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Synthesis of selenium conjugated insects' protein in *Hermetia illucens* larvae as poultry feed

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Abstract. Selenium (Se) is an essential trace element for human health and livestock growth. The element can be fed in inorganic or organic forms, depending on the diet's chemical makeup and how it influences the accumulated amount of Se in the tissues of animals. Insects are known to be natural bio-accumulators of various nutrients, but research on the capacity of insects to be bio-enriched with Se in feed is still limited. The aims of this study were to synthesise Se conjugated insects' protein and examine how effectively they can deliver selenium to black soldier fly (BSF, *Hermetia illucens*) larvae. In this study, BSF larvae were reared on different substrates with four levels of dietary sodium selenite supplementation (0, 100, 200 and 400 mg/kg). BSF larvae were analysed for biomass yield, nutritional value, and Se content. We revealed that selenium content in the fortified BSF larvae was several orders of magnitude higher than in the unfortified ones. There were significant differences between doses of selenium delivered to BSF larvae by different selenium compounds. Inductively coupled plasma mass spectrometry (ICP-MS) analysis of these BSF larvae has shown that there was a higher accumulation of total selenium content in the treated samples (348 mg Se/g DW) as compared to control (0.64 mg Se/g DW). Se conjugated insects' protein can withstand as high as 400 mg sodium selenite without compromising its growth pattern and BSF larvae biomass yield and nutritional value. Collectively, the current study proposes that, Se conjugate BSF larvae with high organic Se content and can serve as selenium feed additives.

1 Introduction

Selenium (Se), an essential trace element, is an important part of selenoproteins, which are involved in several physiological processes in production animals [1]. In addition to being a necessary nutritional trace element for many species, selenium also strengthens the body's antioxidant defence system, preserving both human and animal health and normal physiology. It is involved in the synthesis of at least 25 selenoproteins [2]. Consequently, selenium supplementation through diet is required to maintain human health [3]. There are

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both organic and inorganic forms of selenium. Compared to inorganic selenium, organic selenium is more bioavailable and safer [4]. Numerous studies have produced organic selenium by using plants, animals, and edible microbes. Nevertheless, a few sources of selenium have disadvantages, such as a low level of organic selenium, a lengthy production time, and a high price [5]. As a result, a novel selenium-enriched technique that enables a high yield of organic selenium in a brief production time and at a reasonable cost is still needed [6].

Livestock can be fed supplements containing selenium, as the chemical form of the element affects the accumulated amount of selenium in animal tissues. Although it is well known that insects are naturally occurring bioaccumulators of various nutrients, no research has been done on the possibility of using insects as a source of selenium in feed in the future [7]. Feed ingredients include marine products, nuts, seeds, animal manure, and other protein-rich foods that are often included in a balanced diet. Nowadays, environmentally conscious enterprises in animal husbandry are becoming more and more interested in using insects as feed ingredients for protein sources [8]. For environmental sustainability in animal production, it is therefore feasible to boost insect production on a wide scale and use it as an alternative feed additive [9]. A novel and extremely promising substitute protein source for animal feed is insects [10]. The ability of the black soldier fly (BSF; *Hermetia illucens L.*) to transform organic waste into high-quality protein, suppress some dangerous bacteria and insect pests, supply possible chemical precursors to produce biodiesel, and be used as feed for a range of animals has all been investigated. The nutritional worth of BSF larvae is examined, together with the impact of biotic and abiotic elements on the body composition and functionality of the larvae [11]. There needs to be a study on the potential use of Se with the method of bioconversion of Se conjugated insect protein using BSF larvae as poultry feed.

2 Materials and Methods

2.1 Materials

BSF larvae raised on growth media supplemented with selenium (sodium selenite) served as the study's source material. BSF eggs, yeast *Saccharomyces cerevisiae*, growth media (made of bran, soybean meal, and molasses), and sodium selenite are the components employed. The Blitar Regency's community farms' BSF hatcheries are the source of BSF larvae eggs. In BSF culture, growth media consists of 60% rice bran, 30% soybean meal, and 10% molasses. The growth media is fermented with 1% yeast *Saccharomyces* cerevisiae and combined with food waste organic waste in a 1:1 ratio. Scales, buckets, and a plastic tube (Biopon) are the utilised tools.

2.2 Preparation of selenium-enriched samples

The experimental enrichment of selenium levels in BSF larval growth media is the methodology employed in this work, as illustrated in Figure 1. A biological test with a Complete Randomised Design (CRD) comprising four treatments and three repeats—P0 being control media, P1 being media containing 100 mg/kg, P2 being media containing 200 mg/kg, and P3 being media containing 400 mg/kg—was the research methodology employed. The process of enhancing sodium selenite through two biotransformation processes to produce Insect Protein Conjugated Selenium is based on research conducted by Qiu et al. (2021) [12]. First, using yeast *Saccharomyces cerevisiae*, ferment the BSF larval development medium that has been supplemented with selenium. Sodium selenite is added to bran and soybean meal as raw materials based on the treatment. To generate selenium conjugated on yeast (selenium yeast) based fermentation, yeast *Saccharomyces cerevisiae* is used under ideal fermentation conditions [13]. Second, to synthesise BSF protein-conjugated selenium, fermented growth media containing *Saccharomyces cerevisiae* (a yeast rich in selenium) is fed to BSF larvae. Conjugated selenium BSF flour is created by grinding and drying selenium conjugated BSF protein, and it is then kept dry until the next testing procedure [7].

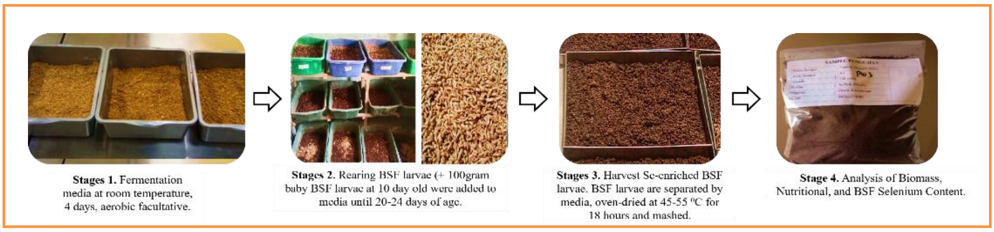


Fig. 1. Procedure for selenium-conjugated BSF protein synthesis.

2.3 Determination of nutritional and selenium content

The moisture, crude protein (CP), crude lipid (CL), and ash content of the BSF flour were measured in accordance with the guidelines provided by the Association of Official Analytical Chemists (AOAC) (2005). According to Commission Regulation No. 152/2009, samples were dried in an oven at 130 °C for two hours to ascertain their moisture content. The Kjeldahl method was used to measure the CP content (proc. 2001.11; AOAC 2005). A muffle furnace operating at 550 °C was used to measure the ash (proc. 942.05; AOAC 2005). Additionally, an inductively coupled plasma mass spectrometer (ICP-MS) was used to quantify the selenium concentration, following (Liu et al. 2020) [14]. The BSF protein attached selenium was morphologically and size verified using scanning electron microscopy (SEM) as per (Souza et al. 2022) [15]. The functional groups on the surface of BSF flour that took part in the biosorption of selenium (Se) were determined using FTIR analysis. For the raw BSF flour, this analysis was carried out. Identifying the distinctive peaks linked to the complex matrix of BSF Flour, which includes protein, carbohydrate, and lipid fractions along with functional groups involved in the metal ion biosorption, is the primary benefit of this approach [16].

2.4 Statistical analysis

In order to clarify the impacts of dietary Se levels, one-way analysis of variance (ANOVA) was performed on all the data. When the treatment difference was significant ($P < 0.05$), Duncan's test was employed to separate the means. $P < 0.05$ was used as the threshold for statistical significance.

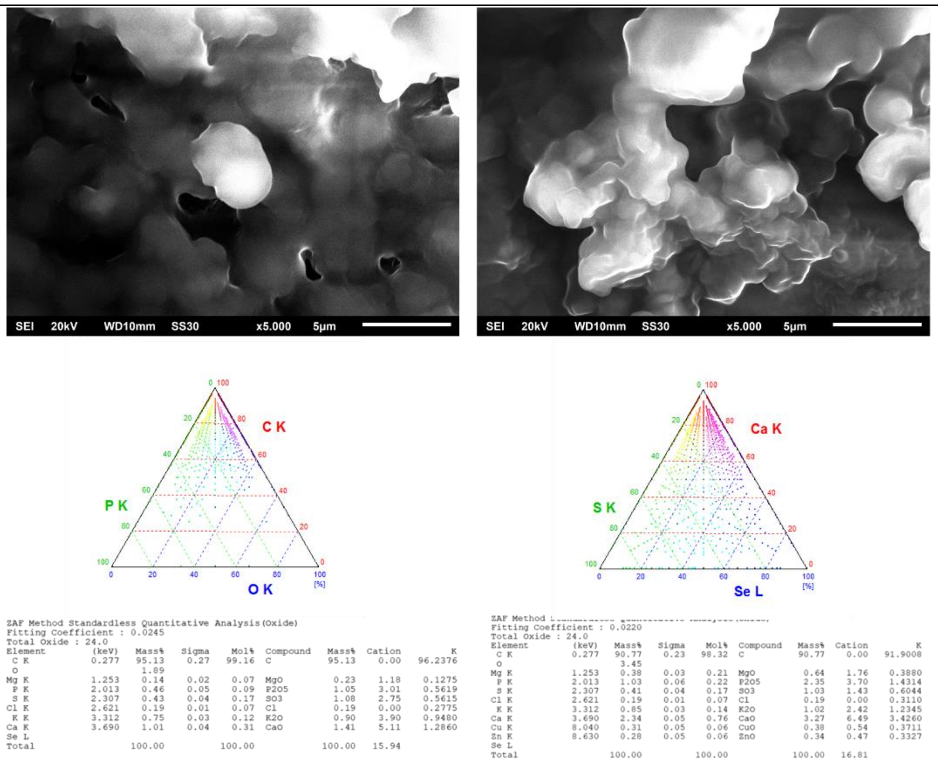
3 Results and discussion

Total biomass produced, nutritional and selenium content under different substrates were shown in Table 1. The group given sodium selenite supplementation had greater total selenium levels. We discovered that enriched BSF larvae had five times more selenium than non-enriched larvae. The dosages of selenium that various selenium compounds gave to BSF larvae varied significantly. These BSF larvae were analysed using an inductively coupled plasma mass spectrometer (ICP-MS), which showed that the treated samples had accumulated more total selenium content (378.17 mg Se/kg DW) than the control samples (1.43 mg Se/kg DW). Previous research has shown *Tenebrio molitor* larvae's total selenium level increased 83-fold to 54.21 ± 1.25 µg/g after being fed 20 µg/g of sodium selenite. Nearly 97% of this content was organic selenium [6].

Table 1. Biomass, nutritional and selenium content of Se-enrichment BSF

Variables	**Level of Se-enrichment**			
	0 mg/kg	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**
Fresh Larva Weight (g)	1327	1258	1286	1172
Dry Matter (%)	28,32	33,62	30,84	34,3
Moisture (%)	6,23	6,09	4,70	4,70
Ash (%)	9,26	9,31	8,84	11,27
Crude Protein (%)	42,04	42,62	43,49	42,61
Crude Lipid (%)	28,18	29,48	32,34	29,82
Selenium Content (mg/kg)	0,64[a]	42,4[b]	223,33[c]	348[d]

Significant variations (P<0.05) between dietary groups are shown by different superscripts.



(a) (b)

Fig. 2. Morphological and size verification of BSF flour (a) and selenium-enriched BSF flour (b) was performed using Scanning electron microscopy (SEM) and analysis of EDX elementary oxide.

The morphology and size of the produced BSF and BSF protein attached selenium flour were examined using SEM, as illustrated in Figure 2. The FTIR of BSF flour and selenium enriched BSF flour are shown in Figure 3.

Qiu et al. (2021) observed that laying hen performance and egg quality were enhanced by adding organic selenium (selenium conjugated to insect protein, or SCIP) to their meals, even at the 10 mg/kg inclusion level, without causing any adverse effects on toxicity. Yellow mealworms and super mealworms fed on vegetables and yeast protein high in selenium produced SCIP. After being dried and crushed, SCIP was turned into powder, and it was shown to have 4,480 mg/kg of selenium [12]. Ferrari et al. (2022) revealed that the concentration of Se in the three meals given to the insects had an impact on the amount of Se in BSF prepupae. SeMet levels in the Se group were greater than in the control group, suggesting that BSF prepupae can convert inorganic selenium to organic selenium [7].

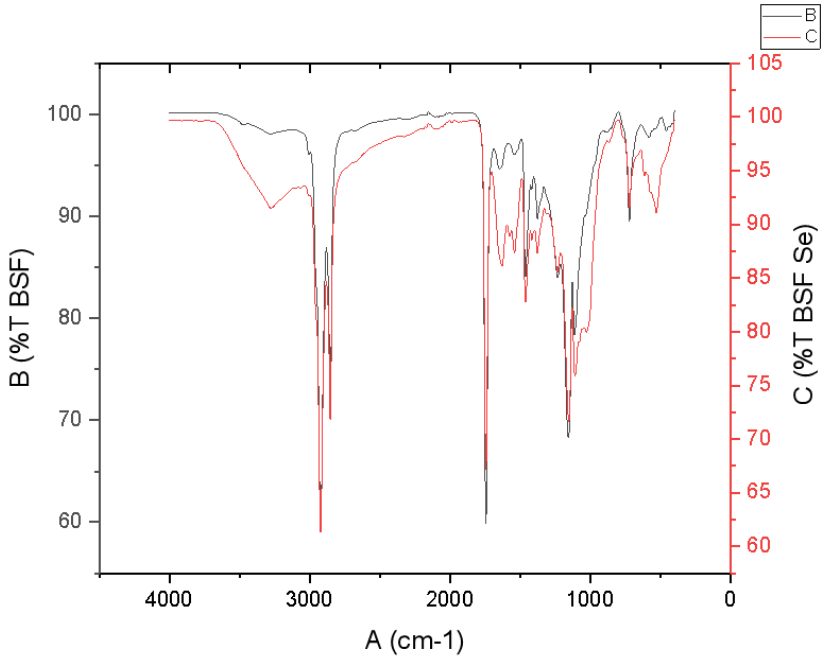


Fig. 3. FTIR of BSF flour (a) and selenium enriched BSF flour (b).

4 Conclusion

The BSF product's organic Se conjugated insect protein may withstand up to 400 mg of sodium selenite without compromising the larvae's nutritional value, growth pattern, or biomass yield. Overall, the current study indicates that high organic Se content Se conjugated BSF larvae may be used as a selenium feed addition for chickens.

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