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Randomized Control Trials

Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial☆

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SUMMARY

Background: Disturbance in the equilibrium of the gut microbiota has been involved in the pathophysiology of depression. Probiotics have the potential to healthfully modulate the gut microbiome. Prebiotics could also be effective by stimulation of growth of some bacterial species in the gut microbiota.
Objective: The aim of this double blind clinical trial, was to compare the effect of supplementation with the probiotic and prebiotic on the Beck Depression Inventory (BDI) score as a primary outcome as well as the kynurenine/tryptophan ratio and tryptophan/branch chain amino acids (BCAAs) ratio as secondary outcomes in patients with major depressive disorder (MDD).
Design: One hundred and ten depressed patients were randomly assigned to receive the probiotic (*Lactobacillus helveticus* and *Bifidobacterium longum*), prebiotic (galactooligosaccharide) or placebo for 8 weeks. Serum tryptophan and BCAAs were measured by HPLC, and kynurenine by ELISA kit. Dietary intake and physical activity of the participants were recorded at baseline.
Results: A total of 81 subjects (aged 36.5 ± 8.03 y; mean (95% CI), 2.27 (1.76–2.93) y of depression duration) completed the trial (28 in the probiotic group, 27 in the prebiotic group, and 26 in the placebo group). From baseline to 8 weeks, probiotic supplementation resulted in a significant decrease in BDI score (18.25–9.0) compared to the placebo (18.74–15.55) and prebiotic (19.43–14.14) supplementation ($p = 0.042$). Inter-group comparison indicated no significant differences among the groups in terms of serum kynurenine/tryptophan ratio and tryptophan/BCAAs ratio. However, the kynurenine/tryptophan ratio decreased significantly in the probiotic group compared to the placebo group after adjusting for serum isoleucine ($p = 0.048$). In addition, the tryptophan/isoleucine ratio increased significantly in the probiotic group when compared to the placebo group ($p = 0.023$).
Conclusion: Overall, 8 weeks of probiotic supplements to subjects with MDD resulted in an improvement in BDI score compared with placebo whereas no significant effect of prebiotic supplementation was seen. Study was registered in IRCT.ir under IRCT2015092924271N1.

Abbreviations: BDI, beck depression inventory; MDD, major depressive disorder; BCAAs, branch chain amino acids; RCT, randomized controlled trial; IPAQ, international physical activity questionnaire; MET, metabolic equivalents; BMI, body mass index; ITT, intention to treat; PP, per protocol.

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1. Introduction

Major depressive disorder (MDD) is a common disorder linked to comorbidity, poor health, and mortality [1]. According to the World Health Organization, depression is a major contributor to the overall global burden of disease and it is the leading cause of disability [2]. The global prevalence of MDD is 4.7% [3]. In recent years, an emerging field of research has revealed a link between depression and gut microbiota [4]. There is now a great body of evidence that indicate that pathophysiological pathways involved in the pathogenesis of depression are influenced by disturbance in the equilibrium of the gut microbiota [5,6]. The human gut is colonized by nearly 100 trillion microorganisms [7], that can be classified as pathogenic, neutral or beneficial to the host. One of the mechanisms by which the gut microbiome affects depression is the regulation of tryptophan metabolism both directly and indirectly [8]. The beneficial bacteria indirectly impact tryptophan availability and serotonin synthesis by decreasing the activity of enzymes responsible for tryptophan degradation along the kynurenine pathway [9]. The gut microbiota can also directly synthesize tryptophan via enzymes such as tryptophan synthase, whereas certain bacterial strains harbor a tryptophanase enzyme that degrades tryptophan, thereby potentially limiting its availability to the host [8]. Oral delivery of probiotics that are then integrated into the gut ecosystem has the potential to healthfully modulate the gut microbiome [10]. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [11]. Prebiotics are also dietary non-viable components that can act indirectly through the selective stimulation of growth and/or activities of one or a limited number of bacterial species in the gut microbiota that confer health benefits to the host [12].

Branch chain amino acids (BCAAs), which include leucine, isoleucine, and valine, compete with tryptophan for a transporter involved in the uptake of both tryptophan and BCAA from the blood to the brain [13], and the gut microbiome has the potential to affect serum BCAAs [14]. A negative correlation between BCAAs concentrations and the severity of MDD has been reported and a decrease in blood levels of BCAAs has been demonstrated in patients with MDD after treatment with antidepressants [13]. Since the tryptophan circulating levels do not directly represent the availability of tryptophan to the brain, it is better to measure serum levels of tryptophan/BCAAs ratio rather than total serum tryptophan concentrations.

Since not all probiotics are beneficial in all circumstances, careful selection of appropriate strains of bacteria based on the desired clinical outcome is of great importance [15]. Therefore, we selected *Lactobacillus helveticus* and *Bifidobacterium longum*, which have been shown in a recent review study to improve emotional behavior in animals and psychological outcomes in humans [16].

The human trials verifying the impact of probiotics and prebiotics on psychological outcomes are few and they have been conducted on healthy humans. The potential effects of probiotic and prebiotic on mood may be mediated by several variables potentially involved in gut-to-brain communication. To our knowledge, two studies have investigated the effect of probiotics in depressed subjects. However, the strains of probiotics used in one of these studies were not chosen on the basis of desired outcome and in the other study they were used as a primary treatment. In addition, none of them measured the kynurenine to tryptophan ratio. To the best of our knowledge, no study has compared the effects of probiotics and prebiotics on improvement of depression. Furthermore, no study has measured the serum tryptophan to BCAAs ratio. Therefore, the aim of the current double blind RCT was to compare the effect of probiotics and prebiotic versus placebo on decreasing the Beck Depression Inventory (BDI) score as a primary outcome, and the kynurenine/tryptophan ratio along with the tryptophan/BCAAs ratio as secondary outcomes in adult subjects with mild to moderate MDD.

2. Method

2.1. Study design and participants

This study was a three-arm parallel design, placebo-controlled, double-blind Randomized Controlled Trial (RCT). Ethics approval was obtained from the Research Ethics Committee of Tehran University of Medical Sciences, IR.TUMS.REC.1394.1190 and the study was registered in IRCT.ir under IRCT2015092924271N1. The participants were referred to the project by a psychiatrist and faculty of Tehran University of Medical Sciences in Bahman Hospital, Tehran. The research including screening, recruitment, and the trial period, was carried out from July 2016 to April 2017. To be eligible, patients had to meet the inclusion criteria and provide written informed consent before enrollment. Inclusion criteria included mild to moderate major depressed patients aged 18–50 years who took the anti-depressant drugs: sertraline, fluoxetine, citalopram or amitriptyline for 3 months or more prior to beginning the trial. Exclusion criteria were as follows: history of renal, hepatic, cardiovascular, or respiratory diseases; pregnancy and lactation; regular intake of probiotics during last 2 months before recruitment for the study; intake of antioxidant or omega 3 supplements less than 6 weeks before the beginning of the study; alcohol intake; smoking cigarettes (more than 5 during last 6 months) or tobacco (pipe or hookah at least one time during last month); any addiction to opiates; history of heart attack or stroke; following a specific diet; participation in another study during last two months; any significant change in diet and life style; any change in drug regimen; inflammatory diseases which lasted for more than one week during the study; intake of antibiotics during the study. Participants were instructed not to consume any other probiotic supplements during the course of the trial.

2.2. Randomization and blinding

Randomization was done by a research assistant not otherwise involved in the study. Randomization was stratified by age ($\geq$35 vs <35). Within each stratum, patients were randomly assigned to experimental groups (1:1:1) in blocks of 6 using www.randomization.com. Sachets (probiotic, prebiotic and placebo) were pre-packaged according to the randomization code. This meant that participants, clinicians and raters remained blind to the allocated group of each participant until the database was unlocked and data analysis was completed.

2.3. Interventions

The probiotic product was a formula developed and provided by Lallemand Health Solutions (Mirabel, Quebec, Canada), which contains freeze-dried *L. helveticus* R0052 and *B. longum* R0175 (CNCM strain I-3470) bacteria at a dosage of ten billion colony-forming units ($\geq 10 \times 10^9$ CFU) per 5 g sachet. Excipients used were as follows: xylitol, maltodextrin, plum flavor and malic acid. The placebo product contained only the excipients, and the prebiotic product was composed of galactooligosaccharide and 0.2% Plum flavor. The placebo sachet was matched to the study prebiotic and probiotic products for taste, color, and size; it was room temperature stable and in the form of orally dispersible powder in plain sachets.

2.4. Study procedure

Fasting blood was collected from the participants between 0800 and 0900 at baseline and at the end of the study. Serum samples were stored at −80°C until required for assessment. The participants completed self-report questionnaires at baseline and at the end of the study. They were allocated to the next sequentially numbered bag. The sachets were given to them two times: at baseline and at day 30. Participants were asked to take one sachet at the same time each day for two months, preferably before a meal, by pouring the orally dispersible powder from the sachet directly into the mouth where it rapidly dissolved. They were monitored every 2 weeks by telephone contact. Compliance with probiotic, prebiotic, and placebo sachets was also monitored by asking participants to return the medication containers. Participants were considered compliant if they consumed ≥80% of the supplements. At the start of the study, individuals were requested to follow their routine physical activity and dietary intakes during the study. Moreover, they were requested to record their dietary intakes for three non-consecutive days (two usual days and one weekend day) at baseline and the end of the study. To obtain nutrient intakes of participants based on the three-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods. The dietary records were based on estimated values in household measurements. Physical activity was recorded using the short international physical activity questionnaire (IPAQ) at baseline and at the end of the study. It was described as metabolic equivalents (METs) in hours per day. To compute the METs for each subject, we multiplied the times (in hours per week) reported for each physical activity by its related METs coefficient using standard tables. Body weight and height were determined by the use of a digital scale (Seca, Hamburg, Germany) at baseline. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared.

2.5. Outcomes

The primary outcome was the change in BDI score. BDI is a self-compiled questionnaire of 21 items in multiple-choice format [22]. Under each item, there are four statements, and the subjects were instructed to choose the one that best described their situation during the last 2 weeks. The declarations were given the scores 0, 1, 2, and 3, with 0 for the normal or least depressive statement and 3 for the most depressive statement. We calculated the total BDI score by adding together the scores of each item.

The secondary outcomes included changes in the serum levels of both the kynurenine/tryptophan ratio and the tryptophan/BCAAs ratio. Serum tryptophan and BCAAs were measured using high-performance liquid chromatography, and serum kynurenine was measured using a commercially available enzyme-linked immunosorbent assays kit (Cusabio Biotech, Wuhan, China).

2.6. Statistical analysis

Statistical Package for Social Science version 17 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Analysis of the Beck score was completed on an intention-to-treat (ITT) basis. Analyses of secondary outcomes were undertaken using the per protocol (PP) set, defined as those participants who completed the study. We used ANOVA/ANCOVA to compare the outcomes between groups adjusting for corresponding baseline values for all outcomes and BCAA and isoleucine for kynurenine/tryptophan ratio. For pairwise comparison of the groups, ANOVA/ANCOVA was also used. To check the normal distribution of variables, we used the Kolmogorov–Smirnov test. Skewed data were log10 transformed (tryptophan, kynurenine and kynurenine/tryptophan ratio) or square root (BDI score) transformed. Box-cox transformation (using STATA version 12) was used to normalize the isoleucine/tryptophan ratio. STATA was used to find the best transformation for non-normal variables. For transformed data, mean and 95% confidence intervals were derived from the ANCOVA models for each treatment group and then were back transformed for display in the table. Data for non-transformed variables were summarized by mean differences and 95% confidence intervals. When significant main effects were detected, the Bonferroni multiple comparisons test was applied to compare data between groups. Sample size was determined based on mean reductions in BDI score. A sample size of 81 participants (27 in each group) was chosen to provide at least 80% power with 2-sided type I error of 0.05 to detect a mean difference of 5 (minimal important change difference) between intervention and the placebo groups. At first, we predicted a probable loss to follow up of 10%, therefore, we considered a sample size of 30. However, as the missing rate was more than expected during the study, we recruited 110 patients with MDD in total to compensate for the loss to follow-up. All tests were two tailed and p-values <0.05 were considered significant.

3. Results

Out of 230 referred patients, 110 subjects (32 men and 78 women) with mild to moderate depression were enrolled and assigned to the probiotic group (n = 38), prebiotic group (n = 36) or placebo group (n = 36). Mean (SD) age was 36.47 (±8.03). Ten people from the probiotic group, nine from the prebiotic group and ten from the placebo group dropped out prior to the trial completion (Fig. 1). All the patients were on antipsychotic treatment and the baseline characteristics were similar for all three groups (Table 1). Table 2 shows dietary intake and physical activity of participants at baseline and at the end of the study.

3.1. Primary outcome

The ITT analysis of the BDI score demonstrated significant change of this outcome between the groups (p = 0.04). In the pairwise analysis, the mean BDI score significantly decreased in the probiotic group compared to the placebo (p = 0.008) over 8 weeks. The decrease in the mean BDI score induced by the prebiotic, was not significant compared to the placebo (p = 0.39) or the probiotic groups (p = 0.26) (Table 3).

3.2. Secondary outcomes

In the PP analysis of the serum kynurenine/tryptophan ratio, no significant change was seen between the groups (p = 0.124). However it became significant after adjusting for serum isoleucine (p = 0.048) (Table 3). In pairwise analysis of the groups, a significant decrease in the kynurenine/tryptophan ratio was seen only in the probiotic group compared to the placebo group (p = 0.036) (Table 4). Also, while the change in the serum tryptophan/BCAAs ratio was not significant between the groups (p = 0.065), the change in the tryptophan/isoleucine was significant between them (p = 0.026) (Table 3). In pairwise analysis of the groups, a significant decrease in the tryptophan/BCAAs ratio was seen only in the prebiotic group compared to the placebo group (p = 0.031) (Table 4).

3.3. Adverse events and compliance

Some types of adverse events judged as possibly related to the intervention occurred among 15 participants: gastrointestinal complaints in 6 participants (4 prebiotic + 2 probiotic), nausea in 2

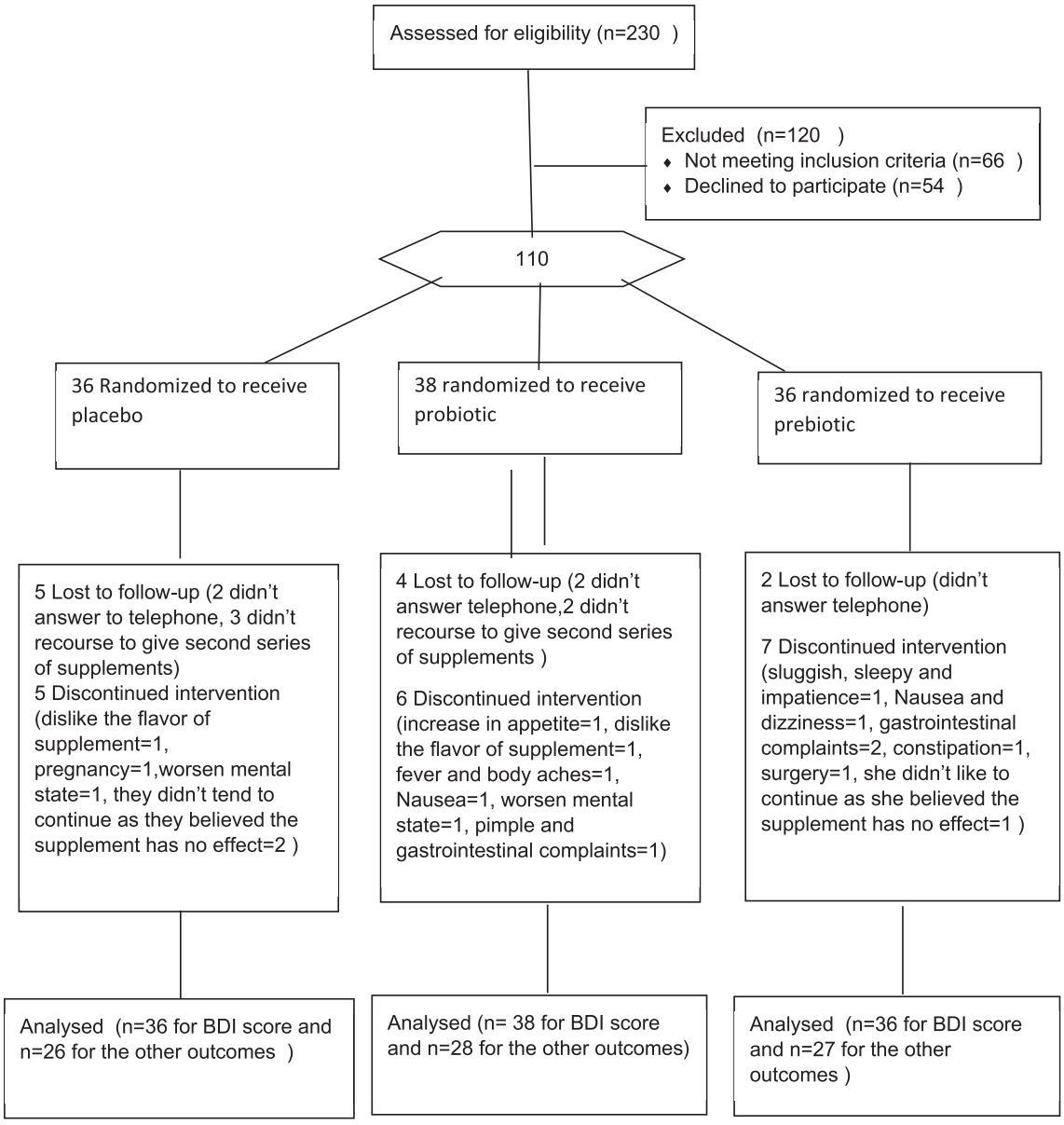

Fig. 1. Summary of patient flow diagram.

Table 1
Baseline characteristics.

Characteristic	Probiotic (n = 38)	Prebiotic (n = 36)	Placebo (n = 36)	Total (n = 110)
Age, mean (SD), Y	36.15 (7.85)	37.35 (7.97)	36 (8.47)	36.47 (8.03)
Male n (%)	11 (28.9)	9 (25)	12 (33.3)	32 (29.1)
BMI, mean (SD)	26.11 (4)	26.9 (5.1)	26.61 (4.97)	26.5 (4.61)
Weight, mean (SD), kg	71.7 (11.8)	72.8 (15.6)	73.2 (14.1)	72.5 (13.6)
Education n (%)				
No high school certificate	2 (7)	5 (15)	3 (11.5)	10 (9)
Completed high school	10 (28.5)	9 (26)	10 (30.7)	29 (26.4)
Undergraduate degree	17 (32.1)	13 (29.6)	16 (30.7)	46 (41.8)
Postgraduate degree	9 (32.1)	9 (29.6)	7 (23.9)	25 (22.7)
Married state n (%)				
Married	20 (52.6)	24 (66.7)	22 (61.1)	66 (60)
Never married	15 (39.5)	10 (27.8)	13 (36.11)	38 (34.5)
Other	3 (7.8)	2 (5.5)	1 (2.8)	6 (5.5)
Duration of depression,[a] mean (95% CI), Y	1.9 (1.2–2.96)	2.7 (1.8–4.17)	2.3 (1.4–3.84)	2.27 (1.76–2.93)
Duration of antipsychotic treatment,[a] mean (95% CI), Y	1.45 (0.91–2.31)	2.17 (1.37–3.44)	1.66 (0.95–2.9)	1.72 (1.31–2.27)

[a] Data was log transformed as distribution was not normal and retransformed to display as mean (95% CI), N: number; Y: year.

Table 2
Dietary intake and physical activity at baseline and at the end of the study.

	Probiotic		Prebiotic		Placebo		p Value
	Baseline	End	Baseline	End	Baseline	End	
Energy	1537 (317)	1662 (334)	1467 (409)	1405 (421)	1505 (565)	1547 (570)	0.06
Protein (%)	13.3 (2.4)	12.6 (2.3)	14.5 (2.3)	14 (3.3)	14.9 (2.2)	14.8 (4.4)	0.12
Fat (%)	28 (7.8)	29.9 (6.2)	30 (7.9)	28.9 (7.7)	30 (5.2)	32 (6.4)	0.16
CHO (%)	58.4 (7.9)	58 (6.3)	55 (7.5)	56.4 (8.3)	54.8 (6.2)	52.4 (8.1)	0.22
Fiber	13.4 (7)	11.8 (5.7)	10.4 (4.6)	11.6 (6.1)	11.9 (4.6)	11.2 (4.8)	0.7
Cholesterol	155.1 (96.3)	175.3 (101.6)	164.9 (115.5)	140.2 (99)	152.3 (128.6)	136 (129.4)	0.24
Mg	157 (59.3)	161.5 (46)	138.3 (43.5)	164.5 (62.5)	150.9 (68.8)	143.2 (58.4)	0.22
Zn	5 (1.7)	5.2 (2.5)	5.5 (2.1)	5 (2.7)	6 (3.4)	6.4 (3.2)	0.32
Se	0.07 (0.05)	0.06 (0.03)	0.06 (0.03)	0.07 (0.05)	0.06 (0.05)	0.08 (0.04)	0.05
VitE	2.8 (2.3)	2 (2.3)	1.94 (1.89)	2.26 (1.46)	2.1 (2.96)	2.46 (3.1)	0.75
VitC	44.5 (64.9)	63.5 (122)	61.4 (65.4)	81.9 (69)	53.8 (53.7)	58.3 (60.8)	0.87
MET	23 (15.3–3.16)	17.43 (12.63–24.05)	19.9 (13.35–25.77)	15.28 (11.87–19.68)	20.65 (13.5–24.05)	16.94 (11.47–25.02)	0.96

Data are presented as mean (SD) for dietary intakes and mean (95% CI) for Physical activity; METs, metabolic equivalents. p Value is for the final values.

Table 3
Mean (95% CI) of biomarker levels at baseline and post 8 weeks.

	Probiotic		Prebiotic		Placebo		Partial Etha square	p Value (adjusted)
	Baseline	End	Baseline	End	Baseline	End		
BDI score	18.25 (14.15–21.62)	9.0 (7.43–14.12)	19.43 (15.42–24.57)	14.14 (9.55–19.62)	18.74 (14.11–23.13)	15.55 (11.36–21.26)	0.09[a]	0.042[a]
Kynurenine (nmol/L)	757.59 (661.27–867.95)	722.47 (621.3–840.08)	913.34 (797.73–1045.71)	909.19 (746.11–1107.9)	772.89 (627.44–952.06)	798.49 (717.06–1002.67)	0.05[a]	0.15[a]
Tryptophan µg/dL	67.46 (59.73–76.19)	75.42 (63.43–89.66)	81.36 (67.21–98.5)	80.62 (69.55–93.46)	69.55 (53.94–89.68)	65.19 (55.76–76.23)	0.038[a] 0.093[b]	0.259[a] 0.036[b]
Kynurenine/ tryptophan ratio (nmol/µg)	11.23 (9.17–13.76)	9.58 (7.64–12.01)	11.22 (8.82 to 14.28	11.27 (9.03 to 14.08	11 (8.8–13.75)	12.4 (9.66–15.99)	0.059[a] 0.086[b]	0.124[a] 0.048[b]
Tryptophan/BCAAs (µg/µg)	0.146 (0.131–0163)	0.154 (0.140–0.168)	0.164 (0.149–0.179)	0.168 (0.151–0.185)	0.149 (0.128–0.172)	0.138 (0.121–0.155)	0.076[a]	0.065[a]

Beck score was square transformed, kynurenine, tryptophan and kynurenine to tryptophan ratio were log transformed and then retransformed to display as mean (95% CI); BDI, beck depression inventory; BCAAs, branch chain amino acids.
[a] Adjusted for baseline.
[b] Adjusted for baseline and isoleucine; p value and partial etha square obtained from anova/ancova.

Table 4
Pairwise comparison of the groups.

	Probiotic vs Placebo		Prebiotic vs Placebo		Probiotic vs Prebiotic	
	ES[a] d	p Value	ES[a] d	p Value	ES[a] d	p Value
BDI score	0.54	0.008	0.18	0.242	0.34	0.135
Kynurenine (nmol/L)	0.19	0.231	0.09	0.833	0.10	0.610
Tryptophan (µmol/L)	0.39	0.171	0.09	0.095	0.27	0.939
Kynurenine/Trp ratio	0.51	0.036	0.21	0.453	0.26	0.124
Tryptophan/BCAA	0.41	0.096	0.34	0.031	0.10	0.546
Tryptophan/Isoleucine	0.15	0.018	0.07	0.025	0.06	0.77

[a] Cohen's d effect size: measured as the mean difference in change divided by the pooled standard deviation of the change, based on values adjusted for baseline. BDI, beck depression inventory.

participants (1 probiotic + 1 prebiotic), fever and body aches in 1 participant (probiotic), and increased appetite in 6 participants (5 probiotic + 1 prebiotic). Two participants claimed worse mental state that was judged unrelated to the study participation (1 placebo + 1 probiotic). Compliance was confirmed by counting returned supplements. At the end of the study, participants achieved a mean (SD) compliance rate of 91.9% (5.53%).

4. Discussion

In this randomized clinical trial of adult subjects with mild to moderate MDD, the probiotic supplement for 8 weeks compared to the placebo resulted in a greater decrease in BDI score while the decrease caused by the prebiotic was not significant compared with either placebo or probiotic. Previous studies which investigated treatment options for depression have demonstrated the clinical importance of the between-group difference in BDI score of 18% [17] that was found in this trial only between probiotic and placebo.

The results of this study are consistent with earlier clinical trials investigating the effect of probiotic supplements on mood outcomes [16,18,19], however most of these studies have been conducted on healthy subjects. Similarly to our study, two recent trials have assessed psychological outcomes of probiotic administration in depressed patients. In one of these trials administration of the same strains of probiotic bacteria used in our study over 8 weeks revealed no significant change in psychological outcomes [20]. An important feature of this study was the use of probiotics as a primary treatment. The other study which used probiotics in addition to antidepressant drug treatment, demonstrated beneficial effects of probiotics on BDI scores compared with placebo in a sample of 40 participants over 8 weeks [21]. Schmidt et al. also investigated the effect of two prebiotics of fructooligosaccharides and galactooligosaccharides compared with the placebo over 21 days in healthy subjects [22]. In line with our results regarding prebiotic, no significant difference was found in psychological outcomes between experimental groups in this trial. In another study, supplementation with 7 g/d galactooligosaccharide in patients with irritable bowel syndrome did not decrease anxiety and depression scores except in a subgroup of patients who were diarrhea predominant [23]. The main mechanisms by which probiotics may

improve depressive symptoms include modulation of neurotransmitters and inflammation [4, 24].

Our results also showed that probiotic supplementation decreased the serum kynurenine/tryptophan ratio significantly compared to the placebo while prebiotic supplementation did not make a significant change compared to the placebo. In comparing the three groups, the significant change in the serum kynurenine/tryptophan ratio was seen only when adjusted for serum isoleucine. The only study which investigated the effects of probiotics on the kynurenine/tryptophan ratio in relation to psychological outcomes, reported no significant changes in the kynurenine/tryptophan ratio after 6 weeks of probiotic administration in healthy subjects [19]. To our knowledge, no study has investigated the effect of prebiotics on the serum kynrenine/tryptophan ratio. Tryptophan is mainly metabolized by two pathways: the serotonin [25] and the kynurenine [26] pathways. A tryptophan shunt towards production of kynurenine leads to the serotonin deficiency [27]. Probiotics drive tryptophan along the serotonin pathway by reducing the activity of enzymes responsible for conversion of tryptophan to kynurenine. Therefore, the decrease in the kynurenine/tryptophan ratio in the probiotic arm may be a mechanism for the observed effects on depression [8].

The probiotic did not make a significant increase in the tryptophan to BCAAs ratio compared to the placebo while the prebiotic increased it significantly compared to the placebo. No significant change in the tryptophan to BCAAs ratio was seen between the groups. However, the tryptophan to isoleucine ratio increased significantly in the probiotic group compared to the placebo group. In two previous studies by Umeharaa [28] and Setoyam [29] on MDD patients, a significant positive correlation was found only between isoleucine concentration from BCAAs and depression severity. To our knowledge, no study has assessed the effect of probiotics on BCAAs. Only a few studies have identified the strains of bacteria with the ability to biosynthesize BCAAs [14,30,31]. Measuring BCAAs in studies investigating the effect of probiotics on tryptophan is highly important as they compete with tryptophan for passing through the blood–brain barrier [13] and they are produced by some strains of gut microbiome that could be influenced by probiotics [14].

The present trial has several limitations. First, there is a lack of fecal microbiome analysis. Since the gut microbiota of humans is highly variable, this may affect the response of subjects to the probiotic. Second, the recruitment phase lasted for a long time (about one year), therefore the intervention was conducted at different times of the year (seasons). Although the condition were identical for all three experimental groups, the changes in lifestyle, diet, vitamin D status etc. along with the seasons might dilute the effect of the probiotic. Third, the antidepressant drugs that the participants had taken were not identical. It would have been better to include only the patients who had received the same drug. The strength of this study lies in selecting the strains of probiotics and the prebiotic that have been indicated in the previous studies to affect mood disorders.

In conclusion, among subjects with MDD, probiotic supplementation resulted in an improvement in BDI score compared with placebo whereas no significant effect of prebiotic was observed. Although the change in the kynurenine to tryptophan ratio was not significant between the groups, it decreased significantly when adjusted for serum isoleucine. Also, it decreased in the probiotic group compared to the placebo. These findings suggest that the probiotic may exert at least part of its effects on depression through the kynurenine to tryptophan ratio. More trials assessing BCAAs as well as the kynurenine to tryptophan ratio may provide better insights regarding the mechanisms involved in the effect of the probiotic on depression.

Conflict of interest

None of the authors reported a conflict of interest related to the study.

Authors' contributions

AK, KD and AAN: designed the research; AK and AAN: conducted the research; KA: analyzed the data; AK and KD wrote the article; KD had primary responsibility for the final content; and all authors: read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.04.010.

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