# Estimation of applicability of protein hydrolysate of black soldier fly (*Hermetia illucens*) larvae prepared using enzymatic hydrolysis as a fish feed component

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**Abstract**. The special advantage of black soldier fly (BSF) (Hermetia illucens) larvae for use as feed is their high protein content and low chitin content, which makes the larvae easier to digest, making them nutritious for fish, farm animals and birds. It has been reported that BSF larvae contain all the amino acids necessary for animal protein, including the essential amino acids, also macro- and micronutrients. The technological parameters of BSF larvae protein hydrolysis procedure were optimized. Some physicochemical parameters of the hydrolysate and the yield of hydrolysis were estimated for the hydrolysate prepared according to the optimized parameters. The sterlet (Acipenser ruthenus) starter feed having 10% of its fishmeal portion substituted with BSF larvae protein hydrolysate has been tested. The BSF larvae protein hydrolysate may be included in fish feed formulations, especially in starter feeds.

## 1 Introduction

To ensure food security and promote sustainable development of agriculture in general and aquaculture in particular, it is necessary to develop the feeding basis. Currently, there is a shortage of protein raw materials for feed production in the world, and therefore the search for new sources of feed protein is relevant. Components with high protein content are necessary to meet the needs of animals for protein, and therefore for their complete development and highest productivity with less feed costs. In the domestic feed industry, the volume of production of feeds of animal origin has been decreasing, and their price is increasing. New high protein content components may reduce these concerns.

According to many researchers, insects may be of particular interest as protein raw materials, while in the last decade there has been a transition from the use of insects as a whole to their processing. Insects make up to about 80% of the global diversity of species,

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and they are also the most abundant class of animals in terms of quantity and biomass, which are distinguished by high fecundity and the ability to mass reproduction [1 – 3].

The most promising option for industrial production is the breeding of black soldier fly (BSF) (Hermetia illucens). It is reasonable due to the biological characteristics of this species, which are its advantages in cultivation. It takes 20 days to grow a larva; a full cycle of cultural reproduction takes one and a half months. Insects need an average of 2 feed units to produce 1 kg of biomass. For their cultivation, various wastes can be used: food and agricultural waste, slaughterhouse waste, the organic part of municipal solid waste, sewage sludge from domestic and agricultural waters, cattle, swine, or poultry manure, etc.

The larvae contain an average of 40% protein and 35% fat (by dry weight), are distinguished by a rich chemical composition of fatty acids and amino acids. The larvae are completely safe and harmless to humans: adults are inactive, do not eat, do not bite, and do not spread diseases. Moreover, they secrete substances inhibiting growth of bacteria, viruses, and other insects. Larvae, pupae and adults are not considered pests. Larvae, like earthworms, play an important role in the decomposition of organic substrates and contribute to the return of nutrients from these substrates back to the soil. The larvae can be used for composting household, food and agricultural waste. They are used as feed. BSF larvae, pupae and prepupae are eaten by poultry, fishes, pigs, cattle and other animals. This insect is one of the few insect species approved for use as feed in agriculture in the EU [4, 5].

The advantages of the BSF include environmental safety and the high speed of processing food waste and waste products from livestock farms and poultry houses. In terms of the intensity of waste processing into zoohumus, they are more than 6-fold more efficient than earthworms and compost worms; what the worms process in three months, the larvae will do in one week.

The BSF larvae recrement (i.e. zoohumus, or zoocompost) contains macro- and micronutrients, the full composition of the necessary organic compounds to enrich the soil, which helps to increase crop yields.

The special advantage of larvae for use as feed is their high protein content and low chitin content, which makes the larvae easier to digest, making them a useful food for fish, farm animals and birds. They increase immunity, natural weight gain, and egg production (in birds). The high concentration of the essential amino acid methionine in the larvae distinguishes this protein feed from other types of feed. The inclusion of BSF larvae or prepupae in the diet is an alternative to the inclusion of synthetic methionine. The high content of protein and other organic compounds in the larvae (higher than in fishmeal) would reduce the amount of fish caught for fodder purposes, which is important due to declining fish stocks [6, 7].

It has been reported that BSF larvae protein contains all the amino acids necessary for animal protein, including the essential acids: aspartate (8.25 ± 3.56%), threonine (3.97 ± 1.62)%. serine (4.49 ± 3.29%), glutamate (12.85 ± 2.01%), proline (6.02 ± 1.27%), glycine (5.63 ± 0.84%), alanine (7.25 ± 1.54%), valine (5.12 ± 0.78%), methionine (1.85 ± 0.16%), cystine (0.85 ± 0.43%), isoleucine (5.58 ± 0.88%), leucine (12.24 ± 1.8%), tyrosine (6.32 ± 0.73%), phenylalanine (4.35 ± 0.85%), histidine (4.21 ± 1.33%), lysine (5.75 ± 1.28%), arginine (4.20 ± 0.70%) [8]. According to our previously published data, the BSF larvae reared on fermented milk industry waste contain by dry weight 3.00 ± 0.04 g/kg of K, 3.05 ± 0.01 g/kg of Ca, 1.55 ± 0.04 g/kg of Mg, 44 ± 2 mg/kg of Fe, 11.0 ± 0.5 mg/kg of Cu, 185 ± 12 mg/kg of Mn, 39 ± 2 mg/kg of Zn, 37,5 ± 1.4 μg/kg of Co, 88 ± 10 μg/kg of Se, and therefore meet the fish needs for K, Ca, Mg, Cu, Mn и Zn [9].

Thus, it is reasonable to suggest that BSF larvae protein hydrolysate would also contain a valuable set of amino acids, macro- and micronutrients. Hydrolysis process increases the rate of absorption of nutrients, which especially helpful for fish fry with yet underdeveloped enzymatic system of the gastrointestinal tract.

## 2 Materials and methods

The BSF larvae (supplied by EcoBelok, Moscow, Russia) were homogenized and hydrolysed at 45 °C for 4 h with trypsin at pH = 7.2. The hydrolysate was filtered and dried in a spray drier. Some physicochemical parameters of the hydrolysate and the yield of hydrolysis were estimated. Amino acid composition of the hydrolysate was determined using an HPLC-based amino acid analyzer.

The samples of starter feed for sterlet (Acipenser ruthenus) were manufactured according to the standard accepted in Russia [10]; 10% of fishmeal portion was substituted with BSF larvae protein hydrolysate or the previously tested European mink (Mustela lutreola) flesh hydrolysate [11] as control. The sterlet larvae (0.025 g mean initial weight) were incubated in 1800 L tanks with 4 m2 water surfaces (10000 larvae per tank) with water recirculation at native temperature for 40 days (from May 12 till June 20). Water temperature, oxygen concentration and pH were controlled. The larvae were fed 14 times a day from 7 am till 8 pm with the amount of tested or control feed equal to 1.2% of fish biomass weight. After the experiment, the fish survival rate and weight gain were estimated. The sterlet starter feed testing was made in triplicate.

## 3 Discussion

The technological parameters of hydrolysis procedure were optimized during a series of preliminary laboratory trials; the optimal parameters are listed in the Materials and methods section. Then, the procedure was scaled up on a pilot installation. Some physicochemical parameters of the hydrolysate and the yield of hydrolysis were estimated for the hydrolysate prepared at the pilot installation (Table 1).

**Table 1.** Values of physicochemical parameters of BSF larvae protein hydrolysate and yield of hydrolysis.

| Parameter | Value (mean ± SD; n = 4) |
|---|---|
| Appearance | Brown chips |
| Water solubility | Partial |
| pH of 1% water solution | 7.11 ± 0.81 |
| Water content (% by weight) | 2.62 ± 0.64 |
| Free amino nitrogen content (% by weight) | 6.03 ± 1.16 |
| Yield of hydrolysis (% by weight) | 38.7 ± 8.2 |

The values of amino acid content are presented in Table 2.

**Table 2.** Amino acid composition of BSF larvae protein hydrolysate.

| Amino acid | Content (mg/g) (mean ± SD; n = 4) |
|---|---|
| Asp | 33.35 ± 2.67 |
| Thr | 13.78 ± 1.65 |
| Ser | 11.05 ± 0.99 |
| Glu | 45.09 ± 3.16 |
| Pro | 28.96 ± 2.03 |
| Gly | 20.13 ± 1.41 |
| Ala | 22.17 ± 1.55 |
| Val | 21.22 ± 2.76 |
| Met | 6.36 ± 0.51 |
| Ile | 17.10 ± 2.05 |

| | |
|---|---|
| Leu | 27.79 ± 3.61 |
| Tyr | 15.14 ± 1.82 |
| Phe | 15.51 ± 1.09 |
| His | 9.41 ± 0.75 |
| Lys | 18.69 ± 1.31 |
| Trp | 37.82 ± 4.92 |
| Arg | 10.43 ± 0.73 |
| Total | 353.99 ± 19.47 |

During the whole sterlet starter feed testing, the feed with BSF larvae protein hydrolysate had been attractive for the fish and eaten willingly. The results of the experiment are presented on Figure 1.

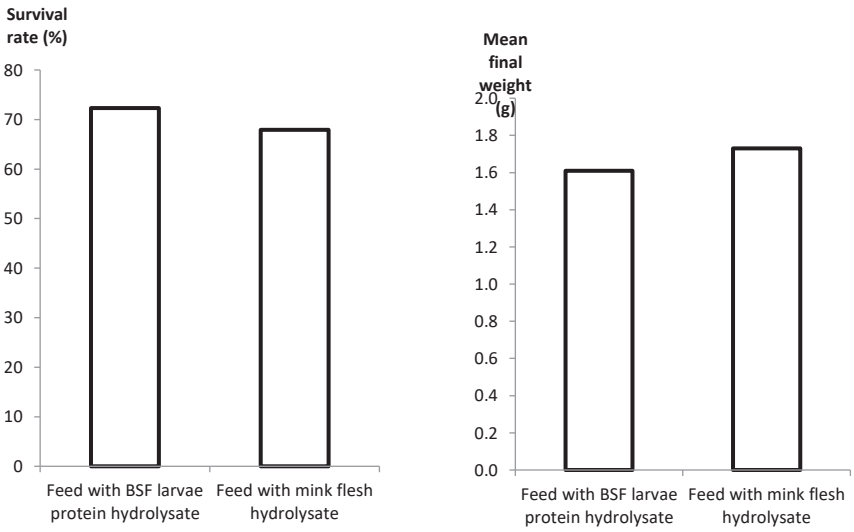


**Fig. 1.** Sterlet starter feed testing results: (A) survival rate of the fish after the experiment; (B) mean final weight of the fish (the mean initial weight is 0.025 g for both groups). *$p < 0.05$ relative to the control group (Mann–Whitney *U* test); n = 3

The mean final weight of the fish fed with the feed being studied was less than that in the control group; the possible reason is that survival rate in the test group was higher than that in the control one, so the stocking density in the test group at the end of experiment was higher.

## 4 Conclusions

Protein hydrolyzate of black soldier fly larvae can be recommended for use as a component of the starter feed formulation for sturgeon, and generally satisfies the needs of larvae for amino acids, macro- and microelements. Hydrolyzate is an accessible raw material for the production of feed, alternative to existing diets based on fishmeal and other sources of animal protein.

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