

INVESTIGATING THE EFFECTS OF PROBIOTIC SUPPLEMENTATION ON COGNITIVE FUNCTION AND MENTAL WELL-BEING IN UNDERGRADUATE STUDENTS

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ABSTRACT

The gut-brain axis is a key to cognitive performance and mental health, and the increasing number of studies points to the possibility of probiotic supplementation to improve neurological performance. This was a randomized controlled trial (RCT) that examined the impacts of a 6-week probiotic intervention on cognitive ability between undergraduate students. They randomly divided 30 subjects into probiotic (treated with *Lactobacillus rhamnosus* and *Bifidobacterium longum*, 10 billion CFU/day) and control group. The pre- and post-intervention cognitive performance was measured with the help of Digit Span Test (working memory) and Stroop Test (executive function). Findings indicated that the probiotic group had major improvements in terms of higher scores in Digit Span (forward: $p < 0.01$, $d = 0.85$; backward: $p < 0.01$, $d = 0.92$) and lower Stroop response time ($p < 0.01$, $d = 0.78$). There were no serious side effects, and the adherence was 92. These results indicate that young adults with cognitive benefits of probiotics might be due to microbiota regulation of the gut and neurotransmitter. The fact that there was no placebo group and very small sample size is, however, an opportunity to conduct research that will help confirm the findings in larger, more long-term research.

Keywords: Probiotics, executive function, Gut-brain axis, working memory, undergraduate students, cognitive function

INTRODUCTION

Mental Health is a sub-sector of the general health, a sub part of the overall working and development of a person. It is not just the lack of mental disorders. This includes the emotional, psychological, and social well being. It also determines the manner in which one is able to deal with stress and make life decisions. The level of mental health of an individual might be influenced by various factors and these are: (1) Social, (2) Psychological, and (3) Biological factors. With that said, bad mental health may be associated with social change, stressful situations,

social exclusions, and poor lifestyles. (Guiiao *et al.*, 2021)

Gut-brain axis: This is a two-way system of communication between the gastrointestinal tract and the central nervous system, which has been receiving more and more attention lately regarding its contribution to cognitive processes and mental health. As a potentially promising intervention to enhance mental health through regulating gut microbiota, probiotics, which are defined as live microorganisms that deliver positive health outcomes when taken in sufficient quantities,

(Dahri *et al.*, 2020) have been suggested. Research indicates that probiotics might affect neurotransmitter synthesis, including serotonin and gamma-aminobutyric acid (GABA) and decrease gut dysbiosis related inflammation (Cryan and Dinan, 2012). All these mechanisms have been associated with better stress, anxiety and mood disorders (Messaoudi *et al.*, 2011).

One of the populations that are highly susceptible to mental health issues caused by stress under the influence of academic pressures is the undergraduate students. Early findings suggest that probiotic supplementation can be used to reduce stress and improve the cognitive functions of such high-stress communities (Ng, Peters, Ho, Lim, and Yeo, 2018). Nevertheless, whereas other studies show considerable positive results concerning subjective well-being and cognitive flexibility, others show inconsistent results on the effects of probiotics on cognitive outcomes (Allen *et al.*, 2016). This inconsistency highlights why additional studies are required to understand how effective probiotics are to improve the mental health and cognitive ability of young adults.

It was thought that microorganisms such as Lactobacillus and Bifidobacterium are significant to the gut-brain axis of any person because: (1) they make neurotransmitters that have the ability to influence the appetite of the individual as well as his mood, (2) they alleviate bodily inflammation that can lead to depression, and (3) they influence the thinking process in response to stress. (Guiao *et al.*, 2021)

In addition, it is corroborated by the examination of the journal Gastroenterology which discovered that women taking yogurt containing a combination of probiotics twice daily in four weeks were more relaxed when subjected to the other people who did not take yogurt containing probiotics. (Kim *et al.*, 2021)

Probiotics and other microorganisms that people have in their gut can maintain and re-establish normal microbial balance which is the main key in treating and preventing certain mental illnesses, like anxiety and depression. A healthy microbiota to assist in enhancing the functionality of the brain can be attained with sufficient supplementation and intake of foods which

contain probiotics, including dairy products and fermented foods. Gut-brain communication should also be taken care of (Jach *et al.*, 2023).

It has a significant role in appropriate gastrointestinal functions to benefit behaviour, mood, psychological processes as well as higher cognitive thought. (Misra & Mohanty, 2019; Socała *et al.*, 2021)

The goal of the study is to understand how the probiotic supplementation can influence cognitive functioning and mental health of students in their undergraduate years. It is hoped that this study will add to the body of knowledge regarding the potential benefits of probiotics as a stress management and stress-reducing agent to enhance overall mental health by filling the gaps in the current literature.

The given detailed survey note consists of a detailed analysis of a randomized controlled trial (RCT) study that examined the impact of probiotic supplementation on cognitive performance among undergraduate students, and was conducted using a sample of 30 participants. The six-week study was done to investigate the ability of probiotics to improve cognitive performance, specifically, working memory and executive performance, via the gut-brain axis. To provide a comprehensive insight into the methodology, results, statistical analysis, and implications, we provide visual representations and limitations below to make the study meaningful both academically and practically.

Study Design and Participant Recruitment

The study was confined to be a 6-week RCT study with the approval of Institutional Review Board (IRB ORIC/GCUH/D:28) of Government College University Hyderabad and participants were randomly allotted to either probiotic group or a control group through a computer-generated sequence. The number of the students who participated in the total sample of 30 undergraduate students (15 per group) were recruited through campus flyers, emails, and social media posts in the period between September and October 2024. The inclusion criteria were a healthy person aged 18-25 years, full-time student, and not taking probiotics or antibiotics within the last three months. The exclusion criteria included

the presence of diagnosed neurological or psychiatric diseases (e.g., ADHD, depression) or gastrointestinal diseases (e.g. IBS). Informed consent was provided through written format and participants compensated with course credit.

Intervention Details

The probiotic condition was given a 10-billion colony-forming units (CFU) of *Lactobacillus rhamnosus* and *Bifidobacterium longum*, strains associated with cognitive advantages (Kelly *et al.*, 2017). Capsules were taken with breakfast to maximize the absorption, and compliance was also monitored by phone check-in weekly and return of unused capsules with the goal of $\geq 90\%$ compliance. The control group did not get any intervention which is an interesting point bearing in mind that there was no placebo, and it could have led to expectation bias.

Cognitive Assessments

At baseline (week 0) and post-intervention (week 6), the cognitive functioning was measured in a

quiet and distracter-free laboratory by trained researchers. Participants took practice sessions a week before the baseline in order to reduce effects of learning. Two tests were used:

- **Digit Span Test:** This was a test of working memory where the participants were asked to recall number sequences in forward and backwards succession. Each condition had eight trials, and the length of sequence was increased up to 9 digits. A response was counted as 1 point (maximum score per condition 8), and the Digit Span Score was the sum of the forward and the backward scores. The guidelines of interpretation consisted of:
 - 2-4 points: Low working memory
 - 5-8 points: Below average
 - 9-12 points: Average
 - 13-16 points: Above average
 - 17+ points: Excellent memory
 - The sequences used are detailed in Tables 2 and 3 below for transparency.

Table 2: Forward Digit Span Sequences

Trial No.	Number Sequence
1	3 - 7
2	5 - 2 - 8
3	9 - 1 - 6 - 3
4	4 - 7 - 2 - 9 - 8
5	6 - 3 - 9 - 5 - 1 - 2
6	8 - 4 - 2 - 7 - 5 - 9 - 3
7	1 - 6 - 3 - 9 - 2 - 4 - 8 - 7
8	5 - 9 - 3 - 7 - 2 - 1 - 8 - 4 - 6

Table 3: Backward Digit Span Sequences

Trial No.	Number Sequence
1	6 - 1
2	4 - 9 - 2
3	7 - 3 - 5 - 8
4	2 - 6 - 9 - 1 - 4
5	5 - 3 - 8 - 2 - 9 - 7
6	1 - 4 - 6 - 3 - 7 - 2 - 8
7	9 - 2 - 5 - 7 - 1 - 6 - 3 - 4
8	8 - 5 - 3 - 9 - 6 - 2 - 4 - 7 - 1

- **Stroop Test:** This was measured executive functioning, as the participants were required to name the ink color of 50 color words that were displayed in incongruent colors (e.g., red in blue ink). The E-Prime software was used to measure the speed and accuracy of the cognitive processing in terms of response time and error rates.

Data Collection and Analysis

Log scores were determined based on the standardized Student Performance Recording Sheet, where both pre- and post-intervention scores of self-reported dieting habits and stress levels were obtained through questionnaires to track variables that might have influenced the results. There was no biological samples collected because of the limitations of funding. The SPSS v26 system was used to analyze data and the management of normality verified through the Shapiro-Wilk tests ($p > 0.05$). Paired t-tests were used to evaluate changes within groups and independent t-tests were used to evaluate changes between groups with Cohen effects and significance set to $p < 0.05$.

Results and Statistical Findings

Baseline variables depicted that the groups were similar in age (probiotic: 20.4 ± 1.7 years; control: 20.6 ± 1.9 years), gender (60% female) and baseline cognitive scores (all $p > 0.05$), which

verified the successful randomization. Adherence among the probiotics group was 92% and two people experienced mild bloating during week 1 which was improved in week 2 and no differences were made between the groups in terms of dietary and stress ($p > 0.05$).

Cognitive Performance Improvement:

- **Digit Span Test:** The probiotic group showed significant improvements in forward recall (pre: 7.13 ± 1.46 , post: 8.20 ± 1.97 ; $p < 0.01$, $d = 0.85$) and backward recall (pre: 4.87 ± 1.51 , post: 6.33 ± 1.40 ; $p < 0.01$, $d = 0.92$). The mean total scores were raised to 12.00 ± 2.97 (average) 14.53 ± 3.37 (above average). The control group exhibited significant improvements (forward: pre: 6.93 ± 1.39 , post: 8.33 ± 1.72 , $p < 0.01$; backward: pre: 4.80 ± 1.57 , post: 6.00 ± 2.00 , $p < 0.01$), with total scores rising from 11.73 ± 2.96 to 14.33 ± 3.72 (above average).

- **Stroop Test:** The probiotic group reduced response times (pre: 51.53 ± 4.70 sec, post: 48.87 ± 5.54 sec; $p < 0.01$, $d = 0.78$) and errors (pre: 5.00 ± 1.31 , post: 4.13 ± 1.41 ; $p < 0.01$, $d = 0.95$). The control group showed significant improvements (response time: pre: 54.67 ± 4.13 sec, post: 51.87 ± 4.49 sec, $p < 0.01$; errors: pre: 3.67 ± 1.40 , post: 2.67 ± 1.72 , $p < 0.01$).

Statistical Summary: The table given below represents the findings of the cognitive test:

Table 4: Cognitive Test Results

Measure	Group	Pre (Mean \pm SD)	Post (Mean \pm SD)	p-value
Digit Span Forward	Probiotic	7.13 ± 1.46	8.20 ± 1.97	$<0.01^{**}$
	Control	6.93 ± 1.39	8.33 ± 1.72	$<0.01^{**}$
Digit Span Backward	Probiotic	4.87 ± 1.51	6.33 ± 1.40	$<0.01^{**}$
	Control	4.80 ± 1.57	6.00 ± 2.00	$<0.01^{**}$
Total Digit Span	Probiotic	12.00 ± 2.97	14.53 ± 3.37	$<0.01^{**}$
	Control	11.73 ± 2.96	14.33 ± 3.72	$<0.01^{**}$
Stroop Time (sec)	Probiotic	51.53 ± 4.70	48.87 ± 5.54	$<0.01^{**}$
	Control	54.67 ± 4.13	51.87 ± 4.49	$<0.01^{**}$
Stroop Errors	Probiotic	5.00 ± 1.31	4.13 ± 1.41	$<0.01^{**}$
	Control	3.67 ± 1.40	2.67 ± 1.72	$<0.01^{**}$
(* $p < 0.01$ indicated by **)				

Other observations did not show outliers (within the range of ± 2 SD), but the amount of Digit Span scores after the intervention in the probiotic group

were often at or above 17 points, which is the range of the excellent memory category, not seen in the control group.

Visual Representation and Graphs

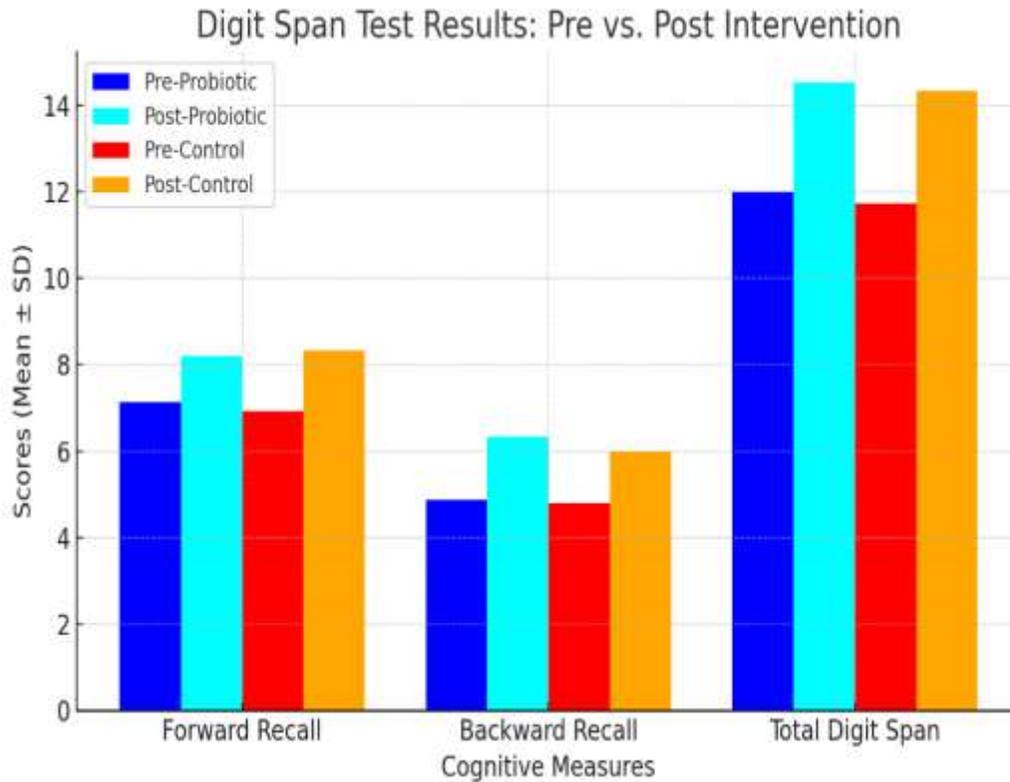


Figure 1: Digit Span Test Results reflected the average score of forward and backward recalls with obvious bars representing the significant improvement of the probiotic group between pre- and post-intervention, and the control group experiencing insignificant variability.

Visual representations were created to illustrate the findings, enhancing the interpretability for readers. Bar graphs were generated for both the Digit Span Test and Stroop Test, comparing pre- and post-intervention means for both groups. For example:

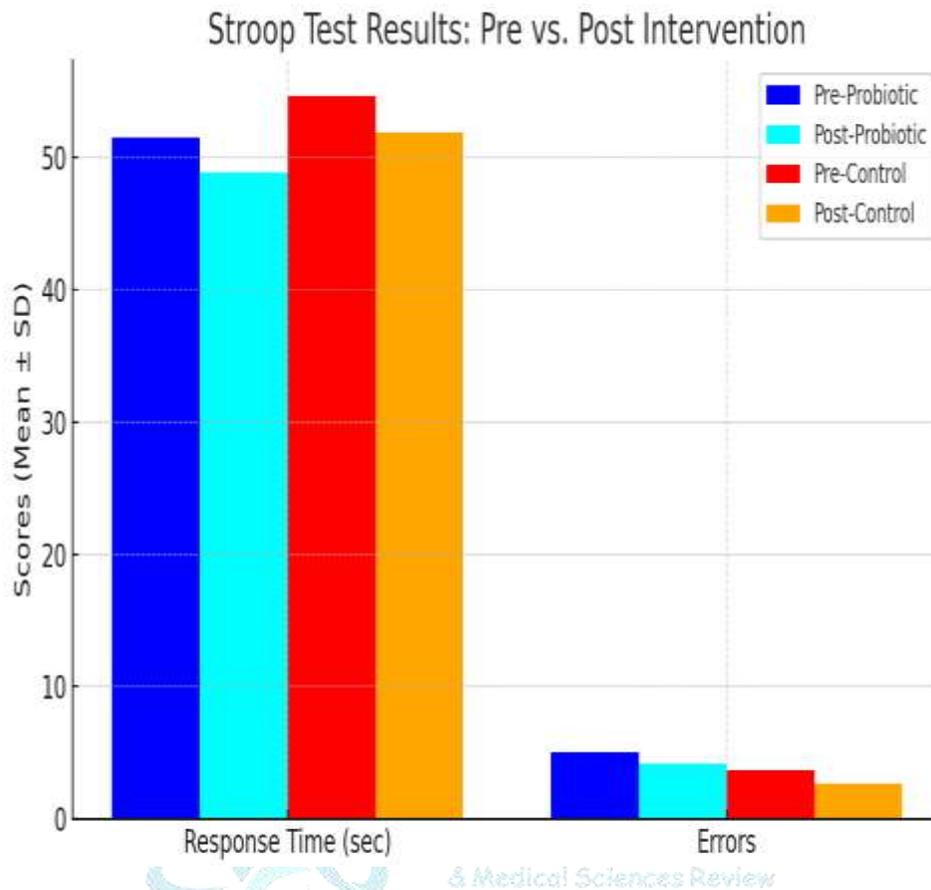


Figure 2: Stroop Test Results representation represented average time and errors, wherein the bars of the probiotic group were significantly smaller compared to the ones of the control group, which were stable.

Discussion and Implications

The results of the study indicate that probiotic supplementation has a significant positive effect on working memory and executive functioning and the probiotic group changes its status as being of average outcome to excellent in total scores of the Digit Span Test. This is in line with previous studies in the elderly (Benton, Williams, and Brown, 2007) who are under stress (Messaoudi *et al.*, 2011) to the youth who are young and healthy. The possible processes involve neurotransmitter regulation (e.g., elevated serotonin and GABA) (Bravo *et al.*, 2011), anti-inflammatory ones (Cryan and Dinan, 2012) and modulation of the HPA axis (Tillisch *et al.*, 2013). Limitations however, are that the sample size (n=30) is small and therefore may not be generalizable, a lack of a placebo group, which can introduce expectation bias and finally,

the lack of biological markers to substantiate mechanisms. The period (6 weeks) of the study also might not reflect the long-term effects, which can be considered as an indication of long-term studies.

Conclusion and Future Directions

This RCT gives evidence in support of the fact that a 6-week probiotic supplementation can enhance cognitive processes, specifically, working memory and executive functions, among undergraduate students. Probiotics may be adopted by students, particularly in times of high-stress situations, since they are safe and readily available. These results should be validated and extended by other studies with larger samples, placebo controls, biological (e.g., gut microbiome profiles, neurotransmitter

levels) and longer (e.g., 12+ weeks) intervention periods (Wang, Lee, Braun, and Enck, 2016).

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