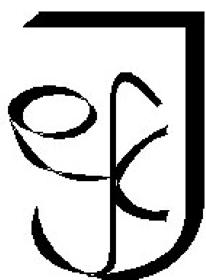

Nutritional supplement for control of diabetes.

Vladimir Vsevolodovich Sadovoy[a,b], Magomed Aslanovich Selimov[c*], Tatiana Viktorovna Shchedrina[a], Andrey Ashotovich Nagdalian[c]

[a]North Caucasus Federal University, Ukrainskaya Street 56 A, Pyatigorsk, Stavropol Krai, Russia
[b]Stavropol Institute of Cooperation (a branch of Belgorod University of Cooperation, Economics and Law.) Goleneva Street 36, Stavropol, Stavropol Krai, Russia
[c]North Caucasus Federal University, Prospekt Kulakova 2, Stavropol, Stavropol Krai, Russia

Received: February 1, 2017; *Accepted:* April 10, 2017

Original Article

ABSTRACT

The analysis of diabetic patients' dietary habits indicate that there is an imbalance of selected vitamins and minerals. A nutrient supplement composition, intended for prophylactic administration, was developed to correct this imbalance. It was composed of dried eggs and a yeast component (60%), vitamin B1 (0.02%) and nicotinamide (0.04%), chromium chelate (0.02%), "selen-active" (0.02%) and lecithin (39.9%). The dried eggs and yeast component were prepared by the homogenization of lysozyme rich chicken eggs and yeast followed by storage and drying at a pre-defined temperature and to a set water content respectively. The nutritional supplement was incorporated at 5% concentration into a sausage recipe which was subsequently cooked into animal feed. An assessment of the safety and efficacy of the prophylactic nutritional supplement was performed in an alloxan induced diabetic rat model. The developed composition, as a part of the animal feed, improved metabolic processes, increased antioxidant activity, reduced lipid peroxidation, decreased blood cholesterol, and improved carbohydrate metabolism in the experimental animals.

KEY WORDS: Diabetes, brewer's yeast, lecithin, chromium chelate, "selen-active", vitamins

INTRODUCTION

In addition to medication, adherence to a diet and dietary habits forms the mainstay of diabetic control and treatment (1). Depending on the type and severity of the disease, the treatment options consist of supplementary insulin, special diet and/or drugs that modulate blood sugar. Patients may also decide to use biological and active/nutritional supplements, i.e., drugs from natural components (2).

The limitations of the diet, in conjunction with metabolic disruptions, can result in a reduced absorption of several essential nutrients in diabetic patients. As a result, this population often exhibits vitamin and mineral deficiencies.

[*]Corresponding author: Magomed Aslanovich Selimov, North Caucasus Federal University, Prospekt Kulakova 2, Stavropol, Stavropol Krai, Russia, Tel: +79 288 200 432,
E-Mail: selimovma@mail.ru

In particular, levels of vitamins *A*, B_1, and *PP* (pellagra preventive factor also known as niacin or vitamin B_3) can be reduced to between 6 and 22% from normal levels. A deficiency of calcium (11-26%), selenium (54-77%), iodine (58%), zinc (41-58%) and chromium (25%) has also been shown (3).

Lecithin, eggs and a semi-finished yeast component, chromium chelate, "selen-active" and vitamins B_1 and *PP* were selected for this study to try and correct vitamin and mineral deficiencies in diabetic patients. Lecithin is an essential chemical compound required for the normal functioning of the nervous system, the formation of intercellular space, the normalization of brain cell activity and neccessary for liver function. Lecithin has been shown to reduce blood cholesterol and to repair damaged cells (4) and has previously been recommended for diabetic patients (5).

Brewer's yeast has been shown to stimulate and regulate the production of insulin (6). It is a natural product with high concentrations of *B1, B2, B5, B6, D* and *PP* vitamins (7). In addition, Brewer's yeast also provides essential minerals and micronutrients (8).

Chromium chelate regulates sugar levels in the blood of insulin resistant diabetic patients, improves muscle tone, normalizes metabolic processes and reduces blood cholesterol (9).

Selenium in the composition "selen-active" is an antioxidant, protecting the cell from the harmful effects of excess free radicals and supports the body's natural defense system by modulating the formation of antibodies. A deficiency of selenium causes a pathological change in the pancreas (10-11).

The main purpose of this research was to develop a prophylactic nutritional supplement for the control of diabetes by providing typical micronutrients, vitamins and minerals that are known to be deficient in diabetic patients. The developed product contained lecithin (acceptable level is 7g per day), chromium (acceptable level is 50 μg per day), selenium (acceptable level is 55 μg per day), and vitamins in allowed concentrations (acceptable level of B1 is 1.7 mg, acceptable level of PP is 15 mg per day). The product is therefore expected to be safe for adults (12-13). The nutritional supplement developed for this study was added to cooked sausage (5 kg of nutritional supplement in 100 kg cooked sausage).

MATERIALS AND METHODS

Materials

Chromium chelate (Altera Holding, Archon Vitamin Corporation, USA) is a digestible form of chromium. It is a ligand, represented by the tetradentate compound of chromium with picolinic acid. "Selen-active" (DIOD «Moscow Factory of Ecofood», Russia) is a tableted dietary supplement. One tablet (0.25 g) contains 200 mg sorbitol, 50 mg ascorbic acid and 210 μg Celexa™, an organic compound of selenium complexed with xanthene. Soy lecithin was obtained from YUVIKS-Pharm, Russia, vitamin *B1* from Thiamine Hydrochloride, 99%, TLC, "WIRUD GmbH", Bad Homburg, Germany, vitamin *PP* (Nicotinamide, 99.5%) from HPLC, Shandong Kunda Biotechnology Co., Ltd. China and the brewer's yeast (*Saccharomyces cerevisiae*) from Ecco Plus, Yeast Technology, Russia.

Methods

The brewer's yeast was processed using lysozyme from chicken eggs. The yeast capsules were disrupted by high-velocity homogenization using a Heidolph Silent Crusher (Heidolph Instruments, Schwabach, Germany). The optimal homogenization parameters were determined by the multidimentional scaling method (MSM): homogenizer rotation speed was 15700–24600 RPM, homogenization time was between 2.0 and 9.4 minutes. After homogenization the mixture was poured into glass tubes for

separation (3-4 hours standing) into an emulsion layer, liquid fraction and sediment. The height of each fraction was measured. The homogenization time varied depending on the amount of yeast that remained intact.

The number of microorganisms was determined using a microscope (optical microscope Carl Zeiss, Germany, magnification 10x40) in a Goryaeva counting chamber.

The measurement of blood RBC, leukocytes, lecithin and hemoglobin content was performed using a hematological analyzer CELL DYN 1700™ (Abbott, USA) (14). Total protein, albumins, aspartate and albumin were identified and quantitated with a photocolorimeter KFK–2MP™ (Russia) with an associated test-kit (15).

Glucose, cholesterin and total lipids content in the blood serum of the experimental animals were determined using a chemset ERBA XL 200 (ERBA Lachema, Czech Republic) per referenced methods (16). The measurement of triglycerides was carried out using a spectrophotometer UNICO 2800 UV/VIS™ (Spectral range 190-1100 nm, Germany), at a wavelength of 505 nm after saponification of the triacylglycerols by potassium hydroxide in glycerin according to the instructions for use of reagent kits for determining the content of triglyceride levels in the serum and blood plasma (Approved by Roszdravnadzor from 18.12.2009 No: 10377-P / 09 RU No: FSR 2009/06423 of 18.12.2009).

The multicomponent food additive consisting of the yeast biomass, chromium chelate, selenium complex, vitamin *B1*, vitamin *PP* and lecithin was added to the animal feed. The investigation of the recipe's efficacy and safety was studied in a diabetes induced rat model (17-18). The supplement's composition was designed to reverse the lack of essential nutrients in prophylactic feed (19-20).

The protein content in the nutritional supplement was determined using Kjeldahl's method (P 4.1.1672-03 Manual on quality control methods and safety of biologically active additives to food. Adopted 06.30.2003.) The lipid determination was carried out by the Soxhlet method (continuous boiling for 5 hours) (P 4.1.1672-03 Manual on quality control methods and safety of biologically active additives to food. Adopted 06.30.2003). The method for determining the ash content was based on the determination of non-combustible inorganic substances residue remaining after burning and calcining raw materials (GOST 24027.2-80 Methods for determination of moisture, ash content, extractive and tannin materials, essential oil. Accepted 01-01-1981).

The investigation of the water content was carried out using the chemical analyzer OHAUS MB 45 (Ohaus Corporation, USA). The samples (3 g) were dried in automatic mode, following the manufacturer's instructions.

Malondialdehyde (MDA) quantification (21) was used to measure the magnitude of serum lipids peroxidation. The MDA reacts with thiobarbituric acid (TBARS) forming a complex of active products. An increase of the TBARS quantity in animal's tissues is one of indicators of an oxidation-reduction imbalance and is indicative of an aberrant metabolism. For the MDA measurement, 5 ml of blood with 1.5 ml of sodium citrate was cooled and separated by centrifugation (12 minutes at 1500 RPM). After centrifugation to 0.25 ml of received serum, 3 ml of 1.4% orthophosphoric acid and 1 ml of 0.5% TBARS were added. The solution was immersed into a water bath for 45 minutes and cooled with the addition of 4 ml butanol mixing until a suspension was formed. The absorbance of the supernatant was measured with a spectrophotometer at wavelengths of 535 nm and 570 nm against a control sample in a cuvet with optical path length of 1 cm (21).

For the processing of the data obtained in this

study, standard mathematical software packages were used Statistic v.8.0, v.10.0, Statistic Neural Networks (SNN) v.4 and Statistic Automatically Neural Networks Code Generator (SANN) v.8 (22-23). MSM allows a search and interpretation of non-supervising variables, thereby explaining similarities between objects in origin space of signs with set parameters. To determin an optimal combination of factors, the input variables were scaled in 2D and functional indexes were scaled in 1D. The significance level of the experimental data was evaluated using the Statistic Neural Networks program with usage of Case Error module. Error per case did not exceed 0.01 (e.p.c. ≤ 0.01). Significance level of results of blood biochemical analysis was determined by the Wilcoxon (p) test after mathematical treatment of data by variation statistics method with usage of "Attestat" module in Microsoft Excel. Wilcoxon criterion did not exceed 0.01 ($p \leq 0.01$).

Analysis of microbiological indexes was carried out in accordance with requirements for food quality (Medical biological requirements and sanitary quality norms for food raw materials and food products) (24).

All animal experiments were approved by the local ethics committee and conformed to directive 86/609/EEC (on the protection of animals used for scientific purposes). The animals were kept in a vivarium of the Stavropol State Medical University, on the standard diet in accordance with the Sanitary Rules and Regulations 2.2.1.3218-14 (25); rules of laboratory practice (26), the requirements of the International Science Committee (27) and the rules of work with the use of experimental animals (28).

Evaluation of safety and efficacy of the developed feed was performed on laboratory animals (white rats of both genders weighing 180-250 g). The animals were divided to 3 groups: 1st group was Control 1 (usual feed), 2nd group was Control 2 (induced diabetes+ usual feed) and 3rd group was Experimental (induced diabetes + usual feed + developed supplement). Changes in height-weight and hematological indexes were recorded for each group.

The rats were divided into units of 8 animal per group and isolated in boxes with dry spreading from wood chips which was renewed every day after daily diuresis evaluation. All groups had access to standard feed and unlimited clean water. Water was dispensed into automatic drinking bowls so that the consumption could be recorded for each group. Feeding was performed once a day in the morning and daily feed consumption was recorded. To increase carbohydrate in the diet, the water in the drinking bowls was changed to 20% sucrose solution in water. Diabetes was induced in the Control 2 and Experimental groups by injecting alloxan under the skin (110 mg/kg).

Alloxan damages β-cells of pancreatic islets. Except for diuresis, water and feed consumption and weight changes, rats from all groups at the 7th, 14th, and 28th and 40th day of the experiment were analyzed for carbohydrate metabolism and lipid peroxidation. Blood was taken from the tail vein. The animals were decapitated in compliance with the international requirements for the humane treatment of animals under ether anesthesia.

RESULTS AND DISCUSSION

The purpose of optimization was determining the homogenization parameters (2D diagram) such as the influence of the rotational speed in RPM (x) and the duration of homogenization in minutes (y) on the response variables that included the maximal emulsification ability, protein and solids content. The optimal zone was identified by the projection of 1D response surface (result of functional indexes multidimensional scaling). Multidimensional scaling is used for latent (directly not observable) variables, which helps users explain

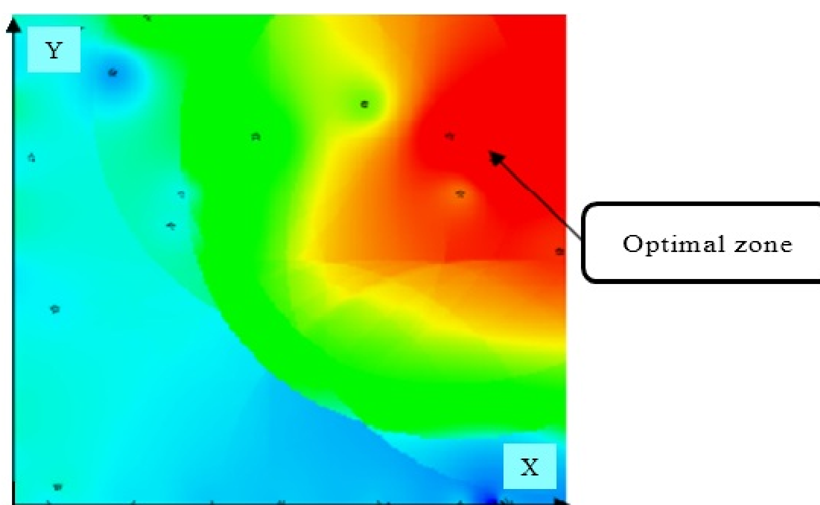

Figure 1 Optimization of homogenizing parameters for yeast biomass

similarities between objects with fixed points in origin space of dimensions shown in Figure 1.

The effective disruption of the yeast's capsules was caused by using fresh chicken eggs with a 3.5% lysozyme content (29). According to the optimal parameters obtained by the MSM method, the weight ratio of brewer's yeast to the fresh chicken egg material was 3:1. This composition mix was homogenized and stored at a temperature of 36-37°C (optimal temperature of lysozyme chemical activation) for 1 hour.
Comparative microscopic analysis was performed before and after the homogenization of the sample. The results indicate the effectiveness of the destruction of the cell walls using these process parameters. Microscopic analysis showed that >95% of the cell walls of the brewer's yeast remained intact initially , shown in Figure 2. Homogenization using the optimal parameters destroyed approximately 77% of the cell membranes. After storage for 1 hour, the mixture was dried at 80°C until the water content was <8%. The other components, vitamins *B1* and nicotinamide, chromium chelate, the biopesticide "selen-active" and lecithin were then added. The chemical composition of the dried product from brewer's yeast and chicken eggs with biological active filler is shown in Table 1. The composition was 34.3 g proteins, 35.3 g fats, 7.8 g water and 17.3 g minerals. The cream colored powdered supplement had an innocuous smell

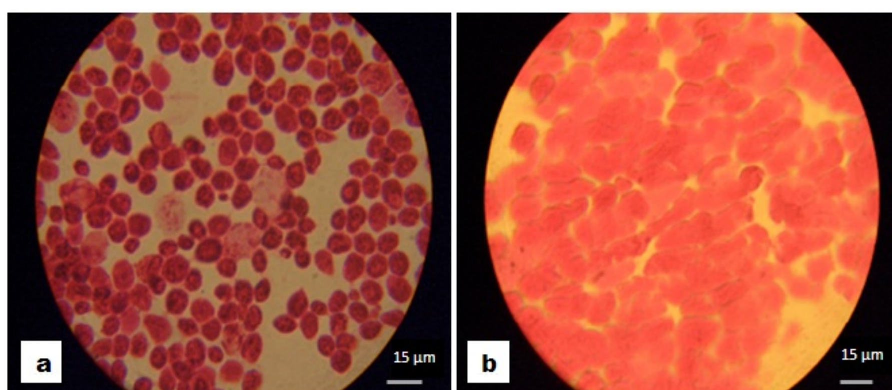

Figure 2 Microscopic analysis of yeast biomass before processing (Figure 2a) and after processing (Figure 2b)

and taste.

Table 1 Components of biological active supplement

SUPPLEMENTS COMPONENT	CONTENT %
Dried eggs and yeast semi-finished product	60.00
Vitamins:	
B1	0.02
Nicotinamide	0.04
Minerals:	
Chromium chelate	0.02
"Selen-Active"	0.02
Lecithin	39.90

Microbiological analysis (at "zero time" and after six months of storage at room temperature in polyethylene packing) showed that the pathogenic micro flora of the supplement did not exceed the required index as defined: coliforms (in 0.1 g), *E. coli* (in 1 g) and pathogenic microorganisms, including salmonella (in 10 g). The quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM, CFU/g) at time zero and after six months was 2.1×10^3 and 3.2×10^3 respectively. This was below the regulatory level of 1×10^4 CFU/g. These results are lower than maximum acceptable levels for biological active supplements derived from yeasts and lysates (Russian Sanitary Regulations and Standards SanPiN 2.3.2.1078-01) (30).

The study of physiological conditions showed that the feed (with, or without, the dietary supplement) contributed to a 5.4% and 5.2% increase in leukocytes in the second and third groups respectively compared with the first group. Blood albumin of the experimental group after 40 days on the diet increased 8.05% in the first group, 2%; in the second group and 4.3% in the third group. The glucose level in the experimental group decreased by 8.5%.

Preliminary biochemical analysis of the glucose content in the rats showed that the β-cells retained normal function until the 3rd day after the alloxan injection (data not shown). Therefore, biochemical analyses were performed on the 7th, 14th, 28th and 40th day. The results of the investigation of glucose concentration in the blood are shown in Table 2.

Table 2 Glucose concentration in blood

GROUP (n=8)	DAY			
	7	14	28	40
	Glucose concentration, mmol/l			
Check 1	4.29±0.52	4.25±0.18	4.23±0.52	4.25±0,33
Check 2	8.32 ± 0.12	19.07±0.43	15.41±3.65	18.40±0.51
Experimental	8.80±0.34	11.84±0.37	6.93±0.40	7.05±0.32

The blood glucose decreased in the Experimental group by 61.7% (at day 40) when compared with Control 2. It was however higher than in Control 1 (>65%), but was within the upper limits for normoglycemia (< 7.5 mMol/l) (32).

The animals injected with alloxan showed symptoms of polyuria, polydipsia and weight loss. The water consumption in the 3rd group decreased to the 40th day. There was no statistically significant difference in feed composition between the three groups.

From the 14th to the 40th day the MDA level in the blood of the animals injected with alloxan (Control 2) was significantly greater than that of Control 1. In the group fed with the nutritional supplement, the MDA level decreased significantly (41.1% by day 40) during this period. However, it did not return to the control level (> 9% at day 40) but was within the upper limits for MDA levels in non-diabetic rats (33). This confirms with previously reported results (31), which concluded that free radicals can be a primary factor in diabetes progression (Table 3).

Table 3 Malondialdehyde concentration in blood

GROUP (n=8)	DAY 7	DAY 14	DAY 28	DAY 40
	Level nMol/ml			
Check 1	3. 13 ±0.35	2.73 ± 0.46	2. 73 ± 0.45	2.77± 0.48
Check 2	4.97 ± 0.75	5.07 ± 0.54	4.87 ± 0.48	5.13± 0.31
Experimental	5.18±0.34	3.58±0.29	3.18±0.19	3.02± 0.16

The prophylactic feed fed to the experimental animals reduced the cholesterol levels in their blood (31.8% at day 40) compared to Control 2. The cholesterol levels in the Experimental group was however, greater than Control 1 (> 66% at day 40) shown in Table 4.

Table 4 Cholesterol levels in the blood

GROUP n=8	DAY 7	DAY 14	DAY 28	DAY 40
	Level nMol/ml			
Check1	1.58 ±0.32	1.56 ± 0.52	1.71 ± 0.63	1.65 ± 0.37
Check 2	3.55 ± 0.65	3.77 ± 0.57	3.98 ± 0.76	4.03 ± 0.34
Experimental	3.80 ± 0.64	2.90 ± 0.61	2.80 ± 0.34	2.75 ± 0.22

The lipid content in the experimental group also decreased when compared to Control 2 (23.3%) but was higher than in the Control 1 group (>38%) (Table 5).

Table 5 Lipids content in the blood

GROUP n=8	DAY 7	DAY 14	DAY 28	DAY 40
	Content, g/l			
Check1	3.50 ± 0.18	3.72 ± 0.28	3.49 ± 0.21	3.40 ± 0.18
Check 2	5.07 ± 0.43	5.67 ± 0.41	6.09 ± 0.33	6.13 ± 0.25
Experimental	4.84 ± 0.37	5.24 ± 0.39	4.89 ± 0.43	4.70 ± 0.30

CONCLUSION

The introduction of a feed with an added prophylactic supplement (Experimental group) caused an increase in antioxidant activity, a reduction of lipid peroxidation and an improvement of carbohydrate metabolism in a diabetes induced rat model when compared with feed alone (Control 2). While none of these attributes returned to baseline when compared with non-diabetic rats (Control 1), the glucose and MDA levels decreased to normal and there was significant reduction in total lipid and cholesterol levels. The data indicates that the developed nutritional supplement normalizes or reduces diabetes induced biomarkers in a rat model and could be useful as a nutritional supplement for the control of diabetes in humans.

REFERENCES

1 Gurvich M.M. Nutritional care for patients with diabetes, M.: TERRA, 2007, 288 p.

2 Tarasenko N.A., Baranov Z.A. Meals as diabetes prevention factor. Proceedings of the higher educational institutions. Food technology, 2015, No: 5-6 (347-348), P 6-9.

3 Bobreneva I.V. On the question of the power of functional foods M.: Meat Industry, 2002. – № 11. – P. 12-14.

4 Miranda, DT Soy lecithin supplementation alters macrophage phagocytosis and lymphocyte response to concanavalin A: a study in alloxan-induced diabetic rats, DT Miranda, VG Batista, FC Grando, FM Paula CA, Felício, GF Rubbo, LC Fernandes, R. Curi, A. Nishiyama. Cell Biochem Funct. 2008 Oct 8.

5 Wilson, TA Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate, TA Wilson, CM Meservey, RJ Nicolosi. Atherosclerosis. 1998 Sep;140 (1):147-53.

6 Caballero-Córdoba, G. M. and Sgarbieri, V. C. (2000), Nutritional and toxicological evaluation of yeast (Saccharomyces cerevisiae) biomass and a yeast protein concentrate. J. Sci. Food Agric., 80(3) P. 341–351.

7 Hee Jeong Chae, Hyun Joo, Man-Jin In. (2001), Utilization of brewer's yeast cells for the production of food-grade yeast extract. Bioresource Technology, Vol. 76(3), P. 253–258.

8 Vieira E., Brandao T., Ferreira, Isabel M. P. L.V.O. (2013) Evaluation of brewer's spent yeast to produce flavor enhancer nucleotides: influence of serial repitching. J. Agric. Food Chem., 2013, 61 (37), P. 8724–8729.

9 Chen WY, Chen CJ, Liu CH, Mao FC. Chromium supplementation enhances insulin signalling in skeletal

muscle of obese KK/HlJ diabetic mice. Diabetes Obes Metab. 2009 Apr;11(4):293-303.

10 Rozanov G.N., Voevodin D.A., Logatchev M.F., Shiryaev T.Y. Application of dietary supplements in diabetes in children, Diabetes, 2000, No: 2.

11 Tagirova P.R., Kasyanov G.I. Nutritional supplements from the seeds and skins of grapes. Proceedings of the Kuban State University of Technology. 2015. No: 9. S. 281-296.

12 Manual P 4.1.1672-03. quality and safety control methods Manual of biologically active food additives, Ministry of Health of Russia, Moscow 2004.

13 Guidelines 2.3.1.2432-08. The norms of physiological requirements in energy and nutrients for different groups of the Russian population.

14 Nazarenko G.I, Kiskun A.A. Clinical evaluation of laboratory results. Medicine, 2006, 543 p.

15 Pimenova L.N. Fotokolorometriya. Methodical instructions on discipline "Physical and chemical methods of investigation", Tomsk: Publishing house. Tomsk State Architectural University, 2007, 22 p.

16 Clin. Lab., 1999., 45., P.617-622. LDL Cholesterol: Dont guss. Measure it. A critical examination of the Friedewald formula

17 Etuk, E.U. Animals models for studying diabetes mellitus, E.U. Etuk, Agric. Biol. J.N. am, 2010, 1 (2). P. 130-134.

18 Macedo C.S. Experimental model of induction of diabetes mellitus in rats, Plastic surgery, laboratory of plastic surgery: Sao Paulo, Paulista School of Medicine, 2005, P. 2-5.

19 Musina O.N., Lisin P.A. An approach to the choice of alternatives of the optimized formulations. Foods and Raw Materials. 2015. T. 3. № 2. P. 65-73.

20 Sadovoy V.V., Selimov M.A., Slichedrina T.V., Nagdalian A.A. Usage of biological active supplements in technology of prophilactic meat products. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016. T. 7. No: 5. P. 1861-1865.

21 Uchiyama M., Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 1978. Vol. 86, No: 1. P. 271–278.

22 Borovikov, V.N. Statistic: the art of data analysis on the computer. Professional, V. Borovikov, SPb .: Peter, 2001, 656 p.

23 Neural networks: STATISTICA Neural Networks: Per. from English, M.: Hotline, Telecom, 2001, 182 S. ISBN 5-93517-015-9.

24 Medical biological requirements and sanitary quality norms for food raw materials and food products, No: 5061-89, M.: 1990.

25 Sanitary Rules and Regulations 2.2.1.3218-14 «Sanitary and epidemiological requirements for design, equipment and maintenance of experimental biological clinics (vivariums), (SP 2.2.1.3218-14 “Unified Sanitary Epidemiological and Hygienic Requirements for Goods Subject to Sanitary and Epidemiological Control”)

26 Rules Laboratory Practice, Russian Ministry of Health Order N 199 n from 1 April 2016. (Rules of laboratorian practice// Order of Minister of Health of the Russian Federation N 199 from 1.04.2016)

27 Requirements of the International Science Committee on use of laboratory animals in experimental studies, Bulletin IKLAS, 1978, No: 24, P. 4-5.

28 Ministry of Health of the USSR Order No: 755 from 12.08.1977 “Rules of work with the use of experimental animals”.

29 Glebova Yu., Vertiychuk A. Study of the effect of cholesterol hens of eggs on people / Scientific works of Vinnytsia National Agrarian University. Series: Agriculture. 2014. № 2 (86). P. 104-109.

30 Food staples and foodstuff. Hygienic requirements of safety and nutrition value of foodstuff. Sanitary and epidemiologic rules and standards, SanPiN 2.3.2.1078-01.

31 Chesnokova N.P., Ponukalina E.V., Bizenkova M.N. Sources of free radicals and their importance in the biological system under normal conditions // Modern high technologies, 2006, No: 6, P. 28-34.

32 Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. Estimation of the normal range of blood glucose in rats, Wei Sheng Yan Jiu, 39(2), 2010, 133-137.

33 Lovric J, Mesic M, Macan M, Koprivanoc M, Kelava M, Bradamente V, Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods. Periodicum Biologorum, 110(1), 2008, 63-67.