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The Effect of Short-Term Probiotic Consumption on Aerobic and Anaerobic Capacity and Delayed Onset Muscle Soreness in Football Players

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ABSTRACT

Introduction: The present study aimed to review the effect of short-term probiotic consumption on aerobic and anaerobic capacity and delayed-onset muscle soreness in football players.

Methods: A total of 30 male football players in the youth age group were selected by purposive sampling and randomly assigned to two supplement and placebo groups. Blood samples were gathered before and after the football simulation workout to measure the creatine kinase levels. In addition, muscle pain was measured 24, 48, and 72 hours after the workout. In addition, aerobic and anaerobic capacities were measured 24 hours before the pre-test and post-test. The subjects participated in the supplementation program specific to their group between the pre-test and the post-test for two weeks so that one group received a probiotic supplement and the other group received a placebo (starch powder) in the same way (two caps received a dose of 2×10^8 microorganisms per day).

Results: Short-term consumption of probiotic supplements led to a slight increase in aerobic capacity (P=0.078) and anaerobic capacity (P=0.11) in football players, but this increase was not statistically significant. However, a significant reduction in muscle pain (P=0.001) and serum levels of creatine kinase (CK) (P=0.001) was observed in the supplement group compared to the placebo group.

Conclusion: Based on the results, short-term consumption of probiotic supplements reduced DOMS after intense exercise in young football players, probably due to probiotics' antioxidant properties.

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Introduction

Currently, football is one of the most popular sports in the international arena (1). Physical fitness is essential in football, which is mainly aerobic and anaerobic so that the athlete can perform various activities, including speed activities, agility, change of direction, tackling, individual techniques, and tactics (2). Sports such as football and futsal use all three energy supply systems due to consuming a large amount of energy. In addition, the intense and exhausting training and competition in these sports leads to muscle damage and, as a result, increases in mediators and inflammatory enzymes in the plasma. Therefore, increased plasma inflammatory and oxidative stress factors are associated with increased enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH). Thus, these enzymes are introduced as functional enzymes in metabolism (3).

As a result, researchers have investigated the influential factors in increasing the performance of soccer players, which can be referred to as nutritional factors and the use of supplements, including probiotic supplements (2). Probiotics bind and settle in the digestive system, inhibit the growth of pathogenic bacteria, and improve the intestinal microbial balance and the digestive system's mucosal barrier (4). Probiotics also

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control the transmission of food antigens and stimulate the host's systemic and mucosal immune system. The effects of probiotics are increasing the body's immunity and resistance to infections, producing organic acids, vitamins, and nutrients, reducing allergic reactions, and increasing antioxidant activity. Probiotics have been proposed as a new therapeutic strategy for dealing with infectious and inflammatory conditions because they modulate the immune system. Probiotics are also used to treat and prevent infectious and chronic diseases, such as inflammatory bowel disease and other diseases, as well as acute and chronic muscle soreness and inflammation following exercise (5).

Research has been conducted on the effect of prebiotics on markers of muscle damage. In a study, probiotic supplements were found to reduce inflammation and muscle damage markers after muscle damage and prevent muscle strength decline after muscle damage (6). Another study showed a significant decrease in CK, blood urea nitrogen (BUN), improved sports performance, and finally accelerated recovery in 48 and 72 hours after exercise in one leg (7). Even though probiotics have been limited in their ability to reduce injury and inflammation following exercise, a study recently discussed the importance and role of probiotics in sports performance. The study noted that even though probiotic supplementation improved athlete performance after intense training, it had not been thoroughly studied as a supplement for athletes (8). On the other hand, intense training in football sports increases the production of reactive oxygen species (ROS), causing cell damage, disruption of immune cells, the occurrence of an open window or URTI, digestive disorders, and reduction of aerobic and anaerobic capacity as well as increase delayed onset muscle soreness (DOMS). In addition, intense exercises cause a decrease in the function of the immune system in the first hours after the activity (9). The probability of URTI and digestive disorders increases with the increase in cortisol secretion, which is a response to very intense exercise (1). Athletes' physical performance is also reduced due to inflammation and DOMS. On the other hand, the results of recent research on whether probiotic supplements are beneficial in reducing these disorders and increasing the physical performance of athletes, including football players, are discussed (10).

Investigating the role of anti-inflammatory and antioxidant supplements in improving the athlete's performance in a competitive environment is essential because the effect of probiotics has been limited in the performance of athletes; more studies are needed in this field. Moreover, probiotics are known as an anti-inflammatory and metabolism-improving supplement. Introducing it as a supplement can improve sports performance and reduce muscle damage caused by intense sports activity. Therefore, according to the characteristics stated concerning probiotic supplements, the current research was conducted to review the effect of short-term consumption of probiotic supplements on aerobic and anaerobic capacity and DOMS of soccer players.

Materials & Methods

Statistical Sample (Subjects)

A total of 30 youth football players of the Shiraz Premier League aged 17-19 years were selected and randomly assigned to two supplement (N=15) and placebo (N=15) groups. Before starting the research, the nature, goals, and risks of this study were explained to the subjects in a face-to-face meeting, and written consent was obtained from them to participate in this study. All the ethical principles were conducted under the supervision of the Ethics Committee in Biomedical Research of Islamic Azad University, Morvdasht Branch, with the approved code IR.IAU.M.REC.1402.083. First, the entire research process (supplementation, physical activity, and sampling), along with its goals and possible risks, was explained to the subjects in a meeting, and their demographic information was also obtained from them along with written voluntary consent (Figure 1). Then, the subjects participated in a session with aerobic, anaerobic, and simulated football physical activity tests one week before the start of the research (Figure 1). Twenty-four hours before blood sampling, the subjects performed aerobic and anaerobic tests, and their record was recorded as the pre-test record. After 24 hours, the initial blood samples were gathered (before doing simulated football physical activity). Then, both groups performed the simulated physical activity of football, and immediately after its completion, a second blood sample was gathered to measure the levels of creatine kinase. Then, the muscle pain was measured 24, 48, and 72 hours after the

simulated football physical activity (recovery period). After that, the subjects participated in the supplement program specific to their group for two weeks, so that one group received a probiotic supplement and one group received a placebo (starch powder). The probiotic supplement and placebo were in the same capsules. A 24-hour nutrition reminder questionnaire was used to measure the subjects' diet during these two weeks because it was impossible to control the nutrition precisely. After two weeks of receiving the interventions, both groups had a post-test session exactly like the pre-test session according to the stated protocol.

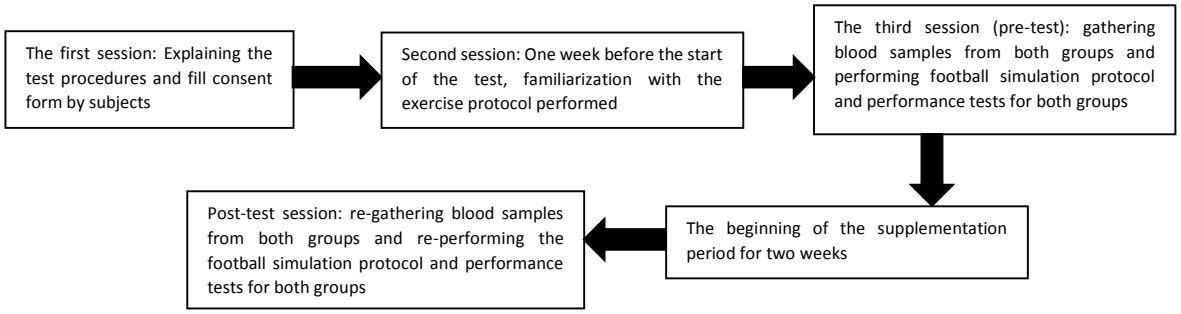

Figure 1. The research implementation method in an overview

Aerobic Capacity

Aerobic capacity was measured through the Tuxworth and Shahnawaz step test, which is one of the practical methods of VO_{2max} measurement (11). Each subject went up and down the standard 40 cm high steps 125 times within 5 minutes (25 times per minute; each going up and down the step is counted once). After 5 minutes, the subject's heart rate was taken three times in a row for every 30 seconds of rest, 30 seconds. Then, the b index was calculated using Equation 1.

(1)

$$b = \frac{[(150 - 180) - (90 - 120) - (30 - 60) - Heart\ rate)] \times 2}{\text{Weight (kg)}}$$

Additionally, the b index is used below to determine the VO_{2max} or aerobic capacity of subjects (11).

$$(VO2max = -0.378b + 4.67)$$

Anaerobic Capacity

Anaerobic capacity was measured through the 30-second Wingate test and the Monarch bicycle (model 894). The software gave the researcher indicators such as peak power, average power, and minimum anaerobic power as outputs. The fatigue index is based on delaying fatigue in the distance between maximum power and minimum power, which was calculated through the following formula (12).

$$\text{Fatigue index} = (maximum\ power - minimum\ power) \times 100 / maximum\ power$$

Football Simulated Physical Activity Program

According to Figure 2, the subjects performed physical activity after 15 minutes of relaxation, including football simulation exercises in the pre-test and the post-test. This exercise consisted of six 15-minute rounds of special exercises, including walking, dribbling the ball through obstacles, running backward, running at speed on four straight lines for a distance of 50 meters, and back and forth, performed on the grass field. Subjects were allowed to drink water after every 15 minutes during a 1.5-minute break. The subjects' heart rate was recorded in the 10th, 20th, 30th, and 40th minutes of each half of the exercise. Bishop *et al.* implemented this exercise protocol in 1999. The selection of this protocol is due to the similarity of its stages with the usual techniques of football sport, and researchers use it to transfer the actual load of football training to the subjects (13).

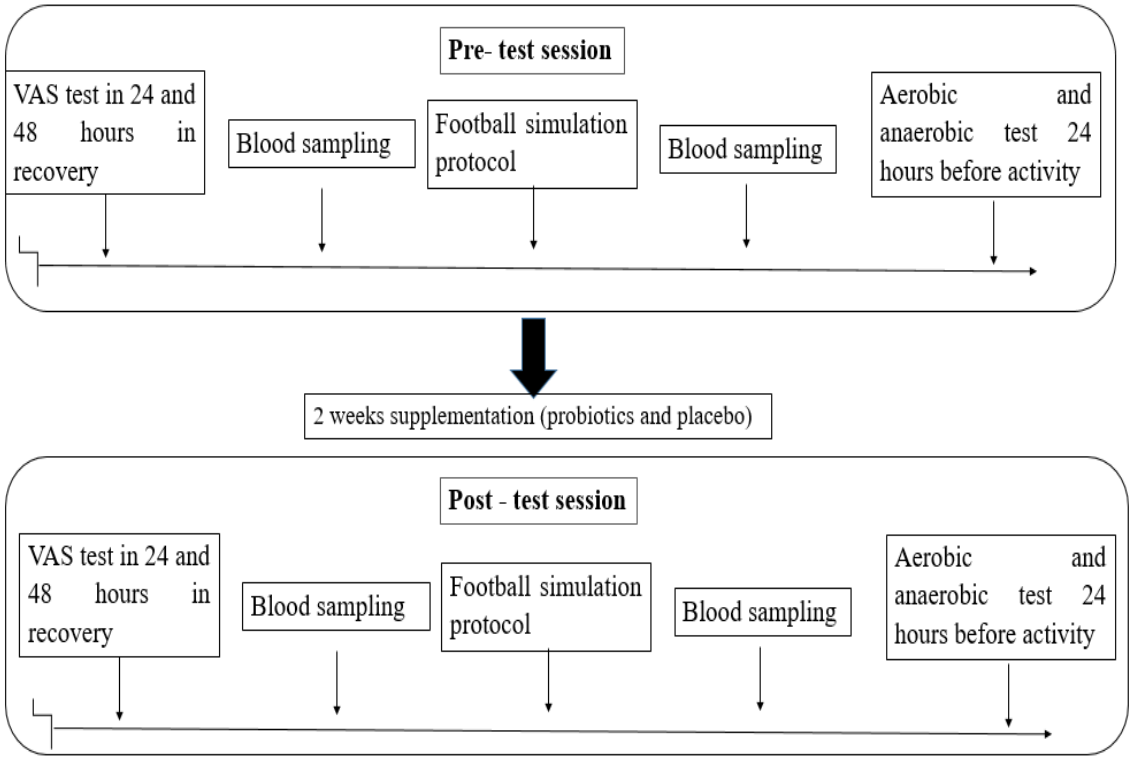

Figure 2. Research design- research method. The time stages of blood sampling for functional tests before and after the test and also the time of the supplementation period

Probiotics and Placebo Consumption

Subjects took 28 probiotic capsules from an English company (Probiotics Healthcare CFU/daily) or placebo with a similar dose of two capsules per day with a dose of 2×10^8 microorganisms for 14 days. The subjects received information about when and how to consume (the best time is one hour after the main meal with water). In addition, all the subjects were asked to avoid consuming yogurt during the research period due to the presence of lactobacillus and any effective supplements to avoid disturbing the evaluation of the investigated factors. The placebo capsules contained starch. The microorganisms present in this probiotic product included Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium Brio, Bifidobacterium longum and Streptococcus.

Blood Sampling and Creatine Kinase Measurement Method

The subjects were placed in a fixed and sitting position, and blood samples were taken from the middle vein (Baslik) with a 5cc syringe. The collected samples were poured into sterile tubes containing K3EDTR. Heparin and EDTA tubes were placed in ice and remained at room temperature for several minutes. Then, serum was separated from plasma by centrifugation for 10 minutes at 3500 RPM. All blood samples were frozen at -20°C until reaching the laboratory, where they were also frozen at -70°C. CK levels were measured using the Pars test kit and auto-analyzer device for each sample.

Measurement of Muscle Pain

A visual analog scale (VAS) was used to measure muscle pain. This is the pain assessment protocol introduced by the University of Melbourne. The VAS method is the most common method used in studies, which determines the level of pain based on behavioral factors (14). The capability and adequacy of this method in measuring postoperative pain has been proven (15,16).

Statistical Analysis

First, the values of each variable were described using the mean and standard deviation. The

Kolmogorov-Smirnov test and the homogeneity of variances were used using the Levine test after confirming the normal distribution of the data. The statistical test of inter-intra-group mixed analysis of variance was used to compare and examine the changes in the variables in the two research groups and at different measurement times. A significance level of $P<0.05$ was considered. All statistical calculations were performed using SPSS version 26 statistical software. Excel 2019 statistical software was also used to draw statistical figures.

Results

Table 1 shows the descriptive information of the subjects (age, height, weight, and BMI). The results of mixed analysis of variance between and within groups with repeated measurements test to compare changes.

Table 1. Descriptive information of subjects

Group	Variable	Time	Number	Highest	Lowest	SD	Mean
Age (Y)	Supplement	Pre- post test	15	19	17	0.16	18
	Placebo	Pre- post test	15	19	17	0.19	17.86
High (cm)	Supplement	Pre- post test	15	186	164	1.62	174.2
	Placebo	Pre- post test	15	189	170	1.57	179.6
Weight (kg)	Supplement	Pre- post test	15	85	65	2.13	74.6
	Placebo	Pre- post test	15	88	69	1.62	77.53
BMI(Kg/m^2)	Supplement	Pre- post test	15	33.91	22.86	0.67	24.59
	Placebo	Pre- post test	15	25.36	22.47	0.72	23.99

The results of repeated measures analysis of variance showed that time significantly affects changes in aerobic capacity (P=0.005, F=9.38, Eta: 0.25). However, the interaction of time and group in aerobic capacity changes is insignificant (P=0.71, F=0.13, Eta:0.005). Further, the Bonferroni post hoc test results showed that the aerobic capacity of the groups during the post-test was significantly higher than the pre-test (P=0.005) (Figure 3).

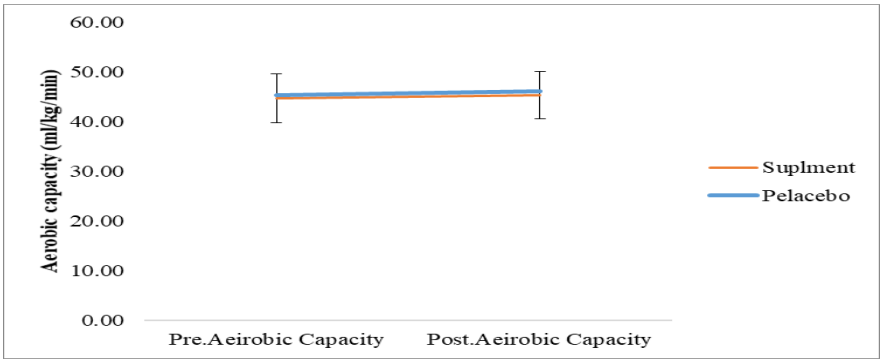

Figure 3. Aerobic capacity changes in each group according to pre-post-test time, there is no significant difference between any of the groups (P=0.07).

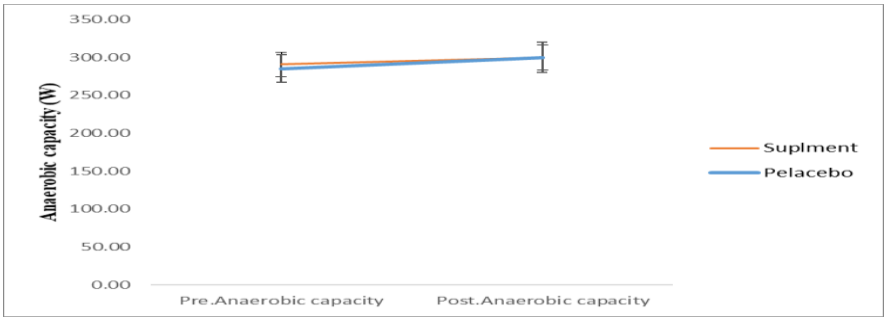

Figure 4. Anaerobic capacity changes in each group by pre-post test time, there is no significant difference between any of the groups (P=0.11).

The results showed that the time factor has a significant effect on anaerobic capacity changes (P=0.02, F=6.09, Eta: 0.17), but the interaction of time and group in anaerobic capacity changes was not significant (P=0.53, F=0.39, Eta:0.014). The results indicated an increase in anaerobic capacity in the post-test period compared to the pre-test (P=0.02) (Figure 4).

The results showed that the time factor (P=0.001, F=376.25, Eta: 0.93) and the interaction of time and group (P=0.001, F=73.75, Eta: 0.72) have a significant effect on the changes in the muscle pain. Further, the results showed that muscle pain levels in the supplement group were significantly lower than in the placebo group at the same time (P=0.001). The muscle pain during the pre-supplementation test was significantly lower than the 24 hours after resistance training at 48 (P=0.001) and 72 (P=0.001) hours after resistance training. At this stage, at 72 hours, it was significantly lower than at 48 hours (P=0.001). In the post-supplement stage, muscle pain was significantly lower at 48 (P=0.001) and 72 (P=0.001) hours after exercise than 24 hours after exercise. In addition, this was significantly lower at 72 hours after resistance training than 48 hours later (P=0.001). During the post-test, the pain relief supplement at 24 (P=0.001), 48 (P=0.001), and 72 (P=0.03) hours after the activity in the supplement group was significantly lower than the time were similar in the placebo group (Figure 5).

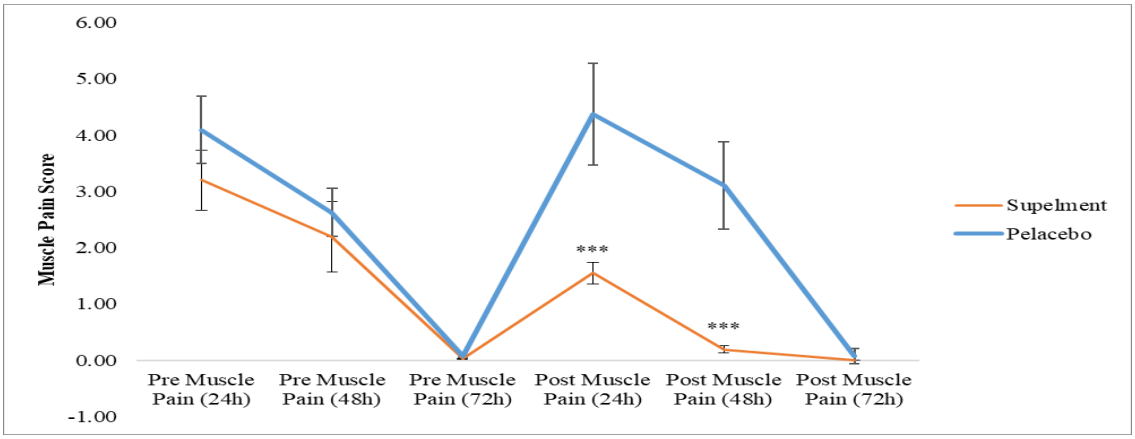

Figure 5. Muscle pain changes due to VAS report
***: Significant decrease in muscle pain in probiotic group compared to placebo group (P=0.001)

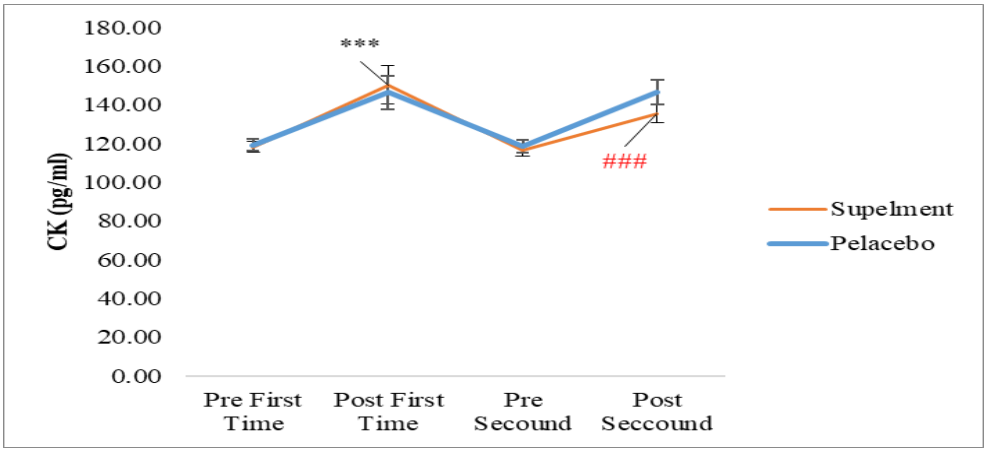

Figure 6. Changes in creatine kinase
***: Significant increase in creatine kinase in the first time in probiotic group compared to the placebo group (P=0.001),
###: Significant decrease in creatine kinase in the secound time in probiotic group compared to the placebo group (P=0.001)

The results showed that the time factor (P=0.001, F=684.28, Eta: 0.96) and the interaction of time and group (P=0.001, F=27.28, Eta: 0.49) significantly affected CK changes. Further, the results showed that the CK levels in the post-test period were significantly higher than in the pre-test in the stage before probiotic supplementation (P=0.001). Also, the CK levels in the post-test were significantly higher than in the pre-test after the probiotic supplementation (P=0.001). When comparing the probiotic supplementation group with the placebo group over the post-probiotic supplementation phase, CK values were significantly lower in the probiotic supplementation group (P=0.001) (Figure 6).

Discussion

This study aimed to review the effect of short-term consumption of probiotic supplements on aerobic and anaerobic capacity and delayed onset muscle soreness of football players. The results of the present study regarding the lack of significant increase in aerobic and anaerobic capacity are inconsistent with previous findings (17). Short-term consumption of probiotic supplements for two weeks led to a slight rise in football players' aerobic and anaerobic capacity, but this increase was not statistically significant. Insufficient consumption duration and the subjects' training status are the reasons for probiotics' lack of significant effect on aerobic and anaerobic capacity. The number of published articles on the use of probiotics in endurance athletes is small and has possible biases or limitations. Only three cases out of nine studies showed a direct relationship between consuming probiotics as an energy aid and improving the performance of endurance athletes, improving training management, and adapting to exercises due to the reduction of muscle damage after exercises (18). However, probiotic supplements have improved aerobic capacity and VO_{2max} (19). In addition, in teenage female swimmers, the intervention with probiotics caused a significant improvement in VO_{2max} (20). The current research findings showed a non-significant increase in football players' aerobic and anaerobic capacity compared to the placebo group. The reason for its lack of significance compared to other background research in this field probably depends on the amount of probiotic supplement consumption and the duration of its consumption, as well as the type of subjects, their field, and exercise history. However, the same non-significant increase in aerobic and non-aerobic capacity in the probiotic supplement group compared to the placebo caused a significant decrease in the fatigue index in the supplement group compared to the placebo. In endurance runners, fatigue was improved in a warm environment with probiotics (21). The findings of Marshall et al. (2017) did not show a significant difference in the VO_{2max} level between a group of athletes who consumed probiotics and placebo (22). The findings of this study were consistent with the findings of the present research. In Jäger et al. (2016), anaerobic performance increased slightly in the studied probiotic group, while it decreased in the placebo group during the Wingate test. It has also been reported that muscle damage decreases after taking probiotics and delays fatigue. In the current research, even though the anaerobic capacity increased insignificantly, the level of fatigue in the probiotic supplement group was significantly reduced. This is probably because the intestinal microbiota plays a more significant role in the rapid regeneration of energy and ATP production (23). The study by Ebrahim et al. (2018) evaluated the effect of probiotic supplementation on anaerobic power, and there was no significant difference between the probiotic group and the placebo group regarding maximum anaerobic power level during the Wingate test (24). The findings of these researchers were also consistent with the current research findings. According to these findings and the current research results, although the consumption of probiotic supplements increases aerobic and anaerobic capacity insignificantly, its positive effects in improving sports performance and reducing fatigue are very significant.

The present study showed that short-term consumption of probiotics significantly reduced muscle pain and serum creatine kinase levels after football activity simulated intense exercise in football players. It has been shown that muscle pain caused by intense activity is caused by increased inflammation and oxidative stress in active skeletal muscles. Since the probiotic supplement group reported a significant reduction in muscle pain compared to the placebo group, the complementary role of probiotics can reduce inflammation and

oxidative stress. Although inflammatory and oxidative factors were not measured in the current research, they can be considered as part of the limitations of the current research. The consumption of probiotics reduced oxidative stress in triathletes (25). Martarelli et al. showed that probiotic supplements increase the level of antioxidants in athletes' blood samples and thus neutralize reactive oxygen species. According to the previous findings, high-intensity exercise causes oxidative stress, and probiotic supplementation increases plasma antioxidant levels, thereby neutralizing reactive oxygen species (26). The activity of malondialdehyde as an indicator of oxidative stress creates a disturbance and will protect the human body from cell damage. Probiotic supplements prevent lipid peroxidation and act as a biological marker to improve oxidative stress. Probiotics have significant effects on reducing the amount of malondialdehyde in the body (27). Ejtahed *et al.* (2012) reported a significant decrease in malondialdehyde following probiotic supplementation (28). Moreover, Vaile *et al.* (2008) showed that lactic acid decreases significantly following the consumption of probiotics. They stated that the accumulation of lactic acid during intense exercise causes muscle cramps and pain. The reduction in lactic acid that occurs after consuming probiotic supplements means, however, that lactic acid is being transported to the process of fuel production and ATP production, which reduces the accumulation of this acid in the muscle, accelerating recovery and reducing muscle pain (29). In sedentary people who participated in endurance exercise, probiotic supplementation improved blood lactate clearance during one hour post-exercise recovery (30). The lactic acid concentration, however, has not been investigated in the present study and, therefore, cannot be cited with certainty, only based on probabilities. Therefore, it can be considered as one of the limitations of the current research. The effects of probiotic supplementation on performance capacity during the post-exercise recovery period in triathlon athletes were studied by (6). This study showed that consuming probiotic supplements for three weeks improved the anaerobic and aerobic capacities in 48 and 72 hours and improved the fatigue index during the recovery period after the triathlon compared to the placebo group (30). In the athletes who participated in the full triathlon championship, the probiotic and placebo groups did not significantly differ in blood creatine kinase levels immediately after the competition. However, in the probiotic group, the creatine kinase levels were significantly lower three hours after exercise compared to the placebo group (30). Probiotic supplementation for two weeks improved perceived recovery status scores 24 and 72 hours after muscle-damaging exercise (31). Probiotics plus casein also reduced perceived muscle soreness 72 hours after exercise compared to casein alone. A tendency to decrease circulating creatine kinase levels and improve performance (as measured by the Wingate test) was observed after muscle-damaging exercise following probiotics and casein supplementation compared to casein supplementation alone (32). High-intensity activity increases ATP consumption from anaerobic and aerobic pathways, and creatine kinase is a key enzyme in the production of ATP from the anaerobic pathway. The reason for the decrease in creatine kinase in the probiotic supplement group compared to the placebo in the recovery period was interpreted this way. The effects of the probiotic absorbed creatine kinase from the blood and transferred it into the muscles, which led to a reduction in creatine kinase concentrations compared to placebos. Thus, the probiotic supplement causes an increase in the acceleration of recovery and regeneration of intra-muscular ATP. Therefore, this type of supplement plays a role in reducing inflammation and oxidative stress. As a result, muscle pain is reduced by high-intensity exercise, energy regeneration, and ATP regeneration of active skeletal muscles, directly and indirectly. The present findings are consistent with some of the previous research results mentioned. The short-term consumption of probiotic supplements is probably for two weeks, significantly increasing aerobic and anaerobic capacity in young football players and can cause delayed onset muscle soreness. The lack of significant improvement in aerobic and anaerobic capacity may be due to the insufficient duration of probiotic consumption or the training status of the subjects. The subjects of the present study were trained football players in favorable conditions regarding their training status.

Conclusion

These people's initial aerobic and anaerobic capacity seemed such that further improvement was impossible in just two weeks. In this regard, additional studies should be done in future research. On the contrary, it seems that the short-term consumption of a probiotic supplement for two weeks reduces delayed-onset muscle soreness after intense exercises in young football players, probably due to the antioxidant and anti-inflammatory properties of the probiotic supplement.

Declarations

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Authors Contributions

In this manuscript, M, E has been responsible for the research implementation and financial support. Also, M, Z and H N, M have been responsible for supervising the implementation of the research. H N, M and S, O R were responsible for data analysis. In addition, S, O R has been responsible for the translation and editing of the manuscript.

Ethical Considerations

All the ethical principles were conducted under the supervision of the Ethics Committee in Biomedical Research of Islamic Azad University, Morvdasht Branch, with the approved code IR.IAU.M.REC.1402.083.

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Conflict of Interest

The authors declare no conflict of interest.

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