Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production. 5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate

Беспозвоночные Сибири как перспективный источник животного белка для инновационного производства кормов и продуктов питания.

5. Изменения состава питательных веществ у червей и сверчков после частичного обогащения кормового субстрата

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Abstract. This study explores the prospect of regulating the nutrient composition in the biomass of terrestrial invertebrates by enriching the nutrient substrate with precursors of particular nutrients. The experiment was conducted on two model species: the house cricket Acheta domesticus (Linnaeus, 1758) and the earthworm Eisenia fetida (Savigny, 1826). It was established that partial enrichment of the food substrate with precursors of specific nutrients shows non-uniform accumulation of nutrients in the biomass of model invertebrate species. The protein content in crickets did not change, while in worms, it increased by 1.2 times with a single dose and 1.1 times with a double dose of substrate enrichment. Enrichment of the food substrate with minerals also did not show a clear pattern of accumulation of individual minerals in the biomass depending on the addition of precursors to the food substrate. Worms better accumulate minerals, and substrate enrichment can slightly increase the content of individual elements. The level of most B-group vitamins increased in both worms and crickets after single-dose substrate enrichment, and a double dose showed a significant increase in concentration for some vitamins. Fat-soluble vitamins showed a clear pattern of accumulation in the biomass of crickets and worms, with a double dose of precursors increasing their concentration compared to a single dose. The accumulation of vitamin C was most pronounced, with a significant increase in its level with a double dose compared to a single dose.

**Резюме.** В работе исследуется перспектива регулирования нутриентного состава в биомассе наземных беспозвоночных через обогащение питательного субстрата предшественниками конкретных нутриентов. Эксперимент проводился на двух модельных видах: домашнем сверчке *Acheta domesticus* (Linnaeus, 1758) и компостном черве *Eisenia fetida* (Savigny, 1826). Установлено, что частичное обогащение пищевого субстрата прекурсорами конкрет-

ных нутриентов показывает неравномерное накопление пищевых элементов в биомассе модельных видов беспозвоночных. Содержание белка у сверчков не изменилось, у червей его уровень повысился в 1,2 раза при однократной и 1,1 раза при двукратной дозе обогащения субстрата. Обогащение пищевого субстрата минеральными веществами также не показало чёткую картину зависимости накопления отдельных минералов в биомассе от внесения прекурсоров в пищевой субстрат. Черви лучше накапливают минералы, обогащение субстрата может несколько повысить содержание отдельных элементов. Уровень большинства витаминов группы В как у червей, так и у сверчков повысился после однократного обогащения субстрата, двойная доза показала увеличение концентрации, значительное у некоторых витаминов. Жирорастворимые витамины показали чёткую картину накопления в биомассе сверчков и червей, причём двойная доза прекурсоров увеличила их концентрацию даже по сравнению с одинарной дозой. Наиболее отчётливо выражено накопление витамина С с заметным увеличением его уровня при двойной дозе в сравнении с одинарной.

## Introduction

The demand for food containing animal protein is steadily increasing [Van Raamsdonk et al., 2017; Tang et al., 2019; Tobolkova, 2019; Gorbunova, Zakharov, 2021]. The importance of animal protein in the human diet and the need to attract new, non-traditional sources are being seriously discussed and researched [Iannotti et al., 2024; Rueda García et al., 2024]. A sustainable trend is emerging in the search for new sources of animal protein based on terrestrial invertebrates [Mlcek et al.,

2014; Jansson, Berggren, 2015; Zielińska et al., 2015; Kim et al., 2019; Hlongwane et al., 2020; Tshernyshev et al., 2022, 2023b]. In the European Union, four insect species and five mollusks have already been approved for cultivation and commercial use in the food industry, while in countries where terrestrial invertebrates have been traditionally consumed for a long time, the number of edible species exceeds dozens [Regulation (EC), 2004; Hanboonsong et al., 2013; Hlongwane et al., 2020; EU Commission, 2023]. The popularisation of terrestrial invertebrate agriculture is also served by works on the history of culinary traditions of different peoples who use terrestrial invertebrates for cooking [Han et al., 2017; Sun, Jiang, 2017; Olivadese, Dindo, 2023; Orkusz, Orkusz, 2024].

The next step in the prospective development of animal protein production from invertebrates will be selective breeding aimed at selecting the most productive individuals and creating breeds with high consumer properties. One of the essential questions in this case is the possibility of accumulating necessary nutrients in the biomass of cultivated invertebrates. Knowing the features of nutrient accumulation in the bodies of terrestrial invertebrates, it is possible to compose a necessary diet for obtaining enriched biomass.

As part of the project «Invertebrates of Siberia, a potential source of animal protein for innovative human food production», a separate direction was identified to study the possibility of designing the nutrient composition of model species. To universalize the comparison of data, four species of terrestrial invertebrates were selected, belonging to three types: arthropods (Arthropoda), mollusks (Mollusca), and annelids (Annelida). Standard conditions were chosen for all species, and a standardized substrate was developed. During the experiment, the substrate was enriched with precursor substances for the formation and accumulation of nutrients in the organism, and the result of accumulation was considered as the potential for accumulating specific nutrients.

In the previous work [Tshernyshev et al., 2023a], the nutrient composition in the biomass of two invertebrate species, the Speckled cockroach *Nauphoeta cinerea* (Olivier, 1789) and the Giant African land snail *Lissachatina fulica* (Férussac, 1821), were studied and analyzed. Biomass content variation of other two species, the House cricket *Acheta domesticus* (Linnaeus, 1758) and the earthworm *Eisenia fetida* (Savigny, 1826), are studied in the present paper.

The obtained results showed non-uniform accumulation of nutrients both in individual invertebrate species and in representatives of different taxonomic types of animals. The revealed trends in the accumulation of specific nutrients can be used for further designing the composition of nutrients in the biomass of cultivated invertebrates.

### Material and methods

*Experimental design.* Two invertebrate species, the House cricket *Acheta domesticus* (Linnaeus, 1758) and the earthworm *Eisenia fetida* (Savigny, 1826), were chosen as model species.

The experiment was held in five groups for each model species. The first group was the control, individuals being fed with a substrate which lacked enrichment. The second was developed on a substrate enriched with vitamins C and B7, the third with a complex mineral addition for plant (chelate), the fourth with vitamins B1 (thiamin), B3 (niacin) and B9 (folate), and the fifth with fat-soluble vitamins A, D, E, K.

The cultures were raised under laboratory conditions with a temperature of c. +25 °C and humidity of c. 60 % in cricket and c. 80 % in earthworms. Model species were placed in separate plastic containers and provided with a feeding substrate and precursors, or without them in case of the control group. The feeding substrate for cockroaches contained a mixture of grated carrot (12 g), oat flakes (10 g), dried milk (1 g) and dried gammarus (1 g), and for earthworm it contained loose tea leaves (5 g). Also, containers with crickets were provided with Petri dishes with water.

Replacement of substrate and addition of precursors were undertaken three times a week. Under a precursor (a substance inserted into the feeding substrate and shared in metabolism of invertebrates) generated a particular nutrient in the biomass. In the experiment the following substances were chosen as precursors: vitamins C and B7 (biotin) to generate protein, a complex mineral addition for plant (chelate) to minerals, vitamins B1, B3 and B9 for concordant vitamins of B-complex, and vitamins A, D, E and K for fat-soluble vitamins.

Enrichment of feeding substrate was generated in two stages. At the first stage precursors were inserted in minimal doses ranging from 1 to 50 mg per 1 kg of feeding substrate according to the type of input substance. Such a dosage corresponds approximately with recommendations for vitamin and mineral rations provided to agricultural animals to prevent hypovitaminosis. At the second stage doses of precursors were increased twice in proportion to each substance input in the substrate. In this case, doses of precursors should have sufficient enriched biomass up to the required level for metabolism and also accumulate particular nutrients. Quantities of input samples of precursors are given in Table 1.

After 30 days samples of crude frozen biomass (0.4 kg) of each model species were analysed. The analyses were undertaken in the test centre «OOO Sibtest» as a small-scale innovative enterprise of the National Research Tomsk Polytechnic University, Tomsk, Russia in a laboratory accredited with the license «GOSTAk-kreditatsiya», No.GOST.RU.22152.

Sample analyses were aimed at detecting ash, carbohydrates, chitin, proteins including content and ratio of amino acids, lipids, including analysis of fat acids, vitamins B1 (thiamine), B2 (riboflavin), B3 (niacinamide), B9 (folic acid), B12 (cyanocobalamin), A (retinol palmitate), D3 (cholecalciferol), E (α-tocopherol), K (fillokinone), and minerals: iron (Fe), selenium (Se), zinc (Zn), magnesium (Mg), copper (Cu), manganese (Mn), phosphorus (P), lead (Pb), mercury (Hg), molybdenum (Mo), iodine (I), calcium (Ca), sodium (Na), potassium (K), and chlorine (Cl). The calorific values of the biomass for both species were also determined. The protocols of analyses are provided with reference

Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production. 289 5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate

Quantity of precursors added to feeding substrate of model species during experiment Table 1. Таблица 1. Количество прекурсоров, добавляемых в питательный субстрат модельных видов эксперимента

	I stage, singular dose of precursor			II stage, doubled dose of precursor			
Type of precursor	per 1 kilo of food substrate, mg	per food portion of crickets, mg	per food portion of earthworms, mg	per 1 kilo of food substrate, mg	per food portion of crickets, mg	per food portion of earthworms, mg	
С	50	1.2	0.25	100	2.4	0.5	
B7	25	0.6	0.125	50	1.2	0.25	
Minerals(chelate)	5	0.12	0.025	10	0.24	0.05	
B1	2	0.048	0.01	4	0.096	0.02	
B3	30	0.72	0.15	60	1.44	0.3	
B9	1	0.024	0.005	1	0.048	0.01	
A	25	0.6	0.125	50	1.2	0.25	
D	2.5	0.06	0.0125	5	0.12	0.025	
E	20	0.48	0.1	40	0.96	0.2	
K	2	0.048	0.01	4	0.096	0.02	

to GOSTs which are a summary of Russian State standards. Tables of analysis results are provided in the Appendix (p. 3–7).

Statistical analysis. R version 4.0.2 [R Core Team, 2024] was used for statistical analysis of the nutrient parameters, and for multiple comparison the nonparametric statistics Kruskal-Wallis rank sum test (kruskal. test) [Kruskal, Wallis, 1952] was applied. To evaluate the differences between groups, the Dunn's test [Dunn, 1964; Dinno, 2017] was used with correction to multiply comparisons of the Benjamini-Hochberg procedure [Benjamini, Hochberg, 1995], applicable for independent tests. Linear regression was applied for the analysis of nutrient content change, and data analysis with estimated graphs [Ho et al., 2019] was used to evaluate influence of enrichment on the feed substrate during the experiment.

## Results

Nutrient composition in biomass of control group of model species. The nutrient content in the biomass of model species differs even when they are developed on unsupplemented feeding substrates in the control group. Among vitamins of group B, the biomass of crickets is richer in terms of B6 content (0.127 mg/100 g), B9 (0.122 mg/100 g), and B12  $(0.282 \mu\text{g}/100 \text{ g})$ , while worms are richer in B1 (1.3 mg/100 g), B2 (11.7 mg/100 g), and B3 (22.3 mg/100 g). Among fat-soluble vitamins, a higher content of A was recorded in the biomass of crickets  $(4.7-5.2 \mu g/100 g)$  and D3  $(0.058-0.075 \mu g/100 g)$ , and in worms, vitamins E (3.7-5.3 mg/100 g) and K (7.76-8.8 mg/100 g). A higher content of minerals in the biomass of crickets was recorded for Na (232.2–355.4 mg/100 g), P (314.7–791.3 mg/100 g), K (455.1–1089.9 mg/100 g), Mg (45.3–92.0 mg/100 g), Cl (661.5–934.5 mg/100 g), Cu (0.78–2.7 mg/100 g), I (0.062–1.1 mg/100 g), Mn (1.67-3.12 mg/100 g), Mo (0.070-0.314 mg/100 g), and Zn (6.8–15.9 mg/100 g), while in worms, Fe (12.1– 24.6 mg/100 g), Hg (0.0008 mg/100 g), Pb (0.038 mg/100 g), and Se (0.37 mg/100 g) were more abundant.

In crickets, a significantly higher ash content (1.44), fat content (3.44), carbohydrate content (35.0), chitin content (4.8), and fiber content (12.55) were recorded, while in worms, higher protein content (17.6) and water content (61.4) were observed.

Precursor minimal dose application. Minimal enrichment of precursors affected the nutrient content in the biomass of model species. It was found that some nutrients increased and others decreased.

Minimal supplementation of vitamins C and B7 in the food substrate did not have a significant impact on the increase in protein in the biomass of Acheta domesticus (Linnaeus), with its level only slightly increasing from 16.9 to 17.1 %. In contrast, the protein level in worms Eisenia fetida (Savigny) significantly increased: from 17.6 to 21.6 %.

When the substrate was enriched with a single (minimum) dose of precursors, the level of nutrients in the biomass of model species changed, either increasing or decreasing, but the level of some nutrients remained practically unchanged. When a single dose of mineral precursors was administered to crickets, an increase in the levels of Fe (from 0.541 to 0.7 mg/100 g), Zn (from 15.9 to 20.6 mg/100 g), Mg (from 92.0 to 122.6 mg/100 g), P (from 791.3 to 908.7 mg/100 g), Pb (from 0.002 to 0.0037 mg/100 g), Mo (from 0.070 to 0.0081 mg/100 g), Ca (from 90.6 to 108.2 mg/100 g), K (from 1089.9 to  $1126.4 \,\mathrm{mg}/100 \,\mathrm{g}$ ), and Hg (from  $0.0004 \,\mathrm{to}\, 0.0017 \,\mathrm{mg}/100 \,\mathrm{g}$ ) was observed. However, a decrease in the levels of Se (from  $0.099 \text{ to } 0.078 \,\mu\text{g}/100 \,\text{g}$ , I (from  $0.062 \text{ to } 0.046 \,\text{mg}/100 \,\text{g}$ ), and Cl (from 934.5 to 615.1 mg/100 g) was also observed. The levels of Cu (from 2.7 to 2.55 mg/100 g), Mn (from 3.12 to 3.5 mg/100 g), and Na (from 355.4 to 352.9 mg/100 g).

An increase in the levels of Fe (from 24.6 to 29.4 mg/100 g), Zn (from 7.0 to 9.8 mg/100 g), Mn (from 1.1 to 1.8 mg/100 g), Cu (from 0.52 to 0.62 mg/100 g), Mg (from 71.3 to 73.9 mg/100 g), P (from 495.7 to 646.0 mg/100 g), Ca (from 147.7 to 152.7 mg/100 g), and Na (from 268.9 to 376.6 mg/100 g) was observed in the worms. However, a decrease in the levels of Se (from 0.37 to 0.055  $\mu$ g/100 g), Pb (from 0.038 to  $0.010 \,\mathrm{mg}/100 \,\mathrm{g}$ ), Hg (from  $0.0008 \,\mathrm{to}\, 0.0005 \,\mathrm{mg}/100 \,\mathrm{g}$ ), I (from 0.024 to 0.0088 mg/100 g), K (from 874.5 to 806.6 mg/100 g), and Cl (from 153.6 to 135.9 mg/100 g) was also observed. The level of Mo (from 0.034 to 0.032 mg/100 g) remained practically unchanged.

In contrast to minerals, the levels of almost all vitamins increased. In crickets, among the B vitamins, the levels of B3 (from 3.292 to 4.445 mg/100 g), B6 (from 0.127 to 0.202 mg/100 g), B9 (from 0.122 to 0.134 mg/100 g), and B12 (from 0.282 to 0.554 mg/100 g) increased. Only the level of B2 decreased (from 3.226 to  $2.879 \mu g/100 g$ ), while the levels of B7 (from 0.011 to 0.018 mg/100 g) and B1 (from 0.033 to 0.032 mg/100 g) remained almost unchanged. In worms, only the level of B1 remained almost unchanged (from 1.223 to 1.182 mg/100 g), while the levels of all other B vitamins increased: B2 (from 9.369 to 13.610 µg/100 g), B3 (from 22.347 to 31.527 mg/100 g), B6 (from 0.101 to 0.201 mg/100 g), B7 (from 0.022 to 0.038 mg/100 g), B9 (from 0.029 to 0.040 mg/100 g), and B12 (from 0.026 to 0.050 mg/100 g).

The levels of almost all fat-soluble vitamins also increased. In crickets, only the level of vitamin E remained unchanged (from 2.083 to 2.056 mg/100 g), while the levels of vitamins A (from 4.752 to 6.995  $\mu$ g/100 g), D3 (from 0.058 to 0.084  $\mu$ g/100 g), and K (from 6.558 to 7.605  $\mu$ g/100 g) increased. In worms, the level of vitamin K dropped sharply (from 7.765 to 1.829  $\mu$ g/100 g), while the levels of vitamins E (from 3.773 to 5.829 mg/100 g), A (from 0.799 to 1.260  $\mu$ g/100 g), and D3 (from 0.026 to 0.047  $\mu$ g/100 g) increased significantly.

The level of vitamin C increased in both crickets (from 2.908 to 3.162 mg/100 g) and worms (from 3.773 to 6.378 mg/100 g).

**Precursor double dose application.** The increased dose of precursors also had a significant effect on the level of nutrients in the biomass of model species, but there was no linear dependence of the level of nutrient accumulation at the minimum and double doses of precursors.

The doubled dose of vitamins C and B7 through supplementation in the food substrate also had no significant effect on the increase of protein in the biomass of *Acheta domesticus* (Linnaeus), its level only slightly increased from 16.2 to 16.8 %, and in worms *Eisenia fetida* (Savigny), the protein level increased slightly more: from 16.6 to 18.5 %.

The level of mineral substances in the biomass of crickets increased only for Fe (from 2.3 to 2.6 mg/100 g), Mo (from 0.031 to 0.044 mg/100 g), and Ca (from 74.8to 79.2 mg/100 g), remained practically unchanged for Cu (from 0.78 to 0.74 mg/100 g) and I (from 1.1 to 0.9 mg/100 g), and decreased for all others: Se (from 0.10 to  $0.09~\mu g/100$  g), Zn (from 6.8 to 4.3 mg/100 g), Mn (from 1.67 to 0.96 mg/100 g), Mg (from 45.3 to 40.7 mg/100 g), P (from 314.7 to 280.3 mg/100 g), Na (from 232.2 to 195.5 mg/100 g), K (from 455.1 to 338.3 mg/100 g), and Cl (from 661.5 to 609 mg/100 g). In contrast, the level of most nutrients increased in worms: Fe (from 12.1 to 17.0 mg/100 g), Zn (from 3.3 to 3.98 mg/100 g), Mn (from 0.74 to 1.27 mg/100 g), Mg (from 28.7 to 40.2 mg/100 g), P (from 159.6 to 190.6 mg/100 g), Ca (from 82.9 to 107.2 mg/100 g), Na (from 196.3 to 230.5 mg/100 g), K (from 229.1 to 251.3 mg/100 g), and Cl (from 615.5 to 681.6 mg/100 g), remained practically

unchanged for Mo (from 0.030 to 0.025 mg/100 g) and Cu (from 0.23 to 0.20 mg/100 g), and decreased for Se (from 0.09 to 0.05  $\mu$ g/100 g) and I (from 0.054 to 0.044 mg/100 g). The level of lead and mercury was not investigated when the double dose of mineral precursors was applied.

The doubling of the precursor dose had an overall effective influence on vitamin accumulation in model species. The level of B-group vitamins in crickets remained unchanged only for B1 (from 0.052 to 0.055 mg/100 g) and B9 (from 0.38 to 0.38 mg/100 g), increased for B2 (from 3.8 to 4.7 µg/100 g), B3 (from 4.2 to 6.9 mg/100 g), B6 (from 0.19 to 0.48 mg/100 g), and B12 (from 0.33 to 0.56 mg/100 g). In worms, the level of all investigated vitamins increased: B1 (from 1.3 to 1.4 mg/100 g), B2 (from 11.7 to 13.2 µg/100 g), B3 (from 17.5 to 23.3 mg/100 g), B6 (from 0.15 to 0.27 mg/100 g), B9 (from 0.042 to 0.048 mg/100 g), and B12 (from 0.019 to 0.036 mg/100 g). The level of B7 was not investigated when the double dose of vitamin precursors was applied.

The level of fat-soluble vitamins increased after the application of the double dose of precursors in both crickets and worms. Specifically, in crickets, the levels of: E (from 2.2 to 3.4 mg/100 g), A (from 5.2 to 7.7  $\mu$ g/100 g), D3 (from 0.075 to 0.088  $\mu$ g/100 g), and K (from 7.4 to 15.2  $\mu$ g/100 g) increased. In worms, the levels of: E (from 5.3 to 8.9 mg/100 g), A (from 0.85 to 2.24  $\mu$ g/100 g), D3 (from 0.033 to 0.048  $\mu$ g/100 g), and K (from 8.8 to 12.7  $\mu$ g/100 g) increased.

The level of vitamin C also significantly increased in both crickets (from 2.6 to 3.4 mg/100 g) and worms (from 5.0 to 9.4 mg/100 g).

The present work is registered in ZooBank (www.zoobank.org) under LSID urn:lsid:zoobank.org:pub:B2CD73DD-E90F-40E4-9A7B-782A6D46B11F

## **Discussion**

Both minimal and double enrichment of the substrate with vitamins C and B7 had a negligible effect on the protein value in the biomass of *Acheta domesticus* (Linnaeus) crickets, whereas in *Eisenia fetida* (Savigny) worms, the protein level increased by a factor of 1.2 at a single dose and 1.1 at a double dose. It is likely that increasing protein content is challenging in invertebrates, but possible in forms lacking a robust external skeleton.

Enrichment of the food substrate with minerals showed an uneven pattern of substance accumulation in both crickets and worms. Thus, when a single dose of mineral precursors was applied to crickets, the levels of phosphorus (P), molybdenum (Mo), and calcium (Ca) increased by a factor of 1.2, iron (Fe), zinc (Zn), and magnesium (Mg) by 1.3, lead (Pb) by 1.8, and mercury (Hg) by 4.2. In worms, there was an increase in iron (Fe) and copper (Cu) levels by 1.2 times, phosphorus (P) by 1.3 times, sodium (Na) and zinc (Zn) by 1.4 times, and manganese (Mn) by 1.6 times. At double dose of substrate enrichment, iron (Fe) and calcium (Ca) increased by 1.1-fold, and molybdenum (Mo) by 1.4-fold in the biomass of crickets, while in worms, potassium (K) and chlorine (Cl) increased by 1.1-fold, zinc (Zn), phosphorus (P), and sodium (Na) by 1.2-fold, calcium (Ca) by 1.3-fold, magnesium (Mg) and iron (Fe) by 1.4-fold, and

Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production. 291 5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate

manganese (Mn) by 1.7-fold. Thus, only half as many minerals were altered in crickets compared with single supplementation, and their levels were approximately the same as those of these elements at the single dose of precursors. In worms at the double dose of enrichment, 1.5 times more minerals were noted, the level of which increased significantly compared to the experiment with a single dose of precursors.

The levels of a number of minerals decreased after substrate enrichment. Thus, at a single enrichment in crickets, the level of selenium (Se), magnesium (Mg), phosphorus (P), and chlorine (Cl) decreased by 1.1-fold, sodium (Na) by 1.2-fold, potassium (K) by 1.4-fold, zinc (Zn) by 1.6-fold, and manganese (Mn) by 1.7-fold, and in worms, selenium (Se) by 1.8-fold and iodine (I) by 8-fold. When the substrate was enriched twice, selenium (Se) and iodine (I) levels decreased by 1.3-fold, and chlorine (Cl) by 1.5-fold in crickets, and potassium (K) by 1.1-fold, chlorine (Cl) by 1.2-fold, mercury (Hg) by 1.6-fold, iodine (I) by 2.7-fold, and selenium (Se) by 6.7fold in worms. At a single dose of precursors, the levels of copper (Cu) and iodine (I) were practically unchanged in crickets, and at double dose, copper (Cu), manganese (Mn), sodium (Na), magnesium (Mg), calcium (Ca), and potassium (K). In worms, a single dose did not change molybdenum (Mo) levels, and a double dose did not change molybdenum (Mo) and copper (Cu) levels.

There was no direct correlation between the use of precursors in the diet and the accumulation of specific minerals in the biomass of the model species. Worms accumulate minerals better, and enrichment of the substrate with minerals may have the effect of increasing their content in biomass.

In contrast to minerals, the level of most B vitamins increased almost linearly. Thus, at a single dose of substrate enrichment with B vitamins precursors, the level of niacin (B3) increased by a factor of 1.3, pyridoxine (B6) by 1.6, folate (B9) by 1.1, and cobalamin (B12) by 1.9 in crickets, decreased only in riboflavin (B2) by 1.1-fold, and practically did not change in biotin (B7) and thiamine (B1). In worms, only the level of thiamine (B1) was practically unchanged, while all other B vitamins increased: niacin (B3) and folate (B9) by 1.4-fold, riboflavin (B2) by 1.5-fold, pyridoxine (B6) by 2-fold, biotin (B7) by 1.7-fold, and cobalamin (B12) by 1.9fold. The double dose of substrate enrichment increased the levels of riboflavin (B2) by 1.2-fold, niacin (B3) by 1.6-fold, pyridoxine (B6) by 2.5-fold, and cobalamin (B12) by 1.7-fold. The levels of these vitamins increased significantly compared to a single dose of precursors. In worms at the double dose of substrate enrichment, the levels of all vitamins studied increased: thiamine (B1), riboflavin (B2), and folate (B9) by 1.1-fold, niacin (B3) by 1.3-fold, pyridoxine (B6) by 1.8-fold, and cobalamin (B12) by 1.9-fold. No sharp increase in vitamin content was observed compared to the single dose of precursors. At the double dose, the levels of thiamine (B1) and folate (B9) remained unchanged in crickets. Thus, substrate enrichment with precursors gives a significant increase in the concentration of B vitamins in the biomass of model invertebrate species.

A similar pattern of increase in the concentration of fat-soluble vitamins was observed in the biomass

of model species at single and double doses of food substrate enrichment. Thus, at a single dose, the level of retinol (vitamin A) and cholecalciferol (vitamin D3) increased by a factor of 1.5, and phylloquinone (vitamin K) by 1.2. In worms, the level of tocopherol (vitamin E) and retinol (vitamin A) increased by 1.6 times, and cholecalciferol (vitamin D3) by 1.8 times, showing a significant increase. At a double dose of precursors, crickets showed an increase in the levels of tocopherol (vitamin E) and retinol (vitamin A) by 1.5-fold, cholecalciferol (vitamin D3) by 1.2-fold, and phylloquinone (vitamin K) by 2-fold, while in worms: tocopherol (vitamin E) by 1.7-fold, retinol (vitamin A) by 2.6-fold, cholecalciferol (vitamin D3) by 1.5-fold, and phylloquinone (vitamin K) by 1.4-fold. Interestingly, the single dose did not increase vitamin E levels in crickets, and worms showed a dramatic 4.3-fold drop in vitamin K levels. At a double dose of precursors, all fat-soluble vitamins showed an increase in concentration in the biomass of model species, and in some species, the level increased compared to the single dose.

Vitamin C showed the clearest pattern of accumulation in the biomass of model species: its level increased after a single dose in crickets by a factor of 1.1 and in worms by 1.7, and after a double dose of substrate enrichment, the level of ascorbic acid (vitamin C) increased significantly in crickets by 1.3 times and in worms by 1.9 times, which suggests the prospect of enriching the food substrate of invertebrates to increase the concentration of vitamin C in the biomass.

### Conclusion

Partial enrichment of the food substrate with precursors of specific nutrients showed an uneven accumulation of nutritional elements in the biomass of model invertebrate species. It was practically impossible to increase the protein content, except in worms, where its level slightly increased, which can be explained by the morphophysiology of worms having soft cuticles, which can increase with the growth of the individual.

Enrichment of the food substrate with minerals also did not show a clear pattern of dependence of accumulation of individual minerals in biomass on the introduction of precursors into the food substrate. Perhaps, worms are more efficient at accumulating minerals, and enrichment of the substrate may slightly increase the content of some elements, but to a negligible extent.

The levels of most vitamins increased almost linearly in the biomass of model species after substrate enrichment with precursors. This was observed for the majority of B vitamins in both worms and crickets; small differences in the accumulation of some vitamins at a single dose may be deviations in a particular experiment; in general, the double dose confirmed an increase in the concentration of B vitamins, with some showing significant increases. Fat-soluble vitamins showed a clear pattern of accumulation in the biomass of crickets and worms, and the double dose of precursors also increased their concentration compared to the single dose. The accumulation of vitamin C in the model species was particularly pronounced at both single and double doses, with a marked increase in vitamin levels at the double

dose compared to the single dose. Thus, enrichment of invertebrate biomass with required vitamins is quite possible.

The results presented in this work relate to the study of nutrient accumulation due to enrichment of the food substrate with precursors of specific groups of nutrients, namely protein, minerals, B vitamins, fat-soluble vitamins, and vitamin C. The accumulation of specific nutrients during enrichment with the full complex of precursors, as well as the influence of antagonist substances on nutrient accumulation, will be discussed in a subsequent paper.

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**Appendix to the article:** S.E. Tshernyshev, A.S. Babenko, I.B. Babkina, R.T-O. Baghirov, V.P. Modyaeva, M.D. Morozova, K.E. Skriptcova, E.Yu. Subbotina, M.V. Shcherbakov, A.V. Simakova. Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production. 5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate (Euroasian Entomological Journal. 2024. Vol.23. No.5. P.287–292).

**Приложение к статье:** С.Э. Чернышёв, А.С. Бабенко, И.Б. Бабкина, Р.Т-О. Багиров, В.П.Модяева, М.Д. Морозова, К.Е. Скрипцова, Е.Ю. Субботина, М.В. Щербаков, А.В. Симакова. Беспозвоночные Сибири как перспективный источник животного белка для инновационного производства кормов и продуктов питания. 5. Изменения состава питательных веществ у червей и сверчков после частичного обогащения кормового субстрата (Евразиатский энтомологический журнал. 2024. Т.23. Вып.4. С.287–292).

# Tables with results of analysis

### **Control group**

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
		Vitamins			
B1 (thiamine), mg/100 g	0.033±0.002	0.052±0.003	1.223±0.061	1.3±0.1	EN 14122
B2 (riboflavin), mg/100 g	3.226±0.161	3.8±0.2	9.369±0.468	11.7±0.6	EN 14152
B3 (niacinamide), mg/100 g	3.292±0.165	4.2±0.2	22.347±1.117	17.5±0.9	31483
B6 (pyridoxine), mg/100 g	0.127±0.006	0.19±0.02	0.101±0.005	0.15±0.01	EN 14164
B7 (biotin), mg/100 g	0.011±0.001		0.022±0.001		P 50929
B9 (folic acid), μg/100 g	0.122±0.006	0.15±0.01	0.029±0.001	0.042±0.002	31483
B12 (cyanocobalamin), μg /100 g	0.282±0.014	0.33±0.05	0.026±0.001	0.019±0.001	ISO 20634
E (α-tocopherol), mg/100 g	2.083±0.104	2.2±0.1	3.773±0.189	5.3±0.3	32307
A (retinol palmitate), μg/100 g	4.752±0.238	5.2±0.3	0.799±0.040	0.85±0.04	32307
D3 (cholecalciferol), μg/100 g	0.058±0.003	0.075±0.004	0.026±0.001	0.033±0.002	32307
K (fillokinone), μg/100 g	6.558±0.328	7.4±0.4	7.765±0.388	8.8±0.4	EN 14148
C (ascorbic acid), mg/100 g	2.908±0.145	2.6±0.1	3.773±0.189	5.0±0.2	34151
		Minerals			
Fe, iron, mg/100 g	0.541±0.081	2.3±0.6	24.6±3.7	12.1±0.6	ICP MS
Se, selenium, µg/100 g	0.099±0.015	0.10±0.02	0.37±0.06	0.09±0.02	ICP MS
Zn, zinc, mg/100 g	15.9±2.4	6.8±0.5	7.0±1.1	3.3±1.1	ICP MS
Mn, manganese, mg/100 g	3.12±047	1.67±0.03	1.1±0.2	0.74±0.03	ICP MS
Cu, copper, mg/100 g	2.7±0.4	0.78±0.12	0.52±0.08	0.23±0.02	ICP MS
Mg, magnesium, mg/100 g	92.0±13.8	45.3±2.3	71.3±10.7	28.7±1.4	ICP MS 32009
P, phosphorus, mg/100 g	791.3±78.7	314.7±15.5	495.7±74.4	159.6±7.4	ICP MS 32009
Pb, lead, mg/100 g	0.002		0.038±0.004		ICP MS 32009
Hg, mercury, mg/100 g	0.0004		0.0008		ICP MS 32009
Mo, molybdenum, mg/100 g	0.070	0.047±0.005	0.034	0.030	ICP MS 32009
I, iodine, mg/100 g	0.062	1.1±0.2	0.024	5.4	ICP MS 32009
Ca, calcium, mg/100 g	90.6±13.2	74.8±3.7	147.7±22.2	82.9±4.1	ICP MS 32009
Na, sodium, mg/100 g	355.4±53.3	232.2±12.3	268.9±40.3	196.3±9.3	ICP MS 32009
K, potassium, mg/100 g	1089.9±163.5	455.1±22.5	874.5±131.2	229.1±11.2	ICP MS 32009
CI, chlorine, mg/100 g	934.5±140.1	661.5±33.7	153.6±23.0	615.5±30.7	ICP MS 32009

# S.E. Tshernyshev et al.

# Table (continuations)

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
	0	ther nutrients mass fra	action, %		
Ash content	1.44±0.134	1.20±0.12	0.40±0.04	0.72±0.04	27494
Fat	2.03±0.20	3.44±0.17	0.27±0.03	0.35±0.03	23042
Protein	16.9±1.7	16.2±0.8	17.6±1.7	16.6±0.7	25011
Carbohydrate	35.0±3.5	28.0±1.4	10.6±1.1	8.7±0.4	32167
Chitin	4.05±0.40	4.8±0.2	0.08±0.01	1.08±0.01	7636
Cellulose		12.55±0.65		0.96±0.11	31675
Water		35.4±1.7		61.4	13586.5
Caloricity, kcal	226.4	207	115.2	104.4	Calculation мethod

# Biotin+ vitamin C for protein accumulation

Index of content of the nutrient detected	Sample No.320A Acheta domes- ticus (Linnaeus, 1758)	Sample No.295 Acheta domes- ticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
Vitamins					
B1 (thiamine), mg/100 g	0.045±0.002		1.247±0.062		EN 14122
B2 (riboflavin), mg/100 g	3.38±0.167		17.521±0.876		EN 14152
B3 (niacinamide), mg/100 g	3.441±0.172		39.143±1.951		31483
B6 (pyridoxine), mg/100 g	0.234±0.012		0.222±0.011		EN 14164
B7 (biotin), mg/100 g	0.020±0.001		0.042±0.002		P 50929
B9 (folic acid), μg/100 g	0.119±0.006		0.059±0.003		31483
B12 (cyanocobalamin), μg /100 g	0.553±0.028		0.052±0.003		ISO 20634
E (α-tocopherol), mg/100 g	2.330±0.116		4.572±0.229		32307
A (retinol palmitate), μg/100 g	5.995±0.300		1.696±0.086		32307
D3 (cholecalciferol), μg/100 g	0.073±0.004		0.052±0.003		32307
K (fillokinone), μg/100 g	7.766±0.0388		2.418±0.121		EN 14148
C (ascorbic acid), mg/100 g	2.398±0.120		6.213±0.311		34151
Minerals					
Fe, iron, mg/100 g	0.720±0.110		26.4±4.3		ICP MS
Se, selenium, µg/100 g	0.078±0.012		0.41±0.05		ICP MS
Zn, zinc, mg/100 g	20.6±3.1		9.0±1.1		ICP MS
Mn, manganese, mg/100 g	3.5±0.5		1.5±0.2		ICP MS
Cu, copper, mg/100 g	2.55±0.38		0.47±0.05		ICP MS
Mg, magnesium, mg/100 g	122.6±18.4		70.6±10.7		ICP MS 32009
P, phosphorus, mg/100 g	908.7±136.3		566.5±83.0		ICP MS 32009
Pb, lead, mg/100 g	0.004		0.018±0.003		ICP MS 32009
Hg, mercury, mg/100 g	0.0002		0.0005		ICP MS 32009
Mo, molybdenum, mg/100 g	0.081		0.035		ICP MS 32009
I, iodine, mg/100 g	0.046		0.0085		ICP MS 32009
Ca, calcium, mg/100 g	108.2±16.2		142.5±21.0		ICP MS 32009
Na, sodium, mg/100 g	352.9±52.9		289.7±45.3		ICP MS 32009
K, potassium, mg/100 g	1126.4±169.0		844.6±115.2		ICP MS 32009
CI, chlorine, mg/100 g	615.1±92.3		66.2±11.4		ICP MS 32009

Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production.

5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate

### Table (continuations)

Index of content of the nutrient detected	Sample No.320A Acheta domes- ticus (Linnaeus, 1758)	Sample No.295 Acheta domes- ticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
	Othe	er nutrients mass frac	tion, %		
Ash content	1.48±0.14	1.30±0.04	0.64±0.06	0.72±0.04	27494
Fat	2.59±0.26	5.12±0.05	0.23±0.02	0.32±0.03	23042
Protein	17.1±1.7	16.8±0.8	21.6±2.1	18.5±0.7	25011
Carbohydrate	29.9±3.0	28.4±0.4	9.1±0.9	8.9±0.4	32167
Chitin	9.98±0.90	5.14±0.01	0.06±0.01	1.28±0.01	7636
Cellulose		13.88±0.65		0.97±0.11	31675
Water		32.2±1.6		67.4	13586.5
Caloricity, kcal	211.3	227	124.8	112.9	Calculation method

#### **B**-group vitamins

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bou- ché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)			
Dosage	1×	2×	1×	2×	0			
Vitamins								
B1 (thiamine), mg/100 g	0.032±0.002	0.055±0.003	1.182±0.059	1.4±0.1	EN 14122			
B2 (riboflavin), mg/100 g	2.879±0.144	4.7±0.2	13.610±0.680	13.2±0.5	EN 14152			
B3 (niacinamide), mg/100 g	4.445±0.222	6.9±0.3	31.527±1.576	23.3±1.1	31483			
B6 (pyridoxine), mg/100 g	0.202±0.010	0.48±0.03	0.201±0.010	0.27±0.02	EN 14164			
B7 (biotin), mg/100 g	0.018±0.001		0.038±0.003		P 50929			
B9 (folic acid), (mg)/100 g	0.134±0.007	0.38±0.02	0.040±0.002	0.048±0.02	31483			
B12 (cyanocobalamin), μg /100 g	0.554±0.028	0.56±0.004	0.050±0.002	0.036±0.003	ISO 20634			
E (α-tocopherol), mg/100 g	1.877±0.094		6.252±0.313		32307			
A (retinol palmitate), μg/100 g	6.625±0.331		1.633±0.082		32307			
D3 (cholecalciferol), μg/100 g	0.077±0.004		0.043±0.002		32307			
K (fillokinone), μg/100 g	7.205±0.360		2.220±0.111		EN 14148			
C (ascorbic acid), mg/100 g	2.669±0.133		5.361±0.268		34151			
		Minerals						
Fe, iron, mg/100 g	0.34±0.03		25.7±3.2		ICP MS			
Se, selenium, µg/100 g	0.051±0.005		0.042±0.006		ICP MS			
Zn, zinc, mg/100 g	8.5±0.8		7.2±0.8		ICP MS			
Mn, manganese, mg/100 g	2.1±0.2		1.5±0.2		ICP MS			
Cu, copper, mg/100 g	1.72±0.17		0.55±0.08		ICP MS			
Mg, magnesium, mg/100 g	105.3±10.5		67.3±8.2		ICP MS 32009			
P, phosphorus, mg/100 g	857.5±86±7		605.0±93.0		ICP MS 32009			
Pb, lead, mg/100 g	0.0026		0.006±0.001		ICP MS 32009			
Hg, mercury, mg/100 g	0.0006		0.0003		ICP MS 32009			
Mo, molybdenum, mg/100 g	0.0023		0.033		ICP MS 32009			
I, iodine, μg/100 g	0.026		0.0069		ICP MS 32009			
Ca, calcium, mg/100 g	112.2±11.2		128.3±21.0		ICP MS 32009			
Na, sodium, mg/100 g	311.5±31.1		352.2±51.2		ICP MS 32009			
K, potassium, mg/100 g	1080.6±108.0		783.5±111.0		ICP MS 32009			
CI, chlorine, mg/100 g	487.5±48.7		31.4±3.2		ICP MS 32009			

# S.E. Tshernyshev et al.

# Table (continuations)

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295  Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
	Oth	ner nutrients mass frac	tion, %		
Ash content	1.60±0.16	1.17±0.11	0.62±0.06	1.12±0.11	27494
Fat	2.27±0.22	5.52±0.07	0.34±0.03	0.33±0.07	23042
Protein	16.6±1.7	21.3±0.8	17.2±1.7	17.9±0.8	25011
Carbohydrate	34.1±3.4	28.7±0.8	8.0±0.8	8.8±0.8	32167
Chitin	4.03±0.40	4.8±0.2	0.07±0.02	1.12±0.05	7636
Cellulose		12.1±0.6		0.90±0.09	31675
Water		37.3±1.5		63.3±3.3	13586.5
Caloricity, kcal	223.2	249.7	103.9	109.8	Calculation method

## **Fat-soluble vitamins**

					1
Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
		Vitamins			
B1 (thiamine), mg/100 g	0.048±0.002		1.231±0.062		EN 14122
B2 (riboflavin), mg/100 g	3.546±0.177		12.472±0.624		EN 14152
B3 (niacinamide), mg/100 g	3.860±0.193		43.541±2.177		31483
B6 (pyridoxine), mg/100 g	0.205±0.010		0.205±0.010		EN 14164
B7 (biotin), mg/100 g	0.017±0.001		0.033±0.002		P 50929
B9 (folic acid), mg/100 g	0.152±0.008		0.048±0.002		31483
B12 (cyanocobalamin), μg /100 g	0.510±0.025		0.053±0.003		ISO 20634
E (α-tocopherol), mg/100 g	2.056±0.103	3.4±0.2	5.829±0.291	8.9±0.5	32307
A (retinol palmitate), μg/100 g	6.995±0.380	7.7±0.3	1.260±0.063	2.24±0.11	32307
D3 (cholecalciferol), μg/100 g	0.084±0.004	0.088±0.004	0.047±0.002	0.048±0.003	32307
K (fillokinone), μg/100 g	7.605±0.309	15.2±0.5	1.829±0.091	12.7±0.6	EN 14148
C (ascorbic acid), mg/100 g	2.725±0.136	3.4±0.2	5.609±0.280	9.4±0.5	34151
		Minerals			
Fe, iron, mg/100 g	0.39±0.04		24.9±0.280		ICP MS
Se, selenium, μg/100 g	0.062±0.006		0.035±0.006		ICP MS
Zn, zinc, mg/100 g	11.5±0.9		6.9±0.7		ICP MS
Mn, manganese, mg/100 g	3.06±0.28		1.2±0.2		ICP MS
Cu, copper, mg/100 g	2.88±0.28		0.53±0.08		ICP MS
Mg, magnesium, mg/100 g	98.3±9.8		69.8±10.2		ICP MS 32009
P, phosphorus, mg/100 g	785.9±79.6		523.7±73.0		ICP MS 32009
Pb, lead, mg/100 g	0.0021		0.005±0.001		ICP MS 32009
Hg, mercury, mg/100 g	0.0011		0.0006		ICP MS 32009
Mo, molybdenum, mg/100 g	0.005		0.035		ICP MS 32009
I, iodine, μg/100 g	0.062		0.005		ICP MS 32009
Ca, calcium, mg/100 g	1002.4±100.2		142.5±22.0		ICP MS 32009
Na, sodium, mg/100 g	333.8±33.3		315.6±41.5		ICP MS 32009
K, potassium, mg/100 g	10.85.5±108.5		833.8±124.0		ICP MS 32009
Cl, chlorine, mg/100 g	517.8±51.7		153.4±23.2		ICP MS 32009

Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production.

5. Changes of nutrient composition in worms and crickets after particular enrichment of feeding substrate

### Table (continuations)

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294  Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
	Ott	ner nutrients mass frac	tion, %		1
Ash content	1.55±0.16	1.12±0.1	0.64±0.06	1.2±0.1	27494
Fat	1.6±0.16	4.97±0.03	0.46±0.05	0.36±0.02	23042
Protein	17.1±1.7	17.2±0.5	18.2±1.8	17.8±0.*	25011
Carbohydrate	39.2±3.9	28.7±0.8	9.9±0.9	8.5±0.8	32167
Chitin	11.4±1.1	4.5±0.02	0.05±0.01	1.03±0.01	7636
Cellulose		15.0±0.2		0.90±0.09	31675
Water		42.2±2.4		65.2±3.3	13586.5
Caloricity, kcal	239.6	228.7	116.5	108.5	Calculation method

### Mineral substances

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295 Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)			
Dosage	1×	2×	1×	2×	0			
Vitamins								
B1 (thiamine), mg/100 g	0.038±0.002		1.631±0.082		EN 14122			
B2 (riboflavin), mg/100 g	3.215±0.161		13.360±0.668		EN 14152			
B3 (niacinamide), mg/100 g	4.363±0.218		41.717±2.086		31483			
B6 (pyridoxine), mg/100 g	0.224±0.011		0.176±0.009		EN 14164			
B7 (biotin), mg/100 g	0.020±0.001		0.031±0.002		P 50929			
B9 (folic acid), mg/100 g	0.159±0.008		0.060±0.003		31483			
B12 (cyanocobalamin), μg /100 g	0.556±0.028		0.058±0.003		ISO 20634			
E (α-tocopherol), mg/100 g	2.750±0.138		4.874±0.244		32307			
A (retinol palmitate), μg/100 g	7.119±0.356		1.487±0.074		32307			
D3 (cholecalciferol), μg/100 g	0.057±0.003		0.042±0.002		32307			
K (fillokinone), μg/100 g	6.967±0.348		2.335±0.117		EN 14148			
C (ascorbic acid), mg/100 g	3.162±0.158		6.378±0.319		34151			
		Minerals						
Fe, iron, mg/100 g	0.7±0.1	2.6±0.6	29.4±4.4	17.0±0.8	ICP MS			
Se, selenium, µg/100 g	0.078±0.012	0.09±0.02	0.055±0.008	0.05±0.01	ICP MS			
Zn, zinc, mg/100 g	20.6±3.1	4.3±0.2	9.8±1.3	3.98±0.24	ICP MS			
Mn, manganese, mg/100 g	3.5±0.5	0.96±0.03	1.8±0.3	1.27±0.06	ICP MS			
Cu, copper, mg/100 g	2.55±0.38	0.74±2.2	0.62±0.09	0.20±0.10	ICP MS			
Mg, magnesium, mg/100 g	122.6±18.4	40.7±2.2	73.9±11.1	40.2±2.2	ICP MS 32009			
P, phosphorus, mg/100 g	908.7±136.3	280.3±14.2	646.0±96.0	190.6±9.2	ICP MS 32009			
Pb, lead, mg/100 g	0.0037		0.010±0.001		ICP MS 32009			
Hg, mercury, mg/100 g	0.0017		0.0005		ICP MS 32009			
Mo, molybdenum, mg/100 g	0.0081	0.044±0.005	0.032	0.025±0.005	ICP MS 32009			
I, iodine, mg/100 g	0.046	0.9±0.2	0.0088	4.4±0.2	ICP MS 32009			
Ca, calcium, mg/100 g	108.2±16.2	79.2±3.8	152.7±22.9	107.2±5.3	ICP MS 32009			
Na, sodium, mg/100 g	352.9±52.9	195.5±11.7	376.6±56.5	230.5±12.5	ICP MS 32009			
K, potassium, mg/100 g	1126.4±169.0	338.3±33.8	806.6±121.0	251.3±13.8	ICP MS 32009			
CI, chlorine, mg/100 g	615.1±92.3	609±60	35.9±3.5	681.60	ICP MS 32009			

# S.E. Tshernyshev et al.

## Table (continuations)

Index of content of the nutrient detected	Sample No.320A Acheta domesticus (Linnaeus, 1758)	Sample No.295  Acheta domesticus (Linnaeus, 1758)	Sample No.315A Eisenia fetida (Bouché, 1972)	Sample No.294 Eisenia fetida (Bouché, 1972)	Method applied (GOST number)
Dosage	1×	2×	1×	2×	0
	Oth	ner nutrients mass frac	tion, %		
Ash content	1.90±0.19	1.34±0.13	0.58±0.06	0.79±0.11	27494
Fat	2.84±0.28	4.78±0.23	0.94±0.09	0.78±0.07	23042
Protein	17.3±1.7	16.9±0.8	17.8±1.7	17.3±0.8	25011
Carbohydrate	32.7±3.3	28.9±1.4	8.3±0.8	8.9±0.8	32167
Chitin	5.90±0.59	4.2±0.2	0.08±0.02	1.68±0.08	7636
Cellulose		14.72±0.77		0.97±0.10	31675
Water		35.9±1.7		65.3±3.3	13586.5
Caloricity, kcal	225.6	226	112.9	116	Calculation method