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RESEARCH ARTICLE

Invertebrates of Siberia, a potential source of animal protein for innovative food and feed production. 2. Nutrient composition of the two new model species

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Abstract

The use of terrestrial invertebrates occurring in Siberia as a source of nutrients as an innovative form of new quality food production in North Asia is analysied. Two species, big slug *Limacus flavus* (Linnaeus, 1758) and rose chafer *Cetonia aurata viridiventris* Reitter, 1896 have been reared under laboratory conditions and prepared for nutrient analysis. Biomass have been taken from the instar stages of slugs and beetle larvae, animals have been killed by frozen and then presented for analysis. To determine the nutritive value, following macro- and micro- nutrients and dietary fibre have been revealed and determined from the biomass: B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folacin), B12 (cyanocobalamin), E (α- tocopherol), A (retinol palmitate), Fe, Se, Zn, Mn, Cu, Mg, F, lipid, protein, carbohydrate and chitin. In slugs and beetle larvae the mass fraction of protein is 20.6 and 20.8%, the fat percentage is 0.43 and 0.44%, and carbohydrate is 0.22 and 0.27%. Caloricity varied from 98 kilocalories in slugs to 96 in beetle larvae. All species are rich in magnesium (160 mg/100 g and 288 mg/100 g) and phosphorus (320 mg/100 g and 450 mg/100 g) (these elements are represented as macronutrients), and contains high level of iron (3.3 mg/100 g and 5.1 mg/100 g), copper (0.50 mg/100 g and 1.6 mg/100 g), selenium (0.0235 mg/100 g and 0.0334 mg/100 g), zinc (0.80 mg/100 g and 1.9 mg/100 g) and manganese (0.0076 mg/100 g and 0.0112 mg/100 g). Four vitamins detected, namely: A (0.0225 mg/100 g and 0.0337 mg/100 g), E (5.2 mg/100 g and 0.59 mg/100 g), B1 (2.5 mg/100 g) и B2 (5.2 mg/100 g and 0.59 mg/100 g). Almost completely lacking B3, B6, B9 and B12. Biomass both slugs and beetles is similar by nutrient composition and differs in presence of vitamin B3 in slugs, and 1.5 time higher vitamin A content in beetle larvae. The data revealed from the two terrestrial invertebrates show high level of natural nutrients, that could be used for food production, and in combination with not difficult raising hese species are perspective for farming in local conditions of different countries, excepting extremely hot and arid regions. Perspectives of terrestrial invertebrates farming for food production, safety aspects and comparative analysis with other edible species are discussed.

Keywords

Mollusca, Limacidae, *Limacus flavus*, Coleoptera, Scarabaeidae, *Cetonia aurata viridiventris*, West Siberia, food perspective, nutrients, protein, carbohydrate, fat, vitamins, minerals, fibre

Introduction

The edible invertebrates nutrients as new source of food in future

Detection of new resources of food containing a spectrum of nutrients, which are available and cheap to produce, are necessary to support terrestrial invertebrates capable of providing protein production for human consumption (Van Huis et al. 2013; Zielińska et al. 2015; Van Raamsdonk et al. 2017). The food in future should be provided with necessary vitamins, minerals, fibers etc, supplying complete necessary daily meal. Environmental problems occurring under the pressure of agricultural activity like pasture of cattle, planting of wide plantations of crops and grasses for animal feed are seriously impact on biosphere via fragmentation of habitats, destruction of natural biogeocoenosis, and emission of waist included pollutants like methane. All of these are active elements in processes of atmospheric warms that lead to global climate change. Obviously, the new approaches in protein production that could demonstrate low influence to environment are strongly needed.

One of the most important condition that comply the new food production is low expenses for new technologies realization in comparison with traditional agriculture and farming. They are not demanding serious changes in agricultural machinery and answering to the purpose of low pollutant emission to atmosphere, and could stop widening of plowing areas and pastures. For this purpose new terrestrial invertebrates as a source of new protein production can be studied.

A number of publications present nutrient composition of insect, arachnid, mollusk and crustacean species and show reach spectrum of nutrient maintenance in biomass of these animals. Unfortunately, only several species of terrestrial invertebrates are permitted for production and trade in EU, in spite of the fact, that a number of invertebrates have been used traditionally in "folk meal", especially in tropic and subtropic countries.

Raising of terrestrial invertebrates can open good perspectives in gaining of food which is balanced by nutrients. The temperate climate zone of Eurasia, particularly South Siberia, is ideal for such an activity. Hence, a new project, the first of its kind in North Asia, entitled "Invertebrates of Siberia as a resource of animal protein for innovative food production", was launched in the National Research Tomsk State University in 2022.

Perspectives of terrestrial invertebrates farming for food production

The use of terrestrial invertebrates for human consumption has been used for a long time, but only a few species of them have been domestically bred; for example, larvae and pupae of silk moth *Bombyx mori* were eaten as food supplement from the very beginning of silk technology, and currently could be met in shop and stores in countries of silk production, such as Korea, China, USA. Others are shell gastropods of the families Achatinidae and Helicidae (Thompson, Cheney 1996). These species are strongly invasive pests and widespread in countries with a warm climate. The exceptionally large size that these snails reach was one of characters attracted to human attention as a food source. As a result, farms have been adapted to produce snail 'meat' for restaurants and markets; it should be noted that the 'domesticated' slugs were formerly regarded as pests which damaged agricultural activity. At present, the mealworm (*Tenebrio molitor*) larvae and the adult desert locust (*Schistocerca gregaria*) are studied for their potential as food usage (Zielińska et al. 2015). They are common species occurring in high numbers, adapted to a range of environmental conditions, and although registered as pests, they are not problematic in terms of fodder plants or substrates.

Amongst terrestrial invertebrates only insects and molluscs are farmed (Thompson, Cheney 1996; Hanboonsong et al. 2013). Species raised, traded and consumed vary in different countries. For example, 79 Coleoptera species, 13 Heteroptera, 4 Odonata, 5 Hymenoptera, 46 Orthoptera, 1 Isoptera, 3 Lepidoptera and 4 Homoptera species are eaten in Northeast Thailand (Hanboonsong et al. 2013; Rattanapan, 2000), and 6 Coleoptera species, 2 Heteroptera, 1 Odonata, 4 Hymenoptera, 4 Orthoptera and 2 Lepidoptera species are eaten in upper Southern Thailand (Lumsaad, 2001). Amongst snails, 14 species, *Cornu aspersum* (O. F. Müller, 1774) (= Helix aspersa Muller), *Helix pomatia* Linnaeus, 1758, *Otala lactea* (Müller, 1774), *Iberus alonensis* (Férussac, 1821), *Cepaea nemoralis* (Linnaeus, 1758), *Cepaea hortensis* (O. F. Müller, 1774), *Otala punctata* (O. F. Müller, 1774), *Eobania vermiculata* (O. F. Müller, 1774), *Helix lucorum* Linnaeus, 1758, *Helix* (*Pomatia*) *adanensis* Kobelt, 1896, *Helix aperta* (Born,1778), *Theba pisana* (Müller, 1774), *Sphincterochila candidisima* (Draparnaud, 1801), and *Achatina fulica* (Férussac, 1821) are uses as edible in the USA. Since 2021, the European Commission (EC) authorized only three species of insects for sale, farming and novel food consumption, namely: *Locusta migratoria* Linnaeus, 1758, commonly known as grasshoppers, *Tenebrio molitor* Linnaeus, 1758, mealworms, and *Acheta domesticus* Linnaeus, 1758, house crickets (Regulation (EC) 2004; EFSA NDA 2015a-d; EFSA 2015; Lahteenmaki-Uutela, Grmelova 2016). On 4 July 2022, EFSA published an opinion confirming the safety of frozen and freeze-dried larvae of *Alphitobius diaperinus* for human consumption

(EFSA 2022). Approval as novel food in the European Union followed on 6 January 2023 with the EU commission's publication of Implementing Regulation 2023/58 authorising the placing on the market of the frozen, paste, dried and powder forms of *A. diaperinus* larvae (EFSA 2022; EU Commission 2023).

The EC Regulation no. 853/2004 defines edible snails as 5 species terrestrial gastropods: *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* (O. F. Müller, 1774) (= *Helix aspersa* Muller), 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. fulica* (Férussac, 1821), the Giant East African Snail.

Such a restriction for the food industry is explainable by the strict order of "studies on bacteria, viruses, parasites and prions associated with food and feed insects, chemical contamination, allergens, processing, and the potential environmental impact of insect farms" (Belluco et al. 2013; Van der Spiegel et al. 2013; Finke et al. 2015). No doubt, further development of invertebrate farming will solve these problems and significantly widen the list of edible species necessary for the generation new quality food products. Currently, a survey of new species with suitable life cycles and morphology is necessary (FAO 2013; Leser 2013; Van Huis 2013).

Safety aspects

The toxic compounds in insect larvae may arise from substrate and soil (cryptotoxics) or the synthesis of naturally occurring poisons by insects (phanerotoxics) (EFSA Scientific Committee 2015). The defence system of some insects is composed of chemical compounds that could be toxic. Consumption of silkworms may result in neurological disorders that lead to a total loss of control over the body's functions (Elhassan et al. 2019). On the other hand, the toxin concentration of any species might be minor and harmless due to the low quantity, for instance *Zygaena* moths (Zagrobelny et al. 2009). There are no apparent signs that *Limacus flavus* and *Cetonia aurata viridiventris* produce a poisonous chemical compound for human consumption.

Regarding edible insects, the substrate that has been microbially contaminated may lead to the spread of hazardous substances carried by the insects themselves (Turck et al. 2021). The presence of contamination in insects may thus be managed by regulating the amounts of contaminants in the substrate (EFSA Scientific Committee 2015). The analyzed samples of *L. flavus* have got potatoes and *C. aurata viridiventris* the blend of fermented wood split and manure for their feeding. It is worth taking into consideration that Regulation (EC) No. 1069/2009 (Regulation (EC) 2009) prohibits the use of specific substrates, such as manure, catering waste, or previous meat- and fish-containing dishes, for the feeding of insects classified as "farm animals".

Furthermore, heavy metals, dioxins, and antinutrient substances may accumulate in insects (Rumpold, Schlüter 2013 a,b; Van der Fels-Klerx 2016). However, the value of fresh matter and the matter after processing procedures such as washing, drying, heating, and grinding have not been evaluated for *L. flavus* and *C. aurata viridiventris*.

The review research showed numerous incidences of cross-food allergies, for instance when patients with a crustacean allergy might have a cross-reaction with the proteins in yellow mealworm (Verhoeckx et al. 2014). The structural properties of allergens may be significantly changed by food processing, either increasing or reducing their antigenic potential (Hayashida, da Silva 2021; Lepski, Brockmeyer 2013). In addition, more research might be conducted for *L. flavus* and *C. aurata viridiventris* to determine if insect allergies and insect-food cross-allergies are related.

It is necessary to conduct research on the bacteria, viruses, parasites, and prions related to food and feed insects species, as well as chemical contamination, and allergies.

Slugs have never been used and studied as food supplement. Limacidae species are the largest slugs, which can reach the same size as Achatinidae and Helicidae, but lack a shell and and are richer in protein biomass. The synanthropic slug *L. flavus* is widespread and introduced into different countries, including North Asia. It has, for example, been found by us in an underground vegetable store in Siberia and questioned: would this species be effective for protein production when raised under simulated conditions? *C. aurata viridiventris* Reitter larvae are also were explored for the purpose of housing, raising and gaining the biomass for the first time, and showed simple maintenance, cheap feed substrate and a biomass rich in nutrients and contains less chitin than imago or in hemimetabolous species. Thus, we initiated an experiment with the aim of defining the nutrient composition of model species of invertebrates occur in Siberia those would provide a source of animal protein for novel food production (Tshernyshev et al. 2022, 2023 a,b, 2024).

Materials and methods

Model species collection and preparation

For the pilot period of the project, two invertebrate species were taken, the keelback slugs *Limacus flavus* (Linnaeus, 1758) (Gastropoda: Limacidae) and rose chafer *Cetonia aurata viridiventris* Reitter, 1896 (Coleoptera: Scarabaeidae). Initially, the slugs and beetle larvae were taken from nature in the City of Novosibirsk and the suburb (Fig. 1), and then reared under laboratory conditions.

Slug specimens examined by Roman Egorov in Moscow in June 2016 were identified as *L. flavus*. On 31 March 2022, 10 juvenile specimens of this species were taken from an underground vegetable store in Novosibirsk and placed in five numbered plastic containers (1 liter vol.) with closely fitted covers to prevent air exchange. Containers 1 and 3 were maintained at 24–26˚C under daylight, containers 2 and 4 at room temperature and in darkness, and container 5 in a refrigerator at 10–12˚C in darkness. Containers 3 and 4 were also provided with viscose tissue saturated with water to increase humidity (Tshernyshev et all. 2022). Collection locality data are as follow: Russia, City of Novosibirsk, Shamshurina str., underground vegetable store, N55°01', E82°55', March 2022, collected S.E. Tshernyshev.

Of the beetle only larvae were used in the experiment. They were collected in old compost hill containing decaying sawdust, and then also reared in laboratory condition. Collection locality data for the larvae are as follow: Russia, Novosibirskaya Oblast', 6.5 km SE Mochishche vill., Ozernyi vill., N55°08'; E82°54' birch-pine forest, April, 2022, collected S.E. Tshernyshev.

The big slug *L. flavus* adults and the rose chafer *C. aurata viridiventris* larvae were killed by frozen and then presented to laboratory for nutrient analysis.

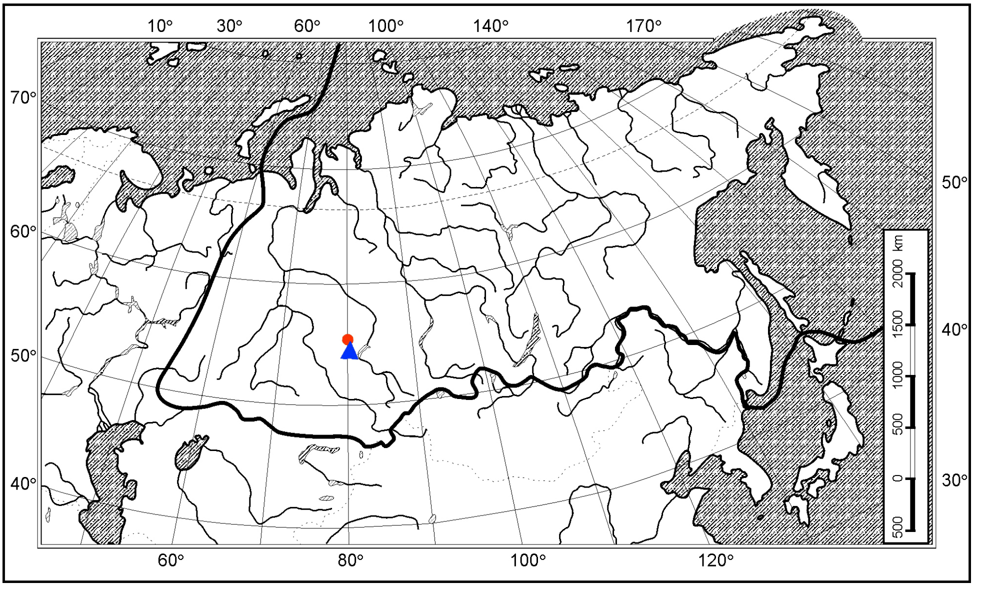

Figure 1. Collection locality of *L. flavus* (red circle) and *C. aurata viridiventris* (blue triangle).

Details of model species nutrient analyses processing

Two samples of raw frozen biomass dozen by 0.4 kg of each model species were presented for analysis. Crude biomass was studied, and indexes revealed were count exactly in respect of it but not dry matter.

The analyses were held in the test center "OOO Sibtest", small-scale innovative enterprise of the National Research Tomsk Polytechnic University, Tomsk, Russia. The laboratory is accredited by voluntary accreditation system "GOSTAkkreditatsiya", the license No.GOST.RU.22152.

The analyses were aimed to detect carbohydrates, proteins, lipids, vitamins B1, B2, B3, B6, B9, B12, A and E, microelements iron (Fe), selenium (Se), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn) and phosphorus (P) in the given sample. Caloricity of these two species biomass was also tested.

The protocols of analyses results were presented in standard tabulate form and provided with reference to GOSTs in term of research method choice. GOST is a summary of Russian State standards, describing methods used to detect necessary matter. To explain what method was applied in case of each nutrient determination, a brief explanation of GOST is provided below with reference to its number given in parentheses.

Carbohydrates determined by the method of mass concentration in terms of glucose. The method is based on ability of reducing carbohydrates formed under acid hydrolysis of sample reduce ferricyanide to ferrocyanide in alkaline medium. Mass concentration of carbohydrates in terms of glucose is determined by titration of ferricyanide surplus in standard glucose solution after reaction with reducing matters (GOST P 53747-2009 2011).

Proteins were revealed by the protein mass fraction using the Kjeldahl method, consists in mineralisation of organic substances of sample with subsequent determination of nitrogen by the quantity of generated ammonia (GOST 25011-2017 2018).

Lipids were determined by using Soxhlet extractor (GOST 23042-2015 2017) as multiply fat extraction by solvent from dehydrated sample in Soxhlet extractor with further elimination of solvent and fat desiccation to the constant mass.

Vitamin B1 was determined using high-yield (high-performance) liquid chromatography, HPLC as total content of thiamine including its phosphorylated derivatives (GOST EN 14122-2013 2015).

Vitamin B2 was also determined by using high-yield (high-performance) liquid chromatography, HPLC, by riboflavin content (GOST EN 14152-2013 2015).

Vitamins B3 & **B9** were determined by the method of capillary electrophoresis traditionally used for water-soluble vitamins. For detection B3 as panteonic acid and B9 as folic acid the method of micellar electrokinetic capillary chromatography was used (GOST 31483-2012 2013). In this case, identification and quantification of vitamins is held using software support.

Vitamin B6 was determined under the method of high-yield (high-performance) liquid chromatography, HPLC, as a sum of mass fraction of pyridoxine, pyridoxal, pyridoxamine, including their phosphorylated products in terms of pyridoxine (GOST EN 14164-2014 2017).

Vitamin B12 was revealed by the method of reversed-phase high performance liquid chromatography with detection in visible spectrum under 550 nanometre wavelength (GOST ISO 20634-2018 2019).

Vitamin A was determined by method of mass concentration of retinol, retinol acetate, retinol palmitate under high-yield (high-performance) liquid chromatography, HPLC (GOST P 54635-2011 2013) with measurement range from 0.5 to 10.0 parts per million (ppa).

Vitamin E also was determined using HPLC method of mass concentration of α-, β-, γ- и δ- tocopherols (GOST EN 12822-2014 2016) and figured in parts per million (ppa).

Iron (Fe), was determined by the method of study the reaction of iron ions with sulfosalicylic acid in alkaline medium with buildup yellow coloured complex compound. Intensity of colouration, proportional to mass concentration of iron, was measured under 400–430 nanometre wavelength with measurement range of iron mass concentration 0.10–2.00 mg/dm^3 (GOST 4011-72 1974).

Phosphorus (P) was determined by the method based on dehumidification of sample with further incineration, cooling and hydrolysis of incineration residue by nitric acid, filtration and further dilution by mixture of ammonium monovanadate and ammonium heptamolibdate resulted to formation of yellow compound which was studied bythe method of photometric scaling of optical density under 430 nanometre wavelength (GOST 32009-2013 2014).

Selenium (Se), **magnesium (Mg)**, **copper (Cu)**, **zinc (Zn)** and **manganese (Mn)** were determined by method of inductively coupled plasma mass spectrometry (ICP MS).

Crude fibre was detected by Genneberg and Shtoman Method (GOST 31675-2012 2020). The method is based on serial processing of sample by acid and alkali solutions resulted to incineration and quantitative determination of organic remains by weighting. Content of crude fibre is figured as percentage of mass concentration or as grams per 1 kilo of dry matter.

Caloricity was determined by calculation of sum of caloricity of each component (carbohydrates, lipids, proteins) of biomass per 100 gram of sample.

Results

Nutrient composition of the two model species

Analysis of daily requirements of nutrients (Tutelyan et al. 2009, 2021) for the biomass of the two model species, *L. flavus* and *C. aurata viridiventris*, are presented in Table 1.

As follows from the Table 1, the biomass of model species contains fiber represented with chitin, amounted 8.4–8.5 g/100 g, presenting a half of daily rate of human nutrition. Fiber enhances intestinal peristalsis and provides microflora with necessary substrate.

Invertebrates contain low amount of carbohydrates, balancing general compliance of food nutrients, in contrast meat of vertebrates lacks them. Also, fats are represented by low indexes 0.14–0.44 g/100 g, whereas in meat of invertebrates their amount is 20–40 times higher.

One of the most important characteristics of food is compound of protein. In the biomass studied it contains considerably higher 20.6–20.8 g/100 g than in meat of vertebrates. This is significant part of daily rate in relation to necessary animal protein.

Having high content of proteins, invertebrates are characteristic by low caloricity, 98–99%, while meat contains 2–3 time higher level of calories. Thus, biomass of invertebrates could be considered as a source of special clinical nutrition.

Table 1. Nutrient components of biomass of *L. flavus* and *C. aurata viridiventris*

Index of content of the nutrient detected	Sample No.0547 *Limacus flavus*	Sample No.0549 *Cetonia aurata viridiventris*	Method applied (GOST number)	Human daily requirement
Vitamins				
B1 (thiamine), mg/100 g	–	2.5 ± 0.2	EN 14122	0.3–1.5 mg/day
B2 (riboflavin), mg/100 g	0.12 ± 0.01	5.5 ± 0.3	EN 14152	1.8 mg/day
B3 (niacinamide), mg/100 g	1.6 ± 0.1	–	31483	20 mg/day
B6 (pyridoxine), mg/100 g	–	–	EN 14164	2.0 mg/day
B9 (folic acid), mg/100 g	–	–	31483	400 μg/100 g
B12 (cyanocobalamin), mg/100 g	–	–	ISO 20634	0.3–3.0 μg/100 g
E (α-tocopherol), mg/100 g	5.2 ± 0.3	0.59 ± 0.03	EN 12822	15 μg/100 g
A (retinol palmitate), μg/100 g	22.5 ± 1.1	33.7 ± 1.7	P 54635	400–1000 μg/100 g
Minerals				
Fe, iron, mg/100 g	3.3 ± 0.3	6.1 ± 0.6	4011	4–18 mg/day
Se, selenium, μg/100 g	23.50 ± 2.35	33.4 ± 3.34	ICP MS	10–70 μg/100 g
Zn, zinc, mg/100 g	0.80 ± 0.08	1.9 ± 0.19	ICP MS	3–12 mg/day
Mn, manganese, μg/100 g	7.60 ± 0.76	11.2 ± 1.12	ICP MS	2 mg/day
Cu, copper, mg/100 g	0.50 ± 0.05	1.6 ± 0.16	ICP MS	0.5–1.0 mg/day
Mg, magnesium, mg/100 g	160 ± 16	288 ± 28.8	ICP MS	55–400 mg/day
P, phosphorus, mg/100 g	320 ± 30	450 ± 45	32009	300–1200 mg/day

Index of content of the nutrient detected	Sample No.0547 *Limacus flavus*	Sample No.0549 *Cetonia aurata viridiventris*	Method applied (GOST number)	Human daily requirement
Fiber mass fraction, %	8.4 ± 0.5	8.5 ± 0.5	31675	20 g/day
Macronutrients mass fraction, %				
Fat	0.43 ± 0.05	0.44 ± 0.04	3042	70 to 154 g/day
Protein	20.6 ± 2.0	20.8 ± 2.0	25011	65–117 g/day with 60% animal protein
Carbohydrate	0.22 ± 0.02	0.27 ± 0.02	P 53747	170–420 g/day
Caloricity, kcal	98	99	96	individually

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, 2021).

Magnesium (160 and 288 mg/100 g) and phosphorus (320 b 450 mg/100 g) are leading amongst all minerals revealed in biomass of model species. This is by a factor of ten higher, than in meat of agricultural animals. Both elements are important for energy metabolism and define its generation in organism. High level of magnesium and phosphorus lends biomass increasing greater importance as a source of power efficient nutrition.

Also, iron (3.3 and 6.1 mg/100 g) and copper (0.5 and 1.6 mg/100 g) content is 2.5–3 times higher, these elements plays significant role in blood formation and endocrine control of people.

Level of other minerals studied is the same as in meat as, for exemplar, selenium (23.5 and 33.4 µg/100 g), or twice lower such as zinc (0.8 and 1.9 µg/100 g) and manganese (7.6 and 11.2 µg/100 g). Other minerals have not been studied.

Amongst vitamins applied for analysis, four B-group vitamins have not been revealed B3, B6, B9 and B12. Vitamins B1 (2.5 mg/100 g in beetle larvae) and B2 (0.12 and 5.5 mg/100 g) are detected significantly higher, than in meat of domestic vertebrates.

High level of liposoluble vitamins A (0.0225 and 0.0337 mg/100 g) and E (5.2 and 0.59 mg /100 g) are reveled, and it being known that meat of agricultural animals lacks vitamin A, and containing of vitamin E lower by a factor of 10. Other vitamins have not been declared for study.

Discussion and comparative analysis

In the western part of Europe these three species the mealworm *Tenebrio molitor*, the migratory locust *Locusta migratoria* and the house cricket *Acheta domesticus* and lesser mealworm beetle *Alphitobius diaperinus* larvae, are currently approved as a Novel food by the European Commission. In the case of edible insects and insect-containing food it must be considered regarding compliance with the provisions of Novel Food Regulation (EU) 2015/2283 (Regulation (EU) 2015; EU Commission 2023).

For comparing studies in the insect research were selected studies regarding the fresh weight of insects biomass. The basic compositions of fresh *L. flavus* and *C. aurata viridiventris* are presented in Table 2. To estimate the appreciation of the potential edible insects for human consumption we inserted the nutrient value of *L. migratoria* (Turck et al. 2021) and *T. molitor* (Siemianowska et al. 2013). The nutrition analysis of the yellow slugs and beetle larvae showed significantly higher contents of protein compared to mealworm and migratory locust. The *L. flavus* and *C. aurata viridiventris* contain 20.6 g and 20.8 g crude protein per 100 g based on fresh matter respectively. As shown in Table 2 and the bar chart (Figure 2) the protein content of *L. migratoria* (14.3%) and *T. molitor* (17.9%) is slightly lower than have been determined by slugs and beetle larvae. Using the nitrogen-to-protein conversion ratio of 6.25 (using the Kejldahl method) may have resulted in an overestimation of the crude protein content of the investigated species, due primarily to the presence of chitin. Therefore, additional research focusing on the examination of this species as a protein source would be of considerable interest.

The fat percentage is 0.43% and 0.44% for *L. flavus* and *C. aurata viridiventris* respectively. An extremely high value of 21.93 g per 100 g of fresh matter was found for the fat content of *T. molitor* (Siemianowska et al. 2013), which is significantly higher compared to the fat content of locusta, slugs, and beetle larvae (Rumpold, Schlüter 2013 a, b).

The difference in carbohydrate content between investigated samples and locusts is minor at about 0.2 g per 100 g. Larvae *T. molitor* contains, on average, a higher carbohydrate content of 7.09 g per 100 g of fresh matter.

Table 2. Nutritional content in g per 100 g (based on fresh matter) *L. flavus* and *C. aurata viridiventris* and edible insects *L. migratoria* and *T. molitor*

	Crude protein	**Fat**	**Carbohydrates**	**Fibre**	**Energie (kcal)**
Limacus flavus	20.6	0.43	0.22	8.4	98
Cetonia aurata	20.8	0.44	0.27	8.5	99
Locusta migratoria (Zielińska et al. 2015)	14.3	10.3	0.2	2.5	158–173
Tenebrio molitor (Van Raamsdonk et al. 2017, Van Peer et al. 2021)	17.9	21.93	7.09	10	214 ± 39

Considering species contain a significant amount of both micro- and macronutrients, *L. flavus* and *C. aurata viridiventris* have potential use in the food and feed industries.

Both species yellow slugs and beetle larvae are good sources of magnesium and phosphorus (Table 3), which are essential to human health. The yellow slug has a phosphorous concentration that ranges around 320 mg, which is comparable to that of larvaes *T. molitor*. The magnesium content is 160 mg per 100 g for *L. flavus* and 288 mg per 100 g for C. aurata viridiventris. The phosphorus content is 320 mg per 100 g for *L. flavus* and 450 mg per 100 g for *C. aurata viridiventris*. The Panel on Dietetic Products, Nutrition, and Allergies (NDA) of the European Food Safety Authority (EFSA) established magnesium of 350 mg/day for males and 300 mg/day for women. Age-dependent varies from 170 to 300 mg/day for children (EFSA NDA Panel 2015b). The Panel determined phosphorus for adults at 550 mg per day. The range for children is between 250 and 640 mg per day (EFSA NDA Panel 2015a). Therefore, further studies focused on the investigation of the digestion of this species as a source of micronutrients would be of great interest.

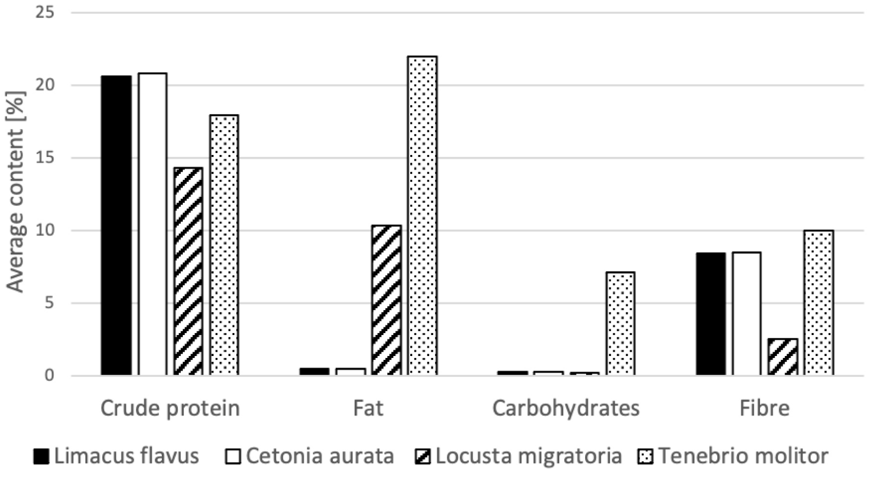

Figure 2. Average nutrient contents (%) (based on fresh matter) of *L. flavus* and *C. aurata viridiventris* and edible insects *L. migratoria* and *T. molitor*.

Table 3. Mineral and vitamin content in mg per 100 g (based on fresh matter) *L. flavus* and *C. aurata viridiventris* and edible insects *L. migratoria* and *T. molitor*

	Limacus flavus	***Cetonia aurata viridiventris***	***Locusta migratoria** (Zielińska et al. 2015)**	***Tenebrio molitor* (Van Raamsdonk et al. 2017, Van Peer et al. 2021)**
Minerals				
Magnesium	160 ± 16	288.0 ± 28.8	15.45	87.5 ± 5.34
Phosphorus	320 ± 30	450 ± 45	127.11	319.4 ± 16.53

	Limacus flavus	*Cetonia aurata viridiventris*	*Locusta migratoria** (Zielińska et al. 2015)	*Tenebrio molitor* (Van Raamsdonk et al. 2017, Van Peer et al. 2021)
Iron	3.3 ± 0.3	6.1 ± 0.6	1.25	3.79 ± 1.362
Zinc	0.8 ± 0.08	1.90 ± 0.19	4.96	4.2 ± 0.21
Copper	0.5 ± 0.05	1.6 ± 0.16	1.05	0.78 ± 0.039
Manganese	0.0076 ± 0.00076	0.0112 ± 0.00112	0.10	0.44 ± 0.019
Vitamins				
B1 Thiamin	–	2.5 ± 0.2	0.02	0.18
B2 Riboflavin	0.12 ± 0.01	5.5 ± 0.3	0.38	1.21
B3 Niacin	1.6 ± 0.1	–	1.97	4.1
B6 Pyridoxin	–	–	0.05	0.7
B9 Folic acid	–	–	–	–
B12 Cobalamin	–	–	0.26	0.3
Vit E (α-Tocopherol)	5.2 ± 0.3	0.59 ± 0.03	0.88	1.9
Vit A (Retinol)	0.0225 ± 0.0011	0.0337 ± 0.0017	0.02	0.0087

Note: * Due to the lack of information for fresh matter, the data for *L. migratoria* were calculated according to the equation in (Nowak et al. 2016).

In addition, our research indicates that slugs and beetle larvae have the ability to provide certain micronutrients, such as iron and copper. The beetle larvae have a high iron content, which comes out to a total of 6.1 mg. The iron content of *L. migratoria* (1.25 mg) and *T. molitor* (3.79 mg) are noticeably lower compared to this specie. This is an essential element for human nourishment and for adult is at a high of 6–11 mg per day (EFSA NDA Panel 2015d). According to EFSA the Acceptable Daily Intake of copper is 1.57 mg per day for men and 1.20–2.07 mg per day for women (EFSA NDA Panel 2015c). The copper content of *C. aurata viridiventri*s is around 1.6 mg, which is sufficient to satisfy daily requirements and is higher than that of both species approved in Europe.

The zinc and manganese content of edible insects *L. migratoria* and *T. molitor* is significantly higher than that of the investigated samples (see Table 3).

The investigated species are good sources of vitamins. The beetle larvae have a high content of thiamin (vitamin B1) and riboflavin (vitamin B2) and showed ranges of 2.5 mg and 5.5 mg per 100 g based on fresh matter respectively. This amount covers the daily requirement for adults (on average, for thiamine 1.0–1.3 mg/day and riboflavin 1.0–1.4 mg/day (DGE)). These values are significantly higher than those found in *L. migratoria*, which contain 0.02 mg of thiamin and 0.38 mg of riboflavin per 100 g of fresh matter. For *T. molitor* the values of thiamin and riboflavin are 0.18 mg and 1.21 mg respectively.

The biomass of slugs and beetles differs in the presence of niacin (vitamin B3). The niacin content is 1.6 ± 0.1 mg per 100 g in beetle larvae. The niacin content of *L. migratoria* and *T. molitor* is slightly higher than have been determined by beetle larvae and showed ranges of 1.97 mg and 4.1 mg per 100 g based on fresh matter respectively. According to the EFSA, the niacin intake for adults is, on average, 11–16 mg NE (1 mg Niacin Equivalent (NE) = 1 mg Niacin) per day (EFSA NDA Panel 2014, DGE).

Pyridoxin (vitamin B6), folic acid (vitamin B9), and cobalamin (vitamin B12) have not been detected in the biomass of slugs and beetles larvae. In comparison to the edible insects *L. migratoria* and *T. molitor*, which contain 0.05 mg to 0.07 mg of vitamin B6 and 0.26 mg to 0.03 mg of vitamin B12 per 100 g, respectively, slugs and beetle larvae cannot be considered a source of vitamins B6, B9 and B12.

L. flavus contains approximately 5.2 ± 0.3 mg of vitamin E (α-Tocopherol) per 100 g of biomass, which is significantly higher compared to the vitamin E content of *C. aurata viridiventris*, *L. migratoria*, and *T. molitor*. The slugs might be regarded as an additional vitamin E source for adults since the daily intake recommended by GNS (DGE) ranges from 11 to 15 mg per day for adults.

The amount of vitamin A found in any of the four species is not particularly high (see Table 3). The daily intake of vitamin A ranges from 0.7 to 1.3 mg per day for adults.

According to (Melis et al. 2019) and (Oonincx, van der Poel 2011) the nutritional composition could be regulated by the diet of species. It is advantageous to understand the metabolic characteristics that determine the growth and body composition of slugs and beetle larvae.

It should be noted that any method of biomass drying, such as by freezing, infrared convection oven, high-frequency heating, or drying in an oven or microwave, does not change the initial characteristics of the nutrients or fatty acid content that are acceptable for nutrient analysis (Keil et al. 2022, Schlussbericht zu IGF-Vorhaben 2020).

Food productivity is characterized by the main term, nutrient bioaccessibility and bioavailability. These terms are defined in several ways, but in pharmacology bioavailability means level of absorption i.e. “the fraction (%) of an administered drug that reaches the systemic circulation” (Hebert 2013). For food supplement bioavailability is defined as quantity absorbed nutrients from the consumed portion (Heaney 2001; Solomons 2003; Sandstead, Zinc 2007). In other words bioaccessibility is the fraction that was released from nutrient portion consumed, and dozen of micronutrient in this portion that is used for organism functioning is bioavailability (Fairweather-Tait, Southon 2003; Marze 2013). The main objectives of the modern agriculture is to maximize and sustaining of nutrient productivity, thus all nutrients available from invertebrates should be examined to their bioavailability. Further researches of nutrients gained from invertebrates should be studied for the purpose to reveal of their bioaccessibility and bioavailability.

Some methods of nutrient detection conditioning limitation in determine of the nutrients. Thus, Kjeldahl method applied for protein detection is based on the quantitative determination of nitrogen contained in sample. Bodies of invertebrates contain considerable level of chitin, that is also include nitrogen. Using the nitrogen-to-protein conversion ratio of 6.25 (using the Kejldahl method) may result to an overestimation of the crude protein content of the investigated species, due primarily to the presence of chitin. Therefore, additional research focusing on the examination of chitin share as nitrogen source to separate its meaning from protein content is necessary. Also, the Kjeldahl method is quite expensive and demands a lot of time to make complete procedure. At present, new methods of matter evaluation are engineered, one of the most simple and fast is the "Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy)". This method allows to measure the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms. Modern devices, infrared spectrometers, could detect different kind of nutrients, such as carbohydrates, protein, fat, vitamins, mineral etc in a moment with precise information on their content. Owing to non traumatic procedure of analysis, infrared spectrometers are used for lifetime study of alive objects. This could be probably used for monitoring of nutrient cumulating in invertebrates bodies.

Conclusion

Analysis of nutrient composition in biomass of model species that were reared in restricted space on artificially prepared food substrates, demonstrate high level of protein, some minerals and vitamins that are presented not less than in traditional animal products, but exceed them. Low caloricity and high level of magnesium and phosphorus provide high energy value to invertebrate biomass. This fact could be interesting for new food product design, aimed to supply people living or working under extreme environment condition. However, deficiency of some vitamins (i.e. B3, B6, B9 и B12) and balance of necessary nutrients could be improved by further study of invertebrate raising characteristics, modelling food substrate in term of its enrichment with necessary elements (Mielgo-Ayuso et al. 2018).

Of course, the species discussed in the present paper are not the only invertebrates that could be studied in terms of raising for food and feed production. Further selection of appropriate species is actual problem in development of new food quality generation.

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