

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

3. Principles of biomass nutrient composition design

Беспозвоночные Сибири как перспективный источник животного белка для инновационного производства продуктов питания. 3. Проектирование нутриентного состава биомассы

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Abstract. The method of providing an invertebrate biomass with particular parameters of a nutrient-dense composition (i.e. supplied with relatively more nutrients than calories) is discussed. An increase in the content of particular nutrients in the biomass of two invertebrate (model) species was achieved via the input of precursor material in the food substrate. The content of protein, minerals, B-group vitamins and liposoluble vitamins A, E, D and K in the biomass of the Giant African land snail *Lissachatina fulica* (Férussac, 1821) and the Speckled cockroach *Nauphoeta cinerea* (Olivier, 1789) were investigated. A minimal input of vitamins C and B7 to the food substrate resulted in only a slight decrease in the level of protein in the biomass of snails from c. 20.4 to 18.8 % and in cockroaches from c. 8.6 to 8.0 %, but an increase in vitamins B1, B2, B3, B4 and B9 was found in the biomass of snails, and B1, B4 and B9 in cockroaches; the content of liposoluble vitamins A, E, D and K increased significantly in the biomass of cockroaches, but A, E and D increased and K decreased in snails. The content of Se, I, Pb, Hg, Mo, Ca, Na, K and Cl in the biomass of snails and of Fe, Se, Zn, Mn, Mg, P, Ca, Na, K and Cl in cockroaches also increased. Clearly, food substrate enrichment by precursor material results in nutrient composition changes in the invertebrate biomass. Further research will clarify the quantity and chemical form of precursor material inserted into a food substrate necessary to determine the most suitable nutrition for invertebrates.

Резюме. В работе обсуждается возможность получения биомассы беспозвоночных с заданными параметрами нутриентного состава, что позволило бы получать насыщенный пищевой продукт с высоким содержанием определённых питательных веществ при общей низкой его калорийности. Исследовался способ повышения содержания отдельных нутриентов в биомассе модельных видов беспозвоночных посредством увеличения концентрации исходных веществ

в кормовом субстрате. Для модельных видов гигантской ахатины *Lissachatina fulica* (Férussac, 1821) и мраморного таракана *Nauphoeta cinerea* (Olivier, 1789) сопоставлены данные по содержанию белка, витаминов группы В, минералов и жирорастворимых витаминов А, Е, D и К при увеличении содержания этих нутриентов в пищевом субстрате. Минимальное увеличение содержания витаминов С и В7 не оказало влияние на увеличение белка в биомассе, его содержание несколько уменьшилось: с 20,4 до 18,8 % у ахатин и с 8,6 до 8,0 % у тараканов. Зафиксировано заметное увеличение содержания витаминов В1, В2, В3, В4 и В9 у ахатин, В1, В4 и В9 у тараканов. Жирорастворимые витамины показали значительное увеличение содержания в биомассе у тараканов и ахатин, за исключением витамина К, снизившегося у ахатин. Зафиксировано увеличение минералов: Se, I, Pb, Hg, Mo, Ca, Na, K, Cl у ахатин, Fe, Se, Zn, Mn, Mg, P, Ca, Na, K, Cl у тараканов. Несмотря на несколько противоречивые результаты, очевидно, что обогащение пищевого субстрата исходными веществами необходимых нутриентов приводит к изменению их содержания в биомассе беспозвоночных. Дальнейшие исследования позволят уточнить количество и формулу вносимого вещества для получения заданных параметров соответствующего нутриента в биомассе.

Introduction

To provide for the increase in food product consumption in the world, the search for new resources of animal protein gain in addition to traditional animal production is necessary [Van Raamsdonk et al., 2017; Tang et al., 2019; Tobolkova, 2019]. New approaches to balanced nutrition demand the generation of functional products, which could provide not only vital organism function,

but also supply it with all necessary nutrients. Intensive development of agricultural technologies can enhance the production of meat, eggs, fish and milk products rich in essential amino acids up to the limit determined by the potential of farmed animals. The burden on ecosystems connected with the production of feed and necessity to use poultry and livestock excrements related to III–V hazard classes, as well as the influence of the processing industry, significantly limits further increases in total livestock numbers [Premalatha et al., 2011; Belluco et al., 2013; Hanboonsong et al., 2013; Mlcek et al., 2014; Assielou et al., 2015; Han et al., 2017; Van Huis, Oonincx, 2017.]. To meet a lack of animal protein avoiding the above-mentioned problems is possible by using the biomass of invertebrates as an alternative to traditional resources of protein [Rumpold, Schluter, 2013; Assielou et al., 2015; Ayensu et al., 2019], but with reference to the safety of reared edible invertebrates [Rumpold, Schluter, 2013; Van Huis, 2013, 2015; Murefu et al., 2019; Tobolkova, 2019].

Currently a trend to raise terrestrial insects, molluscs, arachnids, worms and other invertebrates is actually being developed worldwide [Mlcek et al., 2014; Jansson, Berggren, 2015; Kim et al., 2019; Hlongwane et al., 2020], including Russia [Gorbunova, Zakharov, 2021; Tshernyshev et al., 2022; Skriptcova et al., 2023]. As well as generating technologies to raise and actively work farms to produce a biomass of invertebrates mainly for feed of domestic animals, this work is currently at an experimental level and far from being fully adopted at an industrial level. Nonetheless, four species of insects authorized by the the European Commission (EC) for sale, farming and novel food consumption are already used for the preparation of powder to enrich bread ingredients with animal protein, and the EC Regulation no. 853/2004 defines five species of terrestrial gastropods as ‘edible snails’ to be used in restaurant gastronomy [Regulation EC, 2004; EFSA, 2015, 2022; Lahtenmaki-Uutela, Grmelova, 2016; Van Peer et al., 2021; EU Commission, 2023].

In generating technologies to raise terrestrial invertebrates, it is important that industrial development determines the quality of gained biomass and improvement of its nutrient content and bioavailability [DeFoliart, 1999; Van Huis, 2013, 2015; Drewnowski, Fulgoni III, 2014; Rumpold, Schluter, 2013; Zielińska, et al., 2015; Kouřimská, Adámková, 2016; Gere et al., 2017; Mielgo-Ayuso et al., 2018; Mwangi et al., 2018; Elhassan et al., 2019; Melgar-Lalanne et al., 2019; Patel et al., 2019; Gravel, Doyen, 2020; Gorbunova, Zakharov, 2021; Tshernyshev et al., in litt.]. Invertebrate biomass is rich in large number of nutrients necessary for functional human nutrition; animal protein provides 11 essential amino acids, namely: valine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and phenylalanine. The biomass of invertebrates includes a dense nutrient composition (i.e. supplied with relatively more nutrients than calories) [Drewnowski, Fulgoni, 2014]. In our experiments, the calorific value of the biomass wet matter of two invertebrate (model) species is c. 100 kcal, and

the protein content reached 20.5%, with low content of fat (c. 1.5–3 %) and carbohydrates (0.3–1.5 %) and high level of diversity and value of vitamins and minerals [Tshernyshev et al., in litt.; Morozova et al., 2023]. A low or uneven content of some vitamins (e.g., B1, B3, B7, C, D, K), minerals and fatty acid have been revealed in the biomass of invertebrates [Huis et al., 2013; Zielińska et al., 2015; Oonincx, Finke, 2020; Shan et al., 2022]. Data regarding the nutrient content in biomass gained from raised invertebrates varies for different regions of the World [Ramos-Elorduy et al., 1997]; for example, the biomass of cockroaches and scarabeid beetles larvae reared in Africa contains higher levels of protein, ash, and other characteristics [Hlongwane et al., 2020]. Obviously, the nutrient content in the biomass of raised invertebrates is not constant, and depends on various factors [Oonincx, van der Poel, 2011; Nowak et al., 2016; Payne et al., 2016; Melis et al., 2019]. The present study reveals one of these factors, namely the influence of the application of precursors on the accumulation of nutrients in biomass of model species.

Material and Methods

Two invertebrate species, the Giant African land snail *Lissachatina fulica* (Férussac, 1821) and the Speckled cockroach *Nauphoeta cinerea* (Olivier, 1789) 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 precursors. The second was developed on a substrate enriched with vitamins C and B7, the third with a mineral premix for chickens, the fourth with vitamins B1 (thiamin), B9 (folate) and B3 (niacin), 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 %. 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 achatin snails it contained a mixture of carrot (28.6 g) with dried gammarus (1.4 g). 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 food supplement for chickens (premix) to minerals, vitamins B1, B3, B4 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 2 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), B6 (pyridoxine), B4 (choline), 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 to GOSTs which are a summary of Russian State standards.

Statistical analysis

R version 4.0.2 [R Core Team, 2020] 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.

The present work is registered in ZooBank (www.zoobank.org) under LSID urn:lsid:zoobank.org:pub:DD32E3EB-3C46-43B7-9FA0-0D895463F878.

Results

NUTRIENT COMPOSITION IN BIOMASS OF MODEL SPECIES

The results gained during the experiment showed significant changes in nutrient composition in terms of both an increase and decrease of their content in biomass in comparison with the control groups. Data on the general content of nutrients in the biomass of model species raised in control groups are presented in Table 2.

Precursor minimal dose application

Application of a minimal dose of precursors showed an ambiguous reaction by model species resulting in an increase in some nutrients in the biomass and a decrease in others. The minimal dosage of vitamins C and B7 did not increase the level of protein, but it decreased considerably from 20.4 to 18.8 % in snails, and from 8.6 to 8.0 % in cockroaches.

The following indices of mineral and vitamin content variation after minimal doses of precursors are shown in Figs 1–3. The following minerals content has increased (Fig. 1): Se (from 4.9 to 9.8 $\mu\text{g}/100$), I (from 0.004 to 0.015 mg/100 g), Pb (from 0.0008 to 0.0013 mg/100 g), Hg (from 0.00006 to 0.00009 mg/100 g), Ca (from 250.9 to 278.2 mg/100 g), Na (from 42.8 to 51.5 mg/100 g), K (from 352.4 to 387.4 mg/100 g), Cl (from 6.28 to 8.6 mg/100 g) in snails, and Fe (from 1.8 to 2.4 mg/100 g), Se (from 3.88 to 7.2 mg/100 g),

Table 1. Quantity of precursors (mg) added to feeding substrate of model species during the experiment
Таблица 1. Количество вносимых прекурсоров в экспериментальном кормлении модельных видов

| Type of precursor | I stage, singular dose of precursor | | II stage, doubled dose of precursor | |
|-------------------|-------------------------------------|----------------------|-------------------------------------|----------------------|
| | per 1 kilo of food substrate, mg | per food portion, mg | per 1 kilo of food substrate, mg | per food portion, mg |
| C | 50 | 0.9 | 100 | 1.8 |
| B7 | 25 | 0.45 | 50 | 0.9 |
| premix | 5 | 0.12 | 10 | 0.25 |
| B1 | 2 | 0.048 | 4 | 0.09 |
| B3 | 30 | 0.72 | 60 | 1.4 |
| B9 | 1 | 0.024 | 1 | 0.05 |
| A | 25 | 0.45 | 50 | 0.9 |
| D | 2.5 | 0.045 | 5 | 0.09 |
| E | 20 | 0.48 | 40 | 1 |
| K | 2 | 0.048 | 4 | 0.1 |

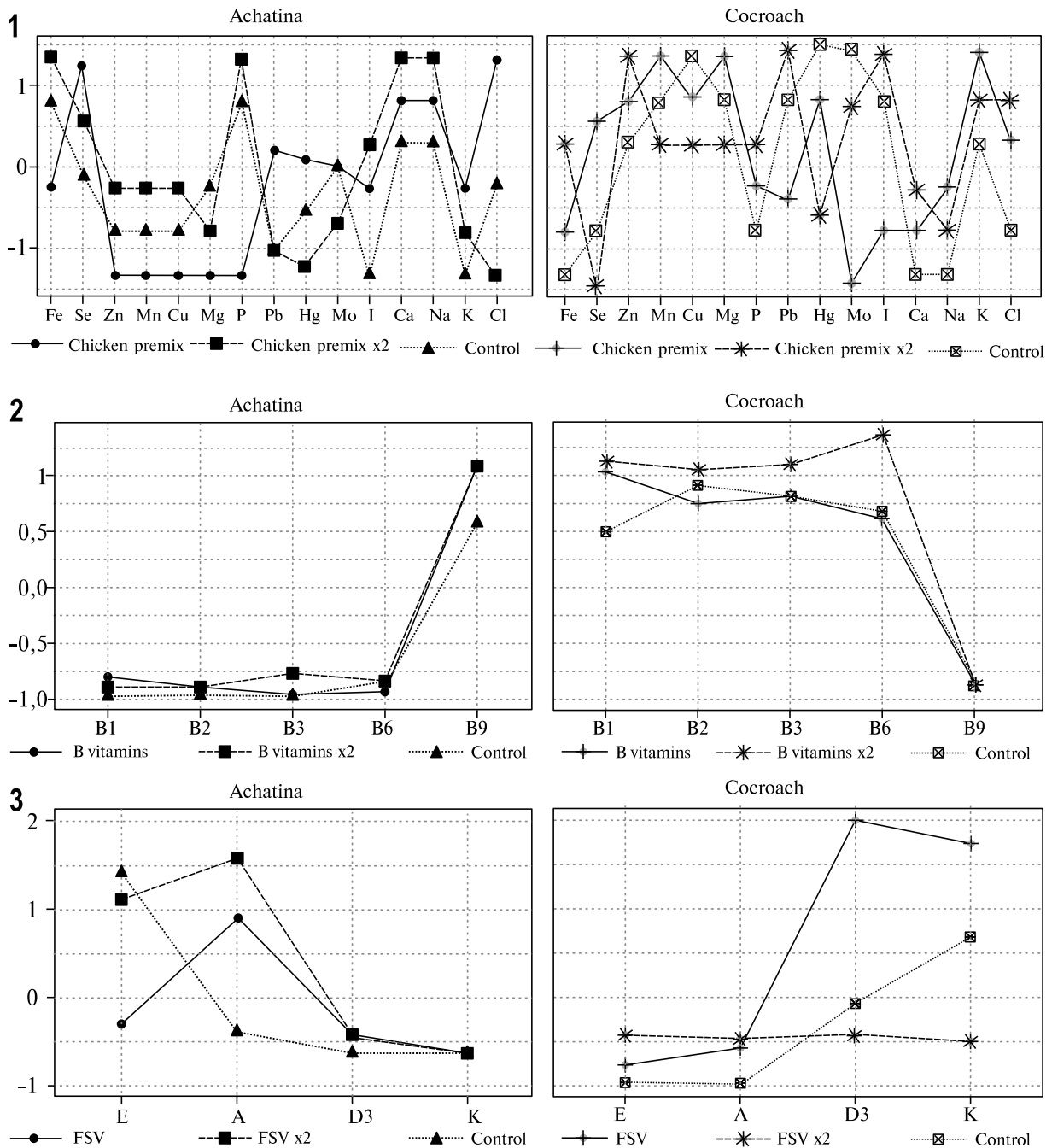
Table 2. Nutrient content of biomass of *Lissachatina fulica* (Férussac, 1821) and *Nauphoeta cinerea* (Olivier, 1789)
 Таблица 2. Содержание питательных веществ (нутриентов) в биомассе *Lissachatina fulica* (Férussac, 1821) и *Nauphoeta cinerea* (Olivier, 1789)

| Index of content of the nutrient detected | Sample No.27A <i>Lissachatina fulica</i> (Férussac, 1821) | Sample No.0549 <i>Nauphoeta cinerea</i> (Olivier, 1789) | Method applied (GOST number) | Human daily requirement |
|---|---|---|---------------------------------|--------------------------------------|
| Vitamins | | | | |
| B1 (thiamine), mg/100 g | 0.094 ± 0.005 | 0.72 ± 0.04 | EN 14122 | 0.3–1.5 mg/day |
| B2 (riboflavin), mg/100 g | 0.071 ± 0.004 | 1.69 ± 0.08 | EN 14152 | 1.8 mg/day |
| B3 (niacinamide), mg/100 g | 1.06 ± 0.05 | 8.7 ± 0.4 | 31483 | 20 mg/day |
| B6 (pyridoxine), mg/100 g | 0.155 ± 0.008 | 0.96 ± 0.05 | EN 14164 | 2.0 mg/day |
| B4 (choline), mg/100 g | 48.9 ± 2.4 | 31.9 ± 1.6 | P 50929 | – |
| B9 (folic acid), mg/100 g | 4.40 ± 0.22 | 0.26 ± 0.02 | 31483 | 400 µg/100 g |
| B12 (cyanocobalamin), mg/100 g | 0.47 ± 0.02 | 0.44 ± 0.02 | ISO 20634 | 0.3–3.0 µg/100 g |
| E (α-tocopherol), mg/100 g | 6.08 ± 0.30 | 3.38 ± 0.17 | 12822 | 15 µg/100 g |
| A (retinol palmitate), µg/100 g | 26.3 ± 1.3 | 22.8 ± 1.1 | 54635 | 400–1000 µg/100 g |
| D3 (cholecalciferol), µg/100 g | 1.92 ± 0.1 | 3.6 ± 0.2 | 54635 | 10 µg |
| K (fillokinone), µg/100 g | 0.090 ± 0.011 | 2.1 ± 0.1 | EN 54635 | 120 µg |
| Minerals | | | | |
| Fe, iron, mg/100 g | 3.9 ± 0.2 | 1.8 ± 0.2 | ICP MS | 4–18 mg/day |
| Se, selenium, µg/100 g | 4.9 ± 0.5 | 3.88 ± 0.35 | ICP MS | 10–70 µg/100 g |
| Zn, zinc, mg/100 g | 0.74 ± 0.07 | 3.8 ± 0.4 | ICP MS | 3–12 mg/day |
| Mn, manganese, µg/100 g | 0.18 ± 0.05 | 0.5 ± 0.1 | ICP MS | 2 mg/day |
| Cu, copper, mg/100 g | 0.13 ± 0.02 | 0.6 ± 0.1 | ICP MS | 0.5–1.0 mg/day |
| Mg, magnesium, mg/100 g | 218.9 ± 1.6 | 45.3 ± 4.6 | ICP MS | 55–400 mg/day |
| P, phosphorus, mg/100 g | 255 ± 5 | 192.9 ± 19.3 | 32009 | 300–1200 mg/day |
| Pb, lead, mg/100 g | 0.0008 | 0.002 | ICP MS | – |
| Hg, mercury, mg/100 g | 0.00006 | 0.00013 | ICP MS | – |
| Mo, molybdenum, mg/100 g | 0.007 | 0.027 | ICP MS | 0.07 mg |
| I, iodine, mg/100 g | 0.004 | 0.03 | ICP MS | 0.15 mg |
| Ca, calcium, mg/100 g | 250.9 | 127.3 | ICP MS | 1000 mg |
| Na, sodium, mg/100 g | 42.8 | 275.6 | ICP MS | 1300 mg |
| K, potassium, mg/100 g | 352.4 | 60.8 | ICP MS | 2500 mg |
| Cl, chlorine, mg/100 g | 6.28 | 3.4 | ICP MS | 2300 mg |
| Other nutrients mass fraction, % | | | | |
| Ash content | 0.52 ± 0.05 | 1.34 ± 0.05 | 27494 | – |
| Fat | 1.43 ± 0.15 | 3.7 ± 0.15 | 3042 | 70 to 154 g/day |
| Protein | 20.4 ± 2.0 | 8.6 ± 0.5 | 25011 | 65–117 g/day with 60% animal protein |
| Carbohydrate | 2.8 ± 0.2 | 13.6 ± 0.2 | P 53747 | 170–420 g/day |
| Chitin | 4.4 ± 0.5 | 3.4 ± 0.5 | 7636 | 20 g/day |
| Caloricity, kcal | 105.7 | 122 | 96 | individually |

Примечание. Расчёт суточной потребности дан согласно «Норм физиологических потребностей в энергии и пищевых веществах для различных групп населения Российской Федерации» 2008 и 2021 гг. [Tutelyan et al., 2009].

Notes. The calculation of daily nutritional requirements is given according to «The norms of physiological requirements in energy and nutrients for various of population in Russian Federation» of 2008 and 2021 years. [Tutelyan et al., 2009].

Mg (from 45.3 to 52.3 mg/100 g), Ca (from 127.3 to 158.9 mg/100 g), K (from 60.8 to 67.2 mg/100 g), Mo (from 0.027 to 0.0031 mg/100 g), Na (from 60.8 to 67.2 mg/100 g), Zn (from 3.8 to 4.2 mg/100 g), Mn (from 0.5 to 0.6 mg/100 g), Cl (from 3.4 to 6.5 mg/100 g) and P (from 192.9 to 216 mg/100 g) in cockroaches.
 In terms of minerals, the following have decreased: Zn (from 0.74 to 0.66 mg/100 g), Mn (from 0.18 to



Figs 1–3. Nutrient content changes in the biomass of the Giant African land snail *Lissachatina fulica* and the Speckled cockroach *Nauphoeta cinerea*. 1 — minerals; 2 — B-group vitamins; 3 — liposoluble vitamins A, E, D and K.

Рис. 1–3. Изменение нутриентного состава биомассы улитки ахатины *Lissachatina fulica* и мраморного таракана *Nauphoeta cinerea*. 1 — минералы; 2 — витамины группы В; 3 — жирорастворимые витамины А, Е, D и К.

0.05 mg/100 g), Mg (from 218.9 to 216.5 mg/100 g), P (from 255 to 128 mg/100 g) in snails, and Pb (from 0.002 to 0.001 mg/100 g), and I (from 0.03 to 0.005 mg/100 g) in cockroaches.

In terms of vitamins, the following have increased: B1 (from 0.094 to 0.158 mg/100 g), B2 (from 0.071 to 0.132 mg/100 g), B3 (from 1.06 to 1.145 mg/100 g), B4 (from 48.9 to 49.5 mg/100 g) and B9 (from 4.40 to 5.78 µg /100 g) in snails, and B1 (from 0.72

to 0.95 mg/100 g), B4 (from 31.9 to 32.5 mg/100 g) and B9 (from 0.26 to 0.30 mg/100 g) in cockroaches. The following have decreased: B6 (from 0.155 to 0.107 mg/100 g) in snails, and B2 (from 1.69 to 1.56 mg/100 g), B3 (from 8.7 to 8.6 mg/100 g) and B6 (from 0.96 to 0.93 mg/100 g) in cockroaches (Fig. 2).

In terms of fat-soluble vitamins, the following have increased significantly: A (from 22.8 to 25.3 µg/100 g), E (from

3.38 to 3.6 mg/100 g), D3 (from 3.6 to 9.8 µg/100 g) and K (from 2.1 to 3.7 µg/100 g) in cockroaches, and also increased for snails for A (from 26.3 to 34.3 µg/100 g) and D3 (from 1.92 to 2.64 µg/100 g), but decreased for E (from 6.08 to 4.10 mg/100 g) and K (from 0.090 to 0.078 µg/100 g) (Fig. 3).

In fact, even minimal enrichment of the feeding substrate with precursors has resulted in notable changes to the nutrient content in the biomass of invertebrates, as in the case of the following minerals. Of those which have been implemented into feed substrate via minimal dose of premix for chickens, the content of elements has increased or decreased, sometimes significantly. In snails, selenium has increased x 3, iodine, lead, mercury and chlorine x 1.5, and calcium, potassium and sodium x 1.1. In cockroaches, chlorine has increased x 1.9, selenium x 1.8, iron x 1.3, calcium x 1.2, manganese x 1.2, and magnesium, sodium, potassium, zinc and molybdenum x 1.1. In snails, there were significant decreases in manganese (3-fold) and phosphorus (2-fold), but insignificant reductions in zinc and magnesium. In cockroaches, the level of lead decreased 6-fold, and for iodine 2-fold.

The minimal addition of precursors has resulted in significant increases of selenium and chlorine, and influenced the accumulation of sodium, potassium and calcium in the biomass. Interestingly the levels of lead and mercury in snails increased considerably, but decreased in cockroaches.

The vitamin content in the biomass also changed in comparison with the control group. Thus, there were significant increases in B2 (x 1.9), B1 (x 1.7) and B9 (x 1.3), but a decrease in B6 (1.4-fold), and insignificant decreases for B3 and B4 in snails. In cockroaches there were increased levels of B1 (x 1.3) and B2 (x 1.1), minor increases in B4 and B9, and stable levels of B3 and B6.

The content of the fat-soluble vitamins also varied in experimental samples and differed from the control; in cockroaches, for example, there were increases for D3 (x 2.7), K (x 1.8), A (1.1) and E (1.1). In snails there were increases for A (x 1.3) and D3 (x 1.4) and decreases for E (1.4-fold) and K (1.1-fold).

After minimal replenishment of feeding the substrate with the precursors the content of almost all nutrients changed, with increasing of some vitamins and minerals in the biomass of snails and cockroaches. The decrease in the level of some nutrients can probably be explained by synergetic effect of the precursors including any antagonist effects. Interestingly, the additional input of vitamins C and B7, which normally generates proteins in the organism, did not affect the increase in protein in the biomass of model species.

PRECURSOR DOUBLE DOSE APPLICATION

Application of a double dose of precursors showed significant nutrient changes in the biomass of model species. A double dose of vitamins C and B7 applied to the feeding substrate increased the protein content in cockroaches from 8.6 % in the control group to 18.8 % in experimental group, whereas in snails it slightly

decreased from 20.4 to 19.5 %. The protein content decreased after a minimal dose of precursors by 18.8 % in snails and 8.0 % in cockroaches.

For a clearer comparison all indices are given over the dashes, for example: (8.6 % (control) — 8.0 % (minimal dose of precursors) — 18.8 % (double dose of precursors)).

After the application of a double dose of the precursor the following indices of mineral and vitamin content have been registered (Figs 1–3). Content of the minerals I (0.004 mg/100 g — 0.015 mg/100 g — 0.017 mg/100 g), Ca (250.9 mg/100 g — 278.2 mg/100 g — 338.4 mg/100 g), Na (42.8 mg/100 g — 51.5 mg/100 g — 63.3 mg/100 g), Cl (6.28 mg/100 g — 8.6 mg/100 g — 12.0 mg/100 g), Fe (3.9 mg/100 g — 3.3 mg/100 g — 4.2 mg/100 g), Cu (0.13 mg/100 g — 0.09 mg/100 g — 0.17 mg/100 g), Zn (0.74 mg/100 g — 0.66 mg/100 g — 0.78 mg/100 g), P (255 mg/100 g — 128 mg/100 g — 270 mg/100 g), Se (4.9 µg/100 g — 9.8 µg/100 g — 7.2 µg/100 g), Mn (0.18 mg/100 g — 0.05 mg/100 g — 0.19 mg/100 g) and K (352.4 mg/100 g — 387.4 mg/100 g — 377.2 mg/100 g) increased in snails, and Fe (1.8 mg/100 g — 2.4 mg/100 g — 3.5 mg/100 g), Mg (45.3 mg/100 g — 52.3 mg/100 g — 255.5 mg/100 g), Ca (127.3 mg/100 g — 158.9 mg/100 g — 162.0 mg/100 g), K (60.8 mg/100 g — 67.2 mg/100 g — 66.2 mg/100 g), Cl (3.4 mg/100 g — 6.5 mg/100 g — 8.1 mg/100 g), Pb (0.002 mg/100 g — 0.001 mg/100 g — 0.005 mg/100 g), I (0.03 mg/100 g — 0.05 mg/100 g — 0.06 mg/100 g), Na (60.8 mg/100 g — 67.2 mg/100 g — 66.2 mg/100 g), P (192.9 mg/100 g — 216 mg/100 g — 251 mg/100 g), Zn (3.8 mg/100 g — 4.2 mg/100 g — 4.56 mg/100 g) increased in cockroaches. Content of minerals, Hg (0.00006 mg/100 g — 0.00009 mg/100 g — 0.00005 mg/100 g), Mo (0.007 mg/100 g — 0.007 mg/100 g — 0.006 mg/100 g) has decreased in snails, and Mo (0.027 mg/100 g — 0.0031 mg/100 g — 0.008 mg/100 g), Mn (0.5 mg/100 g — 0.6 mg/100 g — 0.4 mg/100 g), Cu (6.28 mg/100 g — 0.5 mg/100 g — 0.26 mg/100 g) Hg (0.00013 mg/100 g — 0.0001 mg/100 g — 0.00006 mg/100 g) decreased in cockroaches. Similar meaning of indices of Pb (0.0008 mg/100 g — 0.0013 mg/100 g — 0.0008 mg/100 g) and Mg (218.9 mg/100 g — 216.5 mg/100 g — 218 mg/100 g) are registered in snails, and Se (3.88 µg/100 g — 7.2 µg/100 g — 3.8 µg/100 g) is in cockroaches (Fig. 1).

The contents of B-group vitamins, B1 (from 0.094 mg/100 g to 0.158 mg/100 g — 0.124 mg/100 g), B2 (from 0.071 mg/100 g to 0.132 mg/100 g — 0.153 mg/100 g), B3 (from 1.06 mg/100 g to 1.145 mg/100 g — 1.88 mg/100 g), B6 (from 0.155 mg/100 g to 0.107 mg/100 g — 0.166 mg/100 g), and B9 (from 4.40 mg/100 g to 5.78 mg/100 g — 5.81 mg/100 g) has increased in snails, and B1 (from 0.72 mg/100 g to 0.95 mg/100 g — 0.99 mg/100 g), B2 (from 1.69 mg/100 g to 1.56 mg/100 g — 1.82 mg/100 g), B3 (from 8.7 mg/100 g to 8.6 mg/100 g — 9.94 mg/100 g) B6 (from 0.96 mg/100 g to 0.93 mg/100 g — 1.32 mg/100 g) and B9 (from 0.26 µg/100 g to 0.30 µg/100 g — 0.31 µg/100 g) in cockroaches (Fig. 2).

In cockroaches, the contents of the fat-soluble vitamins K (from 2.1 $\mu\text{g}/100\text{ g}$ to 3.7 $\mu\text{g}/100\text{ g}$ — 29 $\mu\text{g}/100\text{ g}$), A (from 22.8 $\mu\text{g}/100\text{ g}$ to 25.3 $\mu\text{g}/100\text{ g}$ — 25.94 $\mu\text{g}/100\text{ g}$) and E (from 3.38 mg/100 g to 3.6 mg/100 g — 3.98 mg/100 g) has increased, and D3 (from 3.6 $\mu\text{g}/100\text{ g}$ to 9.8 $\mu\text{g}/100\text{ g}$ — 2.52) $\mu\text{g}/100\text{ g}$ has decreased. In snails, content of fat-soluble vitamins A (from 26.3 $\mu\text{g}/100\text{ g}$ to 34.3 $\mu\text{g}/100\text{ g}$ — 38.52 $\mu\text{g}/100\text{ g}$) and D3 (from 1.92 $\mu\text{g}/100\text{ g}$ to 2.64 $\mu\text{g}/100\text{ g}$ — 2.39 $\mu\text{g}/100\text{ g}$) have increased, but E (from 6.08 mg/100 g to 4.10 mg/100 g — 5.71 mg/100 g) and K (from 0.090 $\mu\text{g}/100\text{ g}$ to 0.078 $\mu\text{g}/100\text{ g}$ — 0.067 $\mu\text{g}/100\text{ g}$) — decreased (Fig. 3).

A double dose of precursors applied to the feed substrate presents a more distinctive picture of the mineral content changes in the biomass of model species. As a result there were increases in snails of iodine x 4.3, chlorine x 1.9 times, sodium x 1.5, selenium x 1.5, calcium x 1.4, copper x 1.3, iron x 1.1, phosphorus x 1.08 and zinc x 1.05; and in cockroaches there were increases of manganese x 5.6, chlorine x 2.5, lead x 2.5, iodine x 2, iron x 2, phosphorus x 1.3, calcium x 1.3, zinc x 1.2, sodium x 1.1 and potassium x 1.1. Decreases were only determined in snails for mercury (0.8-fold) and molybdenum (0.8-fold) and in cockroaches for molybdenum (0.3-fold) times and copper (0.4-fold). For B-group vitamins, these increased for B2 (x 2.1), B3 (x 1.8) and B9 (x 1.3) in snails, and for B1 (x 1.4), B6 (x 1.4), B9 (x 1.2), B6 (x 1.1), B2 (x 1.1) and B3 (x 1.1) in cockroaches. The only decrease was for B1 (1.3-fold) in snails. Increases in fat-soluble vitamins in snails were A (x 1.5) and D3 (x 1.2) and in cockroaches were K (x 13.8), A (x 1.13) and E (x 1.2); however, the decreases were found for E (0.9-fold) and K (0.7-fold) in snails and for D3 (0.8-fold) in cockroaches.

In comparison with the minimal dose of precursors, the double dose did not result in a proportionate increase of nutrients in the biomass. Only protein in cockroaches increased (x 2.1) in comparison with the control group, and x 2.3 in comparison with minimal dose of precursors. In snails, the level of protein slightly decreased (0.9-fold) after the double dose in comparison with control group but increased (x 1.03) in comparison with the minimal dose.

The content of some nutrients, such as iodine in snails, after a double dose of the precursor poorly increased in comparison with minimal dose results. Levels of such nutrients as iron, manganese, copper, zinc and phosphorus in snails increased in comparison with the control group, although after a minimal dose of precursors their content decreased. Level of some nutrients was higher after minimal dose of precursors, for example, in snails selenium has increased twice after minimal dose of precursors, and only 1.5 times after double dose, vitamin K in 1.09 and 1.07 times, and in cockroaches potassium increased in 1.7 times after minimal dose and 1.1 times after double dose. Level of molybdenum, lead, magnesium and vitamins B1, B2, B9, D3 in snails, and B3, B6, A and E in cockroaches remained stable and not changed after application of any dose of precursors.

Only a small number of nutrients decreased in the biomass of invertebrates after minimal and double dose

of precursor applications, as for example in the case of vitamins E and K, and protein in snails. This reaction can be explained by the presence of antagonist matter which impeded the accumulation of nutrients.

A further study on the application of the double dose precursors to feeding substrate showed sustainable trend to changes in the nutrient content of the invertebrate's biomass; several nutrients increased after the application of precursors, which stimulated further research into the qualitative and quantitative accumulation of particular nutrients in the biomass of invertebrates, such as *Lissachatina fulica* and *Nauphoeta cinerea* as shown in Figs. 1–3.

Discussion

The present work is a pilot study regarding the possible regulation of nutrient content for the generation of methods to increase the nutritive value of invertebrate biomass. Currently, this can only provide us with the answers to a small range of questions which are important for the industrial development of proteins in terrestrial invertebrates.

One of the main questions is how limited is the spectrum of invertebrate species which could be used for farming and production of a suitable biomass? Raising species which originated from southern territories in regions with temperate climate is quite expensive due to additional charges necessary to supply warm conditions of raising the stock. However, with the correct nutrient composition in the biomass it is possible to raise without extra expenses local species of terrestrial invertebrates that are well adapted to the cold climate of the Temperate Zone. Native individuals are therefore involved in a cycle which provides reproduction of individuals, and a further withdrawal of specimens from nature is not necessary and there is no negative impact on biodiversity.

The second important problem is the selection of terrestrial invertebrate species eligible for processing which are suitable for industrial farming. Thus, for example, as authorized by the European Commission (EC), species for sale, farming and novel food consumption, such as the migratory locust *Locusta migratoria* and house cricket *Acheta domestica* have been chosen. These are hemimetaboly insects with instar stages which look very similar to adults and possess a strong cuticular integument composed from chitin that impedes full protein extraction. The method of directional correction of nutrient composition can help enrich biomass of industrially farmed species with less chitin components in body covers, such as larvae of holometabolic insect species.

The third essential question is related to possible need for nutrient composition design in the invertebrate biomass. As a rule, literature information on nutrient composition in particular invertebrate species is standardized and considered as reference data for further calculation. In fact, values of indices of nutrient composition vary according to feed quality, conditions for farming and level of precursor application. When an effective mechanism for a pre-designed nutrients accumulation in biomass of invertebrates via enrichment of

feed substrate is developed, new kinds of invertebrates, able to act positively to the assimilation of precursors in order to enrich their biomass with much needed animal protein and other necessary nutrients will be selected. In this case, it is essential that positive results are gained without genetic modification of species raised under industrial scales.

Another question refers to the differences in nutrient composition of the same species of invertebrates taken from different regions and occur in habitats functioning under different environmental conditions. Unfortunately, the data gained from specimens taken from nature or raised on non-standardized substrates strongly differs from that given for the same species reared over a long time under stable environmental conditions of laboratories or farms and fed with standard substrates. As a rule, biomass of «wild» specimens is depleted by some nutrients which seriously diverge from referenced data and raises doubt as to the methods employed in biochemical research.

The data presented in our work showed significant variation for nutrient parameters in the biomass of model species after enrichment of feed substrate composition. The results of our experiments demonstrate the necessity of nutrient composition studies clearly associated with reference to conditions of species raising. Differences in nutrient composition or content in biomass of the same species of invertebrates raised under different conditions are normal, but not a mistaken result of a study.

Conclusions

The pilot study of the nutrient composition changes provides significant facts which define the characteristics of raising terrestrial invertebrates, namely:

— Not only traditionally raised southern species, but local species adapted to temperate climatic conditions may be used to gain a highly productive biomass;

— Withdrawal of part of a population of an invertebrate species from a natural habitat in order to establish a farm for raising specimens should not decrease the local biodiversity and in order to implement in a full the life-cycle for the invertebrates to be reared, it should not be necessary to take additional specimens from the natural population;

— Changes of nutrient composition in invertebrate biomass after enriching the feeding substrate with precursors produces a highly productive biomass without genetic modification of the cultured invertebrates;

— The possibility of directional changes of nutrient composition in the biomass of terrestrial invertebrates opens ample opportunities to produce enriched animal protein biomass for the creation of suitable food products.

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