Application of *Paramecium caudatum* for the assessment of energy costs for food raw materials and products digestibility

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Abstract. An analysis of the information contained in the scientific and technical literature shows the potential for the application of Paramecium caudatum culture to the first stage of pre-clinical evaluation of new sources of biologically active substances, functional ingredients and food products. The purpose of the work is to investigate the possibility of estimating the energy costs for digestibility of amaranth flour in a model experiment using Paramecium caudatum ciliates. The objects of the study were two samples of amaranth flour – from native amaranth grain of the Voronezh variety and from grain subjected to preliminary heat treatment (IR heating to a temperature of 100-150 °C for 30-50 sec). Experimental data have been obtained confirming the possibility of using test organism data in the pre-clinical evaluation of food and products: the control of the population size of Paramecium caudatum provides initial data for calculating such important indicators as biotic potential and standardized relative biological value, allowing for an indirect assessment of the energy costs of digesting a prototype (food raw materials or product) in comparison with the reference object. A comprehensive analysis of the experimental data obtained also leads to the conclusion that living systems can function with the use of the test samples. It has been established that the scope of the Paramecium caudatum test object in the pre-clinical evaluation of food raw materials and products can be expanded in the direction of estimating the energy costs of digesting food raw materials or products.

1. Introduction

In-vivo research, involving laboratory animals (mice, rats, guinea pigs, rabbits, etc.), provides high significance of the results obtained in various areas of biotechnology, food and medical technologies, as well as in the field of ecology [1-8].

Before the start of in vivo experiments, a meeting of the Ethics Committee is held, which comprehensively considers the need to perform these studies with the participation of laboratory animals, as well as the possibility (or lack thereof) of obtaining similar results in other more humane ways [9-11]. The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, which is recognized in Europe, the USA, and Latin America, reflects provisions proposing that laboratory experiments be replaced by alternative technologies whenever possible [10-12]. For example, one of the universal and informative methods of biological monitoring is the use of test organisms, in particular, the protozoans [1]. The advantages of using protozoa compared to laboratory animals (mice, rats, etc.) are as follows: the short duration of...
the experiment, its relative simplicity and cheapness, high sensitivity to nutritional and toxic factors, and also the obviousness of the manifestation of the biological effect. Possible directions for using protozoa in biotesting are presented in the form of a diagram in figure 1.

Figure 1. Directions for the use of protozoa in in vivo studies.

A method for identifying Ca ionophores using protozoa Actinosphaerium is described. In the presence of the corresponding ionophore, extracellular Ca passing through the cell membrane affects the microstructure of the protozoa and is easily quantified under a microscope [13].

There is information about the use of some protozoa for monitoring soil and water pollution [8]. For example, Tetrahymena pyriformis has proven to be a useful model for studying environmental and industrial pollutants and biological toxins in fresh water, soil, sewage sludge, and food. In addition, as a single-celled animal model, Tetrahymena has high potential for a wide range of functional, pharmacological, molecular-biological, genetic, and immunotoxic studies. At the same time, general indicators for animal studies can be used, such as growth arrest, respiratory and metabolic inhibition, kinetics and synthesis of specific molecules, as well as their combination [14]. The work [15] presents the results of a study of the biological effect of water contaminated with ultrafine metal particles (zinc, copper, iron, silver, cobalt, titanium, aluminum, molybdenum, nickel) on a living organism. Stylonychia mytilus (wild strain) and Paramecium caudatum cultures were used as test organisms.

There is information about model experiments on Paramecium caudatum, which made it possible to establish effective doses of morphine while reducing side effects in patients treated with this drug [16].

The biotesting method is increasingly used to assess the possible biological effects of not only various components of the environment, but also of feed and food products [17, 18]. The results obtained during testing on protozoa of the genus Paramecium (Paramecium caudatum, Paramecium putrinum, Paramecium aurelia, Paramecium multinucleus) are comparable with data from in vivo experiments on warm-blooded animals, while the correlation coefficients are in the range of 0.77–0.94.
The correctness of interspecific extrapolation of the analysis results on infusoria has been proved by a number of researchers and is due to the similarity of a number of basic metabolic parameters in these organisms and higher animals [20-22].

Earlier, in a model experiment, using a test culture of *Paramecium caudatum*, we studied the biological effectiveness of carrot powder, depending on the drying method [23], the biological effectiveness of flour from chufa tubers, analyzed the effect on the test culture of soy protein isolate, wheat bran, α-tocopherol acetate, lactate calcium and refined sunflower oil [24].

Thus, the analysis of technological literature and the results of our own research allows us to conclude that the use of *Paramecium caudatum* culture is promising for the first stage of preclinical evaluation of new sources of biologically active substances, functional ingredients and food products. It should be noted that there is no information about the experience of using this test organism to assess the energy costs of the digestibility of raw materials and food products. Based on the above, the goal of the work – to investigate the possibility of assessing the energy costs for the digestibility of amaranth flour in a model experiment using *Paramecium caudatum* infusoria is formulated.

2. Materials and methods

Two samples of amaranth flour, native and heat-treated, were used as research objects. Native amaranth flour was obtained by grinding amaranth grain of the Voronezh variety grown in the Voronezh region (Russia) according to the method described in patent RU № 2209233. Heat-treated amaranth flour was obtained in a similar way, but with preliminary IR heating of the feed at the UTZ-4 unit (heating temperature of the feed to 100–150 °C, processing time – 30–50 sec.) [24].

Due to the fact that amaranth flour contains a significant amount of protein, digestibility monitoring was carried out according to this indicator. Guided by the fact that the average recommended level of daily intake of proteins for humans is 75 g, the mass of amaranth flour was recalculated to bring it into the incubation medium taking into account an extrapolation coefficient of 20,000; for the experiment amaranth flour was selected so that the protein content in the culture medium of *P. caudatum* was 4 mg/cm³ [21, 22]. According to the methodological recommendations [26, 27], a series of consistent dilutions was performed from the prepared suspension with a protein concentration of 4 mg/cm³. Thus, amaranth flour was studied at concentrations corresponding to the calculated protein content of 1, 2 and 4 mg/cm³, respectively. The indicated content of amaranth flour is equivalent, taking into account the extrapolation coefficient, to human consumption of 18.75; 37.50 and 75.00 g of protein, respectively [21, 22].

Egg protein (albumin) was chosen as the standard for evaluating the digestibility and biological value of amaranth flour samples. It is known that the greatest amount of energy is spent on the reception, digestion and assimilation of proteins: this requires up to 30-40 % of the total energy value of assimilated proteins. When 1 g of chicken protein is digested, the body gets 4 kcal (17.2 kJ). The average recommended level of daily protein intake for a person is 75 g, so it is possible to get 330 kcal of energy (75 g × 4 kcal = 300 kcal from it), of which 30 % (90 kcal) the body will spend on digestion and assimilation of this protein.

The egg protein content in the control medium was 1, 2, 4 mg/cm³, which corresponds to generally accepted concentrations in determining the digestibility and biological value of the protein. The object of comparison was peeled rye flour according to State Standard Specification 52809-2007 with a calculated protein content in the incubation environment for infusoria of 1, 2 and 4 mg/cm³, respectively. To prepare cultural environment, distilled water was used; no added additional micro- and macronutrients and vitamins.

The biotic potential of a population (BP, unit) is understood as the amount of its growth per unit of time per 1 individual. BP characterizes the internal potential capacity of a population to increase its number. The BP value is calculated as the product of the ratio of the number of organisms in the experiment to the corresponding incubation duration (24; 48; 72 or 96 hours) and the coefficient 1/2000. The standardized relative biological value of the product (SRBV, %) is the ratio of the number
of protozoa grown on the substrate containing the object under study to the number of protozoa in a standard environment with a similar number of proteins, multiplied by 100.

Statistical processing of experimental data was performed in the Microsoft Excel 2010.

3. Discussion of the results
Calculation of the number of infusoria cultivated on a substrate with amaranth flour, as compared with a substrate based on egg protein, revealed a lower generative function at all control points. Moreover, its value varied in the range from 33 to 81 % of the generative function relative to the substrate with egg protein at the studied concentrations. On the substrate containing rye flour, a significant decrease in the generative function of ciliates was noted compared with the substrate containing egg protein – it amounted to only 24–57 % of the generative function noted on the substrate with egg protein (table 1).

Table 1. The population size (M ± m) of P. caudatum, cultivated in an environment based on egg protein and amaranth flour, processed thermally and native (P<0.05)

| Protein content, mg/cm³ | Population size (units) at exposure time (h): |
|------------------------|-----------------------------------------------|
|                        | 24          | 48          | 72          | 96          |
| Egg protein            |             |             |             |             |
| 1.0                    | 21250±1227  | 29700±1190  | 37900±899   | 35200±1050  |
| 2.0                    | 25800±960   | 38650±1300  | 44100±1140  | 41300±1120  |
| 4.0                    | 37200±1100  | 60250±1950  | 56300±1290  | 69400±1350  |
| Peeled rye flour       |             |             |             |             |
| 1.0                    | 7013±538    | 15918±643   | 16929±720   | 16192±611   |
| 2.0                    | 9288±621    | 20727±480   | 19712±802   | 17346±538   |
| 4.0                    | 8928±580    | 32091±496   | 32535±625   | 29148±564   |
| Amaranth flour without heat treatment | | | | |
| 1.0                    | 8713±611    | 20196±583   | 19329±708   | 19360±636   |
| 2.0                    | 10578±499   | 20871±582   | 22932±479   | 20237±687   |
| 4.0                    | 12276±705   | 36753±671   | 37158±635   | 34700±534   |
| Amaranth flour after heat treatment | | | | |
| 1.0                    | 11263±588   | 24057±1115  | 23498±813   | 23584±642   |
| 2.0                    | 13932±613   | 29760.5±684 | 29106±724   | 27671±538   |
| 4.0                    | 22320±710   | 31932.5±922 | 39973±736   | 45110±773   |

An analysis of the data obtained (table 1) shows that the generative function of infusoria cultivated in environment with peeled rye flour is lower than in environments with native and heat-treated flour already with a minimum protein content of 1 mg/cm³ by 16.2 and 29.6 %, respectively. In addition, it should be noted that on a substrate with peeled rye flour, the maximum generative function of infusoria was observed after 72 hours of cultivation (with a protein content of 1 mg/cm³), and on a substrate with native and heat – treated amaranth flour – after 48 hours, which may indicate a high biological value of amaranth flour, which more meets the needs of a living organism. We also observed that in an environment containing 4 mg/cm³ of heat-treated amaranth flour protein, the population of infusoria increased throughout the entire exposure time without a recession. A similar relationship was noted in a sample containing egg white at a concentration of 4 mg/cm³. This may indicate the correlated biological value of amaranth flour and egg protein nutrients.

The biotic potential of infusoria cultivated on a substrate containing rye flour in all studied concentrations was significantly lower than on a substrate containing egg protein throughout the entire life cycle, while the values of its indicators ranged from 0.08 ± 0.010 to 0.33 ± 0.006, respectively.
The biotic potential of infusoria cultivated on a substrate containing native amaranth flour, in contrast to the previous one, was significantly higher in all studied concentrations than on a substrate with egg protein, its values ranged from 0.10 ± 0.007 to 0.50 ± 0.006, respectively (table 2).

Table 2. Biotic potential (M ± m) of *P. caudatum*, cultivated in an environment based on egg protein and amaranth flour, processed thermally and native (P<0.05)

| Protein content, mg/cm³ | Biotic potential at exposure time (h): | Egg protein | Peeled rye flour | Amaranth flour without heat treatment | Amaranth flour after heat treatment |
|------------------------|--------------------------------------|-------------|-----------------|--------------------------------------|-----------------------------------|
|                        | 24  | 48  | 72  | 96  |                        | 24  | 48  | 72  | 96  |                        | 24  | 48  | 72  | 96  |                        |
| 1.0                    | 0.44±0.011 | 0.31±0.012 | 0.26±0.006 | 0.18±0.011 |                        | 0.15±0.007 | 0.17±0.005 | 0.12±0.006 | 0.08±0.010 |                        | 0.18±0.009 | 0.21±0.012 | 0.13±0.008 | 0.10±0.007 |                        |
| 2.0                    | 0.54±0.005 | 0.40±0.010 | 0.31±0.011 | 0.22±0.004 |                        | 0.19±0.010 | 0.22±0.004 | 0.14±0.010 | 0.09±0.011 |                        | 0.22±0.009 | 0.26±0.010 | 0.16±0.005 | 0.11±0.004 |                        |
| 4.0                    | 0.78±0.008 | 0.63±0.007 | 0.39±0.010 | 0.36±0.004 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        | 0.26±0.006 | 0.38±0.010 | 0.26±0.010 | 0.18±0.010 |                        |
|                        | 1.0 | 0.15±0.007 | 0.17±0.005 | 0.12±0.006 | 0.08±0.010 |                        | 0.19±0.010 | 0.22±0.004 | 0.14±0.010 | 0.09±0.011 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        |
|                        | 2.0 | 0.19±0.010 | 0.22±0.004 | 0.14±0.010 | 0.09±0.011 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        |
|                        | 4.0 | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        | 0.19±0.011 | 0.33±0.006 | 0.23±0.009 | 0.15±0.010 |                        |
| Peeled rye flour       | 1.0 | 0.18±0.010 | 0.21±0.012 | 0.13±0.008 | 0.10±0.007 |                        | 0.23±0.009 | 0.25±0.004 | 0.16±0.011 | 0.12±0.006 |                        | 0.23±0.009 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        |
|                        | 2.0 | 0.22±0.009 | 0.22±0.010 | 0.16±0.005 | 0.11±0.004 |                        | 0.29±0.010 | 0.31±0.009 | 0.20±0.009 | 0.15±0.005 |                        | 0.29±0.010 | 0.31±0.009 | 0.20±0.009 | 0.15±0.005 |                        |
|                        | 4.0 | 0.26±0.006 | 0.38±0.010 | 0.26±0.010 | 0.18±0.010 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        |
| Amaranth flour without heat treatment | 1.0 | 0.23±0.009 | 0.25±0.004 | 0.16±0.011 | 0.12±0.006 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        |
|                        | 2.0 | 0.29±0.010 | 0.31±0.009 | 0.20±0.009 | 0.15±0.005 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        |
|                        | 4.0 | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        | 0.47±0.005 | 0.33±0.006 | 0.28±0.012 | 0.23±0.007 |                        |
| Peeled rye flour, as a % of egg protein  | 1.0 | 34 | 55 | 46 | 44 |                        | 34 | 55 | 46 | 44 |                        | 34 | 55 | 46 | 44 |                        |
|                        | 2.0 | 35 | 55 | 45 | 41 |                        | 35 | 55 | 45 | 41 |                        | 35 | 55 | 45 | 41 |                        |
|                        | 4.0 | 24 | 52 | 59 | 42 |                        | 24 | 52 | 59 | 42 |                        | 24 | 52 | 59 | 42 |                        |
| Amaranth flour without heat treatment, as a % of egg protein | 1.0 | 41 | 68 | 50 | 56 |                        | 41 | 68 | 50 | 56 |                        | 41 | 68 | 50 | 56 |                        |
|                        | 2.0 | 41 | 55 | 52 | 50 |                        | 41 | 55 | 52 | 50 |                        | 41 | 55 | 52 | 50 |                        |
|                        | 4.0 | 33 | 60 | 67 | 50 |                        | 33 | 60 | 67 | 50 |                        | 33 | 60 | 67 | 50 |                        |
| Amaranth flour after heat treatment, as a % of egg protein | 1.0 | 52 | 81 | 62 | 67 |                        | 52 | 81 | 62 | 67 |                        | 52 | 81 | 62 | 67 |                        |
|                        | 2.0 | 54 | 78 | 65 | 64 |                        | 54 | 78 | 65 | 64 |                        | 54 | 78 | 65 | 64 |                        |
|                        | 4.0 | 60 | 52 | 72 | 64 |                        | 60 | 52 | 72 | 64 |                        | 60 | 52 | 72 | 64 |                        |

It should be noted that the biotic potential of infusoria cultivated on a substrate containing heat-treated amaranth flour was 81 % of that of infusoria incubated in an environment containing egg protein.

The biological value of amaranth flour were calculated after 48 h of incubation (at protein level in culture environment of 1 mg/cm³), because at this stage, the biotic potential of the population is comparable with the indicators of infusorian grown on environment containing egg protein, and reached the maximum of 0.21±0.012 and 0.25±0.004 in substrates with native and heat-treated amaranth flour, respectively, due to the high biological value of amaranth flour, as well as lower content of anti-nutritional substances during the heat treatment.
The biological value of rye flour was calculated after 72 h of incubation at a protein level in the culture environment also in 1 mg/cm$^3$, because at this stage, the biotic potential of rye flour reached a maximum at a concentration of 1 mg/cm$^3$, which amounted to 0.18 ± 0.006.

The standardized relative biological value (SRBV) of native and heat-treated amaranth flour was calculated in relation to egg protein. According to the results of the study, it was found that the SRBV of amaranth flour without heat treatment is 32 % inferior to the biological value of egg protein, and amaranth flour after heat treatment is 19 %, respectively (table 3). It should be noted that medium rye flour is significantly inferior to egg protein, the difference is 46.4 %. In addition, the SRBV of rye flour is 20.6 and 33.3 % less than that of native amaranth and heat-treated flour, respectively.

### Table 3. Calculation results of SRBV and energy costs for digestion of the studied samples based on evaluation results

| Sample                                      | SRBV, %   | Energy costs for digesting 75 g of protein, kcal |
|---------------------------------------------|-----------|--------------------------------------------------|
| Egg protein                                 | 100±1.7   | 90.0±4.8                                         |
| Peeled rye flour                            | 54±4.1    | 131.9±6.4                                        |
| Amaranth flour without heat treatment       | 68±3.9    | 118.8±5.6                                        |
| Amaranth flour after heat treatment         | 81±4.7    | 99.9±6.1                                         |

Given there is low standardized relative biological value of amaranth flour proteins, the estimated cost of digesting it before heat treatment was 118.8±5.6 kcal, after heat treatment, respectively, 99.9±5.6 kcal, while the estimated cost of digesting rye flour was 131.9±6.4 kcal (table 3).

Based on the analysis of the data in table 3, we can conclude that the digestion of amaranth flour requires 11.0-32.0 % more energy costs of the body compared to pure egg protein. However, compared with the cost of digesting medium rye flour, the body will consume less energy by 7.7-22.4 %.

4. Conclusion

Studies conducted using the *Paramecium caudatum* culture have confirmed the possibility of using these test organisms in preclinical evaluation of food raw materials and products.

The control of *Paramecium caudatum* population size provides initial data for calculating such important indicators as biotic potential and standardized relative biological value, which allow for an indirect assessment of the energy costs of digesting a prototype (food raw material or product) compared to the reference object (in our case, chicken egg protein was used). A comprehensive analysis of the experimental data obtained also allows us to draw a conclusion about the possibility of functioning of living systems when using the studied samples.

Thus, the application scope of the *Paramecium caudatum* test object in the preclinical evaluation of food raw materials and products can be expanded in the direction of evaluating the energy costs of digesting food raw materials or products.

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Conflict of interest

The authors state that they have no conflict of interest.
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