PRECLINICAL STUDIES OF KEFIR PRODUCT WITH REDUCED ALLERGENICITY OF β-LACTOGLOBULIN

R. P. Korzhov, A. N. Ponomarev, E. I. Melnikova, E. V. Bogdanova*

Voronezh State University of Engineering Technologies,
Revolution Avenue 19, Voronezh, 394036, Russian Federation

* e-mail: ek-v-b@yandex.ru

Received April 30, 2015; Accepted in revised form June 15, 2015; Published October 20, 2015

Abstract: The most promising approach for reducing the allergenicity of milk products with high biological value is biocatalytic conversion of whey proteins, producing hydrolysates with the specified molecular-mass distribution and residual allergenicity. The purpose of this study was to evaluate the biological effect of kefir product produced with the use of whey protein hydrolysate. The experimental works were conducted at the A.N. Bach Institute of Biochemistry of Russian Academy of Sciences. The objects of the studies were males of Brown Norway rats of BN/SsNolaHsd line with RT1n haplotype with initial mass of about 150 g, which had been obtained from the centre for Laboratory Animal Breeding of Harlan Laboratories, Inc. Company and had passed quarantine for at least 3 weeks after delivery. A model for testing the bio-functional properties of fermented milk products on the basis of enzymatic whey protein hydrolysates was created. Its essence lies in the induction of IgE-mediated allergic reaction to antigens of dairy products by oral administration with adjuvant – chlorea toxin that increases the permeability of the intestinal wall. Multiple investigations on laboratory animals found that the developed kefir product, produced with the use of whey protein hydrolysate, is characterized by hypotensive, hypolipidemic and hypocholesterolemic effect compared to kefir produced by traditional technology, as well as by reduced allergenicity of β-lactoglobulin. According to the opinion of the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences a status of diet (preventive) food product for adults with symptoms of food allergy to milk proteins was assigned to the fermented milk drink.

Keywords: β-lactoglobulin, allergenicity reduction, hydrolysate, kefir product, in vivo biological effects

DOI 10.12737/13128 *Foods and Raw Materials*, 2015, vol. 3, no. 2, pp. 115–121.

INTRODUCTION

The production of protein products with high content of milk (cheese, curd, technical casein) provides getting whey, which causes a significant economic damage and irreparable harm to the environment [1]. The volumes of the obtained whey reach 90% of the volume of processed milk: almost they are somewhat less because of incomplete gathering and process losses. About 50% of milk solids go into the whey. According to the International Dairy Federation data of 120 million tons of whey, produced in the world (in Russia – more than 15 million tons), up to 15% is discharged into the sewer which leads to the waste of about 400 thousand tons of milk protein and other components of raw milk [2, 3].

The most valuable raw material resource for the dairy industry is cheese whey – a complex biological system. Annually about 20 million tons of cheese are produced in the world, the whey production amounts to more than 160 million tons. In developed countries (USA, Canada, Germany, France, Sweden) the dairy industry processes from 60 to 95% of the whey resources. Russia is characterized by a low level of industrial processing – less than 40% [4, 5].

The most valuable part of this raw product is whey proteins with a high biological value and digestibility. The increase in volumes of whey extraction and large-scale implementation of membrane technologies in fractionation of raw milk components have caused great interest in the use of whey proteins, have led to the study of their functional properties.

The main whey protein fractions, represented by β-lactoglobulin, α-lactalbumin, immunoglobulin are of a globular shape and have a compact structure due to the low content of proline and the disulfide bridges between molecules of cysteine [6].

The widespread use of whey proteins in food technologies is constrained by their residual antigenicity. According to the data of International Union of Immunological Societies, the known food-borne allergens in dairy products include: caseins, immunoglobulins, β-lactoglobulins, α-lactalbumins, bovine serum albumin [7].

Entering the body, these proteins are recognized by the immune system which leads to its sensibilization, or sensitization to a specific allergen containing the antigen. Allergen's re-entering the body leads to the development

of allergic reactions with specific symptoms [8, 9].

According to the results of experimental evaluations of the European Academy of Allergy and Clinical Immunology (EAACI), there has been significant spread of allergic diseases in the last decade [10]. According to the data of the Institute of Immunology of Russia's Federal Medico-Biological Agency, every third inhabitant of our country is affected by allergy [11].

One of the most common forms of allergic diseases (about 80%) is food allergy, in which nutrition, providing for the correction of the patient's diet through the use of specialized products, including ones with reduced content of major allergen milk proteins, is of great importance [12].

Thus, modification of the composition and properties of cheese whey to reduce the residual antigenicity, its protein fractions, in particular, β-lactoglobulin, is particularly relevant. The most promising for this purpose is to apply the bioconversion of whey proteins with high residual antigenicity [13].

A method for producing hydrolysate of whey proteins with reduced allergenicity of β-lactoglobulin, providing for the bioconversion of whey proteins of cheese whey ultrafiltration concentrate, is well-known [14]. The purpose of these studies is to evaluate the biological effect of kefir product produced with the use of whey protein hydrolysate.

OBJECTS AND METHODS OF STUDY

The experimental works were conducted at the A.N. Bach Institute of Biochemistry of Russian Academy of Sciences.

The objects of the studies were males of Brown Norway rats of BN/SsNolaHsd line with RT1n haplotype with initial mass of about 150 g, which had been obtained from the centre for Laboratory Animal Breeding of Harlan Laboratories, Inc. Company and had passed quarantine for at least 3 weeks after delivery. During quarantine and follow-up experiment the animals were kept by 4 in standard cages with an upper wire frame and a plastic drinker at (20 ± 2)°C temperature, 60% relative humidity and 12 h/day lighting cycle duration. The change of bedding and drinking water was carried out daily. Feed and animal drinking water were provided ad libitum. During quarantine and follow-up experiment the animals were kept on complete granular feed (recipe PK-120 for breeding laboratory animals (LLC Laboratorkorm)) that did not include components and ingredients derived from milk (Table 1).

The test results of the physical and chemical parameters of feed ration are presented in Table 2.

Before the experiment, all the animals underwent blood sampling from vena saphenus lateralis to control the absence of circulating antibodies, specific to milk proteins, in blood serum. Then the animals were randomly divided into 5 groups of at least 10 animals each.

The animals of group 1 served as the positive control. On the first day of the experiment the animals were injected with a mixture of 0.2 cm^3 colloidal suspension of 40 mg/cm^3 aluminum hydroxide (Thermo Fisher Scientific, USA) with 0.5 cm^3 solution of skimmed milk powder (0.2 mg/cm^3 in sterile isotonic solution of sodium chloride) intraperitoneally. Then on the 2nd, 4th, 6th, 8th, 10th and 12th days the animals were reimmunized, injected with 0.5 cm^3 solution of skimmed milk powder (0.2 mg/cm^3 in sterile isotonic solution of sodium chloride) intraperitoneally. On the 28th day of the experiment the animals were euthanized by a method of carbon dioxide euthanasia. The flow rate of carbon dioxide was determined by a rotameter at the level of 3.5 dm^3/min. The end of exposure time of the animal in the chamber for carbon dioxide euthanasia was determined visually by the cessation of respiratory movements of animals. Blood samples were collected with a sterile syringe from the heart cavity and were incubated for 1 h at indoor temperature. Blood serum was separated by centrifugation for 10 min in CM6 centrifuge (Elmi, Latvia) (at 3 500 rpm). From the obtained samples of blood serum a combined sample, which was poured into portions of 150 μl, was frozen in liquid nitrogen and stored in low temperature (–80°C) freezer, was prepared. The combined sample of serum was used as the positive control in the analysis of specific antibody titers in blood serum of experimental animals.

The animals of group 2 served as the negative control. During 56 days they were kept on complete granular feed (recipe PK-120 for breeding laboratory animals) with periodic sampling of 0.5 cm^3 blood from vena saphenus lateralis on the 20th, 32nd, 44th and 56th days of the experiment. Blood serum was separated by centrifugation for 10 min in Mini spin centrifuge (Eppendorf, Germany) (at 3 500 rpm). From the obtained samples of blood serum a combined sample, which was poured into portions of 150 μl, was frozen in liquid nitrogen and stored in low temperature (–80°C) freezer, was prepared. The combined sample of serum was used as the negative control in the analysis of specific antibody titers in blood serum of experimental animals.

The animals of group 3 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On the 8th-49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 ultrafiltration concentrate of whey milk proteins intragastrically. On the 21st, 35th and 49th days of the experiment each animal underwent 0.5 cm^3 sampling of blood from vena saphenus lateralis. Blood serum was separated by centrifugation for 10 min in Mini spin centrifuge (Eppendorf, Germany) (at 3 500 rpm). In the obtained blood serum samples the total content of IgE, IgG1 titer, IgE, IgG2α, specific to β-lactoglobulin, α-lactalbumin and caseines, as well as the activity of mast cell-specific protease were determined. On the 56th day of the experiment the animals were injected with 2 cm^3 of 14% aqueous solution of skimmed milk powder intragastrically. After 30 min under

Table 1. Chemical composition of feed ration of rats

Component/parameter	Measurement units	Content
moisture	%	10.00
crude protein	%	22.00
crude fiber	%	4.00
crude fat	%	5.00
crude ash	%	4.36
lysine	%	1.09
methionine+cystine	%	0.70
calcium	%	1.80
phosphorus	%	1.10
sodium	%	0.10
metabolizable energy	kcal/100 g	310
vitamin A	thousand IU/kg	5.00
vitamin D3	thousand IU/kg	0.50
vitamin K3	mg/kg	0.30
vitamin E	mg/kg	30.00
vitamin B1	mg/kg	2.24
vitamin B2	mg/kg	1.20
vitamin B3	mg/kg	6.00
vitamin B5	mg/kg	22.15
vitamin B6	mg/kg	2.33
vitamin B9	mg/kg	6.60
vitamin B12	mg/kg	0.05
vitamin H	mg/kg	0.04
ferrum	mg/kg	18.00
manganese	mg/kg	9.00
zinc	mg/kg	22.50
copper	mg/kg	2.40
cobalt	mg/kg	0.18
iodine	mg/kg	0.24
selenium	mg/kg	0.06

Table 2. Physical and chemical parameters of feed ration of rats

Parameter	Measurement units	Value (M ± SD)	Method of analysis
Mass fraction of moisture	%	5.89 ± 0.26	GOST 13496.3
Mass fraction of total ash on a dry weight basis	%	8.32 ± 0.01	GOST 26226
Mass fraction of protein on a dry weight basis	%	23.16 ± 0.02	GOST 25011
Mass fraction of fat on a dry weight basis	%	5.08 ± 0.04	GOST 17681
Mass fraction of soluble dietary fibers on a dry weight basis	%	7.57 ± 0.32	GOST R 54014
Mass fraction of insoluble dietary fibers on a dry weight basis	%	17.65 ± 0.14	GOST R 54014

aseptic conditions blood sampling from the cavity of the heart was performed. Besides, under aseptic conditions spleen sampling for subsequent ex vivo experiments was carried out. The spleen was placed in a sterile falcon of 15 cm^3 volume of 5 cm^3 RPMI 1640 medium (PanEco, Russia) with 25 mM Hepes, L-glutamine, 1% penicillin-streptomycin (PanEco, Russia), 5% heat-inactivated fetal calf serum and 75 mM mercaptoethanol.

The animals of group 4 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On the 8th-49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 enzymatic whey protein hydrolysate intragastrically. The further course of the experiment is similar to the one described previously for the animals of group 3.

The animals of group 5 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On

the 8th–49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 kefir product with 1% mass fraction of fat produced with the use of enzymatic whey protein hydrolysate intragastrically. The further course of the experiment is similar to the one described previously for the animals of group 3.

Determination of the activity of mast cell-specific protease, the total IgE content and the specific antibody titers in blood serum of experimental animals

To determine the activity of mast cell-specific protease in blood serum of laboratory animals Mast cell protease II ELISA kit (Antibodies-online Gmbh, UK) was used. To determine the total IgE content in blood serum of laboratory animals total rat IgE ELISA KIT (Innovative research, USA) was applied.

Determination of the specific antibody titers in blood serum was performed by the method of noncompetitive enzyme-linked immunosorbent assay (ELISA). Sodium caseinate, α-lactalbumin, β-lactoglobulin (Sigma, USA) were used as the antigens. The antigens – (2 μg/cm^3 by protein, in 50 mM phosphate-buffered saline, pH 7.4) were sorbed in the microplate wells of 100 μl volume for 16 hours at 4°C. After four times of washing with 50 mM phosphate-saline buffer, pH 7.4 in the plate wells 100 μl of 0.1% gelatin solution were administered to prevent nonspecific sorption and were thermostated for 1 hour at 37°C, followed by 4 times of washing with 50 mM phosphate-saline buffer, pH 7.4 with 0.05% Triton X-100. Next, the wells were added with 100 μl dilution of biotinylated mouse antibodies to IgE, IgG1 or IgG2α (BD Biosciences, USA) rat antibodies and were incubated for 1 hour at 37°C. Then the plates were re-washed and were added with 100 μl solution of streptavidin conjugate with horseradish peroxidase (BD Biosciences, USA). After 1 hour incubation at 37°C the plate was washed first with 50 mM phosphate-buffered saline, pH 7.4 with 0.05% Triton X-100 (three times), then with distilled water, and the enzymatic activity, conjugated to a peroxidase label bearer, was detected. In the wells 100 μl of tetramethylbenzidine solution (TMB substrate reagent set, BD Biosciences, USA) were added as the substrate. After 15 min incubation at indoor temperature the reaction was terminated by adding 100 μl of 2M sulfuric acid solution. The optical density of solutions at 450 nm was determined with Synergy 2 photometer-fluorimeter (BioTek, USA). Based on the measurement results a curve for the antibody titration – dependence of the optical density of tetramethylbenzidine oxidation products of blood serum dilution – was plotted.

Determination of the differential blood count in blood of experimental animals

For this purpose 0.5 cm^3 sampled blood were transferred into the microtubes with K3-EDTA (Greiner Bio One, Germany). The smears of blood by the standard method no later than 1 hour after sampling were prepared. The air dried smears were stained by the Romanowsky method. The microscopic examination of blood smears (increase of 100, under immersion) using microscope (Motic BA300, Canada) and differential blood count of 100 cells were performed.

Determination of cytokine production by splenocytes

To obtain splenocyte suspension under aseptic conditions the spleen was rubbed by the sterile syringe plunger of 10 cm^3 volume through 70 μm nylon cellular extractor. Cells were washed 2 times with 5 cm^3 RPMI 1640 medium (PanEco, Russia) with 25 mM Hepes, L-glutamine, 1% penicillin-streptomycin, 10% fetal calf serum and 75 mM mercaptoethanol. Cell mass was separated by centrifugation at 3 500 rpm in 5702R centrifuge (Eppendorf, Germany). After washing the splenocytes were resuspended in 5 cm^3 of the above mentioned medium, and calculation of the number of cells was made in the Gorjaev's chamber. Then 100 μl splenocyte suspension with 4×10^6 cytosis was introduced into the wells of sterile 96-well microplates with high sorption capacity (Greiner Bio One, Germany). In the cultivation medium the sterile solutions of antigens (sodium caseinate, α-lactalbumin, β-lactoglobulin) were added into the wells so that their final concentrations should be 0 (control wells), 0.5 and 1.0 mg/cm^3. Cells were incubated in CO_2 incubator for 96 hours. The similar samples, in which concavalin A in the final concentration of 3.33 mg/cm^3 followed by incubation was added as mitogen for 48 hours additionally, served as the positive control. Upon completion of the incubation the contents of the wells were aspirated and centrifuged for 10 min at 5000 rpm in Mini spin centrifuge (Eppendorf, Germany), and in the supernatant IL-2, IL-12, IL-4, TNFα, IFNγ concentrations using IL-2 Rat ELISA Kit (Life Technologies, USA), IL-12 Rat ELISA Kit (Life Technologies, USA), Rat IL-4 BDOpt EIA ELISA Set (BD Biosciences, USA), Rat TNFα BDOpt EIA ELISA Kit (BD Biosciences, USA), Rat IFNγ BDOpt EIA ELISA Set (BD Biosciences, USA) were determined respectively.

RESULTS AND DISCUSSION

A model for testing the bio-functional properties of fermented milk products on the basis of enzymatic whey protein hydrolysates has been created. Its essence lies in the induction of IgE-mediated allergic reaction to antigens of dairy products by oral administration with adjuvant – chlorea toxin that increases the permeability of the intestinal wall. The list of monitored parameters included the total contents of IgE in blood serum, serum antibody titers of IgE, IgG1 and IgG2α-isotypes specific to the allergenic proteins of dairy products, the activity of mast cell-specific protease in blood serum, which is a specific marker of mast cell degranulation after IgE- mediated allergic reaction, the determination of the products of pro- and anti-inflammatory cytokines

by splenocytes in *ex vivo* culture after exposure of mitogen and allergenic proteins of dairy products.

Multiple investigations on laboratory animals found that the developed kefir product, produced with the use of whey protein hydrolysate, is characterized by hypotensive, hypolipidemic and hypocholesterolemic effect compared to kefir produced by traditional technology (Table 3).

The developed technological scheme of production of kefir product with reduced allergenicity of β-lactoglobulin (Fig. 1) provides modification to the traditional technology, which consists in the introduction of additional operations for getting whey protein hydrolysate and administrating it to the normalized mixture.

TOR 9222-512-00419785-13 technical documentation "Fermented milk products for dietary preventive nutrition", which has passed experimental-industrial testing in conditions of OJSC Dairy industrial complex "Voronezh" has been developed and approved.

The finished kefir product is characterized by qualitatively new consumer properties and reduced allergenicity of β-lactoglobulin. According to the opinion of the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences a status of diet (preventive) food product for adults with symptoms of food allergy to milk proteins has been assigned to the fermented milk drink.

ACKNOWLEDGEMENTS

The work was performed in frames of the Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2007-2013, state contract no. 12.527.11.0008.

Table 3. Biofunctional properties of the investigated products

Product name	*In vivo* biological effects		
	Hypocholesteremic effect	Hypotensive effect	Hypolipidemic and hypocholesterolemic effect
Biokefir with 1% mass fraction of fat produced by traditional technology	—	—	In the use of the product in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypocholesterolemic effect, expressed as decrease in serum concentration of total cholesterol by 15.2% ($p < 0.01$), high- density-lipoprotein-non-atherogenic fraction by 16.9% ($p < 0.001$) and low-density- lipoprotein-atherogenic fraction by 27.9% ($p < 0.05$) compared to the control with high alimentary lipid loading, was shown during 42 days. For the animals a statistically significant decrease in serum concentration of triglycerides by 62.5% ($p < 0.01$) compared to the value of this parameter in no. 2 control group, which indicates the presence of hypolipidemic properties in bio-kefir, was shown.
Biokefir with 1% mass fraction of fat with the introduction of 30% whey protein hydrolysate	The hypocholesteremic effect in exogenous induction of free- radical pathology of liver intoxicated with carbon tetrachloride.The decrease in serum concentration of total cholesterol and high density lipoproteins by 9.1% ($p < 0.07$) and 5.7% ($p < 0.05$) compared to the control respectively.	Hypotensive effect in conditions of high alimentary lipid loading. On normotensive Wistar rats in the use of biokefir functionnal product in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypotensive effect, expressed as decrease in systolic and mean arterial pressure by –19.47 and –13.42 mmHg compared to the control, was shown during 42 days.	Hypolipidemic and hypocholesterolemic effect in conditions of high alimentary lipid loading. In the use of biokefir in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypocholesteremic effect, expressed as decrease in serum concentration of total cholesterol by 15.6% ($p < 0.01$), high- density-lipoprotein-non-atherogenic fraction by 24.8% ($p < 0.001$) and low-density-lipoprotein-atherogenic fraction by 22.2% ($p < 0.01$) compared to the control with high alimentary lipid loading, was shown during 42 days. For the animals a statistically significant decrease in serum concentration of triglycerides by 68.1% ($p < 0.001$) compared to the value of this parameter in no. 2 control group, which indicates the presence of hypolipidemic properties in bio-kefir, was shown.

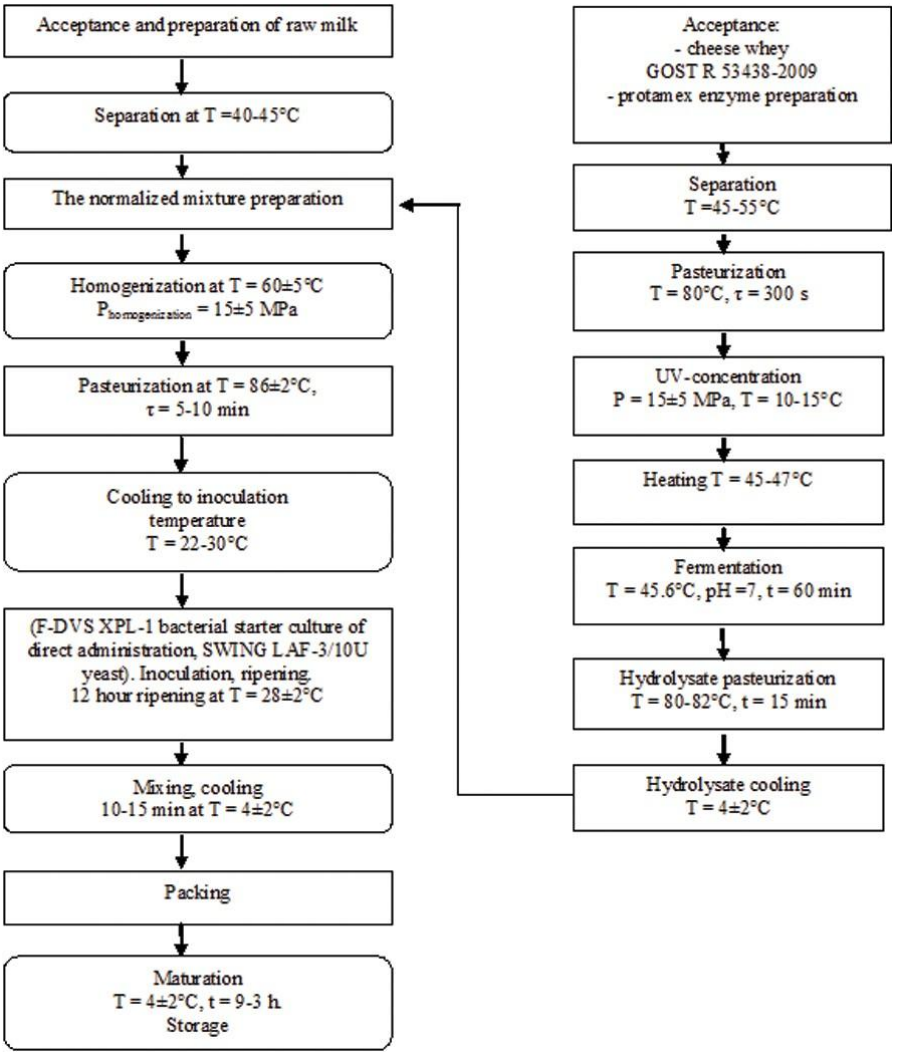

Fig. 1. Technological scheme of production of kefir product.

REFERENCES

1. Musina O.N. The modern state of combined dairy products' biotechnology. *Storage and processing of farm products*, 2010, no. 3, pp. 59–63. (In Russian).
2. Golovach G.N. and Kurchenko V.P. Allergenicity of milk proteins and ways to reduce. *Proceedings of the Belarusian State University*, 2010, vol. 5, no. 1, pp. 9–11. (In Russian).
3. Gavrilov G.B. and Kravchenko E.F. Rational use of whey. *Dairy Industry*, 2012, no. 7, pp. 32–33. (In Russian).
4. Ozhgikhina N.N. and Volkova T.A. Rational processing of whey. *Milk Processing*, 2012, no. 9, pp. 44. (In Russian).
5. Khramtsov A.G., Ryabtseva S.A. and Evdokimov I.A. World trends in whey processing. *Milk Processing*, 2009, no. 5, pp. 18. (In Russian).
6. Tepel A. *Chemistry and physics of milk*. St. Petersburg: Profession Publ., 2012. 832 p. (In Russian).
7. Koroleva O.V., Agarkova E.Yu., Botina S.G., Nikolaev I.V., Ponomareva N.V., Mel'nikova E.I., Kharitonov V.D., Prosekov A.Yu., Krokhmal' M.V. and Rozhkova I.V. Prospects for the use of whey protein hydrolysate in the technology of fermented milk products. *Dairy Industry*, 2013, no. 7, pp. 66–67. (In Russian).
8. Khaitov R.M. and Il'ina N.I. *Allergy*. Moscow: GEOTAR-Media Publ., 2010. 228 p. (In Russian).
9. Frenhani P.B. and Burini R.C. Mechanisms of absorption of amino acids and oligopeptides. Control and implications in human diet therapy. *Arq. Gastroenterol*, 1999, vol. 36, no. 4, pp. 227–237.
10. Johansson S.G., Hourihane J.O., Bousquet J., Bruijnzeel-Koomen C., Dreborg S., Haahtela T., Kowalski M.L., Mygind N., Ring J., van Cauwenberge P., van Hage-Hamsten M. and Wüthrich B. A revised nomenclature for allergy. An EAACI position statement from EAACI nomenclature task force. *Allergy*, 2001, no. 56, pp. 813–824.
11. Novoselova M.V., Borisova G.V., Bondarchuk O.N. and Malova Yu.S. Selection of parameters of enzymatic hydrolysis of casein. *Modern Problems of Science and Education*, 2012, no. 6. (In Russian).
12. Kharitonov V.D., Budrik V.G., Agarkova E.Yu., Botina S.G., Berezkina K.A., Kruchinin A.G., Ponomarev A.N. and Mel'nikova E.I. Perspective directions of struggle with allergy. *Food Processing: Techniques and Technology*, 2012, vol. 27, no. 4, pp. 3–6. (In Russian).

13. Maksimyuk N.N. and Mar'yanovskaya Yu.V. About the advantages of the enzymatic method of obtaining protein hydrolysates. *Fundamental Research*, 2009, no. 1, pp. 34–35. (In Russian).
14. Prosekov A.Yu., Ul'rikh E.V., Mel'nikova E.I., Noskova S.Yu., Budrik V.G., Botina S.G. and Agarkova E.Yu. The getting enzymatic whey protein hydrolyzate using proteolytic enzyme. *Fundamental Research*, 2013, no. 6, part 5, pp. 1089–1093. (In Russian).

Please cite this article in press as: Korzhov R.P., Ponomarev A.N., Melnikova E.I. and Bogdanova E.V. Preclinical studies of kefir product with reduced allergenicity of β-lactoglobulin. *Foods and Raw Materials*, 2015, vol. 3, no. 1, pp. 115–121. doi: 10.12737/13128.