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Invertebrates of Siberia as a promising source of animal protein for innovative feed and food production. 6. Potential of nutrient accumulation in biomass via feed enrichment

Sergei E. Tshernyshev[1], Andrei S. Babenko[1], Irina B. Babkina[1], Ruslan T-O. Baghirov[1], Vera P. Modyaeva[1], Margarita D. Morozova[1], Ksenia E. Skriptcova[1], Elena Yu. Subbotina[1], Mikhail V. Shcherbakov[1], Anastasia V. Simakova[1]

1 *Tomsk State University, 36 Lenina pr., Tomsk, 634050, Russia*

Corresponding author: Sergei E. Tshernyshev (sch-sch@mail.ru)

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Abstract

This study investigates the feasibility of designing the nutrient composition of four model species of terrestrial invertebrates, namely: two insects, the House cricket (*Acheta domesticus* (Linnaeus, 1758)) and the Speckled cockroach (*Nauphoeta cinerea* (Olivier, 1789)), the Giant African land snail (*Lissachatina fulica* (Férussac, 1821)), and the oligochaete compost worm (*Eisenia fetida* (Savigny, 1826)), through the modification of the feeding substrate composition to obtain biomass with specified parameters of nutrients. It was established that the addition of B-vitamins to the feeding substrate leads to an increase in their content in invertebrates, with the greatest effect observed in insects. In some cases, doubling the dose of these vitamins in the feeding substrate yields a positive effect and promotes greater vitamin accumulation in the animal organisms. The study demonstrated that the addition of fat-soluble vitamins to the feeding substrate also contributes to an increase in their levels in the investigated species. Doubling the dose has a positive effect on crickets, snails, and worms, while a single dose is sufficient for cockroaches. No significant changes in the content of micro- and macroelements were observed in any of the four invertebrate species when mineral substances were added to the diet. In terms of protein content, all studied invertebrates were comparable, while insects had significantly higher levels of fats, carbohydrates, and chitin. The addition of nutrients (substrate with a double dose of vitamins B7 and C, a mineral complex additive, fat-soluble vitamins, and B-complex vitamins) increased the content of fats, proteins, and carbohydrates in insects, as well as ash content in snails and worms. Insects are twice as caloric as snails and worms, and the caloric content of insects increases with the addition of nutrients, while that of snails and worms remains unchanged. Our experiments have shown that it is possible to control the content of vitamins, fats, proteins, and carbohydrates in the biomass of invertebrates, as well as the caloric content in insects by enriching the feeding substrate with precursors. In contrast, the addition of mineral substances to the feeding substrate does not lead to changes in the content of micro- and macroelements in the studied species. Insects are more susceptible to changes in nutrient composition, being enriched with a greater variety of vitamins, proteins, fats, and carbohydrates, and they are significantly more caloric. Therefore, they are the most promising candidates for biomass production with specified properties.

Keywords
Terrestrial invertebrates, Siberia, biomass, nutrient design

Introduction

Terrestrial invertebrates are increasingly being used in the development of animal protein production. A notable trend in recent years has been the cultivation of terrestrial invertebrates for commercial use in the food and feed industries (DeFoliart 1999; Premalatha et al. 2011; Belluco et al. 2013; Hanboonsong et al. 2013; Van Huis 2013, 2015; Mlcek et al. 2014; Assielou et al. 2015; Jansson, Berggren 2015; Han et al. 2017; Van Huis, Oonincx 2017; Van Raamsdonk et al. 2017; Ayensu et al. 2019; Kim et al. 2019; Melgar-Lalanne et al. 2019; Tang et al. 2019; Tobolkova 2019; Gravel, Doyen 2020; Hlongwane et al. 2020; Gorbunova and Zakharov 2021; Tshernyshev et al. 2022). Among the cultivated species there are representatives of different taxonomic classes – Arthropoda: Insecta and Arachnida, Mollusca: Gastropoda and Annelida: Clitellata.

Thus, 4 species of insects *Locusta migratoria* (Linnaeus, 1758), commonly known as grasshoppers, *Tenebrio molitor* (Linnaeus, 1758), mealworms, and *Acheta domesticus* (Linnaeus, 1758), house crickets and *Alphitobius diaperinus* Panzer, 1797 and 5 species of molluscs are authorised for use in the food industry in the European Union, *Helix pomatia* Linnaeus, 1758, the Roman snail, or apple snail, lunar, La Vignaiola, the German "Weinbergschnecke," the French "escargot de Bourgogne" or "Burgundy snail," or "gros blanc"; *Cornu aspersum* (Müller, 1774) (= *Helix aspersa* Müller, 1774), also known as the French "petit gris," "small grey snail," the "escargot chagrine," or "La Zigrinata"; *Helix lucorum* Linnaeus, 1758, or Turkish snail, as well as tropical species of the family Achatinidae, including the well-known species *Achatina achatina* (Linnaeus, 1758), the Giant African Snail, and *A. fulic*a (Férussac, 1821), the Giant East African Snail (Regulation EC 2004; EFSA, 2015 2022; Lahteenmaki-Uutela, Grmelova 2016; Van Peer et al. 2021; EU Commission 2023). In countries where terrestrial invertebrates have been traditionally consumed since ancient times, the number of species eaten exceeds dozens (Hanboonsong et al. 2013; Gere et al. 2018; Patel et al. 2019; Hlongwane et al. 2020). In addition, earthworms are traditionally consumed as food in South East Asian countries (Sun, Jiang 2017).

The range of species used to produce animal feed or protein supplements is quite extensive. In particular, the black soldier fly (*Hermetia illucens* (Linnaeus, 1758)) is actively used, together with a by-product of sericulture – the cocoons of the mulberry silkworm (*Bombyx mori* (Linnaeus, 1758)), cultures of earthworms (*Eisenia fetida* (Savigny, 1826), and *E. andrei* (Bouché, 1972)), and many others. Selection of species for successful cultivation is ongoing, with other species being proposed, such as the larvae of cellulose decomposing beetles. (Tshernyshev et al. 2023b).

All this suggests that terrestrial invertebrates, particularly insects, are becoming increasingly interesting for commercial use in the production of animal protein.

The introduction of terrestrial invertebrates into culture and the development of their cultivation require high efficiency and quality of the final product – biomass, especially in terms of nutrient composition. This raises the question: is it possible to control the composition and levels of individual nutrients in the biomass of invertebrates? It is known that the biomass of all cultivated invertebrate species is characterised by high levels of protein, magnesium, phosphorus and iron and relatively low caloric content. The levels of specific nutrients vary between different species (Ramos-Elorduy et al. 1997; Rumpold, Schlüter 2013; Zielińska et al. 2015; Payne et al. 2016; Mwangi et al. 2018; Udomsil et al. 2019; Magara et al. 2021). It would be interesting to investigate which nutrients contribute to an increase in biomass content and which remain stable at their levels. Understanding the characteristics of nutrient accumulation in invertebrate biomass will not only allow the selection of the most labile species in this regard, but also allow the adjustment of nutrient levels in the biomass produced.

The aim of the study is to assess the potential for altering the nutrient composition of model invertebrate species when reared on diets containing different levels of vitamins and minerals.

Materials and methods

Four invertebrate species, the Giant African land snail *Lissachatina fulic*a, the Speckled cockroach *Nauphoeta cinerea*, the House cricket *Acheta domesticus* and the earthworm *Eisenia fetida* were chosen as model species.

The experiment was conducted in five groups for each model species. The first group was the control, in which individuals were fed a substrate lacking precursors. The second was on a diet enriched with vitamins C and B7, the third with a mineral premix for chickens or a complex mineral supplement for plants (chelate) to minerals, the fourth with vitamins B1 (thiamin), B9 (folate) and B3 (niacin) and the fifth with fat-soluble vitamins A, D, E, K.

Cultures were maintained under laboratory conditions with a temperature of approximately +25˚C and humidity of approximately 60%. The model species were placed in separate plastic containers and provided with a feeding substrate and precursors, or without them in the 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). Substrate was changed and precursors added three times a week.

Under a precursor (a substance introduced into the feed substrate and involved in the metabolism of invertebrates), a specific nutrient was produced in the biomass. In the experiment, the following substances were chosen as precursors: vitamins C and B7 (biotin) for protein production, a complex mineral feed supplement for chickens (premix) for minerals, vitamins B1, B3, B4 and B9 for concordant vitamins of the B-complex, and vitamins A, D, E and K for fat-soluble vitamins.

The fortification of the feeding substrate was achieved in two steps. In the first stage, precursors were added in minimum doses ranging from 2 to 50 mg per 1 kg of feeding substrate, depending on the type of input. Such doses correspond approximately to the recommendations for vitamin and mineral rations given to farm animals to prevent hypovitaminosis. In the second phase, the doses of the precursors were increased twofold in proportion to each input substance in the substrate. In this case, the doses of precursors should have sufficient enriched biomass up to the level required for metabolism and also accumulate certain nutrients. The amounts of precursor input samples are given in Table 1.

Table 1. Quantity of precursors (mg) added to feeding substrate of model species during the experiment

Type of precursor	**I stage, singular dose of precursor**		**II stage, doubled dose of precursor**	
	Per 1 kilo of feeding substrate, mg	**Per food portion, mg**	**Per 1 kilo of feeding 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

After 30 days, samples of raw frozen biomass (0.4 kg) of each model species were analysed. The analyses were performed in the testing centre "OOO Sibtest" as a small innovative enterprise of the National Research Tomsk Polytechnic University, Tomsk, Russia, in a laboratory accredited with the licence "GOSTAkkreditatsiya", 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.

Results

A study was conducted to investigate the changes in nutrient composition of four species of invertebrates upon the addition of single and double doses of B vitamins, fat-soluble vitamins, and mineral substances containing micro- and macroelements to the feed substrate.

Adding a single dose of B vitamins to crickets will increase the levels of B12 and B9, while a double dose will increase the levels of all B vitamins. We can therefore increase the levels of B vitamins by incorporating them into the feed substrate.

Adding a single dose of B vitamins to the cockroaches will only increase the level of vitamin B1, whereas a double dose will increase the levels of B1, B12 and B6. So we can only increase the levels of certain B vitamins.

In snails, the B vitamin content does not change, but we can increase the vitamin B12 content by adding a double dose of B vitamins to the substrate.

Worms are the richest in B vitamins. The addition of a single dose increases the levels of all vitamins except B1 and B9. The addition of a double dose is comparable to that of a single dose.

Thus, the addition of B vitamins to the feed substrate generally leads to their accumulation in invertebrates, with the most significant accumulation effects observed in insects (Fig. 1).

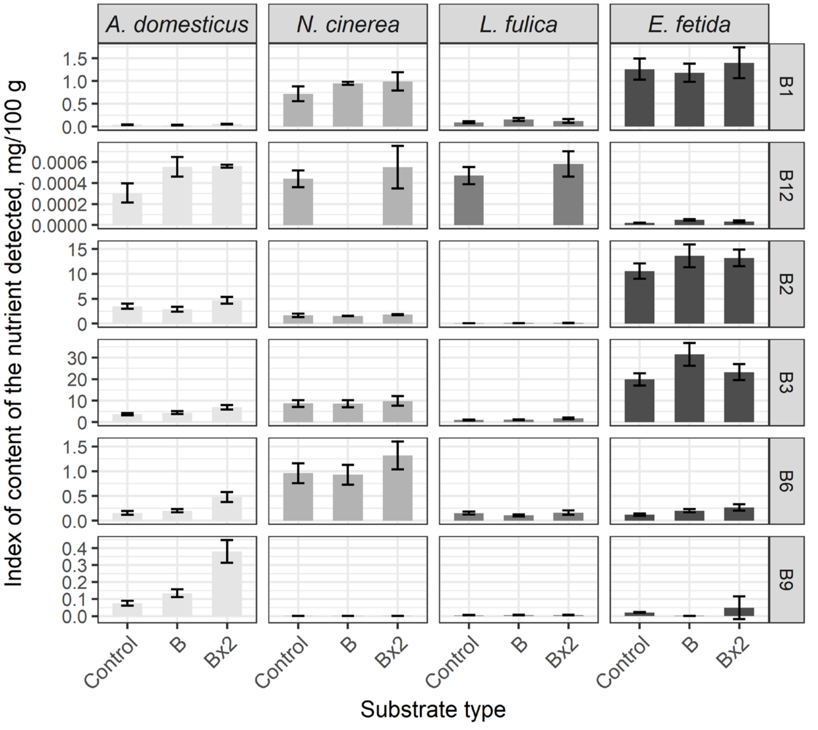

Figure 1. Content of B vitamins in four species of invertebrates studied on different types of nutrient substrates. The graphs show the mean values of the indicators with confidence intervals (adjusted for multiple comparisons CI 99%). Designations: Control – control group; B – substrate with B-complex vitamins (B1, B12, B2, B3, B6, and B9); B×2 – substrate with double dose of B-complex vitamins.

When a single dose of fat-soluble vitamins is added to the diet of crickets, no significant changes are observed. However, when the dose is doubled, a significant increase in vitamins A, E, and K is noted. Thus, the addition of high doses of fat-soluble vitamins to the nutrient substrate leads to their accumulation.

In cockroaches, the levels of vitamins A and E do not change with either a single or double dose, whereas the levels of vitamins D3 and K increase significantly with a single dose and decrease with a double dose. Thus, only a single dose is required to increase the levels of certain fat-soluble vitamins in cockroaches, suggesting that not all vitamins in this group can accumulate.

In achatina snails, the addition of fat-soluble vitamins only leads to an accumulation of vitamin A. In worms, however, the application of a double dose leads to an increase in the levels of vitamins A, E and K.

Thus, cockroaches and achatina snails are the richest in fat-soluble vitamins. The addition of an extra dose of fat-soluble vitamins promotes their accumulation in the bodies of invertebrates. A double dose has a positive effect on crickets, achatina snails and worms, while a single dose is sufficient for cockroaches (Fig. 2).

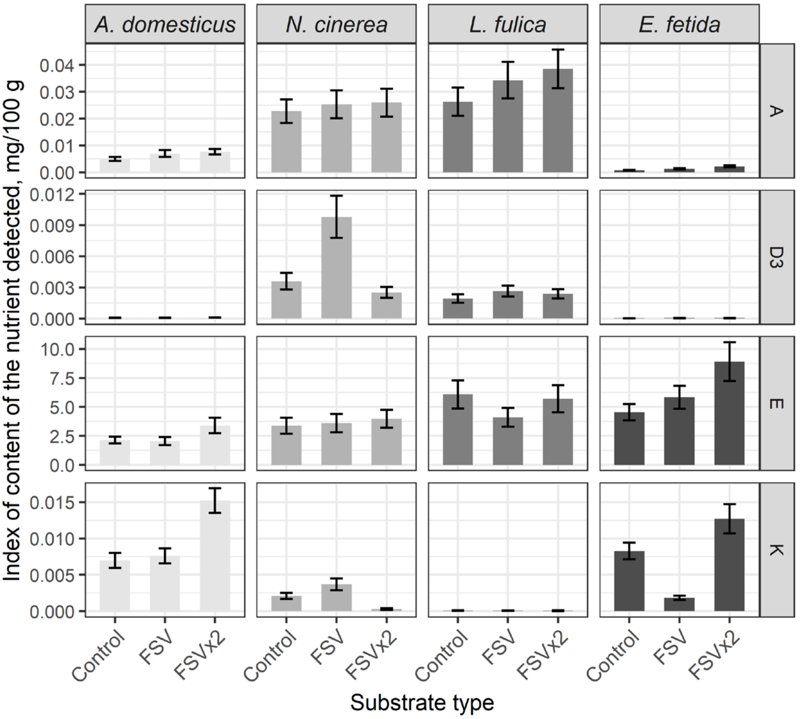

Figure 2. The content of fat-soluble vitamins in four species of invertebrates studied on different types of feeding substrates. The graphs show mean values with confidence intervals (adjusted for multiple comparisons, CI 99%). Designations: Control – control group; FSV – substrate with fat-soluble vitamins; FSV×2 – substrate with double dose of fat-soluble vitamins.

The invertebrates studied have different levels of micro- and macroelements. Crickets are particularly rich in microelements such as Cu, I, Mn, Mo and Zn, while cockroaches have high levels of I and Se, achatina snails are rich in Se and worms contain significant amounts of Fe, Hg, Mo and Pb. In terms of macroelements, crickets are the most abundant, with high levels of all macroelements except Ca. Cockroaches have high levels of Ca and Na, achatina snails are rich in Ca and Mg, and worms have high levels of K and Na. Overall, crickets are more mineral saturated.

The addition of mineral substances to the feeding substrate did not result in any significant changes in the levels of micro- and macroelements in any of the invertebrates (Figs 3, 4).

In crickets, the addition of various nutrients to the feeding substrate (substrate supplemented with a double dose of vitamins B7 and C; mineral complex supplement; and a dose of fat-soluble vitamins) results in an increase in fat content, while the addition of a double dose of B group vitamins results in an increase in protein content.

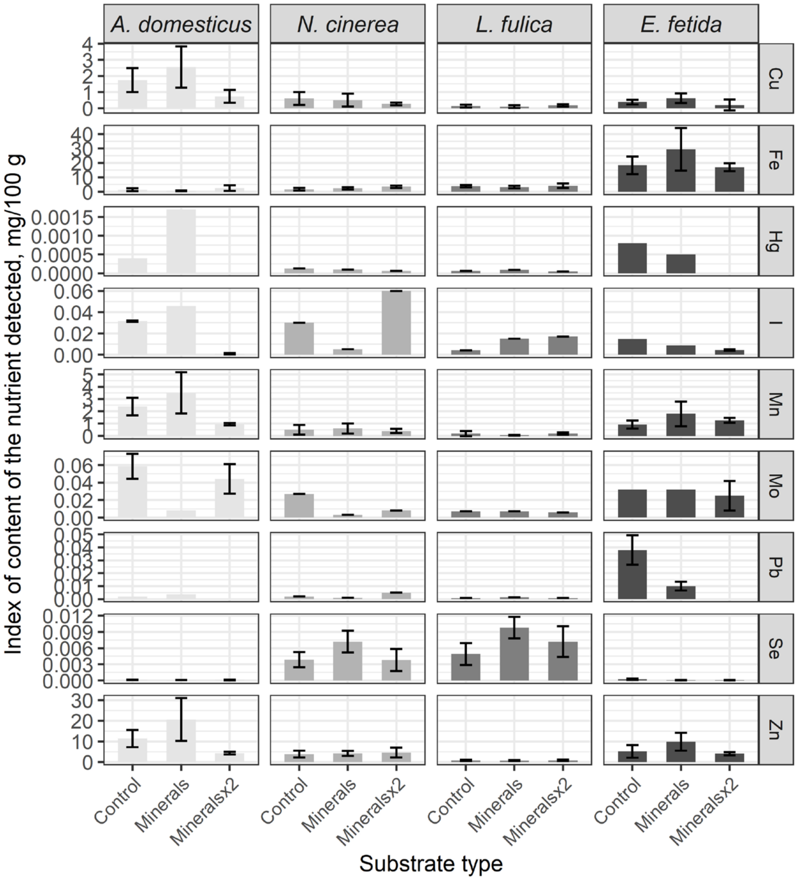

Figure 3. The levels of microelements in four species of invertebrates studied in relation to different types of feeding substrate. The graphs show the mean values of the measurements with confidence intervals (with correction for multiple comparisons at CI 99%). Designations: Control – control group; FSV – substrate with fat-soluble vitamins; FSV×2 – substrate with double dose of fat-soluble vitamins. Designations: Control – control group; Minerals – substrate supplemented with a mineral complex of microelements; Minerals×2 – substrate with a double dose of the microelements mineral complex supplement.

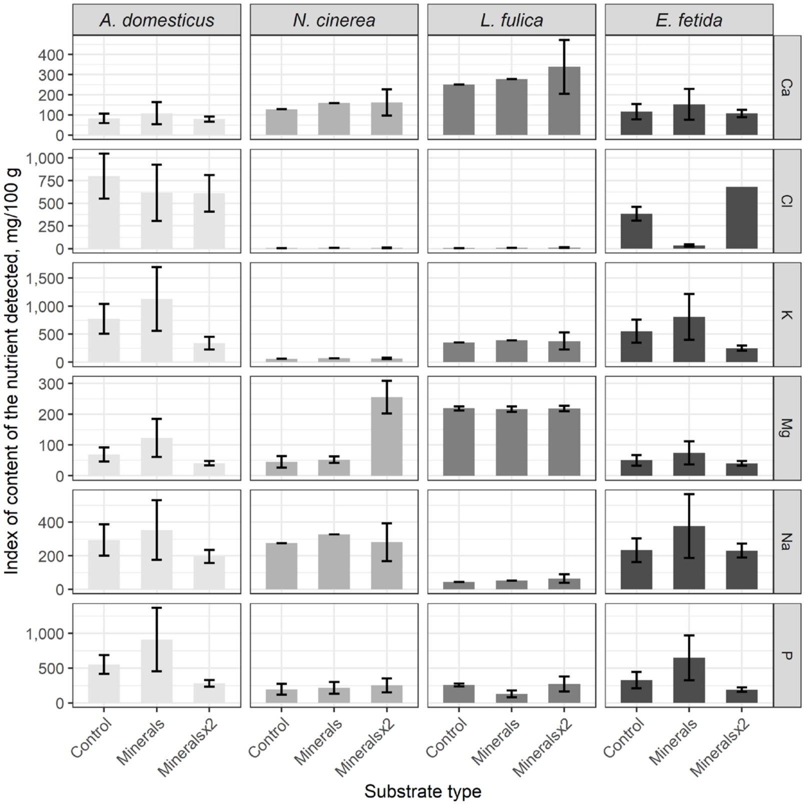

Figure 4. Macroelement content in four species of invertebrates studied over different types of feeding substrates. The graphs show the mean values of the measurements with confidence intervals (adjusted for multiple comparisons with CI 99%). Designations: Control – control group; Minerals – substrate with a complex mineral supplement of macroelements; Minerals×2 – substrate with a double dose of mineral complex supplement of macroelements.

In cockroaches, the addition of nutrients to the feeding substrate results in an increase in all organic matter except ash content.

In achatina there is an increase in ash content and a decrease in chitin content. In worms, both ash and chitin increase (Fig. 5).

This means that we can increase the fat, protein and carbohydrate content of insects and the ash content of achatina and worms. In terms of protein content, all the invertebrates studied are comparable, whereas insects have significantly higher levels of fats, carbohydrates and chitin.

The addition of nutrients (substrate with double doses of vitamins B7 and C; mineral complex supplement; fat-soluble vitamins; B-complex vitamins) increases the caloric content of insects, while the caloric content of achatina and worms remains unchanged. Overall, insects are significantly higher in calories (Fig. 6).

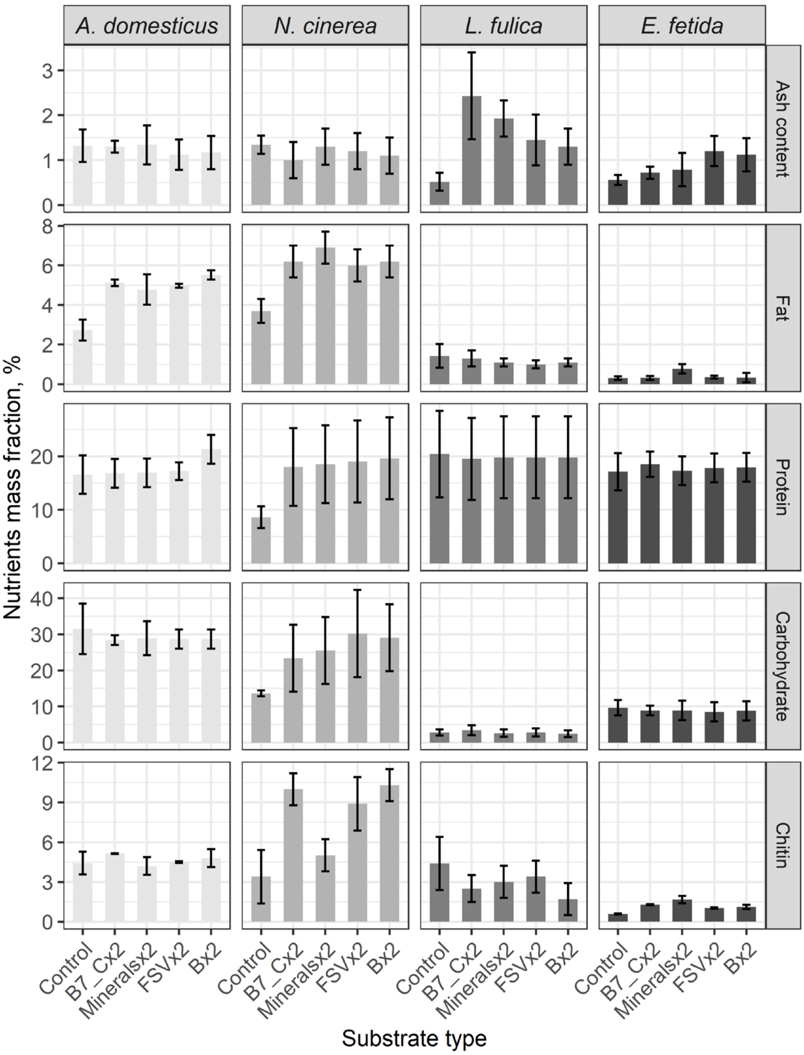

Figure 5. Comprehensive analyses of the biomass of four species of invertebrates studied on different types of feeding substrates. The graphs show the mean values of the parameters with confidence intervals (adjusted for multiple comparisons CI 99.4%). Designations: Control – control group; B7×2_C – substrate with double dose of vitamins B7 and C; Minerals×2 – substrate with double dose of mineral complex supplement; FSV×2 – substrate with double dose of fat-soluble vitamins; B×2 – substrate with double dose of B-complex vitamins.

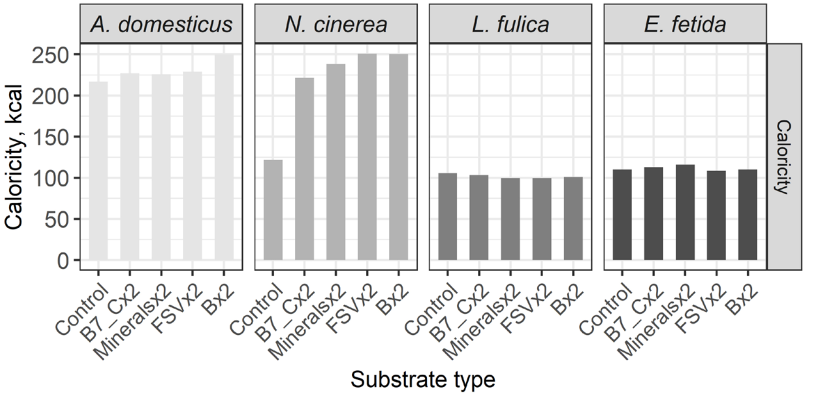

Figure 6. Caloric content of four species of invertebrates studied on different types of feeding substrates. The graphs show the mean values of the parameters with confidence intervals (adjusted for multiple comparisons CI 99.4%).Designations: Control – control group; B7×2_C – substrate with double dose of vitamins B7 and C; Minerals×2 – substrate with double dose of mineral complex supplement; FSV×2 – substrate with double dose of fat-soluble vitamins; B×2 – substrate with double dose of B-complex vitamins.

Discussion

The model species selected for the study are well-known subjects in the field of agriculture and next generation food production.

The house cricket, *Acheta domesticus*, has been approved by the European Commission for commercial food production (Regulation (EC) No 852/2004; EFSA 2015), which has led to a considerable amount of research on the nutrient composition of crickets. Detailed analyses of their nutrient composition, microbiological and toxicological profiles have been carried out (Udomsil et al. 2019; Ververis et al. 2022), and their applications in food range from protein extraction for food fortification to the development of cricket snack products (Combrzynski et al. 2023; Pilco-Romero et al. 2023). The species is considered a potential source of protein for human consumption (von Hackewitz 2018). In agriculture, house crickets are used both as livestock feed (Van Peer et al. 2024) and as an innovative method of 'recycling' unusable meal into a source of animal protein (Paulk et al. 2024).

The speckled cockroach, *Nauphoeta cinerea*, has also found applications in the food industry. Dry powder derived from the speckled cockroach has been used to enrich wheat bread with protein (Lauren Menegon de Oliveira et al. 2017). In agriculture, cockroach biomass is increasingly being used in fish feed formulations (Tubin et al. 2023; Ng et al. 2024; Silveira et al. 2024).

The giant African land snail, *Lissachatina fulica*, is one of 14 species authorised for consumption in the United States and is included in Regulation (EC) No 853/2004 under the local name Giant East African Snail as one of five species of edible snails. The snail is used in the preparation of stuffed shells known as "escargots" and is a major source of protein in some African countries (Tanyitiku 2022). Recently, the food potential of edible snails, including *L. fulica*, has been explored as a sustainable underutilised food resource (Baghele et al. 2022). Due to its rapid growth and ease of cultivation, snail meal has been proposed as a protein supplement in animal feeds (Ferreira et al. 2021). In medicine, the species is known for its use as an anti-inflammatory and skin regenerating agent (Tella 1979; Wiya et al. 2020), and snail secretions are being studied for their anticancer properties (E-Kobon et al. 2016). Furthermore, *Lissachatina fulica* is one of the most popular snail species in the hobbyist community.

The oligochaete compost worm, *Eisenia fetida*, is also used as food in some regions of Southeast Asia (Sun, Jiang 2017), but mainly the nutrient composition of the biomass of *Eisenia* sp. has been studied to justify the effectiveness of using the earthworm in the production of feed for livestock (broilers, fish, pigs, etc.) (Gunya et al. 2019; Isea-León et al. 2019; Castro-Bedriñana et al. 2020). It has been reported that the protein content of the dietary powder obtained from the earthworm E. fotida is six times higher than that of barley flour (66.90% vs. 11.81%), and that feeding broilers with a mixture of traditional feed and the worm powder improves the taste of the meat and increases weight gain (Isea-León et al. 2019); in guinea pigs, it improves energy exchange and feed digestibility by 10% (Castro-Bedriñana et al. 2020); and for fish and crustaceans, it provides the necessary fatty acids (Isea-León et al. 2019).

Studies on the nutrient composition and food value of insects have been carried out repeatedly, and are summarised and thoroughly reviewed in overviews by V. Meyer-Rochow (2019) and V. Meyer-Rochow et al. (2021). These studies have demonstrated changes in the chemical composition of different insect species depending on their developmental stage and preparation methods, and have analysed the composition of nutrients and anti-nutrients, as well as microbial and pathogenic loads. Our research shows clear trends in the accumulation of nutrients in the biomass of invertebrates of different taxonomic types, not just insects, which makes it possible to identify which invertebrate species accumulate nutrients most effectively, gain mass rapidly and have a nutrient-rich composition.

For example, partial enrichment of the feeding substrate with precursors of specific nutrients resulted in uneven accumulation of dietary elements in the biomass of model invertebrate species. There was little success in increasing protein levels in achatina snails and crickets, whereas protein levels increased slightly in cockroaches and worms (Tshernyshev et al. 2023a, 2024a). Comparative analysis shows that no changes in protein levels are observed with comprehensive enrichment of the feeding substrate, suggesting that it is challenging to further increase the already high protein content of terrestrial invertebrates, regardless of their taxonomic affiliation.

Changes in mineral composition during partial enrichment are most pronounced in achatina snails and worms, while cockroaches and crickets only showed significant increases in certain elements (Tshernyshev et al. 2023b, 2024b). Overall, an increase in the mineral composition of invertebrates is possible, but is hindered by extensive enrichment of the substrate. In this case, no significant changes in mineral composition occur.

Interestingly, both partial and comprehensive enrichment were successful in consistently increasing the levels of vitamins, including the B group, vitamin C and fat-soluble vitamins. Thus, in order to achieve the objectives of accumulating specific nutrients in the biomass of invertebrates, it is advisable to adopt different strategies for enriching the feeding substrate: selective enrichment of the substrate for the accumulation of protein and mineral substances, while using comprehensive enrichment for the accumulation of vitamins, as comprehensive enrichment does not inhibit the accumulation of protein or mineral substances.

Conclusions

Among the invertebrates studied, worms are the richest in B vitamins. The addition of B vitamins to the feeding substrate results in an increased concentration of these vitamins in invertebrates, with the most significant effect observed in insects. In some cases, increasing the dosage of these vitamins in the feeding substrate has a positive effect and promotes greater accumulation of vitamins in the invertebrate organisms.

Cockroaches and achatina snails are the richest in fat-soluble vitamins. The addition of fat-soluble vitamins to the feeding substrate helps to increase their concentration in the invertebrate organisms. Doubling the dose has a positive effect on crickets, achatina snails and worms, while a single dose is sufficient for cockroaches.

Crickets have the highest mineral content compared to cockroaches, achatina snails and worms. No significant changes in the levels of micro- and macroelements were observed in any of the invertebrates when minerals were added to the feeding substrate.

In terms of protein content, all the invertebrates studied are comparable, while insects have significantly higher levels of fats, carbohydrates and chitin. The addition of nutrients (substrate with double doses of vitamins B7 and C, mineral complex additives, fat-soluble vitamins and B-complex vitamins) increases the content of fats, proteins and carbohydrates in insects and the ash content in achatina snails and worms.

Insects are significantly higher in calories than achatina snails and worms. The addition of nutrients increases the calorific value of insects, while the calorific value of achatina snails and worms remains unchanged.

Thus, the addition of vitamins to the feeding substrate makes it possible to control their levels in invertebrates. The addition of nutrients can increase the calorific value and the content of lipids, proteins and carbohydrates in terrestrial insects. However, the addition of minerals to the feeding substrate does not lead to changes in the content of micro- and macronutrients in the species studied.

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