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DEVELOPMENT OF THE RECIPE OF THE SAUSAGE WITH PROTEIN ISOLATE FROM VETCH SEEDS AND SELENIUM-PROTEIN DIETARY SUPPLEMENT TAKING INTO ACCOUNT THE SAFETY INDICATOR

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Abstract

Relevance. Today the consumption of sausage products is ineffective from the point of view of nutrition and it can be dangerous to the human body. In this case, the studies *in vivo* aimed at identifying possible toxicological manifestations after consumption of such sausage products become relevant. An additional factor in leveling food threats to the human body is the targeted combination of recipe ingredients. **Aim.** The aim of the present work are the development of a sausage composition with pronounced bioprotective properties using mathematical modeling, detection of possible acute toxicity manifestations from the sausage consumption according to the optimized recipe with vetch seed protein isolate and Selenium-protein dietary supplement and determination of the toxicity class of designed product. **Research methods.** While carrying out the work, the methods of experiment planning, mathematical modeling, research on biological objects (*in vivo*), and the Litchfield-Wilcoxon probit analysis method were used. **Results.** The article examines the method of designing the sausage composition with protein isolate from vetch seeds and Selenium-protein dietary supplement, based on which the main recipe ingredients of the product were selected. An analysis of the consumer value of the designed sausage owing to which the content of macronutrients in the designed recipe was determined (43.06 g per 100 g) is given in the present article. The influence of sausage with vetch seed protein isolate and dietary Selenium-protein supplement on the body of white line rats was investigated. The general fluidity of the clinical picture in the case of acute toxicity in laboratory animals after the introduction of sausage with such additive was studied. The parameters of acute toxicity of the product are calculated. These are indicators of lethal and effective doses and its safety degree. Thus, according to the results of *in vivo* studies, the LD₅₀ indicator for designed sausage was 22.75 g/kg (with calculated values of LD₁₆=8.21 g/kg, LD₈₄=437.61 g/kg, and LD₉₉=1864.28 g/kg), which corresponds to the fifth class of additives' toxicity and proves their practical non-toxicity. **Conclusions.** The safety of sausage consumption with vetch seed protein isolate and Selenium-protein dietary supplement for the body of mammals indicates the possibility of its use in the diets of people who need to correlate their own macronutrient and Selenium statuses, since the designed recipe of the sausage has high nutritional and biological values.

Keywords: design; sausage; vetch seeds; protein isolate; pH-shifting treatment; Selenium-protein dietary supplement; plant protein; toxicity; optimization; recipe.

РОЗРОБКА РЕЦЕПТУРИ КОВБАСИ З БІЛКОВИМ ІЗОЛЯТОМ З НАСІННЯ ВИКИ ТА ДОБАВКОЮ ДІЄТИЧНОЮ СЕЛЕН-БІЛКОВОЮ З УРАХУВАННЯМ ПОКАЗНИКА БЕЗПЕЧНОСТІ

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Анотація

Актуальність. На сьогодні споживання ковбасних виробів є малоефективним з погляду нутриціології та може становити небезпеку для людського організму. У цьому випадку набувають актуальності дослідження *in vivo*, сфокусовані на виявленні можливих токсикологічних проявів після споживання таких ковбасних продуктів.

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Додатковим фактором нівелювання продуктивних загроз для людського організму є цільове комбінування рецептурних інгредієнтів. Мета. Ціллю роботи є розроблення композиції ковбаси із вираженими біопротекторними властивостями за допомогою математичного моделювання, виявлення можливих проявів гострої токсичності від споживання ковбаси за оптимізованою рецептурою з ізолятом білка з насіння вики та добавкою дієтичною селен-білковою, а також визначення класу токсичності такого продукту. Методи дослідження. При виконанні роботи використовувались методи планування експерименту, математичного моделювання, дослідження на біологічних об'єктах (*in vivo*), метод пробіт-аналізу Літчфілда-Вілкоксона. Результати. У статті розглядається методика проєктування складу ковбаси з білковим ізолятом з насіння вики та добавкою дієтичною селен-білковою, на основі якої здійснено добір основних рецептурних інгредієнтів продукту. Наведено аналіз споживчої цінності розробленої ковбаси, в результаті якого встановлено вміст макронутрієнтів у розроблюваній рецептурі (43,06 г на 100 г). Досліджено вплив ковбаси з ізолятом білка з насіння вики та добавкою дієтичною селен-білковою на організм щурів білої лінії. Вивчено загальну плінність клінічної картини при гострій токсичності у лабораторних тварин після введення ковбаси з добавкою. Розраховано параметри гострої токсичності продукту, а саме: показники летальних та ефективних доз, ступінь його безпечності. Так, за результатами проведених досліджень *in vivo* показник ЛД₅₀ для ковбаси з добавкою дієтичною селен-білковою становить 22.75 г/кг (при розрахункових величинах ЛД₁₆=8.21 г/кг, ЛД₈₄=437.61 г/кг, а ЛД₉₉=1864.28 г/кг), що відповідає п'ятому класу токсичності добавок та засвідчує їх практичну нетоксичність. Висновки. Безпечність споживання ковбаси з ізолятом білка з насіння вики та добавкою дієтичною селен-білковою для організму ссавців свідчить про можливість її застосування у харчових раціонах осіб, які потребують кореляції власного макронутрієнтного та селенового статусів, оскільки розроблена рецептура ковбаси має високу харчову та біологічну цінність.

Ключові слова: розробка; ковбаса; насіння вики; білковий ізолят; рН-коригуюча обробка; добавка дієтична селен-білкова; рослинний білок; токсичність; оптимізація; рецептура.

Introduction

Today the manufacturing of various food products for health purposes is rapidly gaining popularity in Ukraine. Food industry enterprises try to introduce such products to the market, because in the case of their regular consumption, many diseases can be avoided, immunity can be strengthened and the body's condition can be improved in general.

Special attention should be paid to the enrichment of meat products. Among the wide range of such food, sausage products are in particular demand by consumers; these are sausages, frankfurters, meat breads. The rapid growth of demand for sausage products determines the feasibility of developing new types with improved functional and technological properties and increased nutritional value.

The analysis of the food market of Ukraine shows a tendency to use semi-finished products and food additives of foreign production or mixtures improving the taste and aroma, structure, color and other quality indicators of sausage products. At the same time, there is a demand for the growth of production and consumption of meat-based sausage products, for the formation and stabilization of which both food additives and dietetics are used. Therefore, it is important to increase the nutritional and biological value of sausage meat products, which can be achieved by enriching them with Selenium-protein substances. The introduction of such products to the consumer market will make possible to improve significantly the nutritional status of modern Ukrainians.

Now the development of new combined products tends to reduce the share of raw materials of animal origin in their composition. At the same time, an increase in the proportion of plant components contributes to the emergence of undesirable technological factors, such as delamination or instability of dispersed systems, reduction of the ultimate shear stress, etc. To eliminate them, stabilizing components are added to the recipe mixture [1].

Today the use of traditional stabilizers, such as flour, starch, gelatin, is ineffective, since they do not have the full range of properties necessary to create the structure of sausage products. To improve the properties of the designed sausage Selenium-protein dietary supplement (SPDS) «Neoselen» was chosen [2]. Its functional and technological properties, aspects of the interaction of its components etc., were revealed in previous studies [3; 4].

The safety of a food or dietary supplement is confirmed only in the case they do not cause acute or chronic toxicity, contain no carcinogenic substances, mutagens, have no teratogenic and gonadotoxic properties [5]. Moreover, the daily amount of such substances entering the body is of decisive importance. Such studies of the SPDS «Neoselen» were carried out by the authors earlier and showed its practical safety. This is confirmed by previous research results [6–14]. Also the perspective of using the protein isolate obtained from vetch seeds (*Vicia sativa L.*) by pH-shifting treatment had been proved. In the previous work the chemical composition of vetch seeds as a raw material for obtaining protein isolate by pH-shifting treatment was investigated.

The obtained protein isolate showed high functional properties and a high-quality chemical composition of proteins and minerals for use in the technology of sausage and confectionery products and also as food for dietetic and vegetarian nutrition [15]. Therefore, the creation of a sausage product enriched with essential nutrients is relevant. The use of vegetable and animal raw materials (protein isolate from vetch seeds and whey proteins, which are part of SPDS) while manufacturing products with a dispersed structure will improve their organoleptic and rheological indicators.

The specialized international organization «FAO/WHO Joint Expert Committee on Food Additives and Contaminants» (JECFA) regulates the activities of food manufacturers in matters of the use of food additives. In Ukraine, these issues fall under the jurisdiction of the State Health Supervision Department of the Ministry of Health.

Since the last decade the majority of scientists are inclined to recognize the fact that the primary stage of hygienic regulation of the content of food additives in products and human diets should be their preliminary toxicological and hygienic assessment [16; 17]. Thus, world studies are being conducted aimed at identifying the parameters of acute toxicological poisoning of living organisms not only due to the consumption of food additives [18; 19], but also determining the extent of the effect of some insecticides on pests of agricultural crops [20], cypermethrin on catfish [21], γ -radiation on some legumes [22], allelopathic phenomenon [23] and so on. To optimize the process of statistical processing of the data obtained experimentally, computer support should be used [24]. To calculate the parameters of the acute toxicity of «Ukrainian with Selenium» sausage produced with protein isolate from vetch seeds and SPDS «Neoselen», the Probit Analysis software package was used, which is recommended for this type of research [23].

Task statement. The parameters of acute toxicity of any substance or product, its potential danger, become key issues during toxicological studies. Studies of these parameters are necessary to establish the degree of danger of the additive, as well as for further studies where information on maximum tolerated doses is needed. The obtained information is necessary for determining the toxicity coefficient of the object under study: the ratio of the LD₅₀ dose to the therapeutic dose. «Ukrainian with Selenium» sausage contains a number of food and dietary additives, each of which, in the case of excessive intake to the body, can cause intoxication processes. Therefore,

scientific research aimed at determining the parameters of acute toxicity of «Ukrainian with Selenium» sausage produced with protein isolate from vetch seeds and SPDS «Neoselen» are relevant. The purpose of the present work is to develop a composition of sausage with pronounced bioprotective properties using mathematical modeling, to identify possible manifestations of acute toxicity from the consumption of sausage according to an optimized recipe with protein isolate from vetch seeds and SPDS and also to determine the toxicity class of such a product. The following tasks were set for achieving the purpose:

- to make the screen of raw materials for sausage production;
- to develop a sausage composition with a high content of nutrients (especially mineral) substances;
- to establish a possible toxicological effect from the consumption of sausage according to an optimized recipe with protein isolate from vetch seeds and SPDS;
- to determine the toxicity class of such product.

The research methods are – the experiment planning, the mathematical modeling on biological objects (*in vivo*), the Litchfield and Wilcoxon probit analysis method. The implementation of mathematical modeling of the sausage composition was solved using linear programming with the MS Excel editor. *The object of the research* is the parameters of acute toxicity of sausage according to an optimized recipe with protein isolate from vetch seeds and SPDS. *The subject of the study* is a recipe-optimized sausage with protein isolate from vetch seeds and SPDS, its composition and toxicological indicators.

Results and discussion

The recipe optimization was carried out in the Excel Solver program, which is designed to solve certain systems of equations, linear and non-linear optimization tasks [24].

As a result of the application of mathematical programming the authors performed the optimal solution. They have found the extremum of the linear objective function under the constraints on the variables that need to be determined. The solution of the system of linear balance equations was carried out using «Solution Search» in Excel.

As a result of the calculation, the program obtained the proportions of recipe components for sausage «Ukrainian with Selenium»:

$$x_1 = 51, x_2 = 9, x_3 = 13, x_4 = 14, x_5 = 5, \\ x_6 = 2.5, x_7 = 4.5,$$

herewith $F(x) = 43.063$.

So, the content of the main nutrients for «Ukrainian with Selenium» sausage was 43.06 g per 100 g.

The clinical picture after the introduction of «Ukrainian with Selenium» sausage into the stomach of white rats in toxic and effective (non-lethal) doses developed after 10...12 hours. Clinical symptoms of acute poisoning of white rats after oral administration were not observed. Aggressiveness and periods of excitement or increased motor activity were not observed in the most individuals.

In the same individuals exposed to the toxic effect, after a short period of excitement, sharply expressed depression and drowsiness developed. It should be noted that no laboratory animal died during the experiment. Some rats only experienced moderate poisoning due to the introduction of maximum doses of «Ukrainian with Selenium» sausage (7.5...9.0 g per one rat).

The obtained results of the Probit analysis allow establishing the degree of safety of «Ukrainian with Selenium» sausage in accordance with the data presented in the Table 1 [25].

Table 1

Toxicity of substances depending on the value of LD ₅₀		
Toxicity class	LD ₅₀ , mg/kg	Toxicity characteristics
1 st	Less than 5	Extremely toxic
2 nd	5...49	Highly toxic
3 rd	50...499	Moderately toxic
4 th	500...4999	Low toxic
5 th	More than 5000	Practically non-toxic

The higher is the LD₅₀ value, and the lower is the acute toxicity of the food additive. According to the results of the experiment, it was established that the average value of LD₅₀ for «Ukrainian with Selenium» sausage corresponds to 22.75 g/kg. Thus, according to the Table 1 its toxicity class is fifth, i.e. it is practically non-toxic.

Experimental part

While developing the sausage composition considerable attention was paid to the nutrient composition of the raw material, its change during technological processing. The basis of the task is developing and optimizing the recipe for the production of sausage by introducing a Selenium-protein dietary supplement (SPDS), which will

ensure an increase in the nutritional and biological value of the final product, as well as an expansion of the assortment. Restrictions on the content of components for the development of a new sausage are presented in the Table 2. The Table 3 presents the data matrix for designing a sausage product recipe. It includes the following blocks: recipe ingredients (RI), the possible range of variation of the RI, the dry matter content of the RI and indexed variables. The Table 4 shows the nutritional value of RI.

During the establishment of restrictions, the physiological needs of a person in biologically active substances were guided.

Table 2

Calculation of the main raw materials for «Ukrainian with Selenium» sausage					
Main raw materials	Quantity of raw materials		Auxiliary raw material	Quantity of raw materials	
	%	kg		%	kg
	100.00	180.00			
2nd grade beef	50.00	90.00	Kitchen salt	2.60	4.70
Fat pork	10.00	18.00	Sodium nitrite	0.008	0.01
Spine lard	15.00	27.00	Black pepper	0.03	0.05
Protein isolate from vetch seeds	15.00	27.00	Drinking water	40.00	72.00
SPDS «Neoselen»	3.00	5.40	Progel 40+	0.60	1.08
Starch	2.00	3.60	Pork aroma	0.10	0.18
Semolina	5.00	9.00	Combi	0.03	0.10
			Output, %	125.00	

Table 3

Restrictions on the recipe content (g per 100 g of the final product). Information matrix of the data for designing the recipe of «Ukrainian with Selenium» sausage

Main raw materials	Quantity of raw materials, %		Index, x_i	Dry matter content in RI, %
	Min	Max		
2nd grade beef	48.0	51.0	x_1	27.6
Fat pork	9.0	11.0	x_2	52.5
Spine lard	13.0	16.0	x_3	89.5
Protein isolate from vetch seeds	14.0	16.0	x_4	95.0
SPDS «Neoselen»	3.0	5.0	x_5	85.0
Starch	1.5	2.5	x_6	85.0
Semolina	4.5	5.5	x_7	89.5

Table 4

Nutritional value of recipe ingredients of «Ukrainian with Selenium» sausage

Nutrients	Nutritional value of the sausage recipe						
	2nd grade beef	Fat pork	Spine lard	Protein isolate from vetch	SPDS «Neoselen»	Starch	Semolina
Proteins	20.1	14.5	3.6	17.0	88.3	0.1	10.6
Carbohydrates	0.1	0.1	0.1	0.1	-	78.2	78.0
Fats	7.4	37.3	85.6	17.3	3.4	-	0.5
Total	27.6	51.9	89.3	34.4	91.7	78.3	89.1

The objective function is the maximum nutritional value of the designed product, defined as the sum of the nutritional value of the constituent parts of the RI, and their mass of which is presented in the recipe. The maximum

nutritional value of the designed sausage with bioprotective effect is defined as the sum of the nutritional value of the constituent parts of the RI, and their mass is presented in the recipe:

$$F(x) = \frac{27.6x_1 + 52.5x_2 + 89.5x_3 + 95x_4 + 85x_5 + 85x_6 + 89.5x_7}{100} \rightarrow \max.$$

On the basis of the information matrix (Table 3) and nutritional value (Table 4), the authors formed a system of linear balance equations as to the content of proteins, fats, carbohydrates in the

recipe, subject to restrictions according to the physiological needs of a person: the presence of protein in the recipe consists at least 15%:

$$20.1x_1 + 14.5x_2 + 3.6x_3 + 17x_4 + 30.25x_5 + 0.1x_6 + 10.6x_7 \geq 15;$$

the presence of carbohydrates in the recipe is not less than 50%, but not more than 65%:

$$50 \leq 0.1x_1 + 0.1x_2 + 0.1x_3 + 0.1x_4 + 39.3x_5 + 78.2x_6 + 78x_7 \leq 65;$$

the presence of fats in the recipe consists no more than 12%:

$$7.4x_1 + 37.3x_2 + 85.6x_3 + 17.3x_4 + 0.5x_7 \leq 12;$$

the ratio of proteins to carbohydrates consists at least 0.2 and no more than 0.3:

$$0.2 \leq \frac{20.1x_1 + 14.5x_2 + 3.6x_3 + 17x_4 + 30.25x_5 + 0.1x_6 + 10.6x_7}{0.1x_1 + 0.1x_2 + 0.1x_3 + 0.1x_4 + 39.3x_5 + 78.2x_6 + 78x_7} \leq 0.3;$$

the ratio of proteins to fats should be equal to 0.25 %:

$$\frac{20.1x_1 + 14.5x_2 + 3.6x_3 + 17x_4 + 30.25x_5 + 0.1x_6 + 10.6x_7}{0.1x_1 + 0.1x_2 + 0.1x_3 + 0.1x_4 + 39.3x_5 + 78.2x_6 + 78x_7} = 0.25;$$

normalization conditions (mixture mass 100 g):

$$x_1 + x_2 + x_3 + x_4 + x_5 + x_6 + x_7 = 100;$$

upper limits of variables (RI):

$$x_1 < 51, x_2 < 11, x_3 < 16, x_4 < 16, x_5 < 5, x_6 < 2.5, x_7 < 5.5;$$

lower limits of variables (RI):

$$x_1 > 48, x_2 > 9, x_3 > 13, x_4 > 14, x_5 > 3, x_6 > 1.5, x_7 > 4.5.$$

The safety of the new sausage composition «Ukrainian with Selenium» was investigated on 170 white linear rats with a body weight of 155...175 g bred in the vivarium of the Dnipro Medical Academy of the Ministry of Health of Ukraine. Experimental animals were divided into 17 groups of 10 heads each. The conditions for keeping animals and providing them with food rations were carried out according to the regulatory requirements of the International Convention: at a temperature of 20...22 °C and an air humidity of 40...60 % [26].

Previously maintained on a fasting diet (for 4...6 hours) the animals were orally injected with sausage using a metal probe with oil on the end. Sausage was given once a day in the maximum

permissible amount for oral administration – 1.0...9.0 g per animal or 6.25...56.25 g/kg.

The animals were observed for two weeks with studying the clinical picture of acute experimental poisoning: continuously during the first day after the introduction of sausage «Ukrainian with Selenium»; later – twice a day for 13 days.

The general status and behavior of the animals, the state of their neuromuscular and vegetative functions, fur coat, feed intake, and water consumption were registered. Special attention was paid to the development of toxicosis signs, their severity and recovery time were assessed. The general clinical picture of the *in vivo* experiment is shown in the Fig. 1.

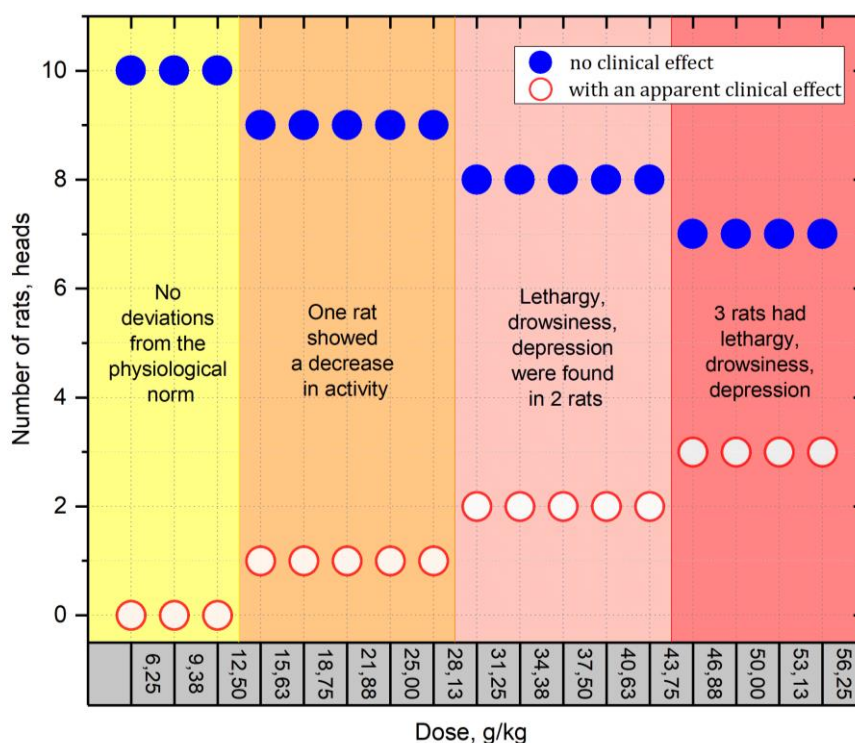


Fig. 1. The general fluidity of the clinical picture of acute toxicity in rats with the introduction of sausage «Ukrainian with Selenium»

The toxicity of «Ukrainian with Selenium» sausage was determined according to the following parameters: maximum tolerated dose LD_0 , average lethal dose LD_{50} , as well as LD_{16} and LD_{84} to establish confidence limits of the average lethal dose LD_{50} and LD_{99} using the computer

program Probit Analysis v.2.0.0.6. Data on the maximum tolerated (effective) dose of LD_0 , the average lethal (effective) dose of LD_{50} , as well as LD_{16} and LD_{84} for establishing the confidence limits of the LD_{50} dose are presented in the Table 5.

Table 5

The results of determining the parameters of acute toxicity of «Ukrainian with Selenium» sausage (in terms of protein isolate from vetch seeds and SPDS) (n = 17, p ≥ 0.05)

Response percentage, (p%), %	SPDS Dose, g/kg	Maximum SPDS Dose (D_{max}), g/kg	Minimum SPDS Dose (D_{min}), g/kg
1.0	0.76	1.76	0.01
16.0	5.32	8.21	3.26
50.0	22.75	48.35	12.09
84.0	97.18	437.61	29.56
99.0	679.60	1864.28	95.22

The figure presented below shows a graph expressing the «dose-effect» relationship after the

consumption of «Ukrainian with Selenium» sausage by rats.

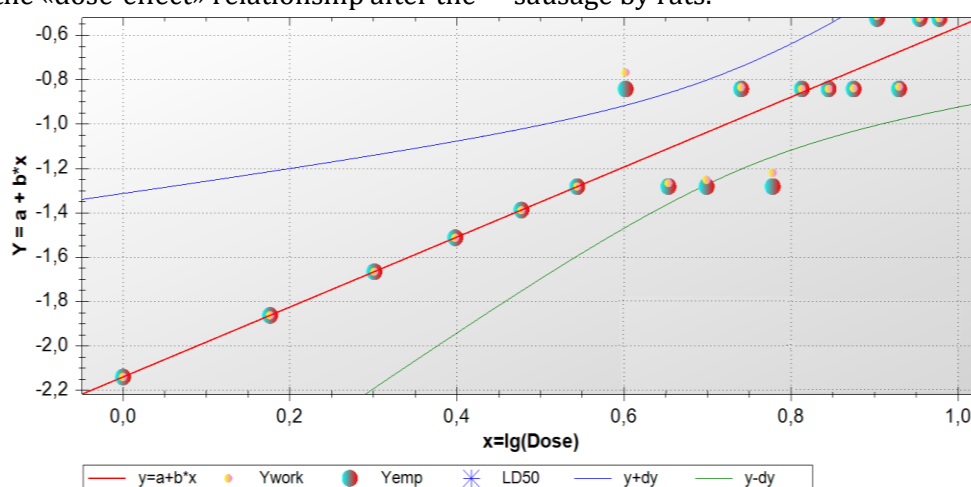


Fig. 2. Dependence of the shown effect after consumption by rats of «Ukrainian with Selenium» sausage stabilizer on quantity of the supplements

Logarithms of SPDS doses are plotted on the abscissa axis, and probit values are plotted on the ordinate axis. A straight line, which is drawn through the found points, by interpolation allows determining the LD_{50} or any other dose at which the effect is observed.

The experimental probit points coincide completely with the calculated points, which confirms the 95 % reliability of the research results.

Conclusions

Available, high-quality and safe raw materials with high biological indicators were chosen for the production of «Ukrainian with Selenium» sausage according to modern HACCP requirements and safety standards for food products and raw materials: beef, pork, lard, starch and semolina. The recommended dose of protein isolate from vetch seeds and SPDS «Neoselen» was taken for composite design. The composition of the sausage product was modeled with the help of the MS Excel spreadsheet. Thanks to the introduction of vetch

seed protein isolate and SPDS "Neoselen" into the recipe composition, final products exhibit pronounced bioprotective properties. Studying the macronutrient composition of the obtained sausage showed that the final product has a high protein content. The content of macronutrients for «Ukrainian with selenium» sausage consists 43.06 g per 100 g. According to the results of in vivo studies, the fifth class of toxicity of «Ukrainian with Selenium» sausage was established, which proves its practical non-toxicity. The «effect-dose» dependence was established after the consumption of «Ukrainian with Selenium» sausage by rats, based on which the parameters of acute toxicity of the product were calculated: indicators of lethal and effective doses, the degree of its safety. The LD_{50} index for the sausage product, based on vetch seed protein isolate and SPDS «Neoselen», was 22.75 g/kg with calculated values of LD_{16} =8.21 g/kg, LD_{84} =437.61 g/kg, and LD_{99} =1864.28 g/kg), which proves its safety in the case of consuming in moderate doses. In general,

no laboratory animals died during the experiment, only some of them experienced moderate poisoning (lethargy, drowsiness, depression) due to the introduction of maximum doses of sausage (7.5...9.0 g per one rat). In turn, this had an impact on the general fluidity of the experiment clinical picture. In general, the safety of vetch seed protein isolate and SPDS «Neoselen» for the mammalian body indicates their possibility of being used in the

technology of manufacturing food products and semi-finished products, in particular sausage ones. The data on the toxicology of vetch seed protein isolate and SPDS «Neoselen», obtained in the course of the research described in the present work, will be used as a basis for determining the indicator of its daily permissible dose and more in-depth clinical studies, for example, biochemical studies of the blood of laboratory animals.

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