (g/kg of DM); Cr_{diet} and $Cr_{digesta}$ are the chromium concentrations of the diet and ileal output, respectively (g/kg of DM); and I_{AAend} stands for the basal ileal endogenous loss of AA (g/kg of DM intake).

In the conduct of the experiment, individual housing was provided for the pigs via the use of metabolism cages measuring 1.2 m × 1 m, which were equipped with a feeder, fully slatted floors, and urinary trays that facilitated the separate collection of urine and feces from each animal. The temperature within the rooms was maintained at 22 °C, and a 20-hour light and 4-hour dark regimen were employed. The experimental diets were specially formulated, as detailed in Tables 2 and 3, and included additions of vitamins and minerals to meet the estimated requirements as outlined by the National Research Council [15]. The pigs were provided with feed at a daily allowance of 2.5 times the estimated energy requirement for maintenance (2.5 × 197 kcal of metabolizable energy/kg of $BW_{0.60}$, [15]), which was divided into two equal meals and offered at 09:00 and 17:00.

The first three days were designated as an adaptation period to the diets, and on the fourth day, a marker (0.5% chromic oxide) was added to the meal. Fecal samples were obtained as a means of identifying the marker present in the excrement. On the seventh day of the study, a secondary marker containing 0.25% chromium oxide was administered during the morning meal, and quantitative fecal collection was continued until the emergence of the second marker, as per the marker-to-marker methodology [13]. Collection of urine began at 09:00 on the eighth day and ended at 09:00 on the thirteenth day, with the urine being collected in a bucket containing 50 mL of 6 mol/L HCl. The entire fecal output and a portion (20%) of the collected urine were promptly preserved at a temperature of -20°C. The digestible energy and metabolizable energy values for each experimental component were determined using the differential technique, with reference to the chromium oxide (Cr) concentration (0.25%) in the feed, digesta, and feces [13]. Fecal samples were air-dried, ground, and analyzed for gross energy via bomb calorimetry (Model 1241, Parr Instrument Co., Molin, IL, US), while urine samples were freeze-dried prior to analysis. The experiment was conducted for 28 d.

Crude protein and amino acids determination

The DM (method 930.15), CP (method 990.03), and ether extract (EE; method 2003.03) of the experimental diets and excreta samples were analyzed in triplicate following the guidelines of AOAC [16]. Chromium concentration was evaluated using an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) through the procedure outlined by Hosseindoust et al. [17]. The AA composition of both feed samples and ileum contents was determined via high-performance liquid chromatography (Waters 486, Waters Corp., Milford, MA) after acid hydrolysis as per the methodology established by Lee et al. [18]. The determination of methionine and cysteine was carried out after oxidation with performic acid in accordance with the technique developed by Kim et al [12].

Statistical analysis

The data was analyzed using SAS (SAS Inst. Inc., Cary, NC, US). A complete Latin square design was employed to compare the AID and SID AA in FM, BSFM, and BSFE treatments. The GLM procedure was utilized with individual pigs as experimental units to conduct the analysis. Statistical significance was determined at $p < 0.05$ and the tendency was considered at $0.05 \leq p < 0.10$. The AA digestibility were analyzed on an individual pig basis.

RESULTS

Apparent ileal digestibility

The AID of protein was higher in the FM treatment compared with the BSFM (Table 3). Among essential AA, the AID of Arg, His, Leu, and Thr were higher in the FM compared with the BSFM and BSFE. A greater AID of Ile and Phe was detected in pigs in the FM treatment compared with the BSFM. The AID of Lys, Met, Trp, and Val was unaffected. The average AA digestibility did not show any difference between treatments. Among non-essential AA, the AID of Ala, Asp, Cys, Glu, Gly, Pro, Ser, Tyr, and average digestibility did now differ among the treatments, however, the AID of Ala ($p = 0.054$) and Glu ($p = 0.064$) tended to be increased in the FM compared with the BSFM.

Standardized ileal digestibility

Among essential AA, the SID of Arg, His, Ile, and Leu were higher in the FM compared with the BSFM (Table 4). There was no difference in the SID of His, Lys, Met, Phe, Thr, Trp, and Val. Moreover, there was no difference in SID of essential AA between the FM and BSFE treatments. The average SID of AA digestibility did not show any difference between treatments. Among non-essential AA, the SID of Ala, Asp, Glu, Gly, Pro, Ser, Tyr, and average digestibility did now differ among the treatments, however, the SID of Cys ($p = 0.074$) tended to be increased in the FM compared with the BSFM.

Discussion

The concept of AID refers to the proportion of the ingested nutrients that are absorbed and utilized by the host organism after passing through the ileum, the final segment of the small intestine. In the context of BSF larvae meal fed to pigs, the AID of the meal various nutrients can be determined experimentally through techniques such as the ileal cannulation method, whereby the contents of the ileum are collected and analyzed to quantify the AID of the nutrients in question. In the current study, the AID of essential AA were relatively more reduced rather than SID in the BSFM treatment. It is noteworthy that the AID of BSF meal in pigs may vary depending on various factors such as the source and quality of the meal, the age and weight of the pigs, as well as the overall dietary regimen. Nevertheless, previous studies have demonstrated that BSF larvae meal constitutes a valuable source of both protein and energy for pigs, with AID values for CP ranging from 70 to 85% [7,19].

In previous research, BSF larvae or prepupae products have been reported to possess an advantageous AA profile in comparison to soybean meal [1]. However, the results of the present study revealed lower AA digestibility in BSF in comparison to FM. Studies conducted earlier on the nutritional value of BSF larvae products in swine have employed dried BSF larvae meal, which is also referred to as dried full-fat BSF larvae meal, which has an average CP content of 42% ranging from 35.9% to 48.1% and an average EE content of 42.5% ranging from 36.8% to 48.1% [2,3,20]. The BSF in our study contained 27.55 % ether extract, which may be responsible for the lower digestibility of AA. However, 6% soy oil was added to the FM diet but it still contained lower total EE rather than the BSFM treatment. The AID of AA in BSF was found to be consistent with previously reported values [2]. The AID of Arg, Ile, and Lys in BSF was relatively higher than the values that are previously reported for BSF in growing pigs [2], however, the relative AID of all non-essential AA including Ala, Asp, Cys, Glu, Gly, Pro, Ser, and Tyr were considerably lower than the AID values of a previous report [2]. Our study showed a high difference between the AID and SID of proline. The proline is known to exhibit high endogenous losses, which are thought to be caused by the poor reabsorption of mucin [21]. However, there is no information in the literature regarding the effect of BSF on endogenous losses. Tan et al. [2] recently conducted a study to investigate the digestibility of AA in full-fat BSF prepupae. According to their findings, the AID coefficients for AA ranged from 0.641 to 0.821, while the SID coefficients ranged from 0.767 to 1.177. However, the AID of lysine tended to decrease as the amount of BSF prepupae in the diet increased. The authors reported a SID coefficient of 0.776 for lysine in BSF prepupae, and suggested that an overestimation of the SID coefficient may have contributed to the reduced AID of lysine in diets containing higher levels of BSF. It is worth noting that there is currently no information available on the impact of BSF on endogenous losses. In comparison to a previous experiment involving pigs of the same age, the AID of AA in the control diets used in Tan et al. [2] study was slightly lower.

As the aim of this study was to test the BSF larva meal in its natural form, we were unable of reducing the fat content. However, the BSF meal was mixed with corn to increase the extrusion performance, which indeed reduced the total fat content. The mechanisms by which high dietary fat influences the digestibility of AA in pigs can be because fat increases the viscosity of the intestinal contents, which slows down the transit time and reduces the contact time between the digesta and digestive enzymes [1,6]. This can decrease the digestibility of AA, particularly in the small intestine, where the majority of digestion and absorption of AA occurs. Additionally, high-fat diets have been shown to stimulate the secretion of bile, which has been suggested to have a negative impact on the digestibility of some amino acids, such as methionine [7,19]. We did not evaluate the abundance of microbiota, however, high-fat diets can induce changes in the gut microbiome, altering the balance of bacterial populations and potentially suppressing the production of digestive enzymes involved in amino acid metabolism [22], which could lead to a reduction in AA digestibility.

Conclusion

In conclusion, while BSF meal exhibits promising potential as a protein source for pigs, further research is necessary to thoroughly understand its suitability as a feed ingredient and to determine optimal inclusion levels in pig diets. The processing and thermal conditioning techniques utilized for BSF larvae meal can significantly impact its quality as a feed ingredient. It is therefore important to carefully consider these techniques to ensure the optimal quality and nutritional value of BSF larvae meal for use in livestock diets. The selection of either BSF larvae meal or fish meal as a protein source in swine diets will depend on the specific dietary requirements of the swine and the accessibility and cost of these feed ingredients. A balanced diet that takes into account the nutritional disparities between these two feed ingredients can support the optimal growth and health of swine.

REFERENCES

1. Kim B, Bang HT, Kim KH, Kim MJ, Jeong JY, Chun JL, et al. Evaluation of black soldier fly larvae oil as a dietary fat source in broiler chicken diets. J Anim Sci Technol. 2020;62:803-811. https://doi.org/10.1186/s40781-020-00383-7

2. Tan X, Yang HS, Wang M, Yi ZF, Ji FJ, Li JZ, et al. Amino acid digestibility in housefly and black soldier fly prepupae by growing pigs. Anim Feed Sci Technol. 2020;263:114442. https://doi.org/10.1016/j.anifeedsci.2020.114459

3. Hong J, Kim YY. Insect as feed ingredients for pigs. Anim Biosci. 2022. 2022;53:563-571. https://doi.org/10.1186/s43793-022-00123-4

4. Lee J-H, Kim T-K, Cha JY, Jang HW, Yong HI, Choi Y-S. How to develop strategies to use insects as animal feed: digestibility, functionality, safety, and regulation. J Anim Sci Technol. 2022;64:409–31. https://doi.org/10.5187/jast.2022.e27

5. Lee S, Choi YS, Jo K, Kim TK, Yong HI, Jung S. Quality characteristics and protein digestibility of Protaetia brevitarsis larvae. J Anim Sci Technol. 2020;62:72-79. https://doi.org/10.1186/s40781-020-00394-4

6. Kim TK, Yong HI, Jung S, Sung JM, Jang HW, Choi YS. Physicochemical and textural properties of emulsions prepared from the larvae of the edible insects Tenebrio molitor, Allomyrina dichotoma, and Protaetia brevitarsis seulensis. J Anim Sci Technol. 2021;63:628-639. https://doi.org/10.1186/s40781-021-00318-7

7. Tansil F, Pezzali JG, Cargo-Froom C, Huber L-A, Kiarie EG, Courtney-Martin G, et al. Evaluation of standardized ileal digestibility of amino acids and metabolic availability of methionine, using the indicator amino acid oxidation method, in black soldier fly larvae (Hermetia illucens) meal fed to growing pigs. J Anim Sci. 2023;101:skac420. Available from: https://doi.org/10.1093/jas/skac420

8. Crosbie M, Zhu C, Shoveller AK, Huber LA. Standardized ileal digestible amino acids and net energy contents in full fat and defatted black soldier fly larvae meals (Hermetia illucens) fed to growing pigs. Transl Anim Sci. 2020;4:144. https://doi.org/10.1093/tas/txaa178

9. Ko HS, Kim YH, Kim JS. The produced mealworm meal through organic wastes as a sustainable protein source for weanling pigs. J Anim Sci Technol. 2020;62:10. https://doi.org/10.1186/s40781-020-00378-4

10. Osunbami OT, Adeola O. Regression method-derived digestible and metabolizable energy concentrations of partially defatted black soldier fly larvae meal for broiler chickens and pigs. Livest Sci. 2022;264:105042. https://doi.org/10.1016/j.livsci.2021.104584

11. Chia SY, Tanga CM, Osuga IM, Alaru AO, Mwangi DM, Githinji M, et al. Black Soldier Fly Larval Meal in Feed Enhances Growth Performance, Carcass Yield and Meat Quality of Finishing Pigs. J Insects as Food Feed. 2020;62:10. https://doi.org/10.1186/s40781-020-00378-4

12. Kim JS, Hosseindoust AR, Shim YH, Lee SH, Choi YH, Kim MJ, et al. Processing diets containing corn distillers' dried grains with solubles in growing broiler chickens: Effects on performance, pellet quality, ileal amino acids digestibility, and intestinal microbiota. Poult Sci. 2018;97:3038-45. https://doi.org/doi:10.3382/ps/pey100.

13. Lee JH, Hosseindoust A, Kim MJ, Kim KY, Choi YH, Moturi J, et al. Effects of hot melt extrusion processed nano-iron on growth performance, blood composition, and iron bioavailability in weanling pigs. J Anim Sci Technol. 2019;61:51-61. https://doi.org/10.5187/jast.2019.61.3.131.

14. Jeon SM, Hosseindoust A, Choi YH, Kim MJ, Kim KY, Lee JH, et al. Comparative standardized ileal amino acid digestibility and metabolizable energy contents of main feed ingredients for growing pigs when adding dietary β-mannanase. Anim Nutr. 2019;5:85-90. https://doi.org/10.1016/j.aninu.2018.10.004.

15. NRC. Nutrient Requirements of Swine: Eleventh Revised Edition. Natl. Acad. Press. 2012. https://doi.org/10.17226/13234.

16. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International. Assoc Off Anal Chem Int. 2007;1. https://doi.org/10.1002/jps.2600530630.

17. Hosseindoust A, Lee SH, Gook Nho W, Song YH, Shin JS, Laxman Ingale S, et al. A dose–response study to evaluate the effects of pH-stable β-mannanase derived from Trichoderma citrinoviride on growth performance, nutrient retention, and intestine morphology in broiler chickens. Ital J Anim Sci. 2019;18:147-154. doi.org/10.1080/1828051X.2018.1500872

18. Lee SH, Hosseindoust A, Laxman Ingale S, Rathi PC, Yoon SY, Choi JW, et al. Thermostable xylanase derived from Trichoderma citrinoviride increases growth performance and non-starch polysaccharide degradation in broiler chickens. Br Poult Sci. 2020;61:57-62. https://doi.org/10.1080/00071668.2019.1673316

19. Kar SK, Schokker D, Harms AC, Kruijt L, Smits MA, Jansman AJM. Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. Sci Rep. 2021;11:1-16. https://doi.org/10.1038/s41598-021-94604-8

20. Ji YJ, Liu HN, Kong XF, Blachier F, Geng MM, Liu YY, et al. Use of insect powder as a source of dietary protein in early-weaned piglets. J Anim Sci. 2016;94:111-116. https://doi.org/10.2527/jas.2015-9555

21. Stein HH, Trottier NL, Bellaver C, Easter RA. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J Anim Sci. 1999;77:1180-87. https://doi.org/10.2527/1999.7751180x

22. Lauridsen C. Effects of dietary fatty acids on gut health and function of pigs pre- And post-weaning. J. Anim. Sci. 2020;98:skaa086. https://doi.org/10.1093/jas/skaa086

Table 1. Ingredient and calculated composition of experimental diets (as-fed diets).

Item, %	N-free	FM	BSFM	BSFE
Ingredients				
Corn starch	81.5	63.43	54.16	27.01
Fishmeal	-	27.37	-	-
BSF-larva meal	-	-	42.76	-
BSF-larva expanded	-	-	-	69.61
Sucrose	10.0	-	-	-
Glucose	5.0	-	-	-
Soy oil	-	6.0	-	-
Choline chloride	0.05	0.05	0.05	0.05
Tri-calcium phosphate	1.68	1.40	1.25	1.25
Limestone	0.62	0.60	0.63	0.63
Salt	0.30	0.30	0.30	0.30
Mineral premix[1]	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.30	0.30	0.30	0.30
Chromium oxide	0.25	0.25	0.25	0.25
Total	100	100	100	100
Analysed composition, %				
ME, kcal/kg (calculated)	3,578	4,248	4,268	4,411
Crude protein	-	18	18	18
Ether extract	-	8.83	11.12	10.59
Calcium	0.71	0.70	0.72	0.70
Total phosphorus	0.66	0.61	0.63	0.64
Phosphorus (calculated)	0.32	0.32	0.32	0.32

[1]Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.
[2]Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin D3, 40 IU vitamin E, 5.0 mg vitamin K3, 5.0 mg vitamin B_1, 20 mg vitamin B_2, 4 mg vitamin B_6, 0.08 mg vitamin B_{12}, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.
*Abbreviation: FM, fish meal; BSFM, black soldier fly meal; BSFE, expanded black soldier fly meal; BSF, black soldier fly.

Table 2. Proximate values and amino acids composition of the protein sources.

Item, %	FM	BSFM	BSFE
Dry matter	92.10	94.05	91.11
Crude protein	65.77	43.78	25.35
Ether extract	10.34	27.55	15.96
Ash	19.41	12.15	6.78
Calcium	25.38	7.56	3.81
Phosphorus	21.25	0.90	0.61
Essential amino acid			
Arginine	3.26	1.95	1.56
Histidine	1.36	1.16	0.58
Isoleucine	2.50	1.63	0.93
Leucine	4.11	2.6	1.70
Lysine	4.35	2.95	1.28
Methionine	1.68	0.65	0.38
Phenylalanine	2.12	1.41	0.90
Threonine	2.41	1.43	0.81
Tryptophan	0.57	0.50	0.38
Valine	2.90	3.06	1.55
Non-essential amino acid			
Alanine	3.58	2.63	1.56
Aspartic	5.05	3.38	1.81
Cystine	0.34	0.50	0.28
Glutamic acid	7.11	4.82	2.69
Glycine	0.39	2.17	1.13
Proline	2.23	2.57	1.47
Serine	2.37	1.57	0.88
Tyrosine	1.55	2.25	0.99

*Abbreviation: FM, fish meal; BSFM, black soldier fly meal; BSFE, expanded black soldier fly meal.

Table 3. Effects of protein sources on apparent ileal digestibility in pigs.

Item[1], %	FM	BSFM	BSFE	SEM[2]	P-value
CP digestibility, %	73.81^{a}	71.60^{b}	72.16^{ab}	0.52	0.001
Essential amino acid digestibility, %					
Arginine	84.11^{a}	78.23^{b}	79.64^{b}	1.16	0.001
Histidine	80.89^{a}	76.50^{b}	77.37^{b}	1.27	0.020
Isoleucine	82.46^{a}	77.40^{b}	79.41^{ab}	1.29	0.001
Leucine	80.19^{a}	75.18^{b}	76.26^{b}	0.86	<0.001
Lysine	79.88	77.34	78.57	1.52	0.212
Methionine	82.50	81.55	81.60	2.16	0.766
Phenylalanine	80.87^{a}	74.70^{b}	76.58^{ab}	2.29	0.037
Threonine	78.93^{a}	74.31^{b}	75.47^{b}	1.28	0.031
Tryptophan	76.92	76.36	77.55	1.72	0.854
Valine	78.35	78.09	76.19	1.26	0.119
Average	80.51	76.97	77.86	1.83	0.413
Non-essential amino acid digestibility, %					
Alanine	76.63	75.00	77.01	0.80	0.054
Aspartic acid	69.83	68.50	69.59	1.24	0.536
Cystine	62.54	59.38	61.42	2.59	0.483
Glutamic acid	79.05	75.16	78.61	1.65	0.064
Glycine	45.83	37.21	43.80	8.64	0.592
Proline	32.61	30.16	32.79	6.37	0.900
Serine	75.00	73.33	73.13	1.90	0.568
Tyrosine	74.29	69.90	70.52	2.44	0.184
Average	64.47	61.08	63.36	1.27	0.233

[1]FM, fish meal; BSFM, black soldier fly meal; BSF-EP, expanded black soldier fly; CP, crude protein.

[2]Standard error of means

[a-b]means different superscript letters indicate significant differences (p <0.05).

Table 4. Effects of protein sources on standardized ileal digestibility in pigs.

Item[1], %	FM	BSFM	BSFE	SEM[2]	P-value
Essential amino acid					
Arginine	90.90^{a}	85.91^{b}	87.99^{ab}	1.16	0.005
Histidine	87.42	84.95	85.47	1.27	0.219
Isoleucine	89.49^{a}	85.48^{b}	86.48^{ab}	1.29	0.015
Leucine	87.13^{a}	84.34^{b}	85.55^{ab}	0.86	0.028
Lysine	87.21	84.34	85.83	1.52	0.172
Methionine	88.22	85.47	86.39	2.16	0.434
Phenylalanine	88.00	84.52	85.03	2.29	0.286
Threonine	85.24	84.46	82.95	1.28	0.324
Tryptophan	85.28	84.36	84.96	1.72	0.838
Valine	85.93	86.23	84.13	1.26	0.142
Average	87.48	85.01	85.48		
Non-essential amino acid					
Alanine	84.97	84.31	85.10	0.80	0.584
Aspartic acid	81.35	79.69	79.89	1.24	0.365
Cystine	76.07	69.68	73.78	2.59	0.074
Glutamic acid	86.89	84.39	85.64	1.65	0.342
Glycine	62.08	64.83	68.15	8.64	0.784
Proline	53.68	50.23	55.28	6.37	0.725
Serine	81.91	84.00	81.47	1.90	0.387
Tyrosine	84.49	80.84	80.16	2.44	0.196
Average	76.43	74.75	76.18		

[1] FM, fish meal; BSFM, black soldier fly meal; BSF-EP, expanded black soldier fly.

[2]Standard error of means

$^{a-b}$means different superscript letters indicate significant differences (p <0.05).