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Study of the National Cooked Smoked Meat Products While Tests With Laboratory Animals at the Pathology Models With the Purpose to Confirm the Set of Biocorrective Features

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Abstract

Conducted physico-chemical and biological studies of Goji berries and national products made it possible to obtain information on the bioavailability of nutrients included in the product, to evaluate their food value and nutritional value, and their functional orientation. The study of the processes of digestibility and absorption of the developed products on the gastric invitro model and the monolayer of the intestinal wall epithelium showed high absorption and assimilability of the low molecular weight (from 30 kDa and below) protein fraction of the studied product. The detected acceleration of the absorption of nutrients of the tested products was reflected positively on the dynamics of changes in the biochemical composition of the blood, which, in turn, will lead to an acceleration of the biocorrecting action of functional biologically active substances. As a result of the experiment it was established that Goji berries, characterized by high catalase activity, have a prominent antioxidant effect, helping to control free radical oxidation in the rat organism with hyperlipidemia by decrease of MDA concentration (to 30%) and improvement of blood serum antioxidant activity (to 40 %). A prominent hipolipidemic (lipid-lowering) effect of berries has been established, which consists in normalization of the blood serum lipid profile of rats with hyperlipidemia 28 days after the beginning of feeding (decrease in total cholesterol (by 1.5 times), low density lipoproteins (to 40%) and blood serum atherogenity index (by more than 50%) with increase of high-density lipoproteins (up to 45%).

Article History

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Keywords

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Introduction

Currently the world population is about 6 billion people. Within 25 years according to FAOSTAT forecast the world will be inhabited by nearly 9 billion people. And all of these people will need food. Today much more attention is paid to the healthy life-style than before, and one of the main components of such life-style is healthy eating. In this connection the consumers pay attention not only to the taste and to the nutritive value of the product, but also to fat and cholesterol content, presence of vitamins, macro- and micro-elements[1].

Meat and poultry are the primary protein source in Western omnivorous diets. Intake of beef, pork, lamb and poultry is around 220, 275, 240 and 140 g/day/person in the US and Australia, Spain, UK and Norway, respectively (based on FAOSTAT protein intake data, 20% protein in lean meat). Trend analyses over the last decades indicate that the consumption of meat products in most European countries is relatively constant and that consumers are becoming more critical with regard to health and safety aspects of food in general, including meat products. As a consequence, the market segment labeled as 'light and healthy' is indeed the main segment of meat products that showed considerable growth during the last 10–15 years[2].

Chronic diseases (diabetes, cardiovascular disease and diabetes) contribute to 60% of all deaths in the world. Worryingly, their global prevalence is still increasing worldwide, creating a heavy economic burden for societies. It is well-known that nutrition is a major modifiable contributing factor to these diseases. Diet modifications have strong effects on health throughout life. They may not only influence current health but may determine whether or not an individual will develop chronic diseases much later in life. The benefit of consuming particular food products, such as fruits and vegetables, has largely been emphasized. There is strong scientific evidence in favour of a constructive affiliation between the intake of food mainly vegetables and fruits and the possible risk factors for the development of diabetes, cardiovascular diseases and cancer[3].

For assessment of nutrition value and functional tendency of the food products it is necessary to have the information on bioaccessibility of nutrients included in the product composition. The nutritive substance will manifest its directional effect only in the condition of bioavailability that is when it is released from food and efficiently ingested by the gut walls.

Digestion is the process of the substances hydrolysis to the assimilable form there of. Absorption is the process of substances admission from gastrointestinal tract lumen to blood channels.

The digestive system takes a central place in various processes associated with the circulatory, endocrine and nervous systems, and therefore it plays an important role in the food products and ingredients researches. The substances coming through the gastrointestinal tract, in the process of absorption appear in the blood circulatory system and spread throughout the entire organism. In its turn, from the blood circulatory system, the substances necessary for nutrition and secretion are supplied to the digestive organs[4].

Due to the complexity of the gastrointestinal tract, most studies do not consider complex approaches to modeling of the digestive system, i.e. they do not take into consideration the relationship with other systems and functional disorders of the organism. For more exact modeling, the approaches that reproduce not only the physicochemical conditions of all stages of digestion but also the presence of gastric and intestinal microbial flora are required. *In vitro* systems should be improved in such a way as to take into account the additional pathological and physiological disorders of human digestion. In recent years, much attention has been paid to application of invitro-invivo correlation (IVIVC).

In recent years, the study of the bioavailability of nutrients in the invitro model in the experiments on the cultures of $Caco_2$ cells in the Glahnetal modifications becomes more and more popular. And Laurentetal. Initially, $Caco_2$ cells have been extracted from human intestinal adenocarcinoma.

Shokhin and other have formulated the main advantages and disadvantages of intestinal permeability modeling on $Caco_2$ cells culture[5].

The advantages include: a high correlation level of the results with invivo data, the method rapidness, its low cost per one examination and absence of need in the highly qualified personnel.

The main disadvantage is limitation of modeling. The model does not reproduce all intestine conditions (the presence of bile, slimes and etc.). In addition, according to the opinion of Sun JinHur, who carried out meta-analysis of invitro models for digestibility study, the method of modeling with the cell culture till the recent time has been used for assessment of iron preparations absorption. In this connection it is interesting to try this method with protein products[6].

The purpose of this work is to study the processes of Goji berries digestibility and absorption while the long-term experiment with laboratory animals (186 days) invivo, and the product containing the berries with the Digestion model *in-vitro*.

Materials and Methods

The laboratory researches of test samples have been carried out in the scientific-research laboratory of the Gorbatov's All-Russian Meat Research Institute (Moscow). In order to make the test samples of the cooked smoked meat products, the meat of a two-year-old Kazakh two-humped camel was used, the camel meat was purchased from the camel breeding farm "Daulet-Beket" LLP. Horsemeat, beef and mutton has been purchased from the farm "Kainar" LLP. Fresh goji berries were purchased at the local market.

The test samples have been made in the scientific center for meat processing of Almaty Technological University (Almaty, Kazakhstan). A chilled horse meat, beef, camel meat and mutton first category of fatness was used.

The meat products were cooked according to traditional technology. The meat products was boiled in cooking-smoking chambers to a temperature in the centre of 74-75 ^{0}C for 2–2.5 h until the temperature at the centre of the product had reached 72 ^{0}C. The boiled product was chilled and smoked for 30 min at a smoke temperature of 40 ^{0}C. After cooking meat products was cooled down to 10–12 ^{0}C and was vacuum-packaged before sampling.

Meat products have been packed in polyethylene bags at vacuum (-1.0 bar) using a vacuum packaging Henkelman Boxer 42. Vacuum packed meat products have been examined upon expiration of 6 days.

Physico-chemical studies. State standard 9793-74 Meat products. Methods for determining moisture. State standard 23042-86 Meat and meat products. Methods for determining fat. State standard 25011-81 Meat and meat products. Methods for the determination of protein. State standard 31727-2012 (ISO 936: 1998) Meat and meat products. Method for determining the mass fraction of total ash. Meat and meat products. State standard 23041-78 Meat and meat products. Method for the determination of hydroxyproline. The content of tryptophan according to Zhuravskaya N.K.[12,13,14,15,16,17].

The fatty acid composition has been determined by lipids detachment in the samples by chloroform / methanol extraction using the Folch method. The purity of the extracted lipids has been checked by thin-layer chromatography. Determination of the fatty acid composition was made on a gas chromatograph HP 6890 "HewlettPackard"[21,22].

The content of phenolic compounds in Goji berries was determined according to method of Denisenko and other. The antioxidant and energy potential of berries was determined based on activity of catalase and superoxide dismutase, assuming the quantity of hydrogen peroxide (mmol) decomposed per 1 min with the addition of supernatant, obtained by extracting of 1 g of berries as the unit of catalase activity (E / g); and as the unit of superoxide dismutase activity (E / mg) the ability of supernatant to inhibit 50% of the pyrogallol auto-oxidation reaction is assumed[23,7,8].

Protein quality index (PQI) is determined by calculation of tryptophan content to hydroxyl-proline content ratio.

Absorbance has been determined at the monolayer of the $Caco_2$ (American Type Culture Collection (ATCCÒHTB-37TM) cell culture by content of substances before and after the monolayer passage. The sample (200 g) has been homogenized in the distilled water (200 ml). Then it has been cooked in microwave for 3 minutes at 1.5 minute intervals.

It has been homogenized after heat treatment and crushed, lyophilized, then lyophilizate has been stored at minus 20C till tests commencement. 9 g of lyophilizate was incubated in 0.01 mol / LHCl solution at 37 °C, and then it was followed by centrifugation at 4000 g. Above the sediment liquid was passed through the column with Sephadex G-25, the fractions were collected for investigation as described (EunChul Huh, 2014)[9].

Digestibility in vitro has been determined by successive impact on protein substances of the studied object with proteinases a system consisting of pepsin and trypsin, with continuous removal of the hydrolysis products from reaction medium by dialysis according to the method of Pokrovskiy and Yertanov in the modification of the Gorbatov's All-Russian Meat Research Institute[10].

Study of the functional properties of Goji berries in long-term experiment with laboratory animals has been carried out on the basis of the Minutes of the meeting of the Bioethics Commission of the Gorbatov's All-Russian Meat Research Institute[11,20].

Modeling of hyperlipidemia and atherosclerosis: The animals of the experimental and control groups are kept on a balanced diet, calculated based on weighing results, the lipid component is mixed to the ration (alternation of melted pork and lamb fat, and cholesterol). In a day, the animals of the experimental and control groups are orally administered with vitamin D2 in quantity of 35000 ME / kg of body weight. Intact animals for the entire experiment period take the general vivarium ration, calculated based on the weighing results.

The experiment studying biological activity of Goji berries was carried out on male rats of Wistar stock age of 10-12 months (380 ± 20) g, obtained from Andreyevka branch of FGBNU "SCBMT" FMBA of Russia named after V.M. Gorbatov" (Federal State Budget Scientific Institution "Scientific Center of Biomedical Technologies" of Federal Medical Biological Agency of Russia). After 5 days quarantine, the rats have been randomly devided to intact (n = 10), throughout the entire experiment taking a balanced diet (full feed mixed fodder according to GOST R 50258) and the trial ones exposed to modeling of alimentary hyperlipidemia (AH).The variation in the group was (± 10) g. AH was reproduced by enriching of the ration with cholesterol in the quantity of 0.5-2.0%, animal fat 5-20%, and vitamin D2 in the quantity of 35,000 IU / kg of weight for 100 days[12,19].

After AH modeling, the trial rats have been randomly divided to two groups: the 1st group consisted of control rats with AH, during the subsequent period taking the balanced ration (control), the 2nd group – the ration of which included Goji berries or a meat product with Goji berries in the quantity of 20% of carbohydrate or 25% of the protein component of the ration.

For 86 days the rats have been kept in standard conditions of vivarium, the animals have been provided with food and water adlibitum.

The experiment has been carried out in compliance with the requirements of the Order of the MHC RF "On Approval of Laboratory Practice Regulations" (№ 267 as of 19.06.2003), of the Declaration of Helsinki (2000), European Community Guidelines 86/609EEC[21].

On the 128-th and 186-th day of the experiment the animals have been exposed to euthanasia in CO_2-chamber (VetTech, United Kingdom) and took blood samples from the right ventricle of heart.

A general clinical study of blood samples was carried using an automatic veterinary hematological analyzer Abacusjuniorvet 2.7 with reagent sets of Diatron company (Diatron Messtechnik GmbH, Austria), 18 indicators were determined. Biochemical researches have been carried out using an automatic biochemical analyzer BioChem FC-360 (USA), using the reagents sets of HighTechnology (USA).

Biochemical study of blood serum has been made on a BioChem FC-360 analyzer (HTI, USA), in accordance with the methods attached to the reagents (HTI, USA). The serum atherogenicity index (IA) was determined by calculation: IA = (OXC-XC HDLP) / HDL cholesterol.

The antioxidant activity (AOA) of blood serum was determined by recording of the oxidation rate of

the reparative form 2,6-dichlorophenolindophenol (2,6-DCPIP) with oxygen dissolved in the reaction medium on a BioChem SA photometer (HTI, USA). The AOA indicator was the inhibition constant value (Ci) of the auto-oxidation of 2,6- DCPIP. The content of malondialdehyde (MDA) in the blood serum was determined by the color reaction with 2-thiobarbituric acid (2-TBA) on the photometer KFK-3-01 (Russia).

The statistical processing was made using the STATISTICA 10.0 software package. The results are presented in form of "the weighted average ± standard deviation" (M ± m), "interquartile range" (P25-P75), minimum (Min) and maximum (Max) values. The reliability of the differences in the average values, satisfying the conditions of normal distribution and the equality of the dispersions has been estimated by the single-factor dispersion analysis (ANOVA) using Duncan criterion. The critical significance level of the null statistical hypothesis (p) was assumed to be 0.05.

Results

The Results of Physical and Chemical Researches of the National Meat Products are provided in Table 1.

Table 1: Content of Main Macronutrients in the National Kazakh Meat Products

Sample No.	Moisture,% state standard 9793	Fat, % state standard 23042	Protein, % state standard 25011	Ash content,% state standard 31727
Boiled and smoked meat product from horse meat	49,5±0,2	25,7±2,1	20,4±0,1	1,44±0,20
Restructured boiled-smoked meat product from camel meat	66,3±0,1	11,2±1,7	19,1±0,2	2,91±0,39
Smoked boiled-smoked mutton meat product	65,0±0,1	14,2±2,0	15,3±0,1	1,89±0,26
Smoked-boiled whole-muscular meat product from camel	70,35±0,15	2,2±0,3	21,6±0,1	3,83±0,50

The table analysis showed that the cooked smoked meat product made of lamb have the lowest protein content. The calculated indices of the energy value per 100 g of product constituted 312.9; 177.2; 189, 0 and 106.2 kcal, which is primarily conditioned by high fat content and low moisture in the horse meat product. More comparable results of the energy value of the product can be obtained on dry basis: 619.6; 525.8; 540 and 358.2 respectively.

The amino-acid product composition evidences their high biological value as shown in Figures 1-4. Protein quality index values constituted 5.57; 5.11; 5.65 and 9.81 respectively. Surprisingly that the lowest hydroxyproline content was detected in the camel meat, 0.048%, which is 1.6 times lower than in whole-muscle products made of horse and lamb meat, while these products did not differ significantly by tryptophan content.

In general, it is necessary to mention that the product made of mutton muscle tissue has lower essential amino-acids content than other products. The share thereof is 33.3% against 38.5 and 38,3 in whole-muscle products made of horse and camel meat, respectively.

The results of the meat products digestibility research are shown in Fig. 5. Nutrient attack by enzymes of the gastrointestinal tract in the invitro experiment simulate the conditions of food product digestion first in the stomach (pepsin, pH 2.3) and then in the duodenum (trypsin, pH 7.8), in the process of which nutrients, including biologically active low-molecular compounds, are released.

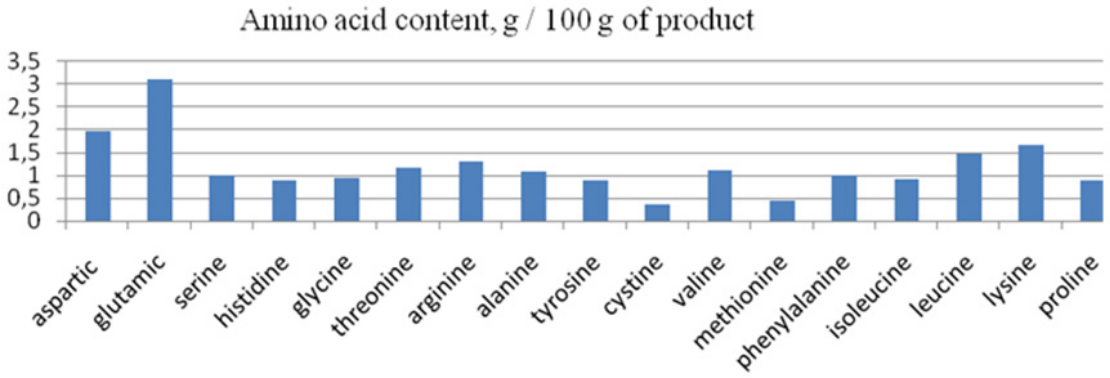

Fig. 1: Amino acid profile of the national meat product from horse meat

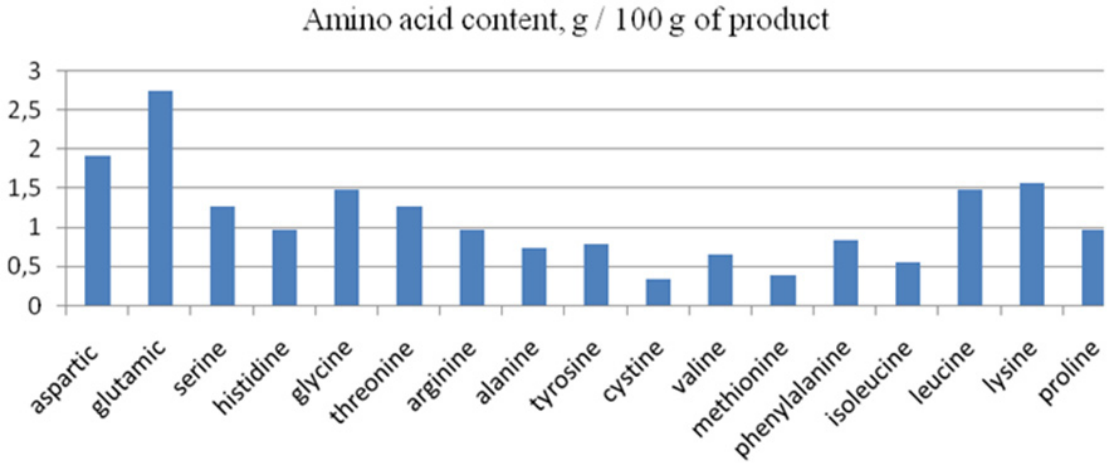

Fig. 2: The amino acid profile of the national meat product from camel (re-structured)

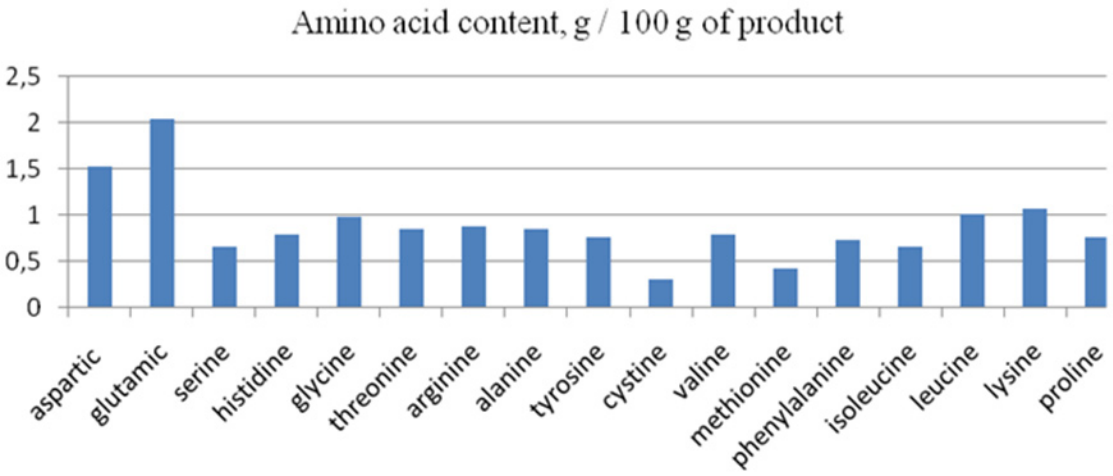

Fig. 3: Amino acid profile of the national meat product from lamb

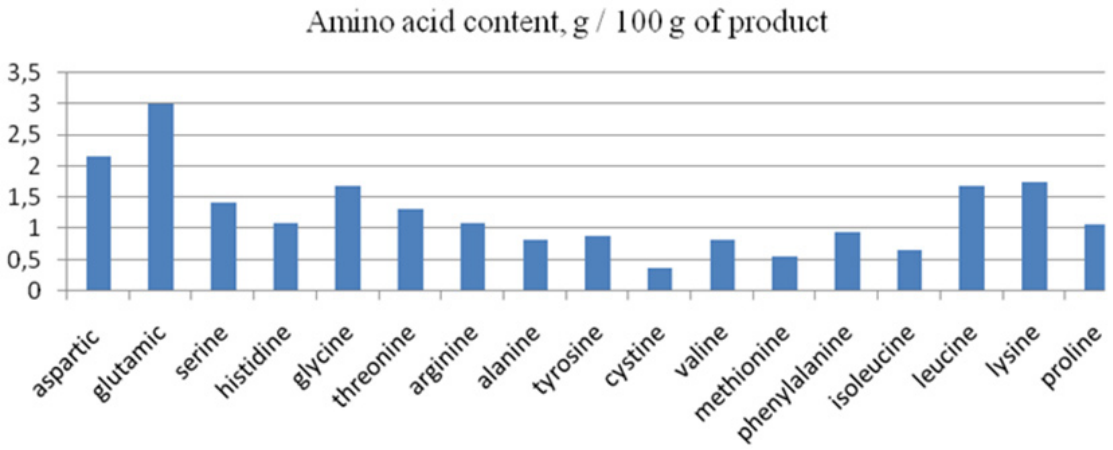

Fig. 4: Amino acid profile of the national meat product from camel (whole-muscular)

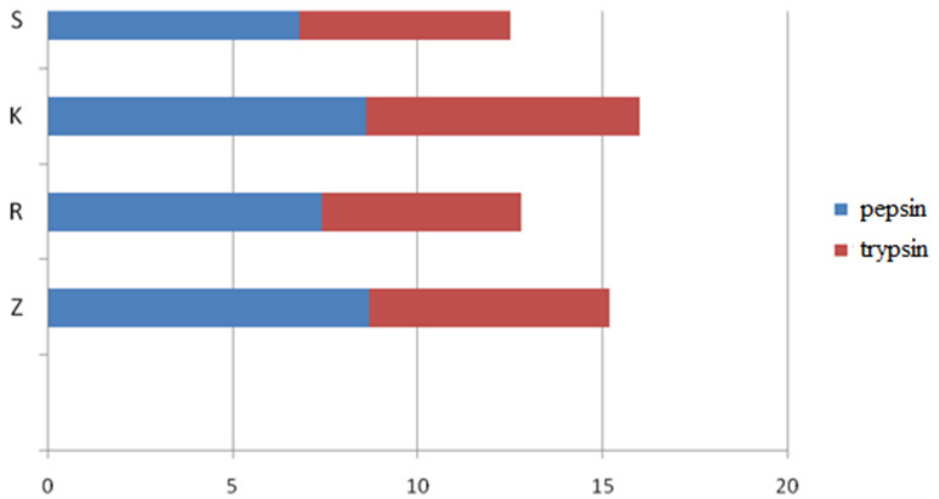

Fig. 5: meat Product Protiens Attack by Enzymes of the Gastrointestinal Tract (Digestibility), Z - boiled smoke whole-muscular lamb meat product with the addition of Goji berries; R - boiled smoke whole-muscular meat product from camel meat; K - Restructured boiled-smoke meat product from camel with addition of Goji berries; S - boiled-smoke whole-muscular meat product from horse meat

The process of digestion is influenced by various factors, in particular, the product fat content. Therefore, the horse meat products, characterized by a significant fat content, digestibility is lower, on average by 23.7%, if compared to other whole-muscle products.

Pepsin, first of all, affects the muscle proteins - myosin, myogen, albumins and globulins, splitting them by peptide bond with increase of free carboxyl and amine groups quantities. Pepsin mainly impacts the internal peptide bonds. It is interesting that pepsin splits the protein molecule into approximately equal length particles. Pepsin actively hydrolyses peptide bonds between aromatic amino acids and worse – between leucine and dicarboxylic amino acids. The maximum content of aromatic amino acids is in the horse meat product, 2.24 mg / 100 g of product, which is 1.2 times higher than in the mutton product.

The active form of trypsin is formed in the intestine with participation of enteropeptidase enzyme, secreted by intestinal cells. Enteropeptidase segregates

trypsinogenasexapeptide from N-terminal, resulting in change of the molecule conformation and generation of an active trypsin center. Trypsin acts similarly to pepsin, breaking the internal, that is, distant from the terminal peptide bonded proteins to polypeptides of the average length.

The obtained results evidence high digestibility of the studied products, which is important for assessing of availability thereof for different population groups, for example, aged people, for sarcopenia prophylactics. It is known that entry of even insignificant quantities of protein molecules into the bloodstream can result in organism allergic reactions. There is also the assumption that the intake of undigested protein to large intestine can provoke tumors (carcinogenesis)[24].

Study of the digestibility and absorption of Goji berries in a long-term experiment showed the following. Hematological analysis of whole blood (Table 3) of controlled rats (2nd group) on the 128th day of the experiment revealed the poor-quality data on increase of leukocytes concentration by 18%, in comparison with the intact (1st group), mainly due to monocytes - by 4, 8 times (P<0,05) and granulocytes – by 1.8 times (P<0,05). At the same time, the reliable significant decrease of lymphocyte content by 42% has been noted. On the 186th day the control animals had the leukocytes and lymphocytes concentration, which did not much differ from the indices of the intact group, but increase in the monocyte and granulocyte content by 2.9 times (P<0,05) has been observed. It is necessary to mention that on the 128th and 186th days, the share of lymphocyte has significantly decreased by 26% and 32%, while proportional increase of monocytes (by 6.4 and 2.2 times) and granulocytes (by more than 2 times, P<0,05), respectively, has been noted in comparison with that of the intact animals.

Introduction of Kazakh Goji berries into the ration for 28 days contributed to a statistically significant decrease of monocytes concentration (up to 90%) and granulocytes (up to 60%) in comparison with the indices of control rats from the 2nd group. In such case, in comparison the intact group indices, decrease of lymphocytes concentration to 30% (P<0,05). On the 186th day, the rates of the 3rd group showed significant decrease of monocyte concentration (by 74%) and granulocytes (by 55%) in comparison with the control group, the concentration of leukocytes and lymphocytes had not changed significantly. From the part of relative leukocytes content in the blood of the rats of the 3rd group, in comparison with control rats of the 2nd group, on the 128th and 186th days, the increase of lymphocyte share (by 35% and 36%, P<0,05) along with a statistically significant decrease of monocytes share (by 86% and 73%) and granulocytes (by 46% and 37%) has been detected.

The ratio of granulocytes to lymphocytes (GRAN/LYM) on the 128th and 186th days with control animals significantly exceeded the indices of intact rats by 3 and 3.4 times, respectively, while the animals of the 3rd group fed by Goji berries, this index decreased in comparison with the control group by 60.7% and to 50% (P<0,05) (Table 3).

Table 2: Hematological Indices Characterizing Condition of the Experimental Animals Leukocytes

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
	128 days		
Leucocytes, 109/l	10,64±0,25	8,73±1,66	7,63±0,92
Lymphocytes, 109/l	8,79±0,20	5,14±0,97*	6,16±0,81*
Monocytes, 109/l	0,08±0,01	0,38±0,11*	0,04±0,00#
Granulocytes, 109/l	1,78±0,09	3,21±0,67*	1,43±0,21#
Lymphocytes, %	82,63±0,70	60,86±2,71*	82,08±2,97#
Monocytes, %	0,66±0,07	4,24±1,06*	0,58±0,02#

Monocytes, %	0,66±0,07	4,24±1,06*	0,58±0,02#
Relative content of granulocytes,%	16,70±0,70	34,90±1,96*	19,03±1,95#
GRAN/LYM	0,20±0,01	0,61±0,07*	0,24±0,03#
	186 days		
Leucocytes, 109/l	6,75±1,07	8,93±1,05	9,66±2,97
Lymphocytes, 109/l	5,16±0,89	4,61±0,46	7,77±2,99
Monocytes, 109/l	0,20±0,04	0,58±0,10*	0,15±0,03#
Granulocytes, 109/l	1,36±0,18	3,88±0,68*	1,75±0,28#
Lymphocytes, %	77,63±1,14	52,59±3,29*	71,72±6,81#
Monocytes, %	2,89±0,52	6,50±1,48*	1,75±0,41#
Relative content of granulocytes,%	19,49±0,80	42,34±3,83*	26,52±6,58#
GRAN/LYM	0,25±0,01	0,85±0,11*	0,43±0,13#

Note, henceforward: * - significant difference from intact group,
- significant difference from control group.

Analysis of hematological indices characterizing the erythrocytes condition of the experimental animals (Table 4) showed that on day 128 the control animals of the 2nd group, the concentration of erythrocytes, hemoglobin, hematocrit level, the average erythrocyte volume, hemoglobin content in erythrocyte and the width of erythrocytes distribution has not significantly changed. At the same time, the significant decrease of hemoglobin content in erythrocytes by 5% in comparison with that of the intact rats of the 1st group. On day 186 the rats of control group showed that concentration of erythrocytes, hemoglobin, the average erythrocyte volume and hemoglobin content in the erythrocyte has not change significantly, but a statistically significant increase of hematocrit level concentration (by 10%) and the width of erythrocytes distribution (by 9%), in comparison with the intact animals, subject to decrease of hemoglobin content in erythrocytes by 5% (P<0,05).

The animals that eaten Goji berries for 28 days had the concentrations of erythrocytes, hemoglobin, hematocrit level, average erythrocyte volume, hemoglobin content in erythrocyte and the width of erythrocyte distribution that do not differ significantly from the indices of the control and intact group, but increase of hemoglobin content in erythrocytes by 3,4% (P<0,05) in comparison with the indices of the control group. 86 days after commencement of Goji berries entering into the ration, the rats of the 3rd group, in comparison with the intact and control groups, demonstrated a significant decrease of erythrocytes concentration (by 14% and 19%), hemoglobin (by 18% and 21%) and hematocrit level (to 17% and 24%). In this connection the average erythrocyte volume and erythrocyte distribution width decreased in comparison with that of the control group by more than 5% (P<0,05), and the average concentration of hemoglobin in erythrocytes, on the contrary, has significantly increased by 3.4%.

On the 128th day of the experiment, the animals of the 2nd group showed a 15% decrease (P<0,05) in comparison with that of the intact group, the average volume of platelet and platelet distribution, to the contrary, have significantly increased by 9% and 3%, respectively. On day 186 platelet concentration in the blood of control rats, plateletcrit level, the average platelet volume and platelet distribution have increased up to 15% (low-quality data), 30% (P<0,05), 14% (P<0,05), and 6% (P<0,05), respectively, in comparison with the intact animals.

A significant decrease in the platelet concentration (by 35%) and the plateletcrit level (to 30%) has been established in the indices of the animals that eaten Goji berries for 28 days after alimentary hyperlipidemia (AH) development, subject to significant increase of the average platelet volume (by 9%) and platelet distribution (by 4%), in comparison with the indices of the intact animals. It is important to mention that the animals from the 3rd group had platelet concentration and plateletcrit level decreased by more than 20% (P<0,05) in comparison with that

of the animals from control group. On the 186th day of the experiment, a statistically significant decrease of platelet concentration (by 36%), plateletcrit level (by more than 40%), average platelet volume (to 9%) and platelet distribution by 3% has been detected in the blood of the 3rd group animals in comparison with that of the control group.

Table 3: Hematological Indices Characterizing Condition of the Experimental Animals Erythrocytes

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
128 days			
Erythrocytes, 1012/ l	7,87±0,16	8,09±0,18	7,55±0,28
Hemoglobin, g/ l	144,1±1,9	140,3±3,3	131,0±3,7
Hematocrit, %	40,31±0,70	41,39±1,15	37,34±1,01
The average volume of erythrocyte, mkm3	51,20±0,29	51,33±0,69	49,83±1,42
The average content of hemoglobin in erythrocyte, пг	18,32±0,17	17,33±0,20	17,40±0,28
The average concentration of hemoglobin in erythrocytes, g/l	357,7±2,8	339,3±2,7*	350,8±5,2#
Width of distribution of erythrocytes, %	16,59±0,26	16,43±0,15	16,50±0,28
186 days			
Erythrocytes, 1012/ l	8,05±0,08	8,59±0,21	6,95±0,75*,#
Hemoglobin, g/ l	135,8±0,4	141,0±4,1	111,2±10,2*,#
Hematocrit, %	39,70±0,18	43,35±1,39*	33,12±3,30*,#
The average volume of erythrocyte, mkm3	49,30±0,60	50,38±0,86	47,83±0,70#
The average content of hemoglobin in erythrocyte, пг	16,91±0,20	16,41±0,30	16,13±0,34
The average concentration of hemoglobin in erythrocytes, g/l	342,7±2,0	325,6±1,5*	336,7±3,3#
Width of distribution of erythrocytes, %	15,91±0,24	17,33±0,55*	16,40±0,15#

Table 4: Hematological Indices Characterizing Condition of the Experimental Animals Platelets

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
128 days			
Platelets, 109/l	1020,3±35,4	863,3±32,9*	666,2±34,9*,#,+
Thrombote, %	0,66±0,03	0,61±0,02	0,47±0,02*,#
Average platelet volume, mkm3	6,42±0,10	7,02±0,09*	7,02±0,23*
Distribution of platelets,%	32,36±0,31	33,38±0,29*	33,62±0,39*
186 days			
Platelets, 109/l	883,3±23,1	1014,6±55,7	651,7±65,6*,#,+
Thrombote, %	0,58±0,02	0,75±0,08*	0,44±0,05#,+
Average platelet volume, mkm3	6,50±0,06	7,39±0,13*	6,75±0,04*,#
Distribution of platelets,%	32,45±0,34	34,40±0,40*	33,3±0,32#

Upon analysis of biochemical parameters charac terizing protein metabolism of rats (Table 6), no statistically significant differences have been detected in the experimental groups with respect to total protein and albumin on the 128th day of the experiment. The control rats in comparison with the intact rats have statistically significant increase in creatinine (by 8%) and a decrease in urea (by 21%) providing that on the 186th day of the experiment decrease of total protein (to 10%) has been observed. The rats having Goji berries in ration for 28 days, in comparison with the intact rats, show a significant decrease of urea concentration by 24% with creatinine increase by 7%. On the 186th day a significant decrease of total protein to 8%, with an increase in urea by 15% has been noted in the indices of the rats of the 3rd group.

Table 5: Biochemical Indices, Characterizing the Protein Metabolism Condition of the Experimental Rats

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
128 days			
Total protein, g/l	71,34±1,81	71,68±0,95	70,83±0,28
Albumen, g/l	39,48±0,61	37,97±1,72	38,87±1,92
Creatinine, mmol / l	41,40±0,62	44,67±0,83*	44,50±0,87*
Urea, mmol / l	6,99±0,32	5,52±0,14*	5,31±0,18*
186 days			
Total protein, g/l	75,42±1,03	68,45±1,20*	70,80±0,46*
Albumen, g/l	40,98±0,93	36,63±2,56	39,60±1,88
Creatinine, mmol / l	49,70±0,62	49,63±1,98	47,67±0,44
Urea, mmol / l	5,41±0,11	5,98±0,14	6,20±0,63*

It was determined that on the 128th day, the control group animals had glucose concentration increased by 52% (low-quality data) in comparison with the intact animals, while the activity of amylase, to the contrary, decreased by 32.5% (P<0,05). Introduction of Goji berries to the animals ration for 28 days has not contribute to decrease of glucose concentration (it exceeded the intact level by 82%, P<0,05). It is interesting that activity of amylase in the blood serum of the 3rd group animals has significantly increased in comparison with that of the control group by 43% (Table 7). On the 186th day, the glucose concentration in the blood of the 2nd and 3rd group has not statistically changed, while the activity of amylase in the blood of control rats decreased by 28% (P<0,05). It is interesting, that the activity of amylase in the blood serum of animals that eaten Goji berries for 86 days also exceeded the control values by 30%, but it is unreliable data. Because, there is not enough statistical data to confirm.

Table 6: Biochemical Indices, Characterizing Carbohydrate Metabolism Condition of the Experimental Rats

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
128 days			
Glucose, mmol / l	12,90±1,26	19,63±2,55*	23,50±5,24*
Amylase E / l	612,0±14,6	412,8±31,8*	591,33±178,7#
186 days			
Glucose, mmol / l	14,20±2,73	14,25±1,34	14,33±2,80
Amylase E / l	416,4±16,0	301,8±33,3*	392,3±67,5

Analysis of lipid metabolism indices (Table 8) of control animals revealed increase of cholesterol concentration (by 60%, P<0,05) and triglycerides (by 24%, unreliable) on the 128th day compared to in comparison with that of the intact animals. In addition, we noted that the animals of the 1st group, in comparison with the intact group animals, had redistribution of lipoprotein fractions towards a significant increase in Low Density Lipoprotein cholesterol (LDL) (over 50%) and non-LDL and non-LDL cholesterol (by 2.5 times). On the 186th day in the animals of the control group cholesterol concentration exceeded the intact level by 46% (P<0,05), LDL cholesterol by 16% (unreliable), non-LDL and non-LDL cholesterol by 2.8 times (P<0,05).

Introduction of Goji berries into the ration for 28 days contributed to decrease of cholesterol concentration (by 44%, P<0,05) and triglycerides (by 12%, unreliable) in comparison with that of the control group. The HDL cholesterol did not differ significantly from the indices of the control and intact groups, in this connection significant decrease in LDL cholesterol concentration (by 36%) and non-LDL cholesterol and non-LDL (by 3.4 times) has been established in comparison with that of the control group. It is interesting to mention that the rats of this group on the 186th day showed statistically significant increase of HDL cholesterol levels (to 45%) in comparison with the intact and control groups.

Redistribution of lipoprotein fractions affected the atherogenicity index: on the 128th day of the experiment, the animals of the 1st group had the indices exceeding that of the intact animals by 77% (P<0,05), on the 18th day – by 2.1 times (P<0,05). Introduction of Goji berries to the ration of animals decreased their atherogenicity index on the 128th day by 42% (P<0,05), on the 186th day –by 53% (P<0,05), respectively.

Table 7: Biochemical Indices Characterizing Lipid Metabolism Condition of the Experimental Rats

Indicators	Group of animals			
	1 (intact)	2 (control)	3(Goji berries)	4meat product
128 days				
Cholesterol, mmol / l	1,64±0,20	2,63±0,10*	1,47±0,09#,+	1,68±0,11#,+
Triglycerides, mmol / l	1,74±0,32	2,16±0,54	1,90±0,59	2,09±0,49
LDL cholesterol, mmol / l	0,63±0,03	0,95±0,04*	0,61±0,07#,+	0,68±0,10#,+
HDL cholesterol, mmol / l	0,61±0,06	0,67±0,05	0,56±0,09	0,64±0,09
Cholesterol non-LDL and non-HDL, mmol / L	0,40±0,13	1,01±0,03*	0,30±0,09#,+	0,54±0,11#,+
atherogenicity index	1,68±0,15	2,97±0,13*	1,71±0,30#	1,62±0,38#
186 days				
Cholesterol, mmol / l	1,76±0,11	2,58±0,19*	2,48±0,42	2,36±0,33#,+
Triglycerides, mmol / l	1,38±0,11	1,28±0,42	1,40±0,06	1,23±0,19
LDL cholesterol, mmol / l	0,62±0,04	0,72±0,05	0,90±0,19*,+	0,78±0,07#,+
HDL cholesterol, mmol / l	0,69±0,04	0,61±0,07	0,98±0,15*,#	0,86±0,05
Cholesterol non-LDL and non-HDL, mmol / L	0,45±0,04	1,26±0,14*	0,60±0,19#	0,74±0,08#,+
atherogenicity index	1,57±0,07	3,34±0,30*	1,58±0,49#	1,74±0,30#

On the 128th day the rats of the 2nd group, alanine aminotransferase (ALT) and alkaline phosphatase activity has not changed significantly in comparison with the intact animals. In this case, the activity of aspartate aminotransferase (ACAT), Gammagrutanil transferase (GGT) and lipase in control rats increased by 34% (P<0,05), 46% (unreliable), and by 2.4 times (P<0,05), respectively, in comparison with the intact animals. Introduction of Goji berries to the ration for 28 days promoted increase in ALT activity (by 70%

and more than 50%, P<0,05), alkaline phosphatase (by 33%, unreliable, and by 32 %, P<0,05) and GGT (by 67%, P<0,05 in comparison with the intact group) in comparison with the intact and control groups. At the same time some decrease of ACAT activity (by 11%, unreliable), in comparison with that of the control group. The lipase activity exceeded the intact levels by more than 2 times (P<0,05). No significant changes in lactate dehydrogenase (LDH) and creatine phosphokinase (CKF) activity of the animals from all experimental groups have been noted.

On the 186th day of the experiment, ALT and GGT activity of the indices of the experimental animals of 2-3 groups has not changed significantly in comparison with that of the intact group. The control rats showed significant increase of ACAT activity (by 72%), alkaline phosphatase (to 30%), CKF (by 2.1 times) and LDH (75%, unreliable)

Introduction of Goji berries to the ration for 86 days promoted insignificant decrease of ACAT activity of by 18%, LDH to 50% and CKF to 10%, however, alkaline phosphatase activity statistically significantly exceeded the intact indices by 32%, in comparison with the indices of the controlgroup.

Table 8: Experimental Rats Blood Serum Enzymes Activity

Indicators	Group of animals		
	1 (intact)	2 (control)	3(Goji berries)
128 days			
ACAT, E / l	87,2±8,3	117,3±5,5*	104,3±9,0
ALT, E/l	48,20±1,20	53,67±3,29	82,00±15,5*,#
Alkaline phosphatase, E/l	171,7±10,1	173,7±16,9	229,1±33,4
GGT, E/l	1,80±0,44	2,63±0,35	3,00±0,40*
Lipase, E/l	73,4±11,3	173,1±33,4*	152,1±53,3*
Creatine kinase, E/l	503,2±92,3	584,2±35,2	446,2±115,2
LDH, E/l	231,8±41,7	297,0±24,1	303,8±57,4
186 days			
ACAT, E / l	62,3±3,6	107,2±22,0*	87,47±4,72
ALT, E/l	35,40±2,38	37,50±7,19	38,00±6,00
Alkaline phosphatase, E/l	124,9±10,3	160,8±11,1*	166,0±14,2*
GGT, E/l	2,98±0,14	2,49±0,44	2,41±0,70
Lipase, E/l	189,0±52,5	330,7±57,2*	299,2±28,9
Creatine kinase, E/l	160,5±22,6	335,7±77,1	178,1±32,1

On the 128th day no significant changes in concentrations of potassium, calcium, chlorine, phosphorus and magnesium have been detected in the indices of animals of all experimental groups. On the 186th day animals of all experimental groups have shown no significant changes in calcium concentrations. The animals of the 2nd group, in comparison with intact animals, demonstrated significant increase in chlorine content by 4%, the content of potassium, phosphorus and magnesium has not changed significantly. Introduction of Goji berries into the ration contributed to a significant decrease of potassium concentration (by 20%), chlorine (by 3%), phosphorus (by 14%) and magnesium (by 30%) in comparison with control group animals (Table 10). The blood serum of animals, having meat product in the ration, was characterized by lower calcium content than that of animals from other groups, but the differences were equivocal.

On the 128th day the control animals of the 2nd group, in comparison with the intact animals, showed increase in malonic dialdehyde (MDA)concentration (by 25%, P<0,05) and a decrease in antioxidant activity of blood serum (by 2.1 times, P<0,05)

(Table 12). On the 186th day the rats of the control group, in comparison with the intact group, showed increase of MDA concentration by 2.2 times (P<0,05), accompanied by decrease in antioxidant activity of blood serum by 56% (P<0,05).

Table 9: Assimilability of Mineral Substances, Determined on the Basis of the Mineral Balance of the Experimental Rats Blood Serum

Indicators	Group of animals			
	1 (intact)	2 (control)	3(Goji berries)	4meat product
128 days				
Potassium, mmol / l	6,02±0,38	6,01±0,15	6,46±0,65	6,12±0,25
Calcium, mmol / l	3,80±0,10	3,83±0,18	3,77±0,57	2,87±0,62
Chlorine, mmol / l	95,60±1,86	99,50±1,28	97,67±1,20	95,67±1,68
Phosphorus, mmol / l	2,70±0,23	2,32±0,08	2,67±0,37	2,71±0,21
Magnesium, mmol / l	1,32±0,08	1,31±0,06	1,13±0,03	1,26±0,05
186 days				
Potassium, mmol / l	6,21±0,36	6,65±0,29	5,30±0,41	5,96±0,56
Calcium, mmol / l	3,24±0,07	3,28±0,05	3,10±0,10	2,98±0,06
Chlorine, mmol / l	98,40±1,21	102,00±0,71	99,00±0,58	101,00±0,83
Phosphorus, mmol / l	2,54±0,10	2,65±0,10	2,27±0,15	2,63±0,11
Magnesium, mmol / l	1,46±0,11	1,37±0,16	0,96±0,05	1,34±0,09

Table 10: Indices of Antioxidant-Energy Potentials of Berries *in vitro*

Indicator name	Goji berries
The content of phenolic compounds, mg / 100 g	142±12,6
Catalase activity, E / g	27,69±5,07*
Activity of superoxide dismutase, E / mg	144,14±15,64
Antioxidant activity, Ki*l/(1000*ml*min)	7,02±0,81

Discussion

Entering into the ration of the animals of the 3rd group of Goji berries contributed to decrease of MDA concentration by 43% (P<0,05) in comparison with that of the control group and by 30% (P<0,05) in comparison with that of the intact group, the antioxidant activity of the blood serum also increased, exceeding the control values by 44% (P<0,05), but was lower than the same index of the intact animals by 32% (P<0,05). On the 86th day the rats of the 3rd group had decrease of MDA concentration by 13% (P<0,05), in comparison with that of the control group, but this index exceeded the intact group indices by 94% (P<0,05). The antioxidant activity of the blood serum of animals having Goji berries in their ration for 186 days has also increased, exceeding the control values by 8.5% (unreliable), decreasing by 2.1 times (P<0,05) in comparison with that of the intact animals.

Thus, as a result of the experiment it was established that Goji berries, characterized by high catalase activity, have a prominent antioxidant effect, helping to control free radical oxidation in the rat organism with hyperlipidemia by decrease of MDA concentration (to 30%) and improvement of blood serum antioxidant activity (to 40%). A prominent hipolipidemic (lipid-lowering) effect of berries has been established, which consists in normalization of the blood serum lipid profile of rats

with hyperlipidemia 28 days after the beginning of feeding (decrease in total cholesterol (by 1.5 times), low density lipoproteins (to 40%) and blood serum atherogenity index (by more than 50%) with increase of high-density lipoproteins (up to 45%).

Biochemical indices of lipid and carbohydrate metabolism showed positive dynamics in the digestibility of food rations on the protein and carbohydrate basis. The acceleration of the tested products nutrients absorption has been detected, which affected the positive dynamics of the biochemical blood composition. Since it is known that nutrients are delivered to the target organs spreading in the bloodstream with blood, it can be assumed that acceleration of the tested products absorption will lead to acceleration of the biocorrecting effect of the functional biologically active substances.

Conclusion

The delicate mechanisms of some proteins synthesis and decomposition regulation have not yet been established, however, general trends and distinctive features are being studied. For example, pepsin actively hydrolyses peptide bonds between aromatic amino-acids (phenylalanine, tryptophan, tyrosine) and worse – between leucine and dicarboxylic amino-acids. The obtained results testify to high digestibility of the studied products, which is important for assessment of availability thereof for various population groups, for example, the aged people for sarcopenia prevention.

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Conflict of Interest

The authors have no conflict of interest to any financial, personal or other relationships with other people or organizations that can influence their work.

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