

Modern probiotic functional products in the practice of training athletes

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ANNOTATION

Target The target of the study: to substantiate performance criteria and safety of the use of modern probiotics in sports (on the example of Laminolact Sport – Enterococcus faecium L3).

Methods. Data analysis of modern scientific literature and the author's own data.

Results. In a placebo-controlled study The study assessed the effect of the probiotic functional product "Laminolact Sport" based on the Enterococcus faecium L3 strain on the parameters of the body's homeostasis, which form the metabolic basis for maintaining and stimulating physical performance, as well as on the state of the cardiovascular system. A positive effect of the course use of this probiotic on the indicators of special physical performance of representatives of different groups of sports (cyclic and strength) in the dynamics of the training process was shown. Athletes did not notice any side effects when taking the probiotic functional product Laminolact Sports - Enterococcus faecium L3.

Conclusion. The data obtained substantiate the need for use, as well as the methodology for assessing the effectiveness and safety of the use of modern probiotic agents in the practice of training athletes.

Keywords: sports, physical work ability, athletes, weightlifters, probiotic functional foods, cardiovascular system, homeostasis.

ABSTRACT

Objective The objective of the study: to substantiate criteria of efficiency and safety of using modern probiotics in sport (by the example of "Laminolact Sport, Enterococcus faecium L3").

Methods. Analysis of data of current scientific literature and those of the author.

results. In placebo controlled study the influence of probiotic functional product «Laminolakt Sporting» on the basis of Enterococcus faecium L3 strain on parameters of the body homeostasis that form metabolic bases for physical work capacity maintenance and stimulation as well as upon the state of cardiovascular system has been evaluated. Positive influence of course usage of this probiotic on indices of special work capacity of athletes of various sports (cyclic and strength) has been demonstrated in the dynamics of training process. Side effects during intake of "Laminolact Sport" have not been noted.

Conclusion. Obtained data indicate the necessity of usage as well as the methods of estimating the efficiency and safety of usage of modern probiotic means in practice of athletes' preparation. **Keywords:** sport, physical work capacity, track and field athletes, weightlifters, probiotic functional products, cardiovascular system, homeostasis.

Statement of the problem and analysis of publications. In recent years, the human body has been exposed to a complex of unfavorable factors that affect the normal functioning of the basic systems of life. On the one hand, it is a deteriorating environmental situation, the increasing number of stressful situations, and on the other hand, the massive uncontrolled use of various medicines, including antibiotics, which is also typical for performance sports and is accompanied by suppression of the immune system [41, 50]. In this regard, a serious problem arose regarding the ways of constructing and restoring the optimal microflora, i.e. microecology and endoecology of the macroorganism [33]. For highly qualified athletes, due to constant physical and emotional overload, frequent changes in climate and time zones, taking a significant number of pharmacological agents whose cross-action is impossible to predict, and other factors that negatively affect homeostasis, maintaining an optimal balance of microflora, closely related to immune system stress, is of particular importance [32, 34].

Moreover, according to the data obtained after examination of the contingent of Ukrainian highly qualified sportsmen (315 people), 65 (20,64%) of them have laboratory attributes of chronic pancreatitis, clinically and according to ultrasound investigation, confirmed in 78,46% of cases (51 sportsmen of 65). Elevated activity of marker liver enzymes (alanine and aspartate aminotransferase, γ -glutamyl transferase), unrelated to the preceding load was revealed in 52 athletes (43.35%), the diagnosis was confirmed in 47. Elevated alkaline phosphatase activity, often indicating biliary dyskinesia of the hypotonic type, was detected in 10.16 % of the athletes [7]. All these factors are very often associated with the presence of an imbalance in the intestinal microflora [20] and limit the physical performance, sports longevity, and quality of life of athletes. The presence of a gut microflora imbalance is often associated [20] and limits physical performance, athletic longevity, and quality of life of athletes [7].

One of the modern trends in maintaining the microbiological homeostasis of the body is the use of probiotics [3]. The term "probiotics" was coined by D. M. Lilly back in 1965 to denote natural adjuvants - live microorganisms [44], the introduction of which into the body contributes to the restoration and maintenance of the biological balance of its normoflora [40]. WHO characterizes probiotics as agents based on intestinal commensals (communities), capable of exercising biological control in the body and possessing regulatory and triggering (triggering) properties. Probiotics not only prevent the occurrence of dysbacteriosis, but also have the ability to produce biologically active substances - vitamins, amino acids, antitoxins, etc., as well as control the pH level of the environment in which they are located [31].

Publications on the use of probiotics as medicines in sports did not appear more than 10-15 years ago [45], and their number in the literature is very limited, especially works that would study the effect of probiotics on homeostasis parameters in comparison with the parameters of physical performance. Therefore, information on the appropriateness and effectiveness of the use of these pharmacological agents at the training stages is mainly taken by athletes and coaches from the Internet [53-55]. However, this information lacks objective criteria for the effectiveness and safety of the use of probiotics by athletes.

In recent years, works have appeared that have significantly expanded the range of criteria for assessing the biological activity of representatives of normal microflora, proposed for the introduction of those or other strains into probiotics [4, 9, 19, 28].

In most cases, probiotics use live cultures of microorganisms, primarily various non-pathogenic strains of *Streptococcus*, *Lactococcus*, *Enterococcus*, *Propionibacterium*, *Leuconostoc*, *Lactobacillus*, *E. coli* M-17 [6, 28].

In recent years, due to its absolute harmlessness to humans and its presence in the normal intestinal microflora, the *Enterococcus faecium* L3 strain has become widespread as a probiotic [30, 31]. Some strains of enterococcus (*E. faecalis*, *E. avium*, *E. casseliflavus*, and some others) lead to the appearance of opportunistic infections, but this does not apply to the *E. faecium* L3 strain, since it is isolated from milk sourdough [35, 38]. Back in 1997, it was found that enterococci isolated from foodstuffs or probiotic strains were practically free of genes for pathogenicity factors [37]. Currently, due to the absence of pathogenicity of *Enterococcus* strains, many of them are used even in the food industry [43].

All of the above determined the feasibility and possibility of using the *E. faecium* L3 strain in the production of dosage forms of probiotics. One of the most well-known representatives of probiotic strains of *E. faecium* is Linex, whose high probiotic efficacy has been confirmed by a long experience of clinical use, but the content of active strains of *Enterococcus* bacteria in it is not too high [27, 51].

What are the selection criteria for the use of the new probiotic Laminolact Sport based on the *E. faecium* L3 strain in the practice of sports training? First, one of the most important criteria for the effectiveness of probiotic therapy is its immunomodulatory effect, which is largely due to stimulation or production of endogenous interferon [23]. With regard to the studied strain *E. faecium* L3, a pronounced immunomodulatory effect was shown [47]. In particular, the synthesis of interleukins of classes 1 and 10 by epithelial cells and leukocytes increased significantly, the concentration of immunoglobulins of all classes, with the exception of E, increased, and circulating of immune complexes in the blood serum, the bactericidal and lysozyme activity of the latter increased [22, 25]. Secondly, for the manifestation of probiotic activity, pronounced antagonism to pathogenic and conditionally pathogenic flora is important, which is very pronounced in the *E. faecium* L3 strain. Thirdly, the strain has the properties of vitamin formation - in comparison with other probiotic strains, the increase in the content of vitamins B and B, C and PP is increased several times, and vitamin A is even an order of magnitude [39].

Fourth - The technology of bacterial preservation without lyophilization makes it possible to produce a complex of pre- and probiotics in the form of dragees and powders. And, finally, the *E. faecium* L3 strain is characterized by high viability in a wide temperature range (from -20 to + 45 °C), as well as resistance to acids and bile [4, 6, 35, 42].

In connection with the above characteristics, the products of the Laminolact series based on the probiotic strain *E. faecium* L3 can be very promising for maintaining homeostasis and, accordingly, indirectly stimulating the performance of athletes. The advantages of Laminolact when used in sports lie in the fact that it also contains prebiotics (i.e. precursors of biologically active substances). These are, first of all, strains of live vitamin-forming bifidobacteria and lactobacilli useful for humans in the amount of 10⁶CFU•G-1, which are representatives of the

normal intestinal microflora and have a positive effect on various metabolic processes in the body [10, 29, 56].

The composition of the dragee, in addition to the above strains of beneficial bacteria, also includes a variety of plant components, which allows the probiotic functional product "Laminolact Sports" to combine the properties of pro- and prebiotics [10, 56]. The product contains vegetable protein, natural fruit pectin and sea kale, but it does not contain glucose and therefore can be used in the presence of diabetes. There are many varieties of "Laminolact", and the specific properties of each of them are manifested by the addition of specially selected medicinal plants and extracts from natural ingredients. For example, the "Sports" variety additionally includes carrots, rose hips, vitagmal (cell extract of the subtropical medicinal plant *Poliascis philicifolia*, which has a powerful antioxidant effect), psyllium juice, calcium lactate; variety "Energy Formula" – nettle, ginger, cardamom [57, 58]. Dragee "Laminolact" is a synbiotic, i.e. combines a probiotic (live culture) and prebiotics - a complex of bifidogenic and lactogenic factors. Therefore, unlike other probiotic products containing pure cultures, the microflora in Laminolact has substrate support when it enters the intestine, and the conditions for adaptation in the intestine are obviously better.

As for the effect of Laminolact on the body, in particular, its beneficial effect has been shown in the treatment of anemia and chronic hepatitis, allergic reactions, and also to increase immunological reactivity [22–25]. In addition, today it has been established that the probiotic functional product "Laminolact" has an indirect positive effect on the cardiovascular system [12, 13].

Based on the complex of these criteria, among many probiotics, we have chosen the probiotic functional product "Laminolact" in the modification "Sports" (in the form of a dragee, which is convenient for taking under conditions of training and competitive process).

In this regard, the assessment of the effect of such a probiotic on homeostasis and physical performance of highly qualified athletes is very relevant.

The purpose of the study was to substantiate the feasibility and effectiveness of using the probiotic functional product "Laminolact Sport" based on an assessment of its effect on immunity, lipid metabolism, prooxidant-antioxidant balance (PAB) in cell membranes, the functional state of the cardiovascular system, as well as indicators of physical performance among representatives of different groups of sports in the dynamics of the training process.

Methods and organization of research. For the study, the representatives of two groups of sports, different in terms of the mechanism of energy supply of muscle activity, are power and cyclic.

The studies were carried out at the special preparatory stage of the preparatory period with the participation of 25 qualified male weightlifters (CMS - 11, MS - 14 athletes) aged from 18 to 24

years (average age 18.5 ± 5.5 years) , 14 of which were included in the main group. The rest of the athletes made up the control group.

Studies at the same stage of the annual macrocycle were also carried out with the participation of 36 male athletes specializing in the running disciplines of athletics (CMS - 21, MS - 15 athletes), aged 18 to 23 years (average age $20, 2 \pm 2.3$ years). Athletes were divided into two equal in number (18 people each), age and qualification groups (main and control) by random sampling.

Athletes from both main groups received four tablets 3 times a day for 30 days as part of a standard pharmacological regimen corresponding to the sport and training period. Representatives of the control groups as part of an identical scheme of pharmacological support received a placebo instead of a probiotic (capsule with starch). Since the athletes were not aware of who was taking the probiotic functional product and who was taking the placebo, this study can be considered a blind, placebo-controlled study. All participants in the study signed an "Informed Consent" to participate in the evaluation of the effectiveness of the use of the probiotic. Athletes were informed that "Laminolact" has a pharmacological registration on the territory of Ukraine, has a positive effect on homeostasis and does not contain prohibited substances; the participants in the study, in turn, pledged to regularly take the capsules given to them.

The duration of the study was determined by the availability of known data from S. V. Belmer regarding the effectiveness of probiotic therapy: therapy courses lasting at least 21 days have the maximum efficiency; after 7 days, the effectiveness of treatment is only 74.1%, after 14 days - 85.2% and after 21 days - 88.9% [2].

Pedagogical, biochemical and electrocardiographic (ECG) studies were carried out before and after the end of the study (21-day mesocycle). For comparison,

similar indicators of biochemical and immunological homeostasis and ECG were also studied in 10 healthy untrained individuals (donors) of the same sex and age.

As indicators of physical performance in weightlifters, the parameters of special speed-strength training were used - the height of the jump from a place and the height of lifting the barbell in a snatch, as well as the time of performing exercises according to the method of V. M. Abalakov [1, 11, 14, 17]. Athletes performed three attempts to jump up from a place, the analysis included the average values of the results of exercises for each athlete.

In middle-distance runners at the special preparatory stage of the preparatory period, the training process was aimed at developing special endurance, which was assessed using a load test (2×400 m) in two series. Between the series, the rest time was 12 minutes, and between runs - 1 minute. The heart rate (HR) was also recorded after each run and after rest.

In the dynamics of the study, to study the effect of probiotics on homeostasis indicators, athletes received blood from the cubital vein in the amount of 3 ml as standard at rest, on an empty stomach, without previous physical activity. As parameters of biochemical homeostasis, the prooxidant-antioxidant balance in cell membranes was studied using washed shadows of erythrocytes, which are a suspension of their membranes, as a model [21]. Shifts in the system of prooxidant-antioxidant balance (PAB) in cell membranes were studied spectrophotometrically

by changes in the content of malondialdehyde (MDA) and reduced glutathione (GSH) [15], with the calculation of the T prooxidant-antioxidant coefficient (K) as a ratio of the above indicators. The toxicity coefficient (K) was also determined,

The immune response was assessed using first-level tests, in particular, by the number of leukocytes, lymphocytes, the content of immunoglobulins (IgA, IgM, IgG) and circulating immune complexes (CIC) in blood serum [18].

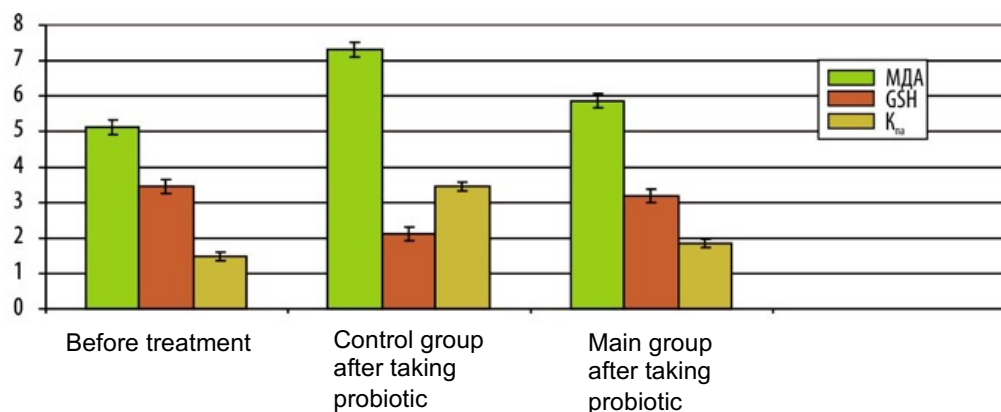
In addition, using a standardized method on a biochemical analyzer "Humalyzer 3000" (Human, Germany), we studied the content of lipid metabolism in the blood serum - total cholesterol and triacylglycerols [15] using authentic expendable and control materials. An electrocardiographic study was performed in 12 standard leads using the diagnostic complex "Cardio+" (Ukraine) in a state of relative muscle rest.

The obtained data were statistically processed by conventional methods using licensed computer programs GraphStatInPad and Statistica 6.0. During the statistical processing of data, the arithmetic mean (\bar{x}) and standard deviation

(S) were calculated. To compare the significance of differences, Student's t-test (t) was used, when the distribution of the sample corresponded to the normal law, which was checked χ^2 - Pearson's criterion. The level of reliability was set to $p = 95\%$ (the error probability was 5%, i.e., the significance level was $p = 0.05$).

Research results and discussion.

At the first stage of research in weightlifters, the antioxidant effect of the Laminolact Sports product was established; at the same time, PAB was normalized in cell membranes, in particular, erythrocyte membranes (Fig. 1).



PICTURE 1 -The influence of the product "Laminolact Sports" on the content in cell membranes MDA and GSH, as well as the K coefficient for weightlifters (the y-axis has no dimension, since it simultaneously reflects the absolute values of all the studied indicators)

In accordance with this, the calculated K practically returned to the values typical for healthy untrained individuals. In parallel with the improvement in the functional state of the erythrocyte membrane, the content of toxic substances - SMP - in the blood serum decreased, i.e. the severity

of the syndrome of endogenous intoxication, which is characteristic of intense physical activity, decreased [46] (Table 1).

TABLE 1 -The influence of the product "Laminolact Sports" on the severity of endogenous intoxication in representatives of different groups of sports

Index (~x ± S)	Non-training bath faces (n=10)	Group			
		control		main	
		Start mesocycle	ending mesocycle	Start mesocycle	ending mesocycle
Weightlifters (n=25)					
Content SMP, conv. units	0.25±0.02	0.41±0.06*	0.72±0.05**	0.43±0.7*	0.58±0.6** ***
TO T	4.8±0.3	8.8±0.3*	11.3±0.6**	8.9±0.4*	6.4±0.6** ***
Athletes (n = 36)					
Content SMP, conv. units	0.25±0.05	0.39±0.03*	0.58±0.04**	0.40±0.04*	0.44±0.03** ***
TO T	4.8±0.3	7.2±0.3*	9.5±0.2**	7.4±0.5*	5.7±0.4** ***

* p < 0.05 compared with data from donors; ** p < 0.05 compared to the data at the beginning of the mesocycle; *** p < 0.05 compared with the data at the end of the mesocycle in the corresponding control groups.

A decrease in the manifestations of endogenous toxicity of the body, in turn, leads to an improvement in myocardial function and an increase in exercise tolerance, as well as to stimulation of immunity and an increase in resistance to viral and bacterial infections [36, 49].

At the same time, among weightlifters, the content of SMP, as markers of the severity of intoxication of the body, and the toxicity coefficient were significantly higher than in athletes. Since athletes from different groups of sports were at the same stage of the annual macrocycle of training, were of the same age and sex, it can be assumed that it is the energy supply mechanism of the testing load (predominantly lactate anaerobic) that affects the value of the toxicity index in weightlifters.

Unidirectional changes in the studied biochemical parameters in the membrane changes in erythrocytes and blood serum were also observed in track and field athletes, except that, due to the predominantly aerobic nature of training loads, the severity of PAP shifts in them is more noticeable (Fig. 2).

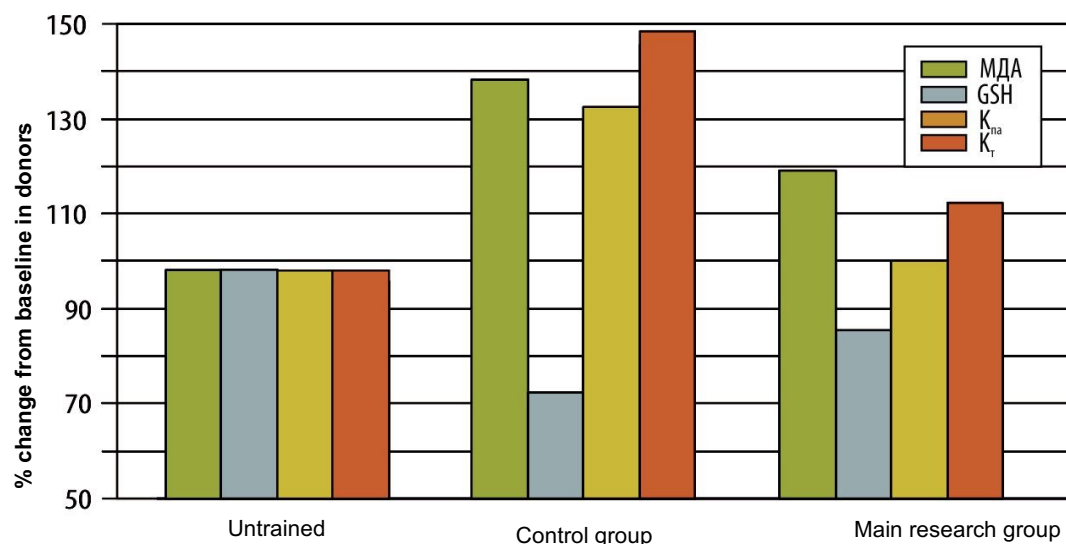


FIGURE 2 -The influence of the product "Laminolact Sports" on the content in cell membranes

The use of the probiotic functional product "Laminolact Sport" was also accompanied by an improvement in the parameters of the blood lipid spectrum in representatives of different sports. If in the control group the levels of total cholesterol and triacylglycerols were increased against the values in healthy untrained individuals, then probiotic therapy led to a decrease in the content of these factors in the blood serum (Table 2).

TABLE 2 -Influence of the product "Laminolact Sports" on the lipid spectrum blood serum ($\bar{x} \pm S$) in representatives of different groups of sports

Index	Group				
	donors (n=10)	control		main	
		Start mesocycle	ending mesocycle	Start mesocycle	ending mesocycle
Weightlifters (n=25)					
total cholesterol, μmol·l ⁻¹	4.63±0.35	5.79 ± 0.21*	5.73±0.36	5.83 ± 0.19*	4.03 ± 0.17**
triacylglycerols, mmol·l ⁻¹	0.99±0.10	2.13 ± 0.08*	2.45±0.39	2.21 ± 0.15*	1.43 ± 0.14**
Athletes (n = 36)					
total cholesterol, μmol·l ⁻¹	4.63±0.35	5.04±0.09	5.08±0.07	5.11±0.7	4.56 ± 0.06**
triacylglycerols, mmol·l ⁻¹	0.99±0.16	1.91 ± 0.04*	2.16 ± 0.11	1.87 ± 0.06	1.02 ± 0.04**

* p < 0.05 compared to donors; ** p < 0.05 compared to similar data at the beginning of the mesocycle.

It should be noted that in weightlifters, compared with representatives of athletics, lipid metabolism parameters were higher both at the beginning of the mesocycle and in the dynamics of the study, which is associated with the nature of the loads and diet [5, 8]. It was also shown that, in comparison with the data in the control group, under the influence of taking "Laminolact" in track and field athletes, the content of immunoglobulins of classes A and M increases in the blood serum (by 13.7 and

11.8%, respectively) and the level of circulating immune complexes and immunoglobulin M - by 12.4 and 18.7%, respectively (Fig. 3).

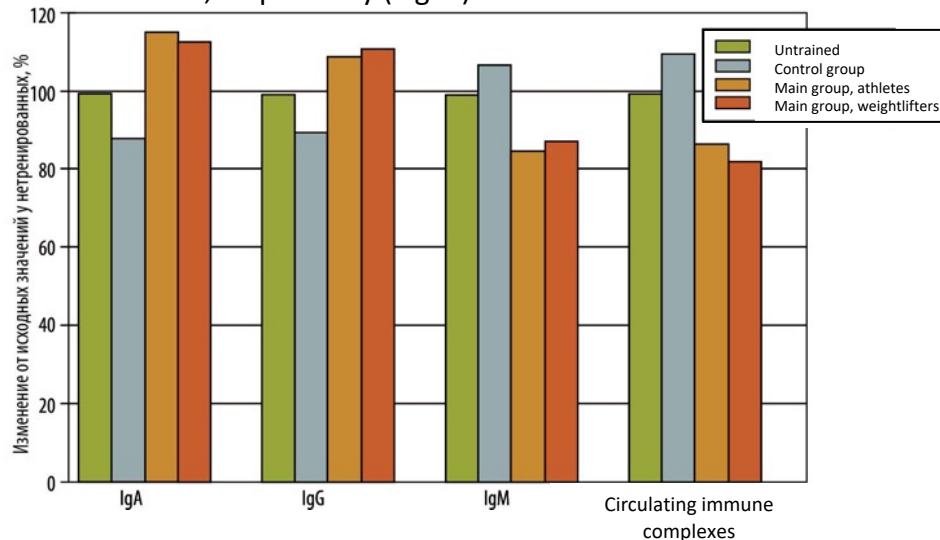


FIGURE 3 -The influence of the product "Laminolact Sports" on the immunogram parameters in athletes (a) and weightlifters (w)

Similar changes in the dynamics of the mesocycle are observed under the influence of Laminolact in representatives of weightlifting. It should be noted that four athletes of the main group, in whom the initial level of leukocytes was below the reference values, amounting to $(3.1 \pm 0.3) \cdot 10^9 \cdot l^{-1}$, when taking Laminolact, its normalization was noted, i.e. The probiotic also has a modulating effect on leukopoiesis. In parallel with the positive shifts in homeostatic parameters on the ECG in athletes (in both main groups of athletes and weightlifters) who received Laminolact Sports during the mesocycle, compared with the generalized data in both control groups of athletes, changes in the ST segment were significantly less common. (depression by 1–1.2 mm) - by 13.4% and widening of the QRS complex (by 9.9%), as well as by 7.9%, the frequency of detection of early repolarization syndrome (ERS) decreased, which indicates normalization of the metabolic and oxygen supply of the heart, and, consequently, helps to improve the contractility of the myocardium and the functional state of one of the main limiting performance of body systems - the cardiovascular system - as a whole (Fig. 4).

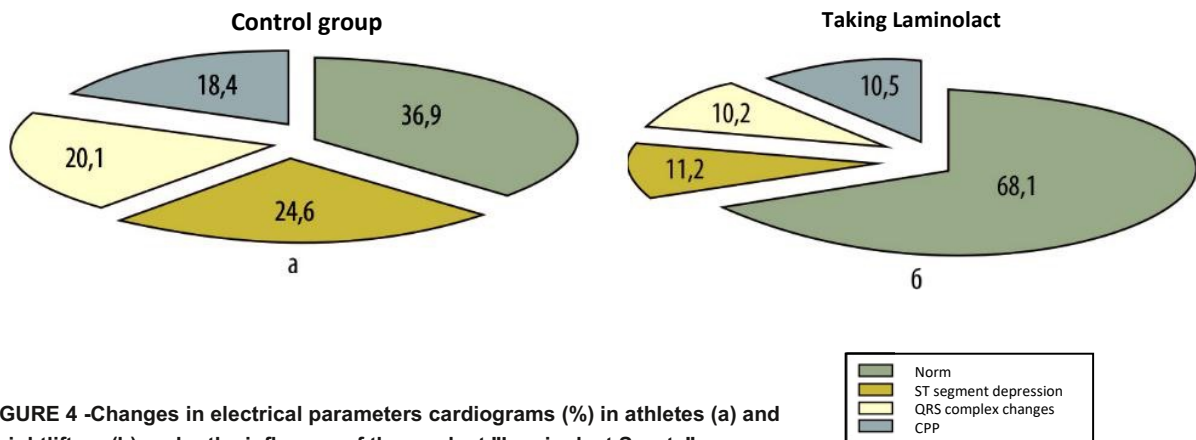


FIGURE 4 -Changes in electrical parameters cardiograms (%) in athletes (a) and weightlifters (b) under the influence of the product "Laminolact Sports"

If before the start of probiotic therapy only in 36.9% of athletes (in general, in representatives of both sports) the electrocardiogram corresponded to the norm for athletes, then after taking "Laminolact Sports" this figure almost doubled - to 68.1 %. In accordance with the improvement of indicators of homeostasis and the functional state of the myocardium in the dynamics of the mesocycle, there was a persistent trend towards an increase in the indicators of special fitness of weightlifters, which gives reason to judge about the improvement and overall physical performance of highly qualified athletes when used in the scheme of pharmacological support of the probiotic product (Table 3).

TABLE 3 -The influence of the product "Laminolact Sports" on the indicators of special fitness level of weightlifters

Athlete groups and research period	Jump up from a place ($\bar{x} \pm S$)		Jerk pull ($\bar{x} \pm S$)	
	Height, cm	Time, ms	Height, cm	Time, ms
Before the start of the mesocycle	56.12 \pm 2.11	0.54 \pm 0.05	81.3 \pm 3.78	0.66 \pm 0.05
Checklist end of mesocycle	58.31 \pm 4.82	0.51 \pm 0.08*	81.65 \pm 2.57	0.63 \pm 0.04*
Basic by end of mesocycle	59.14 \pm 2.25	0.48 \pm 0.05 **	83.76 \pm 2.18	0.59 \pm 0.06**

* p < 0.05 compared to the data before the beginning of the mesocycle; ** p < 0.05 compared with the data in the control group at the corresponding stage of the study.

Analyzing one of the parameters of the special performance of athletes, namely the running of model segments (2 \times 400 m) in two series, it should be noted that the initial results of the studied indicator in the main and control groups of runners specializing in middle-distance running did not differ from each other. By the end of the studied mesocycle, this indicator of physical fitness in the main group under the influence of "Laminolact Sports" slightly (by 7.3%), but significantly increased compared to the data in the control group. In addition, there was an increase in the stability of the speed of running the model segments in each individual run, which corresponded to the set tasks of the control training.

Thus, in a placebo-controlled study, a positive effect of the modern probiotic functional product "Laminolact Sports" on indicators of special performance was established, which is based on the improvement of various homeostasis parameters that are involved in the formation of the ergogenic properties of the body athletes. It is important to note that there were no negative

effects from the use of the probiotic by athletes, which confirms its safety. In our opinion, taking into account the deterioration of the environmental characteristics of the environment, the often uncontrolled intake of antibiotics by athletes, as well as the need to reduce the drug load on the body, the inclusion of probiotic products created on the basis of modern, temperature- and gastrointestinal-tract-content-resistant strains should be mandatory, especially in the dynamics of the special preparatory phase of the preparatory period, as well as in the recovery period.

Conclusions

When choosing probiotic products, one should pay attention to the strains of microorganisms that are included in its composition. It is desirable to supplement the microbiological component with various components of medicinal plants that enhance the effect of strains.

The course intake of the probiotic functional product "Laminolact Sports" based on the *Enterococcus faecium* L3 strain in qualified representatives of different groups of sports is accompanied by a positive effect on the severity of the endogenous intoxication syndrome, which is one of the

essential metabolic factors of physical performance decrease.

The probiotic functional product "Laminolact Sports" has a positive effect on the prooxidant-antioxidant balance in cell membranes, which improves the course of all metabolic processes in the athlete's body without exception.

The course intake of the probiotic functional product "Laminolact Sports" helps to improve the functional state of the cardiovascular system, which is realized indirectly through the improvement of metabolic parameters, primarily endogenous toxicity, oxide homeostasis at the membrane level, lipid metabolism.

The probiotic functional product "Laminolact Sports" indirectly, through the above-described metabolic and functional changes, leads to an increase in the indicators of the special performance of athletes.

Thus, the results obtained give grounds for using the product "Laminolact Sports" in elite sports in the annual training cycle, especially at the special preparatory stage of the preparatory period, to maintain the proper level of metabolic processes and physical performance of representatives of different groups of sports, which is based on the positive effect of this probiotic on the main limiting links of performance.

Prospects for further research in this direction in a more in a point sense, they consist in the search for the most effective and safe probiotics for sports, and in a narrower sense, in an in-depth study of the subtle mechanisms of the influence of probiotics on the effectiveness of training and competitive activities of qualified athletes.

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