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Pre-sleep casein ingestion with probiotic strains improves anaerobic power and lower-body-specific strength and power performance in soccer players

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ABSTRACT

Background: Probiotics comprise various strains that offer numerous health benefits. Researchers have recently focused on the relationship between probiotic consumption and improved gut microbiota function, enhanced digestion, increased nutrient absorption, and enhanced sports performance. Therefore, the present study investigated the effects of pre-sleep casein intake, coupled with probiotic strains, on soccer players' anaerobic power, lower-body-specific strength, and power performance.
Methods: A randomized, double-blinded, and placebo-controlled study was conducted with forty-four male soccer players (Age: 22.81 ± 2.76 years, Height: 177.90 ± 6.75 cm, Weight: 67.42 ± 8.44 kg). The participants underwent the isokinetic strength, Wall-squat, and running-based anaerobic sprint (RAST) tests initially; then, they were randomly divided into four groups: probiotics (PRO), casein (CAS), probiotics with casein (PRO+CAS), and placebo (PLA). The PRO groups were given one probiotic capsule (containing eight bacterial strains: *Lactiplantibacillus plantarum* BP06, *Lacticaseibacillus casei* BP07, *Lactobacillus acidophilus* BA05, *Lactobacillus bulgaricus* BD08, Bifidobacterium infantis BI04, *Bifidobacterium longum* BL03, *Bifidobacterium breve* BB02, and *Streptococcus thermophilus* BT01, with a total dose of 4.5×10^{11} CFU) during dinner, while the CAS groups consumed 20 grams of casein powder 45 minutes before bed. The PRO+CAS group was given one probiotic capsule during dinner and 20 grams of casein powder 45 minutes before bed. The participants in the PLA group were given one red capsule (containing 5 grams of starch) during dinner. All participants were instructed to take the supplements only on training days, three times a week for four weeks. Additionally, isokinetic strength parameters, including absolute peak torque (APT) and average rate of force development (AvRFD), were measured for the knee extensors (ext) and flexors (flx) muscles (concentric phase at angular velocities of 60°/s and 180°/s, using the dominant leg).

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One-way analysis of covariance (ANCOVA) or Quade tests with a significance level of $p < 0.05$ was used to analyze the collected data.
Result: The current study's findings indicated that APT-180°/s (ext) significantly increased in CAS ($p = 0.008$) and PRO+CAS ($p = 0.003$) compared to PLA. Additionally, the AvRFD-180°/s (ext) increased significantly in the PRO compared to the PLA ($p = 0.007$). Also, the AvRFD-60°/s (flx) increased significantly in the PRO+CAS group compared to the PLA ($p = 0.014$), CAS ($p = 0.001$), and PRO ($p = 0.007$). Furthermore, the AvRFD-180°/s (flx) increased significantly in the PRO+CAS compared to the CAS ($p = 0.010$). Moreover, the RAST average power increased dramatically in PRO+CAS compared to PLA ($p = 0.003$) and CAS ($p = 0.02$). Additionally, the Wall-squat test demonstrated a significant increase in PRO+CAS compared to PLA ($p = 0.001$) and PRO ($p = 0.001$). However, there were no significant differences in the APT-60°/s (ext&flx), APT-180°/s (flx), and AvRFD-60°/s (ext) between groups ($p > 0.05$).
Conclusion: The simultaneous consumption of casein and probiotics significantly improved anaerobic power, isokinetic strength, and lower-body muscular endurance in male soccer players. These enhancements were more pronounced than those observed with casein or probiotics alone, as supported by statistical significance and effect sizes. The findings suggest a synergistic benefit of combined supplementation for athletic performance.

1. Introduction

Proper performance in soccer depends on countless factors, including mental, technical/biomechanical, physiological, and tactical areas [1]. For a match with 90 minutes, professional players run about 10 km with an average intensity close to the anaerobic threshold [1]. During this time, they engage in many explosive activities, including jumping, kicking, tackling, turning, sprinting, changing pace, and sustaining powerful contractions. These activities help them maintain balance and control of the ball against defensive pressure [1]. The anaerobic power and the maximal strength of the neuromuscular system, specifically the lower limbs, are responsible for these efforts [2]. Increasing the available force of muscle contraction in the corresponding muscles or muscle groups can improve acceleration and speed in soccer-related skills such as turning, sprinting, jumping, and change of pace [2]. The soccer game involves not only skill but also muscle strength, which helps reduce the risk of injury [3]. As a result, anaerobic and aerobic capacity and muscular function are vital factors for carrying out individual skills, although they stick to team tactics during the match as well [4].

Nevertheless, there is evidence that intense exercise, especially during competitive phases (more intense training periods), can cause upper respiratory and gastrointestinal tract infections and disrupt the homeostasis of the gut microbiota, affecting the athlete's performance and health [5,6]. There is a direct relationship and coordination between the gut microbiota and the host's metabolism in the form of energy utilization and storage [6]. Therefore, maintaining the health of the gastrointestinal tract is particularly important in regulating adaptation to the symptoms of sports and physical activity, especially during long, strenuous competitions. In this context, probiotics (PRO) can be highlighted among the nutritional supplements used in the modulation process of the immune response of athletes [6].

Nowadays, PRO supplements have attracted the attention of the sports community to improve factors such as health, training, and athletic performance [7]. PRO are living microorganisms in the intestines of humans consisting of bacteria, especially lactic acid [7], and are commercially available in the form of capsules or tablets, or powder sachets, in liquid form, and in certain foods such as yogurt [6]. PRO can, directly and indirectly, enhance athletic performance by maintaining gut health, increasing energy availability, improving the structure of the gut microbiota, and enhancing the absorption process of nutrients such as amino acids from proteins [6]. There are different strains of PRO, each of which can influence different physiological parameters. For instance, *L. plantarum* K×041can hold intestinal permeability and enhance antioxidant capacity [6,8]. Besides, some specific strains of *L. plantarum* switch on the signaling pathways, which help cell growth in the intestinal enterocytes. This will increase protein metabolism in the gut [6,9]. Also, *B. coagulans* GBI-30, 6086 improves the health of intestinal mucosal cells by enhancing the absorption of nutrients, including minerals, peptides, and amino acids. This process takes place by reducing inflammation and promoting optimal development of the villus absorption zone. [6,10]. For this reason, some studies suggest that using multi-strain PRO may have more performance measure advantages than single-strain PRO [6].

In addition to PRO, protein intake positively correlates with gut microbiota composition and diversity and is associated with a more favorable inflammatory and metabolic profile [11,12]. Also, due to the nature of soccer and the importance of energy intake and macronutrients, consuming sufficient protein can be necessary [13]. However, soccer players often have a low protein intake, and their diet is not much different from the general diet [13]. One of the most effective strategies to compensate for the lack of protein intake is to take dietary protein supplements after exercise [13]. Common dietary protein supplements include whey protein (WP) and casein (CAS). CAS is often recommended for active individuals as the type of protein to take before bedtime [14]. CAS is insoluble in water and coagulates; this results in the amino acid release being much slower than WP and remaining in the body longer [15,16]. Resulting in a moderate and steady increase in plasma amino acid concentrations [17]. Since CAS prolongs nocturnal hyper aminoacidemia and provides precursors for nocturnal protein metabolism, it appears that CAS is an ideal protein to consume before sleep [18]. In a JISSN position stand and Carbuhn et al. study [6,19] demonstrated that damaging exercise hugely increases muscle soreness and decreases perceived recovery. However, PRO supplementation (*Bacillus coagulans*) at a dose of 1 billion CFU with 20 g CAS significantly improved recovery 24 and 72 hours after exercise and decreased muscle soreness 72 hours after exercise. Moreover, this supplement can help maintain anaerobic performance. The study by Jager et al. [20] proves that PRO supplements combined with CAS reduce muscle damage indicators, improve recovery, and maintain physical performance after intense and damaging exercise. Additionally, the outcomes of the Huang et al. [21] study depicted that the consumption of PRO supplements for four weeks increased plasma branched-chain amino acids significantly (24–69%); in addition, the consumption of PRO significantly reduced oxidative stress and caused a notable increase in the anaerobic performance of triathletes in the Wingate test. Nevertheless, the results of more recent studies are contradictory. For instance, Carbuhn et al. [19] showed no difference between the PRO (*Bifidobacterium longum*) group with a dose of 1 billion CFU and PLA in female swimmers' aerobic and anaerobic performance. Salleh et al. [22] also conducted a study with 30 young badminton players who were given PRO single strain (*Lactobacillus casei*) as a liquid drink at a 3×10^{10} CFU dose for six weeks. Although the intake of PRO improved aerobic capacity, however, it did not influence speed, power, strength, and agility. In another study by Toohey et al. [23] with 23 female athletes, Bacillus subtilis-based PRO supplementation did not affect 1RM strength (chest press, deadlift, and squat) and vertical jump height.

In summary, the studies conducted so far have looked into the impact of PRO (with or without casein) on the performance of athletes other than soccer players, who have different movement and biomechanical patterns from soccer players. Additionally, most studies have utilized only a single strain of PRO. However, consuming multi-strain PRO with CAS positively affects protein digestion and absorption. This, in turn, accelerates recovery and muscle protein synthesis (MPS) and improves the function of the gut-skeletal muscle axis while providing health benefits and balancing intestinal microbial homeostasis. It is likely to enhance the athletic performance of soccer players. Due to the conflicting results of previous studies and the limited study on the effects of simultaneous consumption of PRO with CAS on athletic performance, especially on soccer players, the present study aimed to investigate the impact of pre-sleep casein intake, combined with probiotic strains, on soccer players' anaerobic power, lower-body-specific strength, and power performance.

2. Methodology

2.1. Participants

In the first step, forty-four young male soccer players with approximately three years of experience playing in the First Division League of Iran participated in the current study. Table 1 reveals the demographic information of the participants. The study procedures were explained to the participants in the second step, and their written consent and Physical Activity Readiness Questionnaire (PAR-Q) forms were filled out. They had no history of disease or known medical problems, no history of allergy to PRO and CAS, and were not taking any performance-enhancing drugs. Furthermore, the participants did not smoke or consume alcohol or caffeinated beverages 24 hours before data collection. This study was reviewed and approved by the Research Ethics Committees of the Faculty of Psychology and Educational Sciences, Shiraz University, Shiraz, Iran (ethics approval code: IR.US.PSYEDU.REC.1402.034, Approval Date: 24 May 2023) and carried out by the Declaration of Helsinki.

Table 1. Demographic information of the participants.

Variable	Mean ± SD ($n = 44$)
Age (years)	22.81 ± 2.76
Height (cm)	177.90 ± 6.75
Weight (kg)	67.42 ± 8.44
Body Mass Index (kg/m^2)	21.27 ± 2.09

2.2. Sample size calculation and study design

The sample size was calculated using the G*Power analysis software [24], considering a 5% Type I error rate and 0.80 statistical power. Drawing from data from a previous study on probiotics supplementation [25]. It was estimated that a sample of 10 to 12 participants per group was necessary to identify statistically significant trial effects. Therefore, 44 participants were selected for the current study, with 11 participants in each group.

The current study was conducted in a randomized, double-blind, placebo-controlled format (as shown in Figure 1). Initially, the participants were asked to attend a session where a general practitioner would check their overall health. The participants were also asked to complete a written consent form, which included a detailed description of the implementation method, benefits, risks, and potential side effects. Additionally, they were asked to complete a PAR-Q form and instructed to maintain their habitual diet throughout the study, including on non-training days, to minimize dietary variability. During the pretest phase, all subjects completed a food registration questionnaire to screen for the use of probiotics or supplements that could potentially affect gut microbiota or performance. They were advised to avoid significant dietary changes and refrain from using additional ergogenic or probiotic supplements during the intervention period [26].

Additionally, during this session, the subjects underwent a familiarization session with the equipment, particularly the Biodex device, and were introduced to the isokinetic strength test. Afterward, the participants underwent a pretest, including Wall-squat test, isokinetic of knee extensor/flexor muscles, and the Running-based Anaerobic Sprint Test (RAST). Participants were instructed to avoid consuming caffeinated sources and perform vigorous exercise at least 24 hours before the experiment to control for any acute and side effects on the measured variables. After administering the pretests and measuring the variables, the participants were randomly divided into four groups: PRO ($n = 11$), CAS ($n = 11$), PRO+CAS ($n = 11$), and PLA ($n = 11$). Each group received their supplement and was taught how to maintain and consume it. All participants were fed the same breakfast containing 250 kcal (45 g carbohydrates, 9 g protein, and 5 g fat) one hour and thirty minutes before the exercise test session [27]. The posttest was completed for each participant precisely four weeks after the pretest at the same time of day (8:13:00) with approximately the same environmental conditions (17–21°C). During the trials, participants were allowed to drink water at their discretion. All these steps are shown in Figures 1 and 2.

2.3. Wall-squat test

The wall-squat test was used to evaluate lower-body muscle endurance. The correct posture was sitting, shoulder width straight and attached to the wall, knees at 90 degrees, shoulders to the wall, and arms hanging straight down. For this test, the maximum time to exhaustion was defined as the time interval from the task's start until any of these positions could not be maintained. Participants must do their best to keep the correct position throughout the test while receiving no verbal encouragement [28–30]. The Wall Squat Test was chosen as a practical and reliable method to assess lower-body isometric strength and endurance, targeting critical muscle groups such as the quadriceps, hamstrings, and gluteal muscles. These muscle groups are involved in soccer-specific activities, such as maintaining balance during defensive stances, shielding the ball, and stabilizing

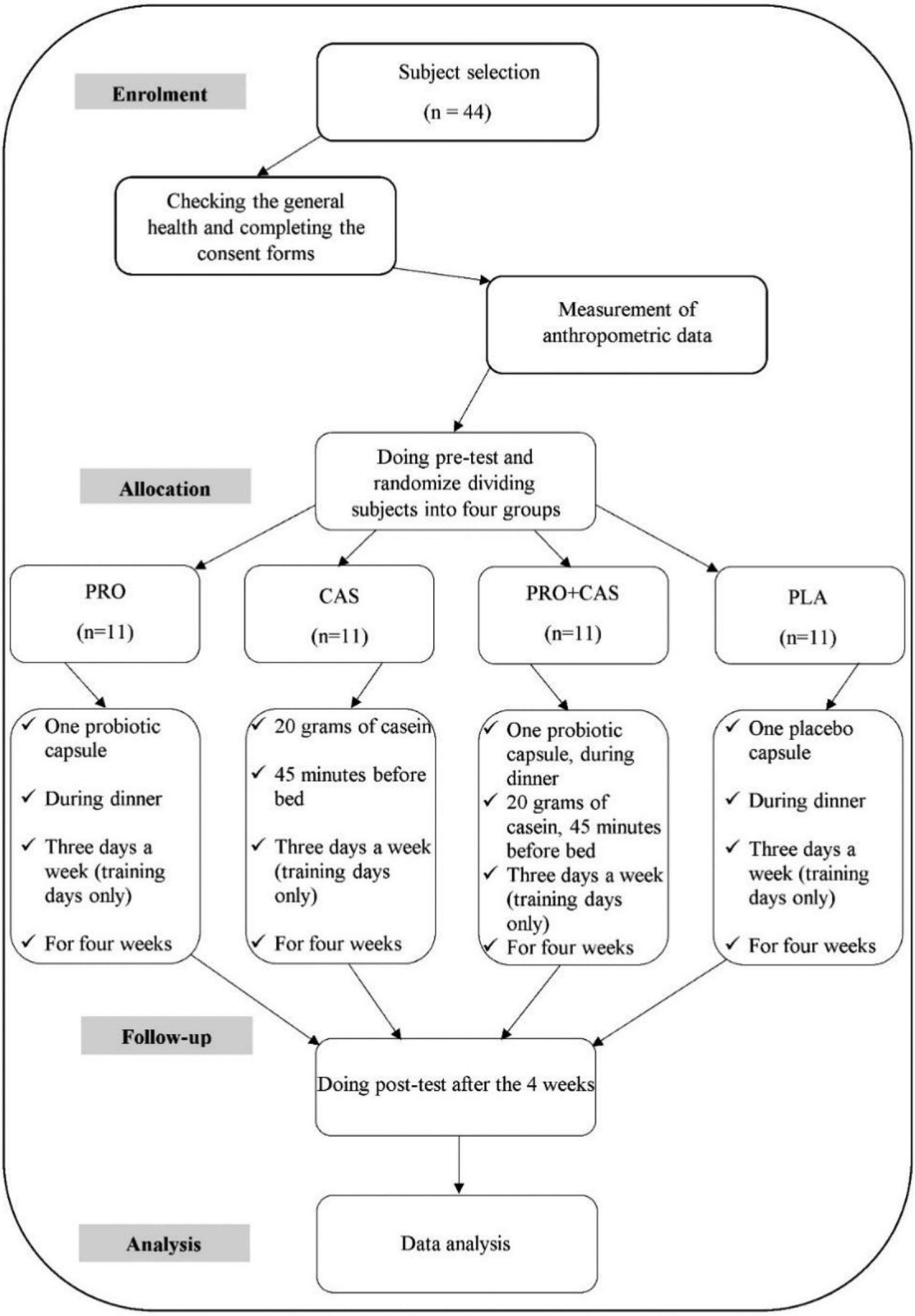

Figure 1. Flowchart illustrating the different phases of the study selection.

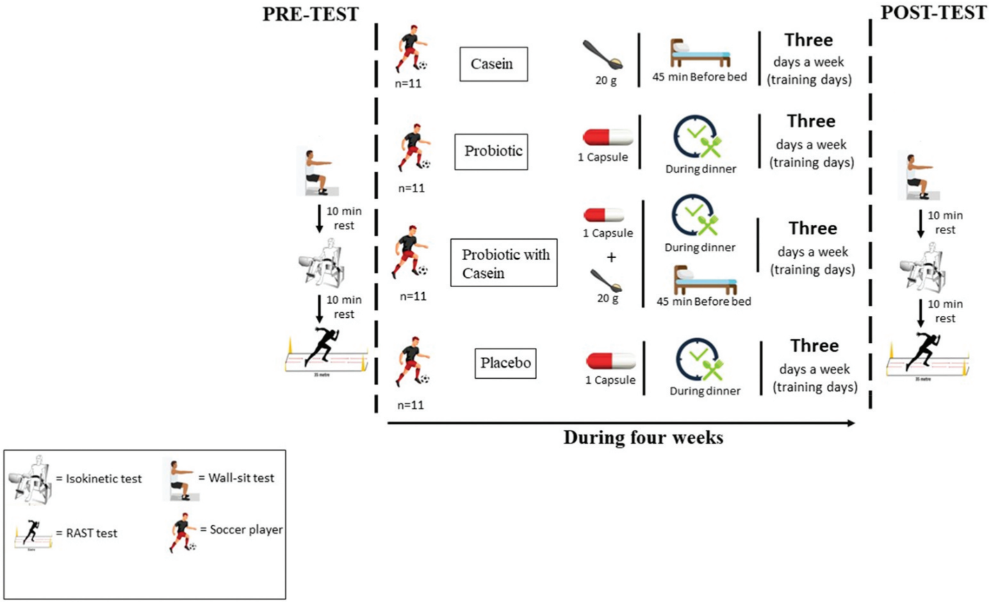

Figure 2. The protocol of taking supplements and performing tests.

the body during sprints and sudden directional changes [31,32]. The test is particularly suitable for measuring the muscular endurance required for prolonged isometric contractions, which is crucial for maintaining stability during gameplay. Given the study's logistical constraints, the Wall Squat Test was selected for its simplicity, accessibility, and ability to provide meaningful insights over the four-week intervention period. Standardized testing conditions and familiarization sessions were employed to ensure consistency and minimize participant variability.

2.4. Isokinetic strength tests

The Biodex isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, Inc., Shirley, New York, USA) measured isokinetic strength parameters. For this purpose, concentric isokinetic strength of knee extensors (ext) and flexors (flx) (dominant leg) was evaluated at 60°/s and 180°/with five consecutive repetitions in the direction of extension-flexion, 60 seconds of rest between each set was considered for recovery. The rotation axis of the dynamometer was fixed with the lateral epicondyle of the femur and the lever arm at 5 cm above the lateral malleolus. The total range of motion allowed for knee flexion-extension during the test was 85°, starting from 90° to 5° (0 = full extension). Finally, gravity's influence was corrected with the limb at 30° of flexion. All these procedures followed the equipment manufacturer's recommendations. Eventually, absolute peak torque (APT), relative peak torque (RPT), average peak torque (AvPT), time to peak torque (TPT), and average rate of force development (AvRFD) were measured (AvRFD was calculated using the APT/TPT equation) [33,34]. The selected velocities were identified to facilitate an evaluation of the following parameters: 60°/s: This lower velocity assesses maximal strength under controlled conditions, offering valuable insights into knee extensors' and flexors' peak torque production capabilities. Such measurements are critical for ensuring stabilization during soccer-specific activities. 180°/s: This increased velocity evaluates dynamic muscle performance and reflects the functional capacity for executing rapid movements, including sprinting and sudden directional changes encountered during soccer matches [2,3,33].

2.5. RAST test

The RAST test measured the anaerobic power of the participants [35]; the test was conducted in such a way that the participants ran a distance of 35 meters with maximum speed and power in 6 repetitions, rested for 10 seconds between each repetition, then according to the time obtained from each repetition using a stopwatch (Q&Q, Q-1model, Japan CBM corp), anaerobic maximum (RAST max power), average (RAST avg power) and minimum powers (RAST min power) (Power = Weight × Distance^2 ÷ Time ^{3}).

The tests were performed in the following order: Wall-squat test, isokinetic strength parameters, and RAST. Participants actively rested (walking) between each test for 10 minutes (Figure 2).

2.6. Training protocol

The participants were members of the one training camp (Shiraz – Iran). The same training regime applied to all the participants. The training program was as follows: 5 training sessions of 90 min per week, including 10 min of warm-up, 20 min of physical training (Core stability, Speed, Agility, and Quickness), 10 min of technical training, 20 min of tactical training, 25 min of the training game, and at the end there was cooling down for 5 min. There was also team training in which strength and power training occurred once per week. It consisted of a combination of plyometric (single leg hops, drop jump, box jump, squat jump: 3 sets × 8 repetitions for each) and resistance exercises (3–4 sets, 10–12 repetitions, 75–80% of a maximum repetition). The same conditions in the form of training program's type, intensity, load, and duration were applied to all participants. Also, one day a week was allocated to friendly matches, in which each participant played for approximately 10–15 minutes.

2.7. Supplementation protocol

During dinner, the PRO group was instructed to consume one PRO capsule (Comflor PRO supplement by Farabiotic Company, Iran). The capsule contains strains of *Lactiplantibacillus plantarum* BP06, *Lacticaseibacillus casei* BP07, *Lactobacillus acidophilus* BA05, *Lactobacillus bulgaricus* BD08, *Bifidobacterium infantis* BI04, *Bifidobacterium longum* BL03, *Bifidobacterium breve* BB02, and *Streptococcus salivarius thermophilus* BT01, with a total dose of 4.5 × 1011 CFU (colony-forming units) [36,37]. Table 2 provides more details about the PRO strains. The CAS group was instructed to consume 20 grams of CAS powder (CAS supplement by EuRho Vital Company, Germany) 45 minutes before bedtime. The powder contains 76.8% pure protein per serving [38]. The PRO+CAS group was instructed to consume one PRO capsule during dinner and 20 grams of CAS powder 45 minutes before bedtime. The PLA group participants were instructed to consume one red capsule during dinner. The capsule contained 5 grams of starch and was used to

Table 2. Strains and dosage per one capsule (200 mg) of the probiotics used in the present study.

Strains	Dosage (CFU)
Lactiplantibacillus plantarum BP06	0.43×10^{11}
Lacticaseibacillus casei BP07	0.65×10^{11}
Lactobacillus acidophilus BA05	0.94×10^{11}
Lactobacillus bulgaricus BD08	0.36×10^{11}
Bifidobacterium infantis BI04	0.57×10^{11}
Bifidobacterium longum BL03	0.73×10^{11}
Bifidobacterium breve BB02	0.62×10^{11}
Streptococcus salivarius thermophilus BT01	0.20×10^{11}
Total	4.5×10^{11}

create a similarity with the red capsules of the Comflor PRO supplement. All participants were instructed to take the supplements only on training days, three times a week (Sundays, Tuesdays, and Thursdays), for four weeks.

2.8. Statistical analysis

The data were analyzed using IBM SPSS software version 28.0 (IBM Corp, Armonk, NY, USA) with descriptive and inferential statistical methods. The results were presented with mean ± standard deviation (SD). The one-way analysis of covariance (ANCOVA) was used to detect differences between the four groups over time, with the baseline values considered. The Quade nonparametric statistical test was used when ANCOVA assumptions were not met. The Bonferroni's post hoc test was then used to find pairwise differences. The normality of the data was checked using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked using Levene's test. The significance level for all hypothesis tests was set at $p < 0.05$.

3. Results

All performance variables before and after four weeks of consuming supplements or placebo are shown in Table 3.

3.1. Knee extensors isokinetic parameters

The results of the ANCOVA statistical test showed no significant difference in the APT-60°/s between groups [F = 0.735, $p = 0.538$, pEta2 = 0.053]. However, there was a significant difference in the APT-180°/s between the groups [F = 4.303, $p = 0.010$, pEta2 = 0.249]. On further analysis, the post hoc test revealed a significant increase in PRO+CAS compared to PLA (MD = 15.88, $p = 0.003$, 95% CI [5.75–26.01]-). Similarly, CAS compared to PLA also showed a significant increase (MD = 13.94, $p = 0.008$, 95% CI [3.78–24.10]). Nonetheless, there was no significant difference between PRO+CAS and PRO (MD = 9.94, $p = 0.053$, 95% CI [−0.12–20]), PRO+CAS and CAS (MD = 1.94, $p = 0.70$, 95% CI [−8.13–12]), CAS and PRO (MD = 8, $p = 0.11$, 95% CI [−2.06–18.07]), and PRO and PLA (MD = 5.94, $p = 0.24$, 95% CI [−4.19–16.07]).

According to the ANCOVA statistical test, there was no significant difference in the RPT-60°/s between the groups [F = 0.43, $p = 0.72$, pEta2 = 0.035]. However, there was

Table 3. Mean and standard deviation of the measured variables.

	variables		PRO ($n = 11$)	CAS ($n = 11$)	PRO+CAS ($n = 11$)	PLA ($n = 11$)	p-Value
Extensors	**APT-60°/s**	**Pre-test**	179.45 ± 36.94	203.3 ± 26.37	204.51 ± 41.93	193.6 ± 39	0.538
	(N-m)	**Post-test**	188.25 ± 20.24	208.4 ± 31.82	221.6 ± 49.74	201.69 ± 31.1	
	APT-180°/s	**Pre-test**	129.94 ± 26.68	128.83 ± 6.22	130.29 ± 28.26	136.17 ± 11.13	0.010
	(N-m)	**Post-test**	134.6 ± 25.28	141.56 ± 15.91	144.87 ± 30.18	134.5 ± 14.24	
	RPT-60°/s	**Pre-test**	267.9 ± 22.88	298.77 ± 60.81	307.76 ± 40.1	281.46 ± 46.36	0.72
	(%)	**Post-test**	294.67 ± 49.85	321.05 ± 43.37	316.18 ± 41.8	295.13 ± 40.3	
	RPT-180°/s	**Pre-test**	192.39 ± 25.82	201.82 ± 40.8	195.78 ± 29.3	197.14 ± 22.17	0.016
	(%)	**Post-test**	195.62 ± 31	215.54 ± 26.41	216.26 ± 22.91	189.83 ± 37	
	AvPT-60°/s	**Pre-test**	178.41 ± 20.62	187.74 ± 28.53	190.53 ± 41.61	171.10 ± 32.49	0.003
	(N-m)	**Post-test**	188.84 ± 11.68	208 ± 30	222.60 ± 36.16	178.40 ± 31.55	
	AvPT-180°/s	**Pre-test**	116.20 ± 32.32	118.36 ± 6.26	127.20 ± 24.4	120.07 ± 17.51	0.001
	(N-m)	**Post-test**	124.03 ± 27.62	137.65 ± 12.45	146.69 ± 25.8	125.91 ± 14.21	
	TPT-60°/s	**Pre-test**	395.4 ± 148.6	319.9 ± 65.8	334.3 ± 32.5	388.1 ± 139.1	0.158
	(ms)	**Post-test**	356.6 ± 95	324 ± 63.4	324.4 ± 65.1	337.7 ± 64.1	
	TPT-180°/s	**Pre-test**	161.8 ± 47.5	153.8 ± 29.1	158.1 ± 27.8	165.4 ± 40.3	0.043
	(ms)	**Post-test**	153.8 ± 43.3	180.6 ± 13.8	172.6 ± 21.3	183.6 ± 30.3	
	AvRFD-60°/s	**Pre-test**	499 ± 189.3	634.4 ± 166.6	606.1 ± 171.8	524.9 ± 95.5	0.88
	(N/s)	**Post-test**	626.9 ± 223.1	665.3 ± 95.6	661.8 ± 104	636.8 ± 98.1	
	AvRFD-180°/s	**Pre-test**	835.8 ± 386	818.5 ± 178.1	834.6 ± 71.6	823.2 ± 339.3	0.046
	(N/s)	**Post-test**	1014.4 ± 387.8	781.9 ± 110.3	824.4 ± 150.9	732.6 ± 28.9	

(*Continued*)

Table 3. (Continued).

	variables		PRO ($n = 11$)	CAS ($n = 11$)	PRO+CAS ($n = 11$)	PLA ($n = 11$)	p-Value
Flexors	**APT-60°/s**	**Pre-test**	121.5 ± 13.24	113.15 ± 21.28	120.5 ± 29.18	120.95 ± 23.11	0.379
	(N-m)	**Post-test**	135.67 ± 17.19	124.9 ± 19.57	139.8 ± 25.64	134.44 ± 12.76	
	APT-180°/s	**Pre-test**	103.73 ± 17.31	91.42 ± 16	93.96 ± 5.95	89.6 ± 27.34	0.956
	(N-m)	**Post-test**	107.31 ± 18.59	101 ± 9	104.98 ± 15.45	101.79 ± 12	
	RPT-60°/s	**Pre-test**	184.66 ± 19.95	167.67 ± 46.72	181 ± 32.92	166.96 ± 45.95	0.735
	(%)	**Post-test**	197.69 ± 22.24	199.62 ± 13.55	201.68 ± 7.35	191.4 ± 29.5	
	RPT-180°/s	**Pre-test**	152 ± 19.58	138.28 ± 32.24	143.77 ± 18	131.4 ± 41	0.297
	(%)	**Post-test**	155.98 ± 23.13	149.8 ± 16.78	163.56 ± 22.82	140.3 ± 33.62	
	AvPT-60°/s	**Pre-test**	115.40 ± 23.24	107.30 ± 20.19	110.13 ± 32.46	110.38 ± 27.15	0.053
	(N-m)	**Post-test**	126.93 ± 17.7	118.80 ± 12.27	132.53 ± 25.52	118.04 ± 25.32	
	AvPT-180°/s	**Pre-test**	88.15 ± 27	88 ± 16.32	92.82 ± 4.63	92.53 ± 15.44	0.698
	(N-m)	**Post-test**	97 ± 23.7	99.2 ± 15	103.70 ± 3	98.84 ± 11	
	TPT-60°/s	**Pre-test**	420.9 ± 163.1	434.6 ± 140.7	454.5 ± 127.2	481.7 ± 75.6	0.029
	(ms)	**Post-test**	475.8 ± 54.8	417.7 ± 56.4	391.6 ± 32.9	420 ± 84.4	
	TPT-180°/s	**Pre-test**	232.4 ± 57.9	180.3 ± 39.2	234.5 ± 104.7	288.9 ± 125.5	0.007
	(ms)	**Post-test**	227.3 ± 34.7	203.8 ± 34.7	168.3 ± 38.4	187.4 ± 48.8	
	AvRFD-60°/s	**Pre-test**	306.1 ± 135.4	297.6 ± 171.9	281.5 ± 101.4	240 ± 111.4	0.002
	(N/s)	**Post-test**	334.5 ± 96.5	294.7 ± 18.1	379.9 ± 45.9	323.3 ± 44.9	
	AvRFD-180°/s (N/s)	**Pre-test**	420.3 ± 241.8	376.6 ± 199.1	470.3 ± 221.5	394.4 ± 262.9	0.010
		Post-test	487 ± 122.5	431.9 ± 111.5	689.63 ± 209.5	560.1 ± 183.2	
Wall-squat test (sec)		**Pre-test**	144.63 ± 36.9	153 ± 51.9	161.54 ± 39.1	141.54 ± 28.1	0.001
		Post-test	160.54 ± 40.2	185.36 ± 53.7	200.09 ± 33.7	155.18 ± 26.3	
RAST-avg-power (watts)		**Pre-test**	625 ± 62	607 ± 62	575 ± 67	565 ± 28	0.018
		Post-test	665 ± 84	625 ± 54	616 ± 62	586 ± 12	
RAS-max-power (watts)		**Pre-test**	767 ± 107	724 ± 74	723 ± 121	707 ± 61	0.001
		Post-test	791 ± 107	738 ± 72	764 ± 131	707 ± 62	
RAST-min-power (watts)		**Pre-test**	520 ± 52	482 ± 47	486 ± 52	467 ± 15	0.964
		Post-test	538.9 ± 46.2	515 ± 37	513.2 ± 52.5	497 ± 35.9	

APT: absolute peak torque, RPT: relative peak torque, TPT: time to peak torque, AvPT: average peak torque, AvRFD: average rate of force development, RAST: running anaerobic sprint test, avg: average, max: maximum, min: minimum, PRO: probiotics, CAS: casein, PRO+CAS: probiotics plus casein, and PLA: placebo, p-Value: ANCOVA and/or Quade test results, N-m: Newton meter, ms: millisecond, sec: second.

a significant difference in the RPT-180°/s between the groups [F = 3.918, p = 0.016, pEta2 = 0.246]. The post hoc test showed a significant increase in PRO+CAS compared to PLA (MD = 27.48, p = 0.026, 95% CI [2.27–52.69]). Nevertheless, there was no significant difference between PRO+CAS and PRO (MD = 17.54, p = 0.37, 95% CI [−7.86–42.95]), PRO+CAS and CAS (MD = 3.83, p = 0.99, 95% CI [−21.48–29.14]), CAS and PLA (MD = 23.65, p = 0.079, 95% CI [−1.65–48.96]), CAS and PRO (MD = 13.71, p = 0.85, 95% CI [−11.79–39.21]), and PRO and PLA (MD = 9.94, p = 0.99, 95% CI [−15.45–35.34]). The study found a significant difference in the AvPT-60°/s between the groups [F = 5.59, p = 0.003, pEta2 = 0.301]. Post hoc analysis indicated that there was a significant increase in PRO+CAS compared to PLA (MD = 30.51, p = 0.001, 95% CI [14.07–46.96]), PRO (MD = 25.23, p = 0.003, 95% CI [9.03-−41.42]), and CAS compared to PLA (MD = 17.88, p = 0.033, 95% CI [1.54–34.21]). However, there was no significant difference between PRO+CAS and CAS (MD = 12.63, p = 0.119, 95% CI [−3.41–28.68]), CAS and PRO (MD = 12.59, p = 0.122, 95% CI [−3.53–-28.72]), and PRO and PLA (MD = 5.28, p = 0.51, 95% CI [−10.81–21.38]). In addition, the results showed a significant difference in the AvPT-180°/s between the groups [F = 6.277, p = 0.001, pEta2 = 0.326]. The post hoc analysis revealed a significant increase in PRO+CAS compared to PLA (MD = 14.82, p = 0.002, 95% CI [5.98–23.67]) and PRO (MD = 13.48, p = 0.004, 95% CI [4.56–22.41]); also, CAS compared to PLA (MD = 13.16, p = 0.004, 95% CI [4.37–21.94]) and PRO (MD = 11.82, p = 0.01, 95% CI [3.03–20.60]). Regardless, there was no significant difference between PRO+CAS and CAS (MD = 1.66, p = 0.70, 95% CI [−7.21–10.54]), and PRO and PLA (MD = 1.34, p = 0.76, 95% CI [−7.46–10.14]).

The study found no significant difference in the TPT-60°/s between the groups [F = 0.158, p = 0.924, pEta2 = 0.013]. However, the Quade statistical test revealed a significant difference in the TPT-180°/s between groups [F = 2.971, p = 0.043]. Therefore, pairwise comparisons illustrated that TPT-180°/s decreased significantly in the PRO compared to the PLA (t = −2, p = 0.045) and CAS (t = −2.9, p = 0.006). Nevertheless, there was no significant difference between PRO and PRO+CAS (t = −1.7, p = 0.098), PRO+CAS and CAS (t = −1.2, p = 0.236), PRO+CAS and PLA (t = −0.38, p = 0.70), and CAS and PLA (t = 0.8, p = 0.416). ANCOVA statistical test showed no significant difference in the AvRFD-60°/s between groups [F = 0.22, p = 0.88, $pEta^2$ = 0.018]. Furthermore, the results showed a significant difference in AvRFD-180°/s between groups [F = 2.906, p = 0.046]. Further, pairwise comparison showed that AvRFD-180°/s increased significantly in the PRO group compared to the PLA group (t = 2.8, p = 0.007). However, there was no significant difference between PRO and PRO+CAS (t = 0.8, p = 0.40), PRO and CAS (t = 1.6, p = 0.104), PRO+CAS and CAS (t = 0.8, p = 0.422), PRO+CAS and PLA (t = 1.9, p = 0.055), and CAS and PLA (t = 1.16, p = 0.25). Figure 3 presents all of the results mentioned above.

3.2. Knee flexors isokinetic parameters

The study found no significant differences in the APT-60°/s, APT-180°/s, RPT-60°/s, RPT-180°/s, AvPT-60°/s, and AvPT-180°/s between the groups (F = 1.054, p = 0.379, pEta2 = 0.075). However, the results showed a significant difference in the TPT-60°/s between the groups (F = 3.323, p = 0.029, pEta2 = 0.204). The post hoc analysis revealed a significant decrease in PRO+CAS compared to PRO (MD = −78.5, p = 0.023, 95% CI [−149.6- −7.4]). Nevertheless, there was no significant difference between PRO+CAS and CAS (MD =

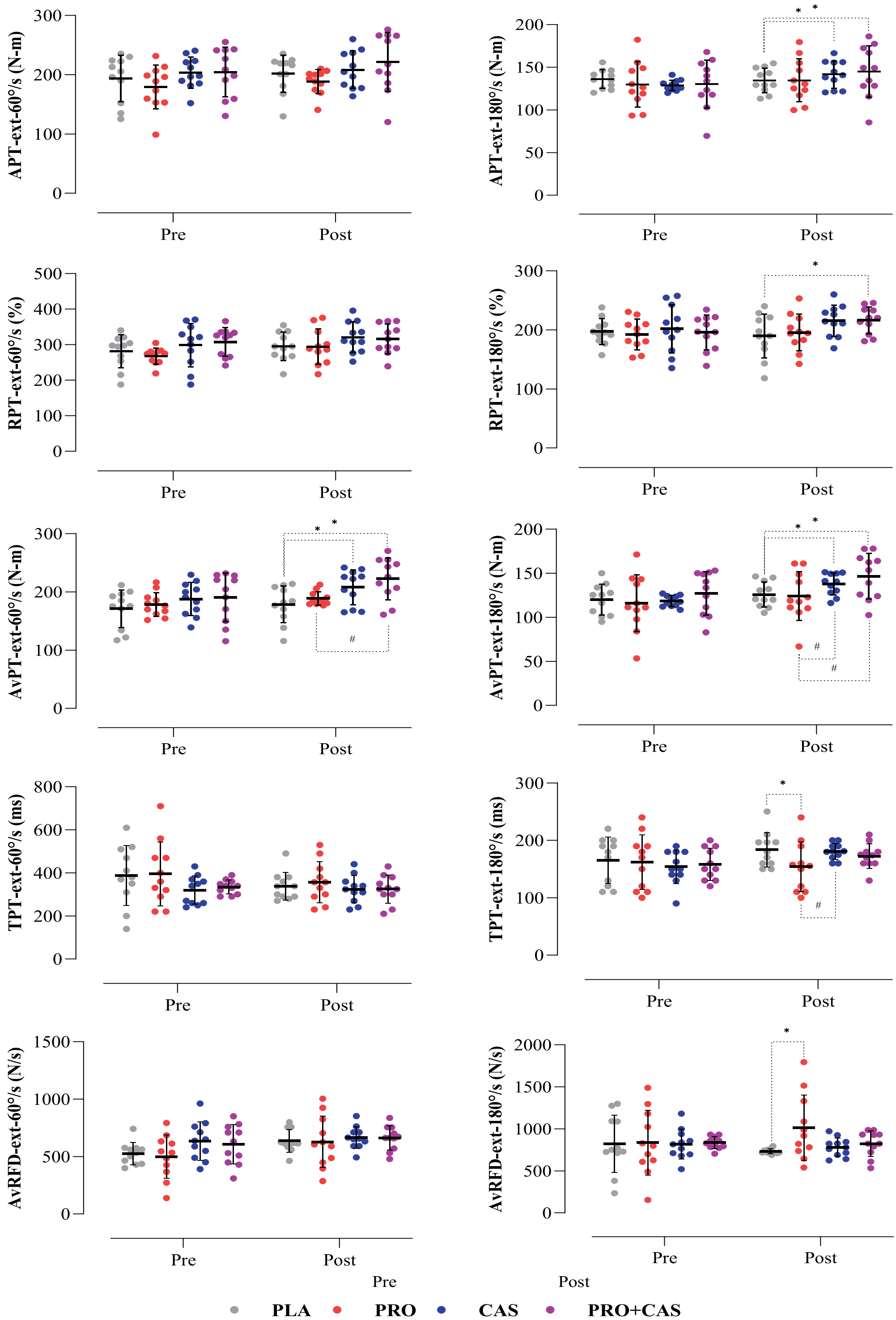

Figure 3. Means and standard deviation (SD) of knee extensors isokinetic parameters in pretest and posttest. *: significant difference compared to the PLA in the posttest. #: significant difference compared to PRO in the posttest. APT: absolute peak torque, RPT: relative peak torque, TPT: time to peak torque, AvPT: average peak torque, AvRFD: average rate of force development.

−25.8, *p* = 0.99, 95% CI [−96.4–44.7]), PRO+CAS and PLA (MD = −23.1, *p* = 0.99, 95% CI [−91.4–45]), CAS and PRO (MD = −52.6, *p* = 0.298, 95% CI [−124.9–19.5]), CAS and PLA (MD = 2.7, *p* = 0.99, 95% CI [−66.7–72.1]), and PRO and PLA (MD = 55.3, *p* = 0.203, 95% CI [−14.6–125.3]). Moreover, the results showed a significant difference in the TPT-180°/s between the groups (F = 4.701, *p* = 0.007, pEta2 = 0.281). On further analysis, the post hoc test revealed a significant decrease in PRO+CAS compared to PRO (MD = −58.8, *p* = 0.001, 95% CI [−99.2- −24.8]), and PRO compared to PLA (MD = −48.4, *p* = 0.009, 95% CI [−84.1- −12.6]). However, there was no significant difference between PRO+CAS and CAS (MD = −35.7, *p* = 0.144, 95% CI [−84.2–12.7]), PRO+CAS and PLA (MD = −10.4, *p* = 0.556, 95% CI [−46.2–25.2]), CAS and PRO (MD = −23.1, *p* = 0.34, 95% CI [−71.6–25.3]), CAS and PLA (MD = 25.2, *p* = 0.31, 95% CI [−24.4–74.9]), and PRO and PLA (MD = 55.3, *p* = 0.203, 95% CI [−14.6–125.3]).

The study results revealed a significant difference in AvRFD-60°/s between the groups [F = 6.052, *p* = 0.002]. Pairwise comparisons demonstrated that AvRFD-60°/s significantly increased in the PRO+CAS compared to the PLA (*t* = 2.578, *p* = 0.014), CAS (*t* = 4.159, *p* = 0.001), and PRO (*t* = 2.838, *p* = 0.007). However, there was no significant difference between PRO and CAS (*t* = 1.321, *p* = 0.194), PRO and PLA (*t* = −0.26, *p* = 0.796), CAS and PLA (*t* = −1.581, *p* = 0.122). Moreover, the study showed a significant difference in AvRFD-180°/s between the groups [F = 4.413, *p* = 0.010, pEta2 = 0.269]. Post hoc tests revealed a significant increase in PRO+CAS compared to CAS (MD = 225.1, *p* = 0.010, 95% CI [40.6- −409.7]). However, there was no significant difference between PRO+CAS and PLA (MD = 92, *p* = 0.99, 95% CI [−91–275]), PRO+CAS and PRO (MD = 170.7, *p* = 0.079, 95% CI [−11.-9.4–353.4]), CAS and PRO (MD = −54.4, *p* = 0.999, 95% CI [−235.9–127.1]), CAS and PLA (MD = −133.1, *p* = 0.290, 95% CI [−315–48.7]), and PRO and PLA (MD = −78.7, *p* = 0.999, 95% CI [−258.7–101.2]). Figure 4 presents all of the results mentioned above.

3.3. Wall-squat test

The study found a significant difference in the Wall-squat test between the groups [F = 12.512, *p* = 0.001, pEta2 = 0.49]. Additionally, the post hoc test revealed that the PRO+CAS group showed a significant increase compared to the PLA (MD = 25.85, *p* = 0.001, 95% CI [15.64–36.05]), and as well as the PRO (MD = 23.43, *p* = 0.001, 95% CI [13.27–33.59]). Furthermore, the CAS group showed a significant increase compared to the PRO (MD = 16.84, *p* = 0.002, 95% CI [6.78–26.91]) and the PLA (MD = 19.26, *p* = 0.001, 95% CI [9.17–-29.35]). However, there was no significant difference between the PRO and PLA (MD = 2.41, *p* = 0.69, 95% CI [−7.62–12.45]), and the PRO+CAS and CAS (MD = 6.58, *p* = 0.19, 95% CI [−3.48–16.65]) (Figure 5).

3.4. RAST test

The study found a significant difference in the RAST-avg-power between groups [F = 3.759, *p* = 0.018]. Pairwise comparisons revealed a significant increase in PRO+CAS compared to PLA (*t* = 3.225, *p* = 0.003) and CAS (*t* = 2.424, *p* = 0.02). However, there was no significant difference between PRO and CAS (*t* = 0.542, *p* = 0.59), PRO and PRO+CAS (*t* = −1.882, *p* = 0.067), PRO and PLA (*t* = 1.343, *p* = 0.187), and CAS and PLA (*t* = 0.80, *p* = 0.428) (refer to Figure 5). Moreover, the study found a significant difference in the RAST-max-

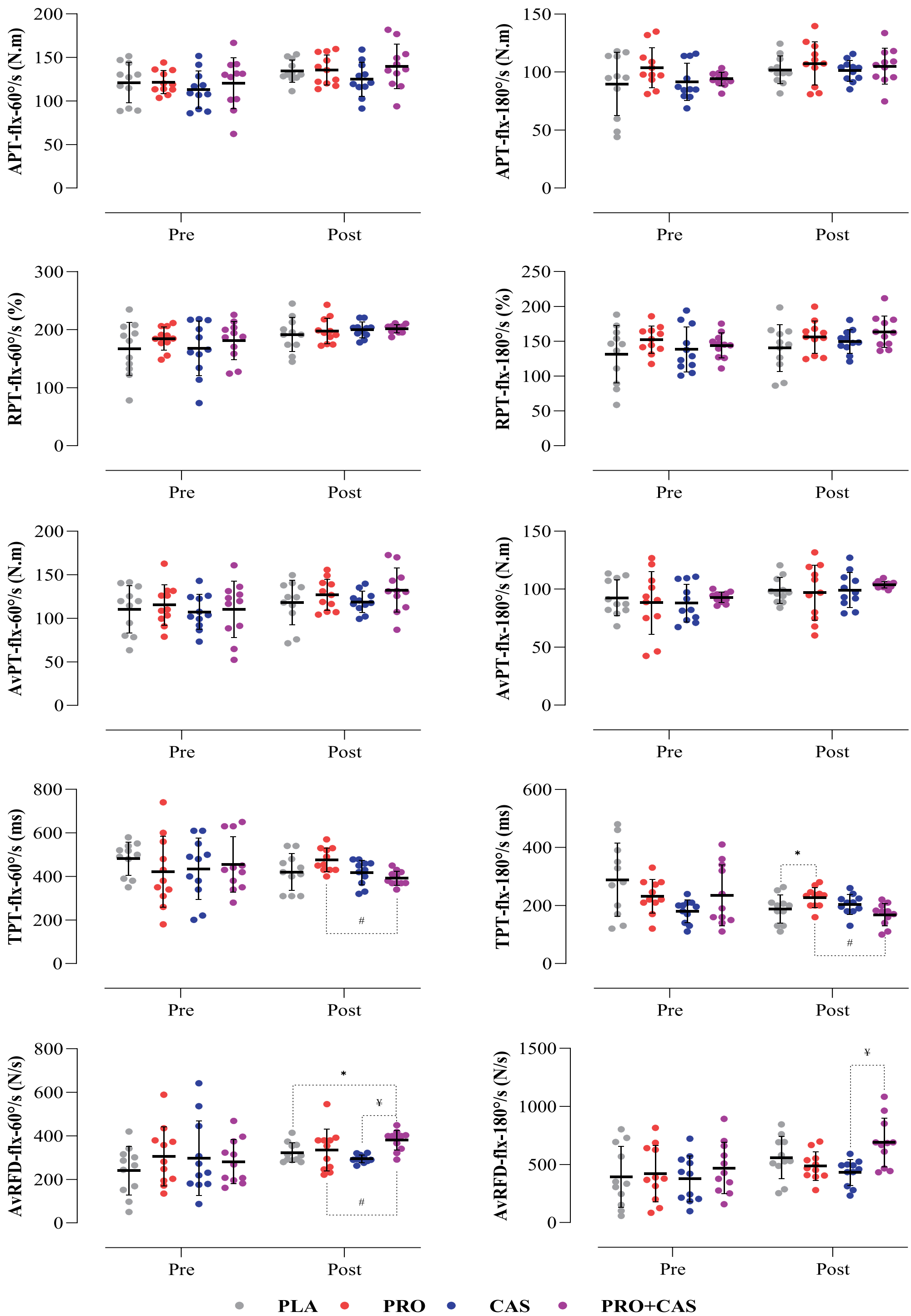

Figure 4. Means and standard deviation (SD) of knee flexor isokinetic parameters in pretest and posttest. *: significant difference compared to the PLA in the posttest. #: significant difference compared to PRO in the posttest. ¥: significant difference compared to CAS in the posttest. APT: absolute peak torque, RPT: relative peak torque, TPT: time to peak torque, AvPT: average peak torque, AvRFD: average rate of force development.

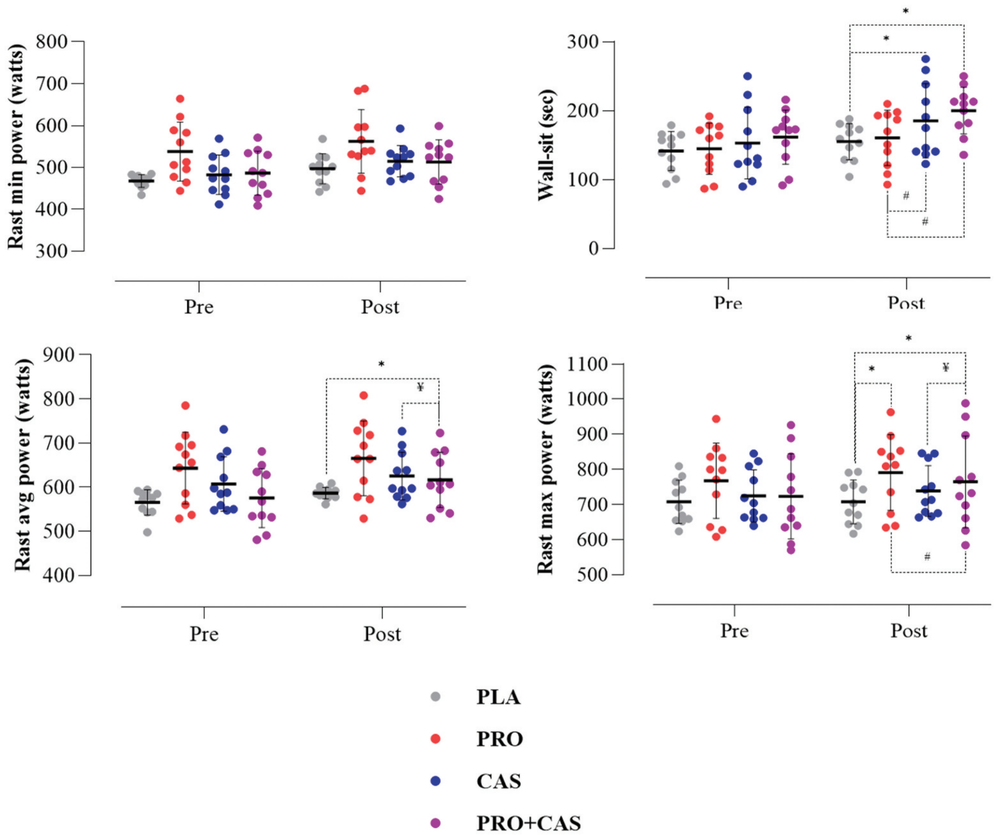

Figure 5. Means and standard deviation (SD) of performance indicators in pretest and posttest. *: significant difference compared to the PLA in the posttest. #: significant difference compared to PRO in the posttest. ¥: significant difference compared to CAS in the posttest.

power between groups [F = 9.571, $p = 0.001$, pEta2 = 0.424]. The post hoc test showed that there was a significant increase in PRO+CAS compared to PLA (MD = 41.42, $p = 0.001$, 95% CI [25.40–57.44]), PRO (MD = 18.53, $p = 0.026$, 95% CI [2.29–34.76]), and CAS (MD = 27.47, $p = 0.001$, 95% CI [11.48–43.46]), and PRO compared to PLA (MD = 22.89, $p = 0.008$, 95% CI [6.46–39.32]). However, there was no significant difference between CAS and PRO (MD = −8.94, $p = 0.27$, 95% CI [−25.16–7.27]), and CAS and PLA (MD = 13.94, $p = 0.086$, 95% CI [−2.08–29.97]) (refer to Figure 5). Lastly, the results showed no significant difference in the RAST-min-power between groups [F = 0.091, $p = 0.964$, pEta2 = 0.007] (Figure 5).

4. Discussion

The results of the current study revealed that taking a multi-strain PRO or CAS supplement alone for four weeks could benefit lower limb muscle endurance, isokinetic strength parameters, and anaerobic power performance. However, the simultaneous consumption of multi-strain PRO (4.5×10^{11} CFU) and CAS (20 grams) supplements significantly impacted these parameters in male soccer players. It is essential to note that producing high-force torque during fast movements is crucial for soccer players. The higher the force produced, the higher the speed, rotation speed, acceleration, and ability to change speed in soccer. In this regard, isokinetic contraction is considered the gold standard for measuring strength in soccer [39].

The results of some studies are in line with the results of the present study. For example, in a preliminary study with the participation of ten resistance-trained men, the effects of taking 20 grams of CAS supplement with *Bacillus coagulans* GBI30, 6086 (BC30) for eight weeks after a periodic resistance training program were investigated, in which the trend of increasing vertical jump power was shown [40]. Another study showed that taking *Bacillus coagulans* (1.0×10^9 CFU) supplements for two weeks caused an increase in the vertical jump power of soldiers (this increase was not significant compared to PLA) [41]. Consumption of PRO *Streptococcus*, *thermophilus* FP4, and *Bifidobacterium breve* BR03 at five bn live cells (AFU) by 15 healthy resistance-trained men for three weeks increased isometric average peak torque (at 24 to 72 h). It increased the resting arm angle (at 24 h and 48 h) into the recovery period following damaging exercises [42]. In the study by Chen et al. [36], which was conducted on mice, it was shown that *L. plantarum* TWK10 (LP10) supplementation for six weeks at 0, 2.05×10^8, or 1.03×10^9 CFU/kg/day significantly increased relative muscle weight, endurance swimming time, grip strength, and the number of type I fibers (slow twitch muscle fibers) in the gastrocnemius muscle. In addition, the study's researchers mentioned above found that blood urea nitrogen, creatinine, serum levels of albumin, and triacylglycerol decreased with LP10 treatment. In another study, in mice that consumed a PRO kefir daily for four weeks, forelimb grip strength was higher, swimming time-to-exhaustion was significantly longer, and ammonia, blood urea nitrogen, serum lactate, and creatine kinase levels were lower after the swimming test [43]. However, the results of some studies are not in line with our findings; for example, Salleh et al. [22] conducted a trial on thirty university badminton players. The players' ages ranged from 19 to 22 years old, and no gender was specified. The players were given a single strain PRO (*Lactobacillus casei Shirota*, dose: drink containing 3×10^{10} CFU) for six weeks. Although PRO supplementation improved aerobic capacity, it did not affect speed, power, leg strength, and agility [22]. This difference in the present study's findings and the study by Salleh et al. was probably due to the difference in the probiotic dosage and strains used. In another study with the presence of twenty-three female athletes (PLA ($n = 12$) and PRO ($n = 11$)), it was shown that *bacillus* subtilis-based PRO supplementation (DE111; 5 billion CFU/day) during off-season training for ten weeks improves body composition, nevertheless; it did not affect 1RM strength (bench press, deadlift, and squat) and vertical jump height [23]. Also, Townsend et al. [40] investigated the effect of daily lower dose B. subtilis (DE111) supplementation on physical adaptation and performance tests of collegiate baseball players; there were no differences between PRO and PLA on measures of strength, performance, and even body composition. The effects of this strain can probably be dose-dependent. In the study by Apweiler et al. [44] done in the morning, thirty-nine active males and females did 100 drop jumps. They followed their usual diet during the day, and then 30 minutes before bedtime, 40 g CAS ($n = 19$) or 40 g carbohydrate-only control (CON) ($n = 20$) was consumed; the results showed that bedtime supplementation with 40 g of CAS is not more beneficial than CON for accelerating recovery after muscle-damaging exercises. Also, there was no considerable difference between CAS and control in MIVC, CMJ, PPT, subjective muscle pain, and brief mood assessment (BAM+) before, 24, and 48 hours after drop jumps. Previous studies have concluded that a daily protein intake of 1.2–1.6 g/kg is likely sufficient to maximize muscle protein turnover and subsequent adaptation [45], so receiving an additional 40 g of CAS had no other benefit, at least for improving acute performance [44]. In the study by Apweiler et al. [44], the participants did not consume PRO, and the amount of CAS received differed from ours.

Vigorous physical activity is associated with neurological impairments at the musculoskeletal junction, the onset of blood lactate and other biochemical metabolites, and energy deficiency, which leads to muscle fatigue and reduced athletic performance [46]. The metabolic activity and composition of the gut microbiota may help digest and absorb nutrients and enhance energy harvest during intense exercise [47]. Recent studies have shown that metabolic activity and pathways related to carbohydrate and amino acid metabolism are increased in the microbiome of athletes compared to sedentary individuals [47]. PRO consumption can reduce the adverse effects of intense exercise in the intestine. Therefore, this ergogenic aid can promote gut health in a way that enhances the intestinal barrier function of epithelial cells through increasing short-chain fatty acids (SCFA) (acetate, butyrate, and propionate) production [48]. Most SCFAs are absorbed from the intestinal tract and contribute to host energy metabolism [49]. In particular, propionate can be used as a precursor for hepatic and intestinal glucose synthesis through gluconeogenesis [50], butyrate can be used as an energy source by epithelial cells in the large intestine, acetate can also increase the neurotransmitter gamma-aminobutyric acid (GABA) [51]. GABA, a primary central nervous system inhibitor, can increase resting serum growth hormone and improve muscle adaptations induced by resistance training. Therefore, SCFAs may affect strength, power, and muscular endurance through different physiological pathways [52]. Due to the current study's limitations, it was impossible to investigate the intestinal barrier function of epithelial cells and gut microbiome diversity. Therefore, the following possibilities can also be considered according to previous studies on probiotic supplementation and its effect on the intestinal microbiome and amino acid absorption. The increase in soccer players' strength, power, and performance in the current study may result from improved intestinal microbiota function by PRO, improved amino acids and other nutrient absorption, and mainly SCFA functions. This matter should be further investigated in future studies for a better and definitive interpretation and conclusion.

On the other hand, CAS releases amino acids continuously over several hours, and protein intake and exercise have been shown to activate mTOR (The protein kinase mechanistic target of rapamycin). mTOR is a critical factor that helps increase the repair process of skeletal muscles by limiting protein breakdown and increasing protein synthesis [53]. For this reason, this process can lead to an increase in the rate of training adaptation followed by hypertrophy and improved recovery after intense exercise. As a result, the performance of soccer players increases in the indicators of strength, power, and muscular endurance.

The present study's findings showed that the simultaneous consumption of multi-strain PRO and CAS for four weeks caused a significant increase in the maximum and average maximum anaerobic power of soccer players, while no effect was observed on the minimum anaerobic power. According to the present study's findings, the survey conducted by Jager et al. showed [12] that damaging exercise tremendously increased muscle soreness and decreased perceived recovery. However, PRO supplementation (*Bacillus coagulans* GBI-30, 6086) at a dose of 1 billion CFU with 20 g of CAS significantly increased recovery at 24 h, and there was a reduction in pain 72 hours after exercise compared to the group that only consumed protein. Also, the simultaneous consumption of PRO and CAS preserved anaerobic power performance. Also, Huang et al. [21] reported the positive effect of *L. plantarum* PS128 (1.5×10^{10} CFU) on anaerobic capacity using the Wingate test – also, *L. plantarum* PS128 supplementation (combined with exercise), hugely increased plasma branched-chain amino acids. In addition, *L. plantarum* PS128 significantly reduced oxidative stress, such as thioredoxin, creatine kinase, and myeloperoxidase indices after triathlon exercise. Despite this, the study by Carbuhn et al. [19] showed that there was no difference between PRO (*Bifidobacterium longum* 35624; 1 billion CFU.d^{-1}) and PLA in the aerobic and anaerobic performance of female swimmers during six weeks of off-season training. However, Imanian et al. showed that co-ingestion of PRO and CAS can relatively improve the aerobic capacity of male soccer players. Nevertheless, simultaneous consumption of probiotics and casein had a more pronounced effect on aerobic capacity indicators, especially time to exhaustion [26].

Competitions performed by athletes at maximum intensity require an efficient energy supply through anaerobic metabolism [54]. There may be several explanations for how multi-strain PRO supplementation and its combination with CAS increased anaerobic capacity in soccer players. In the present study, increasing anaerobic performance may be due to a significant increase in knee extensor muscle performance and a significant increase in explosive power, as well as a significant increase in the maximal isometric endurance of the lower body muscles, as shown in many previous studies that the performance of the neuromuscular system, especially knee extension strength, is considered an essential component in anaerobic performance [55,56]. During anaerobic exercise, gut microbiota may play a critical role in the body's energy system when lactate produced by host skeletal muscle enters the gut lumen (via circulation), a selective advantage for lactate-consuming species that live in the large intestine provides [57,58]. As a result, during intense exercise, fuel in the form of lactate is offered to certain bacteria, which produce metabolites such as propionate, which benefit the athlete's performance [59,60]. Moreover, high-intensity exercise increases serum non-esterified fatty acids (NEFA), which increases the level of tryptophan in the brain and stimulates the synthesis and release of 5-hydroxytryptamine (the neurotransmitter serotonin-5HT). The increase of 5HT, which is associated with sleepiness and fatigue, leads to fatigue of the CNS and reduced physical performance [61]. Improving the absorption of amino acids, especially branched-chain amino acids by PRO, may reduce the serum (or plasma) tryptophan ratio that competes with branched-chain amino acids for transfer to the brain. As a result, the synthesis and release of 5HT may be limited and prevent a decline in physical performance [62]. As mentioned, it has been found that the gut microbiota plays a vital role in the gut-brain connection by producing some neural molecules. For example, Lactobacillus strains produce γ-aminobutyric acid (GABA) [51]. It is, therefore, clear that gut bacteria have the potential to alter the activity of neurotransmitters, thus interacting with the host nervous system to regulate mental health and, consequently, metabolism and exercise capacity [11]. Also, one of the indirect effects of PRO consumption that can be mentioned is that intense training (aerobic and anaerobic) is accompanied by oxidative stress, and the use of PRO supplements may lead to a decrease in oxidative stress by increasing the plasma antioxidant level [57]. Therefore, according to the above, it seems that the simultaneous consumption of multi-strain probiotics with casein protein increases sports performance, especially anaerobic performance, by activating the neurotransmitters and reducing the level of tryptophan in the brain. However, further research is needed to confirm this hypothesis.

The findings of this study demonstrated significant improvements in isokinetic strength, anaerobic power, and jumping performance, particularly in the PRO+CAS group. These results align closely with existing literature on the relationship between strength, power, and injury prevention in soccer players. For instance, enhanced lower-body strength, as observed in increased isokinetic torque at both 60°/s and 180°/s, plays a vital role in stabilizing the knee joint during high-intensity actions such as sprinting, cutting, and landing. Previous research has highlighted a strong correlation between maximal squat strength and critical performance metrics like sprint performance and vertical jump height, underscoring the role of strength training in reducing injury risks [31]. Additionally, balance between quadriceps and hamstring strength, improved in this study, is critical to preventing knee injuries such as anterior cruciate ligament (ACL) tears [2].

Improving anaerobic power further supports injury resilience by enabling soccer players to perform explosive movements with reduced biomechanical stress. This aligns with findings that demonstrate the impact of strength and power training on mitigating overuse and acute injuries associated with repetitive high-intensity actions [32]. These results highlight the practical importance of integrating strength and power development with optimized nutritional strategies, as demonstrated by the PRO+CAS group's superior outcomes, to enhance performance and prevent injuries in soccer players.

This study had several limitations that should be acknowledged. Firstly, due to financial constraints, we were unable to measure the probiotic content of participants' intestines or assess key biomarkers influencing muscle protein synthesis (e.g. mTOR) through muscle biopsies. Additionally, a daily supplementation protocol was not feasible, and the study was limited to male soccer players, which restricts the generalizability of the findings. Although a placebo group was used, a crossover or within-subject design could further enhance internal validity and reduce inter-individual variability. Finally, the current study did not investigate the dose-response relationship of probiotic and casein supplementation, which may influence the magnitude of physiological adaptations. Given these limitations, future studies are encouraged to explore the dose-effect relationship of probiotics and casein on anaerobic performance and lower-limb strength, using varied dosages and durations while incorporating mechanistic biomarkers and broader athletic populations to enhance the applicability and depth of findings.

5. Conclusion

The findings of this study demonstrate that the simultaneous consumption of multi-strain probiotics and casein significantly enhances anaerobic power, isokinetic strength, and lower-body muscular endurance in male soccer players. These improvements – particularly in average anaerobic power, knee extensor torque, and wall-squat performance – were more pronounced than with either supplement alone, indicating a synergistic effect. This supplementation strategy may serve as an effective and practical ergogenic aid for athletes aiming to optimize performance and recovery.

Future research should investigate the dose-response relationship of probiotic and casein supplementation, explore mechanistic pathways such as the gut-muscle axis and mTOR signaling, and assess long-term effects across different sports and athletic populations to broaden applicability and deepen understanding.

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Authors' contributions

All authors were involved in designing the study. All authors were involved in interpreting the data, and critically revised, read and approved the final manuscript.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Research Ethics Committees of the Faculty of Psychology and Educational Sciences at Shiraz University in Shiraz, Iran, thoroughly reviewed and approved the study. The ethics approval code for this study is IR.US.PSYEDU.REC.1402.034, Approval Date: 24 May 2023. The study was conducted in accordance with the Declaration of Helsinki, and all participants provided written consent.

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