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EFFECTS OF PROBIOTIC SUPPLEMENTATION ON SELECTED HEALTH INDICES AMONG A GROUP OF COMPETITIVE CYCLISTS (EFFECT OF PROBIOTIC THERAPY ON HEALTH INDICES)

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D. Data interpretation
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Abstract:

Introduction: In scientific reports from recent years, a significant relationship is clearly indicated between the intestinal microbiota and chronic diseases, and the role of selected probiotic strains is emphasized not only in maintaining good health, but also in achieving optimal physical fitness among athletes.

Aim: The aim of the study was to assess the impact of supplementation with multi-strain probiotics on selected health indices among professional road cycling competitors.

Material and methods: The study group comprised cyclists aged 18-26 (N=26), randomly assigned to 2 subgroups: experimental (n=13) and control (n=13), who were administered a multi-strain probiotic or a placebo for 4 months. The experiment was a double-blinded, randomised trial. Assessment of health status, within the context of analysing selected values of haematological and biochemical indices of the blood, urine and faeces, was performed before the examination, after 1 month, 3 months and following completion of the experiment.

Results: The 16-week intervention of supplementation with a probiotic preparation was associated with a change in some biochemical blood indices of the studied men. Decreases in the concentration of total protein and alkaline phosphatase were found. The applied 4-month supplementation did not affect the levels of haemoglobin, glucose, keratin kinase or total cholesterol, HDL, LDL and triglycerides. Probiotic supplementation contributed to inhibiting the growth of intestinal pathogenic bacteria, simultaneously influencing the growth of selected strains of symbiotic bacteria. The changes in the values of the indices measured before and after the end of the experiment and between the groups, were not statistically significant.

Conclusions: The assessed blood indices in both subgroups were within the reference ranges, both before starting the probiotic/placebo and after the supplementation period. The demonstrated changes in the index values suggest a beneficial effect of the probiotic preparation on the composition of the faecal microbiota.

1. Introduction

Health status is an important factor indirectly influencing exercise capacity. Physical activity has numerous health-promoting effects, but it can cause side effects, e.g. gastric disorders, upper respiratory tract infections, chronic inflammation and injuries. The indicated ailments cause great discomfort and contribute to the reduction of exercise capacity. Within this context, research is undertaken regarding the influence of probiotics on the modu-

lation of the intestinal microflora and the occurrence of disorders of the digestive system and respiratory system infections, which intensify during the training period, especially in the fall-winter and spring season [1].

Ilya Mechnikov made the first observations about the beneficial effect of fermented milk products on human health at the beginning of the 20th century. He pointed to the relationship between the increased life expectancy and health condition of peasants from the Balkans and the consumption of fermented milk [2, 3]. In 1965, Lilly and Stillwell [4] referred to Miecznikov, using the term probiotic to describe factors produced by microorganisms facilitating the growth of other microorganisms. According to the definition proposed by Sperti [5], probiotics were understood as a tissue extract stimulating bacterial growth. In 1974, Parker [6] defined probiotics as "organisms and substances contributing to the balance of the intestinal microflora", and in 1989, Fuller [7] proposed the definition: "a nutritional supplement containing viable microorganisms that beneficially affects the animal host by improving its microbiological intestinal balance".

The applicable definition, presented by the WHO, refers to living organisms as probiotics, which when dosed in appropriate amounts have beneficial effects on the host organism [8]. These are organisms recognised as safe by relevant institutions. Therefore, they must meet a number of requirements, including survival during passage in the digestive system and attachment to intestinal cells; they must not adversely affect the host organism; they must be resistant to technological processes and retain their properties during appropriate storage. In addition, they must be organisms already inhabiting the human intestinal flora; have a pro-health and antagonistic effect against pathogenic microorganisms inhabiting the human microbiome, and must be void of side-effects [9].

The role of selected probiotics in maintaining the health of athletes seems to be more and more noticeable and appreciated in the sports environment. Scientific research conducted in recent years allows to formulate the conclusion that living microorganisms, when provided in the proper amount, can have a positive effect on the health of the host (athlete). The use of probiotics (*Lactobacillus casei*) may shorten the duration of upper respiratory tract infection [10]. In other studies, it has been shown that the use of *Lactobacillus fermentum* may contribute to the reduction of the incidence of gastrointestinal symptoms in physically active people [11].

The influence of probiotic substances on health indices, especially in athletes, is not fully understood. Within the context of maintaining health in active people, the integrity of the intestinal barrier is very important, which, in turn, is modulated by the influence of intestinal bacteria, including those probiotics introduced into the body. There are few scientific publications regarding the effect of probiotic supplementation on various health indices among healthy individuals.

The following functions should be distinguished among the mechanisms of action concerning probiotic strains: protective, digestive, immune and anti-cancer. The protective function is based on antagonistic activity against numerous pathogens, including: adherence to the intestinal mucosa (intestinal barrier), occupation and enzymatic modification of pathogenic bacteria receptors, production of compounds with bacteriostatic/bactericidal activity (bacteriocins, organic acids, hydrogen peroxide, compounds of the lactoperoxidase system, etc.), competition for nutrients, modification of the intestinal environment unfavourable for the development of detrimental microorganisms (lowering pH) [12]. Probiotics have a positive effect on: intestinal peristalsis, maintaining the continuity of the gastrointestinal mucosa, stimulating the secretion of mucin sealing the intestinal epithelium, the production of short-chain fatty acids and polyamines (regeneration of the epithelium and influence on cell maturation) and stimulation of mucus production [12]. The digestive function also concerns synthesis of B vitamins, vitamin K, and increasing the pool of digestive enzymes. The immune function of probiotics is the stimulation of the immune system, favourable modification of the cellular and humoral response by stimulating the synthesis of antibodies; increasing the production of anti-inflammatory cytokines, inhibiting the production of pro-inflammatory cytokines and enhancing the phagocytosis process. The anti-cancer function is connected with the elimination of mutagenic compounds by binding mutagens in the gastrointestinal tract, decomposition of potentially carcinogenic compounds (nitrosamines, amino acid pyrolysates), selective inhibition of bacterial growth synthesising enzymes involved in carcinogenesis and the production of enzymes that destroy catalysts engaged in carcinogen formation [12].

The Polish Olympic Committee recommends the use of probiotics in all professional athletes, especially among runners, triathletes, cyclists and rowers. Rebuilding the intestinal microflora and strengthening the intestinal barrier are especially significant in intensively training athletes reporting gastrointestinal disorders (flatulence, diarrhoea, nausea, irritable bowel syndrome), antibiotic therapy, taking non-steroidal anti-inflammatory drugs or proton pump inhibitors. In addition to a balanced and nutritious diet, supplementation based on appropriately matched strains of probiotic bacteria should be implemented [13].

The aim of the study was to assess the influence of 4-month supplementation with multi-strain probiotics on health, assessed on the basis of blood, urine and faeces morphology and biochemical indices.

2. Research materials and methods

The study comprised 26 cyclists training road cycling, representing clubs from the Małopolska region, who took part in the randomised, double-blinded research. The cyclists' training plan was related to the preparatory period and included mainly training aimed at shaping aerobic endurance, with a predominance of aerobic exercises (up to 90% of the total load). The cyclists undertook 1.48 ± 0.8 training every day, lasting about 104.28 ± 5.34 minutes, which was about 6 training units per week, with a total length of about 11 hours (Tab. 1). The main criterion for inclusion in the study group was competitive cycling for 5 years, good health (assessed during medical qualification), male gender, age 18-26 years, high sports level (participation in national and international cycling competitions).

The cyclists were randomly divided into 2 groups: probiotic supplementation (P), and control (C), placebo. The multi-strain probiotic preparation taken in the amount of 1 capsule a day contained 20 billion bacteria belonging to the following strains of probiotic bacteria: *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus, Lactobacillus, Lactobacillus, Lactophilus, Lactobacillus, Lactobacillus* and *Lactobacillus Lactobacillus rhamnosus, Lactococcus lactis subsp. lactis, Streptococcus salivarius subsp. thermophilus*. The preparation also contained fructo-oligosaccharides - substances that feed probiotic bacteria, stimulating the proper development of the intestinal flora.

The average age of the athletes was 22.26 ± 0.98 years and was comparable in both groups (23.25 ± 3.95 years and 21.28 ± 4.45 years, respectively for the P and C groups; ($p>0.05$). The cyclists from neither of the groups differed in terms of body height ($p>0.05$), the mean values of which were 173.3 ± 5.48 cm and 174 ± 5.42 cm. The cyclists from group P were characterised by significantly lower BMI value (20.80±1.1 kg/m^2 vs. 21.79±2.04 kg/m^2; $p>0.05$), than in the c group, the with all participants having a body mass index within the norm.

Before starting supplementation (probiotics and placebo), the health status was assessed in terms of haematological indices (haematocrit, haemoglobin concentration, red and white blood cell as well as platelet count), biochemical indices (lipid profile - total cholesterol, HDL, LDL, triglycerides) and Biernacki Reaction (ESR). In addition, stool samples were collected from fasting athletes for bacteria and fungi and for the presence of *Helicobacter pylori* and *Giardia intestinalis* antigens. Urine samples were also collected from the competitors (in the morning before the meal on the day of performing all measurements). Determinations were conducted before the start of the experiment and 1, 3 and 4 months later in the "*Diagnostyka*" laboratory and at the Department of Microbiology of the University Hospital in Kraków.

The MS EXCEL 2007 spreadsheet and the PQStat statistical package, version 1.6.8.312, were used for statistical analysis of the results and their graphical interpretation. Basic descriptive statistics of the studied variables were calculated: arithmetic mean ($\bar{x}$) andstandard deviation (SD). Comparing the effect of supplementation with a multi-strain probiotic preparation on changes in the analysed indices among cyclists, analysis of variance with repeated measurements (ANOVA) was used. The distribution of the inoculation results depending on the research group was compared with the Chi ^ 2 test and the Fisher's exact test. Distribution of the culture results depending on the term was compared with the Browker-McNeemar test. The test probability level of $p<0.05$ was considered statistically significant, while $p<0.01$, highly significant.

Table 1. Characteristics of the group of cyclists.

Index	Probiotic group			Placebo group		
	$\bar{x}$	SD	SE	$\bar{x}$	SD	SE
Age [years]	23.25	3.95	1.39	21.28	4.55	1.87
Body mass [kg]	63.52	3.77	1.05	65.97	7.55	2.09
Body height [cm]	173.3	5.48	1.52	174	5.42	1.55
BMI [kg/m^2]	20.80	1.11	0.31	21.79	2.04	0.57
Training experience [years]	10.57	4.07	1.44	9.17	4.71	1.87
Number of training units during the week	5.43	0.50	0.18	5.5	0.50	0.20
Number of hours on training per week [hrs. in 7 days]	10.57	0.75	0.26	10.67	0.75	0.30

$\bar{x}$ - mean; SD – standard deviation; SE - standard error

3. Results

The 16-week supplementation intervention with a probiotic preparation was associated with changes in some biochemical blood indices of the males under study. Decrease in the concentrations of total protein and alkaline phosphatase were found. The applied 4-month probiotic supplementation did not affect the levels of haemoglobin, glucose, keratin kinase, total cholesterol, HDL, LDL and triglycerides (Tab. 2).

The mean urine pH level of the cyclists before initiating the study in the group receiving the probiotic was 5.88±0.82, while in the placebo group, this was 11% lower. After 1 month of probiotic supplementation, pH totalled 5.65±0.8, after 3 months it reached 6±0.68, and after 4 months, it was at the level of 5.96±0.69. In the placebo group, the mean pH level at the corresponding measurement points was equal to: 5.77±1.09, 6.31±1.01 and 5.54±0.69, and these changes did not demonstrate statistical significance. There were no significant differences in urine pH levels between the 1st and 4th measurements. The mean specific urine weight for the entire experiment period in the probiotic and placebo groups was 1.02.

Prior to the study, the urinary creatinine concentration in the P group was 12.74±5.48 mmol/l, and in the placebo group, it was 18.5±8.21 mmol/l. After 4 weeks of supplementation with the probiotic preparation, it equalled 13.14±4.28 mmol/l, after 12 weeks, 15.46±6.49 mmol/l, and after 16 weeks, the total was 13.28±4.66 mmol/l. In the placebo group, the mean urinary creatinine concentrations for the 2nd and 3rd measurements were 15.91±8.53 mmol/l, and 11.13±5.14 mmol/l, 13.41±5.89 mmol/l, and the differences not statistically significant. There were no statistically significant differences in the concentration of creatinine in urine between the 1st and 4th sampling.

None of the cyclists had glucose or protein in their urine throughout the study.

In the stool culture tests for the presence of fungi, no yeast-derived fungi were found in 20% of the athletes taking the probiotic preparation at the beginning of the experiment, and the presence of *Candida albicans* was found in 80% of the competitors. At the end of the experiment, these fungi had not been isolated in half of the competitors from group P. After 3 months of taking the probiotic preparation, yeasts of the genus *Geotrichum species* were isolated in 20% of the subjects (Tab. 21). In the first measurement, no significant differences were found between the probiotic and placebo group ($p=0.13$). Similarly, comparing the results of the culture after 4, 12 and 16 weeks of taking the probiotic supplement, no significant qualitative changes were found in the material from either groups of competitors.

In the stool culture tests for bacteria, *Escherichia coli* was isolated in all athletes from the group receiving the probiotic preparation, before the research and after the experiment. Among 30% of competitors in this group, *Klebsiella* bacteria were identified in the 3rd test, while after 16 weeks of probiotic supplementation, *Klesbsiella* bacteria were not isolated in any of the athletes. In 70% of the competitors from the group taking supplements, at the beginning of the experiment, bacteria of the genus *Enterococcus* were also identified, which after 16 weeks of probiotic intervention, were present only in 20% of the competitors, and at the end of the experiment, in 10%. *Hafnia alvei* and *Pseudomonas aeruginosa* bacteria were also identified in 10% of the participants from the group with the supplementation intervention during the 4th test. Pre-experiment, in the 4th and 16th week of the study, no significant differences ($p=0.72$) were found between the groups, while after 12 weeks of probiotic intervention, a highly significant difference ($p=0.0022$) was found between the study groups. In the probiotic group, the cultures were richer in bacteria than in the placebo group. Comparing the results before and after 4, 12 and 16 weeks of supplementation, no significant changes were found in the group receiving the probiotic preparation.

Giardia intestinals and *Helicobacter pylorii* antigens were not detected in any of the athletes throughout the duration of the supplementation intervention.

4. Discussion

In recent years, the issue of probiotic effects on the human body has been increasingly taken into account when designing research and supporting exercise capacity. People responsible for the optimal preparation of athletes during the competition period are looking for measures that can improve health and indirectly increase exercise capacity. Since oxygen is transported by haemoglobin contained in red blood cells, an increase in its content may analogously lead to an increase in the ability to deliver oxygen to cells and its more efficient utilisation. Blood oxygen content is directly proportional to haemoglobin concentration, therefore, athletes may expect improvement in aerobic fitness. One of the studies aimed at determining the effect of probiotic supplementation with lactic acid bacteria on the haemoglobin content and other haematological indices was conducted in South Africa in 2012. The sample comprised 50 males undertaking moderate physical activity, divided into 2 groups: experimental - supplementing probiotics, and the control - receiving placebos. The research was randomised and double-blinded. It has been shown that the 42-day supplementation with lactic acid bacteria did not have a statistically significant effect on haemoglobin content

Table 2. Mean value of selected biochemical indices, ESR and lipid profile at given measurement points during the supplementation intervention among road cyclists

Index/Measurement		Probiotic group		Placebo group		*p* (Anova)
		$\bar{x}$	SD	$\bar{x}$	SD	
Haemoglobin [g/dl]	1	15.54	1.12	14.81	0.9	NS
	2	15.26	0.82	14.85	0.9	NS
	3	15.42	0.79	14.77	0.77	NS
	4	14.88	0.93	15	0.69	NS
ESR. [mm/h]	1	4.54	4.54	6	6.32	NS
	2	4.54	3.55	6.77	6.82	NS
	3	4.54	0.77	5.69	4.84	NS
	4	3.69	3.71	4.62	2.69	NS
Glucose [mmol/l]	1	4.84	0.61	5.09	0.25	NS
	2	4.86	0.48	4.98	0.38	NS
	3	4.7	0.33	5	0.32	NS
	4	4.63	0.22	5.06	0.27	NS
Total protein [g/dl]	1	78.13	3.15	78.62	3.93	NS
	2	75.11	1.79	77.12	3.2	NS
	3	75.58	3.88	77.78	4.14	NS
	4	75.32	3	78.55	2.68	p=0.0001
Creatine kinase [U/l]	1	170	44.94	198.3	60	NS
	2	118.5	80.75	123.2	47.7	NS
	3	90.3	51	112.2	32.6	NS
	4	181.8	91.88	195.5	73.5	NS
Alkaline phosphatase [U/l]	1	79.38	10.73	72.92	28.77	NS
	2	76.31	9.99	72.23	24.66	NS
	3	70.38	10.44	73.62	24.21	NS
	4	65	10.83	72.42	26.46	p<0.0001
Total cholesterol	1	4.41	0.68	4.36	1.22	NS
	2	4.39	0.66	4.44	1.41	NS
	3	4.41	0.61	4.72	1.17	NS
	4	4.08	0.57	4.64	1.2	NS
HDL-chol [mmol/l]	1	1.67	0.18	1.35	0.32	NS
	2	1.64	0.27	1.54	0.35	NS
	3	1.75	0.32	1.52	0.35	NS
	4	1.54	0.37	1.53	0.35	NS
LDL-chol [mmol/l]	1	2.36	0.7	2.48	0.99	NS
	2	2.29	0.68	2.59	1.08	NS
	3	2.28	0.63	2.74	0.99	NS
	4	2.13	0.54	2.83	0.95	NS
Triglycerides [mmol/l]	1	0.86	0.26	1.16	0.79	NS
	2	0.98	0.45	1.02	0.54	NS
	3	0.83	0.3	1	0.53	NS
	4	0.91	0.26	1.19	0.63	NS

1 - prior to supplementation, 2 - 4 weeks of supplementation, 3 - 12 weeks of supplementation, 4 - 16 weeks of supplementation; $\bar{x}$ - mean; SD - standard deviation;

or any of the other analysed haematological indices [14]. The results reached in this study are consistent with this report. The haemoglobin level after 4 months of probiotic supplementation decreased, but these changes were not statistically significant. A similar effect was observed by Kekkonen [15], who demonstrated a statistically insignificant decrease in haemoglobin level after 3 months of probiotic therapy. An interesting double-blinded study was carried out at the University of Helsinki. Marathon runners (n=141) were randomised into 2 groups: the experimental group, who received a probiotic drink containing *Lactobacillus rhamnosus* GG for 3 months, and the control group, who received the same drink, but free from probiotic bacteria. After 3 months of supplementation, a decrease in the mean haemoglobin concentration was observed in both groups. However, this change was not statistically significant [15].

Biernacki Reaction increases with inflammation, and testing in this direction provides a measurable indication of inflammation in the body. In the authors' own research, the mean values of ESR at the end of the experiment decreased in both groups, while the difference was greater in the control group. Assessing the impact of probiotic supplementation on the ESR index in athletes did not provide clear conclusions, however, there are reports in which a potential beneficial effect of probiotics on selected indicators of inflammation in humans is indicated. In the work by Valentini [16], it was shown that 8-week implementation of the Mediterranean diet reduces the ESR rate in people aged 65-85 ($p=0.02$), while the combined use of the VSL#3 probiotic (2 capsules a day) contributed to a greater degree decrease in ESR index ($p=0.05$) [16]. In this study, the ESR index decreased in both groups, nonetheless, these changes were not statistically significant within the groups or between groups at any stage of the experiment.

In a study conducted by Salehzad among a group of 30 men who consumed yogurt enriched with probiotic bacteria for a period of 14 weeks, a decrease in blood glucose levels was observed, but this change was not statistically significant [17]. The results reached in the above research are consistent with those obtained in the authors' research. The probiotic preparation used in the experiment included the *Lactobacillus rhamnosus* strain, also implemented by Kekkonen [15]. In a study conducted by Abbasi among a group of 14 bodybuilders, supplementing their diet with a probiotic preparation [(*Lactobacillus casei* (109 × 5.1 CFU/g), *Lactobacillus acidophilus* (109 × 2 CFU/g), Lactobacillus C. (109 × 5.1 CFU/g), *Lactobacillus bulgaricus* (108 × 2 CFU/g), *Bifidobacterium breve* (1010 × 2 CFU/g), *Bifidobacterium longum* (109 × 7 CFU/g), *Streptococcus thermophilus* (109 × 5.1 CFU/g)] for 28 days, no effects on glucose level were noted [18]. In the presented study, the mean blood glucose concentration in the group consuming the probiotic preparation decreased after 4 months, but did not exceed the reference range. Furthermore, the change was not statistically significant ($p>0.05$). However, significant differences between groups ($p=0.038$) were observed, with higher glucose values in the placebo group.

Total protein in the serum is one indicator of the state of protein nutrition. It reflects nutritional status, the liver's efficiency in the production of proteins and the assessment of the loss of these compounds by the kidneys, skin and gastrointestinal tract. Total protein in the serum allows to determine the condition of these organs. It can be a signal of an ongoing neoplastic process. In the study by Kekkonen, the total protein concentration after 3 months decreased in the placebo group, while in the experimental group, it experienced an increase, but the changes were not significant [15]. There was no statistically significant difference between the groups of cyclists in total protein concentration at the beginning of the study. In the group with probiotic supplementation, its level decreased after 4 months, while in the control group, it increased slightly after 3 months to finally reach the baseline value. The changes after 16 weeks of probiotic supplementation were significant, in contrast to the Kekkonen study. It should also be noted that the mean values of total protein in both groups were within the reference range.

IgA immunoglobulins, secreted by the intestinal lymphoid tissue (GALT, i.e. gut-associated lymphoid tissues) [19], as well as intestinal alkaline phosphatase, play an important regulatory role in maintaining intestinal homeostasis, involved in the dephosphorylation of phosphate lipopolysaccharide residues, affecting the ability of pathogenic bacteria to adhere to enterocytes and penetrate intestinal epithelial cells [20]. Although the exact mechanism of action regarding alkaline phosphatase *in vivo* is not yet clear, it is likely that dephosphorylation of liposaccharides occurs at the plasma membrane level and/or in the gut lumen [20]. In the present study, after 16 weeks of probiotic supplementation, a significant increase in IgA was observed between the 1st and 4th measurement in the group with the supplementation intervention. This allowed to conclude that the implemented supplementation resulted in a significant reduction of alkaline phosphatase in the blood serum. The current data indicate that alkaline phosphatase is a component of the intestinal mucosa defence system [19, 20, 21]. Produced by enterocytes, it is likely to be protective at many locations, including inside the intestinal epithelial cell, in the lumen of the gut, and it may possibly have whole-body, systemic influence as well. It should be noted that a lower concentration of alkaline phosphatase was observed only in the group supplemented with the

multi-strain probiotic preparation. It may concluded that the supplementation used in intestinal alkaline phosphatase prevented bacterial invasion through the intestinal mucosa barrier.

The lipidogram test is a basic diagnostic tool used to measure the level of total cholesterol and its fractions, as well as triglycerides in the blood, allowing to determine the presence or absence of disorders in the body's lipid metabolism. In the authors' research, a positive effect of 3-month probiotic supplementation was shown on the HDL cholesterol fraction, as after 12 weeks of using the probiotic, the HDL fraction level increased significantly. A similar effect was observed in the experiment conducted by Cox's team [22]. Although statistical significance was not achieved, the level of HDL cholesterol increased after 5 months of supplementation. In the presented studies, the level of HDL cholesterol fraction in the experimental group, compared to baseline, decreased at the end of the experiment, but this change was not statistically significant (1.67 ± 0.18mmol/l vs. 1.54 ± 0.37mmol/l). In the group supplementing the probiotic, a decrease in the level of total cholesterol and LDL fraction was also observed, while in the placebo group, there was an increase, however, the changes were not statistically significant. In the study by Cox [22], the level of total cholesterol in the group supplementing probiotics slightly increased. In the control group it also experienced an increase, which correlates to the results obtained in this work. This may have been influenced by the dose and the amount of probiotic bacteria used in the experiment. It is difficult to make unequivocal conclusions about the influence of a probiotic on selected indices if the dose, type of strain and duration of its supplementation differ. In the trial by Abbasi [18], after 28 days of supplementation with a probiotic preparation, there was significant ($p=0.0008$) reduction in the level of total cholesterol and triglycerides in the experimental group. In a meta-analysis published in 2015, it was indicated that an intervention in the form of probiotic therapy contributes to the reduction of total cholesterol and LDL cholesterol levels, while longer probiotic therapy (> 4 weeks) has a stronger effect on lowering total cholesterol and LDL fraction than that short-term (≤ 4 weeks). Moreover, positive changes are more visible in individuals with elevated levels of total cholesterol than in those with normal levels. It was noticed that *Lactobacillus acidophilus* strains and a probiotic called "Gaio", containing *Enterococcus faecium Streptococcus thermophilus* have significant effects. In the authors' study, the above-mentioned bacteria were part of the implemented probiotic preparation, thus, the achieved reduction in the level of total cholesterol and LDL fraction (although statistically insignificant) may be related to their positive effects on the host organism. It should be mentioned that the data analyses of lipid profile did not always reach statistical significance, which may be related to the small size of the group, suggesting the need to continue research on a larger sample of athletes. Analysis of changes in the concentration of triglycerides in the blood of the cyclists showed a slight, statistically insignificant increase in both groups. These results partially correlate with those obtained by Cox [22]. The level of triglycerides in the group supplementing the probiotic slightly increased, both in the group supplementing the one- and two-strain preparations. On the other hand, in the control group, the level of triglycerides decreased. However, these changes did not reach the level of statistical significance, as in the presented study. Similar results were reported in the study carried out by Salehzadeh [17]. In a group of 30 athletes who consumed yogurt enriched with probiotic bacteria for 10 weeks, a reduction in the level of triglycerides, total cholesterol and LDL cholesterol, as well as an increase in HDL cholesterol, were noted.

Urinalysis provides information about overall health and helps in diagnosing diseases of the genitourinary tract. As a result of catabolic processes, acids excreted by the kidneys are produced. The analysis of changes in urine indices may indicate possible health problems. In the presented study, the urine pH value was higher in the probiotic supplementation group, both before and after the experiment. In both groups, mean urine pH increased at the end of the experiment, but these changes were not significant, and the results were within the reference range. It should be noted that the observed changes (pre and post) were small. The experiment allowed to show regularities in the absence of glucose, proteins and bilirubin in the urine. There was no presence of glucose, proteins or bilirubin in the urine of the participants at any stage of the examination, which proves the proper functioning of the glomeruli. Although vigorous physical exertion may cause a physiological increase in protein excretion, this phenomenon did not take place in the presented study.

Ketone bodies appear in the urine with disorders of lipid and carbohydrate metabolism, diabetes, diabetic acidosis, diarrhoea, low-carbohydrate and high-fat diets, after vomiting and during fasting, and in ethanol poisoning. Nitrites in urine are the result of nitrate reduction by bacteria, which are the cause of urinary tract infections, and are therefore used as an indirect indicator of these infections. In the presented study, no nitrite was detected in the urine of any of the competitors from either of the groups. Leukocytes that could indicate urinary tract infection were not detected in the majority of subjects. Supplementation did not affect functioning of the urinary system, which may be related to its proper condition before testing.

In probiotic therapy, many strains or species of bacteria are used, thanks to which it affects various levels of the digestive tract. This, together with the prevention of

disorders related to exercise, should be viewed in terms of comprehensive treatment of the athlete's health, aimed at causal treatment, modulating the microbiota of the digestive tract rather than symptomatic therapy. Among the infectious factors associated with gastritis, the most common, occurring in over 90% of cases, is *Helicobacter pylori*. It is estimated that it occurs in 50% of the general population, and in Poland, in 84% of adults and 32% of children [23]. This bacterium is a risk factor for the development of peptic ulcer in the stomach (70%) and duodenum (75-90%), gastritis and even cancers such as gastric adenocarcinoma or MALT lymphoma. It is estimated that 10-20% of those infected with *H. Pylori* develop clinical disease. Symptoms of *Helicobacter pylori* gastritis may be non-specific, sometimes the disease is asymptomatic. Usually, patients develop various ailments related to the digestive system, such as epigastric pain, nausea, vomiting, acid regurgitation, flatulence or a burning sensation in the retrosternal area. In the advanced stage of chronic disease, weight loss, anaemia, frequent fever and blood in the stool are common [23]. Digestive system diseases are a common problem for people with *Helicobacter pylori* infection. The risk of infection with this bacterium is increased in people with type-2 diabetes. Those infected with *Helicobacter pylori* more often report defecation disorders and a feeling of fullness in the epigastric region [24]. Treatment regimens for *Helicobacter pylori* infections are based on antibiotic therapy and the use of proton pump inhibitors. Often the bacteria is resistant and treatment leads to many side effects. It is demonstrated in studies that some bacterial strains can have inhibitory activity against the *Hp* bacterium, and also reduce the incidence of side-effects caused by antibiotic therapy. In prospective studies, it is suggested that some probiotics, such as *Saccharomyces boulardii* or *Lactobacillus johnsonii* La1, may reduce bacterial infection, but not completely remove it. Supplementation with *Saccharomyces boulardii* yeast may be a useful combination therapy with a standard eradication protocol. Probiotic strains, such as *Saccharomyces boulardii, Lactobacillus reuteri* and *Lactobacillus rhamnosus* GG, reduce the side-effects associated with antibiotic therapy [25]. Many strains of the *Bifidobacterium* and *Lactobacillus* genus reduce the adverse effects of antibiotic therapy, stabilising the intestinal ecosystem and improving the health of patients with *H. pylori* infection. In a meta-analysis of 14 randomised trials, it was suggested that the use of probiotic bacteria during antibiotic therapy increases the degree of *H. pylori* eradication [26].

In 16 subjects infected with bacteria, it was shown that during antibiotic therapy, *Lactobacillus casei* Shirota ($2x10^{10}$j cfu/day) administered in fermented milk for 6 weeks, hindered the increase in *H. pylori* by 64% in the group after the administration of the probiotic, and by 33% in the control group [27]. Similar results were obtained in studies using *Bifidobacterium animalis* subsp. *lactis* Bb12 and *Lactobacillus acidophilus* La5 [28]. In the presented study, the *Helicobacter pylori* antigen was not detected in any of the athletes, which does not allow to draw conclusions regarding the possible impact of the supplemented probiotics on *Helicobacter pylori* eradication.

Giardia is an invasive gastrointestinal disease, the etiological factor of which is *Giardia lamblia* (*Giardia intestinalis*), a protozoan residing in the small intestine in spore (cyst) and vegetative (trophozoite) form. *G. lamblia* cysts are an invasive form of the parasite and are resistant to environmental factors and some disinfectants. Infection can occur via the faecal-oral route, when a cyst is ingested in contaminated water or food. A high probability of infection occurs in the case of poor sanitary and hygienic conditions, consumption of contaminated food and anal-oral sexual contact. Infected people develop diarrhoea, nausea, vomiting, flatulence, abdominal pain after meals, fermentation or fatty stools with abundant gases. In chronic giardiosis, the stools are loose and the gases emitted have a characteristic sulphur odour. These symptoms may be regular or disappear for a while to then reappear causing the body to gradually degenerate. In the presented study, the *Giardia intestinalis* antigen was not detected in any of the athletes, which proves that most likely, they were not infected with this protozoan.

Candida albicans is a fungus that constitutes the physiological flora of the gastrointestinal tract in 40-80% of the population. It can cause infections in immunocompromised individuals. The use of antibiotics, non-steroidal anti-inflammatory drugs or COX-1 blocking painkillers may contribute to abnormalities in the functioning of the intestinal microflora and *promote the multiplication of pathogenic bacteria and yeasts, including Candida albicans*. There are indications that proper probiotic therapy helps restore the balance in the intestines caused by the above-mentioned factors. A growing body of research into the effects of commensal microbiota on host function shows very promising results. The gut microflora has been indicated to influence pain sensation in mice. Disturbances in the intestinal microflora among these animals mean a lower pain threshold, and a leaky intestinal barrier is associated with longer regeneration time [29]. If the same results were obtained in humans, supplementation with appropriately selected probiotic strains could potentially eliminate these disorders, indirectly contributing to the results obtained by athletes. In this study, *Candida albicans* was isolated in 25% of athletes from the probiotic supplementation group, and 25% of subjects from the placebo group. In the group taking the probiotic preparation, only 7.6% showed yeast growth after 16 weeks of supplementation. Therefore, probiotic therapy had no

effect on the amount of yeast in the intestinal microflora of those subjected to probiotic supplementation. *Geotrichum species* was isolated in 15.6% of the competitors in the probiotic group, and in 15.6% of the placebo group. No growth of these fungi was observed in the following months of mycological culture. It seems that these fungi may have colonised the intestine as a result of the athletes consuming both groups, milk and dairy products. The fact that these fungi were observed in 80% of all the study participants, and the growth did not occur in the following months, proves a lack of probiotic supplementation effects on the quantitative changes of these fungi in the gut microflora of athletes.

Already during the intrauterine life, a mother's intestinal microbiota affects a child's immune system. Although the bacterial microbiota of adults is partially modified (diet, harmful factors), its main profile remains unchanged throughout life. Moreover, 90% of the bacteria in the human intestinal flora are anaerobic bacteria that produce lactic acid and volatile fatty acids. Due to competition, these bacteria inhibit the growth of pathogenic organisms such as, for example, *Clostridium difficile* or *Candida*. They also prevent infections caused by transient pathogens. The microbiota in the intestines produces bacteriocins (substances produced by numerous Gram- and Gram+ bacteria, capable of inhibiting the growth of related organisms or even killing them) and other compounds such as mucopolysaccharides and secreting IgA antibodies. This creates a protective barrier against the invasion of other, undesirable microorganisms in the intestinal mucosa. The intestinal microbiota eliminates harmful products formed during inactivation and elimination of pathogenic bacteria criteria, neutralising the action of toxins and proteolytic enzymes, allergens and food components with antigenic properties. Microbiota bacteria also deal with the processing of food, bile acids and the synthesis of molecules such as B vitamins, vitamin K and short-chain fatty acids (SCFA), i.e. butyrate, which covers 70% of the energy requirements of colonocytes and fulfils an important role in the absorption of electrolytes and water in the large intestine [30]. There are studies in which the influence of different eating habits on the microbial composition of the gut is confirmed. Research carried out on a group of children from Burkina Faso and from European countries revealed a different composition of the microbiota. African children showed significant enrichment in *Bacteroidetes* and depletion of *Firmicutes* ($p<0.001$), with a unique affluence of *Prevotella* and *Xylanibacter* bacteria known to have a set of bacterial genes for cellulose and xylan hydrolysis completely absent in European children. Additionally, significantly more short-chain fatty acids (SCFA) were found in African children compared to European adolescents ($p<0.001$).

Enterobacteria (*Shigella* and *Escherichia*) were also less represented in the group of African children than in those from countries of the European Union ($p<0.05$). Researchers assumed that the gut microbiota co-developed with the polysaccharide-rich diet of African children, thereby maximising the use of fibre energy while creating protection against inflammation and non-infectious bowel disease [31]. Thanks to an efficiently functioning digestive system, a proper degree of nutrient assimilation is possible. *Escherichia coli* naturally inhabits the human digestive tract. It is a gram-negative, relatively anaerobic bacterium whose physiological role in the human body is the production of B and K vitamins as well as food fermentation. There are about 108 organisms of this bacteria in 1 gram of stool. While *Escherichia coli* is not harmful when living in the large intestine, it is pathogenic in other systems. In the authors' research, *Escherichia coli* bacteria were isolated in all athletes from both groups, which may constitute the physiological microbiota of the gastrointestinal tract. Participants in the group consuming the probiotic did not complain about symptoms of acute gastritis, such as: indigestion, flatulence, epigastric pain (resembling ulcer pain), nausea, vomiting, fever, worsening of well-being, diarrhoea, thus it was probably not a pathogenic strain for these individuals. In the experimental group, this bacterium was isolated in all competitors and there was an increase compared to the culture before beginning probiotic supplementation. It is possible that the growth of this bacterium was due to the supplementation of probiotics from other strains present in the probiotic preparation. Such a conclusion may be drawn from the fact that probiotic bacteria have the ability to inhibit the growth of pathogenic bacteria in the intestine, as well as to create conditions for the growth of indigenous bacteria [32]. Supplementation with a probiotic preparation could contribute to the inhibition of pathogenic bacteria in the intestines of athletes from the experimental group, and thus, positively influence the growth of other beneficial bacteria, such as *Escherichia coli*. Research allows to suggest that modification of the intestinal microflora may be related to diet composition [33]. Taking this important factor into account, in future research, the fact that type of food consumed may also have a significant impact on the development or inhibition of intestinal bacteria growth should be considered. Additionally, there are more and more reports on the effects of processed food additives on the integrity of tight junctions between intestinal epithelial cells [34]. Future studies should be focused on the influence of food additives with regard to the functionality of the intestinal barrier of athletes, and thus, quantitative and qualitative changes in the microbiota of athletes, as they are the group most vulnerable to changes in intestinal permeability and therefore, various health problems.

Enterococcus bacteria are recognised as probiotic organisms [35]. *Enterococcus faecium* occurs naturally in the intestines of humans, but it can also be a pathogenic factor, causing urinary tract infections or endocarditis [36]. In the authors' own research, bacteria of the *Enterococcus* species were isolated in 70% of athletes from the group receiving the probiotic preparation. After the completion of probiotic therapy, this strain was isolated in only 20% of the participants. In addition, *Hafnia alvei* and *Pseudomonas aeruginosa bacteria* were also identified in 10% of subjects from the group undergoing the supplementation intervention. It was observed that after a 12-week intervention with a probiotic preparation, the faecal mass was much richer in bacteria than in the placebo group.

It has been reported in many studies that intestinal microbial diversity is much greater in athletes compared to individuals leading a passive lifestyle [37, 38, 39]. Clarke [38] found differences in the composition of the intestinal microbiome of professional, male rugby players and that of the amateur control group. The athletes showed a much higher intestinal microbial diversity, accompanied by a greater content of the symbiotic anaerobic bacteria of the genus *Akkermansia*. In another study, Petersen [39] assessed the intestinal microbiome of cyclists, noting that 30 out of the 33 subjects had increased *Akkermansia*. Barton et al. [37] investigated the effect of 8-week whey protein supplementation on the composition of faecal mass in a group of 90 adults leading an inactive lifestyle, showing no differences between the group with the supplementation intervention or the placebo. The researchers emphasized that the composition of the gut microbiota is not easy to change, and further observations should be made to investigate the possible impact [39].

In the research by Jang [40], the intestinal microbiota of bodybuilders and distance-runners was compared (n = 45). Each group followed a different diet specific to the given discipline. From the obtained results, it was found that bodybuilders consuming a high-protein and -fat diet had a relative abundance of *Faecalibacterium*, *Sutterella*, *Clostridium*, *Haemophilus*, and *Eisenbergiella*, but decreased the amount of *Bifidobacteria* and *Eubacterium*. In a study by Scheiman [41], it was found that the the *Veillonella* genus was enriched in the gut microbiome of marathon runners compared to non-runners. Furthermore, in a mouse model, *Veillonella atypica* was shown to increase endurance, reduce inflammatory cytokines and convert lactate to acetate/propionate. In current research, it is suggested that exercise may beneficially influences changes in the gut microflora and therefore, may be advantageous for athletic performance. The effects of probiotic therapy are strain-dependent and rely on the dose and duration of its use.

Further research should be focused on determining the dose of a given strain demonstrating effectiveness, the time of supplementation necessary to achieve a specific health effect, and assessing whether a given bacterial strain is able to synergistically interact with other strains on the human body.

5. Conclusions

1. The 4-month supplementation of the diet with a multi-strain probiotic preparation did not significantly affect the general health of the group of competitive cyclists, as assessed by haematological and biochemical indices of blood, urine and faeces. However, reduction in the level of total protein and alkaline phosphatase was shown in the group supplementing probiotics.
2. Supplementation with a probiotic preparation contributed the growth inhibition of intestinal pathogenic bacteria, simultaneously influencing the growth of selected strains of symbiotic bacteria. Nonetheless, the observed changes were not statistically significant.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee

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