Cooked sausage enriched with essential nutrients for the gastrointestinal diet

Vladimir V. Sadovoy1,2,*, Tatiana V. Shchedrina1, Irina A. Trubina1, Anna V. Morgunova2, Evgenia P. Franko4

1 North-Caucasus Federal University, Stavropol, Russia
2 Belgorod University of Cooperation, Economics and Law, Belgorod, Russia
3 Stavropol State Agrarian University, Stavropol, Russia
4 Belarusian State Agrarian Technical University, Minsk, Belarus

* e-mail: vsadovoy@yandex.ru

Received June 19, 2020; Accepted in revised form December 03, 2020; Published online October 15, 2021

Abstract: Introduction. People with gastrointestinal disorders should have a sparing diet with a balanced chemical and amino acid composition including all essential components. Based on formulations of meat products, we identified a number of essential nutritional components that could improve the diet for gastrointestinal pathologies. In this study, we aimed to develop a formulation for cooked sausage enriched with deficient essential nutrients.

Study objects and methods. Our study object was cooked sausage. First, we analyzed the diet for people with gastrointestinal disorders. Then, we formulated a meat-based product (cooked sausage), determined its chemical and amino acid compositions, as well as vitamin and mineral contents, and assessed the balance of amino acids. Finally, we evaluated the biological value and safety of the formulated sausage on laboratory mice.

Results and discussion. The chemical and amino acid compositions of a daily gastrointestinal diet in medical institutions revealed a deficiency of some water-soluble vitamins, vitamin A, calcium, magnesium, and iron, as well as an imbalance of amino acids. To replenish the deficiency, we formulated a meat-based product composed of trimmed beef and pork, beef liver, egg mix, food gelatin, chitosan succinate, rice flour, and soy fortifier. The product was classified as a meat and cereal cooked sausage of grade B, in which most amino acids were used for anabolic purposes. Its daily portion of 100 g eliminated the deficiency of potassium and iron, almost completely replenished magnesium, calcium, and vitamin A, as well as reduced the deficiency of dietary fiber by 4.8 g. The cytological studies of the blood of laboratory animals, whose basic diet contained the formulated sausage, proved its high biological value and safety.

Conclusion. We found that the formulated meat and cereal sausage can be included in the diet for patients with gastrointestinal diseases and used in medical institutions to eliminate the deficiency of essential nutrients.

Keywords: Diet, essential components, raw meat, optimization, formulation, chemical composition, amino acid composition, biological value

Please cite this article in press as: Sadovoy VV, Shchedrina TV, Trubina IA, Morgunova AV, Franko EP. Cooked sausage enriched with essential nutrients for the gastrointestinal diet. Foods and Raw Materials. 2021;9(2):345–353. https://doi.org/10.21603/2308-4057-2021-2-345-353.

INTRODUCTION

According to Russian official statistics, gastroenterological disorders rank third among all diseases. Nutrition is key to their prevention and treatment. Its factors include the chemical composition of the ingredients, the ratios between particular components, the frequency of meals, the calorie content of the daily diet, and others. The treatment of gastrointestinal diseases involves changing the chemical composition of the diet by removing or adding certain components based on their energy value, cooking method, and consistency. Balanced nutrition has a beneficial effect on the metabolism and regulatory systems [1–3].
The development of rational nutrition or adequate diet is still a relevant issue [4, 5]. Such nutrition can include dietary supplements that regulate the chemical composition of foods, stabilize their functional and technological properties, and improve their sensory characteristics and digestibility. The economic indicators are also important, therefore food formulators need to create inexpensive products by adjusting their composition and properties [6, 7].

People with gastrointestinal disorders need a diet that has a balanced composition of micro- and macronutrients, protects the mucous membrane of the gastrointestinal tract, restores natural biocenosis, and reduces gastrointestinal inflammation. Dieticians should take into account the medicinal properties of each food component and their effect on the metabolism and other regulatory systems [1, 3, 5, 8].

Fermented milk products with kefir starter culture, as well as bifidobacteria and lactobacilli, can also benefit people with gastrointestinal diseases. The dietary supplements that maintain and restore the gastrointestinal functions include vitamins, macro- and microelements (Fe, Ca, Zn, etc.), polysaccharides, and dietary fiber. Dietary fiber, which acts as a sorbent, normalizes the motor and evacuation functions of the gastrointestinal tract. Its main feature is high water-retention capacity that accelerates intestinal transit and peristalsis. Dietary fiber also acts as a prebiotic that helps the intestinal microflora by increasing the number of beneficial microorganisms and suppressing the development of coliforms and putrefactive microflora [9, 10].

There is scientific evidence that extracts of bovine abomasum polypeptides, both native and thermally treated, have a therapeutic effect on the damaged gastric mucosa of male Wistar rats, namely gastroprotective and antiulcer effects [11]. Extracts of abomasum polypeptides were also used in the production of pâté for children suffering from gastritis.

It should be noted that bovine abomasum is a potential source of tissue-specific peptides that have a selective effect on the gastric mucosa cells. When formulating a meat product, dieticians should make up for the deficiency of essential components according to the daily requirements for nutraceuticals and dietary fiber, as well as ensure a balanced amino acid composition.

Taking into account the chemical and amino acid composition of meat of slaughter animals, we proposed to formulate a cooked sausage for people with gastrointestinal disorders that can make up the deficiency of essential components.

Cow liver is highly recommended to people with gastrointestinal disorders since it contains significant amounts of vitamin A, iron, copper, and other trace elements and hormonal substances [4, 5, 8, 12]. Beef with a high content of connective tissue and lean pork are also good for their diet [8]. Based on literature, we found the following sources of essential components for our formulation: chitosan succinate, egg mix, soy fortifier, rice flour, and food gelatin [4, 13–15].

Chitosan succinate, a chitin processing product, restores intestinal motility, removes toxic components (cholesterol, heavy metals, bile acids, etc.) from the body, revitalizes lymphatic cells, and helps prevent cancer and diabetes. When using it in cooked sausages, we should take into account that chitosan dissolves in acidic solutions and exhibits its gel-forming and emulsifying properties. It can be used as preliminarily dissolved in milk whey. Chitosan succinate (a complex compound of chitosan and succinic acid) has regions with high and low electron density on the surface of a polymer molecule. Therefore, it is capable of dissolving and exhibiting its functional and technological properties in a wide range of active acidity [12, 13]. When used in meat products, it does not change the active acidity of ground meat.

Rice flour is a source of B vitamins that have a beneficial effect on the nervous system. It contains silicon that normalizes metabolic processes in the human body [14, 15]. Also, rice flour is rich in dietary fiber and biotin, as well as minerals (sodium, potassium, phosphorus), which prevent gastrointestinal diseases [4, 16].

Soy fortifier is a soy milk product that contains a significant amount of dietary fiber, potassium, and ferrous digestible iron. It has a positive effect on the cardiovascular system and stimulates the formation of hemoglobin and certain groups of enzymes [15].

Many cooked sausages contain egg mix that is a source of unsaturated fatty acids, phospholipids, vitamins (A, D, E, B₃, B₅, etc.), phosphorus, iron, and calcium. Also, egg mix enhances the sensory characteristics of the sausage [16, 17].

Gelatin is used as a gelling agent. Up to 30% of connective tissue (primarily collagen-containing) proteins in meat products does not reduce their biological value. Gelatin stimulates blood clotting, acts as dietary fiber, and plays an essential role in the colon motility [18].

Thus, we aimed to develop a balanced meat-based product that could improve the daily intake of essential nutrients for people with gastrointestinal disorders.

STUDY OBJECTS AND METHODS

Our study objects included trimmed beef with connective and adipose tissues under 12%, trimmed pork with fat under 10% (State Standard R 34424-2018 “Meat industry. Classification of trimmed meat for production of meat products for child nutrition”), beef liver (State Standard 32244-2013 “Processed meat by-products. Specification”), soy fortifier (Specification 9146-020-00361809-2001 “Soy food fortifier. Technical conditions”), chitosan succinate (Specification 9284-027-11734126-08 “Chitosan succinate”), egg mix (State Standard 30363-2013 “Products egg dry and liquid food. Specification”), food gelatin (State Standard 11293-89 Specification”), food gelatin (State Standard 11293-89 Specification”).
“Gelatin. Specifications”), and rice flour (State Standard 53495-2009 “Flour for baby’s nutrition. Specifications”).

The chemical composition of the meat-based product (grade B cooked sausage) was determined on a FoodScan analyzer. The amino acid composition of the sausage samples, which were pre-hydrolyzed in alkaline and acidic solutions, was determined by liquid chromatography on an AAA-400 automatic amino acid analyzer. The mineral composition was studied using a Spectroscan Max GV universal analyzer. The vitamin composition was determined on an LCMS-10EV liquid chromatograph.

The product’s biological value was studied on BALB/C white mice (males and females) with an initial weight of 20–30 g. The animals were kept on litter in vivarium cages with standard lighting at about 20°C. The experiment complied with the sanitary, epidemiological, and hygienic regulations for laboratory practice. Lipids, triglycerides, cholesterol, and protein in the blood of experimental animals were determined on an Coabas C-111 biochemical analyzer (Roche Diagnostics) [19–23].

The formulation was optimized using multidimensional scaling and cluster analysis in Statistic Neural Networks. To exclude errors, data were obtained in 3–5 repetitions and processed with the Statistic Neural Networks. Multidimensional data sets were created on an Cobas C-111 biochemical analyzer (Roche Diagnostics) [19–23].

Diet therapy for gastrointestinal disorders is based on regular split meals of foods that leave the stomach quickly, do not stimulate gastric secretion, and do not irritate the mucous membrane [3, 5, 8]. To study a daily gastrointestinal diet, we determined the chemical and amino acid compositions and compared the results with the recommended requirements. In particular, we analyzed Diet No. 1 recommended for diseases of the esophagus, stomach, duodenum, and sometimes the small intestine [5, 8, 26, 27].

The calculations were performed in Excel for the diet with and without meat products (Tables 1, 2).

We compared our data for Diet No. 1 (Table 1) with the recommended requirements for people with gastrointestinal disorders and found a number of discrepancies. In particular, the diet had a deficiency of dietary fiber (15.1 g per day – 75.5% of the recommended intake); vitamins: A (0.75 mg – 83.3%), B6 (7.8 mg – 39.0%), B12 (0.7 mg – 46.7%), B3 (1.0 mg – 55.6%); as well as magnesium (23 mg – 7.7%), potassium (87 mg – 4.0%), calcium (207 mg – 25.9%), and iron (3 mg – 16.7%). Since the deficiency of water-soluble vitamins (B6, B3, PP, and C) can be replenished with plant ingredients, and the sodium and

Table 1 Chemical composition of the gastrointestinal diet*

| Name of product | Weight, g | Proteins, g | Carbohydrates, g | Fats, g | Dietary fiber, g | Minerals, mg | Vitamins, mg |
|-----------------|----------|-------------|------------------|--------|----------------|--------------|--------------|
| **Breakfast 1** |          |             |                  |        |                | Na           | K            | Mg           | P  | Fe  | B1 | B2 | PP | C | A |
| Rice porridge   | 250      | 6.3         | 65.8             | 0.5    | 0.5            | 973          | 48           | 36           | 25   | 85   | 1.0 | 0.1 | 0.05 | 1.2 | –  | –  |
| Cocoa           | 200      | 3.8         | 25.8             | 3.9    | 0.7            | 50           | 242          | 122          | 18   | 120  | 0.6 | 0   | 0    | 0.2 | –  | –  |
| Cake ruskies    | 60       | 5.1         | 31.5             | 6.5    | 0.1            | 264          | 85           | 19           | 25   | 62   | 1.1 | 0.1 | 0.06 | 1.1 | –  | –  |
| **Breakfast 2** |          |             |                  |        |                |              |              |              |      |      |     |     |      |    |    |
| Black currant kissel (jelly-drink) | 200 | 0.1    | 28.0             | 0.2    | 0.2            | 7            | 59           | 8            | 4    | 9    | 0.2 | 0   | 0    | 0.1 | 24.0| 0  |
| Cake ruskies    | 60       | 5.1         | 31.5             | 6.5    | 0.1            | 264          | 85           | 19           | 25   | 62   | 1.1 | 0.1 | 0.1  | 0.1 | 0  | 0  |
| **Lunch**       |          |             |                  |        |                |              |              |              |      |      |     |     |      |    |    |
| Grain soup      | 500      | 6.6         | 50.1             | 5.8    | 1.8            | 1277         | 744          | 75           | 79   | 445  | 2.1 | 0.2 | 0.2  | 2.2 | 11.5| 0  |
| Beef patty      | 50       | 5.0         | 6.8              | 8.7    | 0              | 358          | 99           | 11           | 14   | 65   | 0.7 | 0.04| 0.06 | 0.7 | 0   | 0.02|
| Cranberry jelly | 200      | 2.7         | 24.1             | 1.2    | 0.1            | 2            | 21           | 44           | 5    | 19   | 0.2 | 0   | 0    | 0.03| 1.7 | 0  |
| **Afternoon snack** |         |             |                  |        |                |              |              |              |      |      |     |     |      |    |    |
| Fresh apple     | 120      | 0.4         | 9.8              | 0.6    | 0.6            | 26           | 248          | 16           | 9    | 11   | 0.6 | 0.03| 0.02 | 0.3 | 16.0| 0  |
| **Dinner**      |          |             |                  |        |                |              |              |              |      |      |     |     |      |    |    |
| Currd pudding   | 200      | 29.8        | 43.0             | 8.4    | 0.7            | 889          | 381          | 201          | 46   | 332  | 1.7 | 0.03| 0.41 | 1.1 | 0.4 | 0.13|
| Boiled pike perch | 75     | 15.1        | 1.0              | 1.0    | 0              | 493          | 111          | 26           | 13   | 124  | 1.1 | 0.07| 0.06 | 0.6 | 1.5 | 0.02|
| Cake ruskies    | 60       | 5.1         | 31.5             | 6.5    | 0.1            | 264          | 85           | 19           | 25   | 62   | 1.1 | 0.1 | 0.06 | 1.1 | 0  | 0  |
| Rosehip drink   | 200      | 0.4         | 31.0             | 0.0    | 0              | 1.6          | 4            | 8            | 3    | 2    | 4.4 | 0   | 0    | 0.2 | 120| 0  |
| **Daily total:** | –        | 85.5        | 378.9            | 47.8   | 4.9            | 4869         | 2212         | 604          | 291  | 1398 | 16  | 0.8| 0.9  | 13.9| 175.1| 0.17 |
| **Daily total without meat products:** | – | 75.4 | 372.1 | 39.1 | 4.9 | 4511 | 2113 | 593 | 277 | 1333 | 15 | 0.8 | 0.8 | 12.2 | 175.1 | 0.15 |
| **Recommended daily requirements [25]:** | – | 85–90 | 300–330 | 70–80 | 20 | 4000–6000 | 2200–2500 | 800–1000 | 300–500 | 800–2000 | 18–40 | 15–50 | 18–60 | 20–60 | 90–900 | 10–30 |

* The meat product used for the chemical and amino acid analysis was “Kotlety derevenskiye” (Country-style patties) with connective and adipose tissues under 20% (State Standard 52675-2006. “Semi-prepared meat and meat-contained products. General specifications”)
phosphorus contents were within the permissible limits, these components were outside our focus during the formulation.

Since we aimed to formulate a meat-based product, we analyzed the chemical composition of Diet No. 1 with the meat product (beef patty) excluded. We found a discrepancy between the amino acid contents in the diet and the recommended requirements based on the FAO/WHO standards.

All essential amino acids in Diet No. 1, except for tryptophan, were below the recommended daily intake (Table 2).

Having compared the recommended and actual daily intake of essential components, we calculated the desired chemical composition of our meat product for people with gastrointestinal disorders (Table 3).

Experimental calculations were performed in the planning matrix. Each factor had a range of action. The plan included 255 experiments, with each factor analyzed according to the mixture design simplex centroid (Table 4).

It was important that our meat product complied with the FAO/WHO standards. The use of essential amino acids for anabolic purposes was based on the limiting amino acid.

The optimal formulation was identified using modern data mining methods based on artificial intelligence. For this, we used the following algorithm:

- calculation of the chemical and amino acid compositions for each planning matrix experiment;
- development of a neural network to adequately describe the process;
- compilation of an array of input variables in the algorithmic Pascal language;
- filling in functional (output) variables using the developed neural network (multilayer perceptron); and
- identification of the optimal variant by multidimensional scaling and cluster analysis.

The formulation parameters were determined using a neural network approximation: the scaled functions were superimposed on the scaled factors (Figs. 1 and 2).

### Table 2 Amino acid composition of the daily diet

| Essential amino acids | Actual amino acid consumption, g per day | Amino acid composition of the diet, g/100 g protein | FAO/WHO standard, g/100 g protein | Amino acid score of the daily diet, % |
|-----------------------|------------------------------------------|-----------------------------------------------|---------------------------------|-----------------------------------|
| Tryptophan            | 0.88                                     | 1.03                                          | 1.0                             | 102.9                             |
| Leucine               | 4.57                                     | 5.35                                          | 7.0                             | 76.4                              |
| Isoleucine            | 2.25                                     | 2.63                                          | 4.0                             | 65.8                              |
| Valine                | 2.97                                     | 3.47                                          | 5.0                             | 69.5                              |
| Threonine             | 3.22                                     | 3.77                                          | 4.0                             | 94.2                              |
| Lysine                | 3.69                                     | 4.32                                          | 5.5                             | 78.5                              |
| Methionine + cystine  | 2.38                                     | 2.78                                          | 3.5                             | 79.5                              |
| Phenylalanine + tyrosine | 4.66                                   | 5.45                                          | 6.0                             | 90.8                              |

### Table 3 Chemical composition of the formulated cooked sausage for people with gastrointestinal disorders

| Components | Recommended daily intake for people with gastrointestinal disorders [29] | Actual daily consumption without meat products | Nutrient deficiency in the daily diet | Formulated product |
|------------|--------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------|-------------------|
| Dietary fiber, g/day | 20.0                                                        | 4.9                                           | 15.1                                  | 15.1             |
| Vitamin A, mg/day   | 0.9–3.0                                                                | 0.15                                          | 0.75                                  | 0.75–2.85        |
| Calcium, mg/day     | 800–1000                                                               | 593.0                                         | 207                                   | 207.0–407.0      |
| Magnesium, mg/day   | 300–500                                                                | 277                                           | 23                                    | 23–223           |
| Potassium, mg/day   | 2200–2500                                                              | 2113                                          | 87                                    | 87–387           |
| Iron, mg/day        | 18.0–40.0                                                              | 15.0                                          | 3.0                                   | 3.0–25.0         |

### Table 4 Fragment of the planning matrix for the cooked sausage formulation

| Formulation component, % | Experiment No. |
|--------------------------|----------------|
|                         | 1              |
|                         | 37             |
|                         | 93             |
|                         | 170            |
|                         | 240            |
|                         | 254            |
|                         | 255            |

| Formulation component, % | Experiment No. |
|--------------------------|----------------|
|                         | 1              |
|                         | 37             |
|                         | 93             |
|                         | 170            |
|                         | 240            |
|                         | 254            |
|                         | 255            |

Sadovoy V.V. et al. Foods and Raw Materials, 2021, vol. 9, no. 2, pp. 345–353.
The formulation components (input variables) were scaled in two dimensions, while the functional indicators (chemical and amino acid compositions) were one-dimensional. The color gamut of the functional indicators (Figs. 1 and 2) indicated the diversity of compositions and process complexity. Only modern data mining modules can ensure an optimal formulation with the required parameters.

Having analyzed the clusters in different color zones (Clusters 72, 16, and 65 are given as examples in Fig. 2), we found Cluster 16 to have reference indicators \( Y = -0.02448, X = -0.12303 \).

The compositional analysis of Cluster 16 showed that some options had unacceptable ratios between formulation components, often with insignificant amounts of meat. Also, some options had very similar compositions. Therefore, we analyzed the input variables (components) for Cluster 16 (Table 5).

The most optimal composition of the meat product formulated to prevent gastrointestinal disorders is shown in Table 6. This formulation was based on Experiment 86 (Table 5).

The most optimal composition became a basis for our cooked sausage formulation. Chitosan succinate is readily soluble in water and can be directly added to the ground meat. Soy fortifier is a source of high-grade, easily soluble protein that contains 7.9% of plant-based dietary fiber of origin. Egg mix has good binding and emulsifying properties due to the presence of lecithin. It contains digestible proteins and fat-soluble vitamins and is widely used in cooked sausage formulations.

Lebedeva\(^1\) studied the use of rice flour in cooked sausage formulations. She found that preliminary treatment of rice flour in water heated up to 80°C

---

**Table 5** Fragment of the array of optimal cluster formulations

| Experiment No. | Formulation component, % |
|---------------|--------------------------|
|               | Trimmed beef with connective tissue under 12% | Trimmed pork with fat under 10% | Beef liver | Chitosan succinate | Soy fortifier | Egg mix | Food gelatin | Rice flour |
| 1             | 56.9 | 24.4 | 16.3 | 0 | 1.6 | 0 | 0.8 | 0.0 |
| 2             | 75.7 | 8.4 | 12.6 | 0 | 2.5 | 0 | 0.8 | 0.0 |
| 3             | 64.8 | 32.3 | 0 | 0.1 | 1.6 | 0.4 | 0.8 | 0.0 |
| 4             | 73.3 | 8.1 | 12.2 | 0 | 2.4 | 0 | 2.4 | 1.6 |
| ...           | ... | ... | ... | ... | ... | ... | ... | ... |
| 31            | 69.5 | 7.0 | 8.4 | 0.4 | 2.7 | 1.1 | 4.9 | 6.0 |
| 32            | 59.8 | 20.3 | 7.1 | 0.4 | 2.2 | 0.8 | 4.2 | 5.4 |
| ...           | ... | ... | ... | ... | ... | ... | ... | ... |
| 83            | 29.2 | 14.6 | 24.8 | 21.9 | 6.9 | 0.5 | 1.6 | 0.4 |
| 84            | 68.8 | 0 | 14.8 | 0.2 | 3.9 | 0.5 | 3.9 | 7.9 |
| 85            | 61.0 | 20.3 | 6.8 | 0.1 | 3.4 | 0.3 | 2.7 | 5.4 |
| 86            | 62.8 | 12.1 | 9.6 | 0.6 | 2.4 | 1.2 | 5.3 | 6.0 |
| ...           | ... | ... | ... | ... | ... | ... | ... | ... |

**Table 6** The most optimal composition for the formulated cooked sausage*

| Formulation component, % |
|--------------------------|
| Trimmed beef with connective tissue under 12% | Trimmed pork with fat under 10% | Beef liver | Chitosan succinate | Soy fortifier | Egg mix | Food gelatin | Rice flour |
| 63.0 | 12.0 | 10.0 | 0.6 | 2.4 | 1.0 | 5.0 | 6.0 |

* The component contents are rounded to whole values

---

\(^1\) Lebedeva LI. Razrabotka tekhnologii ehmulʹgirovannykh myasnykh produktov s ispol'zovaniem modifitsirovannoy risovoy muki [The development of technology for emulsified meat products with modified rice flour]. Cand. eng. sci. diss. Moscow: V.M. Gorbatov All-Russian Research Institute of the Meat Industry, 2003. 133 p. (In Russ.).
increases the water-binding, fat-binding, gelling, and stabilizing capabilities of the ground meat emulsion. Gelatin swells but does not dissolve in cold water. It dissolves in hot water (60–80°C) and, when cooled, turns into jelly [10, 18]. Since chitosan succinate, egg mix, and soy fortifier are not exposed to heat treatment, a food additive of gelatin and rice flour (1:1.2) was mixed with 80°C water (1:3) and homogenized for 3–4 min. Then, it was chilled and introduced into the meat at the final stage of cutting. Our formulation of cooked sausage with a food additive is shown in Table 7.

The sausage yield was 117 kg/100 kg of unsalted raw materials. State Standard 23670-2019. “Cooked meat sausage products. Specifications” classifies the formulated sausage as a hybrid (meat and cereal) product according to the content of meat ingredients (53.6%), and as belonging to grade B according to the content of muscle tissue (43.0%).

The sensory evaluation of the sausage was performed by 10 panelists. The average score was 4.9 points, with slight discrepancies in the color evaluation. The chemical composition of the cooked sausage sample included 60.8% water, 4.4% fat, 10.3% protein, and 3.2% minerals ($P \leq 0.05$). With a beef patty excluded from the diet, the protein deficiency was 9.6–14.6 g/day (Table 1). Therefore, the diet should contain at least 100 g of cooked sausage to replenish the protein deficiency.

To assess the biological value of the formulated sausage, we determined the amino acid composition of a beef patty using the reference book [4] and calculated the daily intake of amino acids for a diet with cooked sausage and a diet with a beef patty (Table 8).

As can be seen in Table 8, the score of the limiting amino acid (isoleucine) in the sausage (70.1%) was higher than the score in the patty (65.8%). This means that in the diet with cooked sausage, more essential amino acids will be used for anabolic purposes. There was no need to assess the balance of amino acids in the daily diet, since it consisted of split meals consumed at different times.

We compared amino acid contents in the cooked sausage and beef patties (Table 8) and found that the formulated meat product had a well-balanced composition of essential amino acids. Based on Table 8 and using the method of Lipatov, we calculated the mutual balance and rationality of essential amino acid contents in the formulated sausage and beef patties (Table 9) [28, 29].

As can be seen in Table 9, the rationality coefficient for cooked sausage was much higher than for a beef patty. Cooked sausage had a low content of amino acids used for the biosynthesis of nonessential amino acids ($\sum EAA$), compared to a beef patty. The proportion of essential amino acids used as energogenic material ($\sum EAA$) was almost the same for both samples. Thus,
The observations lasted 30 days. The hematological parameters of mice blood at the end of the experiment are given in Table 11.

We found a slight increase in leukocytes, hemoglobin, and erythrocytes in the blood of the experimental mice over the entire feeding period (30 days).

The contents of total protein and albumin increased in the control group B and in the experimental group by 6.6 and 6.0 g/L, respectively. The level of glucose was within the normal range (4.11–4.18 mol/L) for all the animals in the entire period, regardless of the diet.

The activity of aspartaminotransferase (AST) and alanine aminotransferase (ALT) in blood serum is indicative of general health and the state of specific organs. For example, a deviation from the AST norm (26–77 U/L for mice) can indicate liver pathology, heart problems, abnormalities in protein structures and bile production, etc. ALT is responsible for the movement of alanine from one cell to another. Its normal level (54–269 U/L for mice) strengthens the immune system and normalizes metabolic processes. Increased ALT indicates blood disease and disorders in the work of heart, liver, and blood vessels, as well as other pathologies. Decreased ALT is usually caused by a deficiency of vitamin A. Our study showed that the levels of the AST and ALT enzymes were within the normal range in all the groups of mice, and their ratio (de Ritis coefficient) corresponded to the required level (0.91–1.75) [30].

Thus, the hematological parameters pointed to the biological value and safety of the formulated sausage.

### CONCLUSION

We analyzed Diet No. 1 used in medical institutions for people with gastrointestinal disorders and found that its daily contents of essential ingredients (proteins, vitamins, minerals) were significantly higher than the requirements of the FAO/WHO standard. The biological value and safety of the developed formulated sausage in all the groups of animals was confirmed. The experimental mice divided into the control (group A) and experimental (group B) groups, fed on the basic diet with or without a preventative supplement, showed a significant increase in the content of total protein and albumin in the blood serum.

The formulated sausage had a well-balanced amino acid composition. The contents of some deficient minerals and vitamins in the formulated product are shown in Table 10.

Based on Tables 10 and 3, we found that 100 g of the cooked sausage could replenish a daily deficiency of potassium and iron, and significantly reduced the deficiency of magnesium, calcium, and vitamin A. The sausage sample contained 1.2% of insoluble plant-based dietary fiber (cellulose, hemicellulose, and lignin) and 3.6% of animal-based dietary fiber (gelatin). Thus, 100 g of the cooked sausage could reduce the deficiency of dietary fiber in people with gastrointestinal disorders by 4.8 g. We find it advisable to recommend including dietary fiber in the formulations of other products in the diet.

The biological value and safety of the developed sausage was determined on BALB/C white mice in vivo. The experiments complied with the sanitary, hygienic, and epidemiological standards and requirements. The experimental mice were divided into 3 groups of 5 animals: mice fed on the basic diet (control group A), mice fed on the basic diet + cooked sausage without a preventative supplement (control group B), and mice fed on the basic diet + cooked sausage with a preventative supplement (experimental group).

### Table 10 Some minerals and vitamin A in cooked sausage, $P < 0.05$

| Content, mg/100 g of product | K      | Ca     | Mg     | Fe     | Vitamin A |
|------------------------------|--------|--------|--------|--------|-----------|
| 290                          | 185    | 21.8   | 3.2    | 0.73   |

### Table 11 Hematological parameters of mice blood before and after feeding, $P < 0.05$

| Name                          | Erythrocytes, 10$^9$/L | Leukocytes, 10$^9$/L | Hemoglobin, g/L | Total protein, g/L | Albumin, g/L | Glucose, mol/L | AST, U/L | ALT, U/L | ALT/AST (de Ritis coefficient) |
|-------------------------------|------------------------|-----------------------|-----------------|-------------------|--------------|----------------|----------|----------|-----------------------------|
| A day before feeding (7th day of observations) | 8.16                  | 8.64                  | 126.00          | 59.60             | 36.62        | 4.11           | 51.40    | 67.85    | 1.32                      |
| 30th day: Basic diet           | 8.64                  | 8.94                  | 128.60          | 61.40             | 39.40        | 4.18           | 52.00    | 62.92    | 1.21                      |
| Basic diet + sausage without preventative supplement | 8.78                  | 9.32                  | 129.40          | 66.20             | 42.80        | 4.16           | 57.20    | 68.068   | 1.19                      |
| Basic diet + sausage with preventative supplement | 8.50                  | 9.32                  | 129.01          | 65.60             | 42.40        | 4.16           | 53.80    | 62.95    | 1.17                      |

The activity of aspartaminotransferase (AST) and alanine aminotransferase (ALT) in blood serum is indicative of general health and the state of specific organs. For example, a deviation from the AST norm (26–77 U/L for mice) can indicate liver pathology, heart problems, abnormalities in protein structures and bile production, etc. ALT is responsible for the movement of alanine from one cell to another. Its normal level (54–269 U/L for mice) strengthens the immune system and normalizes metabolic processes. Increased ALT indicates blood disease and disorders in the work of heart, liver, and blood vessels, as well as other pathologies. Decreased ALT is usually caused by a deficiency of vitamin A. Our study showed that the levels of the AST and ALT enzymes were within the normal range in all the groups of mice, and their ratio (de Ritis coefficient) corresponded to the required level (0.91–1.75) [30].

Thus, the hematological parameters pointed to the biological value and safety of the formulated sausage.

### Table 9 Mutual balance and rationality of essential amino acids (EAA) in cooked sausage and beef patties

| Name                           | Limiting amino acid score, % to FAO/WHO standard ($C_{\text{min}}$) | Content of essential amino acids in protein, % ($\Sigma$EAA) | Utility coefficient ($U$) | BSEAA used for non-essential amino acids (EAA) used for anabolic purposes, g/100 g protein | Estimated rationality coefficient ($R_e$) | Desirable rationality coefficient ($R_d$) |
|--------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------|---------------------------|------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------|
| Cooked sausage                 | 8.64                                                                | 4.63                                                            | 0.028                     | 0.14                                                                     | 0.87                                      | 1                                         |
| Beef patty                     | 0.55                                                                | 0.362                                                           | 0.55                      | 15.91                                                                  | 28.9                                      | 0.365                                    |
|                                |                                                                     |                                                                |                           |                                                                          |                                           |                                           |

The sausage sample contained 1.2% of insoluble plant-based dietary fiber (cellulose, hemicellulose, and lignin) and 3.6% of animal-based dietary fiber (gelatin). Thus, 100 g of the cooked sausage could reduce the deficiency of dietary fiber in people with gastrointestinal disorders by 4.8 g. We find it advisable to recommend including dietary fiber in the formulations of other products in the diet.
dietary fiber, vitamins, and some minerals) did not meet the recommended intake. In particular, we identified an imbalance of amino acids in the meat product used in the diet (a beef patty). In order to eliminate this nutritional deficiency, we formulated a meat and cereal product of grade B – cooked sausage. Apart from meat, it contained chitosan succinate, egg mix, soy fortifier, food gelatin, and rice flour. Our product had a well-balanced composition of amino acids, with tryptophan as the limiting amino acid (amino acid score – 86.0%, the utility coefficient – 0.87). 100 g of the formulated cooked sausage can completely eliminate the deficiency of potassium, calcium, and iron, as well as significantly reduce the lack of magnesium and vitamin A. Since it can only reduce, rather than fully replenish, the deficiency of dietary fiber, we recommend that foods rich in dietary fiber are included in the diet.

The experimental studies of the hematological parameters in the laboratory mice confirmed the nutritional value and safety of the formulated cooked sausage. Therefore, it can be included in the diet for people with gastrointestinal disorders and used in medical institutions.

CONTRIBUTION

The authors were equally involved in conducting the research and writing the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Nichipuruk E. The Tract. Moscow: Tochka; 2019. 408 p. (In Russ.).
2. Khan TN, Ansari TH, Zulkifle M. Concept of transformation and incorporation of nutrient in the body: A review. International Journal of Food Science and Nutrition. 2019;4(3):93–96.
3. Nikityuk DB, Pogozheva AV, Sharafetdinov KhKh, Baturin AK, Bogdanov AR, Gapparova KM, et al. Standarty lechebnogo pitanija [Standards of medical nutrition]. Moscow, 2017. 313 p. (In Russ.).
4. Spisok produktov (Khimicheskiy sostav) [A list of products (chemical composition)] [Internet]. [cited 2020 May 20]. Available from: http://frs24.ru/himsostav.
5. Baranovskiy AU. Diętologiia. 5-e izd. [Dietology. 5th edition]. St. Petersburg: Piter; 2017. 1104 p. (In Russ.).
6. Sadovoy VV, Selimov MA, Shchedrina TV, Nagdalian AA. Nutritional supplement for control of intake of diabetes. Journal of Excipients and Food Chemicals. 2017;8(2):31–38.
7. Sadovoy VV, Shchedrina TV, Melentyeva VV, Khamsitaeva AS. Forecasting the molecular properties of dietary supplement used in the recipe of foodstuff for diabetes mellitus prevention. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018;9(4):1620–1625.
8. Aljutaily T, Huarte E, Martinez-Monteagudo S, Gonzalez-Hernandez JL, Rovai M, Sergeev IN. Probiotic-enriched milk and dairy products increase gut microbiota diversity: a comparative study. Nutrition Research. 2020;82:25–33. https://doi.org/10.1016/j.nutres.2020.06.017.
9. Simpson T, Deleuil S, Echeverria N, Komanduri M, MacPherson H, Suo C, et al. The Australian Research Council Longevity Intervention (ARCLI) study protocol (ANZCTR12611000487910) addendum: Neuroimaging and gut microbiota protocol. Nutrition Journal. 2019;18(1). https://doi.org/10.1186/s12937-018-0428-9.
10. Chernukha IM, Bogatyrev AN, Dydykin AS, Aslanova MA, Fedulova LV. Effect of polypeptides isolated from cattle abomasum on stomach regenerative processes in rats. Problems of Nutrition. 2014;83(5):26–32. (In Russ.).
11. Aslanova MA. Funktsional'nyy pashchet dlya pitaniya detey, stradayushchikh gastritom [Functional pâté for children with gastritis]. Meat Technology. 2016;157(1):39–41. (In Russ.).
12. Zhu J, Zhang Y, Wu G, Xiao Z, Zhou H, Yu X. Inhibitory effects of oligochitosan on TNF-α, IL-1β and nitric oxide production in lipopolysaccharide-induced RAW264-7 cells. Molecular Medicine Reports. 2015;11(1):729–733. https://doi.org/10.3892/mmr.2014.2643.
13. Azuma K, Osaki T, Minami S, Okamoto Y. Anticancer and anti-inflammatory properties of chitin and chitosan oligosaccharides. Journal of Functional Biomaterials. 2015;6(1):33–49. https://doi.org/10.3390/jfb6010033.
14. Ol’khovatov EA, Ponomarenko LV, Kovalenko M.P. Ispol’zovanie soi v pishevykh i meditsinskikh tselyakh [The use of soy for nutritional and medical purposes]. Young Scientist. 2015; 95(15):231–235. (In Russ.).
15. Antipova LV, Mishchenko AA, Ospova NA. Rastitel’nye istochniki dlya proizvodstva produktov pitanija funktsional’nogo naznacheniya [Vegetable sources for functional food production]. Molodezhny innovatsionnyy vestnik [Youth Innovative Bulletin]. 2018;8(3):37–38. (In Russ.).
16. Korrol’ AE, Drozdova LI. The mélange as a product of the food industry. Molodezh’ i nauka [Youth and science]. 2017;(3):27–32. (In Russ.).
17. Ratushnyy AS, Aminov SS. Vsyo o ede ot A do Ya: Ehntsiklopediya [Everything about food from A to Z: Encyclopedia]. Moscow: Dashkov and K; 2019. pp. 433–434. (In Russ.).

18. Esimbekova EN, Kratasyuk VA, Govoron AE, Lonshkova-Mukina VI. Gelatin and starch: what better stabilizes the enzyme activity? Doklady Rossijskoj Akademii Nauk. Nauki o Zhizni. 2020;491(1):151–154. (In Russ.). https://doi.org/10.31857/S2686738920020110.

19. Sanitarno-veterinarnye pravila soderezhanniya laboratornykh zhivotnykh [Sanitary and veterinary rules for keeping laboratory animals] [Internet]. [cited 2020 May 20]. Available from: https://dommedika.com/medicinskaia_mikrobiologiya/soderzhanie_laboratornih_zivotnykh.html.

20. Denisov AV, Cheprakova VA, Anisin AV, Bezrukov SL. Ethical aspects of modern use of animals in experimental studies. Bulletin of the Russian Military Medical Academy. 2018;63(3):238–242. (In Russ.).

21. Sadovoy VV, Shchedrina TV, Selimov MA. Biologically active composition for regulation of lipolysis process in the organism under obesity. Problems of Nutrition. 2017;86(6):74–83. (In Russ.).

22. Berezin I, Chupakhina L, Popov A. Modern aspects of sanitary-epidemiological normalization of working conditions at work with laboratory animals. Veterinariya sel’skokhozayastvennykh zhivotnykh [Veterinary medicine of farm animals]. 2021;(5). (In Russ.).

23. Garber JC, Barbee RW, Bielitzki JT, Clayton LA, Donovan JC, Kohn DF, et al. Guide for the care and use of laboratory animals. Moscow: IRBIS; 2017. 304 p. (In Russ.).

24. Gromyko GL. Teoriya statistiki [Theory of statistics: practical exercises]. Moscow: Infra-M; 2018. 544 p. (In Russ.).

25. Goreeva NM. Statistika v skhemakh i tablitsakh [Statistics in diagrams and tables]. Moscow: Ehksmo; 2017. 414 p. (In Russ.).

26. Kodentsova VM, Vorhesinskaya OA, Nikityuk DB, Tutelyan VA. Vitamin status of adult population of the Russian Federation: 1987–2017. Problems of Nutrition. 2018;87(4):62–68. (In Russ.). https://doi.org/10.24411/0042-8833-2018-10043.

27. Kodentsova VM, Risnik DV, Nikitiuk DB, Tutelyan VA. Multivitamin-mineral supplementation in medical nutrition. Consilium Medicum. 2017;19(12):76–83. (In Russ.).

28. Yanova MA. Standartizatsiya i sertifikatsiya myasa i myasnykh produktov [Standardization and certification of meat and meat products]. Krasnoyarsk: Krasnoyarsk State Agrarian University; 2020. 51 p. (In Russ.).

29. Borisova T. Quality control of foodstuff and food raw materials. Shimadzu Solutions. Analytics. 2016;28(3):64–71. (In Russ.).

30. Krasnikova ES, Krasnikov AV, Radionov RV, Artemyev DA, Okolelov VI. Blood biochemical parameters of rats – wistar line under the blv experimental infection. Innovations and Food Safety. 2019;24(2):69–75. (In Russ.). https://doi.org/10.31677/2311-0651-2019-24-2-69-75.

ORCID IDs
Vladimir V. Sadovoy https://orcid.org/0000-0002-0182-9318
Tatiana V. Shchedrina https://orcid.org/0000-0001-5201-7961
Anna V. Morgunova https://orcid.org/0000-0001-6312-7963