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STUDY ON THE BENEFICIAL EFFECTS OF PROBIOTICS FOR SWIMMERS

ŠTUDIJA O KORISTNI UPORABI PROBIOTIKOV PRI PLAVALCIH

ABSTRACT

The study aimed to explore the effects of probiotic products on blood and immunological parameters of athletes undergoing intense training. The study sample consisted of 20 swimmers of both sexes (10 men and 10 women), aged 15 to 20 years. During a 4-month observation period, each athlete was administered probiotics for one month, followed by a similar preparation without a probiotic culture (placebo) for another month.
The results show that in the training period under consideration, an increase in erythrocyte production was observed, mainly due to respectively higher figures for the men's group. The reason for increased haematopoiesis among athletes is usually intense training, whereas during a steady training process there may be some effects that cause a decline in inflammation factors, thus suppressing haematopoiesis and boosting production of inflammatory cells (monocytes, lymphocytes, eosinophils and basophils). Our results show that in the period after consuming the active substance, inflammatory stimulation of lymphocytes decreased. The direct effect of probiotics on lower inflammation levels as the result of training should be explored in further studies.

Keywords: training, probiotics, athletes, infection

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IZVLEČEK

V študiji smo želeli preveriti učinke uporabe probiotičnih izdelkov na krvne in imunološke parametre pri športnikih v procesu konstantnega treninga. V preiskovani skupini je bilo 20 plavalcev različnih spolov (10 moških in 10 žensk), starih med 15 in 20 let. Vsak športnik je med 4 – mesečnim opazovanjem prejemal 1 mesec pripravek probiotika, 1 mesec pa enak pripravek, vendar brez vsebnosti probiotične kulture (placebo).
Rezultati kažejo, da je v opazovanem obdobju treninga prišlo do povečane tvorbe eritrocitov predvsem na račun povečane tvorbe eritrocitov pri moških. Vzrok za povečano hematopoezo je pri športnikih običajno povečan trening, pri enakomernem treningu pa so lahko tudi vplivi, ki zmanjšajo vnetne dejavnike, ki sicer zavirajo hematopoezo in povečujejo produkcijo vnetnih celic (monocitov, limfocitov, eozinofilcev, bazofilcev). Naši rezultati kažejo, da je v obdobju po jemanju učinkovine prišlo do manjše vnetne stimulacije limfocitov. Neposreden vpliv probiotika na zmanjšanje vnetja, povzročenega s treningom, pa bi morale dokazati nadaljnje študije.

Ključne besede: trening, probiotiki, športniki, infekt

INTRODUCTION

In recent years, the use of probiotic products has attracted the attention of different sports institutions and communities working to improve athletes' endurance and overall health during periods of training and/or high physical loading (Nichols, 2008; Pyne, et. al. 2015). Probiotics can reduce the risk of respiratory and gastrointestinal diseases during periods of stressful training and competition. The clinical advantages of probiotics are related to changes in the intestinal flora and strengthening of the intestinal and respiratory mucosa.

A number of studies have been published in recent years describing the use of probiotics among professional athletes or highly physically active individuals. The purpose of such study is to evaluate probiotic effects on clinical signs of disease and the functioning of the immune system, while also using placebos in a control group. The most frequently used probiotic cultures include *Lactobacillus casei, Lactobacillus fermentum, Lactobacillus Acidophilus* and *Lactobacillus rhamnosus.*

The first to describe the use of probiotics by athletes were British researchers (Clancy et al., 2006) who aimed to evaluate the effect of the *Lactobacillus acidophilus* probiotic culture on athletes showing signs of fatigue and exhaustion. Poorer athletic performance and chronic fatigue are usually related to recurring infections and lower mucosal immunity. Long-term, high intensity training can lead to a chronic decrease in the concentration of IgA antibodies in saliva. Moreover, top athletes are particularly prone to infections of the upper respiratory tract. These are often caused by the Epstein Barr virus (EBV). Intensive training and other stressful circumstances are likely to trigger immunity control mechanisms that are responsible for suppressing the virus. Among others, specific cytotoxic T-lymphocytes are activated in the upper respiratory tract, along with accelerated production of IgA antibodies. Recent research in mice has shown that the selected isolates of the *Lactobacillus acidophilus* bacteria strengthened the function of T-lymphocytes, thus protecting the body against a mucosal infection. However, the study results did not show any statistically significant differences between healthy and exhausted athletes in terms of concentration of the IgA antibodies and IFN-y cytokines in saliva as well as secretion of IL-4 and IL-12 cytokines in the blood. However, significantly lower blood counts of INF-y cytokine were established in athletes showing signs of exhaustion compared to healthy subjects. Cytokine, which is excreted by helper CD4+ cells, plays the main role in the body's defence against various viruses, as it triggers the effector mechanisms that help control their replication and spread. Secretion of blood IFN-y increased after treatment with *Lactobacillus acidophilus* probiotics, whereas concentration of such reached levels close to those measured in the control group of healthy athletes.

Repeated intensive training can cause the suppression of certain immunological parameters, such as neutrophil function, serum and mucosal immunoglobulin levels, antigen-presenting macrophags, production of cytokines in macrophages and lymphocytes, lymphocyte proliferative response to different mitogens and, most likely, also suppresses the cytotoxic activity of NK cells. It is known that repeated intensive trainings may cause gastrointestinal disorders and upper respiratory diseases, thus exerting a strong effect on athletes' physical condition and diminishing their endurance. Therefore, Kekkonen and others (Kekkonen et al., 2007) investigated the effect of the *Lactobacillus rhamnosus* GG (LGG) probiotic bacteria on the frequency of upper respiratory infections and symptoms of gastrointestinal disorders among marathon runners during a training period and again two weeks after a running a marathon. The study concluded that *Lactobacillus rhamnosus* GG did not affect the frequency of upper respiratory infections and symptoms of GI disorders among marathon runners, but did shorten the duration of infectious diseases.

The probiotic activity of the *Lactobacillus fermentum* culture was studied by Australian researchers (Cox et al., 2010) who aimed to establish whether the abovementioned probiotic induces defensive mucosal immune response and decreases the incidence, intensity and duration of respiratory diseases among endurance athletes. Their 14-week study included 20 highly trained runners who were administered placebo capsules for one month and probiotic capsules for another month. The most important study results include a significant decrease in the number of days suffering from symptoms of respiratory diseases and a downward trend in the intensity of the disease among the test group members who were taking the *L. fermentum* VRI-003 probiotic, thus also demonstrating the positive effect of probiotics on a person's health. Moreover, a slight increase in IFN-y cytokine and IgA1 concentrations in saliva among the test subjects was established; the effect, however, was not sufficiently statistically reliable.

Regular intake of probiotics should have a beneficial effect on intestinal flora and functioning of the immune system, but such positive effects are specific for an individual strain of probiotic bacteria. Gleeson and others (Gleeson et al., 2011) investigated the activity of the *Lactobacillus casei* Shirota probiotic bacteria, exposing 42 test subjects over 4 months of winter training and competitions to a daily dose of a commercially available fermented dairy drink that contained the abovementioned Gram-positive probiotic bacteria. The results showed a 50% decrease in the average number of upper respiratory diseases in the test group over the control group; however, there was no difference observed between the groups in terms of the duration nor the intensity of the disease. The incidence of gastrointestinal disorders did not differ between the groups, and only some differences in terms of duration were observed. Namely, disorders lasted 33% less time in the test group compared to the control, thus indicating new efficacy of the probiotic intake. Nevertheless, these results could not be connected with the immune function, as probiotics did not visibly affect blood counts of lymphocytes, neutrophils, monocytes or lymphocyte subpopulations.

Researchers from the School of Medicine of Griffith University in Australia (West et al., 2011) wanted to examine the effect of a probiotic intake containing the *Lactobacillus fermentum* (PCC®) bacteria on the occurrence of symptoms related to infections of the upper respiratory tract (URT) and gastrointestinal (GI) tract in a group of healthy, physically active individuals over a 15-week winter training period. Moreover, they aimed to check the effect of the same probiotic on faecal microbiological parameters and key elements related to immunity at rest, as well as their response to exercise and exhaustion. They found that probiotic intake resulted in a lower incidence of instances of URT infection and GI disorders during highly intensive training, as well as decreased use of medications against cough and flu, but only in male subjects. The same study with female subjects did not show statistically significant differences between the test and control groups. Measurements of variables of systemic immunity revealed ~20–75% lower concentrations of pro- and anti-inflammatory cytokines in men and women receiving probiotics.

Some probiotic products contain not only one probiotic bacterial culture but several types of microorganisms as well. One such product was tested by Austrian researchers (Lamprecht et al., 2012) who used the *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Enterococcus faecium* W54, *Lactobacillus acidophilus* W22, *Lactobacillus brevis* W63 and *Lactococcus lactis*

W58 cultures. The purpose of the study was to explore the effect of probiotics on the permeability of the wall of the digestive tract as well as on different variables that are determined in venous blood. They identified carbonyl proteins, malondialdehyde (MDA), total oxidation status of lipids (TOS), tumour necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6). In order to evaluate the permeability of the digestive tract wall, they checked for presence of the zonulin protein, as its higher concentration can modulate the condition of tight junctions between cells and consequently increase gastrointestinal permeability. The latter facilitates the passing of antigens out of the GI tract, inducing an inflammatory immune response and oxidative stress. After 14 days the test groups' zonulin values were statistically lower than those of the placebo control group. The pre-exercise value of carbonyl proteins exceeded the normal values in both groups by 15% to 25%. After the exercise, the concentration statistically significantly increased in both groups, whereas analysis showed a slight drop in pre- and post-exercise concentrations in the test group on probiotics, yet the difference was not statistically significant. The values of other parameters did not reveal any statistically significant differences.

MATERIALS AND METHODS

Sample of subjects

The study sample consisted of 20 swimmers of both sexes (10 male and 10 female), aged between 15 and 20 years. During a 4-month observation period, each athlete took a probiotic preparation (Acidosalus plus) for one month and then received the same preparation without a probiotic culture (placebo) for another month.

Acidosalus® is one of a few products with such a high concentration of the beneficial active bacteria LACTOBACILLUS ACIDOPHILUS (1 ml of preparation contains 10 exp.9 to 10 exp.11). It comes in liquid form and does not contain any preservatives.

First we tested which of the Acidosalus products our swimmers could take on an empty stomach, before morning training, without it causing them any trouble during exercise. We decided to test the Acidosalus plus probiotic drink, which is based on cow's milk, with the basic formula enriched with other probiotic cultures.

Immunological tests were conducted every month from February to April 2015. The swimmers were members of three different swimming clubs.

Work methods

Concentrations of lymphocyte populations were measured using **flow cytometry.** The test is based on the binding of monoclonal antibodies marked with different fluorochromes. Cells can be marked with several fluorescent colours at the same time. Basically, they can be marked with two, three or more types of monoclonal antibodies so as to indicate which antigen combinations are expressed by individual cells. Binding of monoclonal antibodies to lymphocyte-specific surface antigens (CD antigens) facilitates recognition of different types of lymphocytes, along with definition of their share and concentration. Monoclonal antibodies were bound to antigen CD3 (T lymphocytes), CD19 (B lymphocytes), CD4 (helper T cells), CD8 (suppressor T cells), CD56 (NK cells) and HLA-DR/CD3 (activated T lymphocytes). The main elements of the flow cytometer include a source of light, a flow chamber with an optical assembly of mirrors, lenses and filters, an electronic device converting light impulses into electrical and these into digital, and a PC for collecting, analysing and aligning data as well as operating the device. A laser serves as the light source. The cells run through the flow chamber in an isotonic solution. As the cell passes through the laser beam, it generates a signal pulse. Two photo-detectors measure light reflection or refraction; one in the direction of the source, i.e. forward-angle light scatter (FALS), and the other in the direction of the incoming light, i.e. right-angle light scatter (RALS). FALS is important in establishing the size of the cells, whereas RALS receives the light reflected from the cells depending on the granulation and surface structure of the cells. The emitted light is measured in one or more detectors equipped with coloured filters. All collected data is arranged and analysed by the computer. Results are presented mathematically and graphically.

LYMPHOGRAM

A **lymphogram** was made so as to measure the concentration of lymphocyte populations using flow cytometry. This test is based on the binding of monoclonal antibodies that are marked with different fluorochromes. Binding of monoclonal antibodies to lymphocyte-specific surface antigens (CD antigens) facilitates recognition of different types of lymphocytes, while also enabling definition of their share and concentration. The process consists of screening the shares and concentrations of T lymphocytes (CD3), B lymphocytes (CD19), helper T cells (CD4), suppressor T cells (CD8), activated T lymphocytes (HLA-DR), natural killer (NK) cells (CD16 + CD56) and establishment of the ratio between the helper T cells and suppressor T cells.

Method protocol – lymphogram

After 20 μL of Lymphogram was pipetted into a 100 μL blood sample, the sample was vortexed and incubated in darkness to allow reaction between specific blood components and the Lymphogram. Then 2 mL of mild hypotonic (Lysing) solution was added and the sample was again incubated in darkness. The sample was centrifuged at 1,600 rev/450 g and the entire supernatant was poured out. The sample was then irrigated with 2 mL PBS. The sample was centrifuged again at 1,600 rev/450 g and the entire supernatant poured out. At the end, 0.5 mL of PBS was added. The sample was thus prepared for flow cytometer analysis.

HLA-DR TEST

A **HLA-DR test** was performed. HLA-DR is an MHC class II cell surface receptor, whose primary function is to present peptide antigens to the immune system. HLA-DR antigen is expressed in B lymphocytes, macrophags, activated T lymphocytes and activated NK cells.

Method protocol – HLA-DR test

After 10 μL of CD3/Anti – HLA-DR was pipetted into a 100 μL blood sample, the sample was vortexed and incubated in darkness to facilitate reaction between specific blood components and CD3/Anti – HLA-DR. Then 2 mL of mild hypotonic (Lysing) solution was added and the sample was again incubated in darkness. The sample was centrifuged at 1,600 rev/450 g and the entire supernatant was poured out. The sample was then irrigated with 2 mL PBS. It was centrifuged again at 1,600 rev/450 g and the entire supernatant poured out. At the end, 0.5 mL of PBS was added. The sample was thus prepared for flow cytometer analysis.

RESULTS

Observation of the entire group of subjects revealed a visible and time-related dynamic in the sense of increasing erythrocyte counts in the first 3 months. This was followed by a significant increase in the concentration of reticulocytes (immature erythrocytes), which normally coincide with higher erythrocyte production due more intensive stimulation of haematopoiesis (e.g. due to training, height etc.). Concurrently with the observed higher erythrocyte production, consumption of iron also increased and, consequently, in Months 3 and 4, serum iron concentration decreased and transferrin concentration increased.

The reason for the increased haematopoiesis in athletes usually lies in more intense training, whereas during a steady training process there may be some effects that cause a decline in inflammation factors, thus suppressing haematopoiesis and boosting production of inflammatory cells (monocytes, lymphocytes, eosinophils and basophils). Our results show that after taking the active substance, inflammatory stimulation of lymphocytes would decrease in relation to training, since the latter itself incites inflammation. We noticed that after intake of the active substance the study subjects had statistically significantly lower concentrations of activated T lymphocytes (109 x 106/mm^3 vs. 140 x 106/mm^3; $p = 0.037$); also, a lower concentration of cytotoxic T lymphocytes (461 vs. 514 x 106/mm^3) was close to significant values.

After the probiotic and placebo intakes, statistically significant differences were established in women in terms of erythrocyte and haematocrit counts, reticulocyte counts, S-TIBC and MCV. In women, more evident and time-related dynamics were observed in terms of increased erythrocyte counts over the last two months. This was followed by a significant increase in the concentration of reticulocytes (immature erythrocytes), which normally coincides with higher production of erythrocytes due to stronger stimulation of haematopoiesis. In the last two months, haematocrit concentrations also grew, along with erythrocyte counts. Haematocrit is the ratio between the volume of erythrocytes and total blood volume. This dynamic is accompanied by a higher mean corpuscular volume (MCV) and total iron-binding capacity (S-TIBC).

After the probiotic and placebo intakes, statistically significant differences were established in men in terms of erythrocyte and haematocrit counts, reticulocyte counts, S-TIBC, S-UIBC, MCV, MCH, MPV, MCHC, monocytes, basophils, ferritin index and serum iron concentration. With men, a decrease in erythrocyte counts was established in Months 2 and 4 and an increase in Month 3. This was accompanied by a significant increase in the serum iron concentration in Month 2 and a decrease in Month 3, as well as lower reticulocyte counts in Month 2 and higher one in Month 3. Concurrently, a decrease in the haemoglobin and haematocrit counts was observed in men in Months 2 and 4, along with an increase in Month 3. The lower erythrocyte counts in Months 2 and 4 can be linked to higher basophil counts during the same months. Basophils are inflammatory cells that reduce inflammatory factors, which is particularly important for athletes, as inflammation can interfere with training and muscle growth. On the negative side, inflammatory cells decrease haematopoiesis. When the basophil count dropped in Month 3, the erythrocyte count grew. This dynamic was accompanied by a higher mean corpuscular volume (MCV) in Month 3 and a lower mean volume in Month 2.

Table 1: Statistically significant differences between women and men, by month

	WOMEN			MEN		
	T-test (January : February)	T-test (January : March)	T-test (January : April)	T-test (January : February)	T-test (January : March)	T-test (January : April)
Ferritin index	0.412510594	0.149668985	0.149668985	0.002286755 ↓	0.799485207	0.277560521
S-iron	0.154415265	0.518797546	0.385519423	0.313248192	0.031762206 ↓	0.877895727
S-UIBC	0.096589675	0.183395284	0.077179128	0.056977945	0.028036646 ↑	0.015793881 ↑
S-TIBC	0.046649047 ↓	0.037631787 ↑	0.649063894	0.03001501 ↓	0.816046291	0.247702041
K-Erci	0.042742965 ↓	0.453755746	0.937325598	0.003872665 ↓	0.017474362 ↑	0.823624153
K-Hb	0.087006548	0.530985482	0.615035135	0.048454321 ↓	0.032737825 ↑	0.266593228
K-Ht	0.037081353 ↓	0.187134833	0.250391041	0.000646258 ↓	0.015718962 ↑	0.10143753
MCV	0.506272479	0.046467584	0.000855814 ↑	0.036979414 ↓	0.918365642	0.005123198 ↑
MCH	0.848858543	0.740454477	0.043814272	0.174372644	0.22644733	0.002750255 ↑
MCHC	0.863172322	0.594379357	0.106392283	0.03899744	0.312288105	0.000667042 ↑
RDW	0.229378125	0.10355171	0.000659081 ↑	0.199871222	0.560423228	0.345889929
MPV	0.081640297	0.743600403	0.654205491	0.082911027	0.011967257 ↓	0.092676762
Monocytes	0.094790965	0.21157734	0.267506545	0.020475233 ↓	0.460218647	0.097673129
Eosinophils	0.238128233	0.249137306	0.648089169	0.615737854	0.713121616	0.501253716
Basophils	0.189365211	0.10870436	0.61762214	0.495817419	0.030360131 ↓	0.749731377
K-Erc-Reticulocytes	0.027167889 ↑	0.275491712	0.353783863	0.028358736 ↑	0.025231788 ↓	0.477766071
K-Erc-Reticulocytes%	0.016698891 ↑	0.330666066	0.258097559	0.01201704 ↑	0.043617341 ↑	0.489589745
Reticulocytes-MCVr	0.127916549	0.375170042	0.825294611	0.849720619	0.015964888 ↓	0.061433394
Reticulocytes-CHr	0.055841514	0.407066198	0.009921513 ↑	0.383018956	0.059894499	0.001219943 ↑
%HYPO Erc	0.74338359	0.350616663	0.275345549	0.295119042	0.791445913	0.037012321 ↓

After the probiotic intake (active substance 1)

Statistically significant differences in erythrocyte counts, MCH, MCV, S-TIBC and S-UIBC were recorded for the entire group of subjects following the probiotic intake. After taking probiotics, the erythrocyte count decreased, which is highly important for athletes, as erythrocytes transport oxygen in the body. Along with erythropoietin, many other elements are necessary for erythrocyte production, the most important among them including iron, vitamin B12 and folic acid (vitamin B9). The reason for the decrease in erythrocytes could be a lack of any of these elements in the athletes. This dynamic was followed by a decrease in the total iron-binding capacity (S-TIBC) and transferrin saturation (S-UIBC). Mean corpuscular volume (MCV) increased, along with mean corpuscular haemoglobin (MCH). The reason for the increase in MCH could be a vitamin B12 deficiency.

The value of the parameters where statistically significant differences were observed both before and after probiotic intake also differed by gender.

After the probiotic intake, statistically significant differences were established in women in terms of erythrocyte and haemoglobin counts, eosinophils, reticulocytes and MCV. After receiving probiotics, the erythrocyte count decreased, which is highly important for athletes, as erythrocytes transport oxygen in the body. We also observed a lower haemoglobin concentration after the probiotic intake. Haemoglobin is a compound in erythrocytes that binds oxygen and carries it in the blood. The amount of haemoglobin in the blood can decrease due to an erythrocyte deficiency or if haemoglobin levels in erythrocytes is below normal. Normal blood haemoglobin levels differ by gender: women have a slightly lower haemoglobin value due to menstrual blood loss, which results in a lower level of haemoglobin in the blood. The lower haemoglobin concentrations could be the result of women athletes' menstrual cycles. An increase in eosinophil concentrations was also observed. Eosinophils are inflammatory cells that suppress inflammation and reduce haematopoiesis, thus causing a decline in erythrocyte and haemoglobin concentrations.

After the probiotic intake, statistically significant differences were established in men in terms of MCH, MCHC, S-TIBC, CD4 and monocytes. In steady training regimes, the production of inflammatory cells, i.e. monocytes, increases and reduces inflammatory factors, which is particularly important for athletes, as inflammation interferes with training and muscle growth. Our results indicate that after taking the active substance, we should see a decrease in inflammatory stimulation of lymphocytes, which is related to training, since lymphocytes incite inflammation.

After placebo intake (active substance 2)

After the placebo intake, statistically significant differences were established in the entire group of subjects in terms of erythrocyte, monocyte and basophil counts, S-TIBC, MCHC, MCH and ferritin. After receiving the placebo, the erythrocyte count decreased. Ferritin is a protein that

Table 2: Statistically significant differences between women and men, before and after the probiotic intake

	WOMEN	MEN
	T-test **BEFORE** intake : **AFTER** intake **PROBIOTIC** (Jan, Mar : Feb, Apr)	T-test **BEFORE** intake : **AFTER** intake **PROBIOTIC** (Jan, Mar : Feb, Apr)
S-TIBC	0.126654	0.031689 ↓
K-Erci	0.037411 ↓	0.071796
K-Hb	0.030008 ↓	0.519385
MCV	0.007082 ↑	0.432183
MCH	0.595218	0.009463 ↑
MCHC	0.924376	0.012847 ↑
RDW	0.003533 ↑	0.787088
Monocytes#	1	0.043111 ↑
Eosinophils	0.004442 ↑	0.155102
Reticulocytes-MCVr	0.014153 ↑	0.377422
Reticulocytes-CHr	0.049713 ↑	0.920488
CD4 [%]	0.568531	0.041795 ↓

carries iron in the blood, and whose concentration in the process grew – when the body needs more iron, ferritin levels in the blood increase. This development was accompanied by an increase in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The increase in MCH and MCHC could be the result of a vitamin B12 deficiency. There was an increase in basophils, i.e. inflammatory cells that suppress inflammation and thus reduce haematopoiesis, which is reflected in lower erythrocyte counts.

Parameter values differ between men and women where statistically significant differences occurred before and after placebo intake.

After the placebo intake, statistically significant differences were established in women in terms of MCH, MCHC, ferritin index, S-TIBC, and share of hypochromic erythrocytes as well as basophil and monocyte counts. There was an increase in basophils, i.e. inflammatory cells that suppress inflammation and thus reduce haematopoiesis. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) also increased.

After the placebo intake, statistically significant differences were observed in men in terms of MCH and MCHC as well as erythrocyte, haematocrit and monocyte counts. After receiving the placebo, the erythrocyte count decreased. This was accompanied by an increase in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC). We also observed a decline in haematocrit, which is the volume, measured as a percentage, of erythrocytes (accounting for nearly all of the cell's volume) in blood. With lower erythrocyte counts diminished haematocrit is logical and to be expected.

DISCUSSION

As in the case of some other studies, we wanted to evaluate the effect of probiotics on blood and immunological parameters. Observation of the entire group of subjects revealed a visible and time-related dynamic in the sense of increased erythrocyte counts in the first 3 months.

Table 3: Statistically significant differences between women and men, before and after the placebo intake

	WOMEN	MEN
	T-test **BEFORE** intake : **AFTER** intake **PLACEBO** (Jan, Mar : Feb, Apr)	T-test **BEFORE** intake : **AFTER** intake **PLACEBO** (Jan, Mar : Feb, Apr)
Ferritin index	0.000916 ↓	0.784882
S-TIBC	0.002107 ↓	0.318139
K-Erci	0.584899	0.024248 ↓
K-Ht	0.806	0.027788 ↓
MCH	0.009656 ↑	0.026352 ↑
MCHC	0.001982 ↑	0.009566 ↑
Monocytes	0.046767 ↓	0.004134 ↓
Basophils	0.017019 ↑	0.271807
%HYPO Erc	0.032559 ↓	0,917024

This was followed by a significant increase in the concentration of reticulocytes (immature erythrocytes), which normally accompanies increased erythrocyte production due to greater stimulation of haematopoiesis (e.g. due to training, height etc.). Concurrently with the observed higher erythrocyte production, consumption of iron also increased and, consequently, serum iron concentration decreased and transferrin concentration decreased in Months 3 and 4.

We noticed gender-related differences in the time-related dynamics of blood and immunological parameters. In women, time-related dynamics in the sense of increased erythrocyte counts in the last two months was observed. This was followed by a significant increase in the concentration of reticulocytes (immature erythrocytes), which normally accompanies increased production of erythrocytes as the result of greater stimulation of haematopoiesis.

Time-related dynamics in men was slightly different: we observed a decrease in the erythrocyte count in Months 2 and 4, as well as an increase in Month 3. This was accompanied by a significant increase in serum iron concentrations in Month 2, and a decrease in Month 3, as well as lower reticulocyte counts in Month 2 and higher counts in Month 3.

The immune response and inflammation parameters did not show any significant changes or trends in either men or women.

Observation of the entire group of subjects showed that after receiving probiotics, erythrocyte counts decreased, which is highly important for athletes as erythrocytes transport oxygen in the body. Along with erythropoietin, many other elements are necessary for erythrocyte production, the most important among them including iron, vitamin B12 and folic acid (vitamin B9). The decrease in erythrocytes could be the result of a lack of any of these elements in the athletes. This dynamic was also accompanied by a decrease in the total iron-binding capacity (S-TIBC) and transferrin saturation (S-UIBC). The mean corpuscular volume (MCV) increased, along with the mean corpuscular haemoglobin (MCH). The increase in MCH could be the result of a vitamin B12 deficiency.

Lower erythrocyte counts after the probiotic intake was due mainly to the results for the female subjects. The men's erythrocyte counts did not drop after the probiotic intake, but did decrease after the placebo. It can be concluded that the probiotic intake in men prevented a reduction in the erythrocyte count, as was seen already in the case of placebo. After the probiotic intake, erythrocyte counts decreased in women, the reason for which could be the lack of any of these elements. We also observed lower haemoglobin concentrations after the probiotic intake. Normal blood haemoglobin levels differ by gender: women have a slightly lower haemoglobin value due to menstrual blood loss, which results in a lower level of haemoglobin in the blood. The lower haemoglobin concentrations could be the result of women athletes' menstrual cycles.

The overall results show that in the training period under scrutiny, erythrocyte counts increased, mainly as the result of higher erythrocyte production in the male subjects. Increased haematopoiesis in athletes is usually the result of particularly intense training, whereas during a steady training regime there may be some effects that lead to a decline in inflammation factors, thus suppressing haematopoiesis and boosting production of inflammatory cells (monocytes, lymphocytes, eosinophils and basophils). Our results indicate that after taking the active substance, there should be a decrease in inflammatory stimulation of lymphocytes, which is related to training, since lymphocytes incite inflammation. We noticed that after the intake of the active substance the study subjects showed statistically significantly lower concentrations of activated

T lymphocytes (109 x 106/mm^3 vs. 140 x 106/mm^3; p = 0.037); lower concentrations of cytotoxic T lymphocytes (461 vs. 514 x 106/mm^3) were also close to statistically significant.

Despite the described time-related increase in erythrocyte counts in the probiotic intake period, we did not notice any direct influence on growing erythrocyte counts. During the month the subjects were administered probiotics, erythrocyte counts decreased in women and remained unchanged in men. It is possible that probiotic activity, which should primarily stabilise the immune response, was only reflected – more than a month after the fact – in higher erythrocyte production. In order to test and prove this hypothesis, groups of study subjects should take probiotics for a longer period of time, i.e. for several months.

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