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Sodium selenite enhanced the selenium content in black soldier fly

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This study focuses on the effects of different levels of sodium selenite on the growth, selenium content, and antioxidant capacity of black soldier fly (*Hermetia illucens*). The experiment used different doses of sodium selenite for treatment, including a basic diet with no supplements (control) and diets supplemented with 10 mg/kg (Se10), 20 mg/kg (Se20), 30 mg/kg (Se30), and 40 mg/kg (Se40) sodium selenite, and results show that sodium selenite supplementation significantly increases selenium content and improves selenium utilization and antioxidant capacity ($P < 0.05$). However, it also negatively affects growth performance and the utilization of other nutrients. The optimal level of sodium selenite supplementation depends on the ration of selenium enrichment with the overall health and productivity of black soldier fly.

Keywords Black soldier fly, Selenium metabolism, Nutrient utilization, Antioxidant capacity, Growth performance

The black soldier fly (BSF; *Hermetia illucens*) has emerged as a key player in sustainable agriculture due to its extraordinary ability of converting organic waste into high-value nutrients. BSF larvae can degrade various organic materials, like food waste and manure, and accumulate essential nutrients such as protein, fat, and minerals, which turns them into a promising source of sustainable animal feed and bioactive compounds[1,2]. Additionally, BSF larvae are recognized for their ability to bioaccumulate trace elements, including heavy metals and selenium (Se), presenting opportunities for bioremediation and functional food development[3].

Selenium is an essential trace element with numerous biological functions, including antioxidant defense, immune modulation, and thyroid hormone metabolism[4]. Selenium is incorporated into selenoproteins such as glutathione peroxidase and thioredoxin reductase, which protect cells from oxidative stress[4]. Dietary selenium supplementation is critical for preventing selenium deficiency and enhancing health outcomes in both animals and humans[5]. However, the gap between optimal supplementation and toxicity thresholds highlights the need for precise dose optimization. Although selenium can be supplemented in organic and inorganic forms, inorganic sources such as sodium selenite are widely used for their cost-effectiveness despite their potential toxicity at high doses[6].

Recent studies on selenium supplementation in insects have primarily focused on its effects on bioaccumulation and antioxidant capacity[3,7]. However, comprehensive investigations concentrated on the impact of selenium supplementation on growth performance, nutrient utilization, and overall metabolic efficiency in BSF larvae are limited. This study addresses this gap by systematically evaluating the effects of varying levels of sodium selenite on selenium enrichment, growth performance, and nutrient metabolism in BSF larvae.

The novelty of this work lies in its integrated approach to understanding the trade-offs between selenium utilization and larval growth. Unlike previous studies, we explore the optimal selenium levels which can maximize selenium enrichment while maintaining efficient growth and nutrient utilization. By providing a detailed analysis of selenium's impacts on BSF larvae, this study contributes to the broader goals of sustainable agriculture and functional food development.

Materials and methods

All procedures of this experiment were approved (ethical committee number: AK2024008) by the Animal Ethics Committee of Anhui Science and Technology University (Fengyang, China).

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Items		Dietary Selenium Levels (mg/kg)	Sodium selenite content (mg/kg)
Control group	Control	0.04	0
Treatment group	Se10	10.04	21.90
	Se20	20.04	43.80
	Se30	30.04	65.71
	Se40	40.04	87.61

Table 1. The design of experiment group.

Items	Content(%)
Wheat bran	10
Food waste	90
Total	100
Nutrient levels (%)	
Dry matter	32.20
Crude protein	9.88
Crude fat	1.79
Calcium	0.31
Phosphorus	0.31

Table 2. Composition and nutrient levels of basal diets.

Experimental design and dietary composition

The eggs of the black soldier fly were incubated at 30 ℃ in the laboratory and cultivated to 5 days old. In this experiment, 300 g larvae of 5-day-old black soldier fly were divided into 30 treatments of 10 g each according to weight. The single-factor completely randomized experiment was randomly designed and divided into 5 groups with 6 replicates per group. The experimental group design was shown in Table 1. The selenium level of 0.04 mg/kg in the control diet reflects the baseline natural selenium content in the base diet. The control group did not add sodium selenite; Therefore, sodium selenite content is reported to be 0 mg/kg. The experiment used different doses of sodium selenite for treatment. Basic diet, no supplements (control); Basic diet plus 10 mg/kg Se (Se10); Basic diet plus 20 mg /kg Se (Se20); Basic diet plus 30 mg/kg Se (Se30); Basic diet plus 40 mg/kg Se (Se40). Black soldier fly were all bred in the animal science laboratory of Anhui University of Science and Technology. The feed for the black soldier fly is prepared from kitchen waste and wheat bran. The composition and nutritional level of the basic feed are shown in Table 2.

Feeding and management

The breeding experiment of black soldier fly was conducted in the laboratory. Five groups of larvae were placed in 30 numbered petri dishes and fed with different dietary levels of sodium selenite. The culture experiment was carried out for 7 days at 30 ± 2 °C. During the breeding period, weigh the amount of feed fed and record it.

Sample collection, processing and determination indexes

At the end of the culture experiment, black soldier fly larvae were randomly selected from each replicate. The larvae were cleaned, dried in an oven at a constant temperature of 103 ± 2 °C until reaching a stable weight, and then grounded into a fine powder using a grinder. Samples were placed in a zip lock bag, numbered and stored in a dryer for analysis.

In each repeat, the medication spoon accurately collected the 3 g test sample in the conical flask, add 10 mL of nitric acid-perchloric acid mixture (9 + 1), and several glass beads, covered the surface dishes and digested them at room temperature overnight. The next day in the electric heating furnace heating, and timely added nitric acid. When the solution became clear and colorless and had white smoke, then heated it until the remaining volume was about 2 mL. After cooling, add 5 mL of hydrochloric acid solution (6 mol/L) until the solution became clear and colorless with white smoke. Transfer to a 10 mL volumetric flask, added 2.5 mL potassium ferricyanide solution (100 g/L), filedl with water and mixed well for testing. The content of trace elements was determined by VARIAN SpectrAA-240 atomic absorption spectrophotometer, and the reagent blank test was performed. Method validation was conducted to ensure accuracy and reliability[8,9], including the following parameters:

Limit of Detection (LOD): The LOD was calculated as 3 times the standard deviation of the blank divided by the slope of the calibration curve. For this study, the LOD for selenium was determined to be 0.01 mg/kg.
Limit of Quantification (LOQ): The LOQ was established at 10 times the standard deviation of the blank divided by the slope. The LOQ for selenium was calculated as 0.03 mg/kg.
Recovery Rates: Recovery experiments were conducted by spiking known amounts of selenium standard solutions into blank samples. The average recovery rate was 98.7%, with a standard deviation of ± 1.5%.

Reference Materials: Certified reference materials (NIST SRM 1573a: Tomato Leaves) were used to validate the accuracy of selenium measurements. The measured selenium concentration was within 95% confidence intervals of the certified value.
Calibration: A multi-point calibration curve was prepared using selenium standards at concentrations of 0.01, 0.05, 0.10, 0.50, and 1.00 mg/L. The correlation coefficient (R^2) of the calibration curve was 0.999, indicating excellent linearity.
Quality Control: Reagent blanks and duplicate samples were analyzed to monitor contamination and repeatability.

Weigh a certain amount of sample (e.g. 5 g) in each replicate and accurately record its mass (wet weight). Put the sample into a known mass drying container, place it in a constant temperature oven, and dry it to a constant weight at 100–105 °C. Take out the dried container and sample, cool them to room temperature in a dryer, and then weigh them (dry weight). Measure and calculate the dry matter content. Nitrogen was measured by FOSS Kjeltec 8400 and crude protein was calculated as N*6.25.and fat (AOAC procedure 7.052) in the feed and feces were determined employing instruments Kjeltec Auto 1030 Analyser and Soxtec 1043 from Tecator Comp. Calcium was estimated by the titrimetric method, number 6.011 of AOAC, and phosphorus by a colorimetric method.

Accurately weigh the crushed sample, add 9 times the volume of normal saline at the ratio of weight (g) : volume (mL) = 1:9, and prepare 10% homogenate under the condition of ice water bath, and take the supernatant to be measured at 2500 RPM for 10 min. The total antioxidant capacity (NTKO, A015-1-2) and glutathione peroxidase (GSH-Px, A005-1-2) content were measured using colorimetric method; Determination of hydrogen peroxide (CAT, A007-1-1) content using visible light method; Measure the content of total superoxide dismutase (T-SOD, A001-1-2) using the hydroxylamine method; Simultaneously, the concentration of homogenate protein (TP, A045-2-2) was measured using the Coomassie Brilliant Blue method.

Statistic analysis

Data were analyzed using the general linear models (GLM) Procedure of SAS.The following model was used:

$$Yij = \mu + di + \varepsilon ij,$$

where Yij is the observation; μ is the general mean; di is the effect of sodium selenite level ($i = 1,\ldots,5$); εij is the random error.

Tukey tests were used to detect statistical significance between treatment groups. Linear and quadratic effects due to sodium selenite level were determined. Correlations were tested with Pearson's correlation procedure. Significant differences were accepted if $P \leq 0.05$.

Results and discussion

Production performance

As shown in Table 3, there were significant differences in larval weight gain, feed weight loss and feed/insect ratio. The control group, with no or standard sodium selenite, had a weight gain of 132.63 g, feed loss of 320.97 g, and a ratio of 6.15. As selenium levels rose from Se10 to Se40, weight gain fell from 126.21 g to 99.51 g, suggesting potential harm at high selenium doses. Feed loss initially rose then fell, and the feed-insect ratio increased with selenium, indicating less efficient biomass production. Statistical analysis revealed highly significant linear and quadratic effects for all metrics ($P = 0.0001$, indicating a complex relationship between selenium dose and growth, with potential for an optimal selenium level.

Many studies have shown that excessive selenium can also cause damage to the animal body and cause some diseases, so it is very important to control the intake of selenium in animals[10,11]. Therefore, it is normal to have different research results. High concentration of sodium selenite can affect the proliferation and differentiation of porcine skeletal muscle satellite cells and the expression of Selenoprotein W (SelW) and Selenium-binding protein 1 (SBP1). The potential toxicity threshold of selenium (Se) for black soldier fly (BSF) larvae has not been

Items	Larva weight gain (g)	Feed weight loss (g)	Feed insect ratio
Control	132.63 ± 8.39^{a}	320.97 ± 33.95^{ab}	6.15 ± 0.36^{c}
Se10	126.21 ± 5.50^{b}	358.03 ± 41.79^{a}	6.43 ± 0.25^{c}
Se20	123.38 ± 9.72^{b}	295.40 ± 60.80^{ab}	6.58 ± 0.48^{c}
Se30	110.21 ± 3.24^{c}	263.40 ± 8.02^{c}	7.28 ± 0.19^{b}
Se40	99.51 ± 2.46^{c}	255.58 ± 22.29^{c}	7.99 ± 0.18^{a}
Linear	0.0001	0.0083	0.0001
Quadratic	0.0001	0.0285	0.0001

Table 3. Effect of dietary sodium selenite levels on the growth performance of black soldier fly. The amount of feed added in each replicate is 2500 g (65% moisture content), and the initial weight of fresh insects in each replicate is 10 g; In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$), The same as below.

precisely defined due to limited studies. However, findings from this study suggest that selenium levels exceeding 20 mg/kg in the diet start to negatively affect larval growth and nutrient utilization. Higher doses (30–40 mg/kg) leed to significant reductions in weight gain, feed efficiency, and nutrient absorption, indicating the onset of toxic effects.

Selenium toxicity likely results from oxidative stress induced by excessive incorporation of selenium into selenoproteins, disrupting protein synthesis and enzyme functions. The bioaccumulation of selenium in larvae also suggests efficient uptake mechanisms, which could increase susceptibility to toxicity at higher doses.

Studies on other insects, such as mealworms, indicate that selenium concentrations exceeding 10–15 mg/kg can adversely affect growth and reproduction[12]. This threshold aligns with the levels observed in BSF larvae, suggesting similar sensitivity among insects.

It is important to note that the nutritional composition of the diet, including vitamins and other key nutrients, plays a crucial role in mitigating the toxic effects of selenium. Vitamins such as vitamin E, a known antioxidant, may help alleviate oxidative stress induced by selenium toxicity[13]. Furthermore, essential amino acids, fatty acids, and minerals like zinc and copper contribute to maintaining cellular function and enzyme activity, which can be disrupted by excessive selenium. A balanced diet that provides these nutrients in adequate amounts is vital to support overall health and resilience against environmental stressors, including the adverse effects of selenium overload. Proper formulation of the insect diet, considering the synergistic effects of these nutrients, could help enhance growth performance and reduce the potential risks associated with selenium toxicity.

Selenium content

Table 4 shows that black soldier fly larva and decidual selenium content has a significant increase in the body. The selenium content in the body and molt of black soldier fly larvae showed a linear ($P=0.0001$) and quadratic ($P=0.0001$) increase with the increase of dietary sodium selenite level, suggesting a non-linear accumulation pattern that may peak at optimal selenium levels, beyond which further increases could lead to reduced benefits or toxicity. The data showed that the selenium content of larvae and their molts was significant and increased with the increase of dose. Larval bodies saw a rise from 0.05 ± 0.03 mg/kg in the control group to 32.13 ± 2.52 mg/kg in the Se40 group, while the molts exhibited an increase from 0.02 ± 0.00 mg/kg in the control to 10.99 ± 0.67 mg/kg in the Se40 group. This indicates efficient bioaccumulation and excretion of selenium in the black soldier fly biomass.

Black water fly larvae serve as a food source with important nutritional potential, rich in protein, fat, minerals and vitamins[14]. The present study extends this by focusing on selenium utilization, a vital nutrient with various health benefits. Furthermore, Wen et al.[15] discussed selenium-enriched proteins, emphasizing their preparation, bioavailability, and bioactivities, which are pertinent to the current study's observations on selenium metabolism.

The implications of selenium utilization in black soldier fly larvae are multifaceted. Selenium is a crucial component of selenoproteins, which play a vital role in various physiological processes, including antioxidant defense and immune function[16]. Therefore, the ability of black soldier fly larvae to accumulate selenium could make them a valuable dietary source of this essential nutrient, potentially benefiting both animal and human nutrition.

Digestibility

Table 5 shows that dietary sodium selenite level has a significant effect on nutrient utilization of black soldier fly larvae. The utilization rate of crude protein uptake by black soldier fly larvae (linear), $P=0.0001$; quadratic, $P=0.0003$), phosphorus utilization (linear, $P=0.0027$; quadratic, $P=0.0127$), crude fat utilization (linear, $P=0.0001$; quadratic, $P=0.0001$) and Ca utilization rate (linear, $P=0.007$; quadratic, $P=0.0075$) decrease linearly and quadratic with increasing dietary selenium levels. In contrast, selenium utilization significantly improved with sodium selenite supplementation, rising from $7.06 \pm 4.23\%$ in the control group to $3.51 \pm 0.22\%$ in the Se40 group. These findings suggest that while sodium selenite may impede the utilization of certain nutrients, it enhances the utilization of selenium.

The current study's observations align with Zhang et al.[17], who investigated the impact of dietary copper methionine concentrations on growth performance and nutrient digestibility in broilers. Their findings, along with the present study, underscore the complex interplay between dietary supplements and nutrient utilization. Furthermore, Yan et al.[6]explored the optimal doses and forms of selenium in maintaining reproductive health, highlighting selenium's regulatory role in gut microbiota and testicular redox. This is pertinent to the current study, as it emphasizes the importance of selenium in biological systems.

Items	Insect body (mg/kg)	Larval moult (mg/kg)
Control	0.05 ± 0.03^{e}	0.02 ± 0.00^{e}
Se10	4.75 ± 0.45^{d}	2.01 ± 0.19^{d}
Se20	10.11 ± 1.18^{c}	4.13 ± 0.22^{c}
Se30	18.43 ± 0.65^{b}	7.10 ± 0.18^{b}
Se40	32.13 ± 2.52^{a}	10.99 ± 0.67^{a}
Linear	0.0001	0.0001
Quadratic	0.0001	0.0001

Table 4. The effects of dietary sodium selenite on selenium metabolism of black soldier fly.

Items	Crude protein	Crude fat	Phosphorus	Calcium	Selenium
Control	24.64 ± 1.64^{a}	94.86 ± 10.70^{a}	16.83 ± 0.88^{a}	27.11 ± 2.12^{a}	7.06 ± 4.23^{b}
Se10	23.46 ± 1.17^{a}	88.91 ± 5.19^{a}	16.88 ± 1.11^{a}	25.51 ± 2.72^{a}	2.58 ± 0.32^{a}
Se20	22.18 ± 2.17^{ab}	87.07 ± 1.65^{a}	14.90 ± 2.07^{ab}	23.95 ± 2.75^{a}	2.71 ± 0.51^{a}
Se30	20.37 ± 1.53^{bc}	74.90 ± 4.10^{b}	12.25 ± 2.78^{b}	23.95 ± 1.59^{ab}	2.95 ± 0.12^{a}
Se40	18.36 ± 1.20^{c}	66.38 ± 4.28^{b}	13.31 ± 0.30^{b}	20.19 ± 1.94^{b}	3.51 ± 0.22^{a}
Linear	0.0001	0.0001	0.0027	0.0017	0.1248
Quadratic	0.0003	0.0001	0.0127	0.0075	0.0266

Table 5. Effect of dietary sodium selenite levels on nutrient utilization of black soldier fly.

Items	Total antioxidant capacity (U/mg)	Catalase activity (U/mL)	Total superoxide dismutase (U/mL)	Glutathione peroxidase (U/mL)
Control	2.79 ± 0.55a	10.02 ± 2.18	65.19 ± 13.93	1023.12 ± 129.88a
Se10	9.15 ± 2.99b	8.38 ± 1.09	74.17 ± 7.28	1321.56 ± 162.67b
Se20	5.26 ± 1.45a	8.67 ± 1.87	80.29 ± 8.30	1352.70 ± 210.93b
Se30	4.58 ± 1.21a	8.06 ± 2.61	76.29 ± 13.39	1470.57 ± 141.38b
Se40	4.05 ± 1.49a	8.47 ± 1.93	75.11 ± 13.44	1441.41 ± 93.33b
Linear	0.6886	0.3224	0.2945	0.0045
Quadratic	0.1727	0.4584	0.2635	0.0049

Table 6. The effects of dietary sodium selenite on antioxidant indices in black soldier fly.

The implications of these findings are significant, as they demonstrate the trade-offs associated with dietary selenium supplementation in black soldier fly larvae. While selenium enhancement improved selenium utilization, it concurrently reduced the digestibility of other essential nutrients. This highlights the need for careful consideration of selenium supplementation levels in diets to optimize nutrient utilization and overall larval growth performance.

Antioxidant index

Table 6 shows that dietary sodium selenite can increase antioxidant indexes of black soldier fly larvae. The glutathione peroxidase (GSH-Px) of black soldier fly larvae increases with the increase of dietary sodium selenite levels (linear, $P = 0.0045$; quadratic, $P = 0.00491$), GSH-Px activity significantly increased with sodium selenite supplementation. The total antioxidant capacity (T-AOC) exhibited a significant increase with sodium selenite supplementation, rising from 2.79 ± 0.55 U/mg in the control group to 9.15 ± 2.99 U/mg in the Se10 group, and remained elevated across all treatment groups. Catalase (CAT) activity did not significantly vary with the addition of sodium selenite. Total superoxide dismutase (T-SOD) activity saw a slight uptick with selenium supplementation but did not achieve statistical significance. These findings suggest that sodium selenite supplementation enhances the antioxidant defenses of black soldier fly larvae, particularly by boosting GSH-Px activity.

The observations from the current study are consistent with recent research that has shown selenium-rich black soldier fly supplementation to enhance serum indexes and egg selenium content in laying hens[18]. Additionally, studies have demonstrated that selenium-enriched Cardamine violifolia can increase selenium content and reduce cholesterol concentrations in broilers[19], highlighting the positive impacts of selenium on antioxidant status and animal health. The implications of these findings are notable, suggesting that dietary selenium supplementation can bolster the antioxidant defenses of black soldier fly larvae, potentially enhancing their resilience to oxidative stress and improving their survival and growth. The results further emphasize the critical role of selenium in biological systems and its potential benefits in animal nutrition and health.

Conclusion

In summary, The addition of sodium selenite to the diet of BSF larvae significantly increases selenium content, improves selenium utilization, and enhances antioxidant capacity. However, while moderate selenium supplementation can be beneficial, excessive levels have been shown to negatively impact growth performance and the utilization of other nutrients. The potential selenium toxicity threshold for black soldier fly (BSF) larvae is approximately 20 mg/kg in the diet. Selenium levels exceeding this amount begin to negatively impact larval growth and nutrient utilization. At higher doses (30–40 mg/kg), these negative effects become more pronounced, indicating the onset of toxicity. This toxicity is likely due to oxidative stress, as excessive selenium incorporation into selenoproteins disrupts protein synthesis and enzyme functions, impairing cellular metabolism. These findings emphasize the need for careful regulation of selenium levels in insect diets. The optimal level of sodium selenite supplementation should balance selenium enrichment with overall health and productivity. Future research should focus on determining the precise threshold for selenium toxicity in BSF larvae and explore how other essential nutrients, such as antioxidants (e.g., vitamin E) and minerals (e.g., zinc), can mitigate oxidative

stress and support nutrient utilization. Understanding these interactions will be key to formulating diets that enhance selenium benefits while avoiding negative impacts on larval growth and nutrient absorption.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

Yifan Li and Han Chen: Conceptualization, Data Curation. Yunting Zhang: Methodology. Shoukang Cao: Software, Visualization. Huihui Wang: Resources. Zhentao Lu: Visualization. Wu Xuezhuang*: Funding acquisition, Writing—review & editing.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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