

УДК 57.017

The Giant African Land Snail *Achatina fulica* (Bowdich, 1720) as a Candidate Species for Bioregenerative Life Support Systems

**Nicolay S. Manukovsky,
Vladimir S. Kovalev*, Alexander A. Tikhomirov,
Galina S. Kalacheva and Anzhelika A. Kolmakova**
*Institute of Biophysics SB RAS
50/50 Akademgorodok, Krasnoyarsk, 660036, Russia*

Received 12.09.2014, received in revised form 22.10.2014, accepted 26.01.2015

*The capability of snails to consume and convert inedible plant biomass and kitchen waste was tested. Inedible biomass of wheat and cabbage and also potato peels as a food were worse than lettuce, which is an ordinary feed for snails. In order to describe the growth of *Achatina fulica* its logistic function was fitted to the experimental data. It was found that calculated specific growth rate and carrying capacity, as constants of the logistic function, are 1.06 month⁻¹ and 250 g of wet weight correspondingly. Mass ratio shell/whole body in terms of wet weight was 18-21 % irrespective of snail age. Snail meat was characterized by the low content of fat – 6.0 % DM. Essential fatty acids constituted 16.6 % of the total sum. Linolenic and linoleic acids dominated in a pool of essential fatty acids. The scores of essential amino acids, except sulfuric amino acids, exceeded 100 %. To estimate nutritious properties of snail meat, a computer program was developed. It was observed that the maximum intake of snail meat can reach 497 g/crewmember day. Addition of snail meat to a basic diet enabled increasing food independence of bioregenerative life support system to 97 %.*

Keywords: snail, feed, growth, chemical composition, diet, bioregenerative life support system (BLSS).

© Siberian Federal University. All rights reserved

* Corresponding author E-mail address: kovalev49@mail.ru

Гигантская африканская наземная улитка *Achatina fulica* (Bowdich, 1720) как вид-кандидат для биорегенеративной системы жизнеобеспечения

**Н.С. Мануковский, В.С. Ковалёв,
А.А. Тихомиров, Г.С. Калачева, А.А. Колмакова**
Институт биофизики СО РАН
Россия, 660036, Красноярск, Академгородок, 50/50

*Изучена способность улиток потреблять и перерабатывать несъедобную биомассу растений и пищевые отходы. Несъедобная биомасса пшеницы и капусты, а также картофельные очистки оказались менее пригодными, чем салат, которым обычно кормят улиток. Для описания роста *Achatina fulica* проведено приближение логистической функции к экспериментальным данным. Установлено, что удельная скорость роста и максимальная масса особи как константы логистической функции составляют 1.06 месяц⁻¹ и 250 г влажного веса соответственно. Отношение веса раковины к общему весу улитки составляло 18-21 % независимо от возраста. Мясо улитки характеризовалось низким содержанием жира – 6.0 % в пересчёте на сухой вес. Содержание незаменимых жирных кислот составило 16.6 % от их общего количества. В пуле незаменимых жирных кислот доминируют линоленовая и линолевая кислоты. Скоры незаменимых аминокислот, за исключением серосодержащих аминокислот, превышали 100 %. Чтобы оценить питательные свойства мяса улитки, была разработана компьютерная программа. Рассчитано, что максимальное потребление мяса улитки может достигнуть 497 г на одного члена экипажа в сутки. Добавление мяса улитки к основной диете позволяет увеличить продовольственную независимость биорегенеративной системы жизнеобеспечения до 97 %.*

Ключевые слова: улитка, корм, рост, химический состав, диета, биорегенеративная система жизнеобеспечения (БЖО).

Introduction

It is known, that two-thirds of protein in a space diet should have an animal origin (Cooper et al., 2011). At least a part of animal food could be produced in Bioregenerative Life Support System (BLSS). In that case, one should reduce the production costs. Small forms of animals such as silkworms (Yu et al., 2008; Yang et al., 2009), yellow worms (Li et al., 2013), and snails were considered as candidate species to solve the problem. A space diet based on the use of rice,

soybean, lettuce, strawberry, and land snails *Helix pomatia* L. was developed (Midorikawa et al., 1993). The calculated amount of snail meat was equal to 110 g/crewmember day according to the Japanese dietary requirements of that time. Snails also took part in waste processing. According to the scheme of the BLSS material balance, the production of plant wastes was calculated as 2583 g/crewmember day and 690 g of this amount was earmarked for the snail feed. Undoubtedly, the search for new animal-

candidates for BLSS is an important task. There are some cultivated land snails besides *Helix pomatia*. One of them is the giant snail *Achatina fulica* (Bowdich, 1720) (Cobbinah et al., 2008). As compared with *Helix pomatia*, the giant snail has a greater growth rate. For example, the snails *Achatina fulica* that are 5 month old had a whole body weight of about 33 g (Upatham et al., 1988), whereas the snails *Helix pomatia* which were 1 year-old weighed only 23-26 g (Toader-Williams, Bentea, 2010). Hence, the introducing of *Achatina fulica* into BLSS could help solve a problem related to animal protein.

The aim of this work was to forecast the practicability of *Achatina fulica* in BLSS. In this connection, the growth, chemical composition and nutritional properties of *Achatina fulica* were studied. The capability of snails to use plant and kitchen wastes as a food was estimated.

Materials and methods

Conditions of snail growing

The object of the research was the giant African land snails *Achatina fulica* Bowdich (Fig. 1). Taxonomic Position: Animalia: Mollusca: Gastropoda: Stylommatophora: Achatinidae. Like many snails, they are hermaphrodites. This means that instead of having male and female snails,

each snail has both male and female reproductive organs. Young snails produce sperm only, but as they grow larger, they will produce both sperm and eggs. The fertilized eggs hatch, and immature snails grow to adulthood in about six months.

The adult snails in an amount of 25 individuals were cultivated in a rectangular container (L×W×H = 140×50×60 cm) and fed *ad libitum*. At the bottom of the container a soil like substrate (SLS) was placed. It looked similar to natural black soil. Unlike natural soils, SLS did not contain aluminosilicate matrix. SLS included (% of dry mass): organic matter 64-68, ash 32–36, humic acids 9.1-9.6, fulvic acids 4.7-5.1, nitrogen 1.63-1.74, phosphorus 0.88-0.95, potassium 1.80-2.18, calcium 1.70-1.78. SLS was produced by processing plant wastes by oyster mushrooms and worms *Eisenia foetida* (Savigny, 1826) (Manukovsky et al., 1997). The thickness of the SLS layer was equal to 15 cm; bulk density – 785 g/l; moisture – 72 %, moisture capacity – 88 %, pH of water extract – 6.8.

The juvenile snails in an amount of 10 individuals lived separately from adult individuals in the rectangular container (L×W×H = 20×16×10 cm). An air temperature in the breeding containers was equal to 25 °C, and the relative air humidity was 80 %.



Fig. 1. The young and adult snails of *Achatina fulica*

Feed testing

In an experiment studying the consumption of various feeds by one year old snails, we used leaves of lettuce (*Lactuca sativa* L.), straw of wheat (*Triticum aestivum* L.), roots of cabbage (*Brassica oleracea* L.), and peels of potato (*Solanum tuberosum* L.). Each feed was tested separately in three sets of one-day (24 hours) trials. In each trial eight snails were used. All trials were performed in the test container (L×W×H = 37×32×18 cm). Table 1 shows the chemical composition of these feeds.

Mass of consumed feed was calculated by a difference between masses of the submitted and residual feed. The rate of feed degradation was calculated by the following formula:

$$RFD=100 (Feed_c - Feces)/Feed_c, \quad (1)$$

where $Feed_c$ – consumed feed in terms of DM; $Feces$ – produced feces in terms of DM.

Studying of snail growth

As a feed, leaves of lettuce (*Lactuca sativa* L.), taproots of carrot (*Daucus carota* L.), leaves of the Peking cabbage (*Brassica sinensis* L.), fruits of tomato (*Solanum lycopersicum* L.) and fruits of vegetable marrow (*Cucurbita pepo* L.) were

used. Table 2 shows the chemical composition of these feeds.

The clay and sand were added to the feed to improve the digestion. Calcium was added in the form of chalk. There was a shallow cup with potable water in the growth chamber.

Whole body mass of 28 snails was measured during the process of their growth from oviposition to one year by weighing them at least once a week. To describe a snail's growth, a logistic function was used:

$$M_b=M_0M_a/(M_0+(M_a-M_0)e^{-kt}), \quad (2)$$

where M_b – whole body mass at the time t ; M_0 – initial whole body mass at the time 0; M_a – upper boundary of whole body mass (carrying capacity); and k – specific growth rate.

The equation (2) was fitted to the data obtained in the measurements of whole body mass by using the least-squares method in Excel 2007.

Chemical assays

After studying of snail growth (see above) the same 28 individuals were used for chemical analyses. The objects of chemical analysis were pedal mass (snail meat), shell, feces and eggs. Ash content of samples was measured by ashing

Table 1. Chemical composition of lettuce and plant wastes

Components	Content in biomass, %			
	Lettuce	Wheat straw	Cabbage roots	Potato peels
Water	95.21	11.49	73.50	81.74
Ash	0.68	5.62	2.66	1.03
Cellulose	1.64	40.35	6.27	1.21
Total lipids	0.27	1.65	0.05	0.14
Total nitrogen	0.24	0.62	0.48	0.27
Total phosphorus	0.04	0.06	0.13	0.05
Potassium	0.22	1.10	0.80	0.46
Calcium	0.04	0.15	0.13	0.03

Table 2. Chemical composition of the vegetable biomass used as feed

Components	Content in plant biomass, %			
	Carrot roots	Cabbage leaves	Tomatoes	Vegetable marrow
Water	89.11	93.57	95.21	93.05
Ash	1.14	1.07	0.64	0.62
Cellulose	1.20	1.29	0.80	1.35
Total lipids	0.19	0.18	0.22	0.13
Total nitrogen	0.20	0.21	0.15	0.11
Total phosphorus	0.05	0.03	0.04	0.03
Potassium	0.28	0.15	0.24	0.29
Calcium	0.04	0.05	0.01	0.02

a sample in a muffle furnace at 600 °C. Moisture was measured by the conventional oven-dry method, in which the samples were dried in an oven at 105 °C for 24 h and then quickly weighed. The nitrogen was determined by Kjeldahl method (Volynets, 1977). To calculate a content of crude protein we multiplied the nitrogen content of corresponding sample by a conversion coefficient of 6.25.

The content of lipids was determined gravimetrically. Extraction of lipids was done by the method of Folch (Folch et al., 1957). Briefly, lipids from samples were extracted thrice with chloroform:methanol (2:1, v/v) simultaneously with mechanical homogenization of the tissues with glass beads. Methyl esters of fatty acids were produced after acid methanolysis of lipids (Makhutova et al., 2013). Methanolysis of lipids was carried out in the mixtures of methanol and sulfuric acid (50:1 v/v) at a temperature of 90 °C within two hours on a water bath, using the backflow condenser. Methyl esters of fatty acids were analyzed on a chromatograph with the mass-spectrometer detector 6890N/5975 (Agilent, USA). Conditions of analysis were as follows: gas carrier – helium, speed – 1.2 ml/min, capillary column HP-FFAP, of length 30 m, of diameter – 0.32 mm, temperature of inlet – 230 °C; starting temperature – 120 °C,

temperature increase up to 190 °C with a speed 3 °C/min, an isothermal mode for 5 min, next temperature increase to 220 °C with 10 °C/min, finally, the isothermal for 20 min, electronic ionization with 70 eV, and scanning mode from 45 to 580 *m/z* at 0.5 sec/scan. Identification of fatty acids was carried out on their mass spectra, and a comparison with those of standards. As standards, we used the saturated, branched, and monounsaturated fatty acids with the length of a chain from 10 to 24 carbon atoms, and also linoleic, α -linolenic, γ -linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids (“Serva”, Germany and “Sigma”, USA). Calculation of the relative content of fatty acids was carried out by a method of internal normalization. The location of double bonds in the unsaturated fatty acids was determined on the spectra of dimethyl oxazoline derivatives (DMOX) of fatty acids. DMOX were prepared as follows (Spitzer, 1997): 0.2 ml of 2-Amino-2-methyl-1-propanol was added to the saponified lipids. Helium was passed through the mixture. The flask was densely closed and heated to approximately 190 °C within 2 hours. To the reactionary mixture, 2 ml of the distilled water were added and the solution was acidified. DMOX were extracted with a hexane-acetone mixture (96:4 v/v).

Identification of amino acids was performed using A0326V2 analyzer (Knauer, Germany). Macro and microelements were determined by the methods described by Kalacheva et al., 2002.

Modeling use of snail meat in BLSS diet

Each food product P_j is presented as a column vector:

$$P_j = \begin{bmatrix} m_{1j} \\ \vdots \\ m_{ij} \\ \vdots \\ m_{uj} \\ mp_j \end{bmatrix}, \quad (3)$$

where m_{ij} – mass of nutrient i in 100 g of product P_j ; u – the number of monitored nutrients; i – sequence number of nutrient; $1 \leq i \leq u$; j – sequence number of product; mp_j – intake of product j , g/crewmember day;

A set of v column vectors P_j (3) corresponds to the “nutrient-product” matrix R with size $(u+1) \times v$. The first column vector P_i of matrix R presents the nutritional characteristics of snail meat. Daily intake of nutrient i is calculated by the formula:

$$mn_i = \sum_1^v \frac{m_{ij} mp_j}{100}, \quad (4)$$

Caloric value of food (kcal) is calculated by the formula:

$$E = 4mn_2 + 9mn_3 + 4mn_4, \quad (5)$$

where mn_2 , mn_3 , mn_4 – protein, fat, and carbohydrate intake correspondingly, g/crewmember day.

The model is intended to estimate the benefit of using the snail meat as a food in BLSS. To do that, a basic diet and a similar one with snail meat are optimized. A basic diet is composed by a user or adopted from any published work. The

efficacy of optimization is estimated according to the following indices:

- Intake of total food mass (IFM_t), g/crewmember day:

$$IFM_t = \sum_1^v mp_j, \quad (6)$$

IFM_t comprises food intake from stores (IFM_s) and food intake from BLSS agriculture and snail rearing facility (IFM_{in}).

- Food independence:

$$FI = 100IFM_{in}/IFM_t, \quad (7)$$

- Daily intake of snail meat (mp_1), g/crewmember day.
- Sum of nutrient imbalances (SNI) – number of nutrients in which intakes do not correspond to the daily requirements.

In addition, amino acid scores of food protein were estimated according to the FAO/WHO standard (FAO/WHO, 1990). The amino acid score was calculated using the ratio of a content of the individual amino acid in the snail meat (mg/g of protein) to the content of same amino acid in a reference pattern (mg/g of protein) multiplied by 100. The scoring patterns suggested by the FAO/WHO were used for this purpose (FAO/WHO, 1990).

These indices, besides SNI , were also used as objective functions of diet optimization

$$IFM_{min} = \min(IFM)_t, \quad (8)$$

$$FI_{max} = \max(FI), \quad (9)$$

$$mp_{1max} = \max(mp_1), \quad (10)$$

Diet optimization is carried out by using a Solver Add-in for Excel 2007 with the following parameters:

- independent variables – masses of food products (mp_j), g/day
- constraints – lower and upper bounds on the independent variables; daily nutrient intakes recommended by NASA (Cooper et al., 2011).

The most important index is the daily edible mass of snail (mp_i). The use of different objective functions (8-10) is intended to study their influence on the daily intake of snail meat (mp_i) and other indices mentioned earlier.

Solver options used in the study: MaxTime: = 300, Iterations: = 1000, Precision: = 0.000001, Tolerance: = 5 %, Convergence: = 0.0001, AssumeLinear: = False, AssumeNonNeg: = True, Estimates: = Tangent, Derivatives: = Forward, Search: = Newton.

To validate the model, we used a space diet intended for a lunar base (Liu et al., 2008). The number of food products in that diet is equal to 21. Data on chemical composition of “lunar base” products were imported from the open access databases (USDA, .SELFNutritionData, Danish Food Composition Databank).

It was suggested that a part of these products could be produced in BLSS: wheat grains, rice grains, pepper, carrot, coriander, tomato, cabbage, salad, radish, oyster mushroom, soy (sprouts out), pumpkin, pumpkin seeds saute, onion, garlic, and peanut oil. The remaining products: sardine, scad, seafood sauce, beef sauce savory, and sugar could be obtained from stories. In our study the number of food products was increased up to 22 because of the addition of snail meat to the basic

space diet. The number of monitored nutrients in the study was equal to 40. Therefore, “nutrient-product” matrix R had the size 41×22.

Results

Data on feeding

The lettuce leaves (0.23 g DM/snail day) were the most used feed (Table 3). This feed also had the greatest extent of degradation – 67 %. Worst of all, the snails consumed the cabbage roots – 0.07 g DM/snail day. However, cabbage roots *RFD* (60.4 %) was more than the *RFD* of straw (26.0 %).

Characteristics of snail growth

The upper boundary of whole body mass M_a was calculated as equal to 250 g and growth rate was equal to 1.06 month⁻¹ (Formula 2). At the age of 11 months, a snail was in a stage of active growth. The calculated whole body mass was 174 g (Fig. 2). Mass ratio shell/whole body mass was within 18-21 % (Fig. 2).

Chemical composition of snail meat

Water occupied 80.8 % of total meat mass. Protein dominated in snail meat in terms of dry matter (78 %). The content of fats was found to be 6.0 %, ash – 7 % and carbohydrates – 9 % (calculated by difference) (Fig. 3).

The score of sulfur amino acid was found to be 47 (Fig. 4). Other essential amino acids had a score above 100.

An example of a chromatogram of fatty acids composition in snail meat is given in Fig. 5.

Table 3. Results of feed testing

Indices	Sources of feed			
	Lettuce	Wheat straw	Cabbage roots	Potato peels
Mass of consumed feed, g DM/snail day	0.23	0.17	0.07	0.16
Rate of feed degradation (<i>RFD</i>), %	67.0	26.0	60.4	50.3

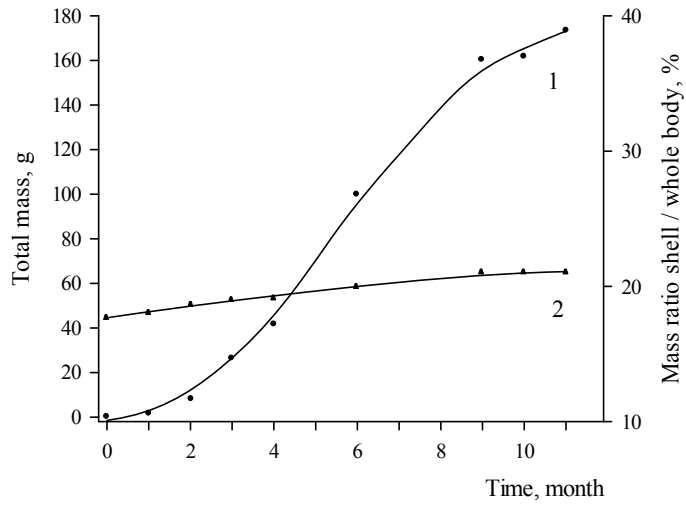


Fig. 2. Characteristics of snail growth: 1- total mass, 2 – mass ratio shell/whole body

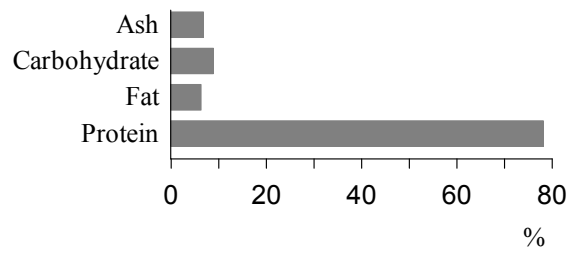


Fig. 3. Contents (% of dry mass) of ash, carbohydrates, fat and protein in snail meat

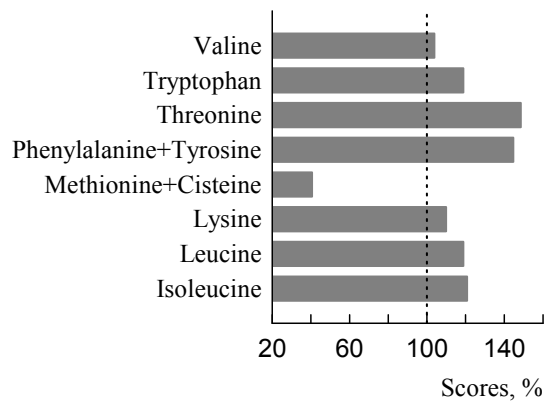


Fig. 4. Scores of essential amino acids of snail meat

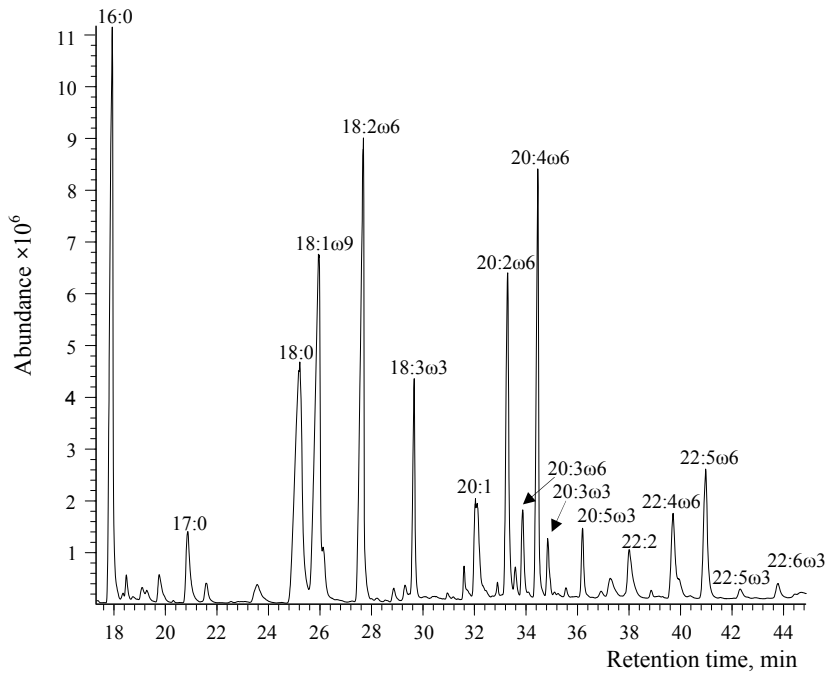


Fig. 5. Chromatogram of the fatty acid methyl esters of lipids of *Achatina fulica*

There are both saturated and unsaturated fatty acids. The mass of essential fatty acids amounted to 16.6 % of the total sum. Among $\omega 3$ fatty acids, 18:3 $\omega 3$ (linolenic) acid prevailed (3.8 %), whereas 18:2 $\omega 6$ (linoleic) acid had the highest content among $\omega 6$ fatty acids (12.6 %) (Fig. 6). The $\omega 6:\omega 3$ polyunsaturated fatty acid ratio was found to be 4.6. According to the recommendation of NASA, that ratio should be within 8.8-12.7 (Cooper et al., 2011).

The greatest content of nitrogen was found in snail meat – 11350 mg/100 g DM (Table 4).

As a part of a shell, calcium dominated – 38843 mg/100 g DM. It is known, that calcium in a shell is mainly a component of calcium carbonate (Saleuddin, Wilbur, 1969). Therefore, taking into account the content of calcium, it is possible to calculate closely the content of calcium carbonate in a shell. It was found to be 97 % of the total shell mass. The other part of a shell fell on the nitrogen-bearing substances and mineral elements. An unexpected result was the

phenomenon of the high iron content in feces – 571 mg/100 g DM and the low content in meat – 7 mg/100 g DM. This testifies as though a snail got rid of excess of iron. A superiority of calcium over nitrogen in eggs could be explained as follows. Calcium is a component of a shell, which almost completely consists of calcium carbonate. Moreover, nitrogen-bearing substances of eggs are distributed in a liquid. It also reduces the content of nitrogen in terms of dry weight.

Efficacy of using snail meat in diet: optimization of basic diet

When minimization of total food intake (IFM_t) was used as an objective function, the indices of diet optimization were as follows: $IFM_t = 3450$ g/day, $FI = 76$ %, $SNI = 4$ (Table 5).

Use of FI_{max} as an objective function yields the following results: $IFM_t = 3512$ g/day, $FI = 94$ %, $SNI = 4$. The increase of FI from 76 % to 94 % occurred due to the decrease of food

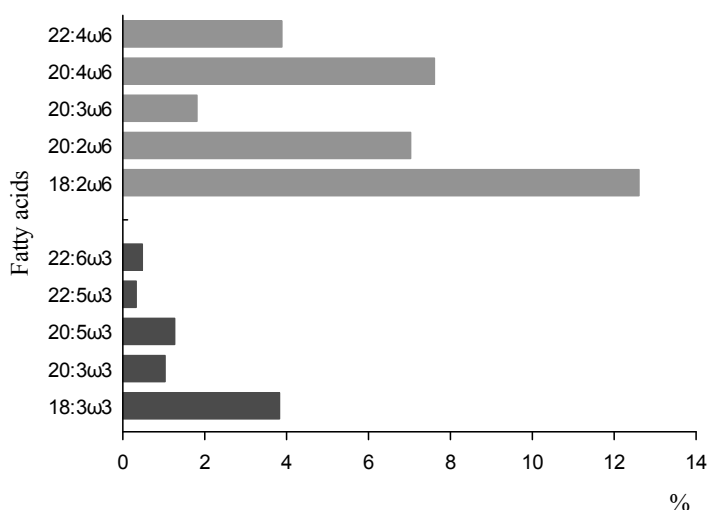


Fig. 6. Percentages of ω3 and ω6 fatty acids of the total FA.

Table 4. Nitrogen and mineral composition of snail meat, shell, feces and eggs

Components	Content of elements, mg/100 g DM								
	N	Ca	Fe	Mg	P	K	Na	Zn	Cu
Meat	11350	52	7	1302	1417	1990	365	5	2
Shell	363	38843	6	13	3	63	158	1	0.4
Feces	4050	3750	571	975	1210	1800	563	38	210
Eggs	4100	23625	2	90	210	228	212	2	2

Table 5. Results of diet optimization

Index	Basic diet		Basic diet + snail meat		
	IFM _{min}	FI _{max}	IFM _{min}	FI _{max}	mp _I max
IFM _t (Intake of total food mass, g/day)	3450	3512	3461	3800	3548
mp _t (Intake of snail meat, g/day)	0	0	0	118	497
FI (Food independence, %)	76	94	74	97	82
SNI (Sum of nutrient imbalances)	4	4	4	4	4

The designations in the table are identified in the "Materials and methods" section

consumption from stories (IFM_s). Accordingly, intake of total food mass (IFM_t) increased at the expense of vegetable food produced in BLSS, because vegetables are less caloric as compared with meat and fish products obtained from stories. The imbalances resulted from the excess of iron and low ω6:ω3 polyunsaturated

fatty acid ratio as well as from a deficiency of vitamins D and K.

Efficacy of using snail meat in diet: inclusion of snail meat in basic diet

The snail meat was not included in diet if minimization of total food intake (IFM_{min}) was

set as an objective function. The other indices were close to ones obtained under optimization of basic diet. On the contrary, when maximization of food independence (FI_{max}) was assigned to be an objective function, the intake of snail meat reached the value of 118 g/day (Table 5). At that value, intake of total food mass (IFM_t) and food independence (FI) increased from 3461 to 3800 g/day and from 74 % to 97 % correspondingly. The intake of snail meat (mp_1) reached the maximal value of 497 g/day when that index was used as an objective function (mp_{1max}). Inclusion of snail meat in diet did not influence the sum (SN) and nature of imbalances.

Discussion

Definition of the feed base of snails is a necessary condition for their inclusion in the BLSS. Natural food base consists of plant species growing in the locality of snail habitat. For example, the snails *Archachatina marginata* preferred *Clerodendrum paniculatum* and *Laportea aestuans* plants which offered the best potassium, sodium, phosphorus, protein, lipid and cellulose contents (Agongnikpo et al., 2010). The diets for snails *Archachatina ventricosa* based on four plant species with additives of Ca mineral were also tested (Otchoumou et al., 2004). Two plant species – lettuce and cabbage – from that work have been already entered to the inventory of candidates for BLSS (Advanced Life Support..., 2004). Of course, the new plant species could be included in BLSS, especially to extend a feed base for animals. It was proposed in case of the use of the silkworm, which consumes the leaves of the mulberry tree (Yang et al., 2009). However, it will cause an extra expense, because a sawn area in BLSS should be increased. In view of saving BLSS resources, inedible plant mass and kitchen wastes could be the best feed base for snails. It is stated that the quality of inedible plant parts available and the quantity of necessary feed for

the snails are well matched (Midorikawa et al., 1993). In that regard, our results seem to stand in contrast to that statement. Inedible plant mass and potato peels turned out to be worse than the edible part of the lettuce (Table 3).

The selection of bedding plays an important role in snail breeding. It was established that the best bedding of four tested for the snails *Archachatina ventricosa* was a ground collected under a cassava plantation with addition of sawdust (Kouassi et al., 2007). As compared with the other beddings that substrate had the greatest organic matter content – 10.81 % DM. In our study, SLS was used as a bedding. Organic matter content in the SLS was much higher – 64-68 %.

It was possible to keep worms in SLS after its preparation. In that case, SLS did not have an unpleasant smell, because the worms consumed the snail feces.

Nowadays, there is no approved algorithm to compare snail-candidates to BLSS. One of the approaches to the development of this algorithm is to formalize snail growth. In this study we have used the logistic function to describe the individual growth of the land snail *Achatina fulica*. A similar description was performed in the case of land snail *Helix aspersa* (Czarneleski et al., 2008).

One can see the value of Ca content of feces in Table 4 – 3750 mg/100 g DM. If a snail eats only calcium carbonate, Ca content can be a lot more. On the other hand, the question arises: what is the lower boundary of Ca content? The answer is of practical importance in view of the savings of calcium carbonate as a feed. In a diet of *Archachatina ventricosa* the optimum calcium content of the feed was found to be 16.01 % (Otchoumou et al., 2004). The lower Ca content is justified because there is a problem of Ca looping in BLSS if snails live in it. To develop Ca looping, the shell and worm casts should be tested as the Ca sources for snails.

The obvious shortcoming of *Achatina fulica* was the deficiency of essential sulfur-containing amino acids in the snail meat. We have determined the score of sulfur-containing amino acids to be 40 (Fig. 3). Snail meat of *Helix pomatia* had a more favorable score – 88 (Midorikawa et al., 1993). However, this value is less than 100. A very high score of 207 was determined for the snail protein of *Helix aspersa* (Cagiltay et al., 2011). A look at the data raises the question: what factor has an impact on the score? It could be taxonomic position and conditions of snail growing. Undoubtedly, this is an issue of further investigations. In our study, the addition of snail meat did not affect the score, because it was surely established in the basic diet. The meat of *Achatina fulica* can be characterized as a low-fat one, because protein and fat content was 78 % and 6 %, respectively. A lower protein and fat content in snail meat was determined earlier (Otchoumou et al., 2010).

The shell of *Achatina fulica* in our study accounted for 18-21 % of the live snail weight (Fig. 2). In the previous study the shell/whole body mass ratio was established as 32.61 and 47.20 % for the wild and cultivated snails, respectively (Otchoumou et al., 2010). This discrepancy of published and present data can be attributed to the differences in snail feeding.

The previous work (Midorikawa et al., 1993) and this work have an identical approach: snail meat was added to a diet and the effect of this operation was estimated. Adding snail meat of *Helix pomatia* in a previous work has eliminated the deficiency of calcium, sodium, vitamin A and vitamin B12 of a basic vegetarian diet. No improvement in the basic diet was obtained if the meat of *Achatina fulica* was used. The effect from the implication of *Helix pomatia* could be explained by the poverty of a basic diet. Foods were produced from only four plant species. On the contrary, in this work, the fully-

variable basic diet for a lunar base was used. Therefore, the implication of *Achatina fulica* enabled us to improve only one diet index – the food independence.

The number of imbalances in the basic diet is 4 and the addition of snail meat has no influence on the index (Table 5). Deficiencies of vitamins D and K are most easy to remove by adding vitamin pills to the diet. The $\omega 6:\omega 3$ polyunsaturated fatty acid ratio in the meat of *Achatina fulica* does not fit into the norm of NASA. However, the contents of polyunsaturated acids in snail meat are small. So, it can hardly be that the inclusion of snail meat in the diet affects the $\omega 6:\omega 3$ polyunsaturated fatty acid ratio. It is not clear how to meet the daily recommended intake concerning iron. The requirement to consume iron within the range of 10-12 mg/crewmember day seems to be very strict. The intake of snail meat (118 g/crewmember day), calculated in our study, is close to the value of 110 g/crewmember day presented in the previous work (Midorikawa et al., 1993). However, these rates of snail meat seem to be too much for the daily intake. According to the menu of the International Space Station, the intake of the same product is allowed twice to thrice during the 8-day menu cycle (Perchonok, Bourland, 2002). Hence, the rate $118 \cdot 3/8 = 44.25$ g of snail meat/crewmember day is more realistic.

Conclusion

The snails *Achatina fulica* can produce a food protein by the use of both edible and inedible plant biomass as a feed. In case of inedible plant biomass the process can be considered as the secondary food production. Snails also consume kitchen wastes such as potato peels. The possibility of feeding snails with only inedible plant biomass and kitchen wastes is a subject of further research. Moreover, the problem to return calcium from feces to the matter turnover of BLSS should be solved. The addition of snail

meat in a well-balanced basic diet does not eliminate nutrient imbalances; however, it leads to an increase in the food independence of BLSS. The data obtained in the study could be used in the simulation of the BLSS snail facility.

Acknowledgments

The work was carried out within the frames of the state task on the subject No 56.1.4 of the Basic Research Program (Section VI) of Russian State Academies for 2013-2020.

References

1. Advanced Life Support Baseline Values and Assumptions Document (2004) Anthony J. Hanford, Ph.D., Editor. Lockheed Martin Space Operations Houston, Texas 77058. URL: http://ston.jsc.nasa.gov/collections/trs/_techrep/CR-2004-208941.pdf.
2. Agongnikpo E., Karamoko M., Otchoumou A. (2010) Diet of the giant African land snail *Archachatina marginata* Swainson, 1821, in Ivory Coast: preferences and estimate of damages. *Revue d'Ecologie (Terre et Vie)* 65: 319-330.
3. Cagiltay F., Erkan N., Tosun D., Selcuk A. (2011) Amino acid, fatty acid, vitamin and mineral contents of the edible garden snail (*Helix aspersa*). *Journal of Fisheries Sciences.com* 5: 354-363.
4. Cobbinah J.R., Vink A., Owuka B. (2008) Snail farming. Production, processing and marketing. *Agrodok-series No. 47*, Wageningen, 78 p.
5. Cooper M., Douglas G., Perchonok M. (2011) Developing the NASA food system for long-duration missions. *Journal of Food Science* 76(2): R40-R48.
6. Czarnoleski M, Kozłowski J., Dumiot G., Bonnet J.-C., Mallard J., Dupont-Nivet M. (2008) Scaling of metabolism in *Helix aspersa* snails: changes through ontogeny and response to selection for increased size. *The Journal of Experimental Biology* 211: 391-399.
7. Danish Food Composition Databank. URL: http://www.foodcomp.dk/v7/fcdb_namesearch.asp
8. FAO/WHO (1990) Protein quality evaluation. Report of joint FAO/WHO expert consultation, Bethesda, MD, 4-8 December 1989. FAO/WHO, Rome, Italy.
9. Folch J., Lees M., Sloane Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of biological chemistry* 226: 497-509.
10. Kalacheva G.S., Gubanov V.G., Gribovskaya I.V., Gladchenko I.A., Zinenko G.K., Savitsky S.V. (2002) Chemical analysis of Lake Shira water (1997-2000). *Aquatic Ecology* 36: 123-144.
11. Kouassi K.D., Otchoumou A., Dosso H. (2007) Les escargots comestibles de Cote d'Ivoire: influence de substrats d'élevage sur les parametres de croissance de *Archachatina ventricosa* (Gould, 1850) en élevage hors sol. *Tropicultura* 25(1): 16-20.
12. Li L., Zhao Z., Liu H. (2013) Feasibility of feeding yellow mealworm (*Tenebrio molitor* L.) in bioregenerative life support systems. *Acta Astronautica* 92: 103-109.
13. Liu H., Yu C.Y., Manukovsky N.S., Kovalev V.S., Gurevich Yu.L., Wang J. (2008) A conceptual configuration of the lunar base bioregenerative life support system including soil-like substrate for growing plants. *Advances in Space Research* 42: 1080-1088.
14. Makhutova O.N., Protasov A.A., Gladyshev M.I., Sylaieva A.A., Sushchik N.N., Morozovskaya I.A., Kalachova G.S. (2013) Feeding spectra of bivalve mollusks *Unio* and *Dreissena* from Kanevskoe Reservoir, Ukraine: are they food competitors or not? *Zoological Studies* 52: 56.

15. Manukovsky N.S., Kovalev V.S., Rygalov V.E., Zolotukhin I.G. (1997) Waste bioregeneration in life support CES: development of soil organic substrate. *Advances in Space Research* 20: 1827–1832.
16. Midorikawa Y., Fujii T., Ohira A., Nitta K. (1993) CELSS nutrition system utilizing snails. *Acta Astronautica* 29: 645-650.
17. Otchoumou A., Dupont-Nivet M., Dosso H. (2004) Les escargots comestibles de Cote d'Ivoire: Effets de quelques plantes, d'aliments concentrés et de la teneur en calcium alimentaire sur la croissance d'*Archachatina ventricosa* (Gould, 1850) en élevage hors sol en bâtiment. *Tropicultura* 22 (3): 127-133.
18. Otchoumou A., Dupont-Nivet M., Ocho Anin L., Dosso H. (2010) Body proportions and chemical composition of wild and reared edible snails of Ivory Coast. *Italian Journal of Food Science* 1(22): 1-6.
19. Perchonok M., Bourland C.B. (2002) NASA food systems: past, present and future. *Nutrition* 18: 913-920.
20. Saleuddin A.S.M., Wilbur K.M. (1969) Shell regeneration in *Helix pomatia*. *Canadian Journal of Zoology* 47: 51-53.
21. SELFNutritionData. URL: <http://nutritiondata.self.com/>
22. Spitzer V. (1997) Structure analysis of fatty acids by gas chromatography – low resolution electron impact mass spectrometry of their 4,4-dimethyloxazoline derivatives –a review. *Progress in Lipid Research* 35: 387–408.
23. Toader-Williams A., Bentea M. (2010) Comparative study on the adaptation and growth dynamics of the *Helix pomatia* and *Helix aspersa* Muller terrestrial snails under different feeding regimes. *Animal Science and Biotechnologies* 43(1): 133-142.
24. Upatham E.S., Kruatrachue M., Baidikul V. (1988) Cultivation of the giant snail *Achatina fulica*. *Journal of the Science Society of Thailand* 14: 25-40.
25. USDA National Nutrient Database for Standard Reference. URL: <http://ndb.nal.usda.gov/>
26. Yu X., Liu H., Tong L. (2008) Feeding scenario of the silkworm *Bombyx Mori*, L. in the BLSS. *Acta Astronautica* 63: 1086-1092.
27. Yang Yu., Tang L., Tong L., Liu H. (2009) Silkworms culture as a source of protein for humans in space. *Advances in Space Research* 43: 1236-1242.
28. Volynets V.F. (1977) Analytical chemistry of nitrogen. Moscow: Nauka, 307 p. (in Russian).