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Evaluation of the Effect of Fruit Juice Containing *Bacillus Coagulans* Probiotic Supplement on the Level of Immunoglobulins A, M and Lymphocytes in Two-Speed Athletes

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Abstract

Probiotics exert beneficial effects on their host health by creating microbial balance in the digestive system. The role of some probiotic strains in strengthening the immune system and reducing the risk of diseases, especially respiratory infections, has been proven in previous studies. Aim: The aim of this study was to evaluate the effect of probiotic supplementation containing *Bacillus coagulans* on the Runner athletes immune system. In this study, the effect of *Bacillus coagulans* probiotic on immunoglobulins A, M and monocytes count 60 male athlete sprints. Evaluates that which were randomly divided into two groups of 30. For 3 months, the experimental group received a daily glass of probiotic juice containing 10^9 cfu / ml containing probiotic supplement and the control group received plain and no supplemental juice. During the study period, once every 2 weeks, One day after exercise (running 200 meters), blood samples were taken from all participants. Then In the collected samples, IgA, IgM and lymphocytes were evaluated. Consumption of probiotic juice containing 2×10^9 f cfu/ml *Bacillus coagulans* probiotic supplement showed a significant difference in the amount of IgA, IgM and Lymphocyte between the experimental group and the control group. The results of this study showed that the consumption of juice containing probiotic supplement *Bacillus coagulans* can increase the level of immune factors IgM, IgA, lymphocytes and prevent the occurrence of diseases, especially respiratory infections, by improving the function of the immune system.

Keywords: IgA, IgM, probiotic juice, immune system, lymphocytes, athletes

1. Introduction

Hard and under pressure training combined with stress caused by attending various competitions by reducing the physical and mental strength of individuals

causes dysfunction in the immune system of professional athletes. Intense training, insufficient rest and improper nutrition are the factors that induce stress in athletes and make them prone to several health complications like immune depression, inflammatory dysregulation, increased respiratory tract infections, and mental stress [1–3]. Many epidemiological studies have reported that the symptoms of respiratory tract infections increase 1 to 2 weeks after strenuous endurance competition. The biological balance of the body's organisms improves host health, improves immune system function, and increases the body's defenses. Microbiota plays an important role in the physical performance of the host [4–6]. Dietary nutrients improve energy gain during exercise, which can bring many metabolic benefits to an athlete during exercise and recovery. Studies have shown that metabolic activity and related pathways increase in the microbiome of athletes compared to sedentary individuals [7–9]. Many studies have shown the benefits of probiotics such as reducing toxins, increasing immunity and resistance to infections, production of vitamins and nutrients, production of organic acids, reduction of allergic reactions and respiratory infections, arthritis and modulation of immune responses. Investigating the link between probiotic use and physical function, which shows that probiotics protect the body against undesirable physiological changes that may be caused by strenuous exercise. Probiotics can improve intestinal barrier properties [10] and antioxidant status [11]. Decreased physical performance and immune system strength following chronic fatigue in athletes who exercise intensively has been proven in many studies. Nieman et al. (2019) showed that regular and continuous exercise increases the strength of athletes' immune systems, while with heavy and intermittent exercise, the opposite results have been reported. The results of some studies show that heavy exercise, although it does not harm the organs, but it disrupts the immune system [12]. Humoral immunity in athletes is often assessed by measuring mucosal immunoglobulins A (IgA) and M(IgM) especially the amount of secretion changes from tissues in sports activities. Decrease in immunoglobulin After the intense sports activities, Increased the possibility of upper respiratory tract infections. Lymphocytes play a very important role in the immune system due to the secretion of antibodies. Increasing the level of immunity and reducing the risk of disease in athletes, in addition to Increase their performance, will reduce treatment costs and improving immune function [13–19], so in this study, the effect of consumption of probiotic juice of *Bacillus coagulans* on the level of some immune system factors such as IgA IgM and lymphocyte Has been studied.

2. Materials and methods

In this study, probiotic juices containing 2×10^9 cfu *Bacillus coagulans* were prepared. 60 male runner athletes with an age range of 18 to 20 years and an average weight of 68 to 72 kg were randomly divided into two groups of 30 people. The criterion for participating in the study of complete physical health and was non-smoking. During the 12 weeks of the study, the experimental group received a glass of probiotic juice containing *Bacillus coagulans* daily, and the control group received fruit juice Without supplemental.

2.1 Collect blood samples

Every once two weeks, One day after exercise (Running a distance of 200 meters) 8 ml of blood was taken from all subjects Blood samples were collected in heparin tubes and immediately transferred to the laboratory. To separate the blood serum, samples were centrifuged at 5000 rpm for 15 minutes.

2.2 Measurement of immunoglobulin A& M levels

In the present study, the levels of immunoglobulins A and M were measured using ELISA kits (ab196263 and ab214568). The test steps were performed according to the protocol recommended by the kit manufacturer (abcam Inc., USA) And light absorption of the samples was read by ELISA reader (Biotech microplate reader ELX800).

2.3 Lymphocyte cell count

Lymphocyte counts were performed by flow cytometry. Blood samples were collected in heparinized tubes and then centrifuged. The cell layer was washed with phosphate buffered saline (PBS) twice, then 10 µl of monoclonal antibody was added and the samples were incubated in the dark for 20 min. To remove excess antibodies, wash twice with buffer. to measure adsorption Optical samples of fluorescent isothiocyanate fluorescein fluorochrome (ftic) were used. Then the tubes containing the sample were placed in a flow cytometer and the results were reported based on percentage.

2.4 Statistical analysis

Analysis of variance (ANOVA) was used to determine the effects of probiotics. Sample t-test was used to identify differences within the group (Beginning and end of the experiment). Values less than 0.05 were considered significant. All statistically The analysis was performed using Graphpad Prism software (version 6).

3. Results

All participants completed the test. Taking a probiotic supplement did not cause any side effects. During the first month of the experiment, no significant change in the level of immune factors was observed. With increasing probiotic supplementation time, the experimental group had a significant increase in the levels of immunoglobulin A, immunoglobulin M and lymphocytes compared to the control group, That was arrangement it IgA (258 vs. 233 ug/dL) (**Figure 1**), IgM (159 vs. 133 ug/dl)

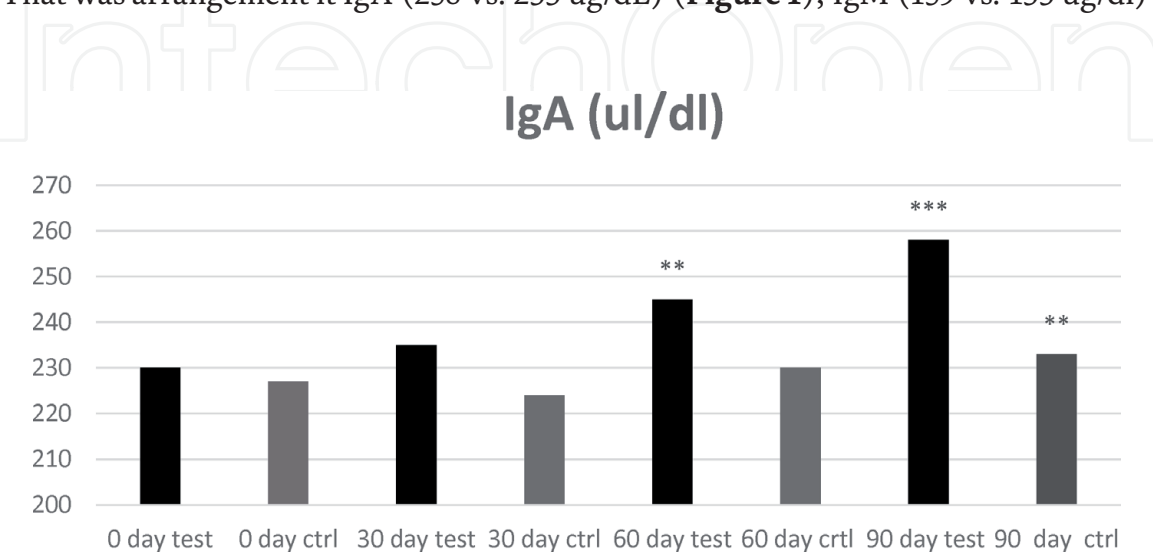


Figure 1. Immunoglobulin A level in experimental and control groups during the study time (0–90 days). * standard deviation, ** means that the difference in the results in each row of values is not significant, *** means that the difference in results in each row is noticeable, (P < 0.05) different.

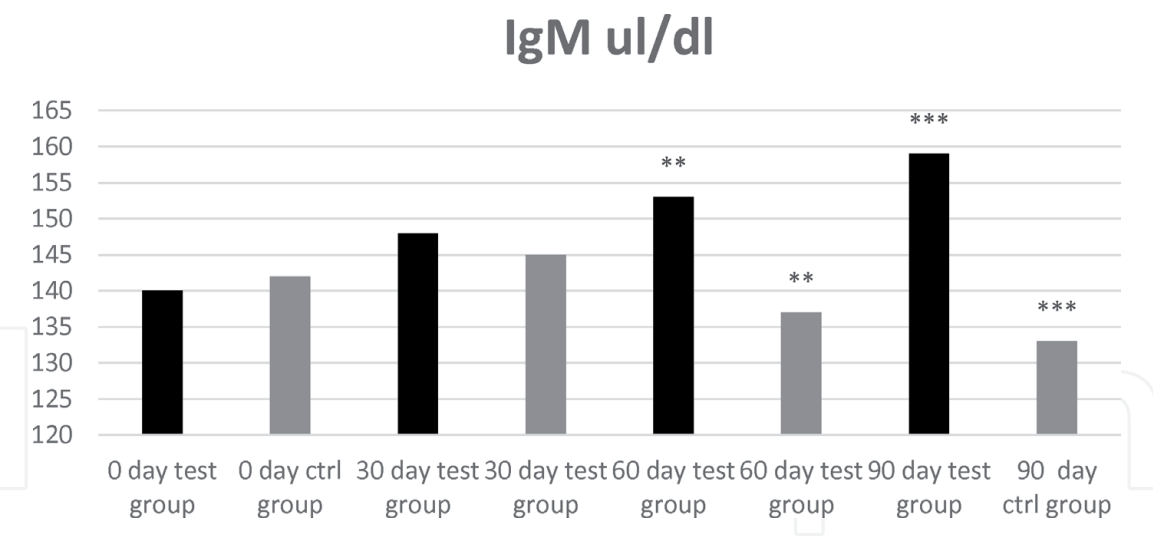


Figure 2. Immunoglobulin M level in experimental and control groups during the study time (0–90 days). * standard deviation, ** means that the difference in the results in each row of values is not significant, *** means that the difference in results in each row is noticeable, ($P < 0.05$) different.

Group	Time (Day)	0	30	60	90
TEST		32 ± 4.1 (Cell/μl)	32.7 ± 2.6 (Cell/μl)	36.8 ± 5.3** (Cell/μl)	37.6 ± 4.6*** (Cell/μl)
Control		32.1 ± 3.9 (Cell/μl)	32.3 ± 3.5 (Cell/μl)	31.0 ± 4.3 (Cell/μl)	33.6 ± 3.1* (Cell/μl)

*Standard deviation.
**means that the difference in the results in each row of values is not significant.
***means that the difference in results in each row is noticeable.
($P < 0.05$) different.

Table 1. Lymphocyte cell count in both experimental and control groups one day after exercise (two 200 m speed) in terms of%.

(**Figure 2**) and lymphocytes (37.6 vs. 33.6 cell/ul) (**Table 1**). In addition, there was a significant reduction in the incidence of respiratory infections among the supplement group compared to the control group.

4. Discussion

Athletes due to their readiness for various competitions endure a lot of physical and psychological stress. These tensions can lead to decreased immune system function and the development of multiple infections. Probiotics are natural compounds with a variety of benefits to increase the quality of life of people by improving their health by improving the function of the immune system [20–22]. The results of this study showed that consumption of fruit juice containing the probiotic *Bacillus coagulans* has a positive effect on improving the health and quality of life of athletes by improving the function of the immune system and reducing the risk of respiratory infections. A 2007 study by Kakonen et al. found that runners who took probiotic supplements were less likely to develop respiratory infections than other athletes [23]. The results of a 2016 study by Ahanchian et al. Also showed the effect of taking probiotic supplements on the prevention of respiratory infections [24]. In this study, it was found that taking probiotic supplements over time shows a positive effect on health. The results of the Zhang et al. study also showed that

taking probiotic supplements *Lactobacillus casei* after 16 weeks has a significant effect on increasing the level of immunoglobulins A [25]. Based on this, it can be concluded that to evaluate the beneficial effects of probiotics, it is necessary to take these natural supplements regularly and intermittently.

Michalikova and colleagues examined the effect of taking *helveticus* Lafti L10 on humoral safety in athletes. The results of this study showed that taking this probiotic supplement for fourteen weeks significantly improves the immune status of athletes [26]. The effect of *L. fermentum* supplement on respiratory and immune system of runners was evaluated by Batatinha and colleagues. The results of this study showed that taking this supplement has a positive effect on athletes' health [27]. Consumption of probiotics restores or maintains the balance of intestinal microbiota, reduces oxidative stress, and improves cardiovascular function in athletes. Strengthening the control system and inhibition of free radicals was observed in athletes using supplements containing several probiotic strains (*L. rhamnosus*® and *L. paracasei*) [28, 29]. Decreased gastrointestinal discomfort and endotoxin content in athletes during multi-component probiotic supplements (*L. acidophilus* CUL-60, *L. acidophilus* CUL-21, *B. bifidum* CUL-20, *B. animalis* subsp. *Lactis* CUL-34) Has been [30]. The results of the study by Strasser et al. Showed that the use of multicomponent probiotic supplements including (*B. bifidum*, *B. lactis*, *E. faecium*, *L. acidophilus* W22, *L. brevis* and *L. lactis*) impaired tryptophan levels during intense exercise. By increasing serotonin levels, exercise induced stress is reduced and athletes' mental state is improved [31].

Probiotics directly affect the function of endocrine immune cells, modulate the immune system and strengthen the immune system, and most importantly, have a positive effect on the immune system without causing a harmful inflammatory response. Therefore, it seems that probiotics are a natural and healthy method that can increase host resistance in the face of injury and stress [32]. The effect of taking the probiotic supplement *L-fermentum* was evaluated in another study in 2014. The results showed that the level of influenza-specific immunoglobulin increased significantly with the use of this supplement compared to the control group [33].

The effect of consuming a mixture of probiotics on increasing the number of immune cells, including lethal t cells, cd4 cells, lymphocytes and monocytes, has been proven in a study by Wu et al. [34]. Considering the importance of maintaining health and improving the performance of athletes during competitions, based on the results of this study and other studies conducted in this field, it was found that taking probiotic supplements will have many positive effects on the health of people, especially professional athletes.

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