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Bioconversion of inorganic selenium to organic selenium in the black soldier fly (BSF) larvae

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ABSTRACT

Selenium is one of the essential micronutrients needed to fulfil livestock nutrition, which can be found in inorganic and organic forms. Black Soldier Fly (BSF) larvae can potentially be used as a natural converter from inorganic selenium to organic selenium. However, the capacity and response of BSF larvae to convert selenium are still unknown. This study aims to determine the effect of inorganic selenium administration on BSF larvae. The research method was the determination of selenium concentration by UV-Vis spectrophotometry method based on the variation in the age of inorganic selenium administration in BSF larvae to the growth of BSF larvae, and the accumulation of selenium in BSF larvae, with variations in age of 0, 4, 8, and 12 days of age given sodium selenite (Na_2SeO_3) and control without administration of sodium selenite from the beginning to the end of rearing. There was no significant difference ($p > 0.05$) in the growth performance of BSF larvae and the accumulation of selenium in BSF larvae in the age variation experiment of BSF larvae when given sodium selenite. Based on the acquisition of larval mass, the growth rate of BSF larvae in the control treatment, with sodium selenite at 1 mg/kg, was 0.129, 0.093, 0.037, 0.156, and 0.128 mg/day at 0, 4, 8, and 12 days, respectively. These results indicate that inorganic selenium administration to BSF larvae can occur during the rearing period. In the experiment of variation in the concentration of inorganic selenium given to BSF larvae, the growth rate of BSF larvae was significantly higher ($p < 0.05$) when given 1000 mg/kg of sodium selenite, which was 0.467 mg/day.

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1. Introduction

Selenium is one of the micronutrients needed by the body, which can catalyse the peroxide conversion reaction to water, thus protecting cells from oxidative damage. Apart from oxidative damage, selenium supplementation also has a protective function by modulating the immune system (Uresti-Rivera et al., 2023), which affects the reproductive system in humans and animals, leading to decreased sperm quality and an increased chance of miscarriage in pregnancy due to low antioxidant protection in fetal DNA and biomembranes (Radomska et al., 2021). In animals, selenium deficiency results in reduced body weight, decreased milk production, and decreased fertility. Selenium deficiency in poultry feed also affects poultry health, including an increase in disease prevalence, reduced immune cell activity, and the emergence of muscle diseases such as white muscle disease (WMD) (Tórtora-Pérez, 2010). Inorganic selenium acts as a simple, short-lived nutritional input, with its sole fate being immediate conversion into selenide to fuel selenoprotein synthesis or swift excretion. Organic selenium, in contrast, functions as both a nutritional input and a biological asset, building a strategic tissue reserve that can be mobilised to support sustained selenoprotein function and physiological resilience in the face of modern production stressors. This capability to create a durable biological safety net is what translates abstract biochemical superiority into tangible improvements in growth, feed conversion, immunity, and reproductive performance (Wickramasuriya et al., 2023; Anizoba et al., 2024).

Naturally, animals obtain selenium from the food chain (Lyons et al., 2007). Plants utilise selenium from inorganic soils as a significant source of selenium in the food chain, absorbing selenium from the soil and converting it into organic selenium using enzymes that facilitate selenium assimilation (Pilon-Smits and Quinn, 2010). The amount of selenium assimilated by plants can be an indicator of inorganic selenium concentrations in the soil where the plant grows. Plant products with the highest concentration of selenium are known to be obtained from Brazil nuts, with a concentration of 544 μg/oz (National Institutes of Health, 2024). Additionally, wheat is recognised as one of the best selenium accumulators (Lyons et al., 2003). The amino acid selenomethionine is a form of selenium that is widely found in Brazil nuts and wheat (Lyons et al., 2007).

The poultry farming industry is one of the key sectors that contribute to meeting global demand for animal protein. According to the FAO, poultry meat is expected to reach a high level of production and consumption, surpassing other ruminant meat products by 2025. In the poultry industry, particularly for chickens, the most significant portion of the overall production cost is the cost of chicken feed, which accounts for approximately 70% of the total production cost (Zampiga et al., 2021). The fulfilment of chicken nutritional needs generally uses rations that have been formulated to achieve nutritional fulfilment in chickens with minimal production costs. The inputs of feed can be grouped into five main ingredients, namely 1) energy sources, 2) protein sources, 3) fats and oils, 4) minerals, and 5) vitamins. However, the formulation of chicken feed primarily focuses on protein and energy sources as key elements based on mass in chicken feed (Belkhanchi et al., 2023), which can lead to selenium deficiency in livestock, compromising animal health and decreasing production efficiency. BSF-derived organic selenium can provide high-quality protein, essential fatty acids, and a rich mineral profile. The combination of these benefits within a single, natural ingredient may create synergistic health effects, rather than a singular additive, offering multiple health and nutritional benefits from a single source (Kurniawan et al., 2024b).

The cost of micronutrient production in the feed formulation industry can be classified as a high-cost input; therefore, it is often used as a cheaper alternative to mineral supplementation, such as salt, or by utilising the mineral content in animal flour ingredients (Heidari et al., 2021; Belkhanchi et al., 2023). The feed formulation industry addresses the potential for mineral deficiencies by selling additional feed ingredients (premixes) that are concentrated from these mineral elements, typically in the form of salts, amino acids, and vitamin biosynthesis (Belkhanchi et al., 2023). The mineral salt forms of selenium are sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4), which are known as inorganic selenium compounds. Inorganic selenium is the first generation of selenium added to chicken feed. However, a study found that administering sodium selenite, in the form of inorganic selenium, to chickens is more toxic than its organic form. Administration of 0.3 ppm sodium selenite to chickens has a pro-oxidant effect that causes structural damage to the chicken intestines due to vacuole degeneration of epithelial cells that protect the duodenum. Excess amounts of sodium selenite cannot be stored in the chicken's body, so it cannot be utilised when the chicken is in a state of oxidative stress. Additionally, sodium selenite cannot be transferred to the embryo in chicken eggs. Based on the aspect of feed formulation, the influence of other feed compositions can also eliminate the effects of sodium selenite; for example, the presence of ascorbic acid can reduce sodium selenite to the element selenium, which cannot be digested by living things and has no biological activity. Pro-oxidant activity and reactivity with other feed components were not found in organic selenium. In organic form, selenium can be found incorporated with amino acids, such as selenomethionine and selenocysteine. Proteins composed of these amino acids are referred to as selenoproteins which carry out the biological function of selenium in the bodies of animals and plants. Naturally, the need for organic selenium is obtained from the consumption of plant and animal products. However, the amount of naturally consumed organic selenium from plant and animal products is not standardised, considering that the availability factor of selenium in the soil where the plant product is produced and the factor of selenium availability in animal feed affect the concentration of organic selenium in both plant and animal products. Based on this description, research and development of organic selenium production techniques for use as animal feed is essential. Developments related to selenium-organic production are leading to the biotransformation of selenium using yeast (Martiniano et al., 2020; Martiniano et al., 2022). However, the disadvantage of this method is that the production process is expensive, as it requires the production of sufficient yeast biomass for large quantities of selenium-organic compounds, including the cost of the medium used and the cost of maintenance during the bioconversion process. Therefore, an alternative is needed in the selenium-organic production process that offers a cheaper process and lower maintenance costs. An alternative method proposed in this study utilises black soldier fly (BSF) larvae as a bioconversion agent for selenium-organic production. Research conducted by Ferrari et al. (2022) reported that BSF larvae fed with selenium at 0.3 mg/kg showed that the accumulation of selenium in the larvae was 5 times greater than the selenium content in the control larvae that were not given selenium intake, with 54% of the selenium in the larvae being selenomethionine. Therefore, it is necessary to conduct further research on the effect of selenium administration on BSF larval growth. Selenium at the molecular level affects the biosynthesis process of selenoproteins, which are proteins that incorporate selenium (Kang et al., 2020). The purpose of this study is to investigate the potential of BSF larvae as a medium for organic selenium production, with the primary advantage of BSF being its dual function as a sustainable protein source and a biological waste converter.

2. Materials and methods

The stages of the research are summarised in the flow chart in Fig. 1.

2.1. Determination of standard curves of selenium measurement in samples

The concentration of selenium in BSF larvae was determined by spectrophotometry (Narayana et al., 2003). The standard curve of Na_2SeO_3 is made with a concentration range of 0-6 mg of selenium/L. A Na_2SeO_3 solution is reacted with 1 mL of 2% (w/v) KI and 1 mL of 2 M HCl, then vortexed until a yellow colour is formed. Then 50 μL of 1% (w/v) soluble starch is added to the vortex, and the solution is adjusted to 10 mL with deionised water. The absorbance of the solution was then measured at a wavelength of 570 nm with a UV-Vis spectrophotometer and compared to a standard curve.

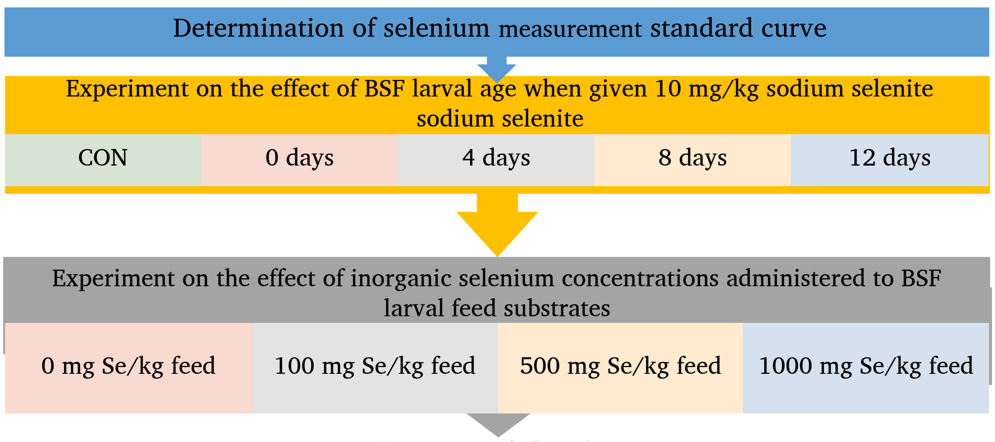


Fig. 1. Research flow chart

2.2. Experiment on the effect of BSF larval age when given inorganic selenium

BSF larvae are divided into several groups as follows: 1) Control treatment (CON): The BSF larvae of the control group (CON) were kept without selenium-inorganic application from the beginning of maintenance to the end of maintenance. The feed substrate in the control group (CON) was mixed with aqueducts in a 1:1 (w/v) ratio. Maintenance is carried out until the larvae are 16 days old. 2) Selenium-0 treatment (Se-0): BSF larvae of the selenium-0 group (Se-0) are larvae that are kept by being given selenium inorganic on the substrate when the eggs are placed in pieces of cardboard on top of the substrate. The feed substrate is mixed with a solution of sodium selenite in a 1:1 (w/v) ratio, resulting in a final concentration of sodium selenite in the feed substrate of 10 mg/kg. The feed substrate mixed with a solution of sodium selenite is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. 3) Selenium-4 (Se-4) treatment: BSF larvae of the selenium-4 (Se-4) group are larvae that are kept by being given selenium inorganic when the larvae of the Se-0 group are 4 days old, calculated from the first day the eggs are laid on the substrate. On the fourth day, 30 larvae were transferred to a new container containing a feed substrate mixed with a 1:1 (w/v) solution of sodium selenite. The concentration of sodium selenite in the feed substrate is 10 mg/kg. The feed substrate mixed with a solution of sodium selenite is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. 5) Selenium-8 (Se-8) treatment: BSF larvae of the selenium-8 (Se-8) group are larvae that are kept by being given selenium inorganic when the larvae of the Se-0 group are 8 days old, calculated from the first day the eggs are laid on the substrate. On the eighth day, 30 larvae are transferred to a new container containing a feed substrate mixed with a 1:1 (w/v) solution of sodium selenite. The concentration of sodium selenite in the feed substrate is 10 mg/kg. The feed substrate mixed with a solution of sodium selenite is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. 6) Selenium-12 treatment (Se-12): BSF larvae of the selenium-12 (Se-12) group are larvae that are kept by being given selenium inorganic when the larvae of the Se-0 group are 12 days old, calculated from the first day the eggs are laid on the substrate. On the fourth day, 30 larvae were transferred to a new container containing a feed substrate mixed with a 1:1 (w/v) solution of sodium selenite. The concentration of sodium selenite in the feed substrate is 10 mg/kg. Maintenance is carried out until the larvae are 16 days old. In this experiment, the larvae receive selenium from day 0 (T_0/hatching). BSF larvae at 12 days are late instar, approaching the pupal stage.

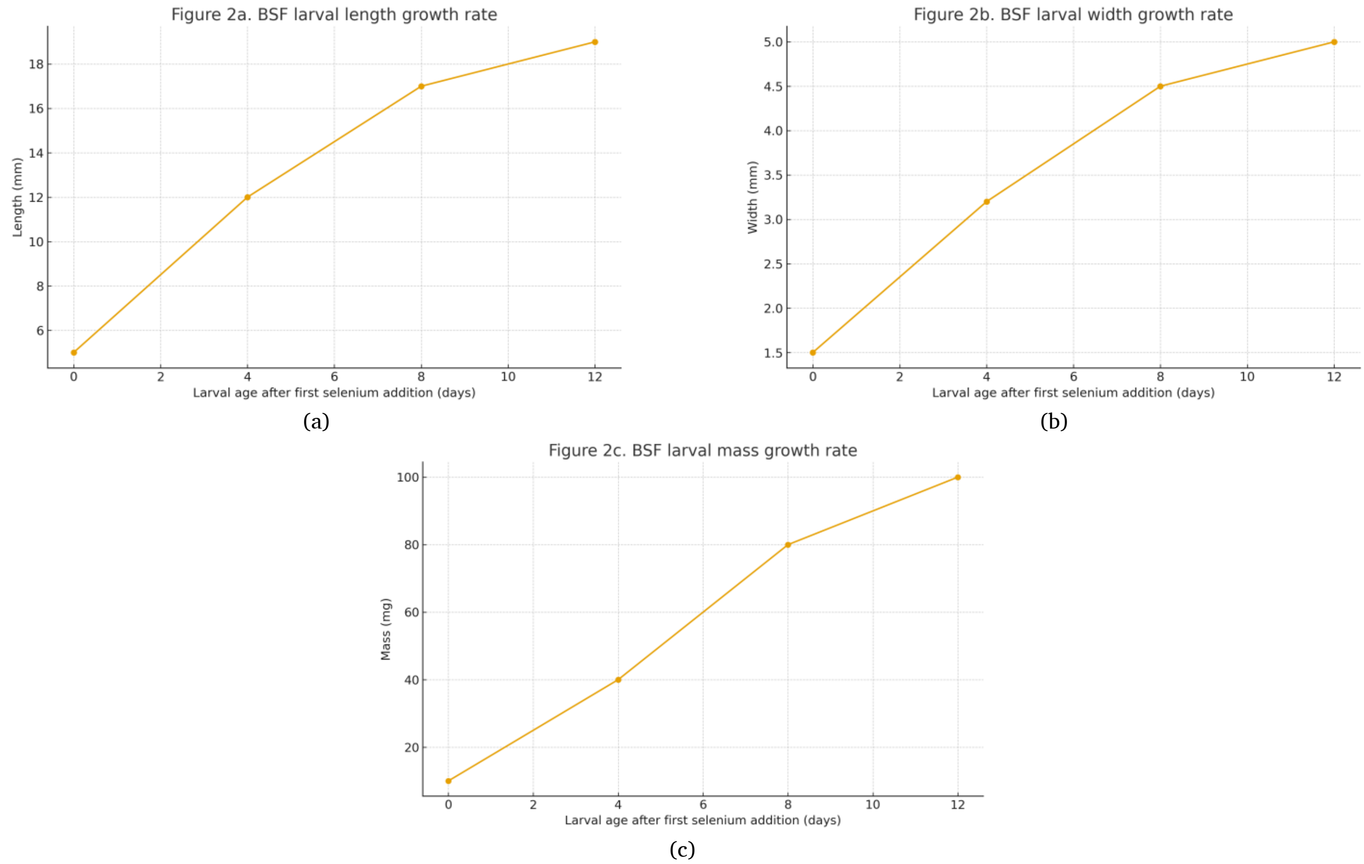


Fig. 2. Growth rate of BSF larvae (a) based on length with variation in larval age when first given 10 mg/kg inorganic selenium. (b) based on width with variation in larval age when first given 10 mg/kg inorganic selenium. (c) based on mass with variation in larval age when first given 10 mg/kg inorganic selenium.

Each treatment group was repeated 6 times. Every 4 days, larvae are collected for measurement of their length, width, and mass. On the 16^{th} day, the larvae of all treatments and repetitions are harvested. The larvae are separated from the substrate residue on the container and washed under running water for ± 1 minute. The larvae are stored at a temperature of -20 °C until the analysis determines the selenium concentration in the larvae.

2.3. Experiment on the effect of selenium-inorganic concentration on BSF larval feed

The BSF larvae are divided into several groups as follows: 1) Control treatment, inorganic selenium concentration 0 mg/kg. BSF larvae in the control group (0 mg/kg of inorganic selenium) were kept without selenium-inorganic administration from the beginning of maintenance to the end of maintenance. The feed substrate in the control group was mixed with aqueducts in a 1:1 (w/v) ratio. The feed substrate is added once every 4 days. Maintenance is carried out until the larvae reach 16 days of age. 2) Treatment of inorganic selenium concentration of 100 mg/kg: BSF larvae of this group are kept by being fed inorganic selenium from the beginning of maintenance to the end of maintenance. The feed substrate in this group is mixed with a solution of sodium selenite in a 1:1 (w/v) ratio. The concentration of sodium selenite in the substrate is 100 mg/kg. The feed substrate is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. 3) Treatment of inorganic selenium concentration of 500 mg/kg: BSF larvae of this group are kept by being fed inorganic selenium from the beginning of maintenance to the end of maintenance. The feed substrate in this group is mixed with a solution of sodium selenite in a 1:1 (w/v) ratio. The concentration of sodium selenite in the substrate is 500 mg/kg. The feed substrate is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. 4) Treatment of inorganic selenium concentration 1000 mg/kg: BSF larvae of this group are kept by being fed inorganic selenium from the beginning of maintenance to the end of maintenance. The feed substrate in this group is mixed with a solution of sodium selenite in a 1:1 (w/v) ratio. The concentration of sodium selenite in the substrate is 1000 mg/kg. The feed substrate is added once every 4 days. Maintenance is carried out until the larvae are 16 days old. BSF larvae at 12 days are late instar, approaching the pupal stage.

Each treatment group was repeated 6 times. Every 4 days, larvae are collected for measurement of their length, width, and mass. On the 16^{th} day, the larvae of all treatments and repetitions are harvested. The larvae are separated from the substrate residue on the container and washed under running water for ± 1 minute. The larvae are stored at -80 °C for molecular analysis.

3. Results and discussion

3.1. Effect of selenium-inorganic given larval age on BSF larval growth rate

The growth rate of BSF larvae was measured based on the length, width, and mass of BSF larvae from each treatment group of BSF larval age variations. Based on the results obtained for the long growth rate (Fig. 2A), a significant variation ($p < 0.05$) was observed in the growth rate of the selenium treatment group. However, when post-hoc tests were conducted, it was observed that no age group of BSF larvae gave a significantly different growth rate compared to other groups. Still, the results of larval growth rate when given inorganic selenium at 4 days of age were significantly different ($p < 0.05$) from the growth rate of larvae when given inorganic selenium for the first time at 8 days of age. For the growth rate obtained from the larval width data (Fig. 2B), a significant difference in variance ($p < 0.05$) was observed in the treatment group. However, based on the post-hoc test, it was observed that there was no significant difference among the treatment groups. This contradiction can occur because the growth rate data calculated by width do not provide sufficient evidence to assume normally distributed data based on the Kolmogorov-Smirnov normality test. In the growth rate of BSF larvae measured based on the mass of BSF larvae (Fig. 2C), there was also a significant difference ($p < 0.05$) among groups of BSF larval age variation based on ANOVA results. Based on the results of the post-hoc test, it was observed that the growth rate of larvae fed with selenium-inorganic for the first time at 4 days of age was significantly different ($p < 0.05$) from the growth rate of larvae fed with selenium-inorganic for the first time at 8 days of age. However, from the growth rate of BSF larvae according to these three parameters, no age group of BSF larvae was found that had significantly different results from all other age groups of BSF larvae, as determined by post-hoc tests.

Based on the visualisation of the growth rate graph (Fig. 2) and the results of the post hoc test, there is a significant relationship between the growth rate of BSF larvae when given selenium inorganic for the first time at 12 days of age and the growth rate of BSF larvae that are not given selenium. The phenomenon occurs because during the rearing period, BSF larvae given selenium inorganic at 10 mg/kg for the first time at 12 days of age receive selenium inorganic only once. The other age variation groups, namely 0, 4, and 8 days, showed higher frequencies of selenium-inorganic during the maintenance period, specifically 4, 3, and 2 times, respectively. Based on these results, it is estimated that there is an indication of a negative influence of selenium-inorganic on the growth rate of BSF larvae when selenium-inorganic is given more than 1 time during BSF larval rearing. Further analysis is needed to confirm these indications. By focusing on the growth rate data based on the length, width, and mass parameters of BSF larvae obtained in this study, it can be statistically concluded that the variation in the age of BSF larvae when first given inorganic selenium has no significant effect ($p < 0.05$) on the growth rate of BSF larvae. However, when larval length reached ~19 mm by day 9, growth was observed to plateau as they transitioned to the pupal stage.

3.2. Effect of larval age when given selenium inorganic on selenium concentration in BSF larvae

The results of inorganic selenium concentration in BSF larvae (Fig. 3) showed a significant difference in variance ($p < 0.05$) between the age groups of the larvae when they were first given inorganic selenium. The selenium concentration of the control group that did not receive inorganic selenium was significantly different ($p < 0.05$) from the selenium concentration of the larval group that was given the first inorganic selenium at 4 days of larval age. However, both data groups still have relevance to the data of other groups, as indicated by post-hoc tests with Tukey's HSD. The data obtained from the lowest selenium concentration in the control group shows the larvae's ability to accumulate selenium inorganic in the feed substrate. However, the age factor of the larvae when first given inorganic selenium did not significantly affect their ability to accumulate the inorganic selenium. Thus, based on the age factor of the larvae being given inorganic selenium for the first time to the larval growth parameters and the concentration of inorganic selenium in the larvae, it can be indicated that inorganic selenium can be given to BSF larvae at any age without providing a significant difference in the aspect of growth performance and selenium concentration in BSF larvae.

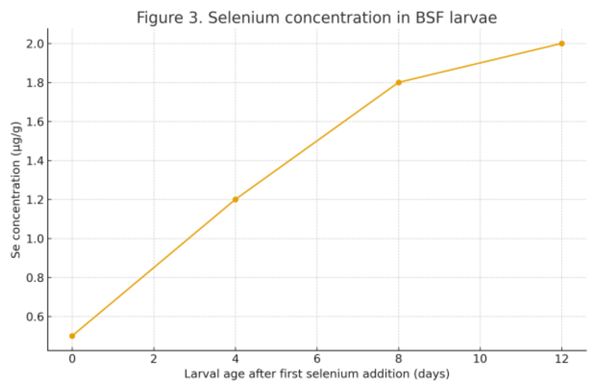
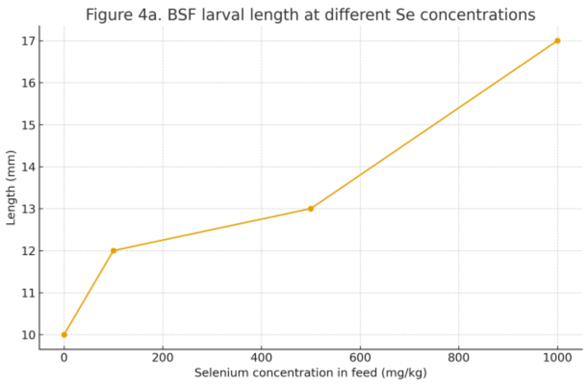
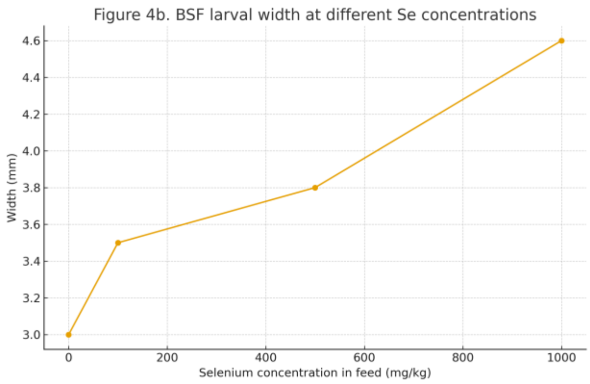


Fig. 3. Selenium concentration in BSF larvae against larval age when first given 10 mg/kg inorganic selenium.

BSF larvae showed the capacity for accumulating selenium from their dietary substrates. However, the evidence suggests that this process is a non-linear, dose-dependent phenomenon that does not conform to strict homeostatic control. Instead, the mechanism is best explained by the chemical similarity between selenium and sulfur, leading to the non-specific incorporation of selenium into sulfur-containing proteins, such as selenomethionine, which bypasses typical regulatory pathways. This non-specific, passive process accounts for the significant accumulation observed at higher dietary concentrations (Wang et al., 2024). The amount of selenium required by the cell must be considered to prevent it from exceeding the toxic threshold and exerting adverse effects on the cells, which can impact the health of the individual, in this case, BSF larvae. The administration of inorganic selenium from the beginning of maintenance is feared to cause toxic effects on larvae due to the accumulation of selenium from the beginning to the end of maintenance. Administering selenium at the beginning of maintenance raises concerns that it may be more likely to cause damage to organs that have not yet fully developed at the start of larval growth. However, the results of this study showed that the development and accumulation of selenium in BSF larvae were not affected by the age of BSF larvae when given inorganic selenium. The results of this study indicate that BSF larvae possess a mechanism of selenium homeostasis that prevents the inhibition of larval growth and the accumulation of excessive selenium by maintaining selenium levels in cells at a specific concentration, regardless of the amount of selenium entering the body. Behne (1989) revealed that the selenium absorption factor from the diet is not a determining factor in the selenium regulatory mechanism; instead, regulation occurs during excretion by the individual. Based on a search of relevant articles, no studies have been found that measure selenium and/or other substance levels in larval urine. However, it is known that insects, including BSF larvae, can sequester heavy metals in their exoskeleton, specifically in the cuticle (Adams et al., 2024). This finding aligns with a study conducted by Proc et al. (2020), which showed that the accumulation of micronutrients such as cobalt (Co), manganese (Mn), and selenium (Se) is higher in the puparia phase, characterised by a thicker cuticle, compared to the larval phase. Thus, administering inorganic selenium to the larvae throughout the rearing period does not affect growth performance or selenium accumulation in the larvae.



(a)



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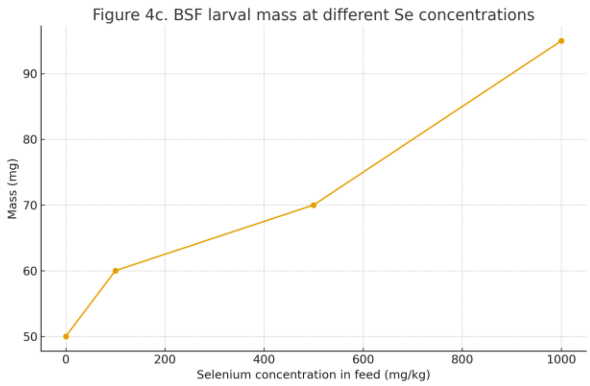

(c)

Fig. 4. BSF larval growth rate (a) based on length with variations in selenium concentrations (0-1000 mg/kg) (b) based on width with variations in selenium concentrations (0-1000 mg/kg) (c) based on mass with variations in selenium concentrations (0-1000 mg/kg)

3.3. Effect of selenium-inorganic concentration on BSF larval growth rate

The growth rate of BSF larvae was measured based on the length, width, and mass of BSF larvae of each given selenium-inorganic concentration group (Fig. 4). Based on the results obtained for the growth rate, both in terms of the parameters of length, width, and mass of BSF larvae, a significant variation ($p < 0.05$) was observed in the growth rate of the treatment group administered selenium. When post-hoc tests were conducted, it was observed that the BSF larvae group given selenium inorganic at 1000 mg/kg showed the highest growth rate and was significantly different ($p < 0.05$) compared to the other groups. This occurs in the length, width, and mass parameters of BSF larvae. These results indicate that the variation in selenium-inorganic concentration given to BSF larvae has a significant effect ($p < 0.05$) on the increase in the growth rate of BSF larvae at 1000 mg/kg, suggesting potential benefit for selenium-enriched BSF larvae.

The results of this study revealed that inorganic selenium in the form of selenium with a concentration of 1000 mg/kg basal diet given by BSF larvae did not cause any toxic effects based on BSF larval growth parameters. In fact, the administration of inorganic selenium with this concentration provided the most significant growth rate of BSF larvae ($p < 0.05$) compared to BSF larvae given a lower concentration of inorganic selenium. Selenium performs its biological function to support growth in its form as a selenoprotein (Zhang et al., 2023). Selenium is available in sufficient quantities in BSF larvae to support the occurrence of these cellular processes, so that they can improve the growth performance of BSF larvae.

The growth rate of BSF larvae when given inorganic selenium with a concentration below 1000 mg/kg did not differ significantly ($p > 0.05$) (Fig. 4). The same results were also found in a study conducted by Kurniawan et al. (2024a), which provided sodium selenite at concentrations of 0, 100, 200, and 400 mg/kg in the basal diet. The mass of fresh larvae from the four groups differed significantly ($p < 0.05$) (Kurniawan et al., 2024a). Thus, the optimum selenium-inorganic concentration, in this case sodium selenite, to support the growth performance of BSF larvae is 1000 mg/kg. It is necessary to analyse larval growth performance when given inorganic selenium at a higher concentration to determine the upper limit of inorganic selenium concentration that can be given to BSF larvae.

The availability of nutrients from BSF broodstock through BSF eggs can meet the nutritional needs of one generation, so that the identification of dietary needs of BSF larvae, especially in terms of vitamins and micronutrients, cannot be observed until two new generations of BSF (Seyedalmoosavi et al., 2022). This study investigated the effect of inorganic selenium administration on a single generation of BSF larvae. Referring to this, the increase in growth rate obtained from BSF larvae receiving inorganic selenium at a concentration of 1000 mg/kg may be due to the adaptive response of 1 generation of BSF larvae to the given conditions. Therefore, further analysis is needed to determine the need for inorganic selenium micronutrients in BSF larvae by evaluating the effects of inorganic selenium administration on subsequent generations.

4. Conclusion

The application of inorganic selenium did not affect the growth performance of BSF larvae and the accumulation of selenium-inorganic in BSF larvae when it was first administered at different larval ages, so that the administration of selenium-inorganic in the production of organic selenium based on BSF larvae could be carried out at the beginning of BSF larval rearing or at the end of BSF larval rearing with no significant difference in selenium-inorganic concentrations in larvae. Thus, while BSF larvae show a positive response to high concentrations of inorganic selenium (1000 mg/kg) in terms of growth performance, they can also bioconvert selenium into organic forms suitable for poultry feed fortification; further chicken feeding trials are needed.

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Conflict of interest

The authors declare no conflict of interest in this research.

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