

Study of the effect of alimentary biocorrectors on the physiological and biochemical parameters of laboratory rats of the Wistar line

*L I Vasilenko*¹, *A V Grebenshchikov*¹, *N S Rodionova*¹, *E S Popov*^{1*}, *E A Pozhidaeva*¹, and *Y A Dymovskikh*¹

¹Voronezh State University of Engineering Technologies, 19, Revolution Avenue, Voronezh, 394036, Russia

Abstract. Physiological and biochemical parameters of blood plasma of laboratory animals were evaluated when using, in addition to the main diet, a food substance with known biocorrecting properties - a probiotic emulsion administered orally, based on wheat germ oil and biomass of a consortium of lacto and bifidobacteria, including *Str. thermophiles*, *B. bifidum*, *B. longum*, *B. adolescentis*, *L. acidophilus*, *L. plantarum*, *L. fermentum*. The object of experimental studies was male and female growing white rats of the Wistar line in the amount of 60 individuals, comprising 2 experimental and 2 control groups of 15 individuals each. Evaluation of the average values of physiological indicators showed an increase in the horizontal and vertical motor activity of the white rats of the experimental group by an average of 16-18 % and 60-70%, an increase in the number of examined minks by 10-12 %, a significant decrease in the level of grooming and defecation. It was established that the clinical and biochemical parameters of the blood plasma of white rats were within the physiological limits. The concentrations of total protein, glucose, high and low density lipoproteins, alanine aminotransferase, aspartate aminotransferase by group differed slightly. It was found that the concentration of total cholesterol and LDL in the test groups of females and males at the end of the experimental studies decreased by 2.1-2.2 % and 4.1-5.8 %. The bilirubinaikreatinin concentration was slightly reduced in the experimental group of females and males by 1.1-1.3 % and 2.1-4.2 %, respectively. A significant increase in hemoglobin and red blood cells by 2.6-7.1 % and 4.8-5.1 % was recorded.

1 Introduction

Alimentary technologies for biocorrection of food status and physiological states of the human body require the development of fundamental and applied scientific research on the biomedical assessment of the safety of new food sources and ingredients, the introduction of innovative technologies, including physicochemical, bio- and nanotechnology, the expansion of the range of new food systems using natural alimentary biocorrectors and the

* Corresponding author: e_s_popov@mail.ru

development of criteria for assessing their effectiveness. The most relevant, effective and promising agents for the correction of food status and the prevention of pathological conditions are natural forms of plant raw materials obtained in the process of their deep processing, in particular wheat germ oil, as well as probiotic microorganisms that allow to enhance the effect of target biologically active substances [1].

The biocorrecting properties of wheat germ oil are due to the high content of vitamins A, D, E, polyunsaturated fatty acids, octocosanol and are confirmed by extensive clinical trials in the treatment of various diseases - cardiovascular, gastroenterological, diabetes, hepatitis, infertility, burn and wound injuries, etc. Wheat germ oil is a valuable source of polycosanol, the content of which is 1,5-8,0 mg/100 g. Polycosanol is a mixture of high-molecular aliphatic alcohols with a long chain of 20 to 36 carbon atoms, the mechanism of action of which is based on modulation of HMG-CoA reductase, on the absorption of bile acids and partial utilization of lactic acid. Polycosanol increases receptor dependent processing of low-density lipoproteins (LDL) by increasing LDL binding to its receptor, improving LDL transport to liver cells, thus significantly enhancing LDL cholesterol breakdown. In addition to lowering LDL-C, polycosanol has been shown to increase high-density lipoprotein (HDL) levels, protect HDL-C from the damaging effects of free radicals, and inhibit excessive platelet aggregation [2].

The biomass of probiotic microorganisms introduced into the diet also has a biocorrecting effect, in particular, cytophobic and energy support of the macroorganism, energy supply of the epithelium, stimulation of the immune system, formation of immunoglobulins, regulation of intestinal motility, participation in regulation, differentiation and regeneration of intestinal epithelium, provision of cytoprotection, detoxification, elimination of endo- and exogenous toxic compounds, destruction of mutagens, activation of drug compounds, formation of signaling molecules (neuro- and transmitters), maintenance of ionic, physical and chemical parameters of homeostasis of the priepithelial zone, supply of substrates for lipo- and glucogenesis [1].

Currently, most scientific schools specializing in ensuring human health are aware of the importance of the use of probiotic foods [3]. The role of probiotics in restoring healthy intestinal microflora (maintaining intestinal homeostasis), improving immunity and combating various metabolic, neurological, autoimmune, cardiovascular, and oncological diseases has been proven [1, 3]. Nevertheless, a number of clinical studies have reported notable differences in the health-promoting effect of probiotics, which can be caused by various reasons, including strain properties, duration of interventions, subject's health, physiology and other environmental factors [3].

Consideration of factors of influence increases the reliability of data and the validity of research results, contributes to the development of individual intervention strategies. Due to the fact that the effects of probiotics depend on the strain and individual characteristics of the body, the use of probiotic drugs as part of drug treatment regimens or as additional therapy should comply with the principles of rationality and have a scientific basis.

The results of experimental work lay the foundation for the development of biotherapeutic drugs, and are also a significant guideline for the production of food products that can have a positive effect on the microbiota of the human gastrointestinal tract.

The purpose of the study is to establish the possibility of increasing the physical strength, endurance, level of stress resistance and efficiency of the cardiovascular system of laboratory animals using alimentary factors (biocorrective food substance).

2 Materials and methods

As a biocorrector, a food substance was studied - a probiotic emulsion, including 2.5 g of wheat germ oil (manufactured by Pulat LLC, RF)) in combination with 2.5 g of biomass of the lacto and bifidobacteria consortium based on strains provided by the bioresource center - the All-Russian Collection of Industrial Microorganisms (BRC VKPM), Research Center "Kurchatov Institute": *Streptococcus thermophiles* (B 8328), *Bifidobacterium bifidum* (AC-1579), *Bifidobacterium longum* (AC-1257), *Bifidobacterium adolescentis* (AC-1245), *Lactobacillus acidophilus* (B-5097), *Lactobacillus plantarum* (B-3962), *Lactobacillus fermentum* (B-2875). In cultured biomass, the concentration of active cells was not less than 10^9 CFU/mL, titratable acidity 80-100 °T, pH 4.61-4.65 (manufactured by FSBEI HE VGUI, RF). Biomass growth was carried out on skimmed milk in the temperature range of 37-42 °C until reaching the pH values of the 4.4-4.7, the standard cultivation time was 8-10 hours. The stability of the emulsions was at least 70-75 % due to the high exopolysaccharide activity of the biomass.

The physical strength, endurance, degree of anxiety and cardiovascular efficiency of laboratory animals were determined *in vivo* in an experiment on 60 growing Wistar rats, divided into 4 groups: 1st (control; n = 15, female, body weight 109.5 ± 3.1 g), 2nd (main; n = 15, females, body weight 111.2 ± 3.0 g), 3rd (control; n = 15, males, body weight 118.4 ± 3.0 g), 4th (main; n = 15, males, body weight 119.7 ± 3.0 g). Studies on animals were performed in accordance with the requirements set forth in GOST 33647-2015 "Principles of Good Laboratory Practice" and GOST 33216-2014 "Guidelines for the Maintenance and Care of Laboratory Animals. In accordance with the adopted Regulation on the Commission for the Control of the Content and Use of Experimental Animals (dated February 12, 2024), the Bioethics Commission operates (Order No. 24 of May 31, 2024). Also equipped with a vivarium for keeping animals and scientific work with them. Persons with veterinary education who have the necessary competencies to work in vivariums are allowed to work with animals. Personnel participating in experiments with animals shall carry out the necessary documentation of procedures, results and information about animals.

The animals participating in the experiment underwent the necessary adaptation period and were kept in appropriate conditions to minimize the possible deterioration of their condition. All procedures with animals were non-lethal and low-traumatic, carried out taking into account their condition to ensure objective and reliable experimental results. At the end of the experiment, the animals were provided with the necessary rehabilitation period under the supervision of experienced veterinary specialists.

Animals of groups 2 and 4 received an additional food source to the main diet of the biocorrector. During 15 days of the experiment, individual indicators of food availability and body weight gain of each animal were determined. From the 14th to the 15th day, food consumption and fecal collection were recorded.

The diet of the animals differed only in the presence of a probiotic emulsion administered orally in the test groups (Table 1).

Table 1. Animal diet.

Component	Content per 100 g of feed, g			
	1st (control group)	2nd (main group)	3rd (control group)	4th (main group)
Probiotic emulsion	-	2.5	-	2.5
Grain mixture	14.0	14.0	14.0	14.0
Bread 2nd grade	4.0	4.0	5.0	5.0
Carrots	8.0	8.0	8.0	8.0
Greens	8.0	8.0	8.0	8.0
Meat	3.0	3.0	5.0	5.0
Fish meal	0.5	0.5	0.5	0.5
Cottage cheese	2.0	2.0	-	-
Fat	Sunflower oil	4.0	4.0	4.5
	Hog grease	6.0	6.0	6.0
Mineral mixture	4.0	4.0	4.0	4.0
NaCl	0.15	0.15	0.15	0.15

3 Assessment of overall animal status

In-life assessment of the general condition of experimental animals was carried out weekly according to the criteria set out in Table 2.

Table 2. Assessment of general condition of experimental animals.

Indicator	Severity of the indicator	Score in points
Motive activity	absence of sign	0
	weak feature	1
	moderate sign	2
	active (corresponding to the norm) sign	3
Reaction to sound stimulus	absence of sign	0
	weak feature	1
	moderate sign	2
	active (corresponding to the norm) sign	3
Reaction to pain stimulus	absence of sign	0
	weak feature	1
	moderate sign	2
	active (corresponding to the norm) sign	3
Eating behavior	does not eat food	0
	consumes food in small quantities	1
	does not eat enough food	2
	normal (normal) food regimen	3
Drinking behavior	animal does not drink water	0
	water drinks badly	1
	water drinks less than the daily norm or significantly more	2
	normal drinking mode	3

The integral severity of the condition of experimental animals was expressed by the arithmetic mean number of points according to the evaluated criteria. For example, motor activity - 2 points, reaction to a sound stimulus - 1 point, reaction to a pain stimulus - 2 points, food regimen - 1 point, severity of the animal's condition – 1.5 points.

The dynamics of the state of experimental animals generally demonstrated a satisfactory clinical state of animals during the experiment (Table 3).

Table 3. Dynamics of general condition of experimental animals.

General condition of animals (points)	Group	
	Females	Males
Start of experiment	2.8±0.45	2.6±0.55
End of experiment	3.0±0	3.0±0

An integral assessment of the condition of animals in the experimental groups revealed a significant deviation.

The open field method is widely used in the study of behavioral reactions in pharmacology, psychogenetics, and consists in the study of the motor component of the indicative reaction and emotional reactivity of animals. The author of the method is Hall C.S. (1934). The installation for the open field technique has many modifications.

A study of the effect on the behavior of rats of introducing a probiotic emulsion into the diet was carried out in an open field test. All experimental procedures were carried out in accordance with international rules for the maintenance and work with laboratory animals. The behavior was considered in a special "open field" installation: a white arena with an opaque side 31 cm high, 62 cm in diameter, drawn into sectors at the intersection of which there were "minks" (holes on the floor). After each experiment, the plant was cleaned. There was no special lighting of the arena, experiments were carried out in the audience in the evening under the light of daytime lamps (illumination - 150 lux) with a noise level within 20 dB. At the same time, a number of elementary motor acts and poses were recorded, the totality of which characterizes holistic behavior in the "open field."

The animal was placed in the center of the arena with its tail to the experimenter and its behavior was observed for 5 minutes. As soon as the rat crossed the sector line, an appropriate mark was made. The number of crossings was noted separately for each minute. Also in the "open field" in addition to horizontal activity (the number of crossed lines along the periphery and center of the arena is total) the number of looks in the "minks" (sticking the head inside the holes) was recorded [6], grooming (the number of approaches of the front paws to the mouth and their licking, cleaning the front of the muzzle, body, as well as the duration of grooming), defecation (amount of excrement), vertical motor activity (the number of lifts on the hind legs with support and support on the wall in total). The results were collected within 5 minutes, and the average value for 1 minute was calculated. The processing of the experimental results was carried out visually, as well as Microsoft Excel.

Total protein-biuret method (VITAL, Russia) was determined in blood plasma, glucose by colorimetric method (VITAL, Russia), AsAT and AlAT by the unified Reitman-Frenkel method (VITAL, Russia), total cholesterol by the enzymatic colorimetric method using the Cholesterol-VITAL kit (VITAL, Russia), low and high density lipoproteins (LDL, HDL) by enzymatic colorimetric method with selective protection (without precipitation) (VITAL, Russia), total bilirubin - by the Endrassik-Grof method (VITAL, Russia), creatinine - by the enzymatic colorimetric method without deproteinization (VITAL, Russia), hemoglobin, red blood cells - by the colorimetric cyanide method (VITAL, Russia).

4 Results

It should be noted that in active animals, exploratory motivation prevails over the emotion of fear of an unfamiliar environment. In the "open field" test, such tentative research activities of animals were recorded as vertical (with and without support on the wall), horizontal motor activity, looking into minks, as well as emotional manifestations - grooming and defecation.

The exploratory and locomotor activity of rats in groups 2 and 4 was found to be higher compared to rats in groups 1 and 3, and the defecation level was found to be lower, which may indicate a higher level of anxiety in rats in groups 1 and 3.

It was found that the total horizontal motor activity of rats from groups 2 and 4 during the experiment averaged 7.5 ± 0.3 and $8,1 \pm 0.2$ crossed lines along the periphery and center per 1 minute, and in rats of groups 1 and 3 this indicator was only 6.3 ± 0.2 and 6.6 ± 0.3 ($p < 0.05$).

Analysis of the number of sightings in minks during the experiment, in terms of 1 minute, showed that in this case the activity of rats of groups 1 and 3 is lower, compared with other groups of rats 5.7 ± 0.3 and 5.8 ± 0.2 , respectively. As in the case of horizontal activity, the performance of rats from groups 2 and 4 remains almost the same at 6.3 ± 0.2 and 6.5 ± 0.3 , respectively, that is, these animals show almost the same degree of both motor and research activity ($p < 0.05$).

The greatest difference is observed in the monitoring of vertical motor activity, where rats of groups 1 and 3 are less likely to climb on the hind legs 0.4 ± 0.02 and 0.5 ± 0.03 lifts per minute, respectively, during the entire experiment, while in rats from groups 2 and 4 these numbers ranged from 0.7 ± 0.2 to 0.8 ± 0.2 ($p < 0.05$).

It is known from literary sources that frequent and short-term "washing" is alarming grooming, and a high level of defecation additionally indicates the animal's anxiety, anxiety, and fear. Thus, a significantly high level of defecation in rats of groups 1 and 3 proves that they have a greater degree of anxiety compared to rats from groups 2 and 4.

Monitoring of food consumption by experimental animals of all groups showed that the food consumption index did not have a significant difference in the experimental and control groups, therefore, the test product for animals in terms of preference did not differ from the usual diet.

In the experiment, the clinical and biochemical status of animals was monitored for a number of parameters (Table 4).

Table 4. Clinical and biochemical status of laboratory animals ($p < 0.05$).

Indicator	Units of measurement	Norm	Group			
			1	2	3	4
Total protein	g/l	69 ± 0.7	73 ± 0.4	68.8 ± 0.4	70 ± 0.4	70 ± 0.4
Total cholesterol	mmol/L	1.9 ± 0.04	1.93 ± 0.1	1.89 ± 0.1	1.92 ± 0.1	1.88 ± 0.1
Glucose	mmol/L	4.2 ± 0.4	4.4 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.4 ± 0.1
High density lipoprotein (HDL) cholesterol	mmol/L	0.84 ± 0.06	0.89 ± 0.05	0.88 ± 0.05	0.85 ± 0.05	0.84 ± 0.05
Low-density lipoprotein (LDL) cholesterol	mmol/L	0.69 ± 0.12	0.74 ± 0.1	0.71 ± 0.1	0.7 ± 0.1	0.66 ± 0.1
Alanine aminotransferase (ALAT)	U/L	56 ± 1	57 ± 0.4	55 ± 0.4	55 ± 0.4	55 ± 0.4
Aspartate aminotransferase (AsAT)	U/L	40 ± 2	39 ± 0.4	41 ± 0.4	39 ± 0.4	42 ± 0.4
Bilirubin total	mg/L	3.2 ± 0.09	3.22 ± 0.1	3.19 ± 0.1	3.21 ± 0.1	3.17 ± 0.1
Creatinine	mg/dL	0.47 ± 0.01	0.47 ± 0.1	0.46 ± 0.1	0.48 ± 0.1	0.46 ± 0.1
Hemoglobin	g/dL	12.05 ± 4.5	14.65 ± 0.2	15.04 ± 0.2	12.89 ± 0.2	13.81 ± 0.2
Erythrocytes	$10^6/\mu\text{L}$	3.60 ± 0.2	3.74 ± 0.2	3.92 ± 0.2	4.15 ± 0.2	4.36 ± 0.2

The concentrations of total protein, glucose, AlAT and AsAT did not differ significantly by group. It was found that the concentration of total cholesterol and LDL in the test groups of females and males at the end of the experimental studies decreased by 2.1-2.2% and 4.1-

5.8%. In this study, in groups 2 and 4 of females and males receiving the probiotic product, a decrease in bilirubin and creatininane levels of 1.1 -1.3% and 2.1-4.2%, respectively.

The highest concentration of hemoglobin was noted in the group of females receiving a probiotic corrector (group 4). Increases in hemoglobin levels after administration of the probiotic product were observed in both gender groups, which amounted to 2.6-7.1%. Group 4 (males, probiotic) had the highest red blood cell counts. A significant increase in red blood cells by 4.8-5.1% was recorded.

5 Discussion

According to the results of an experimental study in animal models, it was noted that additives with probiotic microorganisms reduced indomethacin-induced intestinal inflammation by reducing the production of reactive oxygen species and the production of cytokines IL-4b IL-17 and IFN (interferon). Activation of IL-12 production with the use of Lactobacillus strains with concomitant increased production of interferon was noted in the study by Kim et al. [4]. According to Kim et al., strains of lactobacilli can enhance the release of IL-12, which triggers the activation of natural killer cells (NK cells) and the secretion of IFN, leading to an innate immune response. In particular, the production of immunomodulatory and inhibitory cytokines contributes significantly to the maintenance of tolerance in autoimmune and inflammation-related diseases.

In addition to the above, it has been reported that probiotic microorganisms contribute to the production of a wide range of neuroactive metabolites, including neurotransmitters such as gamma-aminobutyric acid (GABA), norepinephrine and serotonin [5]. In particular, representatives of the genera Bifidobacterium, Lactobacillus and Streptococcus produce GABA [5]. According to the results of studies by Bagheri et al. [5], it is stated that taking probiotics increases the concentration of GABA in the brain. In the autism model in it has been proven that probiotic supplements reduce oxidative stress and increase GABA levels [7]. Behavioral and social impairments are often associated with gastrointestinal disorders, including increased intestinal permeability and inflammation. In a study by Xie et al., a positive correlation was noted between the abundance of Lactobacillus and a number of protective microbes in the lymphoid tissues of the intestines of mice with the level of social interaction of the latter [6].

Taking into account the previously published evidence of the effect on brain function of changes in the levels of neurotransmitters, proinflammatory and anti-inflammatory cytokines in the blood initiated by the microbiota [7], we note the logical, experimentally proven fact of an increase in behavioral and neurobiological markers of emotional resistance on the part of experimental animals consuming probiotic emulsion.

In addition to the above factors, we consider it necessary to note the possible influence of the energy metabolism factor on the decrease in the degree of anxiety of experimental animals. Short-chain fatty acids (SCFAs), such as acetate, butyrate and propionate, derived from the gut microbiota (in particular, as a result of a change in the latter as a result of a probiotic drug) in addition to direct consumption as an energy substrate, increase satiety and reduce food intake, affecting appetite [7]. Short-chain fatty acid propionate can play an important role in appetite regulation. SCFAs increase the secretion of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) by activating GPR41 (G protein-coupled receptors) and GPR43 in enteroendocrine cells of the intestine (L cells) [26, 28]. Increased vagus nerve cell irritation results in a satiety sensation that can be used to reduce energy intake. SCFAs also induce leptin expression in adipocytes and provide another potential mechanism for appetite control [7].

Thus, the gut microbiota can affect central nervous system (CNS) functions through several mechanisms, such as alteration of microbial composition, activation of immunity, vagus nerve, and microbial (neuro) metabolites.

The results of the study indicate the absence of any harm to the organs of animals as a result of taking the probiotic product, including the levels of AlAT and AsAT used to assess liver damage and toxicity of probiotics. The results obtained correlate with the results of the research groups on probiotics when experimenting with changes in immune function in animal experiments, in particular, A. Shehzadi et al. [8], Z. Bibi [9], F. Patra [3]

A decrease in total cholesterol and LDL levels was recorded in experimental subgroups of females and males, which does not contradict the conclusion of Name et al., Which showed, in particular, that the *Lactobacillus plantarum* YS5 strain isolated from homemade yogurt reduces cholesterol levels in rats [10].

The results of the study are consistent with the findings of Dias et al., who reported an increase in the concentration of hemoglobin and red blood cells in Wistar albino rats under oral administration of *L. plantarum* [11]. This result was explained by Korcok et al. [12] activation of hematopoietic organs and certain lactic acid bacteria, such as Lactobacillus, which indirectly increase the availability of dietary iron through a number of processes, including by lowering intestinal pH. Obazelu et al noted that probiotics improve health by increasing hemoglobin levels and red blood cell counts without causing unwanted changes in hematological parameters.

6 Conclusion

In the current study, rats treated with a complex probiotic corrector showed improved health in terms of hematological status, which proves the immunostimulatory effects of the supplement used. Similar results of the study were noted, in particular, according to the results of the study by Z. Bibi et al. [9]. Groups of rats receiving the probiotic product may have a long-term beneficial effect on both sexes, but more so on males than females. The results of the study can be useful in solving the problems of creating new probiotic products, in particular, in the tasks of substantiating the choice of a consortium of probiotic strains, substantiating the prospects for modulating a specific microbiota to prevent negative physiological and psychological consequences associated with chronic stress and depression. The results of the study provide additional material to support the assumption that the gut microbiome may play a regulatory role in the stress response, providing prospects for the development of potential therapeutic tools for the treatment of stress-related mental illness.

Acknowledgement

The study was carried out at the expense of a grant from the Russian Science Foundation No. 23-26-00256, <https://rscf.ru/project/23-26-00256/>.

References

1. B. Chugh, A. Kamal-Eldin, Bioactive compounds produced by probiotics in food products, *Current Opinion in Food Science*, **32**, 76-82 (2020)
2. M. Arruzazabala, S. Valdes, R. Mas, Arruzazabala, M. Effect of policosanols successive dose increase in platelet aggregation healthy volunteers, *Pharmacol. Res.*, **34**, 181-185 (2013)

3. F. Patra, R.K. Duary, Determination and safety aspects of probiotic cultures. Probiotics, prebiotics and synbiotics: technological advancements towards safety and industrial applications, In book: Probiotics, prebiotics and synbiotics, 122-160 (2022)
4. S.Y. Kim, J.S. Shin, K.S. Chung, Immunostimulatory effects of live *Lactobacillus sakei* K040706 on the CYP-induced immunosuppression mouse model, *Nutrients*, **12**, 11, 3573 (2020)
5. S. Bagheri, Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling, *Epilepsy Behavior*, **95**, 43-50 (2019)
6. R. Xie, Oral treatment with *Lactobacillus reuteri* attenuates depressive-like behaviors and serotonin metabolism alterations induced by chronic social defeat stress, *Journal of Psychiatric Research*, **122**, 70-78 (2020)
7. K.G. Jameson, Toward understanding microbiome-neuronal signaling, *Molecular Cell*, **78**, 577-583 (2020)
8. A. Shehzadi, Z. Bibi, M.Q. Sarwar, Effect of probiotics on hematological parameters of male and female Wistar rats, *Saudi Journal of Biological Sciences*, **30**, 1-9 (2023)
9. Z. Bibi, K. Ashraf, A. Shehzadi, Evaluation of isolated probiotics on the efficacy of immune system in male and female Wistar rats, *Saudi Pharmaceutical Journal*, **31**, 1036-1046 (2023)
10. Y. Name, Hypocholesterolaemic activity of a novel autochthonous potential *Lactobacillus plantarum* YS5 isolated from yogurt, *LWT-Food Science and Technology*, **111**, 876-882 (2019)
11. de C. Dias Probiotic supplementation causes hematological changes and improves non-specific immunity in *Bryconamazonicus*, *ActaScientiarum: Biological Sciences*, **42**, 1-9 (2020)
12. D.J. Korcok, Development of probiotic formulation for the treatment of iron deficiency anemia, *Chemical and Pharmaceutical Bulletin*, **66**, 4, 347-352 (2018)