E3S Web of Conferences **363**, 03017 (2022) https://doi.org/10.1051/e3sconf/202236303017
INTERAGROMASH 2022

Nutritional value of black solder fly (*Hermetia illucens*) eggs and larvae reared on fermented milk industry waste as an ingredient of fish feed

Marina Mikhailova[1], *Konstantin* Zolotarev[1*], *Valeriya* Nakhod[1], *Tatiana* Farafonova[1], and *Anton* Mikhailov[1]

[1]Institute of Biomedical Chemistry, 10 bldg. 8, Pogodinskaya Str., Moscow, 119121, Russia

Abstract. Eggs and larvae of the black soldier fly are a new protein and lipid containing component, which seems to be quite promising for inclusion in a diet for fish feeding, since its rearing makes it possible to process wastes from various industries and agriculture. Omnivorous natures of black soldier fly, as well as high activity of amylases, lipases, and proteases are also advantageous. The nutritional value of eggs and larvae reared using fermented milk industry waste as a substrate was assessed by the content of total fat, total protein, water, macronutrients (K, Ca, Mg), micronutrients (Fe, Cu, Mn, Zn, Co, Se), and by amino acid composition as well. Chemical analysis has shown that the larvae are nutritionally valuable in total, but further data on fatty acid analysis and vitamin content are required. Black soldier fly eggs are not nutritionally valuable as a complete feed but may be used as a component of a diet.

1 Introduction

Black soldier fly (BSF) (*Hermetia illucens*) is a species of insects of the Diptera order, originally distributed in Central and South America [1], but in recent decades it has been introduced and has spreaded in Southern Europe, South and Southeast Asia, Africa, Australia, and Oceania [2]. It is caused by the ability of BSF to survive and reproduce in a fairly wide range of natural conditions [3].

Due to the omnivorous nature of BSF, the high activity of amylases, lipases, and proteases in its digestive tract [4], the BSF is of interest concerning the processing of various organic industrial and agricultural wastes into biomass suitable for feeding farm animals. In addition, this fly, unlike most other species, is not capable of flying over long distances at adult stage, which facilitates its industrial rearing. All these factors led to an increased interest of agricultural science in this insect species; the earliest studies were published in the late 1960s. [5]. To date, it has been ascertained that due to the high content of protein and calcium, as well as palatability, BSF larvae are a suitable dietary supplement for feeding, for example, pigs, although it is required to add some of the essential amino acids [6]. In addition, it has been found that replacing 10–20% of soy feed with larvae-

[*] Corresponding author: fireaxe@mail.ru

based feed for broilers (hens [7] and quails [8]) and 50–100% for laying hens [9] does not lead to deterioration in their physiological indicators and productivity. The results of these and other studies, as well as many years of agricultural experience, have led to acceptance of the protein obtained from BSF larvae for use as a component of diets for farm animals in the European Union [10].

As for the productivity and economic effect of using feeds based on BSF eggs and/or larvae for fish, the possibility of their use in aquaculture has not been fully studied and a number of issues need to be clarified [3].

It has been ascertained that chemical composition and nutritional value of BSF larvae varies greatly depending on the substrate that it feeds on. The literature provides data on the chemical composition of larvae grown on cattle, pig and chicken manure, as well as food waste (liver, fruit and vegetable waste, fish waste), but there is no data on the composition of BSF reared using fermented milk products manufacturing waste or expired fermented milk products [3]. Such data are of interest because this type of waste is present in large quantities throughout the world.

2 Materials and methods

Chemical analysis of larvae of BSF reared on fermented milk products manufacturing waste has been carried out. The larvae were reared at 26–28 °C for 2 weeks. The samples of living larvae were kept at -18 °C. Some larvae were left for development till egg laying at adult stage; egg samples were collected and also kept at -18 °C till analysis.

For determination of total fat content in the samples and preparation to further amino acid analysis, the samples were homogenized in a mortar, and then fat and water were removed using 96 % ethanol extraction (8 mL of ethanol to1 g of homogenate ratio) with rigorous shaking for 30 min at room temperature. After extraction, the mixtures were centrifuged at 6000 G for 15 min. The sediment was dried to constant weight. Concurrently, other portions of the samples were dried to constant weight as is; water content (moisture) was determined as a weight loss after such drying. Total fat content was determined as difference between weight losses after ethanol extraction followed by drying and after just drying. The determination was made in triplicate.

Amino acid concentrations in the samples after water and fat removal were measured using chromatographic analysis of their orthophtalic derivatives according to standard amino acid samples. First, 10 mg of the each sample was dissolved in 1 ml of distilled water. The resulting solution was 25× diluted, and 50 µl of the solution were dried up in an ampoule. Then, 100 µl of 6 M HCl was added to it and the ampule was sealed under vacuum. Acidic hydrolysis was performed over 24 hours and at 110 °C. After that, the ampoule was opened and the solution was dried up in the Eppendorf 5301 vacuum concentrator (Eppendorf, Hamburg, Germany). Finally, 50 µl of 0.1 M HCl was added to the dried sediment. The chromatographic separation was done using an Agilent 1200 series chromatographic system equipped with fluorescent detector and ZORBAX Eclipse AAA (5µm; 4.6 x 150 mm) column (Agilent Technologies, Santa Clara, CA). The mobile phases were 40 mM pH 7.8 phosphate buffer solution (Solution A) and 80% water solution of acetonitrile (Solution B). Borate buffer with pH 10.2 and o-phtalaldehyde were used for amino acid derivatization. The amino acid derivatives were eluted at a flow rate of 1 ml min^{-1} with a gradient of the Solution B. A total run time was 41 min including 3 min flushing with 63% Solution B and 2 min re-equilibration to 2% Solution B. The areas under the fluorescent chromatogram peaks of the analyzed samples and of the amino acid standards (Agilent Technologies, Santa Clara, CA) were measured. Some other details of the procedure are described in [11]. The amino acid analysis was made in triplicate. Total protein content was estimated as a sum of all essential and non-essential amino acid concentrations.

Concentrations of macronutrients (K, Ca, Mg) and micronutrients (Fe, Cu, Mn, Zn, Co, Se) in the samples of BSF eggs and larvae were measured with the Agilent 7500ce (Agilent Technologies, Santa Clara, CA) inductively coupled plasma mass spectrometer. About 0.5 g of each sample was digested in HNO_3 using Milestone Start D (Milestone S.r.l., Sorisole, Italy) microwave oven and then 12× diluted with deionized water. Concentrations of the metals were measured in quantitative mode using calibration standards manufactured by Sigma-Aldrich, St. Louis, MO. The final values of the concentrations were calculated using the measured values of concentrations in the diluted solutions, exact masses of samples used for determination of metals, and moisture values.

3 Results and discussion

The values of total water, fat and protein content are presented in Table 1.

Table 1. Total water, fat and protein content of black solder fly (BSF) eggs and larvae reared on fermented milk industry waste, % by weight (mean ± SD).

<table>
<tr><th>Parameter</th><th>Eggs</th><th>Larvae</th></tr>
<tr><td>Water content</td><td rowspan="2">56.1 ± 2.4 (overall)</td><td>55.1 ± 1.8</td></tr>
<tr><td>Total fat content</td><td>20.2 ± 1.3 by dry weight
9.07 ± 0.58 by wet weight</td></tr>
<tr><td>Total protein content</td><td>22.3 ± 1.1 by wet weight</td><td>41.8 ± 3.4 by dry weight
18.8 ± 1.5 by wet weight</td></tr>
</table>

As already mentioned, chemical composition of BSF larvae strongly depends on substrate they are reared on. Total protein content of larvae reared on manure or fruit and vegetable waste [3] is approximately at the same level (40–43% by dry weight) as of the studied eggs and larvae. Total fat content of larvae reared on manure is significantly higher (25–35% by dry weight) than of the studied eggs and larvae. Total fat content of larvae reared on fruit and vegetable waste is significantly lower (6–7% by dry weight) than of the studied eggs and larvae. See Fig. 1 for detailed comparison.

 https://doi.org/10.1051/e3sconf/202236303017

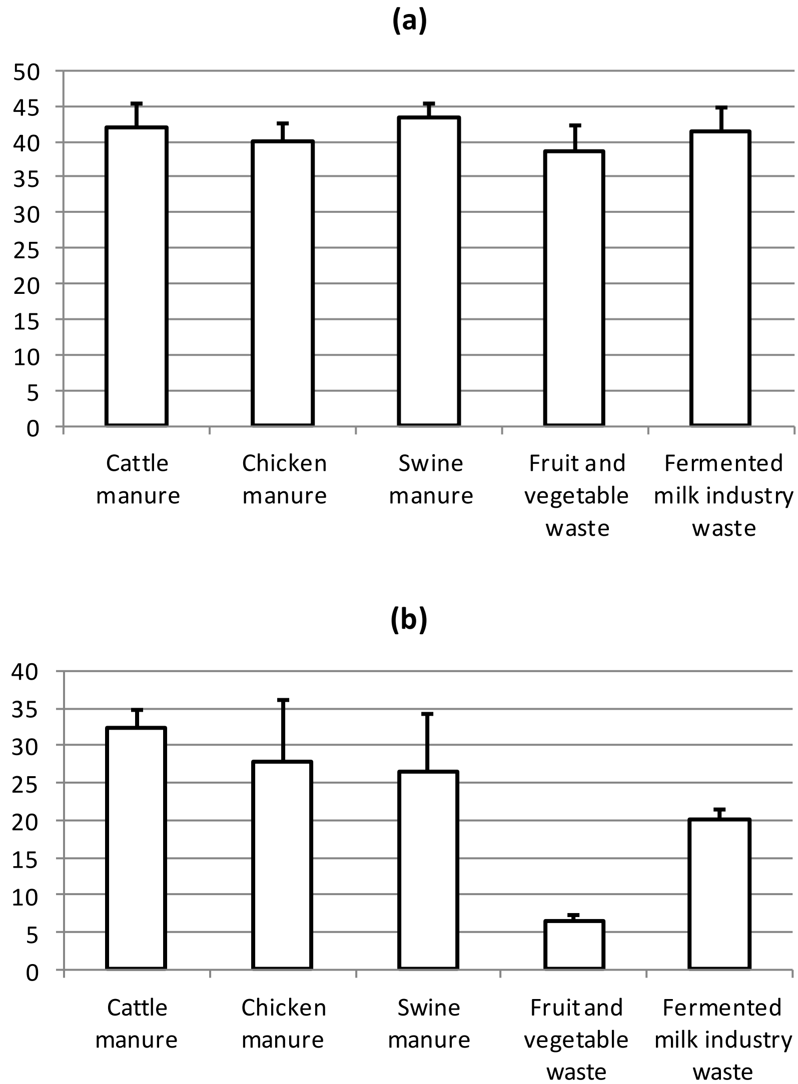

Fig. 1. Chemical composition of BSF larvae reared on manure, fruit and vegetable waste [3] and fermented milk industry waste (mean ± SD): (a) total protein content, % by dry weight; (b) total fat content, % by dry weight.

The values of concentrations of the essential amino acids compared with fish needs are presented in Table 2.

Table 2. Concentrations of essential amino acids in BSF eggs and larvae reared on fermented milk industry waste compared with fish needs, % by dry weight (mean ± SD).

Amino acid	Eggs	Larvae	Fish need [12]
Histidine	0.97 ± 0.10	2.23 ± 0.22	0.56
Threonine	1.02 ± 0.09	1.86 ± 0.20	0.52
Arginine	1.81 ± 0.19	3.47 ± 0.47	1.52
Tyrosine + phenylalanine	2.00 ± 0.20	5.08 ± 0.67	0.48
Valine	1.16 ± 0.12	2.09 ± 0.71	1.24
Methionine	0.26 ± 0.07	1.60 ± 0.88	0.64
Isoleucine	1.17 ± 0.10	1.99 ± 0.24	0.40
Leucine	1.79 ± 0.16	3.09 ± 0.39	0.72
Lysine	2.12 ± 0.18	3.42 ± 0.38	2.12

According to these data, BSF eggs do not meet the needs of fish for two essential amino acids: valine and methionine. The larvae meet the needs of fish for all essential amino acids.

The values of concentrations of macro- and micronutrients compared with fish needs are presented in Table 3.

Table 3. Concentrations of macro- and micronutrients in BSF eggs and larvae reared on fermented milk industry waste compared with fish needs, % by weight (mean ± SD).

Element	Eggs	Larvae	Fish need [12]
K	2680 ± 20	3000 ± 40	1600
Ca	138 ± 8	3050 ± 10	1220–2960*
Mg	590 ± 12	1550 ± 40	330–800*
Fe	59 ± 4	44 ± 2	30–300*
Cu	4.3 ± 0.1	11.0 ± 0.5	4
Mn	3.9 ± 0.3	185 ± 12	13
Zn	29 ± 0.8	39 ± 2	15–40*
Co	0.0005 ± 0.00006	0.0375 ± 0.0014	0.05–1.0*
Se	0.039 ± 0.007	0.088 ± 0.010	0.15–0.5*

*depends on fish species

According to Table 3, the content of macro- and micronutrients of the eggs does not meet the needs of fish for any of the key elements, except for potassium (K) and copper (Cu), but larvae satisfy all the needs, except for needs for iron (Fe), cobalt (Co) and selenium (Se).

Despite the existing need of aquaculture in Russia for feeds and the need of fermented milk industry for waste disposal, it is too early to talk about the potential of using BSF larvae reared on the studied substrate. On the one hand, it is a promising source of total protein, essential amino acids and macronutrients, but micronutrients must be added. In addition, it is required to analyze the content of vitamins, as well as to investigate the fatty acid composition of the larvae. The latter one should expose quantitative and qualitative composition of essential fatty acids and harmful hydrolyzed and oxidized lipids. Palatability and digestibility of BSF larvae-based feeds should also be studied.

Global experience of including black soldier fly larvae in fish feed is rather contradictory. On the one hand, 100% replacement of fishmeal with a protein-chitin concentrate from black soldier fly larvae in the feed for Mozambique and Nile tilapia (45%

in feed) did not affect significant changes in feed productivity and physiological-biochemical parameters of fish [13]. On the other hand, introduction of larvae biomass into the feed (33% in feed) decreased the growth of trout [14] and turbot (Pleuronectiformes); in the latter species, the digestibility of proteins also worsened and the palatability of the feed decreased [15].

Our study has shown that the eggs of BSF reared on fermented milk industry waste may not be used as a single feed for juvenile or adult fish in aquaculture. The eggs may be used only as a component or a supply in a feed for juvenile fish.

Acknowledgements

The work was performed within the framework of the Program for Basic Research in the Russian Federation for a long-term period (2021–2030) (No. 122030100170-5).

References

1. V. V. Gladun, Research Journal of Recent Sciences **4**, 111-113 (2019) doi:10.24189/ncr.2019.063
2. Gujarathi Gayatri R., Pejaver Madhuri K., Research Journal of Recent Sciences **2**, 65-66 (2013)
3. K. B. B. Fonseca, M. Dicke, J. J. A. van Loon, Journal of Insects as Food and Feed **3**, 105-120 (2017) doi:10.3920/JIFF2016.0055
4. W. Kim, S. Bae, A. Kim, et al., BMB Reports **44**, 387-392 (2011) doi:10.5483/BMBRep.2011.44.6.387.
5. C. C. Calvert, R. D. Martin, N. O. Morgan, Journal of Economic Entomology **62**, 938-939 (1969) doi: 10.1093/jee/62.4.938
6. H. P.S. Makkar, G.Tran, V. Heuzé, P. Ankers, Animal Feed Science and Technology **197**, 1-33 (2014) doi:10.1016/j.anifeedsci.2014.07.008
7. J. Zhang, L. Zheng, P. Jin, D. Zhang, Z. Yu, In: Abstract book of the international conference 'Insects to feed the world', 14-17 May 2014, Ede, the Netherlands, 14-17 (2014)
8. M. Cullere, G. Tasoniero, V. Giaccone, R. Miotti-Scapin, E. Claeys, S. De Smet, A. Dalle Zotte, Animal **10**, 1923-1930 (2018) doi: 10.1017/S1751731116001270
9. V. Maurer, M. Holinger, Z. Amsler, B. Früh, J. Wohlfahrt, A. Stamer, F. Leiber, Journal of Insects as Food and Feed **2**: 83-90 (2016) doi:10.3920/JIFF2015.0071
10. Commission Regulation (EU) 2017/893, Official Journal of the European Union **138**, 92-116 (2017)
11. M. V. Mikhailova, K. V. Zolotarev, A. N. Mikhailov, M. A. Sanzhakov, T. E. Farafonova, International Journal of Management and Humanities **4**, 1-5 (2019) doi:10.35940/ijmh.C0442.124419
12. V. Ya. Sklyarov, Feeds and fish feeding in aquaculture, Moscow, Russia: VNIRO Publishing (2008)
13. N. A. Ushakova, S. V. Ponomarev, Y. V. Fedorovykh, A. I. Bastrakov, Izvestiâ Ufimskogo naučnogo centra Rossijskoj akademii nauk **3**, 57-62 (2018) doi:10.31040/2222-8349-2018-0-3-57-62
14. S. St-Hilaire, C. Sheppard, J. K. Tomberlin, et al., Journal of the World Aquaculture Society **38**, 59-67 (2007) doi: 10.1111/j.1749-7345.2006.00073.x

15. S. Kroeckel, A.-G. E. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, C. Schulz, Aquaculture **364-365**, 345-352 (2012) doi: 10.1016/j.aquaculture.2012.08.041