



E-ISSN: 2707-7020  
P-ISSN: 2707-7012  
Impact Factor (RJIF): 5.41  
JSSN 2026; 7(1): 06-11  
[www.allsportsjournal.com](http://www.allsportsjournal.com)  
Received: 05-09-2025  
Accepted: 07-10-2025

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## Assessment of probiotic supplementation on physical performance indicators among women athletes

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**DOI:** <https://www.doi.org/10.33545/27077012.2026.v7.i1a.352>

### Abstract

Women athletes exhibit distinct physiological, nutritional, and psychological characteristics that influence physical fitness and performance outcomes; however, they remain underrepresented in sports nutrition research. The present study aimed to assess the effect of regular probiotic supplementation on selected physical fitness parameters—flexibility, endurance, and muscular strength—among women athletes. A mixed-method research design was adopted, comprising an exploratory baseline assessment ( $n = 150$ ) and a placebo-controlled intervention trial ( $n = 25$ ). Baseline data on anthropometry, training patterns, dietary intake, stress, and fitness parameters were collected using standardized tools. The intervention group received a multi-strain probiotic formulation daily for 20 days, while the control group received a placebo. Pre- and post-intervention assessments were conducted using validated physical fitness and psychological measures. Statistical analysis was performed using SPSS (v22.8), with significance set at  $p < 0.05$ . Results indicated that probiotic supplementation led to significant improvements in flexibility, endurance, and muscular strength compared to baseline and control conditions. These improvements are likely mediated through enhanced gut microbiota balance, improved nutrient absorption, reduced exercise-induced inflammation, and better immune regulation. The study concludes that probiotic supplementation is a safe, non-invasive, and gender-sensitive nutritional strategy that can effectively support physical fitness and performance in women athletes. The findings provide important evidence for integrating probiotics into sports nutrition and athlete recovery programs.

**Keywords:** Probiotic supplementation, women athletes, physical fitness, endurance, muscular strength

### Introduction

Women's participation in competitive sports has increased substantially over the past few decades; however, scientific research addressing the specific physiological, nutritional, and psychological needs of women athletes remains disproportionately limited. Much of the existing sports science literature is derived from male-centric models and is frequently generalized to female athletes without adequate consideration of sex-specific biological differences (da Silva *et al.*, 2019) [8]. Women athletes exhibit distinct hormonal profiles, metabolic responses, stress reactivity, and recovery patterns, all of which directly influence physical fitness, performance outcomes, and overall well-being (Arora *et al.*, 2021) [2]. Consequently, there is a growing need for evidence-based, gender-sensitive interventions that can support optimal fitness and resilience in women athletes. Physical fitness parameters such as flexibility, endurance, and muscular strength are critical determinants of athletic performance and injury prevention. These parameters are influenced not only by training regimens but also by nutritional status, immune function, and gut health. In women athletes, hormonal fluctuations associated with the menstrual cycle can further modulate muscle function, fatigue perception, thermoregulation, and recovery capacity, thereby affecting training efficiency and competitive performance (Liu *et al.*, 2019) [13]. Additionally, women athletes are more susceptible to conditions such as Relative Energy Deficiency in Sport (RED-S), iron deficiency, gastrointestinal discomfort, and immune suppression, which can compromise both health and performance if not adequately addressed.

In recent years, probiotics have emerged as a promising nutritional strategy for enhancing athletic health and performance. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host (FAO/WHO, 2002) [9]. These beneficial microorganisms play a pivotal role in maintaining gut microbiota balance, improving gastrointestinal integrity, enhancing nutrient absorption, and modulating immune

responses (Hill *et al.*, 2014) <sup>[11]</sup>. For athletes, optimal gut health is particularly important, as intense physical training and psychological stress can disrupt intestinal permeability and microbial diversity, leading to inflammation, illness, and impaired performance (Sanders *et al.*, 2019) <sup>[16]</sup>. Beyond gastrointestinal health, probiotics have been increasingly linked to improvements in physical fitness and recovery. Evidence suggests that probiotic supplementation can enhance immune resilience, reduce exercise-induced inflammation, and improve metabolic efficiency, thereby supporting endurance and strength outcomes (da Silva *et al.*, 2019; Arora *et al.*, 2021) <sup>[8, 2]</sup>. Improved nutrient absorption, particularly of micronutrients such as iron and calcium, is especially relevant for women athletes, who face higher risks of deficiencies due to menstrual blood loss and elevated physiological demands. Enhanced gut microbial activity has also been associated with improved muscle function and reduced fatigue, suggesting a potential indirect role of probiotics in supporting flexibility and muscular performance.

Importantly, probiotics exert their effects not only through the gut but also via the gut-brain axis, a bidirectional communication network linking the gastrointestinal system with the central nervous system. This axis plays a critical role in regulating stress responses, mood, and cognitive function. Probiotic strains such as *Lactobacillus* and *Bifidobacterium* have been shown to modulate the hypothalamic-pituitary-adrenal (HPA) axis, reduce cortisol levels, and influence neurotransmitter production, including serotonin and gamma-aminobutyric acid (GABA) (Sarkar *et al.*, 2016; Bansal *et al.*, 2020) <sup>[17, 4]</sup>. Given that psychological stress and mental fatigue can negatively affect physical performance and training adherence, the psychophysiological benefits of probiotics may be particularly valuable for women athletes. Despite the growing global consumption of probiotics and expanding scientific evidence supporting their health benefits, research examining their direct impact on physical fitness parameters in women athletes remains scarce. Most existing studies focus either on general populations or male athletes, with limited attention to female-specific outcomes. Furthermore, while probiotics are widely promoted as ergogenic and wellness-enhancing supplements, empirical data linking regular probiotic consumption to measurable improvements in flexibility, endurance, and strength among women athletes are insufficient.

Against this backdrop, the present study seeks to address this critical research gap by systematically examining the impact of regular probiotic consumption on selected fitness parameters namely flexibility, endurance, and muscular strength in women athletes. By adopting a gender-sensitive and integrative perspective, the study aims to contribute to sports nutrition science, women's health research, and performance optimization strategies. The findings are expected to provide evidence-based insights into the role of probiotics as a safe, natural, and non-invasive nutritional intervention to enhance physical fitness and overall well-being in women athletes.

## Methodology

**a) Study Design:** The present study employed a mixed-method research design comprising two sequential phases: (i) an exploratory cross-sectional baseline assessment, and (ii) a controlled probiotic intervention

trial. This design was adopted to comprehensively evaluate the impact of regular probiotic consumption on selected physical fitness parameters flexibility, endurance, and muscular strength among women athletes, while also accounting for anthropometric, psychological, and lifestyle variables

**b) Study Setting and Participants:** The study was conducted among women athletes registered with various Gymkhanas and sports clubs in Pune City, Maharashtra, India. Athletes actively engaged in regular training and competition were considered eligible. Inclusion criteria included women athletes aged 18-25 years, actively training for at least one year, free from chronic illness, and not consuming antibiotics or probiotic supplements during the preceding one month. Exclusion criteria included pregnancy, diagnosed gastrointestinal disorders, recent illness, or unwillingness to participate.

**c) Phase I: Exploratory Baseline Assessment -** In Phase I, 150 women athletes were selected using a non-probabilistic quota sampling technique to ensure representation across different sports disciplines. Data were collected using a structured and validated interview schedule, which captured information on demographic profile, dietary intake (one-week dietary recall), probiotic awareness and consumption, lifestyle practices, and psychological parameters. Psychological variables such as stress and mood were assessed using the Depression Anxiety Stress Scale (DASS-21). Objective measurements of physical fitness and body composition were conducted using standardized protocols: Flexibility: Zipper test, Endurance: Sit-and-reach test and curl-up test, Muscular strength: Squat test, Anthropometry: Height, weight, BMI, and body fat percentage measured using Karada Scan, This phase established baseline fitness profiles and identified existing associations between probiotic intake, lifestyle factors, and fitness outcomes.

**Phase II: Probiotic Intervention Trial-** Phase II consisted of a placebo-controlled experimental intervention conducted on 25 women athletes, systematically selected from Phase I participants based on eligibility and willingness.

**Participants were randomly allocated into:** Experimental group (probiotic supplementation) Control group (placebo) the intervention duration was 20 consecutive days. The experimental group received a daily probiotic formulation containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, and *Saccharomyces boulardii*, with fructooligosaccharides as a prebiotic component. The total viable count was maintained at clinically relevant levels. The control group received a visually identical placebo. Pre- and post-intervention assessments were conducted using the same fitness, anthropometric, and psychological tools as in Phase I. Compliance was monitored daily, and adverse events were systematically recorded. Outcome Measures The primary outcome variables were changes in: Flexibility, Endurance, Muscular strength, Secondary outcomes included changes in anthropometric characteristics, stress, mood, and lifestyle indicators.

**d) Data Analysis:** Data were analyzed using SPSS version

22.8. Descriptive statistics (mean, standard deviation, frequency) were used to summarize participant characteristics. Inferential statistics included paired t-tests, ANOVA, and chi-square tests to assess pre- and post-intervention differences and group effects. Multivariate relationships were examined using Partial Least Squares Structural Equation Modeling (PLS-SEM) through ADANCO software. Statistical significance was set at  $p < 0.05$ .

- e) **Ethical Considerations:** The study protocol received approval from the Institutional Ethics Committee, Shwas Multispecialty Hospital, Pune. Written informed consent was obtained from all participants prior to data collection. Confidentiality, voluntary participation, and the right to withdraw at any stage were strictly ensured. The probiotic used was classified as Generally Recognized as Safe (GRAS), and participants were monitored for any adverse effects throughout the intervention period

## Results and Discussion

**Table 1:** Baseline Demographic and Training Characteristics of Women Athletes (n = 150)

Variable	Category	Frequency	Percentage (%)
Age (years)	18-25	150	100
Type of Sport	Gymnastics	45	30
	Swimming	30	20
	Running	17	11.3
	Basketball	13	8.7
	Karate/Taekwondo	12	8
	Others	33	22
Daily Exercise Duration	30-60 min	20	13.3
	61-120 min	66	44
	121-240 min	64	42.7

The baseline assessment of 150 women athletes indicated a predominantly young and actively training population. Most participants were engaged in gymnastics, swimming, running, and team or combat sports, reflecting diversity in sport-specific physiological demands. Notably, nearly 87% of the athletes reported exercising for more than one hour per day, indicating a high training load and competitive orientation. Such prolonged training durations are characteristic of performance-focused athletes and are known to place increased physiological stress on musculoskeletal, metabolic, and gastrointestinal systems (Sundgot-Borgen & Garthe, 2011) [19]. This training profile provides an appropriate context for evaluating nutritional interventions such as probiotics, which are increasingly recognized for their role in supporting recovery and physical performance.

**Table 2:** Anthropometric and Body Composition Profile of Women Athletes (n = 150)

Parameter	Category	Percentage (%)
BMI	Severely Underweight	4.7
	Underweight	34
	Normal	57.3
	Overweight	6.7
Visceral Fat	Level 0	80.7
	Level 1-3	16.7
	$\geq 4$	2.6

Anthropometric analysis revealed that the majority of athletes maintained normal BMI values (57.3%), consistent with optimal athletic conditioning. However, a substantial proportion (approximately 39%) fell within underweight or severely underweight categories, highlighting potential risks of low energy availability. This finding aligns with previous research linking restrictive body composition targets in female athletes to energy deficiency and compromised performance (Nattiv *et al.*, 2007; Mountjoy *et al.*, 2014) [15, 14]. Visceral fat levels were remarkably low, with over 80% of athletes showing negligible visceral adiposity, reflecting favorable metabolic profiles. Low visceral fat is commonly associated with regular high-intensity physical activity and reduced cardiometabolic risk (Shen *et al.*, 2004) [18]. Subcutaneous fat distribution across arm, trunk, leg, and whole-body sites demonstrated sport-specific variability but largely remained within healthy or functional ranges. Similar patterns have been reported among trained female athletes, where body fat distribution reflects both training adaptations and sport demands (Heyward & Wagner, 2004; Ackland *et al.*, 2003) [10, 1].

**Table 3:** Baseline Muscular Strength Distribution of Women Athletes (n = 150)

Strength Parameter	Dominant Category	Percentage (%)
Repetition Maximum (RM)	Moderate-High	55.3
Trunk Strength	24-28 units	50
Arm Strength	32-40 units	58.7
Leg Strength	40-44 units	39.3

Baseline muscular performance showed that most athletes clustered within moderate to high strength categories across trunk, arm, and leg muscle groups. Approximately 28% exhibited high repetition maximum (RM) values, indicating well-developed muscular strength, particularly in athletes involved in power- or strength-oriented sports. Moderate-to-high lower body strength was especially prominent, which is critical for locomotion, agility, and endurance-based performance (Comfort *et al.*, 2012) [7]. These findings are consistent with training-induced neuromuscular adaptations observed in female athletes undergoing regular resistance and endurance conditioning (Kraemer & Ratamess, 2004) [12]. However, a smaller subset of athletes fell into lower strength categories, suggesting heterogeneity in training exposure, recovery quality, or nutritional adequacy. Baseline muscular strength clustered predominantly in moderate to high categories, reflecting effective neuromuscular adaptations to regular training. Lower-body strength was particularly well developed, consistent with its central role in athletic performance across sports (Kraemer & Ratamess, 2004; Bompa & Buzzichelli, 2019) [12, 5]. Nevertheless, a minority of athletes with lower strength scores suggests scope for targeted interventions supported by optimized nutrition and recovery.

**Table 4:** Effect of Probiotic Supplementation on Physical Fitness Parameters (Intervention Phase, n = 25)

Fitness Parameter	Pre-Intervention Mean $\pm$ SD	Post-Intervention Mean $\pm$ SD	Direction of Change
Flexibility	Moderate	Improved	↑ Significant
Endurance	Moderate	Markedly Improved	↑ Significant
Muscular Strength	Moderate	Improved	↑ Significant



The probiotic intervention phase demonstrated significant post-intervention improvements in flexibility, endurance, and muscular strength among the experimental group compared to the control group. Athletes receiving probiotic supplementation showed enhanced endurance test scores and improved muscular strength indicators, suggesting a positive physiological response to regular probiotic intake. These improvements may be attributed to enhanced gut microbiota balance, leading to better nutrient absorption, reduced gastrointestinal discomfort, and improved energy metabolism. Previous studies have demonstrated that probiotic supplementation can reduce exercise-induced inflammation, improve immune resilience, and support recovery, thereby indirectly enhancing physical performance (da Silva *et al.*, 2019; Arora *et al.*, 2021) [8, 2]. Improved flexibility outcomes may also be linked to reduced systemic inflammation and muscle stiffness, mechanisms increasingly associated with gut-mediated metabolic regulation (West *et al.*, 2021) [20].

Following the 20-day probiotic intervention, the experimental group demonstrated clear improvements in flexibility, endurance, and muscular strength compared to baseline and control conditions. These findings suggest that regular probiotic intake enhances physical fitness outcomes in women athletes. Improved endurance and strength may result from better nutrient absorption, reduced gastrointestinal discomfort, and enhanced immune regulation, all of which support training efficiency and recovery (da Silva *et al.*, 2019; Arora *et al.*, 2021) [8, 2].

**Table 5:** Repetition Maximum (RM) of women athletes

Repetition Maximum (RM) Category	RM Range	Frequency	Percent (%)
Very Low RM	< 1000	29	19.3
Low RM	1000 - 1099	38	25.3
Moderate RM	1100 - 1199	41	27.3
High RM	≥ 1200	42	28
Total		150	100

Based on the data for Repetition Maximum (RM) among 150 women athletes, the distribution reflects a wide variation in muscular strength capacity, as measured by the highest weight an individual can lift for a given number of repetitions. For analytical clarity, the data were categorized into four groups: Very Low RM (<1000), Low RM (1000-1099), Moderate RM (1100-1199), and High RM (≥1200). The majority of participants, 38.6%, fall within the Moderate RM range (1100-1199). This level of muscular strength suggests that most athletes possess a reasonable capacity for performing sport-specific tasks requiring power, endurance, and resilience. These RM values correspond to balanced neuromuscular conditioning and are ideal for multi-sport athletes. This is supported by literature emphasizing that moderate RM values are beneficial for both strength maintenance and injury prevention (Baechle & Earle, 2008) [3]. A substantial portion 30% belongs to the Low RM group (1000-1099). While still within an acceptable athletic range, this group may benefit from targeted strength training, particularly resistance exercises, to improve muscle force output and endurance under load. Tailored strength programs could help athletes in this group enhance performance and reduce fatigue during prolonged activity.

Interestingly, 20.7% of athletes demonstrated High RM

values (≥1200), indicating superior strength levels. Such high repetition maximums are associated with improved power, explosiveness, and overall athletic capability, especially beneficial in sports demanding short bursts of intense physical activity like sprinting, weightlifting, and contact sports (Kraemer & Ratamess, 2004) [12]. A smaller subset—10.7%—fell into the Very Low RM category (<1000), which could indicate either underdeveloped muscular strength, injury, or other limiting factors such as inadequate training or nutritional deficits. This group may require close monitoring and personalized intervention plans including progressive overload, resistance training, and recovery optimization.

It is further reveals that while the majority of the athletes exhibit sufficient to strong muscular performance levels, a focused training intervention could enhance outcomes for those in the lower ranges. Monitoring RM over time is crucial to track improvements, prevent injuries, and ensure optimal athletic performance.

**Table 6:** Categorized Trunk Muscle Strength Values of Women Athletes

Trunk Muscle Strength Range	Frequency	Percentage
18.0 - <20.0	13	8.70%
20.0 - <22.0	11	7.30%
22.0 - <24.0	15	10.00%
24.0 - <26.0	36	24.00%
26.0 - <28.0	39	26.00%
28.0 - <30.0	9	6.00%
30.0 - <32.0	8	5.30%
32.0 - <34.0	0	0.00%
34.0 - <36.0	0	0.00%
36.0 - <38.0	1	0.70%
Total	150	100.00%

The analysis of trunk muscle strength among women athletes reveals a concentration of values in the mid-range categories, particularly in the 24.0-25.9 (24.0%) and 26.0-27.9 (26.0%) intervals. Together, these categories account for half (50%) of the total sample, indicating a strong clustering of trunk muscle strength in the mid-performance range. This clustering may reflect optimal muscle conditioning commonly achieved through regular training and core-strengthening regimens, which are typical among competitive women athletes (Clark, Lucett, & McGill, 2015) [6]. The next most populated categories are the 22.0-23.9 (10.0%) and 18.0-19.9 (8.7%) ranges, which suggest that a moderate proportion of athletes fall slightly below the mid-range, possibly indicating either early-stage training adaptations or natural variation in muscular strength. On the other hand, the lower frequency in the 28.0-31.9 range (11.3%) and the negligible representation beyond 32.0 points to a limited presence of very high trunk muscle strength values, which may be influenced by physiological ceilings, sport-specific demands, or variability in training intensity.

Interestingly, there are no entries between 32.0 and 35.9, and only one individual (0.7%) falls in the 36.0-37.9 category. These extreme high values might represent elite performance but appear as statistical outliers in this dataset. According to Bompa and Buzzichelli (2019) [5], such high values are rare and often linked to elite-level conditioning or sport-specific hypertrophy in disciplines like gymnastics or weightlifting. This categorization approach helps highlight

the overall muscular performance pattern in women athletes, offering insights useful for training prescription, performance benchmarking, and injury prevention. As noted by Pallant (2020), grouping continuous performance measures into categorical ranges enhances clarity in data interpretation and supports the development of targeted athletic training programs.

**Table 7:** Categorized Arm Muscle Strength Values of Women Athletes

Arm Muscle Strength Range	Frequency	Percentage
19.0 - <24.0	7	4.70%
24.0 - <28.0	13	8.70%
28.0 - <32.0	13	8.70%
32.0 - <36.0	45	30.00%
36.0 - <40.0	43	28.70%
40.0 - <44.0	18	12.00%
44.0 and above	1	0.70%
Total	150	100.00%

The distribution of arm muscle strength among women athletes reveals a clear central tendency, with the majority of values clustered in the 32.0-35.9 and 36.0-39.9 intervals. These two categories alone account for 58.7% of the total participants, suggesting that the arm muscle strength of most athletes falls within a moderately high range, likely due to consistent resistance and strength training practices that are integral to athletic conditioning (Bompa & Buzzichelli, 2019) <sup>[5]</sup>.

The presence of 13 participants (8.7%) each in the 24.0-27.9 and 28.0-31.9 categories indicates a group of athletes who may be in earlier stages of training or belong to sports that do not emphasize upper-body strength. Only a small fraction (4.7%) of athletes fall in the lower range (19.0-23.9), which might include outliers or athletes with lower baseline strength, potentially due to younger age, lower body mass, or sport-specific demands (Clark, Lucett, & McGill, 2015) <sup>[6]</sup>. At the higher end, 12.0% of the athletes fall into the 40.0-43.9 range, reflecting elite or above-average performance. The highest recorded value, in the 44.0+ range, was represented by a single individual (0.7%), likely an outlier with exceptional arm muscle strength. This finding is consistent with research by Kraemer & Ratamess (2004) <sup>[12]</sup>, which highlights that genetic predisposition, specialized training, and sport-specific needs often determine exceptional muscular strength levels. This categorized approach provides a clear understanding of the performance distribution and can help in tailoring training programs, identifying athletes for targeted muscle development, or even screening for physical imbalances or potential injury risks. As emphasized by Pallant (2020), categorized data enhances the visibility of trends in large datasets and aids in practical application across sports science and physical assessment contexts.

**Table 8:** Categorized leg Muscle Strength Values of Women Athletes

Leg Muscle Strength Range	Frequency	Percentage
34.0 - <38.0	9	6.00%
38.0 - <40.0	26	17.30%
40.0 - <42.0	29	19.30%
42.0 - <44.0	30	20.00%
44.0 - <46.0	18	12.00%
46.0 - <48.0	5	3.30%
48.0 and above	3	2.00%
Total	150	100.00%

The distribution of leg muscle strength among the women athletes shows a concentration in the 40.0-43.9 range, with 59 participants (39.3%) falling within the two adjacent categories of 40.0-41.9 and 42.0-43.9. This indicates that a significant proportion of athletes possess moderate to high leg muscle strength, likely a result of regular lower-body training that supports explosive movements, balance, and agility key components in many sports (Bompa & Buzzichelli, 2019) <sup>[5]</sup>. A noteworthy 20.0% of the participants are in the 42.0-43.9 range, suggesting a performance peak. This distribution is consistent with findings from prior sports science literature indicating that lower body strength is a fundamental factor in jumping, sprinting, and endurance (Comfort, Haigh, & Matthews, 2012) <sup>[7]</sup>. Furthermore, this central clustering reflects an effective training threshold for athletic populations. The 38.0-39.9 range accounts for 17.3% of the athletes, likely representing individuals still progressing through structured training or in sports with lower lower-body demands. A smaller proportion only 6.0% fall into the lowest range (34.0-37.9), which may include athletes recovering from injury, beginners, or those in weight-sensitive sports like gymnastics or long-distance running (Kraemer & Ratamess, 2004) <sup>[12]</sup>. At the higher end, 12.0% of athletes show leg strength in the 44.0-45.9 category, and a few outliers extend into the 46.0+ range, including one individual each in the 50.0 and 51.4 kg strength values, possibly indicating elite performance or outliers with genetically high muscle capacity (Zatsiorsky & Kraemer, 2006).

This categorized analysis reveals a normal distribution skewed slightly towards higher performance, aligning with the expectations of trained athlete populations. The categorization also facilitates comparisons across training periods and between different cohorts of athletes (Pallant, 2020).

## Conclusion

The present study provides empirical evidence that regular probiotic supplementation positively influences key physical fitness parameters flexibility, endurance, and muscular strength among women athletes. Baseline findings indicated that while most participants maintained favourable anthropometric profiles and moderate-to-high muscular strength, a considerable proportion exhibited low BMI and potential indicators of low energy availability, underscoring the need for supportive nutritional strategies. The controlled intervention phase demonstrated significant post-supplementation improvements in physical performance outcomes in the probiotic group compared to baseline and control conditions. These improvements are plausibly mediated through enhanced gut microbiota balance, improved nutrient absorption, reduced exercise-induced inflammation, and better immune regulation, collectively supporting efficient energy metabolism, recovery, and neuromuscular function. The findings further suggest that probiotics may offer indirect benefits by modulating stress responses via the gut-brain axis, thereby improving training quality and performance sustainability. Overall, the study establishes probiotics as a safe, non-invasive, and gender-sensitive nutritional intervention with meaningful implications for sports nutrition and women's health. Given the underrepresentation of women athletes in probiotic and performance research, these findings contribute valuable insights and support the integration of probiotic

supplementation into evidence-based training and recovery programs. Future research with larger samples, longer intervention durations, and sport-specific stratification is recommended to strengthen causal inference and refine probiotic guidelines for women athletes

### Acknowledgement

Authors are thankful to Hon. Vice Chancellor, Dean Science & Technology of SNDT Womens University, Mumbai for supporting the seed fund to conduct the research project.

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