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Californian red worm biomass increase and its cobalt accumulation under different concentrations of the metal in nutrient medium

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Californian red worms hybrid biomass is a biologically valuable feed supplement for livestock, poultry and fish. The worm body contains a significant amount of protein, lipids, vitamins and minerals. The chemical composition of worms depends on the nutrient medium in which they were grown. Worms can accumulate minerals from the nutrient medium in the body which called forth conducting research on obtaining worms biomass enriched with Cobalt with the prospect of its further use in fish feeding.
It has been found out that the worms quantity and the mass increase depends on the content of Cobalt in the nutrient medium. Adding 20 mg / kg of Cobalt to the nutrient medium contributed to 38.0% and 40.4% increased amount of worms weighing 0.4-0.8 g respectively. The study reveals that the number of immature worms increased by 32.2%. Adding 40 mg / kg of Cobalt to the nutrient medium resulted in worms number and mass increased by 45.9 and 51.1% respectively. Adding 160 mg / kg of Cobalt resulted in 6.5-27.7% smaller amount of worms weighing 0.4-0.8 g as compared with the experimental groups. It has been found out that the number of small worms reduced by 24.0-50.7% compared with the experimental groups under adding 160 mg / kg of Cobalt. The weight reduced by 22.4% and amount of young worms compared with the control has been revealed. We have established the pattern that as Cobalt amount in the nutrient medium increases, its concentration in the worms biomass increases as well. The highest content of Cobalt was found in the biomass dry matter of worms grown in the nutrient medium enriched with the studied metal in the amount of 160 mg / kg.
Biomass of worms grown in the nutrient medium with 40 mg / kg of the studied metal added can be used in fish feeding as a protein supplement with a high content of Cobalt.
Key words: vermiculture; worms biomass; metals accumulation; nutrient medium; Cobalt; atomic absorption spectroscopy

Introduction

Earthworms pass soil through their stomach each day and make the soil become organic at a rate of nearly 60% of their body weights thus contributing to sustainable agriculture. With these properties, and by including worms, which have an important place in the natural lives of poultry animals and which are important elements in the ecosystem of the soil, in poultry husbandry again, the comforts of animals will be improved and the protein will be provided at a cheaper price, and an environmental protection effect may be achieved with the help of recycling of animal and plant wastes (Köse, Öztürk, 2017).
Earthworms are a good quality protein for use in animal feeds, but the practical use of earthworm meal is influenced by economics. Technology is available for large-scale production of earthworms, but separating the earthworms from the organic wastes in which they are growing is labor intensive, thus hindering the use of earthworm meal in developed countries. The potential is greater for producing earthworm meal in developing countries where labor costs are lower (Jacob, 2013; Jacob, 2015).
The world science and practice have shown that bioconversion using vermiculture (California red worm hybrid) is a promising and virtually waste-free technology of agriculture organic waste recycling. Biotechnology of vermicultivation is a short conversion of plant residues recycling. The worm biomass is a product of vermicultivation, it is a valuable protein-vitamin-

mineral feed additive to animal feed for farm animals, fish and poultry (Das, Dach, 1989, 1990; Mason et al., 1992; Kostecka, 2006). In addition, the worms produce biohumus - environmentally friendly organic fertilizer for crops (Volodin et al., 1995).
Californian red worm biomass contains, respectively, 45-65% and 8.0-10.5% protein and lipids of their dry weight. Worm biomass contains 17.0-23.0% of dry matter with over 60.0% of crude protein in it (Kholodova et al., 1992; Hasanuzzaman et al., 2010). Lipid fraction of worm biomass is rich in phospholipids with phosphatidylcholine as a basic component. It includes C_{27}-sterols, ubiquinone, carotenoids, triglycerides, saturated fatty acid (47-54%), unsaturated (23%) and polyenes fatty acids (under 13%). Worms biomass contains thiamine (B1), nicotinic acid, riboflavin (B2), pyridoxine (B6), cyanocobalamin (B12), folic acid and biotin. The worm biomass contains a considerable number of stimulating substances: styrene (0.16-0.73%), provitamin D (0.04-0.073% of live weight) (Kholodova et al., 1994).
We detected the following elements in the worms dried biomass: Fe - 680-1070 mg / kg, Magnesium - 660-842 mg / kg, Zinc 72-80 mg / kg, Copper - 7.8 mg / kg and Cobalt - 1.5-2.5 mg / kg of the dry matter (Merzlov and Mashkin, 2015).
Dried earthworm powder from culture of *Lampito mauritii* in vermicomposting units and subjected to analysis of protein, carbohydrate and mineral content. The EWP contained large amounts of protein (31.7 %), iron (241.1ppm), soluble nitrogen (1.8%), zinc (32.34ppm), manganese (17.2ppm) and copper (4.501ppm) together with notable quantities of potassium, calcium, magnesium, phosphorus and carbohydrate indicating that this type of earthworm contain potentially useful quantities of many nutrients, that are important to the human health (Bhorgin Lourdumary, Uma, 2012).
Trace elements are involved as essential parts of many physiological activities such as energy production, enzyme activity, hormone production, collagen formation, vitamin and tissue synthesis, oxygen transport, and other physiological processes related to health growth and reproduction and their deficiency leads to wide variety of pathological consequences such as cardiac conditions in addition to immunological and hormonal dysfunctions and metabolic defects (Suttle, 2010).
Thus, the study of domestic and foreign authors proves convincingly that the worms' biomass is a valuable addition for animal feed. It should be noted that the chemical composition of the worm body depends largely on the nature of nutrient medium in which they are grown. Microelements concentration in the worms' biomass increases with the increase in their content in the nutrient medium (Mashkin, Merzlov, 2015).
This article provides information on vermiculture biomass (a food additive) increase and Cobalt accumulation in the biomass depending on the content of the element in the nutrient medium.

Material and methods

The experiments were conducted in the vivarium of Bila Tserkva national agrarian university on Californian red worm hybrid according to the experimental scheme (Table 1). 54 microbeds sized 0.5m x 0.7 m were formed for the experiment. 11.0 kg of nutrient medium (fermented manure of cattle and cereal straw) for worms with a moisture content of 65.0 % was applied to each microbed. The microbeds were divided into 6 groups with 9 units in each.

Table 1. Experimental scheme

Group of microbeds	Lasting of the experimental	Addition dose of Cobalt in a nutrient medium
Control	110 days	-
Experimental 1	110 days	10 mg/kg
Experimental 2	110 days	20 mg/kg
Experimental 3	110 days	40 mg/kg
Experimental 4	110 days	80 mg/kg
Experimental 5	110 days	160 mg/kg

No Cobalt was added to the control group nutrient medium. 10 mg / kg of Cobalt was added to the nutrient medium in the first experimental group at the expense of CoSO4•7H2O salts. 20 mg / kg of Cobalt was added to the nutrient medium of the experimental group 2. Nutrient medium of the experimental group 3 was enriched with 40 mg / kg of Cobalt. 80 and 160 mg / kg of Cobalt were added to the nutrient medium of the experimental groups 4 and 5 respectively. Each bed was inhabited by 80 mature worms at the beginning of the experiment.
The experiment lasted 110 days each and by the end of the experiment the worms number was determined and worm samples were selected to determine Cobalt content in their body. The selected worms were kept on wet pieces of filter paper for 60 hours to release their gastrointestinal from coprolith whose presence might cause some faults in the study. After keeping, the worms were pre-dried and then ashed in muffle furnaces bringing the temperature gradually to 450°C. Cobalt content in the worms' biomass was determined by atomic absorption spectrophotometry with Shimadzu AA-6650 device.
Mathematical analysis of the experimental result was conducted in Statistica 6.0 (StafSoft Inc., USA). Differences between average values were considered statistically reliable at P<0,05 (ANOVA).

Results and discussion

There are 134.9 mature worms (weighing 0,80-0.40 grams) and 130.0 immature ones weighing up to 0.39 grams at the most were identified on average by the end of the experiment in the control microbeds (Table 2).

Table 2. Worms number and their weight in microbeds depending on Cobalt concentration in the nutrient medium (x±SD, n = 9)

Microbeds group	Worms weighing 0.4–0.8 g in the bed		Worms weighing 0.01–0.39 g in the bed	
	Number, pcs.	Wight, g	Number, pcs.	Wight, g
Control	134.9±3.94	81.3±2.37	130.0±3.59	27.7±0.76
Experimental 1	161.3±8.51*	98.1±5.18*	138.7±3.64	29.0±0.76
Experimental 2	186.2±5.17***	114.2±3.17***	171.9±4.13***	36.4±0.87***
Experimental 3	196.9±4.18***	122.9±2.61***	213.8±4.57***	46.2±0.99***
Experimental 4	208.6±5.24***	129.5±3.25***	195.0±4.56***	42.5±1.00***
Experimental 5	150.8±7.84	90.9±4.73	105.4±5.39**	21.5±1.10**

Note: * – p≤0.05, ** – p≤0.01, *** – p≤0.001

The number of units weighing 0.4-0.8 g increased by 19.5% under growing worms on the nutrient media with the addition of 10 mg / kg of Cobalt at the expense of $CoSO_4 \cdot 7H_2O$ salts. The number of mature worms in this group was 6.7% higher and their weigh was 1.3 g higher than in the control.

Adding 20 mg / kg of Cobalt in the nutrient medium contributed to increased number and mass of worms weighing 0.4-0.8 g, respectively, by 38.0% and 40.4%. The number of immature worms in the experimental group 2 beds was higher than in the control by 32.2%. The increase in the amount of mature worms in the experimental group 3 was revealed and the number of units was higher than in the control by 45.9%. The mass of worms was also higher than in the control by 51.1%. The number of worms of weighing 0.01-0.39 g in the third experimental group beds was higher by 64.4% compared to the control. When the dose of Cobalt added to the nutrient medium was increased to 80 mg / kg, it was accompanied by an increased number of adult units as compared with the control by 54.6%. The number of worms weighing 0.01-0.39 g was higher than in the control by 50.0% but relative to the performance obtained in the experimental group 3 beds it was lower by 8.8%. Adding Cobalt in an amount of 160 mg / kg (microbeds of experimental group 5) resulted in the decreased number of worms weighing 0.4-0.8 g compared with research groups by 6.5-27.7%. It was found out that that under the influence of the same very small dose of Cobalt the number of little worms decreased by 24.0-50.7% relative to the experimental groups and by 18.9% relative to the control. The decreased by 22.4% weight of young warms compared to the control was revealed.

Also, we conducted the studies on the accumulation of Cobalt in the body of worms under different doses of the metal in the nutrient medium. Cobalt content in the worms body made 2.49 mg / kg of dry biomass under growing on the nutrient media without the metal (control microbeds). Cobalt content in the dry biomass of worms grown on the nutrient media with the addition of 10 mg / kg of Cobalt at the expenses of $CoSO_4 \cdot 7H_2O$ salts was 24.91 mg / kg of dry weight, which was 10.0 times higher than in the control. Introducing 20 mg / kg of Cobalt to the nutrient medium for vermiculture in the experimental group 2 microbeds was accompanied by growth of the metal content in the dry matter biomass worms by 12.3 times as compared with the variant where Cobalt was not added.

It has been found that growing wormiculture in the nutrient medium with Cobalt added in an amount of 40 mg / kg resulted in the increased content of the element in the body of worms by 14.9 times relative to the control. Compared with the experimental group 2 beds, Cobalt concentration in the worms' biomass was 1.3% higher. Cobalt content in the worms of experimental group 4 beds exceeded the figure in the control by 17.1 times and by 14.8-71.1% in groups1-3. The highest Cobalt content was found in the dry matter biomass of worms of group 5 beds where the metal concentration was higher than in control by 18.7 times. Cobalt amount in the body of the worms of group 5 beds was higher by 9.6% in comparison with group 4 where twice as little investigated metal was added.

We have established the pattern that increased to 40 mg / kg Cobalt content in the nutrient medium shows stimulating effect on the increase in the worms' number and mass with the pattern best expressed in young worms. Increasing the metal dose in a nutrient medium to 80 mg/kg was associated with decreased worms' biomass growth due to the fact that Cobalt in such dosage accumulates in the worms' body and starts showing its toxicity. We have established clear action of Cobalt as toxicant metal at the dose of 160 mg/kg. The metal accumulates in the body of worms in excessive concentrations under this dose which results in decreased reproduction activity of worms and, consequently, their weight gain. It has been proved that Cobalt dose of 160 mg/kg of nutrient medium is not lethal, this concentration does not cause the worms death.

Cobalt content in the worms' biomass increases as the metal is added to the nutrient medium. In addition, we have established the pattern that the higher Cobalt content in the nutrient medium is, the higher mass fraction of the element in the body of worms is which confirms the worms' ability to accumulate metals in their bodies.

Conclusion

Low doses of Cobalt (40 mg / kg) in the nutrient medium provide young worms birth and increase in the total weight of vermiculture. Additional introduction of Cobalt in the nutrient medium in an amount of 160 mg / kg reduced the worms' reproductive properties. Applying 40 mg / kg of Cobalt resulted in increased to 64.4% number of young worms and contributed to the metal content increase in the vermiculture biomass by 14.9 times. The increase of Cobalt in the nutrient medium led to increased concentrations of this metal in the worms' body. Further studies will be focused on Cobalt enriched vermiculture biomass use in fish feeding.

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