

Effects of Daily Probiotic Supplementation on Football Player's Stress and Anxiety

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Abstract - Psychobiotics derived from probiotics which contain psychotropic properties, and with correct administration can positively affect the psychological states. Athletes who go through strenuous training are more vulnerable to undergo stress and anxiety. Thus, the objective of this study is to determine the effect of daily probiotics supplementation on stress and anxiety among the football players. The randomised, double-blinded, placebo-controlled trial was conducted using 20 male footballers (age 19.0 ± 0.72 years) who received either probiotic (*Lactobacillus Casei Shirota* 1×10^9 CFU strain) or placebo supplement over 8 weeks. Competitive state anxiety and perceived stress were measured at the baseline, week 4, and week 8 during the intervention. Statistical analysis using *t*-test revealed that probiotic group significantly decreased the cognitive state anxiety scores (18.20 ± 3.94 vs. 23.11 ± 4.81 , $p = 0.026$), somatic state anxiety (13.00 ± 2.83 vs. 16.78 ± 3.03 , $p = 0.012$) and perceived stress scores (12.30 ± 1.70 vs. 14.67 ± 1.66 , $p = 0.007$) but no statistical significant was found in self-confidence (34.80 ± 3.16 vs. 32.89 ± 3.62 , $p = 0.236$). Therefore, daily probiotic supplementation is a promising approach to regulate stress and anxiety among football players and it may indirectly improve athletic performances.

Keywords: Psychobiotics, anxiety, stress

I. INTRODUCTION

Microbiota-gut-brain axis was revealed in the past decade and recently, many researchers are beginning to explore the depth of this bi-directional relationship between the gut microbiota and brain which means any changes occurred to the brain can affect the gut and vice versa [1,2].

According to scholars, the gut can affect the brain in different ways. One way is through probiotics' ability to secrete neurotransmitters like serotonin, gamma-aminobutyric acid (GABA) and catecholamines, which prevent the communication of nerve impulses in the central nervous system (CNS). As well as probiotics can correct the dysfunctional hypothalamic-pituitary-adrenal axis (HPA axis). When in stressful conditions, the HPA axis gets activated and causes adrenal glands to secrete cortisol; thus, probiotics can alter this dysfunction and helps to reduce stress.

Furthermore, probiotics can act as anti-inflammatory agents. When inflammation occurs, those transmitters send impulses to the brain through the vagus nerve causing anxiety,

stress, and depression but probiotics are capable of minimising the inflammations, thus improving the functions of the immune system [3,4,5].

The human gut colonized by more than 100 trillion microbiota, and some of these living bacteria can provide benefits to the host while some could cause harm and some remain neutral [6]. Probiotics are recognized as good bacteria living inside the gut and through correct administration, it can provide various health benefits to the host [1,7]. Effect of probiotics on human and animal health is evidenced by many types of research. Studies revealed that gut microbiota can provide a positive impact on psychological conditions such as stress, anxiety, irritable bowel syndrome (IBS) and depression [2]. Psychobiotics are probiotics that contain psychotropic properties and if consume right amount it can positively affect the brain by providing benefits to the people who suffer from chronic stress, poor mood, and anxiety-like behaviors [3].

Recent studies have found the effectiveness of probiotics on the stress and anxiety using different study samples such as medical students [8,9], petrochemical workers [10], multiple sclerosis patients [11], irritable bowel syndrome (IBS) patients [12], major depressive disorder patients [13], healthy volunteers [14,15] and volunteers with stress symptoms [16]. In sports, even though athletes are more vulnerable to suffer from stress, anxiety, and depressive disorders due to their high training load and exercise intensity, limited studies have been conducted to determine the effect of probiotics on psychological conditions on athletes.

Mackinnon (2000) [17] stated that the endurance athletes who undergo intense daily training without having proper time outs are more vulnerable to psychological disorders such as stress and anxiety and due to that occurrence of psychological disorders are high among them. These prolonged strenuous athletic training can increase the risk of injuries, infections, and depression, which could lead to common problems like upper respiratory tract infections (URTI), gastrointestinal (GI) disturbances and psychological disorders [7]. According to a study conducted by Wilson [18], perceived stress and anxiety are positively correlated with gastrointestinal distress among athletes. Thus, it is necessary to prevent these conditions among athletes in order to help them achieve their peak performance during competitions [19].

Probiotic supplementation is known to be a promising approach for the immunity improvement of the athletes [7]. Improved immunity and psychological conditions can indirectly help athletes to achieve high performance. Thus, the present study was designed to identify the effect of 8 weeks probiotic supplementation on competitive state anxiety and perceived stress among the football players.

II. MATERIALS AND METHOD

A. Research Design

This intervention was a randomised, double-blind placebo-controlled study. Recruited participants were randomly divided into two research conditions, where the probiotic group (PG) received probiotic supplement daily, while placebo group (PL) received placebo supplement daily over 8 weeks. Stress and anxiety responses of the participants were measured at the baseline, 4th week and 8th week during the intervention. The study received ethical approval from the Human Research Ethics Committee of the Universiti Teknologi MARA, Shah Alam, Malaysia (600-IRMI (5/1/6)) and was conducted in accordance with the guidelines of the International Declaration of Helsinki. In addition, permission was also granted from the management of the UiTM Football Club (UiTM FC). Before recruiting participants, the participants were thoroughly informed about the research procedure and the data collection methods verbally and using a printed document.

B. Participants

Young adult male football players, aged 18 to 21 years, who played competitively for over five years of experiences in national leagues, and are physically and mentally sound were selected for the present study. To minimise any other variables and to maintain the uniformity of the sample, athletes were selected from one team (UiTM FC) where they all received similar training daily in the same setting. All the football players were screened through inclusion and exclusion criteria to be eligible for volunteering to participate in this study.

They were interviewed about their medical history. Participants with psychological disorders such as depression, anxiety and chronic diseases such as gastrointestinal disease, respiratory disease, cardiovascular disease were excluded from the study. Since the supplementation was a probiotic product, participants with known intolerance to probiotics, milk products were excluded from the study. Smokers and participants who have used medicines such as antibiotics in recent days before study begins were also excluded. Inclusion and exclusion criteria in the present study were set after referring the following studies [14] [20][21].

C. Materials and Methods

Commercially available probiotic cultured milk with *Lactobacillus Casei* 1×10⁹ CFU Shirota strain (80ml) mixed with commercially available orange fruit juice (120ml) was used as a probiotic supplement and placebo supplement only contained commercially available orange fruit juice (200ml).

Both drinks are commercially available products in Malaysia and approved beverages by the Ministry of Health, Malaysia. Both groups received the same amount of supplement (200ml) where the colour and smell of the drinks are similar and it supports the double-blinded process.

Kato-Kataoka [8] stated that *Lactobacillus Casei* Shirota strain (LcS) has been approved by the Food and Drug Administration of the USA as 'generally recognised as safe' probiotic strain. A study conducted by the Sheehan [22] showed that orange is one of the best fruit juice to mixed with probiotics and the study revealed that *Lactobacillus Casei* can survive more than 8 weeks after mixed with commercially available orange juice with a good amount of bacteria in it such as 10⁷ CFU. According to Luckow et al. (2006), orange juice can mask the perceptible off-flavor that is given by the probiotics while consuming at longer periods [23]. Thus, in this study, orange juice was selected as the placebo drink and to mix with the probiotic drink for the experiment condition.

D. Instruments

1) Competitive State Anxiety Inventory – 2R (CSAI-2R)

Competitive State Anxiety Inventory – 2R (CSAI-2R), developed by Cox, Martens, and Russell [24], was used to identify competitive anxieties and self-confidence in this study. This multidimensional inventory assesses three parameters such as cognitive anxiety (5 items), somatic anxiety (7 items), and self-confidence (5 items) with 17 items using a four-point Likert scale, where 1 is equal to 'Not at all,' and 4 is equal to 'Very much so'. Scoring is done by summing the item scores in each subscale and divide it by the number of items in each scale and multiply it by 10. Due to that score can range from 10 to 40 in each subscale. Validity and reliability of this inventory were conducted by Cox, Martens, & Russell [24] and Cronbach alpha reliability coefficients for CSAI-2R is 0.81 for cognitive anxiety, 0.81 for somatic anxiety and 0.86 for self-confidence [24]. Since the study sample is from the Malay ethnic group in Malaysia, the Malay version of the CSAI -2R was used in the present study which was translated and validated by Hashim & Zulkifli [25].

2) Perceived Stress Scale (PSS)

Perceived Stress Scale (PSS) developed by Cohen, Kamarck, & Mermelstein [26], measures one's perception about recent stressful situations and their degree rating, using a five-point Likert Scale where 0 is equal to 'Never' and 4 is equal to 'Very Often'. The ten items version of PSS inventory is known to be brief and reliable and was recommended by Cohen et al.[26]. Before calculate the total perceived stress, positively stated items (4, 5, 7, and 8) should score reversely as 0 = 4, 1 = 3, 2 = 2, 3 = 1, and 4 = 0. Then adding up item scores to get the total number, which indicates the individuals perceived stress. This score ranges from 0 to 40 where 0 equals the absence of stress and 40 equals to high stress. Validity and the reliability of PSS were studied by the Cohen & Williamson, (1988) included exploratory factor analysis and Cronbach alpha reliability coefficients for PSS is 0.78 [27]. The Malay version of the PSS was used in the present study, which was translated and validated by Al-Dubaai et al. [28].

3) Anthropometric Measures

Anthropometric, body composition measures such as height, weight, body mass index (BMI), percentage of body fat, body fat mass, and protein mass were taken at baseline and at end of the intervention using InBody270 body composition machine at the Sports Injury Clinic, Faculty of Sports Science and Recreation, UiTM using standard protocols.

4) Diet Records

Even though footballers were allowed to continue their usual diet plans, food intake was administrated using three-day dietary record (3DDR) at the baseline, 4th week and 8th week of the intervention where individuals were asked to record their regular food intake for three consecutive days including two weekdays and one weekend. Instructions were given in writing and verbally. Time of the day, food type consumed, amount using grams or spoon size, brand, and other descriptions were asked to fill in the record. In addition to diet records, athletes were instructed to record all the supplements and medications that they are taking during the study period. Since all participants from the Malay ethnic group in Malaysia, food practices among them assumed to be similar. Participants were instructed to provide every information about their diet, medication and other supplements to make sure the effect was entirely due to the probiotic supplementation.

E. Study Procedure

The intervention was conducted from 20th December 2018 to 14th February 2019 (8 weeks). The supplements were prepared each day one hour before the distribution. Football team trained daily at the UiTM football field throughout the study period, hence that provide a facilitative environment to the administration of the supplements. [29]. According to Coqueiro et al.[30], daily probiotic supplementation is crucial, if an interruption occurs such as discontinuation for more than 8 days probiotics may no longer detect in the gut. Thus, in this study, daily administration was done and records were taken. Data were collected at the baseline, 4th week and 8th week during the intervention. During this period, the football team was at their pre-competition period and had a football match with one of their opponent teams on a weekly basis. Thus, data were collected one day prior to these matches to accurately measure their pre-competitive state anxiety.

F. Sample Size Estimation

The sample size was estimated referring to previous research such as Kelly et al. [31], and Sashihara et al. [32] which are similar to the present study. In the study conducted by Allen et al. [33], stated that to achieve 80% power for a one-way ANOVA, a minimum sample size of 20 was required to demonstrate an effect size of 0.3 at the significance level of 0.05. With referencing to that, the sample size was calculated using G*Power 3.0.10 software. To achieve 80% statistical power with a sample effect size of 0.35, with the significant level of 0.05, the proposed study required 8 participants per group and assuming the 20% dropout rate, 10 participants

were recruited for each condition, with the total sample size of 20 participants.

G. Data Analysis

Data were analysed using SPSS (version 25.0) statistical software. According to Kolmogorov-Smirnov test data were normally distributed due to that parametric tests were used to determine the significant difference between probiotic group and placebo group. Repeated measures ANOVA, independent sample *t*-test and paired sample *t*-test were used. The significance level was set for the 0.05 level. The effect size was calculated using equation 1.

$$Eta\ Squad = \frac{t^2}{t^2 + (N1 + N2 - 2)} \tag{11}$$

III. RESULTS AND DISCUSSION

Twenty participants were recruited after getting their consent and they were randomly assigned to either probiotic group (*n* = 10) or placebo group (*n* = 10). One individual from the placebo group withdrew from the study, due to competition commitments. No any side effects or adverse conditions reported during the probiotic supplementation. All the participants were from the Malay ethnic group and their anthropometric, body composition measures were not significantly different at the baseline (Table 1).

TABLE 1. CHARACTERISTICS OF THE PARTICIPANTS

Parameter	Probiotic Group (<i>n</i> = 10)	Placebo Group (<i>n</i> = 09)
Age (Years)	19 ± 0.81	19 ± 0.66
Training Age (Years)	7.9 ± 2.99	7.5 ± 2.67
Height (Cm)	169.86 ± 5.74	169.05 ± 4.87
Weight (Kg)	62.03 ± 5.31	65.82 ± 7.22
BMI (Kg/M ²)	21.59 ± 2.45	22.58 ± 2.20

Note. (Mean ± SD)

A. Competitive State Anxiety Inventory Responses

CSAI 2R measured three dimensions of anxiety, namely cognitive anxiety, somatic anxiety, and self-confidence. All three dimensions were not significantly different in both groups (*p* < 0.05) at the baseline. After 8 weeks of supplementation, cognitive anxiety showed statistically significant differences between probiotic and placebo groups (18.20 ± 3.94 vs. 23.11 ± 4.81, *t*(15.54) = -2.45, *p* = 0.026) showed in Figure 1. Eta squared was 0.26 showing large effect size according to Cohen's (1988) guidelines.

Similarly, after 8 weeks of intervention, somatic anxiety scores showed a statistically significant difference between two groups (13.00 ± 2.83 vs. 16.78 ± 3.03, *t*(17) = -2.81, *p* = 0.012) showed in Figure 2. Eta squared for the mean difference was 0.31 which again shows a large effect size between both groups.

Self-confidence was also measured using the CASI 2R, which is inversely correlated with both cognitive and somatic anxieties. After 8 weeks of probiotic supplementation, results showed no significant changes in self-confidence between two groups (34.80 ± 3.16 vs. 32.89 ± 3.62 , $t(17) = 1.23$, $p = 0.23$) showed in Figure 3. The magnitude of the mean difference was also smaller for self-confidence showing eta squared of 0.08. The results obtained from CSAI-2R at the baseline, 4th week and 8th week of the intervention were presented in Table 2.

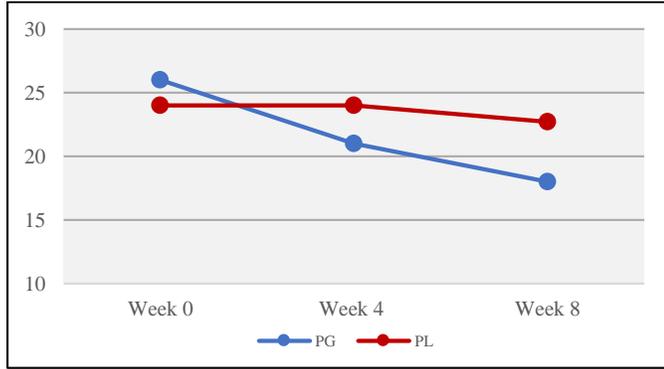


Figure 1. Effects of 8 weeks probiotics and placebo supplementation on cognitive state anxiety (PG=Probiotic Group, PL=Placebo Group)

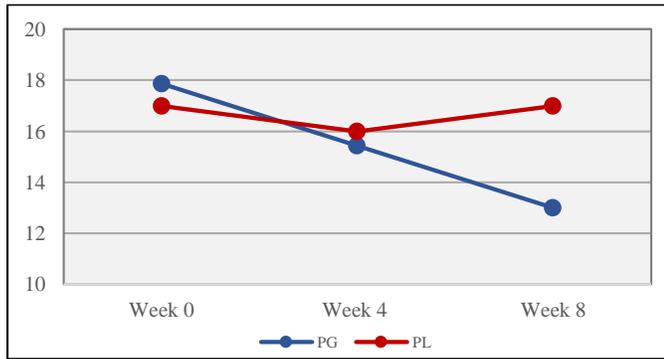


Figure 2. Effects of 8 weeks probiotics and placebo supplementation on somatic state anxiety (PG=Probiotic Group, PL=Placebo Group)

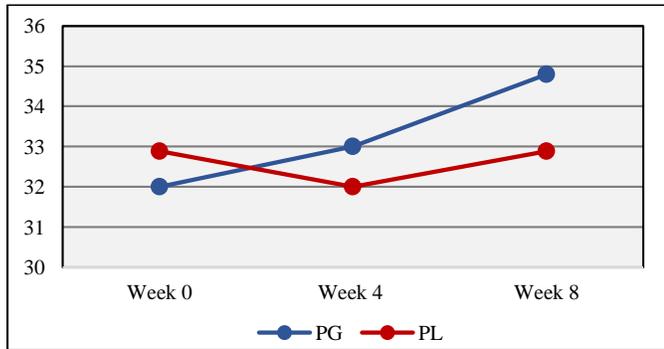


Figure 3. Effects of 8 weeks probiotics and placebo supplementation on self-confidence (PG=Probiotic Group, PL=Placebo Group)

B. Perceived Stress Scale Responses

Perceived stress scores showed statistical significant difference at the end of the intervention (12.30 ± 1.70 vs. 14.67 ± 1.66 , $t(17) = -3.06$, $p = 0.007$). Eta squared was 0.35 which shows large effect size referring to Cohen’s interpretation of effect size magnitude. Mean difference was presented in Figure 4.

C. Anthropometric Body Composition Measurements

Data which was gathered at the baseline and at the end of the trial analysed using independent *t*-test and results revealed that there were no significant changes observed in both baseline and after 8 weeks of either probiotic or placebo supplementation for weight, BMI, body fat mass, body fat percentage and muscle mass between two groups.

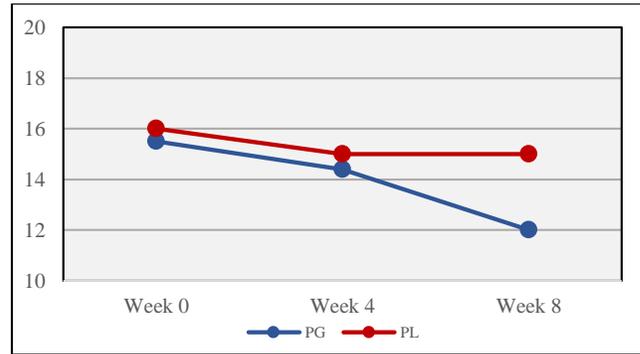


Figure 4. Effects of 8 weeks probiotics and placebo supplementation on Perceived Stress (PG=Probiotic Group, PL=Placebo Group)

Stress is an inevitable aspect of the sports competition [34] and the highest level of competitive anxiety can deteriorate athletes’ performance[35,36]. Eight weeks daily probiotic supplementation significantly decreases cognitive state anxiety, somatic state anxiety, and perceived stress among the football players in the probiotic group compared to the placebo group. Results of the present study open a new window to psychotherapy. Results of the present study can be compared to the study conducted by the Kouchaki [11] using 60 multiple sclerosis patients who underwent 12 weeks either probiotic or placebo treatment and results revealed a significant decrease of Depression, Anxiety and Stress Scale (DASS). Similarly, Sawada [20] reported male students were able to show significant changes in Hospital Anxiety and Depression Scale (HADS) and State-Trait Anxiety Inventory (STAI) after 4 weeks daily probiotic administration.

Slykerman [37] conducted a study using pregnant women over 12 months and revealed a significant decrement in STAI. According to Mohammadi [10], six weeks of probiotic supplementation showed significant improvement in DASS among petrochemical workers. A study conducted by Yang [38] with laryngeal cancer patients, the anxiety level of the patients were lowered significantly before the surgery after exposed to two weeks of probiotic supplementation. Another study conducted by Sashihara [32] using university athletes who daily undergone strenuous training showed significant decrement of tension anxiety scores in profiling mood states

(POMS). Furthermore, Rao [39], conducted a study to find the effect of probiotics on anxiety and depression using chronic fatigue syndrome patients and results were significantly lowered after 8 weeks similar to the present study.

Even though some studies showed decrement of the anxiety, depression and stress parameters after administrating probiotic supplements utilizing a different form of probiotics, dosages and supplement durations, the difference between two

groups is not significant [8,12,16,21,40,41,42]. As well as some studies did not support the present results [9,31,43,44,45,46]. It may be due to the usage of different strains of probiotics, dosages, intervention durations, training intensities, and individual variations among the sample and sample sizes.

TABLE 2. EFFECTS OF PROBIOTIC SUPPLEMENTATION ON COMPETITIVE STATE ANXIETY AND PERCEIVED STRESS AT THE BASELINE, 4TH WEEK AND 8TH WEEK

Parameter	Week 0			Week 4			Week 8		
	PG	PL	p-value	PG	PL	p-value	PG	PL	p-value
Cognitive state anxiety	25.60±6.52	23.78±3.53	0.455	21.20±4.02	23.78±2.54	0.112	18.20±3.94	23.11±4.81	0.026*
Somatic state anxiety	17.80±3.39	16.56±2.79	0.398	15.40±3.72	15.67±3.80	0.879	13.00±2.83	16.78±3.03	0.012*
Self-confidence	32.00±2.82	32.89±4.14	0.588	33.00±4.55	32.00±3.32	0.595	34.80±3.16	32.89±3.62	0.236
Perceived stress	16.10±2.33	16.22±3.86	0.936	14.40±2.01	15.33±2.29	0.357	12.30±1.70	14.67±1.66	0.007*

Note. (Mean ± SD), significant < 0.05*, PG=Probiotic Group, PL=Placebo group

Self-confidence, on the other hand, showed no significant difference between groups. From the baseline, football players showed high self-confidence and with probiotic supplementation, it only showed slight improvement. Perceived stress is not specific to sports but it measures daily life stressors during the one-month period. Daily probiotic supplementation was able to lower the perceived stress significantly among the football players in this study. Messaoudi [47] stated that 4 weeks of probiotic supplementation able to improve HADS and PSS scores significantly in healthy participants and the study conducted by Allen [33] also revealed that 4 weeks probiotic supplementation lowered PSS scores in healthy volunteers similar to the present study.

Lactobacillus Casei Shirota strain combine with other probiotic strains used by some researchers and found out that daily treatment reduces stress and anxiety significantly [13,11] thus, the studies that have used only *Lactobacillus Casei* Shirota strain as probiotic supplement showed reduction of stress and anxiety but none of them was significant [8,9,41] which is contrast to the present study.

Most research has been carried out to find the effect of probiotics to reduce the risk of gastrointestinal disturbances, upper respiratory tract infections and immune disorders associated with high-intensity exercise [48,49]. Very limited studies focus on the psychotropic properties on probiotics to improve psychological conditions among athletes. Prolonged high-intensity training, inadequate recovery period, long traveling, sleep disturbances can increase the risk of infections, immune disorders, GI disturbances which will lead to increase the pre-competitive anxiety, and perceived stress. These conditions can deteriorate sports performance directly or indirectly. Even though the present study provides a promising approach for the athletes with "Psychobiotics" to reduce stress and anxiety during the competition period more research needed to confirm this phenomenon also to

recommend the probiotic dosage, and effective probiotic strain for the athletes.

IV. CONCLUSION

The study was conducted mainly aiming at team players who took part in strenuous daily training with high intensity and moderate volume since they were in their pre-competition period. After 8 weeks, cognitive anxiety, somatic anxiety, and perceived stress showed significant improvement in the probiotic group while anthropometric, body composition and diet records showed no significant changes during supplementation. Self-confidence did not show any significant changes with supplementation. Psychobiotics showed a promising approach as psychotherapy proving the bi-directional communication between gut and brain.

Lactobacillus Casei Shirota strain showed its capacity to work as a Psychobiotic to relieve daily stress and pre-competitive anxiety.

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