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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 713-719
https://doi.org/10.5219/1156
Received: 28 June 2019. Accepted: 6 September 2019.
Available online: 28 September 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

EVALUATION OF THE ADAPTOGENIC PROPERTRIES OF THE QUARK PRODUCT ENRICHED WITH PROBIOTICS, POLYPHENOLS AND VITAMINS

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ABSTRACT

The aim of the study is to evaluate protective properties of the quark product manufactured with transglutaminase and enriched with probiotics, oligomerous proanthocyanidines and vitamins; the biological experiment on the growing laboratory Wistar stock rats has been carried out. The rats of two from three groups subjected within 21 days to the effect of low-frequency weak variable magnetic field received in semi-synthetic diet composition extra experimental and control samples of the quark product. The index of feed intake and the rats' body mass growth was registered within 32 days. At the end of the experiment blood serum biochemical index was evaluated. It was revealed that the animals consuming the experimental product substantially gained the mass before the effect (gain from the 1st up to 10th days made up 12%) as well as after effect (gain from 11th up to 32nd days – 10.3%); upon completion of the experiment the gains of these animals exceeded the gains of the rats consuming the control product by more than 28%. The experiment revealed the lipolipedemic and hypoglycemic effect of the experimental quark product that has been evidenced by the significant reduction of cholesterol (by more than 20%), glucose (up to 40%) in the rats' blood serum. On administration of the experimental dairy product in the animals' diet subjected to the impact of low-frequency weak magnetic field the effect of the broken balance recovery in antioxidant/pro-oxidant system was observed due to reduction of pro-oxidant load at the enzymatic as well as low molecular links of the antioxidant system. The identified antioxidant and adaptogenic effect of the developed dairy product promoting to reduce the intensity of free-radical oxidation at the impact of low-frequency electromagnetic field on the body make it possible recommend it in dietotherapy for correction of antioxidant/pro-oxidant status.

Keywords: curd product; transglutaminase; polyphenols; vitamins, probiotics; LF-EMF; rat

INTRODUCTION

In the modern world, a person faces a large amount of endogenic and exogenic factors negatively effecting his health and welfare. Chronic stresses, unbalanced nutrition, bad habits, radioactive, ultraviolet and electromagnetic radiation cause disbalance in the body antioxidant system that can result in chronic diseases **(Hybertson et al., 2011; Grabowska et al., 2019)**. It is known that all living organisms starting from unicellular and ending with a human are very sensitive to the impact of low-frequency electromagnetic fields (LF-EMF). Low-frequency weak intensity electromagnetic fields of natural origin present the potential threat for people health and stand in one line with such important climatic factors as temperature, pressure, etc. **(Martínez-Sámano et al., 2019; Il'chenko, 2017)**. The accumulated in the world literature data about the important role of antioxidants for prophylaxis of oxidative stress along with information about their insufficient intake in the diet show the advisability of their usage as additives enriching food products **(Tarko et al., 2015)**. Due to this fact, the scientific substantiation and development of the technologies of dairy products enriched with biologically active substances capable more or less protect the body against damaging free radicals is a very actual problem having a scientific, practical interest and social importance.

Usage of food additives complex in the technology of quark products manufacture providing the required functional-technological and prophylaxis properties including fermentative preparations, probiotic cultures, vegetable extracts, and vitamins is of great interest. **Zobkova et al. (2017)** showed that usage of the ferment preparation transglutaminase in the technology of quark production owing to protein modification promotes the increased product yield and lysine content in it.

Among the functional ingredients possessing the pronounced antioxidant properties grape stones extract has been used widely for a long time **(Tomášková et al., 2017)**.

It is generally known the significance of vitamins as antioxidants and molecules predecessor playing the important role redox reactions in cells as well as protectors

and polyphenol synergists **(Kodentsova et al., 2013; Isakov et al., 2018; Kang et al., 2019; Menshchikova et al., 2006)**.

Moreover, antioxidant enzymatic system of probiotic microorganisms enables to consider them as exogenous antioxidant protection of the human organism. The available data testify for the wide spectra of biological activity of *Lactobacillus congener*, particularly, *Lactobacillus acidophilus* which are widely used in probiotic preparations and medical food products. *L. acidophilus* immunomodulatory, antibiotical, antigenotoxical, antioxidant and many other properties have been revealed **(Hardy et al., 2013; Lin and Chang, 2000; Kim et al., 2005; Karamova and Khabibullin, 2013)**. Herewith probiotic cultures fermentation during the product manufacture is more preferably than simple addition as far as in this case besides vegetative cells their metabolites are present.

Scientific hypothesis

The quark product manufactured with transglutaminase enriched with probiotics, polyphenols, and vitamins improve the organism physiological state and increase the laboratory rats natural resistance to low-frequency electromagnetic fields impact.

MATERIAL AND METHODOLOGY

The object of the investigations were quark products – the experimental (enriched) sample prepared with transglutaminase, probiotic cultures, polyphenol-containing extract, and multivitamins premixes; the control sample prepared without the listed above functional ingredients.

The technology of the quark product manufacture

The control samples of the quark product were manufactured from skim quark and fermented product. Experimental samples were manufactured from skim quark and probiotic fermented product. The quark was produced by the acid method by skim milk fermentation with lactococcus with the following whey removal by milk protein coagulate pressing. The experimental samples of the product were prepared from the quark manufactured from skim milk with use of crosslink enzyme - the preparation of microbial tranglutaminase (BioConnecta milk, Prompostavka-M, Ltd., Russia, activity 100 $U.g^{-1}$, dosage 0.15 $g.kg^{-1}$) and probiotic fermented dairy product produced with dry extract of grape stones (Tyaga (Shanghai) Co., China, the total polyphenolics content ≥95%), multivitamin premix 730/4 containing vitamins: A, C, E, D3, B1, B2, B5, B6, B9, PP, biotin (DSM Nutritional Products Ltd., Swiss) using the *Lactobacillus acidophilus* (strain 20T supplied by the Central Laboratory of Microbiology FSANO VNIMI, Russia).

The methods of physical-chemical characteristics investigation

The following parameters were measured in the product samples: the fat – by Gerber butyrometric method (ISO 2446:2008); the total protein - by Kjeldahl method with use of KJELTEC automatic system (ISO 8968-1:2014); the carbohydrate – by IDF 028A:1974; the vitamin C – by AOAC Official method 967.21; the total polyphenolics content in terms of gallic acid – in supernatant by Folina-Ciocalteu method **(Kovarovič et al., 2017)** with use of spectrophotometer Specord M40 (Analytik Jena AG, Germany); the number of Lactobacillus – by calculation of colonies grown on agar-like selective growth media (type MRS manufactured by Pharmacotherapy Research Center, St. Petersburg, Russia) at optimal conditions (ISO 20128:2006).

The laboratory rats biological experiment

The products were studied at white male rats Wistar stock (165 ±15 g) from the Laboratory Animals Nursery "Andreevka" (Federal State Budgetary Scientific Institution "Scientific Center of Biomedical Technologies") at the base of the Experimental Clinic-laboratory of biologically active substances Gorbatov's Federal Research Center for Food Systems of Russian Academy of Sciences (Russia). After adaptation (14 days) the animals were individually marked and grouped at random: group 1 – involved the intact rats (n = 10); group 2 – control rats (n = 10); groups 3 and 4 – experimental rats (I) consuming the control quark product (n = 10) and (II), consuming the enriched quark product (n = 10). The animals of all groups during the whole experiment consumed the standard diet (310 $kcal.100g^{-1}$). The first stage of the experiment – from the first up to 10 days – was aimed at adaptation of the rats of the third and the fourth group to the introduction in the standard diet of the quark products (53 $kcal.100g^{-1}$) which were fed on the basis of 20 g per head using polysulfone vials. At the second stage starting from 11^{th} to 32^{nd} days the animals from 2, 3 and 4 groups were subjected to daily 10 minutes effect of LF-EMF with frequency 8 Hz 5 μT **(Torres-Durán et al., 2007)** at the that rats of group 3 and 4 continued to consume additionally to the diet the quark products. During the whole experiment, the rats of all groups received feed and water ad libitum. The rats were kept in polysulfone cages IV S (Tecniplast, Italy) at the temperature 22 ±3 °C, humidity – 50 – 60%, lighting – in the regime 12/12, daylight hours – from 6.00 to 18.00.

Prior to the investigation start and every fourth day the animals were weighed at the scale Ohaus (Adventurer Pro, USA). The duration of the experiment made up 32 days. At the end of the experiment the rats were put to sleep with carbon dioxide in the chamber for euthanasia (VetTech, Great Britain), sampled blood from the heart for analysis. Blood sampling for hematology research was carried out in tubes with EDTA as an anticoagulant (Aquisel, Spain). Rat plasma was obtained after centrifugation (at 2,000 g for 10 min) of EDTA tubes with blood. Rat blood serum was obtained in conformity with **Chernukha et al. (2018)**.

Hematologic, biochemical blood analysis and parameters of the animals' antioxidant system

Quantitative amount of leucocytes (LEU); limphocetes (LYM), granolucites (GRAN); mixture of monocytes, eosinophils, basophilus and immature cells (MID); erythrocytes (RBC); hemoglobin (HGB); hematocrit (HCT); mean amount of erythrocyte (MCV); mean content of hemoglobin in erythrocytes (MCH); thrombocytes (PLT); thrombocrit (PCT) in the rats' whole blood was

carried out at the automatic hematologic analyzer Abacus junior vet 2.7 (Diatron Messtechnik GmbH, Austria (using Diatron set of assay kit).

The following blood serum index was measured at the automatic analyzer BioChem FC-300 (HTI, USA) using HTI assay kit: total protein, albumin (ALB), creatinine (CREA), aspartate aminotransferase (ASF), alanine aminotransferase (ALT), glucose (GLU), total cholesterol (CHLST), trigliceride (TG).

Moreover, the following index of antioxidant organism protection was evaluated in the rats' blood serum:

- total antioxidant activity was determined by Ferric Reducing Antioxidant Power method **(Merola et al., 2009)**;
- the active products content reacting with tiobarbituric acid (TBA-RP, p.a.), by **Brazhe et al. (2014)** method;
- superoxiddismutase activity was measured (SOD) was determined by **Marklund and Marklund (1974)** method with modification of **Gatellier, Mercier and Renerre (2004)**: reaction mixture – 2850 µL 50 mM of phosphate buffer (pH 8.2), 75 µL of blood serum and 75 µL of 10 mM pirogallols (p.a.); wavelength – 340 nm;
- catalase activity (CAT) was measured by **Beers and Sizer (1952)** and **Iwase et al. (2013)** method;
- glutationperoxidase activity (GPx) was determined by **Paglia and Valentina (1967)**;
- the concentration of reduced glutathione (GSH) was determined using Ellman reagent **(Noctor et al., 2011)**.

Statistic analysis

STATISTICA 10 program was used. The results were presented as "Weighted statistical significance ±Standard deviation" (M ±*SD*). Statistical significance was calculated by one-parametric ANOVA test and Tukey criterion. 0.05 probability was chosen as a significant level.

RESULTS AND DISCUSSION

The basic parameters of the control and enriched quark product samples are specified in Table 1.

The first stage of the biological experiment didn't show any changes in the animals' clinical state in all groups.

The analysis of the consumed feed showed that from the second week in group 1, 2 and 3 the amount of the consumed calories was reduced by 5 – 7 kcal. At the second stage of the experiment the rats of group 4 relative to group 1 consumed in the average by 3 – 5% more feed and from the 2nd to 3rd week their ration caloricity made up in average 50 kcal and then to the 4th week it was reduced to 43 kcal per day. It should be noted that slight consumed calories variability was registered in rats from group 2, 3 and 4 from the second week (Figure 1, a).

Weight gain of the rats from group 2, 3 and 4 authentically didn't differ from group 1. The rats from group 1 and 4 firmly gained weight during the whole experiment. At the second stage of the experiment the weight gain of the rats from group 2 and 3 was reduced but at the 4th week, the rats weight gain was registered.

The weight of the rats from group 2 and 3 starting from the second week and up to the end of the experiment was significantly varied that is stipulated by the individual peculiarities of the organism and development of compensatory reactions in response to LF-EMF impact (Figure 1, b).

Table 1 The quark product samples parameters.

Parameter	Quark product	
	Control (*M* ±*SD*)	Experimental (*M* ±*SD*)
Fat, %	0.4 ±0.06	0.4 ±0.1
Total protein, %	10.7 ±0.3	10.8 ±0.5
Carbohydrate, %	3.3 ±0.15	3.1 ±0.15
Vitamin C, mg %	-	47 ±5
Total polyphenolics content, mg.kg^{-1}	-	246 ±14
Amount of probiotic microorganisms (*Lactobacillus acidophilus*), CFU.g^{-1}	-	8 x 10^6 ÷ 4 x 10^7

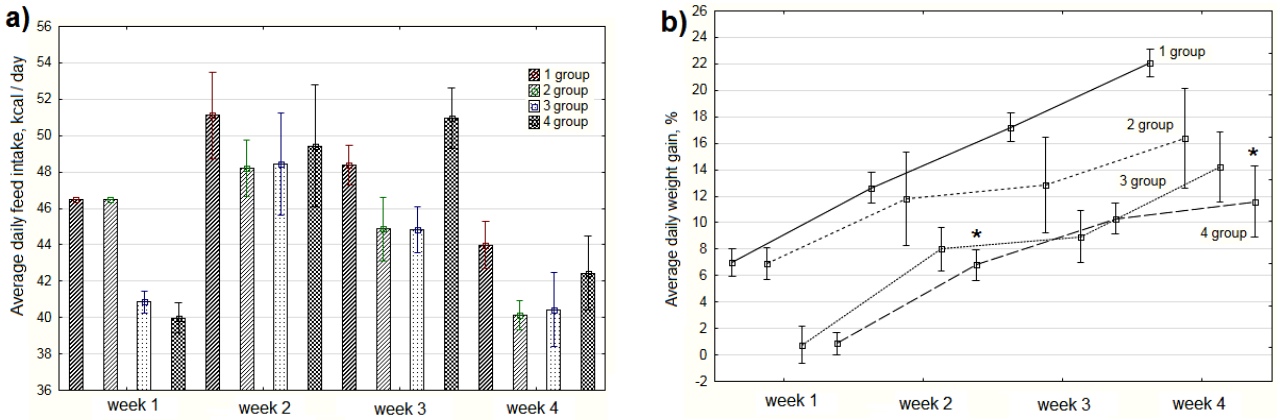

Figure 1 The dynamics of calories consumption by the rats (a) and change of the rats weight (b) during the experiment.
Note: * – significant difference by comparison between group 4 and 2.

It should be noted that at the first stage of the experiment the maximum gains were registered in rats of 1 and 4 group – 14% and 12%, in rats of 2 and 3 group – 10% and 8%.

At the second stage after LF-EMF impact the gains were: in group 1 – 9.9%, in group 2 – 4.8%, in group 3 – 5.4%, in group 4 – 10.3%. The gains at the end of the experiment relating to the initial mass of the rats made up: in group 1 – 25.9%; group 2 – 19.8%, group 3 – 14.7% and group 4 – 20.6%. Evidently, LF-EMF impact on the rats from 2nd up 4th week didn't affect significantly the rats gain from group 4 consuming the enriched product.

Statistically significant reduction of leucocytes and lymphocytes was revealed in blood serum of the rats from groups 2, 3 and 4 relatives to group 1 by 13.6% and 23.5%, 35.0% and 53.5%, 34.2%, and 45.4%, respectively.

Authentically the number of leucocytes and lymphocytes was reduced relative to group 2 in the rats of group 3 by 24.7% and 39.4% and group 4 by 23.9% and 28.6% (Table 2).

Herewith the concentration of granulocytes in the rats' blood of group 4 was reduced relative to the figures of group 1 by 41.1%, relative to the figures of group 3 by 42.7%.

There were no statistically significant changes of erythrocytes, hemoglobin content and hematocrit level in the groups. In group 2 compared to group 1, the valid reduction of MCV by 2.6% was observed. The rats from group 4 showed the valid increase MCV and MCH relative to group 1 by 2.6% and 3.1%, group 2 – by 5.3% and 4.0%, group 3 – by 3.3% and 2.7%. The reduction of thrombocytes number in the rats consuming dairy products compared to group 1 and 2 was revealed: in group 3 – by 6.8% and 8.1%; in group 4 – by 7.6% and 8.9%.

The analysis of rat blood serum biochemical index (Table 3) characterizing protein metabolism showed the lack of variations in the concentration of total protein and urea. Herewith reduction of albumin concentration in blood serum of rats group 3 and 4 was revealed compared to group 1 by 12.8% and 10.0% compared to group 2 – by 12.5% and 9.8% respectively. The rats of group 2 showed an increase of creatinine relation to group 1 by 9.5%; to group 4 – by 10.2% and to group 2 by 18.0%. AST activity in rats groups 2 - 4 was increased compared to group 1 by 22.2%, 65.8%, and 27.1%. AST activity of the rats of groups 3 compared to group 4 was increased by 20%. Herewith ALT activity was reduced in groups 2 and 3 compared to group 1 by 23.3% and 30.6%. Glucose concentration in rats blood serum of group 3 and 4 compared to group 1 by 38%, compared to group 2 up to 40% was registered. Groups 3 and 4 showed cholesterol content reduction compared to group 1 by 17.0% and 27.9%, herewith cholesterol amount in group 4 was reduced for sure compared to group 2 by 20.9%. Triglycerides content was reduced in groups 3 and 4 by 36.7% and 29.2% compared to group 1.

The analysis of antioxidant status of rats' plasma is specified in Figure 2.

It was shown that CAT activity wasn't changed in all rat groups. TBA-RP content was increased in rats of group 2 by 54.5% compared to group 1 while this index was reduced in group 3 and 4 by 31.8% and 20.4% ($p \geq 0.05$) compared to group 2. Reduction of GPx activity in rats' blood plasma of group 4 compared to group 1 by 8.9%, compared to group 2 by 11.6%, compared to group 3 by 10.8% was registered. SOD activity in the rats of group 2 was increased by 7.2% compared to group 1, in rats of group 3 and 4 it was reduced compared to group 2 by 5.2% and 7.0%. GSH concentration reduction was detected in rats blood plasma of group 2, 3 and 4 compared to group 1 by 34.4%, 33,4% and 38.4% respectively while GSH content in group 4 was also reduced compared to group 2 by 6.1% and in group 3 by 7.4%. The level of blood plasms total antioxidant activity was reduced in group 3 and 4 compared to group 1 by 35.9% and 20.2% ($p \geq 0.05$) while this index in group 3 was lower of group 2 figures by 29.4%. It was noted that the level of blood plasms

Table 2 Hematological index of the rats' blood after LF-EMF impact and feeding with fermented dairy products.

Parameter	Group			
	1	2	3	4
	(*M* ±*SD*)	(*M* ±*SD*)	(*M* ±*SD*)	(*M* ±*SD*)
LEU, 10^9/L	12.23 ±0.86	10.56 ±0.70 [1]	7.95 ±0.72 [1,2]	8.04 ±0.63 [1,2]
LYM, 10^9/L	10.41 ±0.61	7.96 ±0.48 [1]	4.82 ±0.53 [1,2]	5.68 ±0.24 [1,2]
GRAN, 10^9/L	2.41 ±0.14	1.78 ±0.15	2.48 ±0.34	1.42 ±0.11 [1,3]
MID, 10^9/L	0.33 ±0.10	0.54 ±0.12	0.28 ±0.05	0.40 ±0.12
RBC, 10^{12}/L	17.47 ±0.80	18.69 ±0.44	28.81 ±1.34 [1, 2, 3]	20.67 ±0.92
HGB, $g.L^{-1}$	156.9 ±1.3	155.1 ±1.9	153.6 ±1.3	156.8 ±1.3
HCT, %	43.08 ±0.42	41.64 ±0.50	41.59 ±0.37	42.68 ±0.35
MCV, mkm^3	46.15 ±0.33	44.95 ±0.38 [1]	45.85 ±0.27	47.35 ±0.20 [1, 2, 3]
MCH, pg	16.88 ±0.10	16.74 ±0.09	16.96 ±0.11	17.41 ±0.08 [1, 2, 3]
PLT, 10^9/L	833.3 ±12.9	844.8 ±16.0	776.3 ±13.1 [1,2]	769.7 ±15.0 [1,2]
PCT, %	0.49 ±0.01	0.50 ±0.02	0.46 ±0.01	0.47 ±0.02

Note 1: LEU – leukocytes, LYM – lymphocyte, GRAN – granulocyte, MID – other types of white blood cells not classified as lymphocytes or granulocytes; RBC – red blood cell; HGB – hemoglobin; HCT – hematocrit; MCV – mean cell volume RBC; MCH - mean cell hemoglobin; PLT – platelet (thrombocyte); PCT – plateletcrit.

Note 2: [1] – significant difference compared to group 1; [2] – significant difference compared to group 2; [3] – significant difference compared between group 3 and 4.

Table 3 The biochemical index of rats' blood serum after LF-EMF impact and the product consumption.

Parameter	Group			
	1 (*M* ±*SD*)	2 (*M* ±*SD*)	3 (*M* ±*SD*)	4 (*M* ±*SD*)
Total protein, g.L^{-1}	76.93 ±0.95	77.12 ±1.78	72.53 ±1.76	72.60 ±1.75
ALB, g.L^{-1}	46.70 ±1.24	46.58 ±0.76	40.73 ±0.39 [1,2]	42,01 ±1.12 [1,2]
CREA, µmol.L^{-1}	59.50 ±1.86	65.14 ±1.49 [1]	59.17 ±1.85	53.43 ±1.55 [1,2]
Urea, mmol.L^{-1}	8.01 ±0.30	7.94 ±0.20	8.32 ±0.31	8.05 ±0.28
AST, U.L^{-1}	85.9 ±10.0	105.0 ±6.9 [1]	142.6 ±8.1 [1,3]	109.2 ±8.0 [1,3]
ALT, U.L^{-1}	87.4 ±7.54	67.00 ±2.48 [1]	60.67 ±5.20 [1]	75.43 ±2.74
GLU, mmol.L^{-1}	19.46 ±1.21	20.14 ±1.94	12.23 ±1.41 [1,2]	12.06 ±1.72 [1,3]
CHLST, mmol.L^{-1}	1.47 ±0.07	1.34 ±0.04	1.22 ±0.07 [1]	1.06 ±0,04 [1,2]
TG, mmol.L^{-1}	0.71 ±0.04	0.58 ±0.04 [1]	0.45 ±0.06 [1]	0.51 ±0.05 [1]

Note 1: ALB – albumin, CREA – creatinine, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GLU – glucose, CHLST – total cholesterol, TG – triglyceride.

Note 2: [1] – significant difference compared to group 1; [2] – significant difference compared to group 2; [3] – significant difference compared between group 3 and 4.

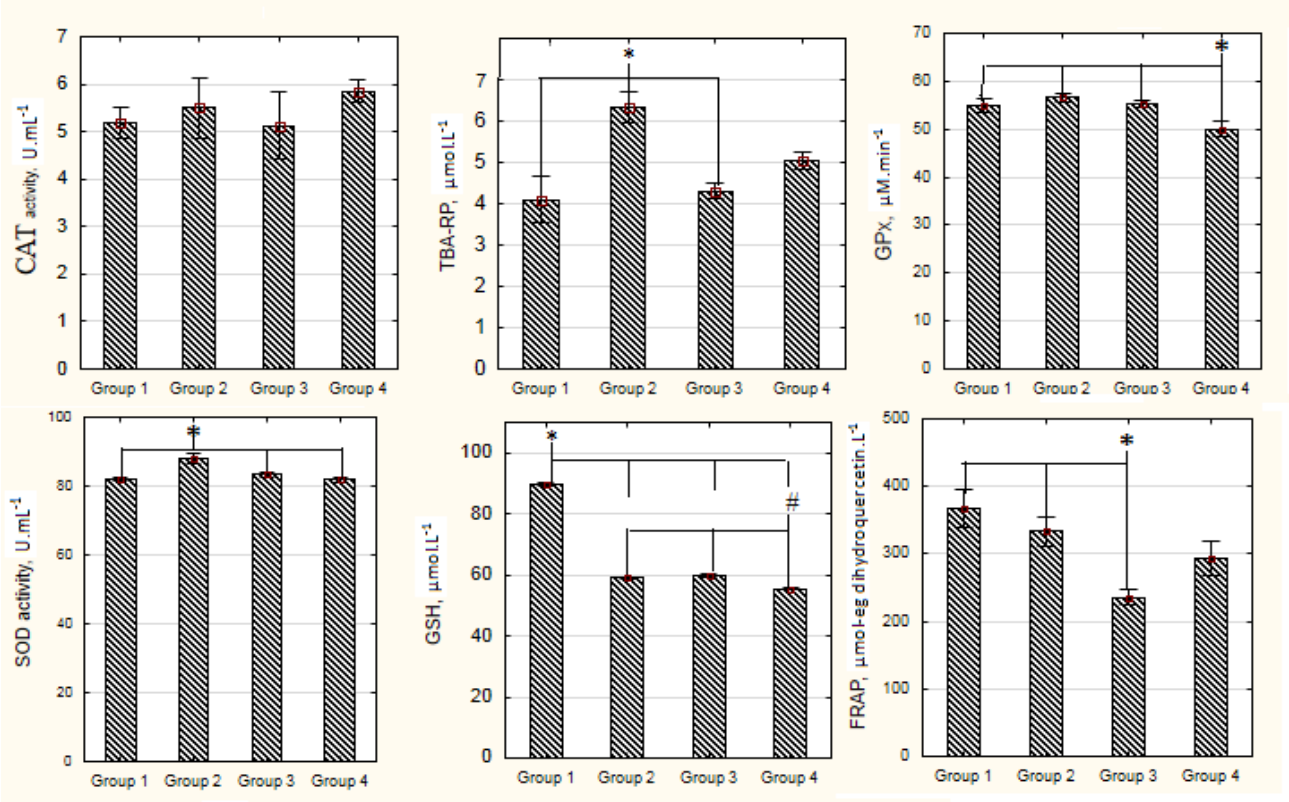

Figure 2 Antioxidant status of rats' plasma.

antioxidant activity of group 4 was increased by 24.6% ($p \geq 0.05$) relative to group 3 index.

CONCLUSION

The dairy product enriched with probiotics, polyphenols, and vitamins presented in this experiment showed the protective effect at rats' damaging impact of LF-EMF. The normalization of protein catabolism and growth rates, blood cells functional activity particularly leucocytes and erythrocytes, reduction of free-radical oxidation at electromagnetic field impact on the body were recorded. Besides, hypo-cholesterolemic (reduction of cholesterol by more than 20% relative to group 2 and 3 rats) and hypoglycemic (reduction of glucose up to 40% relative to group 1 and 2) effects were revealed.

The mentioned recovery of the broken balance in pro-oxidant-antioxidant system obviously occurs due to reduction of pro-oxidant load on enzymatic (reduction of SOD activity up to 5%, GPx by more than 10%) as well as on low-molecular chains of the antioxidant system (reduction of GSH up to 40%). These data evidence high bioavailability of self – antioxidants of milk as well as introduced additionally due to technological processes. Since any food products consumed by a human effect pro-oxidant-antioxidant state balance to a variable extent and different tendency it creates the presuppositions for correction of antioxidant potential without the usage of pharmaceutical preparations at high effectiveness due to long systematic impact of alimentary agents. Thus the enriched dairy product is of great practical interest in dieto-therapy for correction of antioxidant/pro-oxidant status.

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