

Perspectives of hydrolysates from mare's milk use in sport nutrition

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Summary

Modern sport is characterized by intensive physical, psychic, and emotional loads. Means and methods for recovery of athletes' physical efficiency shall arise from character of executed work. One of the very first and the main methods for recovery is nutrition; first of all, it can extend boundaries of adaptation of athlete's organism to the extreme physical loads. General principles of balanced nutrition are considered as the basis of athletes' nutrition strategy; however, there are special objectives as well. They mean the increase of working capacity, removal of time of exhaustion occurrence, and speeding-up of recovery processes after the physical load.

Basis of sport nutrition products majority is a protein, mainly milk protein, including protein fractions as the most balanced based on amino acid profile and level of biological value. Whey protein (lactalbumin, lactoglobulin, and immunoglobulin) has the highest speed of reduction among the whole proteins.

Use of namely mare's milk as the source of low-molecular-weight peptides is justified by its uniqueness of chemical composition. Mare's milk is related to the albumen type and contains, as a woman's, low percent of protein (up to 2 %), which is mainly presented by albumens and globulins. Mares milk, together with albumens, has high level of low-molecular-weight peptides and free amino acids; therefore, mare's milk is a perspective raw material for use as the basis for sport nutrition products construction.

Taking the abovementioned, the purpose of these researches is the comparative assessment of properties of hydrolysates of mare's milk, enriched with complex of vitamins and antioxidants (A, E, C).

Materials and methods. The research was carried out on 35 outbreed male mice with weight of 18.5–24.0 g; mice were divided into 2 groups (experimental – 17 mice and control group – 18 mice). In addition to the vivarium diet, mice of experimental group daily obtained 0.2 ml of mare's milk, enriched with antioxidants of hydrolysate. Mice of control group obtained 0.2 ml of 0.9 % aquatic solution of sodium chloride enriched with antioxidants. Oral supplementation of hydrolysate was carried out by needles for mice feeding under the method (Machholz et al., 2012). Forced swimming with weight, which equals to 10 % of animal body weight, was used for assessment of animals' endurance and working capacity (maximum time of swimming was measured). In 30 days of daily swimming, the indicators of antioxidant status (concentration of malondialdehyde (MDA), dien and trien conjugates as well as Schiff's bases) were estimated after killing. Concentration of lactate, glucose, and urine as well as activity of key enzymes of antioxidant system (superoxide dismutase and catalase) in mice blood serum was determined at the Minitecno biochemical analyser and by application of kits and chemical reactive, made by the Biosystem and Sigma.

Results and conclusions. Hydrolysates of mare's milk proteins, enriched with vitamins A, E, and C, have a favourable impact on the endurance of mice, decrease lactate and urine concentration in the blood, provides decreasing of concentration of transition and final products of lipid peroxidation, activation of antioxidants system enzymes. Hydrolysates can be applied as agents of stimulating effect during working in extremal conditions and in conditions of increased physical load including appliance in sport medicine.

Keywords: hydrolysates of mare's milk, antioxidants, lipid peroxidation processes, ferments of antioxidant system.

Introduction

Modern sport is characterized by intensive physical, psychic, and emotional loads. Process of preparation for competitions as a rule includes two or three-time daily training, having left with less time for rest and recovery of physical efficiency (Лютт, 2006; Азизбемян et al., 2009). Means and methods for recovery of physical efficiency of athletes shall arise from character of executed work. One of the very first and the main methods for recovery is nutrition; first of all, it can extend

boundaries of athlete's organism adaptation to very extreme physical loads. Proper development of food ration of athlete with obligatory supplementing of energy consumption and keeping of organism's water balance is an important requirement during organization of the training process. General principles of balanced nutrition are considered as the basis of athletes' nutrition strategy, however, there are special objectives as well. They mean increase of working capacity, removal of time of exhaustion occurrence, and speeding-up of recovery

processes after physical load (Азизбекян et al., 2008; Тутьельян et al., 2010).

One of the ways for optimization of athletes' nutrition is correction of ration with the help of specialized products and (or) biologically active dietary supplements, which mean the substances with the scientifically proven and exactly balanced composition, produced at observance of all modern production technologies and standards on the newest equipment (Rowan et al., 2005; Круглик, 2007^{a,b}). Specialized nutrition products of athletes can be used for the following purposes: change of quality orientation of daily ration in accordance with the direction of training loads; urgent correction of non-balanced daily ration; increase of nutrition frequency under conditions of 2-3-time training per day; increase of muscle mass of athletes, body weight reduction; as dietary recovery means after training loads of substantial volume and intensiveness; during the recovery period, etc.

Basis of majority of sport nutrition products is protein, mainly milk protein, including protein fractions as the most balanced and based on amino acid profile and level of biological value. Whey proteins have the most valuable biological properties, since they have optimal set of essential amino acids and approached to the amino acid scale of *ideal* protein from the point of view of physiology. Whey protein (lactalbumin, lactoglobulin, and immunoglobulin) have the highest speed of reduction among the whole proteins. Amino acid composition of whey proteins is the closest to the amino acid composition of muscle tissue of a man, and they exceed all the rest proteins of animal and vegetable origin based on composition of irreplaceable amino acids and amino acids with branched chain (valine, leucine, and isoleucine). Besides that, approximately 14 % of whey milk proteins are in the form of hydrolysis products (amino acids, di-, tri-, and polypeptides), which participate in synthesis of essential ferments and hormones (Токаев et al., 2007; Максимюк, Марьяновская, 2009). In order to speed-up the metabolism in organism, proteins shall be provided in the form of peptides and amino acids. One of methods of receiving of short-chain peptides and free amino acids is the fermentative partial or full hydrolysis. In the course of hydrolysis, large protein molecules are divided into separate small fragments that allow them to rapidly and easily digest in the alimentary tract (Severin, Wenshui, 2005).

The following forms of protein compounds are used for production of foods on the basis of milk protein: concentrates of whey proteins – the most common basis of protein sport nutrition products; concentrated hydrolysates of whey proteins as the source of free amino acids and peptides, etc. (Sinha et al., 2007; Рытченко, Красноштанова, 2011).

Hydrolysis of whey proteins is actual because of several reasons; firstly, mixtures of peptides of various lengths are soaked through the alimentary tract faster and completely than the mixtures of amino acids equivalent based on composition. Secondly, composition of protein hydrolysates can have various physiologically active peptides, required for regulation of variety of important organism functions. Thirdly, positive impact of peptides on digestion of some essential micro-nutrients, namely metal ions, is possible. Composition of fermentative hydrolysates of casein and whey protein has the peptides, which can form solid coordination (chelate) compounds with calcium ions and significantly increase efficiency of their soaking. Fourthly, fermentative protein hydrolysates as a part of specialized hypo-allergic products have certain technological (functional) advantages over the mixtures of amino acids. Hydrolysis of whey proteins can be performed by chemical (under impact of mineral acids and alkalies under and elevated temperature) and fermentative (with help of products of protein-degrading enzyme) methods (Vigovsky et al., 2003; Курбанова, 2012).

Positive physiological effect during consumption of hydrolysed proteins can be achieved by the best digestion of short-chain peptides in the alimentary tract in comparison with native proteins and amino acids (Minami et al., 1992).

Use of namely mare's milk as the source of low-molecular-weight peptides is justified by its uniqueness of chemical composition. Mare's milk is related to the albumen type and contains, as a woman's, low percent of protein (up to 2 %), which is mainly presented by albumens and globulins. Mare's milk, together with albumens, has high level of low-molecular-weight peptides and free amino acids; therefore, mare's milk is perspective raw material for use as the basis for sport nutrition products construction (Охлопкова, 2011).

Antioxidant, hypocholesteremic as well as detoxicant effect of products on the basis of mare's milk (Abdel-Salam et al., 2010; Sinyavsky et al.,

2014) has been proven in the work of domestic and foreign scientists clinically and during experiments on animals. As distinguished from milk of other types of agricultural animals, mare's milk differs by high composition of lysozyme, lactoferrin as well as immunoglobulin G that allows its using as modulator of nonspecific immunity (Hoffman et al., 1998; Doreau, Martin-Rosset, 2002). Available data specifies the future of use mare's milk for creation of functional products and biologically active dietary supplements improving quality of life.

Taking the abovementioned, the purpose of these researches is the comparative assessment of properties of hydrolysates of mare's milk, enriched in complex of vitamins and antioxidants (A, E, C).

Material and methods

This work represents the assessment of impact of hydrolysate of mare's milk protein, enriched with a complex of antioxidants (vitamins A, E, C) on mice's organism. Rennet consisting of 70 % of chymosin and 30 % of pepsin was used for hydrolysates deriving. Ferment in the amount of 0.1 % was added into pasteurized mare's milk, then it was being thermostated for 24 hours at the temperature of 40 °C. Degree of proteins hydrolysis was determined in polyacrylamide gel (Стручкова, Кальясова, 2012).

Further derived hydrolysate was mixed with vitamins A, E, and C in the following concentration: retinyl acetate 15 mg/kg, tocopherol acetate 30 mg/kg, and ascorbic acid 1 mg/kg – and was used as additive to mice's nutrition. Research was carried out on 35 outbred male mice with weight of 18.5–24.0 g, which were divided into 2 groups (experimental – 17 mice and control group – 18 mice). Animals were kept in the vivarium in plastic cages with 5–6 mice in each at the air temperature of 20–22 °C in daylight on the beddings made of tree cuttings of hardwood. In addition to the vivarium diet, mice of experimental group daily obtained 0.2 ml of mare's milk, enriched with antioxidants of hydrolysate. Mice of control group obtained 0.2 ml of 0.9 % aquatic solution of sodium chloride enriched with antioxidants. Oral supplementation of hydrolysate was carried out by needles for mice feeding under the method (Machholz et al., 2012).

Forced swimming with weight equals to 10 % of animal's body weight was used for assessment of

animals' endurance and working capacity (maximum time of swimming was measured). Swimming was carried out according to the methodology, described by Ye. Shustov, V. Bolotova (Шустов, Болотова, 2013). The animals were pithed through capitation under brief ether anaesthesia. Initial biochemical indicators were measured in the blood of 12 mice before experiment commencement. In 30 days of daily swimming, the indicators of antioxidant status (concentration of malondialdehyde (MDA), dien and trien conjugates as well as Schiff's bases) were estimated after killing according to some authors (Дерюгина et al., 2010).

Concentration of lactate, glucose, and urine as well as activity of key enzymes of antioxidant system (superoxide dismutase and catalase) in mice's blood serum was determined at the Minitecno biochemical analyser and by applying of kits and chemical reactive, made by the Biosystem and Sigma.

Statistical analysis of obtained results was carried out under the method (Кокунин, 1975).

Results and discussion

Assessment of the average time of swimming, indicators of lipid peroxidation, enzymes of antioxidant system, lactate and urine concentration in mice's blood under the influence of physical loads (swimming with weight) was carried out at the first stage of the experimental research.

The duration of mice's swimming with weight was minimal and equal to 250–260 s for the first two days. For the next days, the duration of animals' swimming was increased step by step and became equal to 275–290 s by the 9th day.

The change of biochemical indicators in mice's blood under the influence of physical load is stated in the Table 1. Concentration of the lipid peroxides was increased in mice's blood during physical load in comparison with state of rest. The concentration of malondialdehyde (MDA), dien, and trien conjugates and Schiff's bases was increased by 31.6 %, 44.7 %, 34.6 %, and 53.3 % respectively. Significant decrease of activity of key enzymes of superoxide dismutase and catalase in mice's blood serum by 68.3 % and 63.4 % was registered accordingly. The increase of lactate and urine concentration in mice's blood, affected by physical load, was registered as 22.6 % and 24.0 % respectively.

Table 1

Change of biochemical indicators in mice's blood under the influence of physical load (swimming with weight)

Investigated indicator	State of rest	During physical load on the 5 th day
MDA, mmol/l	3.95 ± 0.26	5.20 ± 0.39*
Dien conjugate, absorbance unit	0.38 ± 0.03	0.55 ± 0.05*
Trien conjugate, absorbance unit	0.49 ± 0.02	0.66 ± 0.04*
Schiff's bases, absorbance unit	0.15 ± 0.01	0.23 ± 0.02*
Superoxide dismutase, enzyme unit	6.0 ± 0.57	4.1 ± 0.35*
Catalase, enzyme unit	12.3 ± 1.3	7.8 ± 0.7*
Lactate, mmol/l	5.12 ± 0.44	6.28 ± 0.68
Urine, mmol/l	4.53 ± 0.41	5.62 ± 0.51

* – differences are statistically valid $p \leq 0.05$ with regard to the data during state of rest

The duration of swimming of mice was decreased in comparison with initial data of 275–290 s by 8.7 % and 8.0 % respectively in both control and experimental groups after hydrolysate, taken during the first two days. For the following days, the duration of animals' swimming was increased step by step and further increase of swimming time was registered till 30th day in the experimental group, and increasing of swimming time of animals was carried out till 25th day of attendance in the control group. The increase of swimming time was equal to 28.9 % in the experimental group, whereas swimming time of the control group was equal to 17 % (Fig. 1).

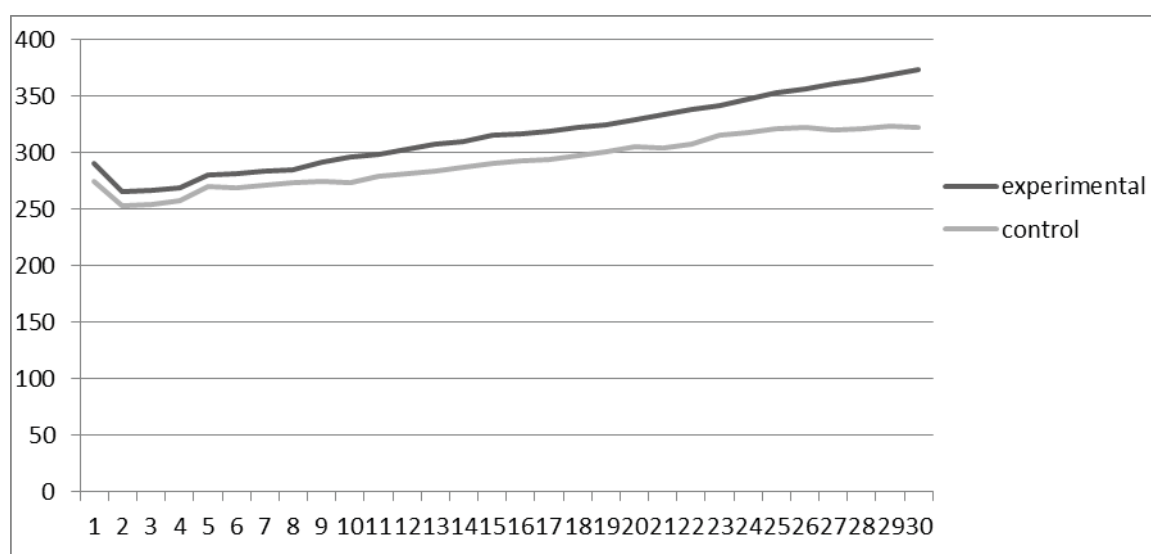


Fig. 1. Change of swimming time of mice during taking hydrolysate of protein in the experimental and control groups

Intensive physical load as stress factor was supported by the activation of indicators of lipid peroxidation. Strengthening formation of lipid peroxides in the organism during muscular exercise can be the evidence of decreasing activity of antioxidant system and insufficient provision of organism with antioxidants. Research of the status of antioxidant protection system can be the one of key mechanisms, providing adaptive processes during muscular exercise.

Change of concentration of final, transitional, and secondary products of lipid peroxides in mice's blood serum is stated in the Table 2.

Table 2

Concentration of MDA, dien, and trien conjugants as well as Schiff's bases and changing of activity of antioxidant system enzymes in mice's blood of the control and experimental group in 30 days after hydrolysate taken

Indicator	Experimental group (n = 17)	Control group (n = 15)
MDA, mmol/l	4.13 ± 0.39	5.22 ± 0.51
Dien conjugate, absorbance unit	0.45 ± 0.04	0.55 ± 0.05
Trien conjugate, absorbance unit	0.56 ± 0.05	0.66 ± 0.06
Schiff's bases, absorbance unit	0.18 ± 0.02	0.23 ± 0.02
Superoxide dismutase, enzyme unit	5.5 ± 0.48	4.1 ± 0.35
Catalase, enzyme unit	11.0 ± 0.9	7.8 ± 0.7*

*- results are statistically valid with regard to the data of the control group

As it is evident from the data, specified in the Table 2, concentration of MDA was increased

by 26.6 %, dien conjugates – by 22.4 %, trien conjugates – by 18.6 %, Schiff's bases – by 31.1 % in mice's blood of the control group in comparison with the experimental group. With regard to changing of activity of superoxide dismutase and catalase, it is required to specify the activation of these enzymes by the end of experiment. The activity of Superoxide dismutase and catalase was increased by 35 % and 42 % respectively in the experimental group in comparison with the control group (Table 2). Obtained changing bears evidence more intensive antioxidant effect of hydrolysates of mare's milk in comparison with taken 9 % aquatic solution of sodium chloride, enriched with antioxidants, by the animals of the control group.

Assessment of urine concentration in blood, first of all, bears an evidence of the state of anabolic and catabolic processes in the organism. Urine is mainly a decay product of muscular proteins. Concentration of urine as a rule increases in the blood during high breakdown of proteins in muscles. It can be an evidence of increased muscles exercises.

Accumulation of lactate in organism during exercises is one of the main factors, which limits the working capacity and effectiveness of sport achievements (especially in cyclic sport). This indicator progressively decreases during endurance increasing.

As it is evident from the data, specified in the Table 3, lactic acid concentration was decreased by 10.2 % and urine concentration – by 12.5 % in the blood of experimental group mice.

Table 3

Change of concentration of lactic acid and urine in blood serum of mice of the control and experimental groups after 30 days of taking hydrolysates of mare's milk proteins

Indicator	Experimental group (n = 17)	Control group (n = 15)
Lactate, mmol/l	5.63 ± 0.51	6.28 ± 0.61
Urine, mmol/l	4.91 ± 0.43	5.62 ± 0.56

It was established that physical load was supported by the activation of the antioxidant protection system, which led to accumulation of lipid peroxides and decreasing of activity of antioxidant system enzymes. Besides, physical load leads to intensification of reactions of anaerobic glycolysis, which is supported by the increase of lactate concentration in mice's blood serum. Concentration of urine increases in the blood, in consequence

of intensifying of proteins catabolism during physical load. With this background, introduction of hydrolysates of mare's milk to nutrition of experimental group animals flattens the above-mentioned metabolic abnormalities and is supported by decreasing of lipid peroxides concentration in the blood, increasing of activity of superoxide dismutase and catalase enzymes as well as decreasing of lactate and urine concentration.

Therefore, introduction of hydrolysates of mare's milk to mice's ration provides increasing of resistance of animals' organisms to physical load.

The results, obtained in this research, can be the basis of wide usage of mare's milk and its products in sport nutrition.

Consequently, hydrolysates of mare' milk proteins, enriched with vitamins A, E, and C, have a favourable impact on the endurance of mice, decrease lactate and urine concentration in the blood, provides decreasing of concentration of transition and final products of lipid peroxidation, activation of antioxidants system enzymes.

Thereby, the results of performed researches bear an evidence that hydrolysates of mare's milk proteins provide increasing of endurance and working capacity of animals, have a favourable impact on antioxidant status and biochemical indicators of animals' blood. Due to this, they can be recommended as agents of stimulating effect during working in extremal conditions, in conditions of increased physical load including for appliance in sport medicine.

Conclusions

1. Hydrolysates of mare's milk proteins, enriched with vitamins A, E, and C, have a favourable impact on the endurance of mice, decrease lactate and urine concentration in the blood, provides decreasing of concentration of transition and final products of lipid peroxidation, activation of antioxidants system enzymes.

2. Hydrolysates can be applied as agents of stimulating effect during working in extremal conditions as well as in conditions of increased physical load, including appliance in sport medicine.

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HIDROLIZATŲ, GAMINAMŲ IŠ KUMELĖS PIENO, VARTOJIMO SPORTININKŲ MITYBOJE PERSPEKTYVOS

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SANTRAUKA

Hidrolizatas – aukščiausia baltymo kategorija, kuriai būdingas maksimalus pasisavinimas organizme, jame yra mažiausiai alergenų, pasižymi didžiausiu poveikiu ugdant liesąją raumenų masę. Šiuolaikinis sportas pasižymi intensyviais fiziniais, psichiniais ir emociniais krūviais. Sportininkų fizinio darbingumo bei atsigavimo būdai ir priemonės turi būti efektyvios ir taikomos laiku. Viena iš svarbiausių organizmo atsigavimo priemonių yra mityba. Būtent ji yra pajėgi išplėsti organizmo adaptacijos prie ekstremalių fizinių krūvių galimybes. Sportininkų mityboje svarbūs yra subalansuotos, racionali mitybos principai, tačiau, be to, jai dar keliami specialūs uždaviniai – turi didinti darbingumą, atitolinti nuovargio atsiradimą, greitinginti atsigavimą po fizinių krūvių.

Sportininkų mityboje svarbūs yra baltymai, visų pirma, pieno baltymai, nes juose yra geriausiai subalansuotos aminorūgštys ir jų didžiausia biologinė vertė.

Ypač šiomis savybėmis pasižymi kumelės pienas. Jame daug albuminų, o bendras baltymų kiekis neviršija 2 procentų. Kumelės piene, be albuminų, yra didesnis kiekis mažo molekulinio svorio peptidų, laisvųjų aminorūgščių ir dėl to šis pienas yra perspektyvus produktas sportininkų mityboje.

Šios aplinkybės padėjo suformuluoti darbo tikslą – įvertinti iš kumelės pieno gaminamo hidrolizato, praturtinto vitaminais (A, E, C), turinčiais antioksidacinį poveikį, įtaką organizmui.

Tyrimo metodika. Tyrimas atliktas su 35 neturinčiomis kilmės vyriškos lyties pelėmis, sveriančiomis 18,5–24 gramus. Jos buvo padalytos į 2 grupes: 17 pelių – į eksperimentinę, 18 pelių – į kontrolinę. Bendrasis eksperimentinės grupės gyvūnų vivariumo dienos maisto racionas buvo papildomas 0,2 ml kumelės pieno hidrolizatu, prisetintu antioksidantais. Kontrolinės grupės pelės gaudavo 0,2 ml 0,9 % natrio chlorido tirpalo. Pelės buvo maitinamos specialia pipete per burną. Gyvūnų darbingumui ir ištvermei nustatyti buvo taikomas plaukiojimas su papildomu 10 % kūno masės svoriu iki leido jėgos.

Po 30 dienų plaukiojimo buvo nustatyta eksperimentinės ir kontrolinės grupės gyvūnų kraujo antioksidacinė būklė (malono dialdehidas – MDA). Laktato, gliukozės, šlapalo koncentracija ir pagrindinių antioksidacinės sistemos fermentų aktyvumas buvo nustatomas pelių kraujo serume. Tyrimas buvo atliktas biocheminiu analizatoriumi *Minitecno* naudojant *BioSystem* ir *Sigma* įmonių reagentų rinkinį.

Darbo rezultatai. Kumelės pieno baltymų hidrolizatai, praturtinti A, E, C vitaminais, teigiamai veikia pelių ištvermę, sumažina eksperimentinių gyvūnų kraujyje laktato, šlapalo koncentraciją, sumažina tarpinių ir galutinių riebalų apykaitos produktų kiekį, suaktyvina antioksidacinės sistemos fermentus. Kumelės pieno baltymų hidrolizatai gali būti vartojami sporto medicinoje kaip medžiagos, stimuliuojančios darbingumą ekstremaliomis sąlygomis atliekant didelės apimties ir intensyvumo fizinius krūvius.

Raktažodžiai: kumelės pieno hidrolizatai, antioksidantai, lipidų peroksidacijos procesai, antioksidacinės sistemos fermentai.