American Journal of Food Science and Health
Vol. 6, No. 1, 2020, pp. 1-11
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

The Effect of Probiotics on Short-term Memory and Continuous Concentration Among Medical Students in Melaka-Manipal Medical College

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Abstract

Probiotics have been used in medicine for the treatment of many disorders, such as IBS, type 2 diabetes and severe burns. Probiotics are now also being studied for their effect on the brain, through the 'gut-brain axis', and have been studied in many psychiatric disorders and neurodegenerative disorders. They are generally considered safe, but their adverse effects in human subjects is still being studied. Our objective is to compare the effect of food probiotics versus probiotics tablets on short term memory and concentration, and also to identify and document any adverse effect due to probiotics consumption, among undergraduate students in Malaysia. A randomized controlled trial (RCT), parallel design was conducted from September 2019 to October 2019. Purposive sampling was used to select 40 students based on inclusion and exclusion criteria, who were then randomized into two groups of 20 students each. Baseline testing was done with the Human Benchmark Test and TestMyBrain continuous concentration. Each group was then given either the food probiotic or tablet probiotic for 5 days, and the tests were repeated after the 5th day. The mean difference between the two groups, as well as before and after the intervention was calculated. Chi square test, unpaired and paired t tests were used. Level of significance was set at $P < 0.05$. Based on the results from our study, there were no significant differences in visual memory, verbal memory, number memory and continuous concentration between intervention and control groups. We found that after 5 days of consuming probiotics, there was an increase in verbal, visual, number memory test scores, as well as continuous concentration scores in both food as well as tablet probiotics. When comparing the scores before and after food probiotics consumption, there were increases in verbal memory, visual memory and number memory, as well as continuous concentration but these were not statistically significant. Additionally, when comparing the scores before and after tablet probiotics, visual memory and continuous concentration had decreased; however, the participants who consumed probiotics for 5 days had higher visual memory and number memory scores than participants in the control group. Based on out study, probiotics can improve short term memory such as verbal, visual and number memory, and continuous concentration, with no significant differences between food and tablet probiotics. The authors recommend food probiotics, due to their improved safety profile and relative economic value, to aid the improvement of short term memory and continuous concentration.

Keywords

Probiotics, Short-Term Memory, Continuous Concentration, RCT

Received: October 24, 2019 / Accepted: December 26, 2019 / Published online: February 10, 2020

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1. Introduction

Probiotics are defined as live organisms that when administered in adequate amounts, confer a beneficial effect for the host [1]. Since 2001, research into probiotics has increased, and part of this upsurge has been due to the fact that probiotics represent new frontiers in treatment: using a combination of microbiology and pharmacology, in order to harness existing microorganisms in order to treat ailing animals and humans [1]. Increased unhealthy eating habits, antibiotic therapy, stress, genetic predisposition as well as an unfavorable environment are all factors that have been implicated in the increased imbalance of our gut flora in the present age, and this has further highlighted the need for probiotics [2]. New fields, such as prebiotics and synbiotics, have also cropped up, highlighting the need for better understanding of these microorganisms, and their possible role in therapeutics [3].

Probiotics are active microorganisms, usually of different strains, and their actual mechanism of action in therapeutics is actively debated [4]. They are usually found in the form of tablets (supplementation) and also food such as yoghurt, kimchi, kefir, kombucha and miso among others. There are various types of probiotics supplements available, which contain strains such as *Lactobacilli*, *Bifidobacterium*, *Acidophilus*, yeasts such as *Saccharomyces boulardii*, and many more. It is postulated that in sufficient quantities, they can play a beneficial role in improving the ecological balance of the intestinal tract. Many studies have shown that these microorganisms play an active role in modulating the synthesis of neurotransmitters and other biologically active factors, thereby exerting a wide array of activities in the human body [5]. Probiotics have been used as therapeutics in a wide array of conditions, which include severe burns, anti-obesity treatment, irritable bowel syndrome (IBS) and diabetes [6 – 9].

Probiotics have also been studied for their effect on the brain, due to the bi-directional signaling between the human gut and brain [10]. The 'gut-brain axis' as it is called, is now being used to study the possibilities of new treatment options in existing diseases, as well as change our preconceptions on the pathogenesis of certain diseases, especially psychiatric illnesses [11, 12]. Psychobiotics is a new term that has been coined to represent probiotics used to treat mental illnesses; preclinical and clinical studies have shown that these probiotics can alter the human brain networks involved in emotional and cognitive responses [13]. Psychiatric disorders such as autism-spectrum disorder and depressive disorders have been linked to perturbations in the gut-brain axis, with many mechanisms being put forward for its pathogenesis, as well as opening up discussions into the use of probiotics as a possible treatment [14-15]. Anxiety has also been linked to disturbances in the gut-brain axis, with dysbiosis of the intestinal flora being associated with anxiety; however, treatment modalities with probiotics has not been discussed [16]. Additionally, probiotics have been put forward as a possible treatment modality for neurodegenerative diseases, due to their ability to modulate gut bacterial composition, and thereby brain biochemistry, thereby delaying the onset and progress of these diseases [17]. Probiotics have also been proposed as a treatment option in stress situations, due to their possible effect on the host immunity, which is another factor that affects an individual's ability to adapt to stressful situations. [18-19].

Using the gut-brain axis hypothesis, a double-blind, randomized control pilot trial is also being carried out to assess the efficacy of probiotics in the treatment of depression and anxiety in pregnant women in the Netherlands [20]. Another meta-analysis looked at probiotics for the treatment of depressive symptoms, due to an anti-inflammatory mechanism, where the authors concluded that probiotics should be considered as an adjuvant treatment to current available medication, but also noted that more research needed to be done [21]. Another randomized trial showed probiotics did not affect the brain unless stress induction had occurred; however, stress-induced participants did show a significant increase in working memory performance after supplementation compared to placebo. The authors concluded that probiotics showed a neurocognitive effect which helped working memory performance [22]. Another meta-analysis showed inconclusive results regarding probiotics and stress-related conditions in human subjects; however, the authors noted that this was due to many studies being underway at the time the meta-analysis was conducted, and planned to conduct the meta-analysis again in 5 years, to assess the progress [23]. This wide range of outcomes highlights the importance of more research into the subject, since it is unclear which form of probiotics (food vs tablet, single-strain vs multiple strains) has the best outcome in patients.

Probiotics are generally considered safe for the consumption, they have been classified by the FDA as "Generally Recognized as Safe (GRAS)". However they have been linked to major adverse effects in people with underlying medical conditions. Usually these patients suffer from immune system dysfunction, and serious adverse effects such as severe sepsis, GI ischemia, fungemia and bacteremia has been reported in RCTs in these patient population [28-30]. In healthy patient populations, metabolism upsetting effects, allergic dermatitis, higher risk for allergic rhinitis, increased risk of atopic sensitization, food poisoning and headaches have been reported [30]. Other adverse effects reported have include gas/bloating, constipation,

abdominal pain, rashes, acne, nausea and malaise [30-31]. More seriously, transfer of virulent properties between bacterial strains and intestinal bacteria overgrowth have been reported in long-term clinical studies [32].

As many studies being done in relation of probiotics in memory enhancement, we decided to study the effect of probiotic tablets and yoghurt culture drinks on short term memory and concentration among the students of Melaka Manipal Medical College, Malaysia. The objectives of this study were to compare the effect of food probiotics (in the form of yoghurt drinks) versus probiotics tablets on short term memory and concentration, and also to identify and document any adverse effect due to probiotics consumption. The hypothesis of our study is do probiotics increase short term memory and concentration in the medical student population.

2. Methods

2.1. Study Design, Study Setting and Study Population

We have carried out a Randomized controlled trial, parallel design to compare the consumption of Probiotics (Tablet probiotics vs Food probiotic/yogurt culture drink) and their effects on short term memory (verbal, number, visual memory) and continuous concentration as well as side effects of probiotics among healthy undergraduate, semester 7 students pursuing Bachelor of Medicine & Bachelor of Surgery (MBBS) in Melaka Manipal Medical College (MMMC), Muar Campus, Muar, Johor, Malaysia. This study was held from September 2019 to October 2019.

2.2. Sample Size

The sample size was calculated based on a previous study as reference using the Sample Calc app. 40 participants were asked to participate in the study. 20 participants were randomized into the probiotic tablet group and the other 20 participants were randomized into the yogurt culture drink/food probiotic group. We then continued to calculate the sample size needed using the Sample Calc app on the smartphone. The standard deviation was 35.09 and the size difference was 33.61. The significance level was set to be 0.05 and the power was 80%. A sample size of 17 in each group to detect a difference of 33.61 points, assuming a standard deviation of 35.09 points, power of 80% and a significance level of 0.05 [21]. 20 people in each group were recruited.

2.3. Sampling and Randomization

In this study, we used the non-probability sampling technique (purposive sampling) to obtain sample from our study population and the sample were selected based on the inclusion and exclusion criteria included in the table below. Our study population consisted of 146 students and 40 students volunteered to be in our sample size for this study. These 40 students were randomized into 2 groups, intervention and active control. We applied a randomization block size of 2, and randomized 20 students in the intervention group and 20 students in the active control group using the online web randomizer software (randomizer.org).

Table 1. Inclusion criteria and Exclusion criteria.

Inclusion criteria	Exclusion criteria
Students of age between 18-30 years old	Students who consumes alcohol regularly
Students of any gender	Students who smokes regularly
Students with any race	Students who are on sedative drugs
Students with any nationality	Students who consume illicit drugs
Students with any ethnicity	Students who are allergic to probiotics
Students who are willing to provide written informed consent	Students who suffer from medical conditions e.g.: flu, headache, migraine.

Table 2. Randomization table.

SET 1	SET 2	SET 3	SET 4	SET 5	SET 6	SET 7	SET 8	SET 9	SET 10	SET 11	SET 12	SET 13	SET 14	SET 15	SET 16	SET 17	SET 18	SET 19	SET 20
2	2	1	2	1	2	1	1	2	1	1	1	2	2	1	1	2	2	2	1
1	1	2	1	2	1	2	2	1	2	2	2	1	1	2	2	1	1	1	2

2.4. Intervention Procedure

The intervention of this research was divided into 2 categories, one group with probiotic tablets (21st Century) and another group with food probiotics (Yakult). The probiotic tablet group consisted of 20 randomized students who received 1 tablet each consisting of 2 billion cfu/100mg of *Lactobacillus acidophillus*, *Lactobacillus salivarius*, *Bifidobacterium bifidum*, *Streptococcus thermophillus*. They were given 1 probiotic tablet each after food at the same time, every day for 5 consecutive days as the intervention. The food probiotic group consisted of 20 randomized students who received 1 bottle each of a yogurt culture drink consisting of 10 billion strains of Lactobacillus case, every day after food at the same time for 5 consecutive days as the intervention. Before the intervention began, all 40

participants were asked to undergo the pre-intervention testing comprised of the Human Benchmark test (www.humanbenchmark.com) and the Continuous concentration test. The Human Benchmark test assessed the short-term Visual memory, verbal memory as well as number memory of the students. They then went on to perform the Test My Brain (www.testmybrain.org) Continuous concentration test which assessed the continuous concentration of the participants for 6 minutes straight and the total time for all the tests roughly took around 15 minutes. All the results were recorded accordingly. After the tests, the intervention for each group was started on the next day for 5 consecutive days. The participants of both groups were also told to not consume any sources of probiotics other than the given probiotics during these 5 days of the intervention. The Post-intervention testing was done 24 hours after the last day of the intervention using the same Human Benchmark test to assess the Visual, Verbal and Number memory as well as the Test My Brain continuous concentration test and all the results were again recorded down and compared by us.

3. Data Collection

3.1. Study Procedure

The independent variables in this study were the tablet probiotics and the food probiotics (Yogurt culture drink) and the dependent variables were short term memory (verbal, visual, number) and continuous concentration. First, we divided all the participants into 2 groups. 20 participants were placed into the probiotic tablet group and the other 20 participants were placed in the food probiotic group (yogurt culture drink). Before the study, all the participants were given the informed consent and they were also informed that they could choose not to participate & quit the study halfway if they experienced major side effects or unable to tolerate the probiotics. Next, the questionnaire was distributed to all participants before starting the test and intervention as to satisfy the inclusion and exclusion criteria. Participants who successfully fulfilled the inclusion criteria then proceeded to do the Human Benchmark test and Test My Brain Continuous Concentration test as a pre-intervention testing and all the scores were recorded down. After that, we started the 5 days of intervention and following the intervention, the participants were again asked to undergo the Human Benchmark test and Test my brain continuous concentration testing. The pre-intervention and post-intervention scores of the participants from both groups were compared to observe the effects of tablet probiotics vs food probiotics on short term memory (Verbal, Visual, Number) and continuous concentration.

3.2. Data Collection Instrument

Human benchmark was an application where an individual can measure one's ability and identify what was his/her strengths and weaknesses. This application tests the brain based 6 categories which are number, reaction time, verbal, visual, hearing and typing memory. For the number memory test, each number will appear for 5 seconds for the participant to memorize. The number starts with 1 digit and an additional digit was added after each level. Participant was required to enter the number appeared immediately after it has disappeared. The score was the number of digits which the participant can remember. As for the verbal memory test, it measures the number of words the participant can memorize at once. The number of words that were needed to remember increased continually. The participant had 3 chances until scores are shown. The score refers to the number of turns while the participant lasted. For the visual memory test, number of white tiles were flashed at every level and the participant was required to pick the same tiles correctly after the tiles reset. The number of white tiles increased with level. The participant lost a life after missing 3 tiles on a level. Each participant had 3 lives in one attempt. The score was the level which the participant reached. Test My Brain was also an online application where the individual can measure continuous concentration. Here, the participant was asked to remember two consecutive numbers, add them, and check whether the resulting sum was greater or less than 10. Then, another number would be displayed, and the participant was required to continue the process until the participant took too long to reply. The resulting score (out of 100) was displayed, along with the average for the population, which was 54.

3.3. Data Processing and Analysis

Microsoft Excel version 2007 and the values were doubled checked to prevent any errors. From Microsoft Excel, the information was then used to statistical calculations, using analytical software Epi Info version 7.2 from Centers for disease control and prevention website (CDC) and Graph Pad.

For descriptive statistical analysis, we included mean, standard deviation (SD), frequency and percentage. Mean and standard deviation were used to analyze the score of visual memory, number memory and verbal memory as well as continuous concentration. Frequency and percentage were calculated for adverse effects of probiotics consumption. The significant level was set at $P<0.05$, in which any values of more than 0.05 were considered not statistically significant. Mean plot was used to represent the numerical data of verbal, visual and number memory with continuous concentration.

Table 3. Statistical test used for data analysis.

Independent Variable	Dependent Variable	Statistical Test
TABLET PROBIOTIC versus FOOD PROBIOTIC	VERBAL MEMORY	UNPAIRED t-test
	VISUAL MEMORY	UNPAIRED t-test
	NUMBER MEMORY	UNPAIRED t-test
	CONTINUOUS CONCENTRATION	UNPAIRED t-test
BEFORE VS AFTER IN TABLET PROBIOTIC GROUP (21st CENTURY PROBIOTICS)	VERBAL MEMORY	PAIRED t-test
	VISUAL MEMORY	PAIRED t-test
	NUMBER MEMORY	PAIRED t-test
	CONTINUOUS CONCENTRATION	PAIRED t-test
BEFORE VS AFTER IN FOOD PROBIOTIC GROUP (YAKULT)	VERBAL MEMORY	PAIRED t-test
	VISUAL MEMORY	PAIRED t-test
	NUMBER MEMORY	PAIRED t-test
	CONTINUOUS CONCENTRATION	PAIRED t-test

4. Ethical Considerations

An Informed consent consist of all relevant information about our studies was distributed to 40 participants. The participants were given the option to participate in study and none were forced into participating. The form also had a concise explanation about the study and its requirements. Furthermore, participants were also informed that all the data collected and gained will be kept confidential and they could choose to withdraw from studies whenever they want to. The study was approved by the Research Ethics Committee, Faculty of Medicine, Melaka Manipal Medical College.

5. Results

A total of 40 students participated in his study and were randomized into two groups, which were receiving food probiotics (n=20) and receiving tablet probiotics (n=20). Table 4 shows baseline characteristics between the food probiotics and tablet probiotics. The mean age of participants in the food probiotics group was 22.20 (SD=1.12), while the mean age for the tablet probiotics group was 22.40 (SD=1.10). As for gender, number of male participants (55.00%) was slightly higher than female participants (45.00%) in the food probiotics group and in the tablet probiotics group, number of both male and female participants were equal. Majority of the participants were Indians and others with a shared percentage of 32.50% in both food and tablet probiotics groups. Most of the participants were Malaysians with 70.00% in food probiotics and 75.00% in tablet probiotics. Generally, the participants were on a non-vegetarian diet consisting of 100% in the food probiotics group and 90% in the tablets probiotics group. Based on the duration of sleep, the participants mostly sleep less than 7 hours in which receiving food probiotics (80.00%) and receiving tablet probiotics (90.00%). For most of the participants, they were not practicing any meditation or mental exercise with 90.00% in food probiotics and 85.00% in tablet probiotics. Lastly, majority of participants were doing physical exercises in both food (80.00%) and tablet (70.00%) probiotics group.

Table 4. Baseline characteristics between tablet probiotics (n=20) and food probiotics (n=20).

VARIABLES		Food Probiotics N (%)	Tablet Probiotics N (%)	Total (n=40)
AGE[a]		22.20 (1.12)	22.40 (1.10)	22.30 (1.14)
GENDER	MALE	11 (55.00)	10 (50.00)	21 (52.50)
	FEMALE	9 (45.00)	10 (50.00)	19 (47.50)
RACE	MALAY	6 (30.00)	4 (20.00)	10 (25.00)
	CHINESE	2 (10.00)	2 (10.00)	4 (10.00)
	INDIAN	5 (25.00)	8 (40.00)	13 (32.50)
	OTHERS	7 (35.00)	6 (30.00)	13 (32.50)
NATIONALITY	MALAYSIAN	14 (70.00)	15 (75.00)	29 (72.50)
	NON-MALAYSIAN	6 (54.55)	5 (45.45)	11 (27.50)
DIET	VEG	0 (0.00)	2 (10.00)	2 (5.00)
	NON-VEG	20 (100.00)	18 (90.00)	38 (95.00)
PROBIOTICS	YES	5 (25.00)	9 (45.00)	14 (35.00)
	NO	15 (75.00)	11 (55.00	26 (65.00)
SLEEP	LESS THAN 7HRS	16 (80.00)	15 (75.00)	31 (77.50)
	BETWEEN 7 TO 9 HRS	4 (20.00)	5 (25.00)	9 (22.50)
	MORE THAN 9HRS	0 (0.00)	0 (0.00)	0 (0.00)
MEDITATION OR MENTAL EXERCISE	YES	2 (10.00)	3 (15.00)	5 (12.50)
	NO	18 (90.00)	17 (85.00)	35 (87.50)
PHYSICAL EXERCISE	YES	16 (80.00)	14 (70.00)	30 (75.00)
	NO	4 (20.00)	6 (30.00)	10 (25.00)

[a]Mean (SD).

Table 5. Probiotic consumption between food probiotics (n=20) and tablet probiotics (n=20).

VARIABLES		Food Probiotics	Tablet Probiotics	TOTAL
PROBIOTICS	YES	5 (25.00)	9 (45.00)	14 (35.00)
	NO	15 (75.00)	11 (55.00)	26 (65.00)
TYPE OF PROBIOTICS	YOGHURT DRINK	4 (80.00)	9 (100.00)	11 (92.86)
	TABLETS	1 (20.00)	0 (0.00)	1 (7.14)
FREQUENCY OF CONSUMPTION	DAILY	0 (0.00)	1 (11.11)	1 (7.14)
	2-3 TIMES/WEEK	0 (0.00)	1 (11.11)	1 (11.11)
	ONCE A WEEK	3 (60.00)	7 (77.78)	10 (71.43)
AMOUNT OF CONSUMPTION	1	3 (60.00)	6 (66.67)	9 (64.29)
	2	2 (40.00)	3 (33.33)	5 (35.71)

Table 5 shows probiotic consumption between food probiotics and tablet probiotics along with the types, frequencies and amount among the participants. For most of the participants, they were not consuming any probiotics with a percentage 75.00% in food probiotics and 55.00% in tablets probiotics. Among the participants consuming probiotics, most of them were having yoghurt drinks with 80.00 in food probiotics group and 100% in tablet probiotics. Participants who were consuming probiotics, 60.00% from food probiotics and 77.78% from tablet probiotics, consumed it once a week. As for the amount, most of them consumed only one in which food probiotics had a percentage of 60 and tablet probiotics of 66.67%.

Table 6. Short term memory and concentration between food probiotics (n=20) and tablet probiotics (n=20) UNPAIRED T TEST (BEFORE).

VARIABLE	MEAN (SD)		MEAN DIFFERENCE (95% CI)	t-STATISTIC (df)	*P* - VALUE
	FOOD N = 20	TABLET N = 20			
VISUAL MEMORY (before)	9.45 (1.88)	10.55 (2.72)	-2.60 to 0.40	-1.49	0.145
VERBAL MEMORY (before)	41.30 (25.73)	52.85 (28.69)	-29.00 to 5.90	-1.34	0.188
NUMBER MEMORY (before)	9.55 (1.79)	9.30 (2.34)	-1.09 to 1.58	0.38	0.707
CONTINUOUS CONCENTRATION (before)	48.60 (10.94)	49.95 (8.26)	-7.55 to 4.85	-0.44	0.662

Table 6 shows the comparison of visual memory, verbal memory, number memory and continuous concentration memory between food probiotics and tablets probiotics before the consumption of probiotics. The mean (SD) of the visual memory score in the food probiotics group was 9.45 (1.88) and in the tablet probiotics group, the mean was 10.55 (2.72). The mean differences (95% CI) and t-statistics of visual memory score between the food probiotics group and tablet probiotics group were -2.60 to 0.40 and -1.49 respectively. The P- value obtained for visual memory score was 0.145, there was no significant difference in the visual memory score between the food probiotics group and tablet probiotics group before consuming them. The mean (SD) of the verbal memory score in the food probiotics group was 41.30 (25.73) and in the tablet group, the mean was 52.85 (28.69). The mean difference (95%CI) and t-statistics of verbal memory score between the food probiotics group and tablet probiotics group were -29.00 to 5.90 and -1.34. The P-value obtained for verbal memory score was 0.188. Hence, there was no significant difference in the verbal memory score between the food probiotics group and tablet probiotics group before the consumption of them. The mean (SD) of the number memory score in the food probiotics group was 9.55 (1.79) and in the tablet probiotics group, the mean was 9.30 (2.34). The mean differences (95% CI) and t-statistics of number memory score between the food probiotics group and tablet probiotics group were -1.09 to 1.58 and 0.38 respectively. The P-value obtained for number memory score was 0.707, there was no significant differences in the number memory score between the food probiotics group and tablet probiotics group before the consumption of probiotics. The mean (SD) of the continuous concentration test in the food probiotics group was 48.60 (10.94) and in the tablet probiotics group, the mean was 49.95 (8.26). The mean difference (95%CI) and t-statistics of continuous concentration score between the food probiotics group and tablet probiotics group were -7.55 to 4.85 and -0.44 respectively. The P-value obtained for total continuous concentration score was 0.662. Therefore, there was no significant difference in the continuous concentration score between the food probiotics group and tablet probiotics group before the consumption of probiotics.

Table 7. Short term memory and concentration between food probiotics (n=20) and tablet probiotics (n=20) UNPAIRED T TEST (after).

VARIABLE	MEAN (SD)		MEAN DIFFERENCE (95% CI)	t – STATISTIC (df)	*P* - VALUE
	FOOD N = 20	TABLET N = 20			
VISUAL MEMORY (after)	10.10 (2.33)	10.15 (1.69)	-1.36 to 1.26	-0.08	0.939
VERBAL MEMORY (after)	56.15 (33.57)	64.40 (31.59)	-29.12 to 12.62	-0.80	0.429
NUMBER MEMORY (after)	9.75 (1.86)	9.90 (1.80)	-1.32 to 1.02	-0.26	0.797
CONTINUOUS CONCENTRATION (after)	49.90 (9.69)	48.80 (7.68)	-4.50 to 6.70	0.40	0.693

Table 7 shows the comparison of visual memory, verbal memory, number memory and continuous concentration memory between food probiotics and tablet probiotics group after the consumption of probiotics. The mean (SD) of the visual memory score in the food probiotics group was 10.10 (2.33) and in the tablet probiotics group, the mean was 10.15 (1.69). The mean differences (95% CI) and t-statistics of visual memory score between the food probiotics group and tablet probiotics group were -1.36 to 1.26 and -0.08 respectively. The P-value obtained for visual memory score was 0.939, there was no significant difference in the visual memory score between the food probiotics group and tablet probiotics group before taking the interventions. The mean (SD) of the verbal memory score in the food probiotics group was 56.15 (33.57) and in the tablet probiotics group, the mean was 64.40 (31.59). The mean difference (95%CI) and t-statistics of verbal memory score between the food probiotics group and control group were -29.12 to 12.62 and -0.80. The P- value obtained for verbal memory score was 0.429. Hence, there was no significant difference in the verbal memory score between the food probiotics group and tablet probiotics group before the consumption of probiotics. The mean (SD) of the number memory score in the food probiotics group was 9.75 (1.86) and in the tablet probiotics group, the mean was 9.90 (1.80). The mean differences (95% CI) and t-statistics of number memory score between the food probiotics group and tablet probiotics group were -1.32 to 1.02 and -0.26 respectively. The P- value obtained for number memory score was 0.797, there was no significant differences in the number memory score between the food probiotics group and tablet probiotics group before the consumption of probiotics. The mean (SD) of the continuous concentration test in the food probiotics group was 49.90 (9.69) and in the tablet probiotics group, the mean was 48.8 (7.68). The mean difference (95%CI) and t-statistics of continuous concentration score between the food probiotics group and tablet probiotics group were -4.50 to 6.70 and 0.40 respectively. The P- value obtained for total continuous concentration score was 0.693. Therefore, there was no significant difference in the continuous concentration score between the food probiotics group and tablet probiotics group before the consumption of probiotics.

Table 8. Short term memory and concentration between pre-test and post-test among food probiotics.

VARIABLE	MEAN (SD)		MEAN DIFFERENCE (95%)	t – STATISTIC (df)	*P* - VALUE
	PRE-TEST	POST TEST			
VISUAL MEMORY	9.45 (1.88)	10.10 (2.34)	-0.65 (-1.79 to 0.49)	1.19 (19)	0.247
VERBAL MEMORY	40.58 (26.23)	56.74 (34.38)	-16.16 (-33.75 to 1.43)	1.93 (18)	0.070
NUMBER MEMORY	9.42 (1.74)	9.74 (1.91)	-0.32 (-1.68 to 1.05)	0.49 (18)	0.633
CONTINUOUS CONCENTRATION	47.94 (11.31)	49.11 (9.90)	-1.17 (-9.10 to 6.76)	0.31 (17)	0.760

Table 8 shows the short-term memory and concentration between pre-test and post-test among food probiotics. The mean (SD) of the visual memory score in pre-test was 9.45 (1.88) and in the post test was 10.10 (2.34). The mean differences (95% CI) and t-statistics of visual memory score between the pre-test and post-test were -0.65 (-1.79 to 0.49) and 1.19. P-value obtained for visual memory score was 0.247. The mean (SD) of the verbal memory score in pre-test was 40.58 (26.23) and in the post test was 56.74 (34.38). The mean differences (95% CI) and t-statistics of visual memory score between the pre-test and post-test were -16.16 (-33.75 to 1.43) and 1.93. P-value obtained for verbal memory score was 0.070. The mean (SD) of the number memory score in pre-test was 9.42 (1.74) and in the post test was 9.74 (1.91). The mean differences (95% CI) and t-statistics of number memory score between the pre-test and post-test were -0.32 (-1.68 to 1.05) and 0.49. P-value obtained for number memory score was 0.633. The mean (SD) of the continuous concentration in pre-test was 47.94 (11.31) and in the post test was 49.11 (9.90). The mean differences (95% CI) and t-statistics of continuous concentration memory score between the pre-test and post-test were -1.17 (-9.10 to 6.76) and 0.31. P-value obtained for continuous concentration score was 0.760.

Table 9. Short term memory and concentration between pre-test and post-test among tablet probiotics.

VARIABLE	MEAN (SD)		MEAN DIFFERENCE (95%)	t – STATISTIC (df)	*P* - VALUE
	PRE-TEST	POST TEST			
VISUAL MEMORY	10.58 (2.80)	10.00 (1.60)	0.58 (-0.82 to 1.98)	0.87 (18)	0.398
VERBAL MEMORY	52.85 (28.69)	64.40 (31.59)	-11.55 (-24.54 to 1.44)	1.86 (19)	0.078
NUMBER MEMORY	9.30 (2.34)	9.90 (1.80)	-0.60 (-1.94 to 0.74)	0.94 (19)	0.359
CONTINUOUS CONCENTRATION	50.26 (8.36)	48.37 (7.63)	1.89 (-4.23 to 8.02)	0.65 (18)	0.524

Table 9 shows the short-term memory and concentration between pre-test and post-test among tablet probiotics. The mean (SD) of the visual memory score in pre-test was 10.58 (2.80) and in the post test was 10.00 (1.60). The mean differences (95% CI) and t-statistics of visual memory score between the pre-test and post-test were 0.58 (-0.82 to 1.98) and 0.87. P-value obtained for visual memory score was

0.398, there was no significance difference between pre-test and post-test on visual memory score. The mean (SD) of the verbal memory score in pre-test was 52.8 (28.69) and in the post test was 64.40 (31.59). The mean differences (95% CI) and t-statistics of verbal memory score between the pre-test and post-test were -11.55 (-24.54 to 1.44) and 1.86. P-value obtained for verbal memory score was 0.078, there was no significance difference between pre-test and post-test on verbal memory score. The mean (SD) of the number memory score in pre-test was 9.30 (2.34) and in the post test was 9.90 (1.80). The mean differences (95% CI) and t-statistics of number memory score between the pre-test and post-test were -0.60 (-1.94 to 0.74) and 0.94. P-value obtained for number memory score was 0.359, there was no significance difference between pre-test and post-test on number memory score. The mean (SD) of the continuous concentration score in pre-test was 50.26 (8.36) and in the post test was 48.37 (7.63). The mean differences (95% CI) and t-statistics of continuous concentration memory score between the pre-test and post-test were 1.89 (-4.23 to 8.02) and 0.65. P-value obtained for continuous concentration score was 0.524, there was no significance difference between pre-test and post-test on continuous concentration test.

Table 10. Adverse events between food probiotics (n=20) and tablet probiotics (n=20).

Adverse effects		Food probiotics	Tablet probiotics	P-value
Gas / Bloating	Yes	0 (0.00)	1 (100.00)	0.311
	No	20 (51.28)	19 (48.72)	
Constipation	Yes	1 (100.00)	0 (0.00)	0.311
	No	19 (48.72)	20 (51.28)	
Headache	Yes	0 (0.00)	0 (0.00)	1.000
	No	20 (100.00)	20 (100.00)	
Abdominal pain	Yes	0 (0.00)	1 (100.00)	0.311
	No	20 (51.28)	19 (48.72)	
Cramps	Yes	1 (33.33)	2 (66.67)	0.548
	No	19 (51.35)	18 (48.65)	
Rashes	Yes	0 (0.00)	1 (100.00)	0.311
	No	20 (51.28)	19 (48.72)	
Acne	Yes	0 (0.00)	1 (100.00)	0.311
	No	20 (51.28)	19 (48.72)	
Diarrhea	Yes	0 (0.00)	2 (100.00)	0.147
	No	20 (52.63)	18 (47.37)	
Brain fog	Yes	1 (50.00)	1 (50.00)	1.000
	No	19 (50.00)	19 (50.00)	
Vomiting	Yes	0 (0.00)	0 (0.00)	1.000
	No	20 (100.00)	20 (100.00)	
Nausea	Yes	1 (100.00)	0 (0.00)	0.311
	No	19 (48.72)	20 (51.28)	
Malaise	Yes	0 (0.00)	0 (0.00)	1.000
	No	20 (100.00)	20 (100.00)	

Table 10 shows the adverse events between food probiotics and tablet probiotics among the participants. For gas / bloating, none of the participants had experience it from the food probiotics (51.28%) and in the tablet probiotics (48.72%). Only one participant from food probiotics experienced constipation (100.00%) and none from tablet probiotics (0.00%). Headache was not experienced in any of the participants from both groups with 100.00%. Besides, majority of the participants did not experience any abdominal pain in the food probiotics group (51.28%) and tablet probiotics (48.72%). Cramps were recorded in 33.33% of food probiotics group and 66.67% in the tablet probiotics. As for rashes, most of the participants did not experience in the food probiotics with a percentage of 51.28% and 48.72% in the tablet probiotics. Acne was only seen in one of the participants from tablet probiotics group (100.00%) and from the food probiotics group (0.00%). Some of the participants experienced diarrhea while consuming the tablet probiotics (100.00%) and none from the food probiotics (0.00%). Brain fog was recorded in one of the participants in each group with a percentage of 50.00%. None of them experienced any vomiting and malaise from both groups (100.00%). Lastly for the nausea, only one participant reported it from the food probiotics (100.00%) and none from the tablet probiotics (0.00%).

6. Discussion

A randomized controlled trial (RCT), parallel design was done among undergraduate students to compare the effects of food probiotics and tablet probiotics on short term memory such as verbal, visual and number, and continuous concentration. In a previous study carried out among healthy volunteers, the authors showed a significant increase in working memory performance after supplementation of probiotics compared to placebo in a stress-induced condition.

The authors concluded that probiotics showed a neurocognitive effect which helped working memory performance [22]. In our study, we found that after 5 days of consuming probiotics, there was an increase in verbal, visual, number memory test scores, as well as continuous concentration scores in both food as well as tablet probiotics, even though these were not statistically significant.

Furthermore, within the groups (i.e. food probiotics and tablet probiotics), there was an increase in all the scores, suggesting that there was an association between consumption of probiotics (whether in food form or tablet form) and short-term memory and continuous concentration. When comparing the scores before and after food probiotics consumption, there were increases in verbal memory, visual memory and number memory, as well as continuous concentration but these were not statistically significant. Additionally, when comparing the scores before and after tablet probiotics, visual memory and continuous concentration had decreased; whereas there was an increase in verbal and number memory scores. This supports the findings in the previous studies carried out in healthy human volunteers [22].

Although probiotics are generally considered safe for consumption, there are common side effects seen in the healthy population [28-31]. In terms of adverse effects, in the food probiotics group, the common adverse effects were constipation (1 participant), cramps (1 participant), brain fog (1 participant) and nausea. In the tablet probiotics group, there were more adverse effects observed, such as gas/bloating (1 participant), abdominal pain (1 participant), cramps (2 participants), rashes (1 participant), acne (1 participant), diarrhea (2 participants) and brain fog (1 participant). As opposed to previous studies, there were no infectious complications observed in our study, but this could be due to the fact that our participants were healthy volunteers. Otherwise, the adverse effects were consistent with previous studies, but were not statistically significant, when the food probiotics group was compared with the tablet probiotics group [28-31]. The different profile of adverse effects seen in the two groups may have been due to variations seen in the composition of the microorganisms in the food probiotic versus in the tablet probiotic. Since these probiotics mainly act on the gut, there is an increased chance of GI- related side effects such as gas and bloating, due to the overgrowth of certain bacterial strains [28-31]. Additionally, it has been postulated that the different micro-environment in the gut, along with immune factors, forces those bacteria to select for genes that would aid their survival, which would lead to development of different virulence factors, which in turn would lead to infectious and immune-system related complications. For example, these bacteria could also worsen an atopic individual's sensitivity to allergens, possibly leading to allergic reactions such as rashes, acne and diarrhea [28-31].

Our study had many limitations that affected our results; for one, the sample size of 40 was too small; increasing the sample size would increase the statistical significance of our results. Another limitation was the time frame in which the intervention was conducted in. Most of the probiotics studies were conducted with a minimum of 30 days of intervention; since we carried out the RCT in 5 days, we were unable to ascertain the long-term benefits of probiotics intake, as well as any long term risks for consumption of food and tablet probiotics [33]. Additionally, our study population consisted of only healthy young adults; this meant our results could not be generalized to the general population, as they could be suffering from conditions that could predispose them to more serious adverse effects of probiotics. Additionally, more adverse effects could be studied in a larger population, thereby increasing the validity of our study.

Our study can be improved by carrying out a long-term study, to better study the adverse effect profile of probiotics, in a larger, more heterogenous population. Furthermore, the comparison of single-strain versus multi-strain probiotics will aid in the determination of which probiotic has more efficacy in the improvement of short term memory and continuous concentration. Thirdly, better tests, such as Brief Visuospatial Memory Test-Revised, Hopkins Verbal Reasoning Test-Revised and the Rey-Osterrieth Complex Figure Test, would further validate the results obtained, allowing better generalizability to the population [34]. Additionally, more tests to assess long-term memory can be used to better understand the effects of probiotics of long-term memory, which was not assessed in this study.

Food probiotics, due to their improved safety profile, compared to tablet probiotics, as well their relative economic value, and hence would be useful for improving short -term memory and continuous concentration.

7. Conclusion

In conclusion, probiotics can improve short term memory such as verbal, visual and number memory, and continuous concentration, with no significant differences between food and tablet probiotics in the aforementioned parameters. Additionally, food probiotics displayed an increase in short term memory, with a more favorable adverse effect profile, as compared to the tablet probiotics, in the sample studied. Our study showed that probiotics can be used to improve the short-term memory of medical students; however further research is required in order to better evaluate the adverse effect profile of the probiotics, as well as to identify whether particular strains

of microorganisms are favorable in the augmentation of short term memory. The authors recommend food probiotics, due to their improved safety profile and relative economic value, to aid the improvement of short term memory and continuous concentration in medical students.

Acknowledgements

We would like to thank all our volunteers for participating in this study and helping us carry out the experiment. We would like to warmly express our appreciation to Prof. Dr. Adinegara Lutfi Abas (Dean of the Faculty of Medicine & Head of Department of Community Medicine, MMMC), Prof. Dr. Htoo Htoo Kyaw Soe (Department of Community Medicine, MMMC), Associate Prof. Dr. Sujata Khobragrade (Department of Community Medicine, MMMC) and Associate Prof. Dr. Mila Nu Nu Htay (Department of Community Medicine, MMMC) as for their guidance throughout the whole study. Last but not least, we would like to thank the Research Ethics Committee, Faculty of Medicine, Melaka-Manipal Medical College (MMMC) for approving our research.

References

[1] Reid G et al., 2019, 'Probiotics: Reiterating What They Are and What They Are Not', *Frontiers in Microbiology*, vol. 10, pg. 424.

[2] Tarasiuk A & Fichna J, 2019, 'Gut microbiota: what is its place in pharmacology', *Expert Review of Clinical Pharmacology*; doi: 10.1080/17512433.2019.1670058.

[3] Davani-Davari D et al., 2019, 'Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications', *Foods*, vol. 8, no. 3 (Epub).

[4] Liu Y et al., 2018, 'Probiotics in Disease Prevention and Treatment', *J Clin Pharmacol.*, vol. 58, supplement 10, pp. S164-179.

[5] Sarkar A et al., 2016, 'Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals', *Trends Neurosci*, vol. 39, no. 11, pp. 763-781.

[6] Saputro ID et al., 2019, 'Effects of probiotic administration on IGA and IL-6 level in severe burn patients: a randomized trial', *Ann Burns Fire Disasters*, vol. 32, no. 1, pp. 70-76.

[7] Ejtahed HS & Hasani-Ranjbar S, 2019, 'Neuromodulatory effect of microbiome on gut-brain axis; new target for obesity drugs', *J Diabetes Metab Disord*, vol. 18, no. 1, pp. 263-265.

[8] Dale HF et. Al, 2019, 'Probiotics in Irritable Bowel Syndrome: An Up-to-Date Systematic Review', *Nutrients*, vol. 11, no. 9, (Epub).

[9] Rittiphairoj T et al., 2019, 'Probiotics for glycemic control in patients with type 2 diabetes mellitus: a protocol for a systematic review', *Syst Rev.*, vol. 8, no. 1 (Epub).

[10] Dilip KC et al., 2019, 'Gut microbiota and health', *Postgraduate Medicine*, doi: 10.1080/00325481.2019.1662711.

[11] Scriven M et al., 2018, 'Neuropsychiatric Disorders: Influence of Gut Microbe to Brain Signalling", *Diseases*, vol. 6, no. 3 (Epub).

[12] Cheng L et al., 2019, 'Psychobiotics in mental health, neurodegenerative and neurodevelopment disorders', *Journal of Food and Drug Analysis*, vol. 27, no. 3, pp. 632-648.

[13] Park C et al., 2018, 'Probiotics for the treatment of depressive symptoms: An anti-inflammatory mechanism?', *Brain Behav Immun.*, vol. 73, no. 115-124 (Epub).

[14] Nikolova V et al., 2019, 'Gut feeling: randomized controlled trials of probiotics for the treatment of clinical depression: Systematic review and meta-analysis', *Ther Adv Psychopharmacol.*, vol. 26, no. 9 (Epub).

[15] Srikantha P & Mohajeri MH, 2019, 'The Possible Role of the Microbiota-Gut-Brain-Axis in Autism Spectrum Disorder', *Int J Mol Sci*, vol. 20, no. 9, pg. 2115.

[16] Umbrello G & Esposito S, 2016, 'Microbiota and neurologic diseases: potential effects of probiotics', *J Transl Med*, vol. 14, no. 298 (Epub).

[17] Yang B et al., 2019, 'Effects of regulating intestinal microbiota on anxiety symptoms: A systematic review', *Gen Psychiatr.*, vol. 32, no. 2 (e100056).

[18] Dantzer R et al., 2018, 'Resilience and immunity' *Brain Behav Immun*., vol. 74, no. 28 (E-publication).

[19] Filosa S et al., 2018, 'Polyphenols - gut microbiota interplay and brain neuromodulation', *Neural Regen Res*., vol. 13, no. 12, pp. 2055-2059.

[20] Browne PD et al. 2019, 'Probiotics in pregnancy: protocol of a double-blind randomized controlled pilot trial for pregnant women with depression and anxiety (PIP pilot trial), *Trials*, vol. 20, no. 1, pg 440.

[21] Jacobs JP & Mayer EA, 2019, 'Psychobiotics: Shaping the Mind with Gut Bacteria', *Am J Gasteroenterol.*, vol. 114, no. 2, pp. 1034-1035.

[22] Papalini S et al., 2018, 'Stress matters: Randomized controlled trial on effects of probiotics on neurocognition", *Neurobiol Stress*., vol. 10: 100141 (Ecollection).

[23] Simkin DR, 2019, 'Microbiome and Mental Health, Specifically as It Relates to Adolescents', *Curr Psychiatry Rep.*, vol. 21, no. 9, pg 93.

[24] Zhang Ning et al., 2019, 'Probiotic supplements for relieving stress in healthy participants: A protocol for systematic review and meta-analysis of randomized control trials', *Medicine (Baltimore)*, vol. 98, no. 2 (e15416).

[25] Reis DJ et al., 2018, 'The anxiolytic effect of probiotics: A systematic review and meta-analysis of the clinical and preclinical literature', *PLoS One*, vol. 13, no. 6 (Epub).

[26] Khalesi S et al., 2019, "A review of probiotic supplementation in healthy adults: helpful or hype?" *European Journal of Clinical Nutrition*, vol. 73, pp. 24-37.

[27] Abbasi J, 2019, "Are Probiotics Money Down the Toilet? Or Worse", *JAMA*, vol. 321, no. 7, pp. 633-635.

[28] Suez J et al., 2019, "The pros, cons and many unknowns of probiotics", *Nature Medicine*, vol. 25, pp. 716-729.

[29] Didari T et al., 2014, "A systematic review of the safety of probiotics", *Expert Opinion on Drug Safety*, vol. 13, no. 2, pp. 227-239.

[30] Costa R et al., 2018, "Infectious complications following probiotic ingestion: a potentially underestimated problem? A systematic review of reports and case series", *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, p 329.

[31] Kothari D et al., 2019, "Probiotics supplements might not be universally-effective and safe: A review", *Biomedicine & Pharmacotherapy*, vol. 113, pp. 537-547.

[32] Cohen PA, 2018, "Probiotic Safety- No Guarantees", *JAMA Intern Med.*, vol. 178, no. 12, pp. 1577-1578.

[33] Lerner A et al., 2019, "Probiotics: If It Does Not Help It Does Not Do Any Harm. Really?" *Microorganisms*, vol. 7, no. 104 (Epub).

[34] Committee on Psychological Testing, Including Validity Testing, for Social Security Administration Disability Determinations; Board on the Health of Select Populations; Institute of Medicine. Psychological Testing in the Service of Disability Determination. Washington (DC): National Academies Press (US); 2015 Jun 29. 5, Cognitive Tests and Performance Validity Tests. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305230/

[35] Supplementary Materials Papalini et al. Stress matters: randomized controlled trial on the effect of probiotics on neurocognition (https://ars.els-cdn.com/content/image/1-s2.0-S2352289518300560-mmc1.pdf)