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ORIGINAL ARTICLE

Effect of different doses of selenium in the nutrient medium on California worm reproduction

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Worms have a unique ability to accumulate trace elements in their bodies from the substrate, which creates the preconditions for obtaining a protein-mineral supplement with high metal content in organic form. Selenium occupies a special place among the seventeen trace elements recognized as vital for the human body, animals, and birds. Selenium, present in a body in small quantities, performs essential functions: catalytic, structural, regulatory. Selenium has antioxidant, immunostimulatory, anticancer, antimutagenic, adaptogenic, antiviral, and radioprotective properties. The aim was to conduct experimental studies on the reproduction of worms, weight gain, and selenium accumulation in their body depending on this element's content in the nutrient medium. To experiment, we formed 54 "microbeds". The size of such bed was 0.5 x 0.7 m. We placed 11.0 kg of a worm nutrient medium in each microbed and divided all microbeds into six groups (one control and five experimental) with nine microbeds. The nutrient medium contained selenium's natural background; an additional trace element was not introduced. In the experimental microbeds, selenium 1, 2, 4, 8, and 16 mg/kg of the nutrient medium was added in the form of sodium selenite $Na_2SeO3{\cdot}5\ H_2O$. Eighty mature hybrids of red California worms were populated in each microbed. At the end of the 110-day experiment, the number of worms was counted in all microbeds, and average worm biomass samples were taken to determine the selenium content. The atomic absorption spectrophotometry determined selenium content in the worm biomass on a Shimadzu AA-6650 ("Shimadzu", Japan). We found that the optimal dose of selenium is 4 mg/kg of medium nutrient weight, while the number and weight of adult worms increase by 86.7% and by 93.2%, and immature ones by 60.2% and by 63.9% as compared to the control. The negative effect on the vermiculture by Selenium16 mg/kg dose of the nutrient medium decreases in adult worms by 38% compared with control microbeds. When growing worms on a nutrient medium with additional application of selenium with doses of 1 mg/kg, 2, 4, 8, and 16 mg/kg of the substrate, the content of this element in the dry vermiculture mass increases, respectively, by 2.4, 4.4, 8.5, 14.9, and 26.8 times. We also established a direct staleness between selenium content in the nutrient medium and a dry mass of the vermiculture.

Keywords: selenium; red California worm's hybrid; the number of worms; worm mass; vermiculture; microelement

Introduction

Selenium takes a special place among the seventeen trace elements that are recognized as vital for the human body, animals, and birds (Łukaszewicz et al., 2011; Prashanth et al., 2015; Hasanato, 2019).

Numerous studies showed that selenium, which is found in a body in small amounts, performs essential functions – catalytic, structural, and regulatory – interacts with proteins, enzymes, vitamins, trace elements, and biological membranes. Selenium has a wide range of pleiotropic action; it has antioxidant, immunostimulatory, anticancer, antimutagenic, adaptogenic, antiviral, and radioprotective properties (Rayman, 2012). It normalizes reproductive function, promotes the removal of heavy metals and several organic compounds from the body, and participates in growth and development (Reich and Hondal, 2016).

The recommended level of selenium intake with food in the United Kingdom (UK) and China is 60 mcg per day for adults, while in the US – 55 mcg per day (Stoffaneller and Morse, 2015). Low selenium levels in the environment lead to its deficiency in most plant foods and animal feeds, which leads to insufficient consumption of the trace element by humans. Thus, many recent studies have focused on food biofortification to enrich human diets with organic selenium forms. Selenium is naturally present in the forms of selenate, selenite, and various organic Selenium compounds, such as selenium methionine (SeMet), selenocysteine (SeCys2), and methylselenocysteine (MeSeCys). Organic Se compounds in food are considered more bioavailable and bioactive for human consumption and are safe or have a higher toxicity threshold as compared to inorganic Se (Amoako et al., 2009). Selenium-enriched foods or selenium-containing supplements are generally recommended for Se deficiency people (Rayman et al., 2008). In recent years, selenium enrichment of food products has become an effective strategy of nutrition. There were developed methods for the use of Se additives to produce a variety of micronutrient-enriched agricultural products, including cereals and legumes, vegetables and fruits (Puccinelli et al., 2017), dairy products (Ling et al., 2017), and mushrooms (Falandysz, 2013).

Previous studies showed that SeMet is the dominant compound of selenium in fish tissues (Jagtap et al., 2016), in yeast (Egressy-Molnár et al., 2016), cereals, and soybeans (Liu et al., 2001). Se in food's bioavailability is often assessed by simulating gastric and intestinal digestion (dialysis method). As a rule, organic selenium compounds are more bioavailable than inorganic ones, which means that a body readily absorbs this form of the element in food. Also, it was previously noted that Se from foods of animal origin is less bioavailable than the trace elements contained in plants (Thiry et al., 2012; Moreda-Piñeiro et al., 2013; Silva et al., 2017). Selenium source and its bioavailability were studied only in some Se-enriched crops; most crops have a low storage capacity of up to 50 mg/kg, so the total Se concentration and bioavailable form of the element in these crops is not sufficient to provide people or animals, living in low biogeochemical zones in this element. Studies of many scientists showed that grain feeds, used for feeding different species and age groups of poultry, are selenium deficient (Thiry et al., 2012). Currently, the high selenium need for poultry can be met only by introducing inorganic or organic forms of this microelement into compound feeds. Selenium deficiency causes about 75 different diseases and pain symptoms (Rayman, 2000). Selenium deficiency is considered a possible etiological factor of 14 cardiovascular diseases, including distal cardiomyopathy (Keshan's disease), atherosclerosis, coronary heart disease, myocardial infarction, hypertension (Benstoem et al., 2015). Some scientists link Kashin-Bek disease (Urov disease) with profound selenium deficiency in soils, plants, and food, and this is a severe human bone disease (mainly affects children), common in the Chita region's Southeastern areas in Russia, North Korea, North-eastern China (Yao et al., 2011). Earthworms consume different organic soil fractions and bioaccumulate some soil contaminants: chemicals, parasite larvae, and cysts (Ernst et al., 2008; Antipov et al., 2018). Such contaminants are trace elements salts, such as Na2SeO3•5H2O. Sizmur et al. (2011) proved that the presence of *Lumbricus terrestris* increases metals' bioavailability in soil.
Therefore, our research aim was to study the effect of different doses of selenium in the nutrient medium on worm reproduction, body weight dynamics, and metal accumulation in worm biomass.

Methods

Our research was conducted based on the Bila Tserkva National Agrarian University vivarium (Ukraine). The biological object in the experiment was red California hybrid worms. For the experiment, we formed 54 so-called "microbeds", the size of which was 0.5 m x 0.7 m. 11.0 kg of nutrient medium (fermented cattle manure and cereal straw) for worms was placed in each microbed. The humidity of the nutrient medium was in the range of 65.0 to 67.0%. All microbeds were divided into six groups (one control and five experimental), with nine microbeds in each.
The nutrient medium contained natural selenium background; an additional trace element was not introduced. Selenium was added to the nutrient medium with 1 mg/kg of nutrient medium in the first experimental group due to sodium selenite Na2SeO3·5H2O. To the nutrient medium of the second experimental group there was introduced 2 mg of selenium per kg. The third experimental group's nutrient medium was enriched with selenium in 4 mg/kg. In the fourth and fifth experimental groups, 8 and 16 mg/kg of the element were added to the nutrient medium. At the beginning of the experiment, 80 mature red hybrid California worms were housed in each microbed. At the end of the 110-day experiment, the number of worms was counted in all microbeds, and average worm biomass samples were taken to determine the selenium content. The selected worm samples were incubated for 60 hours on moistened filter paper to free the gastrointestinal tract from the contents, which may be erroneous when examining the selenium content. After keeping the worms on the filter paper, they were dried in a drying cabinet at a temperature of 45 °C and active ventilation, bringing the temperature to 105 °C. The dry worm biomass was ground in a porcelain mortar, and wet ashing samples were prepared to determine the selenium content. The selenium content of worms' biomass was determined by atomic absorption spectrophotometry on a Shimadzu AA-6650 ("Shimadzu", Japan) instrument. Student's test evaluated the difference between the groups and considered credible with values: * P<0.05, ** P<0.01, *** P<0.001.

Results and Discussion

At the end of the experiment, it was found that in the control group of microbeds, the average number of worms weighing 0.4-0.8 grams constituted 134.9 pieces (Table 1). When cultivating vermiculture on a nutrient medium with an additional selenium content of 1 mg/kg, the number of adults reliably increased by 23.7% (p≤0.001). There was revealed a stimulating effect of additional Selenium application in the amount of 2 mg/kg and 4 mg/kg in the nutrient medium on the amount and growth of vermiculture. The number of worms in the II and III experimental groups of beds was higher than in control by 57.6 and 86.7% (p≤0.001).

Table 1. The worms' number and their mass in microbeds depending on the concentration of selenium in the nutrient medium, n = 9.

Microbed groups	Worms in microbeds with 0.4–0.8 g mass		Worms in microbeds with 0.01–0.39 g mass	
	Number, ps.	Mass, g	Number, ps.	Mass, g
Control	134.9±3.9	81.3±2.4	130.0±3.6	27.7±0.8
I experimental	166.9±4.2***	101.6±2.6***	186.3±3.9***	40.3±0.8***
II experimental	212.7±5.3***	130.4±3.2***	198.1±3.8***	43.8±0.8***
III experimental	251.8±6.2***	157.1±3.9***	208.2±4.4***	45.6±0.9***
IV experimental	142.1±4.4	85.4±2.7	131.2±4.5	27.8±0.9
V experimental	83.7±3.5***	49.6±3.3***	57.8±5.5***	11.9±1.2***

With trace element introduction 8 mg/kg to nutrient medium (IV experimental group of microbeds), there was a tendency to increase the number of adult worms by 5.3%. The use of a 16 mg/kg dose of the studied metal-biotic leads to a decrease in the number of adult worms in the V experimental group's microbeds by 40.0% relative to control ($p \le 0.001$).

After analyzing the adult worms' mass, we found that in the control group of microbeds, the weight of 134.9 individuals was 81.3±2.37 g. The mass of vermiculture from the first experimental group of microbeds was more significant than in control by 25.0%). With selenium introduction into the nutrient medium 2 mg/kg (II experimental group of microbeds), the worm's weight increases by 60.4% ($p \le 0.001$) relative to control. We found that the cultivation of red California worms on a nutrient medium with additional biotic elements with a dose of 4 mg/kg (III microbed experimental group) led to vermiculture weight increase by 1.93 times ($p \le 0,001$) as compared with control data. In the IV experimental group, the addition of selenium in the amount of 8 mg/kg of the nutrient medium was accompanied by increasing the adult worms' weight compared with the control variant. We found a negative effect on the worms' weight by selenium dose of 16 mg/kg of nutrient medium in the V experimental group's vermiculture, where the mass was less than in control by 39.0% ($p \le 0.001$).

We also recorded the number and weight of young, immature worms (up to 90 days old and up to 0.39 g mass). In control microbeds, without sodium selenite, the number of immature individuals was – 130 worms. With the selenium introduction of 1 mg/kg, the number of immature worms in microbeds increases by 43.3% ($p \le 0.001$) relative to control. The element application of 2 mg/kg for worms' cultivation increases the number of individuals weighing up to 0.39 g relative to the control variant by 52.4% ($p \le 0.001$). We also registered an increase in the number of immature worms in the third experimental group of microbeds. This indicator was the highest among the experimental groups and differed from the control by 60.2% ($p \le 0.001$).

By increasing the Selenium dose to 8 mg/kg and counting the number of worms weighing up to 0.39 g in microbeds, we found that their number was almost the same as the control and was only 1.2 pieces (1.0%) higher. The lowest number of immature worms we registered in microbeds from experimental group V. The indicator was 2.25 times lower than in control and amounted to 57.8 worms. Therefore, selenium in optimal doses stimulates the reproduction of worms. Excessive increase in the content of trace elements in the nutrient medium harms, increasing the number of vermiculture individuals. We found that the number of immature worms in the control group of microbeds amounted to 130 pieces, which weighed 27.7 g. Introduction of selenium in the amount of 1 mg/kg of nutrient medium (I experimental group of microbeds) led to vermiculture mass increase by 45.5% ($p \le 0.001$) relative to control. With the element used in the amount of 2 mg/kg, the mass of immature worms increases by 61.1% ($p \le 0,001$) relative to the mass of the control group worms. Selenium at a 4 mg/kg dose led to worms' mass increase by 63.9% ($p \le 0.001$) relative to the control indicator. Relative to the number, the weight of Californian worms in the IV experimental group's microbeds, which were cultured on a nutrient medium with an additional microelement of 8 mg/kg, did not differ from control.

With selenium's introduction at a dose of 16 mg/kg of nutrient medium, immature worms' weight decreased relative to control by 2.32 times. Thus, the application of different doses of selenium in different ways affects the mass of immature worms. In control, the average weight of young worms was 0.213. With the Selenium use of 4 mg/kg of nutrient medium, individuals' average weight was 1.4% higher than in control. The highest dose of the element caused a decrease in the average weight of immature worms relative to this indicator in the control and the III experimental group, respectively, by 4.2% and 5.6% (Table 2). In the dry mass of the control group's worms, which were grown on a nutrient medium without the additional introduction of selenium, the element content was 7.2 mg/kg.

Table 2. Selenium content in the dry biomass of red hybrid California worms, n = 9.

Microbed groups	Selenium mass share, mg/kg
Control	7.2±0.5
I experimental	17.1±0.8***
II experimental	31.4±0.8***
III experimental	61.1±0.8***
IV experimental	107.3±1.2***
V experimental	192.7±1.9***

Thus, worms play an important role in changing soil structure and improving general soil quality. They are widely used as feed for animals, poultry, and fish, due to the high content of proteins, minerals, and other bioactive compounds. Also, earthworms are known to have the ability to accumulate biotic metals, including selenium (Sun et al., 1997; Kostecka and Pączka, 2006; Khan, 2018). The effects of selenium on earthworms were mainly studied by focusing on toxicological aspects. The selenium bioaccumulation coefficient in worms' body was more significant than one in both fields (Richardson et al., 2015) and laboratory conditions (Liu et al., 2001; Stewart et al., 2013; Xiao et al., 2018), which indicates that worms have a specific ability to enrich Se. The average lethal concentration (LC50) of selenate and selenite for *Lumbricus terrestris* is 60 and 31 mg/kg substrate, respectively, while 50 mg/kg $Na2SeO3$ has no significant effect on the mortality of the *E. fetida* (Fischer and Koszorus, 1992). Previous studies were also conducted to study the Se accumulation in *E. fetida* tissues, depending on the time and concentration of selenium in the substrate, with bioaccumulation factor in *E. fetida* increased to 2.09 (Xiao et al., 2018) the maximum concentration of Se in *E. fetida* tissues reached 332.5 mg/kg of dry weight (Liu et al., 2001) with an organic predominance of selenium (Sun et al., 2014).

The chemical composition of vermiculture biomass largely depends on the nature of the nutrient medium in which the worms live. As the content of metals in the nutrient medium increases, their concentration in worms' biomass increases.

Our research showed that the addition of the lowest selenium dose in experimental microbeds leads to a significant increase in this trace element in worms' dry matter. Thus, in the dry biomass from the first experimental group of microbeds, the selenium content was 2.4 times higher than in control (p≤0.001). When growing worms on a nutrient medium to which selenium was added in the amount of 2 mg/kg (experimental group II), the element accumulated by 4.4 times (p≤0.001) more in the dry matter than in control. There was an increase in selenium's content in the dry matter of worms from the third experimental group of microbeds, such content of the element exceeded the control index by 8.5 times (p≤0.001). Thus, the data we obtained indicates that adult worms' number and weight depended on selenium's nutrient medium content. With a micronutrient dose of 4 mg/kg of nutrient medium, the worms' number and weight were the highest and likely to exceed control. The selenium dose of 16 mg per kg of the substrate was toxic, which is confirmed by a decrease in the number and weight of vermiculture worms. These data for selenium's effect on several worms and worm mass is consistent with the data reported by Sun et al. (2014).

The concentration of selenium in the dry mass of vermiculture from the IV experimental group was 14.9 times higher than in the control (p≤0.001). The highest content of the element was in the dry mass of California worms, grown on a nutrient medium to which selenium was added in 16 mg/kg. The metal content in the dry matter of the experimental group's vermiculture was 192.7 mg/kg, which exceeds the control by 26.8 times. Our data for the effect of selenium accumulation in the biomass of worms is consistent with the data reported by Steward et al. (2013).

Thus, as selenium's content in the nutrient medium increases, this element's concentration in worms' biomass increases. The results allow the use of dry biomass of red California worms to feed farm animals, poultry, and fish as a biofortified feed additive for selenium.

Conclusion

Our research confirmed the ability of chemical elements to accumulate in the worm's body on the example of selenium. Depending on selenium's concentration in the nutrient medium, worms' number and weight increase in different ways. The stimulating effect of the microelement on the reproduction of worms is manifested in doses of 1-4 mg/kg of nutrient medium. The addition of selenium at 16 mg/kg toxically affects the worm's body, which led to a decrease in the number of adults by 38.0%.

We found a direct relationship between selenium's content in the nutrient medium and dry mass culture. Thus, selenium's content in the dry matter of worms at the natural conditions was 7.2 mg/kg, while with the introduction of selenium in the doze of 16 mg/kg, this index was 192.7 mg/kg (increased by 26.8 times).

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