T.C.

REPUBLIC OF TURKEY

HACETTEPE UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

Effects of Probiotic and Prebiotic Supplements on Serum BDNF, Kynurenine, Tryptophan Competing Amino Acids and Intestinal Microbiome in Major Depressed Patients

Nazanin HEIDARZADEH RAD

Program of Nutrition and Dietetic

DOCTOR OF PHILOSOPHY THESIS

ANKARA

2020

ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor *Prof. Dr. Hülya GÖKMEN-ÖZEL* and *Prof. Dr. Kurosh DJAFARIAN* and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

Nazanin Heidarzadeh Rad

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my advisor, *Prof. Dr. Hülya Gökmen Özel* for her kind advice, constant support and help.

I would like to express my deep gratitude to my second advisor, *Prof. Dr. Kurosh Djafarian* for the help and support, for his patience and motivation and financial support. This study would not have been possible without his support.

My sincere thanks also to all academic members of the *Nutrıtion and Dietetics Department, Graduate School of Health Sciences of Hacettepe University during my graduate study,* especially those members of my thesis monitoring committee, *Prof. Dr. Gülhan Samur* and *Assoc. Prof. Dr. Emine Yıldız* for their valuable input, discussions and access.

My sincere thanks also to the *Tehran University of Medical Sciences, Institute of Nutritional Sciences and Dietetics*. This work would not have been possible without the financial support of them.

My sincere thanks to *Dr. Asma Kazemi* for her scientific and technical assistance during the thesis work.

I would like to extend my deepest gratitude to my dear friend, *Dr. Negin Almasi* for her great friendship and her understanding and help during my graduate study.

I would like to dedicate this thesis to my loving parents *Parvaneh Gholizadeh* and *Sattar Heidarzadeh*, my siblings *Elmira* and *Babak*; and last but not least to my dearest husband *Maziar Shoaie*. Words cannot express their unconditional love, patience, motivation, understanding and support without which this journey would not have been possible.

ÖZET

Heidarzadeh Rad, N. Prebiyotik ve probiyotik takviyesinin majör depresyonu olan hastalarda serum beyin kaynaklı nörotrofik faktör, kinürenin, triptofan ile yarışan dallı zincirli aminoasitler ve bağırsak mikrobiyomu üzerine etkisi, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Beslenme ve Diyetetik Programı Doktora Tezi, Ankara, 2020. Son zamanlarda yapılan araştırmalar, bozuk bağırsak mikrobiyomunun depresyon gelişimindeki rolünü vurgulamıştır. Probiyotikler ve prebiyotikler, bağırsak mikrobiyomunu dengeleyebildikleri ve bağırsak-beyin eksenini düzenleyebildikleri için depresyonun önlenmesi ve tedavisinde yararlı olabilcekleri ileri sürülmüştür. Bu çift kör randomize kontrollü klinik çalışmanın amacı, *Lactobacillus helveticus* ve *Bifidobacterium longum* probiyotiklerin ve galakto-oligosakkarit prebiyotiğinin majör depresif bozukluğu olan hastalarda serum beyin kaynaklı nörotrofik faktör (BKNF), kinurenin, triptofan ile yarışan dallı zincirli amino asitler (DZAA) ve bağırsak mikrobiyomu üzerine etkilerini karşılaştırmaktır. Çalışmaya İran'daki Bahman Hastanesi psikiyatri kliniğine başvuran düşük ila orta derecede depresyonu olan toplam 110 hasta (20-50 yaş arası 78 kadın ve 32 erkek,) dahil edilmiştir. Hastalar rastgele probiyotik (n=38), prebiyotik (n= 36) ve plasebo (n= 36) gruplarından birine atanmıştır ve 81 hasta çalışmayı tamamlamıştır. Geriye dönük besin tüketim kaydı kullanılarak besin tüketimi alımı ve uluslararası fiziksel aktivite anketi kullanarak fiziksel aktivite seviyesi, başlangıç ve bitiş noktalarında kaydedilmiştir. Ayrıca katılımcıların boy ve ağırlıkları ölçülerek beden kütle indeksleri (BKİ) hesaplandı. Depresyon şiddeti, Beck depresyon ölçeği (BDÖ) kullanılarak değerlendirildi. Müdahaleden önce ve sonra serum BKNF, kinurenin, DZAA ve bağırsak mikrobiyom seviyeleri ölçülmüştür. Serum BKNF ve kinurenin seviyeleri ELISA, amino asitler HPLC yöntemi ve bağırsak mikrobiyomu real-time PCR ile ölçülmüştür. Ortalama yaşı 36,5 ve ortalama depresyon süresi 2,3 yıl olan toplam 81 hasta (28 probiyotik, 27 prebiyotik ve 26 plasebo grubu) çalışmayı tamamlamıştır. Müdahale öncesi ve sonrası ortalama yaş, BKİ ve aktivite açısından üç grup arasında anlamlı bir fark bulunmamıştır. Çalışma sonunda besin ögeleri alımı gruplar arasında plaseboya kıyasla probiyotik grupta daha düşük olan selenyum (P=0.05) haricinde başka fark bulunmamıştır. Probiyotik grubunda, BDÖ puanları (P=0.042) ve kinurenin/triptofan oranı (P=0.048) önemli ölçüde azalmış iken, plaseboya kıyasla triptofan/izolösin oranı (P=0.023) artmıştır. Prebiyotik grubunda bu sonuçlar üzerinde önemli değişiklik görülmemiştir. Ayrıca, BKNF düzeylerindeki artış, hem prebiyotik (P<0.001) hem de plasebo (P=0.02) gruplarına kıyasla probiyotik grupta anlamlı bulunmuştur. Ayrıca probiyotik grubunda prebiyotik gruba göre bağırsak Lactobacillus grubu ve Bifidobacterium spp. anlamlı olarak arttığı bulunmuştur (sırayla P=0.014 ve P=0.025). Bu çalışma, probiyotik desteğinin, muhtemelen bağırsak Lactobacillus grubunu ve Bifidobacterium spp.'yi artırarak depresyon durumunu ve serum BKNF'yi iyileştirdiğini ve kinureninin triptofan oranını azalttığı, ve prebiyotiklerin ise depresyon durumu üzerinde etkisinin olmadığını göstermiştir.

Anahtar Kelimeler: Probiyotik, prebiyotik, majör depresif bozukluk (MDB), beyin kaynaklı nörotrofik faktör (BKNF), bağırsak mikrobiyomu, kynurenin / triptofan oranı

ABSTRACT

Heidarzadeh Rad, N. Effects of Probiotic and Prebiotic Supplements on Serum BDNF, Kynurenine, Tryptophan Competing Amino Acids and Intestinal Microbiome in Major Depressed Patients, Hacettepe University Graduate school of Health Sciences, Nutrition and Dietetics Program PhD Thesis, Ankara, 2020. Recent studies highlighted the role of disturbed intestinal microbiome in development of depression. Probiotics and prebiotics can be useful in prevention and treatment of depression as they can balance the intestinal microbiome and modulate the gut-brain axis. The aim of this double-blind randomized controlled clinical study was to compare the effects of *Lactobacillus helveticus* and *Bifidobacterium longum* probiotics and galacto-oligosaccharide prebiotic on serum brain derived neurotrophic factor (BDNF), kynurenine, tryptophan competing branched chain amino acids (BCAA) and intestinal microbiome in patients with major depressive disorder. A total of 110 patients (78 female and 32 male, aged 20-50 years) with low to moderate depression, who referred to psychiatric clinic of Bahman Hospital in Iran, were included and randomly assigned to one of the probiotic (n=38), prebiotic (n=36) and placebo (n=36) groups and 81 patients completed the study. Dietary intake using dietary recall, and physical activity level using international physical activity questionnaire were recorded at the baseline and endpoint. Furthermore, Height and weight of the participants were measured and body mass indexes (BMI) were calculated. Depression severity assessed using Beck's depression inventory (BDI). Serum BDNF, kynurenine, BCAA, and intestinal microbiome levels were measured before and after the intervention. Serum BDNF and kynurenine levels were measured using ELISA. Amino acids were measured by HPLC. Intestinal microbiome was measured using real-time PCR. Totally, 81 patients (28 in the probiotic, 27 in the prebiotic and 26 in the placebo groups) with a mean age of 36.5 years and mean depression duration of 2.3 years completed the study. There was no significant difference among the three groups in terms of mean age, BMI and activity before and after intervention. Dietary intake was not different between groups at the endpoint except for selenium which was lower in probiotic group compared to placebo (P= 0.05). Probiotic use significantly reduced Beck depression scores (P= 0.042) and kynurenine to tryptophan ratio (P= 0.048) while increased tryptophan to isoleucine ratio (P= 0.023) compared to placebo, whereas prebiotic had no effect on these outcomes. Furthermore the rise in BDNF levels was significant in probiotic group compared to both prebiotic (P<0.001) and placebo (P=0.02) groups. Additionally, the increase in intestinal *Lactobacillus* group and *Bifidobacterium spp.* in probiotic group was significant compared to prebiotic group (P= 0.014 and P=0.025, respectively). This study showed that probiotic supplementation improves depression status probably by increasing intestinal *Lactobacillus group* and *Bifidobacterium spp*. and serum BDNF and reducing kynurenine to tryptophan ratio, whereas prebiotics had no effect on depression status.

Keywords: Probiotic, prebiotic, major depressive disorder (MDD), brain derived neurotrophic factor (BDNF), intestinal microbiome, kynurenine to tryptophan ratio

TABLE OF CONTENT

EK 1. Ethical Approval

EK 2. Beck's Depression Inventory

EK 3. International Physical Activity Questionnaire

Ek 4. Turnitin Report

9. CURRICULUM VITAE

SYMBOLS AND ABBREVIATIONS

AA	Amino acid
ACTH	Adrenocorticotropic stimulating hormone
Akt	Protein Kinase B
BBB	Blood brain barrier
BCAAs	Branched chain amino acids
Bcl2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
CamK	Calmodulin kinase
CES-D	Center for Epidemiological Studies Depression Scale
CFS	chronic fatigue syndrome
CFU	Colony forming unit
CNS	Central nervous system
CREB	Cyclic AMP response element binding protein
CRF	Corticotropin releasing factor
DALY	Disability-adjusted life year
DASS	Depression, anxiety and stress scale
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
ECs	Enterochromaffin cells
ENS	Enteric nervous system
ERK	Extracellular signal related kinase
FOS	Fructo-oligosaccharides
GBA	Gut-brain axis
GBD	Global Burden of Disease
GDS	Geriatric depression scale
GI	Gastrointestinal
GHQ	General health questionnaire
GOS	Galacto-oligosaccharide
GWAS	Genome-Wide Association Studies
HADS	Hospital Anxiety and Depression Scale

HAM-D	Hamilton Rating Scale for Depression
HPA	Hypothalamic–pituitary–adrenal axis
HSCL-90	Hopkins Symptom Checklist
IBS-QOL	IBS quality of life questionnaire
IDO	Indoleamine 2,3-dioxygenase
IgA	Immunoglobin A
IP3	Inositol triphosphate
KYN	Kynurenine
LPS	Lipopolysaccharides
MADRS	Montgomery-Asberg Depression Rating Scale
MAPK	Mitogen-activated protein kinase
MDD	Major depressive disorder
NAD	Nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
PI3K	Phosphatidylinositol-3-kinase
PLCγ	Phospholipase C-gamma
PND	Postnatal day
POMS	Profile of mood states
PSS	Perceived stress scale
SCFA	Short-chain fatty acids
SSRI	Serotonin selective reuptake inhibitor
STAI	State-Trait Anxiety Inventory
TDO	Tryptophan 2,3-dioxygenase
TrkB	Tyrosine kinase receptor B
TRP	Tryptophan
VAS	Visual analog scale
XOS	Xylo-oligosaccharides

FIGURES

TABLES

1. INTRODUCTION

1.1. Theoretical Approach and Scope

Depression is a mental disorder with high prevalence and notable disabilities which reduces the quality of life (1). Untreated depression is considered as a chronic and recurrent disorder along with increasing disability over time (2, 3). Major depressive disorders (MDD) are a main cause of global burden of disease (GBD). It was listed as the third leading cause of the GBD in 2004 and is expected to be the first by 2030 (4). It was projected that depression would be the first leading cause of disability-adjusted life year (DALYs) in 2030 (5). Moreover, in Iran, it is considered as the third disease burden with an overall prevalence of 4.1% (6).

Response to antidepressant medications among MDD patients is low. It has been shown that only half of the patients respond to first and fewer to second and third prescriptions (7). Waiting to see the results in responder patients or switching the medications in non-responders may take several weeks which can be frustrating by itself (8).

Depression has many risk factors such as environmental, genetic and psychological factors (9, 10). Environmental factors such as exposure to stress particularly starting from intrauterine period may change the programming of microbiome colonization which results in elevated stress reactivity and depression in the offspring (11, 12).

Several theories are suggested for mechanisms underlying MDD pathogenesis such as neurotransmission deficiency (13), neurotrophic alterations (14), endocrine-immune system dysfunction (15), neuroanatomical abnormalities (16) and intestinal microbiome alteration (17).

Since the brain's health depends on nutrients from diet, diet may be involved in etiology of depression (18). One mechanism of diet's involvement is through the impact on the intestinal microbial population (19). Diet and intestinal microbiome are related through the pathways by which the nutrient intakes and microbiome can

affect each other (20). There is a complex and bidirectional interaction between brain and gastrointestinal (GI) system, which is called gut-brain-axis (GBA). The GBA involves not only in the central nervous system (CNS) and enteric nervous system (ENS), it is inter-connected by neuro-immune and neuro-endocrine systems (21) with a main node of intestinal microbiome (22).

While a balanced intestinal microbiome is essential for mental health, altered microbiome composition may lead to MDD (23). Intestinal bacterial population affects the serotoninergic system indirectly by inhibiting the tryptophan-degrading enzyme (22). Serotonin is an important neurotransmitter in the GBA. Although, some bacteria have the enzymatic system to metabolize the tryptophan and decrease serotonin levels; some can convert tryptophan to serotonin (22, 24-26).

The intestinal microbiome modulation induced by diet can improve mood and behavior through the bidirectional relation, GBA (27). Several studies indicate that probiotics and prebiotics supplementations improve the intestinal microbiome (28, 29). In several animal models of depression, improvement was observed after administration of some species of probiotic bacteria such as *Lactobacillus helveticus, L. rhamnosus, Bifidobacterium longum and B. infantis* (30-33).

Interventional microbiota studies in depressive population are very few and most of the studies are conducted in animals or healthy individuals (27). Clinical trials of probiotic interventions in psychiatric patients will also be valuable in people with gastrointestinal or mental disorders (23). Administration of probiotics and prebiotics for modification of gut microbiome may serve a solution for the population suffering from MDD which is a global health problem (34, 35).

1.2. Objectives and Assumptions

This study was designed to investigate the effects of probiotics (*Lactobacillus helveticus* and *Bifidobacterium longum)* and galacto-oligosaccharide (GOS) prebiotics on Beck's depression inventory (BDI), serum levels of brain-derived neurotrophic factor (BDNF), kynurenine (KYN), tryptophan-competing amino acids

and intestinal microbiome in patients with depression. With the results obtained from this study, as the following variables were evaluated:

- Comparison of sex and age at baseline within all three study groups and between groups.

- Comparison of weight, height and BMI at the beginning and the end of the study within all study groups and between groups.

- Comparison of physical activity (METs), total energy, carbohydrate, protein and fat, as well as saturated fatty acids, MUFA, PUFA, cholesterol, dietary fiber intake at baseline and end of the study within and between study groups.

- Comparison of Beck Depression (BDI) scores at the beginning and end of the study within and between study groups.

- Comparison of serum levels of BDNF, KYN and KYN to TRP ratio as well as competing amino acids to TRP ratio at the beginning and end of study within and between all three study groups.

- Comparison of intestinal microbiome at the beginning and end of study within and between all three study groups.

The following assumptions were projected in this study:

1. The baseline and clinical variables at the beginning of the study are not significantly different between the study groups.

2. The effects of probiotic, prebiotic and placebo supplementation on physical activity variables, total energy, carbohydrate, protein, total fat, saturated fatty acids, MUFA, PUFA, cholesterol, dietary fiber intakes are different in the study groups.

3. The effects of probiotic, prebiotic and placebo supplementation on mean changes in Beck Depression Inventory scores differ among the intervention groups.

4.	The effects of probiotic, prebiotic and placebo supplementation on the mean kynurenine to tryptophan ratio are different in the study groups.

5.	The effects of probiotic, prebiotic and placebo supplementation on the mean tryptophan-competing branched chain amino acids (BCAAs) are different in the study groups.

6.	The effects of probiotic, prebiotic and placebo supplementation on the serum BDNF levels are different between groups.

7.	The effects of probiotic, prebiotic and placebo supplementation on the intestinal microbiome are different in the study groups

In case of positive effects in treating depression and with the confirmation of future studies, probiotic and prebiotic supplementations can be used as a viable strategy with fewer side effects in reducing the antidepressant dosages, prevention of relapse, progression, and complications of depression.

2.   GENERAL INFORMATION

2.1.   Major Depression Disorder (MDD)

Depressive disorders are common mental disorders that impair individual's normal life (36). They are characterized by persistent feeling of sadness, loss of interest or enjoyment, loss of self-worth or guilty feeling,   sleep or eating disorders, fatigue, inattention or concentration problem. Major depressive disorder (MDD) or depressive episode is a subcategory of depressive disorders that also called as unipolar depression or clinical depression or simply depression. MDD is categorized as mild, moderate or severe (36). These symptoms should persist for at least two weeks, disrupt the daily life including social, occupational and educational activities, not following a medication or physical illness, and cannot be attributed to the mourning of a loss of a loved one. The diagnosis is based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), with a minimum one major depressive episode without a history of mania or hypomanic or mixed episodes. Manic episode is an emotional state for a period of at least one week characterized by high mood and energy associated with increased activity decreased need for sleep and speech pressure (37). The hypomanic episode is the same as the manic with a shorter period (for minimum 4 days and less than a week). The mixed episode is the simultaneous presence of symptoms of depression and mania at the same time (36).

The prevalence of mental disorders is predicted to increase by aging world population (36). On the other hand, the response rate to classic psychiatric treatment is insufficient (38). Clinical, pharmacological, pharmacokinetics and pharmacodynamics factors may involve in resistance to psychotherapeutic drugs (39). In addition to these factors, recent animal findings suggest that abnormal microbiome can possibly change the behaviors and drug metabolism of the host, which may explain the ineffectiveness and other side effects of psychiatric drugs (40). Modern lifestyle, such as antimicrobial medication, vaccination, widely used disinfectant cleaning and dietary changes, have deep and lasting effects on the human microbiome (41).

2.1.1. Epidemiology of MDD in the World and in Iran

MDD affects one in four to six people during some point of life (42) and can start almost at any age but often in youth (4). It may occur once or reoccur in a person's life while the rate of relapse is high (43). Major depression in 20% of the patients tends to become chronic which defines as lasting for minimum two years (44). The more episodes a patient experiences, the shorter the time between relapses and the more severe the depression is (42).

WHO for 20th anniversary of "World Mental Health Day" occasion on October 10, to draw attention of governments and society to address depression as a global and also treatable illness, prepared a report as "Depression: A Global Crisis" as the theme for the year 2012–2013 (4). In this report depression was listed as the third leading cause of the GBD in 2004 and will be the first by 2030. Lifetime prevalence estimates were between 3-16 %, while Japan had the lowest rates; USA was at the top of the list (4). It was also stated that 20% of the mental disorder comorbidities in Europe are caused by depression and this rate is as high as 26% in some other countries (4). MDD is the most commonly seen mental disorder in Europe and 11% of EU citizens have depression in some points of their life (45). Prevalence of MDD in 2015 has been reported to be 4.4% which means 350 million people in the world have depression (36). MDD is more prevalent in women (5.1%) than men (3.6%) (46). According to the GBD report of World Health Organization, MDD has been listed as the fourth leading cause of disability in 2000, however in the latest update report, it has moved up in the ranking to third place in the world and first place in middle- and high-income countries (36). The annual incidence of MDD was estimated to be 3.2% in men and 4.9% in women (47).

The prevalence of depression varies widely around the world. On the other hand, due to the interactive role of genetics and environment, geographical studies are also needed to evaluate the prevalence of depression in different regions. Based on "Mental Health Survey of the Iranian Adult Population", 10.39% of population (11.43% in women and 9.34% in men) are suspected to have depression (48). Iranian female population are found likely to be depressed about 1.95 times more than males

(6). The highest prevalence of MDD has been reported in East Azarbaijan (49) , Gilan (50) and Mazandaran (48) (13%) and the least prevalence was in Sistan and Baluchistan (51) (6.6%) provinces.

2.1.2. Pathogenesis of MDD

2.1.2.1. Genetics

The effect of genetic factors on depression is about 30 to 40% (52). Given that GWAS found no specific genes involved in the pathogenesis of depression, epigenetic mechanisms, that involve both inheritance and environment, have been addressed (53).

2.1.2.2. Neurotransmission deficiency

According to the theory of monoamine deficiency, depression is a result of depletion of neurotransmitters in the CNS such as serotonin, norepinephrine, and dopamine. The serotonin hypothesis of depression is the main hypothesis of pathophysiology of MDD that has been introduced by Coppen in 1967 (54). Most of the serotoninergic, noradrenergic and dopaminergic neurons are located in the midbrain and brainstem nucleus which spread to large areas of the brain. This indicates that the monoaminergic system is involved in regulating a wide range of brain functions including mood, sleep, appetite, reward process, and cognition (9).

2.1.2.3. Hypothalamic-pituitary-adrenal (HPA) axis

Corticotropin releasing factor (CRF) is a neuropeptide secreted from the hypothalamic paraventricular nucleus that induces secretion of adrenocorticotropic stimulating hormone (ACTH) from the anterior pituitary (55). ACTH is a peptide hormone that stimulates cortisol secretion from the adrenal glands. According to the corticosteroid receptor hypothesis of depression, the activity of this axis (hypothalamic-pituitary-adrenal) increases in depression. The hyper-activity of HPA axis appears to be mainly due to the high secretion of CRF. Cortisol normally has negative feedback on CRF secretion. In depression this feedback decreases. In other words, cortisol resistance is seen, thereby CRF secretion increases. There is

concordant evidence that CRF plays a key role in the pathogenesis of some types of depression (55).

2.1.2.4. Inflammatory factors (inflammatory cytokines)

Cytokines are protein molecules produced by immune cells that regulate the immune response (56). The discovery of antibodies against cytokines revealed that other cells, including vascular endothelial cells, some epithelial cells, and adipose tissue are also able to produce some types of cytokines. The biological roles of these protein molecules vary, but are generally divided into inflammatory and anti-inflammatory roles (57). Inflammatory cytokines induce inflammatory reactions directly and indirectly, but anti-inflammatory cytokines suppress inflammatory reactions by blocking the production of inflammatory cytokines and counteracting the activation of cells. The amount of cytokines in the serum depends on the activation of the immune system. The immune system is activated to an infection or damage in the body, leading to inflammation. This response must be controlled by the body. The action of inflammatory cytokines which produce a cascade of inflammatory mediators is counteracted by anti-inflammatory cytokines. Although inflammation is the body's natural response, it can cause illness when it occurs in an uncontrolled manner. The association of immune activation with classic inflammatory diseases such as rheumatoid arthritis has long been known (58). Today, mild activation of the immune system seems to be involved in other diseases as well (57). There is ample evidence that depression is associated with a mild chronic inflammatory response, activation of cellular immunity, and activation of the compensatory anti-inflammatory system (59). Numerous factors that cause depression, including stress, diet, inactivity, smoking, obesity, sleep disturbance and low levels of vitamin D, exert their effect by increasing levels of inflammatory cytokines (56).

Cytokines play roles in pathogenesis of depression in several ways:

1.   Cytokines decrease the synaptic availability of monoamine in several ways, which is known to be the major mechanism in the pathogenesis of depression (60). For example, a study on laboratory animals has shown that IL-1B and TNF increase activity of brain serotonin transporter protein, by enhancing the expression and function of it, through induction of p38 mitogen-activated protein kinase (MAPK). This leads to decreased synaptic availability of serotonin and depression-related behaviors in the animals (61).

2.   Cytokines also form reactive oxygen and nitrogen species which reduce the availability of tetrahydrobiopterin, an important co-enzyme in the synthesis of all monoamines (62).

3.   Th1 Cytokines for example IFNγ, increase the levels of indoleamine 2, 3 dioxygenase (IDO), which break down tryptophan to kynurenine (63).

4.   Cytokines decrease brain-derived growth factor (BDNF) and consequently BDNF-induced neurogenesis that is required for antidepressant response (64).

5.   Increased levels of glutamate in basal ganglia, which is associated with an increase in depressive symptoms, have been demonstrated in patients receiving interferon alpha (65).

6.   Cytokines make changes in the areas of the brain that are involved in regulating motivational behaviors and anxiety (66).

2.2.   The Intestinal Microbiome

Microbiota means a group of microorganisms living in a specific environment which was formerly named as "microflora". The term microbiome refers to all microorganisms of the body and their genetic materials (67). Although these two terms mean completely different, they are used instead of each other .The intestinal microbiome is now known as the major node of the GBA (68, 69). The microbiota of human GI tract consists of more than 10^{14} microorganisms mainly bacteria (mainly

anaerobic), yeast, fungi and viruses (70); in other words prokaryotic cells of human body are 10 times more than eukaryotic cells (71). Numbers are more interesting in terms of genomic content. The intestinal microbiome is approximately have more than 100 times more specific genes than the human genome (72). However, recently Ron Sender et al. (73) have been suggested a revision of the ratio of human to bacteria cell count as 1:1.

Every human individual has specific intestinal microbiome content which consists of mainly two bacterial phyla of *Bacteroidetes* and *Firmicutes*; and also small amounts of *Proteobacteria, Actinobacteria, Fusobacteria, Archaea* and *Verrucomicrobia* (74). The microbiome matures in the first three years of life starting from the intrauterine period (75). Although it has been reported that intestinal microbiome composition changes after age of 60 years, including a reduction of *Bacteroidetes*/*Firmicutes* ratio and *Bifidobacteria* (76), the general composition is stable in adulthood (77).

Although the role of microbiome has not been fully understood yet, it is considered as an important factor in regulating many vital functions in human body such as development of intestinal motility and immune system (78). The intestinal microbiome plays a leading role in metabolism of xenobiotics and undigested/unabsorbed nutrients, production of vitamins (such as intestinal vitamin K) or bioactive molecules and trophic effects on intestinal epithelial cells (75, 79). The intestinal microbiome supports production of short-chain fatty acids (SCFAs), inhibition of growth of pathogens, intestinal barrier integrity and mucosal immune homeostasis (80). Disturbance in the composition of intestinal microbiome results in impaired intestinal function (81).

The intestinal microbiome and nervous system:

The reciprocal link between microbiome and central nervous system was first raised in 2004 (11). The intestinal microbiome and its messenger molecules can reach the ENS and therefore CNS and modulate the functions of brain (82).

The role of intestinal microbiome in development and maturation of both nervous systems has been highlighted in studies on animals with no microbiome (83,

84). Microbiome generates neuro-active molecules which work like local neurotransmitters such as GABA, serotonin and melatonin (85). Some harmful bacteria use tryptophan as their food, thereby reducing its access to the host (86). Some other beneficial bacteria produce tryptophan (87) and even serotonin (88, 89). Serotonin is an important neurotransmitter in the GBA. The CNS levels of tryptophan, the precursor of serotonin, are largely dependent on peripheral availability. The enzyme machinery responsible for serotonin production is not saturated with normal tryptophan levels, thus microbiome play an important role in regulating the synthesis of 5-hydroxytryptophan in the central and enteric nervous systems. This effect is achieved by the effect of the microbiome on the expression of the key enzyme IDO, which breaks down the TRP to KYN (22).

Also some bacteria produces nitric oxide which has immunomodulatory and antibacterial effects (90). Microbial colonization of the intestine also targets muscular contractile proteins' expressions which adjust the sensory-motor functions of GI system, like gastric emptying and intestinal transit (91, 92).

The Microbiome-GBA Concept:

The GBA is defined as the bidirectional link between the "big brain" located in the skull and "little brain" located in the abdomen, which are connected to each other via different nervous systems and mediating molecules(93). In other words, the GBA consists of the CNS including brain and spinal cord, ENS, HPA axis and the autonomic nervous system (ANS) including sympathetic and parasympathetic nerves (afferent and efferent nerves), which are depicted in Fig. 2.1 (94). Afferent nerves carry the sensory information collected from intestine through ENS, vagus nerves and spine to the brain and efferent nerves bring out of the brain to its target in the intestine. HPA (hypothalamic - pituitary - adrenal) axis regulates the body's reactivity to environmental factors such as emotion or stress, since is a part of and driven by the interaction which occurs in the limbic system (95). Limbic system is made up of the amygdala, hippocampus, thalamus, hypothalamus and other structures which together regulate the brain's important functions such as emotions or memory. Pro-inflammatory cytokines and emotional stress elevate the reactivity of

HPA and leads to CRF release from hypothalamus. CRF induce the secretion of ACTH from pituitary which stimulates the adrenal glands to secrete the stress hormone, cortisol. As a result, the brain through nerves and hormones influence the intestinal target cells. Besides, the intestinal microbiome affects these target cells (96) which emerge the new concept of microbiome-GBA. These intestinal target cells include immune cells, epithelium, ENS, smooth muscle cells, Cajal and enterochromaffin cells (94). As mild stress in mice without microbiome causes excessive release of corticosterone, the intestinal microbiome content is essential for the proper development of the stress response (97). On the other hand, some probiotics can decrease plasma cortisol levels that is probably due to the decreased inflammatory cytokines levels which activate the HPA axis (24). Also physiological stress has been associated with higher GI barrier permeability that can be reversed by probiotics (98).
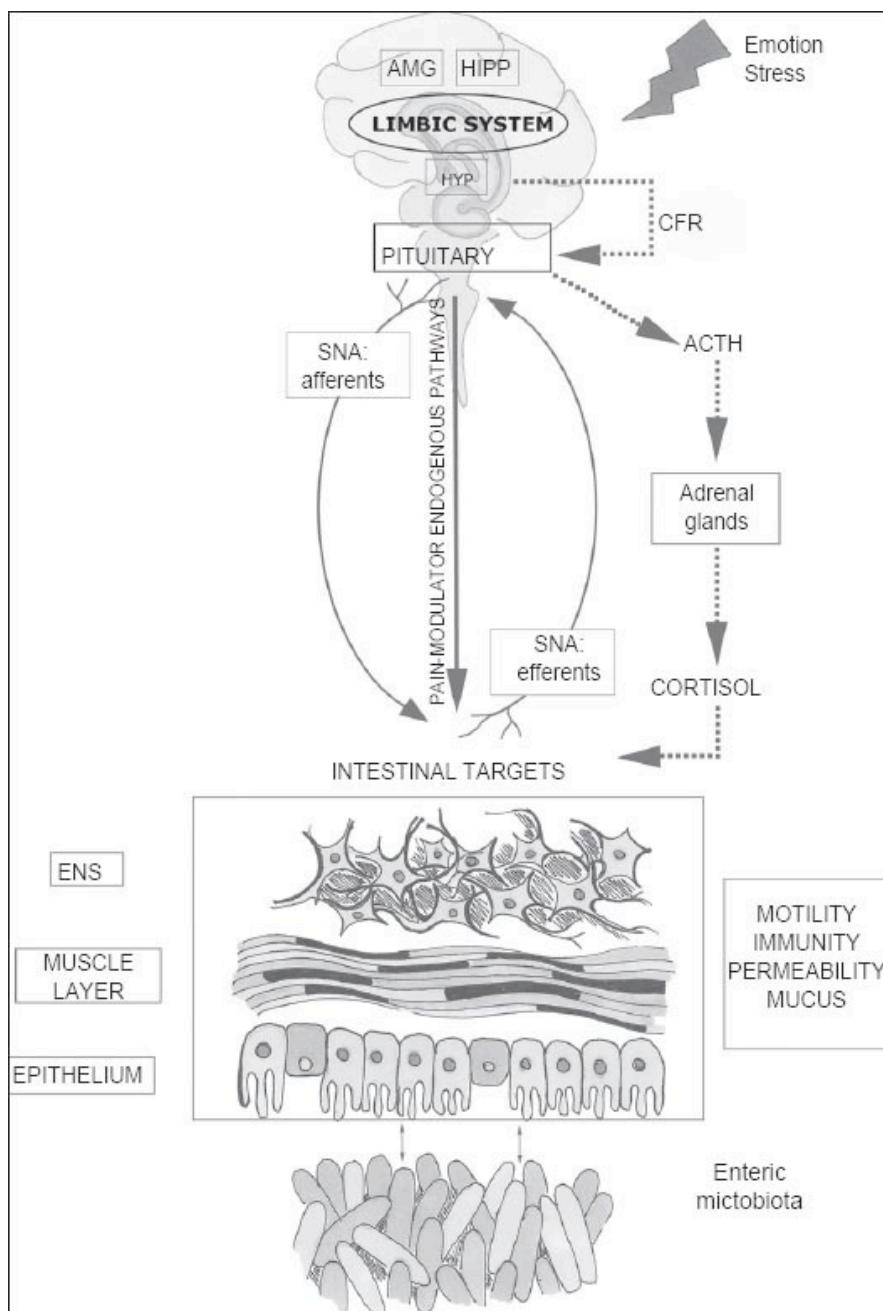
Fig. 2.1- The Microbiome gut-brain axis structure (94)

The intestinal microbiome can interact with the brain through several mechanisms as follows (94):

a.   Production, expression and turnover of neurotransmitters such as serotonin and neurotrophic factors such as BDNF (94). Several studies showed that intestinal microbiome regulates gene expressions of enzymes involved in neurotransmission process in both ENS and CNS (83, 99, 100).

b. Maintaining the intestinal barrier and integrity of tight junctions (94). In depression, intestinal barrier permeability changes. Gram-negative bacteria and their trans-localization are higher in depressed individuals; the immune system is stimulated to overcome with the lipopolysaccharides (LPS) of these bacteria which leads to increased inflammatory cytokines (101). Cytokines trigger activation of inflammatory signaling pathways in the brain, resulting changes in the neurotransmitter system (psychoneuroimmunology) (102). Some probiotic strains can restore integrity of tight junctions in intestinal barrier in rats exposed to stress condition (103). Therefore probiotics reduce inflammatory cytokines by inhibiting pathogens and preventing them from adhering to the intestinal barrier and maintaining an intestinal mucosal integrity (104).

c. Modulation of enteric sensory afferents. For example *Lactobacillus reuteri* has been shown to regulate gut motility by affecting an ion channel in enteric sensory nerves (105)

d. Bacterial metabolites such as SCFA which have been shown to activate sympathetic nervous system (106) through regulation of microglial cells maturation and function (107).

e. Regulation of mucosal immunity partly through the protease enzyme group which are up-regulated in intestinal immunity disorders (94). Manipulation of intestinal microbiome via probiotic administration was able to normalize the visceral hyper-sensitivity and mucosal inflammation (increased substance P) in rats exposed to antimicrobial (108).

The Intestinal Microbiome and Depression:

According to the intestinal microbiome hypothesis in depression, intestinal microbiome is in a close correlation with depressive disorders. It is shown that the composition and diversity of the microbiome changes in depression, which is called "dysbiosis" (17) which is suggestive of a disturbance in the underlying functions of the GBA which are explained above (109).

Preclinical studies have found disturbed intestinal microbiome in depression; stress conditions are used to depict depression in animal studies. For example maternal separation causes depressive-like behaviors and altered microbiome in rats (110). Lower *Firmicutes* and higher *Bacteroidetes* levels are found in depression model of animals (111, 112).

Although the definite difference in the microbiome of depressive and healthy individuals is still under discussion, the diversity and abundance of intestinal microbiome of depressive individuals differ from healthy (113-115). Some of these differences can be summed up as follows (109):

a.   Phylum alterations include: increased Bacteroidetes, Proteobacteria and Actinobacteria and decreased Firmicutes levels (17).

b.   Family alterations include: an increase in *Prevotellaceae* and *Enterobacteriaceae* levels (17).

c.   Genus alterations include: increased *Prevotella* and *Enterobacteriaceae* and reduced *Faecalibacterium* and *Ruminococcus* (17, 116) as well as *Lactobacillus* and *Bifidobacterium* (117).

Intestinal microbiome has been basically targeted via single- or multistrain probiotics treatments which produces beneficial effects on mental health in both animals   and   humans   (23,   118,   119).   Therefore,   probiotic   and   prebiotic supplementations can be promising strategies to modulate intestinal microbiome in mental disorders (120).

2.3.   Probiotics and Their Effects on Mental Health

According to FDA and WHO jointly, probiotics are "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host"(121) . Effects of probiotics depend on the selected strain and using alone or in combination with other strains as well as target group (122). Multi-strain probiotic formulation has been found more effective (123). Mostly reported probiotics which

are effective in alleviating depressive symptoms are lactic acid bacteria such as *Lactobacillus helveticus* (124) and *Bifidobacterium bifidum* (125).

Probiotics equilibrate the gut microbiome in several ways, such as:

1.   Production of bacteriocins, peptidic toxins which inhibit the growth of similar bacterial strains.

2.   Competing with pathogens for the binding site and receptors.

3.   Inhibiting the growth of the pathogen by induction of immunoglobin A (IgA) and β-defensing production by the host.

4.   Maintaining the intestinal mucosal barrier by increasing mucosal expression, reducing bacterial overgrowth, stimulating mucosal immunity (IgA secretion) and producing antioxidant substances (126, 127).

The most commonly used probiotics are Lactobacilli including *Lactobacillus casei, paracasei, rhamnosus, helveticus, fermentum, plantarum, salivarius* and Bifidobacteria including *Bifidobacterium longum*, i*nfantis*, and *bifidum* (128-130).

Dr. George Porter Philips (131) in 1910 for first time reported that Lactic acid bacteria (LAB) consumption can help depressive patients in their symptoms. Since the psychological stress has been related to higher permeability of GI tract (98), it has been suggested that probiotic bacteria may be beneficial on mood (68). Probiotics, by targeting GBA exert their beneficial effects on MDD and modulates the underlying inflammatory process and neurotransmitters (132). Furthermore by supporting the GI tight junctions decrease permeability of intestinal barrier and the inflammation resulting from pathogen's LPS (18).

Studies have shown that administration of probiotics such as *L. plantarum*, *L. helveticus (124)* to the rats, resulted in increased the levels of serotonin (124, 133) and improved depressive-like behaviors (124). Also other studies revealed that *B. longum* and *L. helveticus* when administered alone (134, 135) or in combination (31, 136) decreased anxiety and/or depression in the animals.

Probiotics were first proposed as an adjuvant therapy for MDD in 2005 (137). Probiotics demonstrate their beneficial effects directly or indirectly on the CNS and depression possibly through following mechanisms (Fig. 2.2).

a) Modulation of serotoninergic system including serotonin synthesis, availability of the precursor TRP to the brain.

b) Changing the chemistry of the brain by altering levels of BDNF through the vagus and enteric nerves compartment of the GBA.

c) Effects on endocrine system: probiotics, by decreasing stress hormone cortisol, modulate HPA axis response to the stress, which in turn regulate mood (138).

d) Effects on immune system: probiotics can shift the immune response to reduce the pro- inflammatory cytokines and inflammatory state which in turn affect the endocrine and nervous system (33).

e) Probiotics modify intestinal microbiome by increasing the diversity and composition of beneficial bacteria (139, 140). Colonization of probiotic bacteria in the gut results in products such as SCFAs and tryptophan which are beneficial for mental health (33).
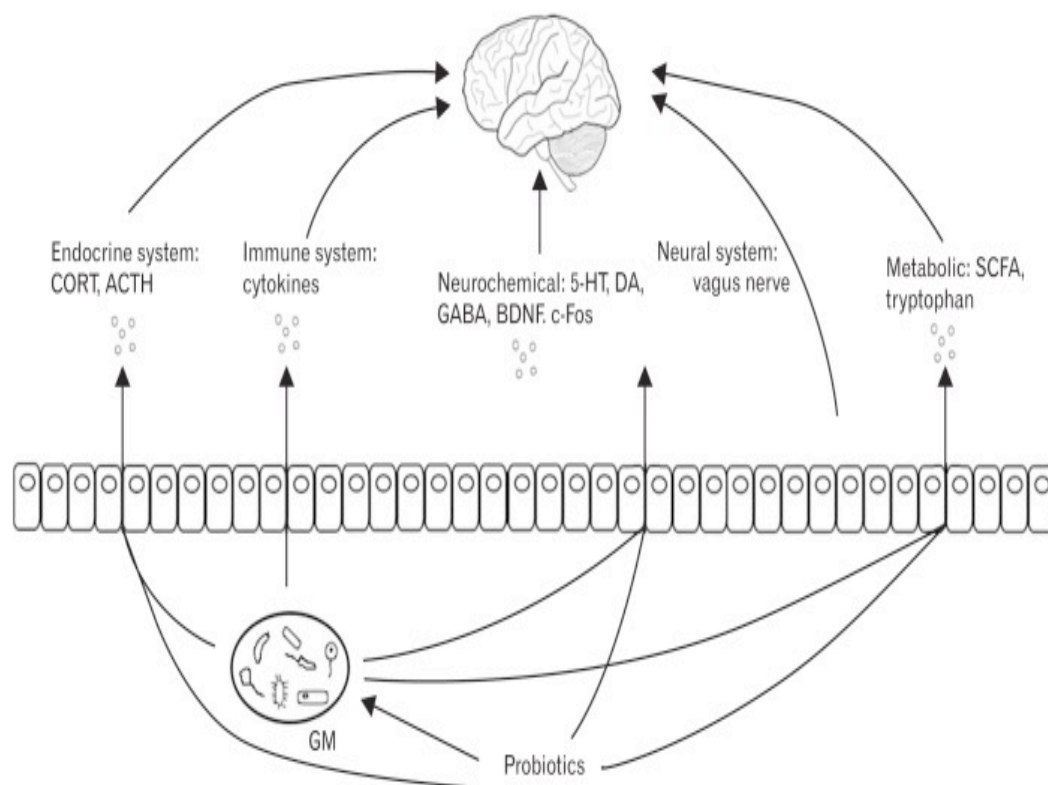
Fig. 2.2- The underlying mechanisms of effects of probiotics on the brain. Probiotics may directly affect the brain by neuro-modulatory molecules such as tryptophan, serotonin and BDNF via vagus nerves and ENS. Probiotics by colonization of beneficial microbiota and fermentation end products may indirectly influence on the brain, as regulate reactivity of HPA axis through suppressing cortisol release as well as pro-inflammatory immune response. (23)

Modulation of Serotonin Synthesis:

Serotonin as a neurotransmitter is a key member of the GBA. Serotonin synthesis in the CNS depends on the available TRP to pass through the blood brain barrier (BBB) via a large transporter. Since majority of the circulating tryptophan is bound to albumin, it is not available to enter the CNS. Central synthesis of serotonin occurs in the neurons of the raphe in the brain stem. Serotonin is also found in ENS the main source of serotonin is synthesized in the GI epithelium- in the enterochromaffin cells, the most common neuroendocrine cell in the GI tract (141).

Factors such as environment, genetics or stress can change tryptophan metabolism and result in depressive symptoms (142). According to "Serotonin Hypothesis" stress leads to altered TRP metabolism resulting serotonin deficiency

mediated depressive behaviors. However this state can be reversed by empowering the serotonin synthesis (143). Intestinal microbiome has been found to increase plasma tryptophan levels and thus potentially facilitate the serotoninergic system in the brain (144).

Tryptophan (TRP) Metabolism

TRP is the precursor of serotonin which is a key neurotransmitter in the gut-brain axis working in both ENS and CNS pathways (145). Tryptophan as an essential amino acid must be obtained through diet, when absorbed from the GI system, circulates in blood stream as free TRP form or binding to albumin (22) and contributes to serotonin synthesize in CNS, by passing through the BBB (146). TRP levels of CNS are highly depending on peripheral availability and which determines the rate of serotonin synthesis in the brain (147). As mentioned above majority of serotonin synthesis from TRP - which accounts for 90% of total serotonin- is performed in the GI tract in the enterochromaffin cells (146).

TRP availability to the brain decreases in presence of stress hormones and inflammatory state. Increased cortisol and pro-inflammatory cytokines such as IFNγ activates the enzymes (which will be discussed below) that lead to catabolism of TRP and lowered its availability to the brain for serotonin synthesis (148-150). The enzyme responsible for producing serotonin (5-hydroxytryptophan- 5HT) is not saturated at normal levels of TRP, and intestinal microbiome plays a key role in regulating 5HT synthesis in the nervous system.

Kynurenine Pathway

Probiotics indirectly regulate the TRP availability for serotoninergic system mainly through KYN pathway (Fig. 2.3) (141). Tryptophan not only utilizes in biosynthesis of serotonin (and melatonin), but also can be metabolized through KYN pathway, which uses 95% of TRP (142). In kynurenine pathway, two enzymes catabolize TRP to kynurenine: 1) tryptophan 2,3-dioxygenase (TDO) or 2) indoleamine 2, 3-dioxygenase (IDO) (151). TDO is induced by glucocorticoids or tryptophan itself, while IDO is stimulated by Th1 immune response (inflammatory factors) (146). Kynurenine is substrate for two enzymes which compete for

kynurenine: 1) kynurenine aminotransferase which result in kynurenic acid, and 2) kynurenine hydroxylase which results in nicotinamide adenine dinucleotide (NAD). Kynurenic acid is an endogenous antagonist of N-methyl-D-aspartate (NMDA) receptors so leads to suppress the glutamatergic system which has anxiolytic effects. Kynurenic acid can limit melatonin synthesis by decreasing N-acetylation of serotonin (152). It is suggested that MDD is associated with increased glutamatergic activity, while NMDA receptors' antagonists such as kynurenic acid result in higher levels of brain serotonin (153). Probiotics by modulation of intestinal microbiome can indirectly suppress the expression of enzyme IDO, tryptophan-degrading enzymes in the kynurenine pathway and thereby increase the levels of tryptophan, which is a precursor of serotonin (154).

Kynurenine-NAD pathway is not desirable due to production of two neurotoxic molecules, quinolinic and picolinic acids, as well as 3-hydroxykynurenine and 3-hydroxyanthranilic acid which are free radical generator. Quinolinic and picolinic acids are agonist of NMDA receptors which lead to hyperglutamatergic status and neuro-excitotoxic which can lead to apoptosis of neurons (155).

Glutamate as a neurotransmitter, with higher concentrations than monoamines, plays a key role in neuroplasticity regulation. Glutamate excitotoxicity is responsible for many CNS disorders. For brain health glutamate should be in balance with its inhibitor GABA. Higher glutamate levels are seen in depressed patients than in controls (156). It has been known that glutamate is essential for dendritic branching development. (157) Hyperglutamatergic state following chronic stress leads to dendritic retraction and spinal loss which plays a role in MDD (158). Higher glutamate levels due to increased NMDA receptor agonists can lead to depressive symptoms (142). On the other hand, the products of kynurenine pathway through hydroxylation route are associated with higher lipid peroxidation and arachidonic acid mediated inflammation and thus higher prostaglandins and leukotrienes (142).

As mentioned above another mechanism of MDD underlies the balance in immunological responses. An imbalanced immune activation results in inflammatory

state which decreases the TRP degrading enzyme IDO, and subsequently alteration in availability of TRP and serotonin, as well as in KYN metabolism. KYN pathways will change in favor of quinolinic acid production which leads to hyperglutamatergic state. Elevated prostaglandin E2 (and COX-2) in depressed patients suggest the inflammatory state of the CNS (153).
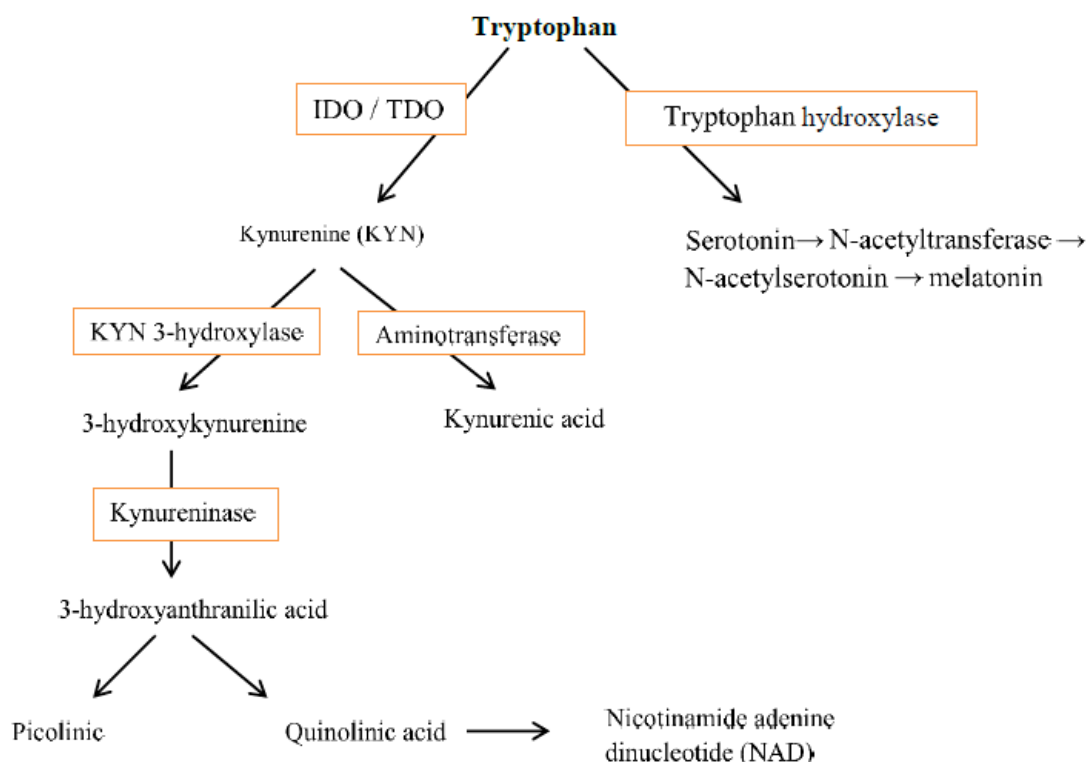
Fig. 2.3- Tryptophan metabolism. IDO: indolamine 2,3-dioxigenase; TDO: tryptophan 2,3-dioxygenase. (142)

Tryptophan Competing Branched Amino Acids (BCAAs)

Branched chain amino acids (BCAAs), are essential amino acids and also important molecules in signaling pathways, includes valine (VAL), leucine (LEU) and isoleucine (ILE). BCAAs compete with TRP for transport across the BBB and thus affect the TRP availability to the CNS for serotonin synthesis (159). Serum TRP levels is not a good representative for its availability for serotonin synthesis in the brain, and measurements of TRP to BCAA ratio can show the most actual availability of TRP to the brain (159).

Former psychiatric research on BCAAs has focused only as a competitor of TRP in transport system across the BBB. While MDD patients have shown deficits in mTOR signaling, recently the role of BCAAs on regulation of mTOR signaling pathway in the pathophysiology of depression has attracted attention. The mammalian target of rapamycin, mTOR, is an evolutionary conserved serine/threonine kinase I3K-related kinase (PIKK) family which regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription (160). It is essential for brain homeostasis due to effects on the neuronal activity by the regulation of autophagy. An abnormality in the regulation of mTOR pathway results in depression (161) through improper synapse inductions in the hippocampus, amygdala, prefrontal cortex and striatum (162) while can be recovered by NMDA receptor antagonists (such as antidepressant agents) (163).

In addition to the role of BCAAs in neuronal cells and astrocytes, high BCAA can interfere with functions of microglial cells. Microglial cells are macrophage population of the brain that scavenge the brain for plaques, damaged or unnecessary neurons and synapses, and infectious agents (164). Improper regulation of these functions may lead to neurotoxicity (165). According to the microglia hypothesis for psychiatric disorders including depression that abnormal activation of microglial cells may induce various psychiatric symptoms (166). It has been suggested that the metabolites of Tryptophan-kynurenine pathway, are involved in brain inflammation and microglial activation (167, 168). The inflammation leads to increased IDO activity which induces higher formation of kynurenic acid. Higher levels of kynurenic acid, which is known to be antagonist of NMDA receptors leads to hyperglutamatergic state (142).

Brain-Derived Neurotrophic Factor (BDNF)

BDNF, one of the most extensively studied neurotrophins, is secreted by neurons and peripheral cells and can be found in most parts of the brain (169). In addition, BDNF is secreted by the bone marrow platelet precursors, the megakaryocytes, and stored in circulating platelets.

The link between BDNF levels and depression emerged in the 90's and yielded the neurotrophic hypothesis of depression (170), which stipulates that the variations in the levels of neurotrophic factors during depression and following antidepressant treatment could be responsible for the atrophy or survival and function of specific stress-vulnerable neurons regulating emotions and mood (170). Sudo et al. (11) have suggested that in hyper-reactivity of HPA axis, corticosterone release in response to stress leads to decrease in BDNF expression.

Specifically, decreased BDNF levels have been found post-mortem in the brain of individuals with MDD (14, 171). Furthermore, several meta-analyses have shown that circulating BDNF levels were decreased in untreated patients with MDD compared to healthy controls, and were normalized after antidepressant treatment (172-174). According to a systematic and quantitative meta-analysis review, circulating BDNF levels can be viewed as a biomarker for diagnosis and treatment efficacy assessment (169) or could be exploited for the clinical improvement of depressive symptoms in individuals receiving antidepressant treatments. (172-175) Indeed, BDNF levels before treatment were shown to correlate with antidepressant treatment response but not with depression severity (176)

BDNF involves in important neurophysiological processes in both the central and peripheral nervous systems such as neuronal survival, neuro-plasticity, neuro-protection and synaptic signaling and augmentation (177, 178). BDNF mediates the neurogenesis by promoting growth and regulating cell proliferation, migration, differentiation and death (179).

BDNF attaches to tyrosine kinase receptor B (TrkB) on target neurons of CNS (i.e. the sub-granular zone of the hippocampus) and support their generation and survival (180) .The interaction of BDNF with its receptor TrkB causes phosphorylation and internalization of the receptor (181). This starts three intracellular signaling cascades as following:

1.   The phosphatidylinositol-3-kinase (PI3K) pathway:

BDNF is responsible for survival of nervous system by regulation of Akt Pathway. Key proteins involved are phosphatidylinositol 3-kinase (PI3K) and Protein

Kinase B (Akt) (182). The PI3k pathway generates phosphatidyl inositides which activates Akt (protein kinase B). Akt is able to regulate cell survival by inhibiting pro-apoptotic proteins such as Bad (Bcl2 associated death promoter) (as well as pro-caspase-9 and Forkhead) and thus promoting the expression of anti-apoptotic proteins like bcl2 (183). Decreased BDNF levels following a stress leads to insufficient anti-apoptotic bcl-2 and reducing neurons survival which may result in depressive disorder (179).

2.   The Phospholipase C-gamma (PLCγ) pathway:

The PLCγ pathway product, IP3 (inositol triphosphate) regulates ion channels. Activation of IP3 receptor, leads to the influx of calcium across the plasma membrane. Binding of Ca2+/Calmodulin, modulates calmodulin kinase (CamK) protein groups which are ample in the brain. Activation of CamK (also known as Ser/Thr protein kinase) increases a Ca2+-responsive transcription factor, CREB (cyclicAMP response element binding protein), which results in neuronal plasticity (181, 184).

3.   The mitogen-activated protein kinase (MAPK) pathway:

The MAPK pathway (ERK pathway) supports cell growth and differentiation. BDNF through triggering the MAPK pathway increases the expression of bcl-2 and CREB which are anti-apoptotic proteins (185). CREB is required by neurotrophin mediated neuronal survival. By activating of extracellular signal related kinase (ERK), MAPK pathway also promotes phosphorylation of synapsin I. Phosphorylated synapsin I involves in regulation of axonogenesis, synaptogenesis and neurotransmitter release (181).

BDNF measurement has no standard method and can be detected in serum, plasma or platelets. Human platelets store and release BDNF upon triggering of a stimulant. While BDNF concentration in plasma reflects the circulating protein which is supplied to the target nervous cells, serum BDNF are total amounts of both plasma and mostly BDNF levels released from platelets by activation (186, 187) . Serum concentration of BDNF is much higher than plasma. While plasma BDNF is usually obtained in the pg/mL range, serum BDNF is in the ng/mL range (187).

Since lifespan of circulating platelets, the reservoir of BDNF, is about 10 days (187, 188), it is suggested that serum levels mirror more actual and detectable BDNF levels while BDNF has a fast clearance from plasma (189).

2.4. Prebiotics and Their Effects on Mental Health

Prebiotics are another strategy to target the intestinal microbiome. Prebiotics are non-living indigestible ingredients of food which provide health benefits by modifying the human gut microbiome, i.e. improve selectively the growth or activity of intestinal microbiota mainly *Lactobacilli* and *Bifidobacteria* (190). Important prebiotics are oligosaccharides or more complex saccharides (101). Inulin, galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and xylo-oligosaccharides (XOS) are examples of commonly used prebiotics (191, 192).

Fermentation of prebiotics by microorganisms of the colon microbiota results in short chain fatty acids (SCFA) such as lactic acid, butyric acid, propionic acid and acetic acid (193) which confer their benefits on mood and mental health via anti-inflamatory properties (194). Acetate, propionate and butyrate with a ratio of 3:1:1 constitute the main content of the intestinal SCFAs (195). SCFAs also directly regulate sympathetic nervous system activity (mediated by GPR41) (106) and release of serotonin from colonic mucosa in the rats (196).

Few human studies have examined the effect of prebiotics on mental health (24). Prebiotic administration has been found to increase the growth of beneficial intestinal bacteria in rodents (29). Also, in a study investigating the effect of FOS and beta-GOS prebiotics in human, B-GOS significantly reduced salivary cortisol levels (24).

Prebiotics have been under-investigated as a supplement in mental disorders. Animal studies showed that prebiotics such as oligosaccharides increase the growth of *Lactobacillus* and *Bifidobacteria* in the intestine and have neurotrophic / anxiolytic effects (29, 197, 198). Sialyllactose, the prebiotic of human milk, has been found to ameliorate effects of stress on gut microbiome and behavior in mice exposed to stressors regardless of immune or endocrine systems (198).

The mechanisms of prebiotics effects on mental health are not yet fully understood. However their modulatory effect on microbiome ecology is a known fact (197) and the bidirectional communication of gut microbiome and brain , GBA, is a necessity to be much explored (21). Prebiotics act by suppressing harmful microorganisms, supporting the intestinal barrier, immune function SCFA production and serotonin metabolism (199).

Some suggested underlying mechanism of effects of prebiotics on mental health and depression are as follows:

a.   Prebiotics have been shown to have antioxidant effects via lowering metabolic endotoxemia and inflamatory mediators which are induced by reactive oxygen species (ROS) (200).

b.   Prebiotics may decrease plasma lipopolysaccharides (LPS) by decresing the permeablity of the intestinal barrier. LPS are found on the external membrane of gram negative bacteria which elicit inflamatory process (through binding to TLR4 and secretion of TNFα, IL6, etc) (201). Cytokine are shown to pass through the BBB and affect neurotransmitter metabolism (202).

c.   Prebiotics fermentation end products, the SCFAs, are found to regulate the immune response. Functions of SCFAs are primarily through suppressing the histone deacetylation of T cells by decreasing histone deacetylase activity and promoting mammalian G protein-coupled receptors activity (GPR41 and GPR43). Activation of GPR43 is found to facilitate neutrophils migration and inhibit inflammatory cytokine production (TNFα) (203). Furthermore, SCFAs by modulating the NF-κB and pro- inflammatory cytokine production and promoting anti-inflammatory IL-10 production results in the dominance of Th2 over Th1 or in other words increasing the IL10 to IFNγ ratio which is disturbed in depression (204). Also SCFAs by inhibiting NF-κB can modulate BDNF and serotonin levels (202). Also, SCFA regulate functions of dendritic cells in both cytokine production and interaction with T cells. For example dendritic cells in presence of butyrate regulate maturation of immature T cell to Tregs (regulatory T cell) rather than IFN-c-

producing cells, through activation of IDO1 and aldehyde dehydrogenase 1A2 (Aldh1A2) (203).

d.   Prebiotics are found to increase neurotransmitters levels by normalizing the intestinal microbiome. Increasing probiotic bacteria such as *Bifidobacterium* can modulate GABA receptors through vagal nerves. (134, 205). Also Bifidobacterium by increasing tryptophan availability to the serotoninergic system improve mental health (33). Some other bacteria like *Clostridia species* and *Bacteroidales fragilis* are found to be associated psychological disorders (206).

2.1.   Literature Review

2.1.1. Studies on the effects of probiotics on depression

Animal studies

Bercik et al. (207) applied T-muris to mice which caused mild to moderate GI inflammation. T-muris infected mice showed anxiety and depression-like behavior which were associated with decreased hippocampal BDNF expression. The researchers showed that 10 days administration *of B. longum* NCC3001 to the mice resulted in behavioral improvement along with restoration of BDNF levels but did not change the cytokine or kynurenine levels. In this study, previously vagotomized mice did not manifest any behavioral change, suggesting that behavior alterations resulting from chronic infection, unlike acute infection, are not mediated by vagus nerves.

Bercik et al.(135) in another study also evaluated effects of supplementation of *B. longum* NCC3001 *on* anxiety in chronic colitis mice. Supplementation of *B. longum* NCC3001 for 14 days had anxiolytic effect and decrease enteric neurons excitability, but did not affect BDNF expression levels. Anxiety-like behaviors were not present in mice with prior vagotomy. It was suggested that *B. longum* require vagus nerve to show its anxiolytic effects which was in contrast with the result of the previous study. The authors explained this contradiction as the difference in the

pattern of inflammation, such that T-muris affects only the colon while in the latter study the infection also reached the small intestine (135).

Javier A. Bravo et al. (134) investigated the effects of *L. rhamnosus (JB-1)* on anxiety and depression in 36 male rats. Anxiety and depression determined by the expression of GABA receptor gene, plasma corticosterone levels, and anxiety and depression-related behavioral tests. The results showed that *L. rhamnosus (JB-1)* changed mRNA of GABA (B1b) in different regions of the brain in rats, including increased in the cortex region (cingulate and prelimbic) while decreased the expression in the hippocampus, amygdala and Locus cereus. The researchers also found expression of GABA (Aa2) mRNA decreased in amygdala and prefrontal cortex and increased hippocampus in the rats. *L. rhamnosus* reduced plasma corticosterone levels and anxiety and depression associated behaviors. However, these effects of *L. rhamnosus* were not found in vagotomized animals suggestive of effects of probiotic through vagus nerve (134).

Michael Messaoudi et al. (136) examined the effects of *Lactobacillus helveticus R0052* and *Bifidobacterium longum R0175* bacteria on anxiety, in 36 male rats for two weeks. The anxiety test was assessed by the conditioned defensive burying test, a screening model for anti-anxiety agents. It was observed that the intervention reduced anxiety-related behaviors in rats.

Smith et al. (208) evaluated effects of supplementation of *L. helveticus R0052* and *L. rhamnosus R0011* (6×10^9 cfu/day) on anxiety and gut microbiome in t lymphocyte deficient mice. Probiotic treatment for 28 days resulted in reduced anxiety-like behaviors and restoration of microbiome to the normal by increasing *Bacteroides, Enterobacteriaceae* and *Firmicutes*.

Liang et al. (124) compared effects of probiotic supplementation containing *Lactobacillus helveticus NS8* in specific pathogen free rats on depressive/anxiety-like behaviors induced by chronic restraint stress. The authors reported improved anxiety and depressive like behavior, higher hippocampal BDNF mRNA levels, restoration of hippocampal serotonin, and lower CORT and ACTH levels in *L.*

helveticus supplemented rats and suggested therapeutic properties of the used probiotic in depression.

Wang et al. (140) tested effects of probiotic *Lactobacillus fermentum NS9* in antibiotic treated rats. To avoid the probiotics harming from antibiotic, they were applied separately during night and day, respectively. It was found that *L. fermentum NS9* administration led to improve anxiety like behaviors, lower CORT and ACTH levels, as well as restoring the gut microbiome composition including increasing *Bacteroides* and *Lactobacillus* and decreasing *C. coccoides and Firmicutes,* which were altered by ampicillin treatment. *L. fermentum NS9* did not change BDNF levels in this study, suggesting that effects of probiotic are strain specific.

Healthy Human studies

Benton et al. (209) investigated the effects of probiotic yoghurt on mood of 124 healthy individuals. The subjects consumed 65 grams of yoghurt containing 6.5 x 10^9 CFU of *Lactobacillus casei* for 3 weeks. The mood status in this study was assessed using profile of mood states (POMS) questionnaire. It was observed that the intervention has significantly improved mood and increased the sense of vitality (209).

Michaël Messaoudi et al. (30) in another study, investigated the effects of *L. helveticus R0052* and *B. longum R0175* on healthy volunteers for 30 days. Depression and anxiety were assessed with the Hopkins Symptom Checklist (HSCL-90) and the Hospital Anxiety and Depression Scale (HADS). Daily administration of *L. helveticus R0052* and *B. longum R0175* probiotics alleviated psychological distress in healthy human volunteers(30).

Steenbergen et. al. (210) in a study on 40 healthy individuals evaluated the effect of taking 2 g of multi-strain probiotic supplement containing 5 x 10^9 CFU on cognitive reactivity of individuals to distressing thoughts. The probiotic supplement contained *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus brevis, Lactobacillus salivarius* and *Lactobacillus lactis.* The response of individuals to the sad mood was assessed using the revised Leiden index of depression sensitivity scale (LEIDS-r). The results

showed that probiotic supplementation for 4 weeks reduced the reactivity to sad mood.

Mohammadi et al. (211) conducted a study evaluating the effects of 6 weeks probiotic supplementation on mental health in 70 petro-chemical industry workers. Individuals were randomized into three groups, one group received a probiotic capsule with conventional yogurt, one group received placebo capsule with probiotic yoghurt, and one group placebo capsule with conventional yogurt. The mental health status of the workers was assessed by DASS (depression, anxiety and stress scale) and GHQ (general health questionnaire). The results showed that both GHQ and DASS scores were significantly improved in both probiotic yoghurt and probiotic capsule groups. In this study, the KYN to TRP ratio and cortisol was also measured, on which probiotic supplementation had no effect (211).

Akito Kato-Kataoka et al. (212) designed a RCT to evaluate the effects of lactic acid bacteria (*Lactobacillus casei Shirota*) on stress related responses and intestinal microbiome among healthy university students under exams stress. The students consumed *Lactobacillus casei* fermented milk (n=23) or placebo milk (n=24) daily from 8 weeks prior to their exam day. It was observed that 8 weeks *L. casei* consumption decreased significantly stress-related salivary cortisol and GI dysfunction, stress feeling measured by VAS (visual analog scale), anxiety measured by STAI (State-Trait Anxiety Inventory) compared to placebo consumption. Intestinal microbiome composition measurements showed *Bacteroidaceae* family was significantly decreased in probiotic group compared to placebo group while *Bacteroidetes* phyla increased slightly in the placebo group (but not significant). The number of species over the supplementation period, in probiotic group was significantly higher than placebo group. Moreover, as another measure of the alpha-diversity index, phylogenic diversity was higher in *L. casei* group than placebo group.

Patients with Nervous System Diseases Other Than Depression

Rao et al. (213) in a randomized controlled pilot study, investigated the effects of probiotic supplementation in 35 patients with chronic fatigue syndrome (CFS). The intervention groups consumed probiotic supplements containing 2.4 x 10^{10} *Lactobacillus casei Shirota* or placebo for eight weeks. Depression and anxiety states and fecal bacteria composition of the participants were evaluated using BDI, BAI (Beck Anxiety Inventory) and culture method, respectively. While probiotic supplements made a significant improvement in anxiety scores, changes in depression scores were not significant. In addition, probiotic supplementation increased fecal *Bifidobacteria spp.* and *Lactobacillus spp.* (in 73.7% and 73.7% of the participants) compared to the placebo (37.5% and 43.8% respectively).

In Simren et al. 's (214) study, 74 patients with IBS consumed 400 mL of fermented milk with yoghurt bacteria (*Lactobacillus bulgaricus and Streptococcus thermophilus)* plus *Lactobacillus casei F19, Lactobacillus acidophilus LA5* and *Bifidobacterium lactis Bb12* for 8 weeks. The bacterial count per mL of milk was 5 x 10^{10} CFU/mL. After intervention no change in the severity of depression based on HADS (Hospital Anxiety and Depression scale) was observed.

Similarly, Dapoigny et al. (215) investigated the effect of *Lactobacillus casei rhamnosus LCR35* (minimum dose of 6×10^8 CFU/day) supplementation in patients with IBS. Four weeks of supplementation had no effect on the severity of depression assessed by HADS. Also in stool samples of 85% of patients who received LCR supplements, *Lactobacillus* was found using PCR analysis, suggesting the survival of the bacteria in GI system.

The lack of observation of the effect of probiotic supplementation on anxiety and depression in individuals with IBS in the last two studies may be related to the heterogeneity of the subjects. Given that the symptoms of IBS are highly heterogeneous, interventions in a specific subgroup of patients to increase homogeneity are recommended (216). The probiotic strains examined in these studies may also be not proper or the duration of intervention may be insufficient.

Pinto-Sanchez et al.(217) evaluated effects of probiotic supplements among 44 subjects with IBS who had mild to moderate depression or anxiety (based on HADS). The supplements were containing 10^{10} CFU *B. longum NCC3001*. Six weeks of probiotic supplementation significantly reduced the BDI depression scores but had no effect on anxiety scores or IBS symptoms or BDNF or fecal microbiota.

Cremon et al. (218) in a pilot study investigated effects of *Lactobacillus paracasei* in patients with IBS. While four weeks of supplementation did not change statistically significant the IBS symptoms compared to placebo, gut microbiome analysis showed decreased *Ruminococcus* and increased SCFA and reduced cytokine IL-15 (a pro-inflammatory cytokine).

Studies in Patients with Depression

In 2016, Akkasheh et al.(125) in a study observed that the administration of 3 probiotic bacteria including *L. acidophilus, L. casei* and *B. bifidum* (with dose rate of 2×10^9 CFU/g for each) for 8 weeks improved the BDI scores and decreased serum hs-CRP levels in 40 patients with major depression. In this study, participants were taking probiotic supplements along with citalopram antidepressant.

Romijn et al. (219) evaluated the effect of probiotics supplementation containing 3×10^9 CFU *Lactobacillus helveticus* and *Bifidobacterium langum* for 8 weeks in 79 depressed patients and observed that probiotic supplementation had no effect on depression indices (based on several depression severity, anxiety and stress questionnaires including Montgomery–Åsberg Depression Rating Scale (MADRS), Improved Clinical Global Impressions scale (iCGI), Quick Inventory of Depressive Symptomatology (QIDS-SR16), DASS-42, Global Assessment of Functioning) and inflammatory factors (IL-1B, IL-6, IL-10 and TNF-α) and BDNF. The main feature of this study was to evaluate the effect of probiotic supplementation as the main treatment and participants did not receive any other treatment.

Recently, Majeed et al. (220) investigated the effects of three-month supplementation of *Bacillus coagulans* (2×10^9 cfu) in 40 MDD patients with IBS. After 90 days, probiotic supplements showed an improvement in depressive

symptoms measured by HAM-D, MADRS, Center for Epidemiological Studies Depression Scale (CES-D) and IBS quality of life questionnaire (IBS-QOL) over the placebo.

Ghorbani et al. (221) investigated the effects of synbiotic supplements in forty patients with MDD. The synbiotic perapats were a probiotics mixture containing *Lactobacillus (casaei, acidofilus, bulgarigus* and *rhamnosus) Bifidobacterium (breve and longum)* and *Streptococus thermophilus*, along with fructooligosaccharide (FOS) prebiotic. The patients after one month of taking flouxetine alone added the synbiotic supplements or placebo to their therapy for 6 weeks. Synbiotic supplementation was associated with a significant improvement of depression of the patients over the placebo, measured by HAM-D scale.

Recently Haghighat et al. (222) conducted a study to compare the effects of a probiotics or synbiotic formulation on depressive symptoms measured by Hospital Anxiety and Depression Scale (HADS) and BDNF levels in 75 hemodialysis patients. Fecal colony counting was also monitored at baseline and end of the trial. The probiotic supplements were containing *Lactobacillus acidophilus T16, Bifidobacterium bifidum BIA-6, Bifidobacterium lactis BIA-7* and *Bifidobacterium longum BIA-8,* each with dose rate of 2.7×10^7 CFU/g. The synbiotic supplements were included the same probiotic formulation with prebiotic fibers (including FOS, GOS and inulin, each 5 g). Over the supplementation period, fecal microbiota colony counting were changed in both probiotic and synbiotic groups including increased Lactobacilli and Bifidobacteria and decreased coliform amounts. Supplementation with synbiotic resulted in decreased depression severity in the group and subgroup of patients with depressive symptoms compared with placebo. In addition, serum BDNF levels were higher in synbiotic group and in the depressed subgroup compared with probiotic and placebo. The authors related the psychotropic ineffectiveness of the probiotic supplementation in to the renal disease of the patient, which have been shown to be associated with intestinal epithelial barrier dysfunction due to ammonia (223). Possibly, microbial dysbiosis has been corrected with synbiotic therapy while not with probiotics alone.

2.1.2. Studies on the Effects of Prebiotics on Depression

Animal Studies

O'Mahony et al. (110) investigated effects of early life stress induced by maternal separation in rat pups. Separation phase lasted from postnatal day 2 (PND) until 12. At week 7-8 the rats were subject to novel stress and showed anxiety-like behavior, higher levels of corticosterone, fecal pellets (suggestive of higher motility of GI in response to the stress) and altered fecal microbiota while the types of bacteria were not identified.

Savignac et al. (29) investigated the effects of GOS and FOS prebiotics on BDNF levels and N-methyl aspartate receptor subunits in rats. The results of five weeks of intervention showed that these prebiotics increased expression of BDNF and NMDAR subunit in rats, while GOS was more effective than FOS.

Andrew J. Tarr et al. (198) tested the effects of human milk prebiotics (Sialyllactose; 6'SL and 3'SL) on behavior and microbiome in mice exposed to social disruption stressor. Feeding prebiotic containing diet (5%) prevented anxiety-like behaviors and microbial dysbiosis in the mice which were manifested in control diet fed mice. Prebiotic pretreatment with prebiotic led to decreased phyla *Firmicutes* and *Cyanobacteria* in both prebiotic fed groups and decreased *Bacteroidetes* and *Verrumicrobia* in 3′SL compared to control group. Also the genera *Bacteroides* and *Coprococcus* were higher in the 3′SL group compared to 6'SL and control groups. Since corticosterone or IL-6 levels were still increased in prebiotic fed mice, the behavior modulating effects of the prebiotics are suggestive of immune/endocrine-independent mechanisms. Sialic acid, in addition proliferation of intestinal microbiota, can possibly pass through the BBB since sialylated glycoproteins and gangliosides were found in the CNS and contribute to brain development (224).

Mika et al. (225) tested the effects of two prebiotics mixture of GOS and polydextrose as well as lactoferrin glycoprotein administration in early life on biological and behavioral responses in later life in male rats. In this 4-week study, rats were divided into 4 groups to receive one of 1) prebiotic GOS and polydextrose,

2) glycoprotein lactoferrin, 3) prebiotic GOS and polydextrose plus lactoferrin glycoprotein and 4) control. At the end of the fourth week, rats were exposed to inescapable stress which produces depression and anxiety like behaviors. All three interventions reduced stress-induced learned helplessness, but corticosterone levels did not decrease in either group. BDNF mRNA was also increased in the prefrontal cortex. Microbial composition analysis showed that GOS plus polydextrose and GOS plus polydextrose plus lactoferrin diets increased *Lactobacillus* species in the rats.

Burokas et al. (197) investigated the effects of FOS, GOS prebiotics and mixture of both on behavior (anxiety and depression) in mice. Three weeks administration of FOS and GOS mixture improved depressive- and anxiety-like behaviors significantly compared with other groups. Furthermore, stress induced behavior, inflammatory factors and microbiome, have been altered through the study. It was found that GOS administered and GOS & FOS administered groups had lower levels of stress hormone cortisol. Hippocampal BDNF expression was higher in GOS & FOS fed mice. In parallel, microbiome composition was changed including increased cecal weight, bacterial and SCFAs content. In phylum level, all prebiotic fed mice had significantly higher levels of *Proteobacteria* and *Actinobacteria* relative to the control group, while FOS & GOS fed mice had significantly higher *Verrucomicrobia* levels compared to other groups. Also prebiotic fed mice had higher levels of anaerobes *Bacteroides* and *Parabacteroides*. Microbiome analysis using qPCR results showed that prebiotic supplementation resulted in higher fecal bacteria content in mice, although Lactobacillus and Bifidobacterium content did not change significantly.

Healthy Human Studies

Smith et al (226) conducted a crossover study to explore the effects of 10g/d FOS enriched inulin intake for two weeks on wellbeing and mood in healthy individuals and found no negative effects, however wellbeing or mood (assessed by battery of questionnaires) were not improved.

Smith et al. (227) in a double-blind RCT also investigated the acute impact of FOS enriched inulin consumption on psychological outcomes in healthy individuals.

The participants were given 5 g of FOS enriched inulin while staying in laboratory for 4 hours and were tested for mood and cognitive performance changes on same day. It was found that on the day of prebiotic consumption the subjects felt happier, and had better recognition memory task and recall performance compared with the placebo group. Mood change was not different between groups.

Childs et al. (228) (2014) designed double-blind RCT to evaluate effects of 21 days consumption of prebiotic Xylo-oligosaccharides (XOS, 8 g/d), probiotic (*Bifidobacterium animalis subsp. Lactis* (Bi-07), 10^9 CFU/d) or synbiotic (XOS + Bi-07) or placebo in healthy individuals. It was observed that consumption of XOS for 3 weeks was associated with increased self-reported vitality and happiness as well as higher amount of fecal *Bifidobacterium* in prebiotic group. Probiotic consumed individuals had higher content of *B. a lactis* in their feces. Synbiotic consumption resulted in higher fecal *Bifidobacterium spp.* compared with placebo. The change of other genera such as *Bacteroides/Prevotella* group (Bac303) and *Lactobacillus/Enterococcus* subgroup (Lab158) was not important.

Schmidt et al. (24) investigated the anxiolytic effect of consumption of 5.5 g/day of a prebiotic mixture consisting FOS and GOS in 45 healthy individuals for 3 weeks. The salivary cortisol response was significantly lower in the group receiving GOS than in the control group. In this study, computer-aided testing of emotional stimulus processing was performed. According to the results of this test, only consumption of GOS reduced attention to negative versus positive information in individuals.

Patients with Disease Other Than Depression

Silk et al. (229) conducted a study to evaluate the effect of prebiotic trans-GOS on 44 people with IBS. In this study, subjects were divided into three groups to receive 3.5 g of GOS (48% purity), 7 g of GOS (48% purity) or 7 g placebo. The result of this cross-over study, which lasted for 12 weeks (2-w baseline, 4-w treatment, 2-w washout and 4-w treatment), showed that only 7 g of GOS supplementation had a significant positive effect on depression and stress. Depression and stress status were assessed using HADS. Fecal microbiome analysis

showed that fecal *Bifidobacterium spp.* in both prebiotic groups was increased and restored to normal levels while in placebo group no change was observed. Also in contrast to placebo, higher *Eubacterium rectale/C. coccoides in* 3.5 g/day prebiotic and lower *Clostridium perfringens - histolyticum* and *Bacteroides-Prevotella Spp.* in 7.0 g/day prebiotic groups were observed which is suggestive of dose-dependent effect of prebiotics.

Azpiroz et al. (230) investigated effects of short chain FOS (scFOS) on gut microbiome and psychological properties in 79 IBS patients. Four-week supplementation of scFOS (5 g) resulted in reduced anxiety scores and depression severity measured by HADS compared to placebo group, however only the change of anxiety scores was statistically important. Fecal analysis showed higher concentration of *Bifidobacteria* in scFOS group approving the bifidogenic effect of the supplements. However concentration of *Lactobacillus, Enterobacteriaceae, Eubacteria* and *Faecalibacterium prausnitzii* were not changed.

Farhangi et al. (205) evaluated effect of 10 g/day of resistant dextrin on depression and anxiety in 55 female patients with type 2 diabetes. After 8 weeks of supplementation, depression status improved in patients who took resistant dextrin (measured by DASS and GHQ) and the ratio of kynurenine to TRP decreased significantly in comparison to placebo group.

Studies in Patients with Depression

To our knowledge no study investigated the effects of prebiotic supplementations in MDD patients so far.

The effects of probiotics and prebiotics on psychology and mental health have been the focus of recent studies. Most of the studies were conducted on healthy subjects and the bacterial strains used in these studies are various. In present study, we investigated effects of two bacterial strains which were effective in most studies, of which mostly conducted in healthy volunteers. Regarding prebiotics, few studies have examined its effects, and so far no study has compared the effects of probiotics and prebiotics together on mood and depression. Only one human study has yet

evaluated the effect of probiotics and prebiotics on BDNF as an underlying mechanism in depression (222).

3. MATERIAL AND METHODS

3.1. Study Design and Participants

Our study was designed as a double blind randomized placebo-controlled trial with 3 parallel arms. We recruited 110 patients (78 women and 32 men) with MDD who have been referred by a psychiatrist in psychiatric clinic of Bahman Hospital located in Tehran, Iran.

Patients between age of 20 to 50 years, diagnosed with a mild to moderate levels of depression, taking anti-depressants were eligible to enter the study. The purpose and methods, as well as benefits and potential risks of the study were explained to all participants. The written informed consent form approved by the Research Ethics Committee of the Tehran University of Medical Sciences was signed by all participants. Ethics approval was provided by the Research Ethics Committee of Tehran University of Medical Sciences, (registration No. IR.TUMS.VCR.REC.1396.4243) (Appendix 1).

General characteristics and medical history and anthropometric measurements were collected. The questionnaires were carefully completed by face to face interview. Inclusion and exclusion criterias were as follows:

Inclusion criteria:

• Patients with mild to moderate depression for at least 3 month diagnosed by a psychiatrist using DSM-IV criteria

• Age: 20 to 50 years old

• Not taking any medication except antidepressant including fluoxetine, citalopram, amitriptyline and sertraline

• Not taking any dietary supplements (such as vitamins, antioxidants, omega-3's) for 4-6 weeks prior to study

• Willing to participate voluntarily

Exclusion criteria:

- Any sensitivity reaction to prebiotic and probiotic compounds,

- Refusal of co-operation, any serious changes in routine diet and lifestyle during the study,

- Any change in antidepressant type or the dosage,

- Long term (at least one week) inflammatory diseases receiving anti-inflammatory drugs,

- Pregnancy

- Antibiotic intake before and during the study

- Priorly diagnosed diseases such as cancer, diabetes, pancreatitis, thyroid, kidney, liver, respiratory, cardiovascular, nutritional allergy by a medical profession

- History of heart and/or brain attack

- Taking medications other than above mentioned antidepressants.

- Patients under shock therapy

- Regular consumption of probiotic products during 2 months prior to the study

- Antioxidant and/or omega-3 supplements intake at least 4-6 weeks prior to the study

- Alcohol consumption (alcoholism according to DSM-IV criteria)

- Smoking (5 cigarettes per day during last 6 months or pipe or hookah at least once during last month),

- Any kind of addiction or substance abuse

- Being on a specific diet (such as vegetarianism or low protein diet)

- Using hormonal drugs

- Participation in another study in less than two months ago.

Participants were instructed to avoid consumption of any other probiotic supplements during the study.

3.2. Randomization and blinding

Patients were randomly assigned to 3 groups of probiotic, prebiotic and placebo. A research assistant not taking part in the project stratified the participants by age ($\geq$35 vs. <35) and then generated permuted block randomization scheme by the method available in *www.randomization.com*. The participants were randomly allocated to the experimental groups (1:1:1) in blocks of 6. The supplement bags were packed and labeled with randomization codes priorly and were distributed.

3.3. Interventions

From 230 participants with mild to severe degree of depression, who referred to the psychiatrics, 110 patients including 78 women and 32 men were included the study. Patients were randomly allocated to receive one of the probiotic, prebiotic or placebo supplements for 8 weeks (Fig. 3.1). First group (n=38) received probiotic supplement sachets, containing 2 strains of bacteria (*Lactobacillus helveticus R0052:* CNCM strain number I-3470 *and Bifidobacterium longum R0175: CNCM strain number I-1722*) at dosage of 5.8 x 10^9 colony forming unit (CFU) per each bacteria (totally >10 billion CFU) plus palm flavor per 5 g sachet (30). Second group (n=36) received prebiotic supplement sachets, containing galactalogosaccharide in powder form with 80% purity plus palm flavor per 5 g sachet. The third group (n=36) received placebo sachets, containing xylitol, maltodextrin, malic acid and palm flavors per 5 g sachet.

All supplements were provided by "Lallemand" company from Canada. Supplements were similar in color, taste and size and were pre-coded by the company. Researchers and the participants were blind to the type of the supplements

until the end of analysis. Due to the high drop rate in participants during the study, the supplements which were primarily sent by Lallemand were not enough until the end of the study; Therefore, the required amount of supplements was sent again by the company.

During the study 29 participants (10 of probiotic group, 9 of prebiotic group and 10 of placebo group) discontinued the study for different reasons as following:

- The probiotic group: 2 subjects did not come to receive the second pack of supplements, 2 subjects did not come for the final test, 6 subjects were discontinued the study because of the complications thought to be associated with supplementation: (not liking the taste (1 subject), Increasing appetite (1 subject), fever and body pain (1 subject), nausea (1 subject), acne and gastrointestinal complications (1 subject)).

- The prebiotic group: 2 subjects did not come to receive the second pack of supplements, 1 subject was excluded from the study due to surgery, 1 person refused to continue the study because he believed that the supplement was not effective, 5 subjects discontinued the study due to the complications they believed to be associated with supplementation: (drowsiness (1 subject), nausea (1 subject), gastrointestinal discomfort (2 subjects), constipation (1 subject).

- The placebo group: 3 subjects did not come to receive the second pack of supplements, 2 subjects did not come for the final test, 1 subject was excluded because of pregnancy, 2 subjects discontinued the study because they thought the supplementation was not beneficial. Two subjects discontinued the study due to complications thought to be associated with supplementation (not liking the taste (1 subject), psychological deterioration (1 subject).
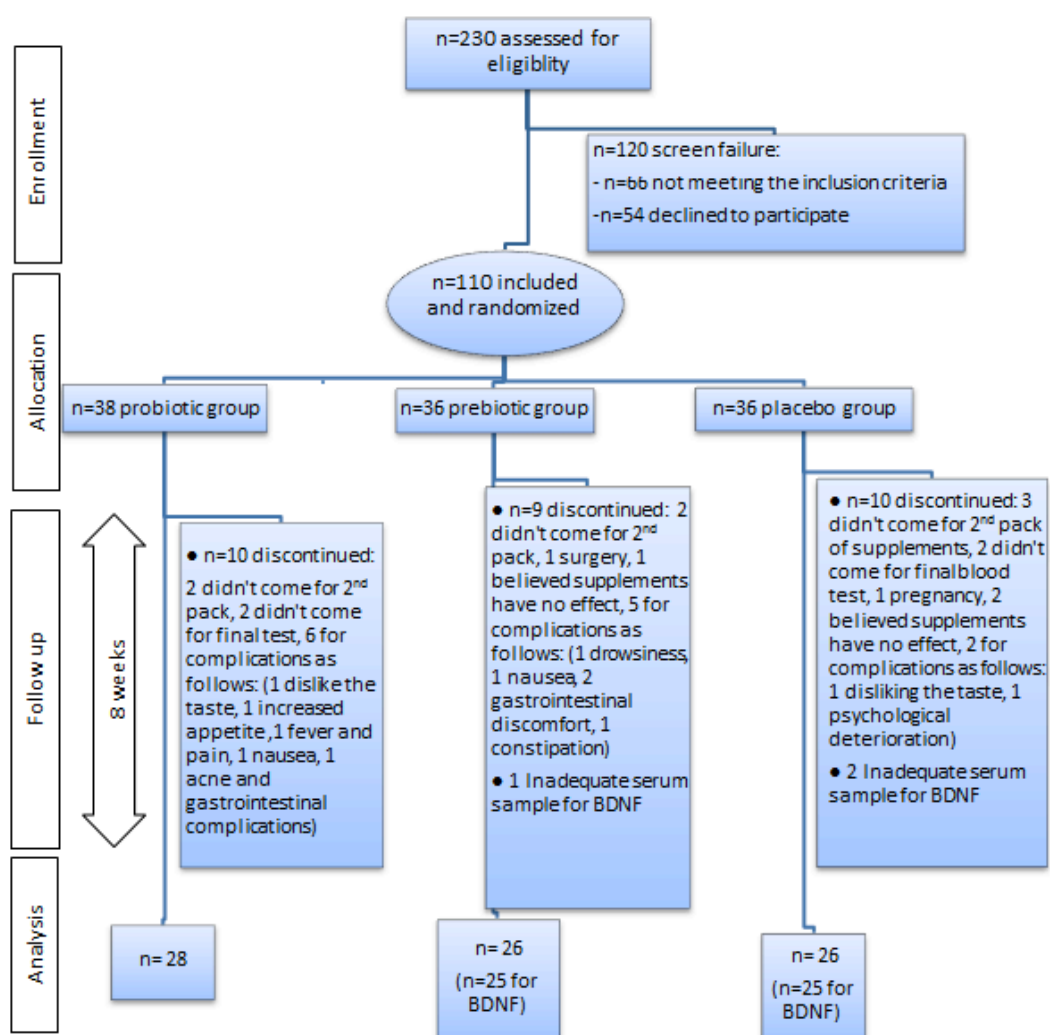
Fig. 3.1- Flow diagram summarizing the enrollment and follow-up of patients

3.4. Study Procedure

The participants were instructed to consume one sachet daily for 8 weeks after lunch by dissolving in a glass of water. Patients were followed by a call or SMS, in a regular basis in terms of regular taking of the supplements. In addition, we asked them to bring the remaining sachets at the end of the study to check their compliance. Consumption of minimum 80% of the sachets was accepted as compliance to the study.

General characteristic of participants as well as other information including anthropometric measurements, nutrition status by 3-day food diary, depression status

using Beck Depression Inventory (BDI-II), physical activity, were inquired at the beginning and end of the study via a face-to-face interview.

Nutritional intake of the participants was evaluated using software Nutritionist 4 (NUT4- First Databank, San Bruno, CA, USA) modified for Iranian cuisine.

BDI is a self-reported scale developed by Beck in 1961 to measure emotional, cognitive, somatic and motivational status. Its main purpose is to comprehensive evaluation of the symptoms of depression; it also allows the evaluation of cognition. The scale is composed of 21 items which are related to a specific symptom of depression. Each item consists of four phrases that express the mildest to the most severe degree of the symptom, with the scores of 0 to 3. The participant must read carefully the phrases and point out the one that best represents his/her status. At the end total scores were calculated to obtain the total score of each participant. The higher total score relates to the more severe grade of depression while the following scores were used to indicate the overall depression level:

• 0 to 13 none or the least depression

• 14 to 19 mild depression

• 20 to 28 moderate depression

• 29 to 63 severe depression

The latest version of BDI-II (Appendix 2) which has been validated among Iranian population was used in this study (231).

Participants' physical activity levels were obtained using international physical activity questionnaire (IPAQ) (Appendix 3) and evaluated as Metabolic Equivalents (METs) (Min/week). MET coefficients of the activity (including for walking 3.3, moderate activity 4, and intensive activity 8) multiplied by duration of the activity (in minutes per day) and the number of days that activity was performed per week, and the sum of all activities was determined as daily physical activity

level. Since the MET distribution was not normal, STATA software was used for the best transformation state which was the inverse square root of the variable ($\frac{1}{\sqrt{MET}}$).

Anthropometric measurements were performed at the beginning and end of the study according to the method proposed by the World Health Organization. Weights with least clothes were measured using a digital scale with 0.01 kilogram accuracy (Seca 803 Clara). Heights without shoes were measured using a wall mounted stadiometer with sensitivity of 0.1 cm (Seca, Germany). Body mass index was also calculated using the formula: BMI (kg/m^2) = weight (kg) / [height (m)]^2.

Fasting blood (10 cc) from participants were collected between 8 to 10 am at first and last day of the intervention. The blood samples were transferred to test tubes and were immediately centrifuged at 3500 rpm for 10 minutes for isolation of the serums. Serum samples were kept at -80°C until further analysis.

Serum concentrations of tryptophan and BCAAs were determined by high performance liquid chromatography (HPLC) method by a private Laboratory (Nour Laboratory). The kynurenine to tryptophan and tryptophan to BCAAs ratios were also calculated to present availability of tryptophan to CNS (degradation).

Serum kynurenine and BDNF levels were measured using enzyme-linked immunosorbent assays kit (Cusabio Biotech, Wuhan, China and Shanghai Crystal Day Biotech, respectively) according to the instruction of the manufacturer (232).

Minimum of 5 grams of stool samples in sterile tubes were collected from subsample of 26 participants (9 probiotic, 8 prebiotic and 9 placebo) at the beginning and 22 participants at endpoint and stored at -80 ° C in the laboratory of the New Technological School, TUMS until processing. The samples did not reach the quantity which was planned due to the lack of cooperation of the participants to provide the samples. Intestinal microbiome of 26 samples (9 from probiotic, 8 from prebiotic, 9 from placebo groups) for baseline and 22 samples (9 from probiotic, 5 from prebiotic, 8 from placebo groups) for endpoint were ready to be analyzed using qRT-PCR.

DNA extraction of stool samples

The bacterial genomic DNA was isolated from 5 g of stool samples using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to manufacturer's protocol. Briefly, approximately 200 mg of stool sample was mixed with 1.4 mL Buffer ASL in a 2 mL microcentrifuge tube till the sample was thoroughly homogenized. After heating (70-90 °C) the suspension for 5 minutes, centrifuged for 1 min to pellet stool particles. 1.2 mL of the supernatant was transferred to a new microcentrifuge tube and 1 InhibitEX tablet was added and the suspension was centrifuged for 3 minutes at full speed. 200 µL of the supernatant was added to a microcentrifuge tube containing 15 µl proteinase-K. After that 200 µL Buffer AL was added and incubated at 70 °C for 10 minutes. Then 200 µL of ethanol (96–100%) was added to the lysate and the lysate mixture was added to QIAamp spin column placed in a 2 mL collection tube and was centrifuged for 1 minute until lysate completely passed through the column. After centrifugation, 500 µL Buffer AW1 was added to the QIAamp spin column and centrifuged for 1 min. The QIAamp spin column was placed in a new 2 mL collection tube and 500 µL Buffer AW2 was added and centrifuged for 3 minutes. Finally QIAamp spin column was placed into a new microcentrifuge tube and 200 µL Buffer AE was added directly onto the QIAamp membrane and was centrifuged at full speed for 1 min to elute DNA. The extracted DNA samples were stored at −20°C for further processing.

Analysis of intestinal microbiome composition:

The intestinal microbiome was measured at the Pasteur Institute of Iran using quantitative real-time PCR (qRT-PCR) method. The prevalence of the bacteria including *Lactobacillus* group and *Enterococcus*, *Bacteroides*/*Prevotella* group, *Bifidobacterium* spp. and *Enterobacteriaceae* were determined by specifically designed primers for amplification of 16S rDNA segment of the bacteria. The primers were previously published and listed in Table 3.1. To ensure that the primers are not homologous and complementary to the nucleotide sequences in other parts of the genome, the designed sequences were tested at the BLAST part of the NCBI BLAST Search tool at http://www.ncbi.nlm.nih.gov/Blast.

Table 3.1. Primer sequence and sizes PCR product estimated by qPCR

Target organism	Primer set	Sequence (5′→3′)	Size (bp)
Bifidobacterium spp. (233)	Bif164F	GGG TGG TAA TGC CGG ATG	438-452 (457)
	Bif601R	TAA GCC ATG GAC TTT CAC ACC	
Enterococcus spp. (234)	F-Entero	CCC TTA TTG TTA GTT GCC ATC ATT	144
	R-Entero	ACT CGT TGT ACT TCC CAT TGT	
Lactobacillus group (235) (Lactobacillus/Leuconostoc/Pediococcus)	F_Lacto 05	AGC AGT AGG GAA TCT TCC A	375
	R_Lacto 04	CGC CAC TGG TGT TCY TCC ATA TA	
Bacteroides / Prevotella (235)	F_Bacter 11	CCT WCG ATG GAT AGG GGT T	131
	R_Bacter 08	CAC GCT ACT GGG CTG GTT CAG	
Enterobacteriaceae(236)	Eco1457F	CATTGACGTTACCCGCAGAAG AAGC	195
	Eco1652R	CTCTACGAGACTCAAGCTTGC	

bp: base pair

The Polymerase Chain Reactions were performed using Roche LightCycler® 96 SW 1.1 device (Switzerland) in duplicate for each sample and the mean concentration were used for analysis. Cycling setting was as follow: 95°C for 10 min, followed by 45 cycles of 95°C for 30 s, 42°C for 10 s, and 72°C for 10 s. The reaction mixture were consisting of 10 μl SYBR Green master mix (Takara Japan), 2 μl forward and reverse primers of specific to each bacterial genus, 7 μl of nuclease free water and 1 μl of DNA sample (extracted). Additionally, two samples of nuclease free water were used as negative control instead of DNA sample. Ten-fold dilution series of DNA of standard with specific concentration were used for standard curve (E.Coli ATCC 25922) (237). The bacterial DNA molecules were calculated according to sample DNA mean concentration (ng) obtained from the standard curves and whole genome size (bp) of the bacteria (at https://cels.uri.edu/gsc/cndna.html) (238, 239). The genome sizes which obtained from NCBI database (Genome project) were as follows: *Lactobacillus* group: 2.3 Mb; *Bifidobacterium spp.*: 2 Mb; Enterococcus spp.: 3Mb; Bacteroides/Prevotella: 4 Mb and Enterobacteriaceae: 4.6 Mb.

3.5. Statistical Analysis

Sample size was determined based on the most important variable of the study, BDI score, with consideration of the mean difference of 11.5 between the intervention and control groups as the least significant difference (240). Accordingly, 27 individuals were calculated for each group with a power of 80%, alpha error of 0.05, that with anticipation of 10% missing cases 30 subjects were considered for each group. But due to the unpredicted losses, 110 people were enrolled in the study.

Statistical analyses of the data obtained in this study were performed using Statistical Package for Social Science (IBM SPSS; version 22.0). Beck scores were analyzed according to Intention to Treat (ITT) method. However analysis of other variables was based on Per Protocol (PP) set. Quantitative variables were presented as mean (± standard deviation, SD) and categorical variables were expressed as frequency (percentage). The normality of the outcomes was analyzed by Kolmogorov–Smirnov test. We used transformation method to normalize the skewed data. STATA software version 12 was used to find the best transformation method.

Data with normal distribution were reported as mean ± SD. For non-normal distributed variables, P value were calculated after transformation, and reported in tables after back-transformation as mean (95% confidence intervals, CI). Beck Depression scores were square root transformed whereas BCAA and isoleucine, tryptophan, kynurenine and kynurenine to tryptophan ratio were logarithmic transformed. Isoleucine/tryptophan ratio was Box-cox transformed. The difference between baseline and endpoint values of the groups was compared using Paired t-test. One way ANOVA was used to analyze all variables and ANOVA / ANCOVA was used to eliminate the effect of confounding variables using baseline values as covariates. Groups were compared using the Bonferroni *post-hoc* multiple comparisons test when a significant difference were found between groups in ANCOVA analysis.

The intestinal microbiome data were reported as median (SEM) and were analyzed by non-parametric tests. The change of intestinal microbiome between groups was compared using Kruskal–Wallis H test Pairwise comparisons were

performed using *post-hoc* Dunn's test for non-parametric Kruskal-Wallis test (adjustment using the Bonferroni correction).

All tests for significance were two-sided and values of $P < 0.05$ were considered significant.

4.   RESULTS

4.1.   General Characteristics of the Participants

General characteristics of the participants by the intervention groups were presented in Table 4.1. The mean ± SD age of the participants was 36.47 ± 8.03 years. While 78% of the participants were women, 32% were men. The mean ± SD BMI of participants was 26.5 ± 4.6. There was no difference in terms of sex, age, weight, BMI, education, occupation, duration of depression and duration of antidepressant therapy between groups at the beginning of the study (P>0.05). Seventy one percent of the participants had university education. The participants had depression for mean (95% CI) 2.27 (1.8-3.0) years while they were taking antidepressant agents for mean (95% CI) 1.72 (1.31-2.27) years.

Table 4.1. General characteristics of the participants at baseline

		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
Sex, n (%)	Woman	27 (71.1%)	27 (75%)	24 (66.7%)	78 (29.1%)
	Man	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 (%)	Pre-high school degree	2 (7)	5 (15)	3 (11.5)	10 (9)
	Completed high school	10 (28.5)	9 (26)	10 (30.7)	29 (26.4)
	Undergraduate	17 (32.1)	13 (29.6)	16 (30.7)	46 (41.8)
	Postgraduate	9 (32.1)	9 (29.6)	7 (23.9)	25 (22.7)
Marriage status, 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)
Occupation, n (% in group)	Manual worker	0 (0)	1 (2.7)	0 (0)	1 (0.9)
	Clerk	12 (31.5)	11 (30.5)	11 (30.5)	34 (30.9)
	Self-employed	8 (21)	7 (19.4)	7 (19.4)	22 (20)
	Housewife	10 (26.3)	11 (30.5)	10 (27.8)	31 (28.3)
	Others	8 (21)	6 (16.7)	8 (22.2)	22 (20)
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 antidepressant therapy [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)
Antidepressant agents, n (%)					
	Citalopram	6(21)	2(8)	8(30)	16 (20)
	Fluoxetine	9(32)	5(18)	4(16)	18 (22)
	Sertraline	9(32)	14(52)	8(30)	31 (38)
	Amitriptyline	4(14)	6(22)	6(23)	16 (20)

The values of the quantitative variables are expressed as mean (±SD) and the difference less than 0.05 considered significant. (P <0.05). [a] Data was log transformed as distribution was not normal and retransformed to display as mean (95% CI), N: number; Y: year.

4.2. Anthropometric Measurements of the Participants

Findings for weight and BMI are shown in Table 4.2. There was no significant difference in weight and BMI between groups at the beginning of the study (Table 4.1). The mean ± SD weight of the participants at the end of the study was 72.8 ± 12.4, 72.8 ± 15.9 and 73.8 ± 13.7 for probiotic, prebiotic and placebo groups, respectively. There was no significant difference between mean weight and BMI in 3 groups (Table 4.2).

4.3. Physical Activity Level of the Participants

Physical activity levels of the participants at the beginning and end of the study were shown in Table 4.3. There was no significant difference in the physical activity level of the participants at the beginning of the study (P>0.05). Although physical activity was reduced in all groups at the end of the study, the difference between the groups was not statistically significant (P>0.05).

Table 4.2. Anthropometric measurements of the participants, Mean ± SD

	Probiotic			Prebiotic			Placebo			Pairwise Comparison			P4
	Baseline	Endpoint	P	Baseline	Endpoint	P	Baseline	Endpoint	P	P1	P2	P3	
Weight (kg)	71.7 ± 11.8	72.8 ± 12.4	0.004	72.5 ± 15.6	72.8 ± 15.9	0.77	73.3 ± 14.1	73.8 ± 13.7	0.8	0.062	0.28	0.88	0.95 [a] 0.27 [b] 0.21 [c]
BMI (kg/m^2)	26.3 ± 4	26.7 ± 4.4	0.005	27 ± 5.1	27.2 ± 5.2	0.69	26.6 ± 4.9	26.9 ± 4.6	0.9	0.053	0.27	0.91	0.92 [a] 0.28 [b] 0.19 [c]

P1: probiotic vs. prebiotic; P2: probiotic vs. placebo; P3: prebiotic vs. placebo; P4: comparison of all 3 groups at endpoint; a: not adjusted; b: Adjusted for baseline values; c: Adjusted for baseline values, antidepressant agent, sex, BMI change and Selenium; ANOVA/ANCOVA analysis.

Table 4.3. Physical activity of the participants (MET / h / week)

GROUPS	MET / h / week			Pairwise comparison			P4
	Baseline	Endpoint	P	P1	P2	P3	
Probiotic	19.6 (14.5-28.1)	15.54 (11.4-22.5)	0.05				
Prebiotic	16.87 (13-22.8)	14.14 (11.5-17.9)	0.25	0.72	0.8	0.87	0.94
Placebo	17.18 (13.1-23.5)	15.25 (11.1-22.3)	0.34				

Data are presented as mean (95% CI); METs, metabolic equivalents. P1: Comparison of probiotic and prebiotic groups; P2: Comparison of probiotic and placebo groups; P3: Comparison of prebiotics and placebo; P4: Comparison of three groups

4.4. Energy, Macronutrients, and Fiber Intakes of the Participants

Findings for energy, protein, fat, carbohydrate and fiber were presented in Table 4.4. To normalize the distribution of protein and fiber the logarithmic transformation was used, and P-value in the table was based on logarithmic transformation. There was no significant difference in participants' intake of energy, carbohydrate, protein, fat, and fiber between groups at the beginning of the study. It was observed that energy intake increased slightly in probiotics group and decreased in prebiotics group, however these changes were not significant. At the end of the study, there was no significant difference in macronutrients intake between the groups except in the amount of fat. Fat intake (g) in the prebiotic group was significantly lower than that of the probiotic and placebo groups, but no significant change was found in fat percentage of total energy.

Table 4.4. Energy, macronutrients, and fiber intake of the participants per day

		Probiotic	Prebiotic	Placebo	P value
Energy (kcal)	Baseline	1573 ± 317	1467 ± 409	1505 ± 565	0.7
	Endpoint	1662 ± 334	1405 ± 421	1547 ± 570	0.06
	P	0.2	0.2	0.07	
Protein (g)	Baseline	53.1 ± 17.4	53.7 ± 19.9	56.4 ± 24.1	0.8
	Endpoint	53.2 ± 13.7	52.11 ± 26	59.2 ± 24.1	0.35
	P	0.96	0.66	0.46	
Protein (%)	Baseline	13.3 ± 2.4	14.5 ± 2.3	14.9 ± 2.2	0.11
	Endpoint	12.6 ± 2.3	14 ± 3.3	14.8 ± 4.4	0.125
	P	0.64	0.81	0.71	
Fat (g)	Baseline	49.9 ± 14.6	50.1 ± 19.7	50.8 ± 20.9	0.9
	Endpoint	54.6 ± 19.5	46 ± 22	55.6 ± 24.7	**0.043 ***
	P	0.24	0.09	0.14	
Fat (%)	Baseline	28 ± 7.8	30 ± 7.9	30 ± 5.2	0.5
	Endpoint	29.9 ± 6.2	28.86 ± 7.7	32 ± 6.4	0.16
	P	0.29	0.21	0.21	
Carbohydrate (g)	Baseline	233.6 ± 56	198.6 ± 67	209 ± 78	0.27
	Endpoint	244 ± 60	199 ± 57	207 ± 82	0.72
	P	0.55	0.97	0.72	
Carbohydrate (%)	Baseline	58.4 ± 7.9	55 ± 7.5	54.8 ± 6.2	0.28
	Endpoint	58 ± 6.3	56.4 ± 8.3	52.4 ± 8.1	0.22
	P	0.85	0.39	0.19	
Fiber (g)	Baseline	13.4 ± 7	10.4 ± 4.6	11.9 ± 4.6	0.23
	Endpoint	11.8 ± 5.7	11.6 ± 6.1	11.2 ± 4.8	0.7
	P	0.21	0.43	0.44	

ANOVA/ANCOVA results presented as Mean (±SD). * P < 0.05 is considered significant.

Pairwise comparison of the groups showed significant differences in energy intake between probiotic and prebiotic groups. Energy intake increased in probiotic group and decreased in prebiotic group but not in comparison to placebo group (Table 4.5). Pairwise comparisons also demonstrated fat intake (g) significantly decreased in prebiotic group in comparison to the both probiotic and placebo groups.

Table 4.5. Pairwise comparison of energy and macronutrients intake of the participants (P value)

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
Energy (kcal)	**0.03**	0.67	0.07
Protein (g)	0.61	0.38	0.45
Fat (g)	**0.04**	0.79	**0.025**
CHO (g)	0.16	0.38	0.99
Protein (%)	0.16	0.14	0.45
Fat (%)	0.17	0.53	0.065
CHO (%)	0.95	0.15	0.11
Fiber (g)	0.56	0.81	0.36

ANOVA/ANCOVA adjusted for baseline values.* $P < 0.05$ is considered significant.

4.5. Saturated, Unsaturated Fatty Acids, and Cholesterol Intakes of the Participants

Findings of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), and cholesterol intakes of the participants were shown in Table 4.6. There was no significant difference in mean PUFA, MUFA, SFA, and cholesterol intake at the beginning of the study. Also, at the end of the study, ANOVA / ANCOVA analysis of the means between groups showed no significant difference. Also, pairwise comparison of the groups showed no significant difference between groups (Table 4.7).

Table 4.6. PUFA, MUFA, SFA, and cholesterol intake of the participants, Mean (±SD)

		Probiotic	Prebiotic	Placebo	P value
PUFA (g)	Baseline	14.9 ± 5.3	13.6 ± 6.9	13.2 ± 5.6	0.75
	Endpoint	15.8 ± 7.8	13 ± 8.2	13.3 ± 6.2	0.43
	P	0.5	0.34	0.99	
MUFA (g)	Baseline	14.6 ± 5.6	15.1 ± 7.5	14.4 ± 6.7	0.95
	Endpoint	15.6 ± 5.8	13.2 ± 7.3	16.4 ± 7.8	0.098
	P	0.23	0.14	0.45	
SFA (g)	Baseline	13.5 ± 9	12.8 ± 7.2	13.8 ± 9.2	0.9
	Endpoint	14.4 ± 12.3	10.8 ± 5.8	15.2 ± 9.1	0.1
	P	0.48	0.06	0.28	
Cholesterol (mg)	Baseline	155.1 ± 96.3	164.9 ± 115.5	152.3 ± 128.6	0.9
	Endpoint	175.3 ± 101.6	140.2 ± 99	136 ± 129.4	0.24
	P	0.5	0.17	0.55	

SFA and cholesterol values were log transformed. ANOVA/ANCOVA adjusted for baseline values. P < 0.05 is considered significant.

Table 4.7. Pairwise comparison of PUFA, MUFA, SFA and cholesterol intake between groups

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
PUFA	0.22	0.55	0.5
MUFA	0.57	0.06	0.1
SFA	0.13	0.82	0.08
Cholesterol	0.15	0.29	0.9

ANOVA/ANCOVA adjusted for baseline values. P < 0.05 is considered significant.

4.6. Micronutrient Intakes of the Participants

Findings for intake of micronutrients including magnesium, selenium, zinc, iron, vitamin B1, B2, B3, vitamin E, and vitamin C were seen in Table 4.8. The distribution of vitamin B_2, B_3, C, vitamin E, and selenium was normalized after logarithmic transformation, and P value in the table is based on logarithmic transformation. There was no significant difference in the micronutrients mean intake between the groups at the beginning of the study. At the end of the study, except for selenium (P = 0.026), there was no difference in mean of other micronutrients in three groups.

Table 4.8. Micronutrient Intakes of the participants, Mean (±SD)

		Probiotic	Prebiotic	Placebo	P value
Magnesium (mg)	Baseline	157 ± 59.3	138.3 ± 43.5	150.9 ± 68.8	0.6
	Endpoint	161.5± 46	164.5 ± 62.5	143.2 ± 58.4	0.22
Zinc (mg)	Baseline	5 ± 1.7	5.5 ± 2.1	6 ± 3.4	0.3
	Endpoint	5.2 ± 2.5	5 ± 2.7	6.4 ± 3.2	0.32
Selenium (µg)	Baseline	0.085 ± 0.047	0.063± 0.027	0.065 ± 0.049	0.25
	Endpoint	0.056 ± 0.028	0.076± 0.44	0.076 ± 0.037	0.026*
Fe (mg)	Baseline	12.15 ± 3.44	11.36 ± 3.97	11.38 ± 4.37	0.77
	Endpoint	13.44 ± 4.41	13.27 ± 5.97	11.53 ± 4.97	0.49
Vitamin B_1	Baseline	1.48 ± 0.31	1.50 ± 0.48	1.40 ± 0.50	0.78
	Endpoint	1.60 ± 0.32	1.45 ± 0.50	1.46 ± 0.59	0.16
Vitamin B_2	Baseline	1.06 ± 0.37	2.13 ± 4.4	1.13 ± 0.68	0.38
	Endpoint	1.19 ± 0.59	1.23 ± 0.99	1.17 ± 0.59	0.98
Vitamin B_3	Baseline	17.04 ± 6.47	24.26 ± 37.64	16.0 ± 6.63	0.48
	Endpoint	15.88 ± 3.94	16.07 ± 7.59	15.43 ± 6.77	0.96
Folate (µg)	Baseline	174.48 ± 90.70	200.9 ± 100.10	171.91 ± 97.09	0.59
	Endpoint	185.70 ± 87.98	180.35 ± 91.66	165.34 ± 74.01	0.76
Vit. E (mg)	Baseline	2.8 ± 2.3	1.94 ± 1.89	2.1 ± 2.96	0.50
	Endpoint	2 ± 2.3	2.26 ± 1.46	2.46 ± 3.1	0.75
Vit. C (mg)	Baseline	44.5 ± 64.9	61.4 ± 65.4	53.8 ± 53.7	0.47
	Endpoint	63.5 ± 122	81.9 ± 69	58.3 ± 60.8	0.87

ANOVA/ANCOVA adjusted for baseline values. * P < 0.05 is considered significant.

Pairwise analysis demonstrated that selenium intake decreased in probiotic group and increased in the prebiotic and placebo groups, however only the difference between probiotic and placebo was significant (P = 0.05). There was no significant

difference in pairwise comparison of other micronutrients between groups (Table 4.9).

Table 4.9. Pairwise comparison of micronutrients intake between groups

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
Mg	0.37	0.36	0.08
Zn	0.26	0.59	0.16
Se	0.05*	0.62	1
Fe	0.98	0.81	0.57
Vit. B1	0.16	0.86	0.58
Vit. B2	0.99	1	0.95
Vit. B3	1	0.99	0.99
Folate	0.95	0.89	0.99
Vit. E	0.58	0.52	0.97
Vit. C	0.75	0.78	0.44

ANOVA/ANCOVA adjusted for baseline values.* $P < 0.05$ is considered significant.

4.7.   BDI Scores of the Participants

Beck Depression Inventory scores of the participants were given in Table 4.10. Square-root transformation was used to normalize the data. ITT analysis of Beck depression scores showed a significant difference between groups ($P = 0.042$). We also checked for PP analysis of BDI scores and similar to the results of ITT analysis, BDI scores were significantly different between the three groups ($P_{PP}=0.044$) (not given in the table).

Table 4.10. Beck Depression Inventory scores of the participants, Mean (95% CI)

Groups	BDI scores			Pairwise comparisons		
	Baseline	Endpoint	P (Partial eta^2)	P1	P2	P3
Probiotic	18.3 (14.2-21.6)	10.9 (7.4-14.1)	0.69 (0.101) [a]	0.135	**0.008***	0.242
Prebiotic	21.1 (15.4-24.6)	16.4 (9.6-19.6)	**0.042* (0.101)** [b]			
Placebo	19.3 (4.1-23.1)	17.2 (11.4-21.3)				

ANOVA/ANCOVA test. P: Comparison of three groups, a: not adjusted. b: adjusted for baseline values. P1: Comparison of probiotic and prebiotic groups; P2: Comparison of probiotic and placebo groups; P3: Comparison of prebiotics and placebo; *$P<0.05$ is considered significant.

Pairwise comparison of the groups was shown a significant BDI score reduction in probiotic group (7.4) compared to placebo group (2.1) (P = 0.008). The BDI scores did not reduce significantly with prebiotic supplementation (4.7) in comparison to placebo (P = 0.242). There was no significant difference between prebiotic and probiotic supplementation (P = 0.135). (Table 4.10)

4.8. Serum TRP, BCAAs, and kynurenine of the Participants

Findings regarding participants' serum TRP, BCAAs, kynurenine and related ratios were shown in Table 4.11. TRP, BCAAs, kynurenine and kynurenine /TRP ratio were logarithmic transformed and retransformed to display as mean (95% CI). The related findings obtained from per protocol analysis.

Serum TRP levels had a slight increase in probiotic, and a decrease in placebo groups, but it was not statistically significant (P = 0.259). Although, the change became significant when adjusted for baseline and isoleucine values (P = 0.036).

The difference between serum TRP/BCAAs of the participants from baseline to the end of the study tended to increase with probiotic and prebiotic and decrease with placebo (P = 0.065). Paired comparisons showed that TRP/BCAA ratio increased significantly in prebiotic group compared with placebo group (P = 0.048). Serum TRP to isoleucine ratio increased in probiotic and prebiotic groups while decreased in placebo group (P = 0.023). Pairwise comparisons showed that only the rise of TRP/Isoleucine ratio with probiotic compared to placebo was significant (P = 0.018) (Table 4.12).

Serum kynurenine to TRP ratio of the participants tended to decrease in probiotic group while it increased in placebo group, but the change was not statistically significant (P= 0.124). However the difference became significant when adjusted for baseline and isoleucine values (P = 0.048). Pairwise analysis confirmed that the decrease in serum kynurenine / TRP in probiotic group was significant compared with the placebo group (Table 4.12).

Table 4.11. Serum TRP, BCAAs and KYN of the participants, Mean (95% CI)

	Probiotic		Prebiotic		Placebo		P_1 (Partial eta^2)	P_2 (Partial eta^2)
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint		
TRP (µg/dL)	67.5 (59.7–76.2)	75.4 (63.6–89.7)	81.4 (67.2–98.5)	80.6 (69.5–93.5)	69.5 (53.9–89.7)	65.2 (55.8–76.2)	0.259 (0.038)	0.036 (0.093)
BCAAs (µg/ dL)	493.8 (438.9 -548.7)	540.4 (459.4- 621.3)	554.4 (446.98-661.7)	529.4 (448.3-610.5)	517.2 (436.9-597.5)	520.9 (425.3-616.4)	0.852 (0.005)	
TRP / BCAAs	0.146 (0.131-0.163)	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)	0.065 (0.076)	
TRP/Leucine	0.49 (0.43-0.55)	0.50 (0.44-0.55)	0.49 (0.43-0.56)	0.47 (0.41-0.53)	0.52 (0.44-0.60)	0.51 (0.45-0.57)	0.589 (0.015)	
TRP/Isoleucine	1.07 (0.81-1.33)	1.53 (1.00-2.06)	0.95 (0.76-1.15)	1.09 (0.78-1.41)	1.01 (0.76-1.26)	0.88 (0.81-0.95)	**0.023*** (0.085)	
TRP/Valine	0.28 (0.25-0.30)	0.28 (0.26-0.31)	0.32 (0.25-0.40)	0.30 (0.24-0.36)	0.28 (0.25-0.31)	0.27 (0.24-0.29)	0.685 (0.011)	
Kynurenine (nmol/L)	757.6 (661.3-868.0)	722.5 (621.3-840.1)	913.3 (797.7-1045.7)	909.2 (746.1-1107.9)	772.9 (627.4-952.1)	798.5 (717.1-1002.7)	0.15 (0.05)	
Kynurenine / TRP	11.2 (9.17-13.76)	9.6 (7.64-12.01)	11.2 (8.82-14.28)	11.3 (9.03-14.08)	11.0 (8.8-13.75)	12.4 (9.66-15.99)	0.124 (0.059)	**0.048*** (0.086)

*P value obtained from ANOVA/ANCOVA analysis, P_1: adjusted for baseline values; P_2: adjusted for baseline and isoleucine values.

Table 4.12. Pairwise comparison of serum TRP, BCAAs, kynurenine, and regarding ratios (P value)

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
TRP	0.939	0.171	0.095
BCAAs	>0.999	>0.999	>0.999
TRP/BCAAs	0.546	0.096	**0.031***
TRP/Leucine	>0.999	>0.999	>0.999
TRP/Isoleucine	0.77	**0.018***	**0.025**
TRP/Valine	>0.999	>0.999	>0.999
Kynurenine	0.610	0.231	0.833
Kynurenine/TRP	0.124	**0.036***	0.453

*P <0.05 is considered significant.   ANOVA/ANCOVA adjusted for baseline values.

4.9. Serum BDNF Levels of the Participants

Findings regarding serum BDNF levels of the participants were given in Table 4.13. For BDNF measurements we had insufficient serum for three patients at endpoint (2 in prebiotic and 1 in placebo groups). The distribution of BDNF values was normalized by logarithmic transformation. The participants were similar in serum BDNF levels at the beginning of the study (P= 0.09). Per protocol analysis of serum BDNF levels at endpoint showed a slight but not significant difference (P=0.09) between the study groups using ANOVA/ANCOVA. However, the difference in BDNF levels turned significant when adjusted to baseline values (P<0.001). The change of serum BDNF (ΔBDNF) from baseline to the endpoint was also tested. ΔBDNF was higher in probiotic group (0.095 (0.056 – 0.134)) and placebo group (0.009 (-0.047 – -0.039)), was lower in the prebiotic group (-0.056 (-0.096 – -0.010)), which was statistically different (P<0.001).

Pairwise comparisons of the groups were shown a significant increase in BDNF levels in probiotic group in comparison to both prebiotic group (P<0.001) and placebo group (P= 0.02) (See Table 4.14). The BDNF levels tended to increase with prebiotic supplementation compared with placebo which was not significant (P = 0.08).

Table 4.13. Serum BDNF levels of the participants, ng/mL, Mean (95% CI)

	Probiotic (n=28)	Prebiotic (n=25)	Placebo (n=25)	P
Baseline	0.392 (0.346 –0.445)	0.425 (0.367 – 0.492)	0.483 (0.419 – 0.558)	0.09
Endpoint	0.483 (0.424 – 0.548)	0.389 (0.335 – 0.453)	0.467 (0.401 – 0.542)	0.09[a]
				<0.001[b]
ΔBDNF	0.095 (0.056 – 0.134)	-0.056 (-0.096– -0.010)	0.009 (-0.047 –0.039)	**<0.001***

BDNF values were log transformed and then back transformed to display as mean (95% CI) P value is based on ANOVA/ANCOVA test; a, not adjusted; b, adjusted for baseline values.

Table 4.14. Pairwise comparison of the serum BDNF level changes (P value)

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
BDNF	**<0.001***	**0.02***	0.08

* P <0.05 is considered significant.   ANOVA/ANCOVA analysis adjusted for baseline values.

4.10. The Intestinal Microbiome

Table 4.15 shows the median (SEM) of the variables related to the intestinal microbiome composition at the beginning and end of the study in all intervention groups. Kruskal- Wallis analysis showed no significant difference in bacterial prevalence between the three groups at in the beginning (P>0.05) and end of the study (P>0.05). However analysis of the intestinal bacteria demonstrated that *Lactobacillus group* and *Bifidobacterium spp.* content changed significantly different during the 8-week supplementation period (Fig. 4.1) as follow: the prevalence of *Lactobacillus group* in all groups increased significantly (P=0.016). *Bifidobacterium spp.* increased only in probiotic supplemented group while in other groups decreased (P= 0.012,). The change of the *Enterococcus spp.*, *Bacteroides/Prevotella* and *Enterobacteriaceae* was not important (Table 4.15).
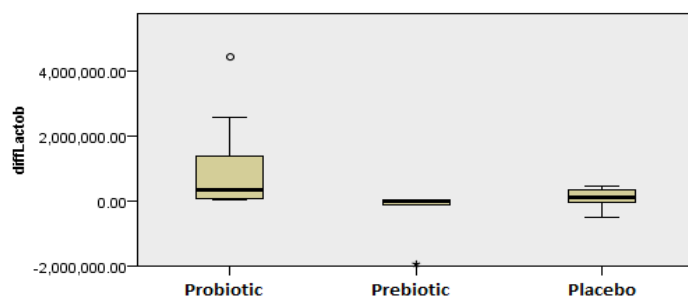
Pairwise comparisons showed that the change of both *Lactobacillus* group and *Bifidobacterium* spp., was significantly different between the probiotic and prebiotic groups (Table 4.16).

**Table 4 .15.** The intestinal microbiome of the participants, CFU, Median (SEM)

Intestinal microbiome composition	Probiotic			Prebiotic			Placebo			P (change)
	Baseline (n=9)	Endpoint (n=9)	Change (n=9)	Baseline (n=8)	Endpoint (n=5)	Change (n=5)	Baseline (n=9)	Endpoint (n=8)	Change (n=8)	
Lactobacillus group*	41000.0 (83697.722)	391000.0 (534965.699)	350000.0 (504013.690)	29750.00 (428495.077)	44100.00 (296987.393)	6500.0 (390253.541)	157000.0 (259201.302)	455000.0 (221851.786)	104000.0 (111828.962)	**0.016**** **χ^2=8.263**
Bifidobacterium spp.	370000.0 (134370.84)	1620000.0 (553805.641)	1250000.0 (473398.394)	655500.0 (362496.674)	567000.0 (230552.271)	-181000.0 (255468.287)	857000.0 (368621.26)	809000.0 (350609.090)	-65000.0 (310060.541)	**0.012**** **χ^2=8.791**
Enterococcus spp.	39700.00 (87108.29)	39400.00 (144416.529)	400.0 (185693.750)	34600.00 (121388.665)	46200.00 (211298.331)	12900.0 (19862.966)	60000.0 (333403.233)	175000.0 (130593.472)	40150.0 (326763.790)	0.963 χ^2=0.075
Bacteroides / Prevotella	152000000.0 (34675060.09)	152000000 (37690650.033)	-1840.0 (36539655.888)	7545.0 (46281942.099)	7700.0 (42755149.05)	-460.0 (30673691.997)	29700000.0 (11088997.489)	18605000.0 (35307044.614)	-2590.0 (25923141.054)	0.790 χ^2=0.472
Enterobacteriaceae	2460000.0 (1588788.47)	3410000.0 (4767815.134)	74000.0 (5036711.280)	1925000.0 (952492.909)	455000.0 (7912241.221)	73000.0 (7643904.853)	6030000.00 (3056468.719)	7020000.0 (2996310.827)	483500.0 (4964156.240)	0.896 χ^2=0.219

Lactobacillus group including *Lactobacillus / Leuconostoc / Pediococcus*. **P <0.05 is considered significant. P value obtained from non-parametric Kruskal-Wallis Test. $\chi 2$; chi-square. SEM, standard error of mean.
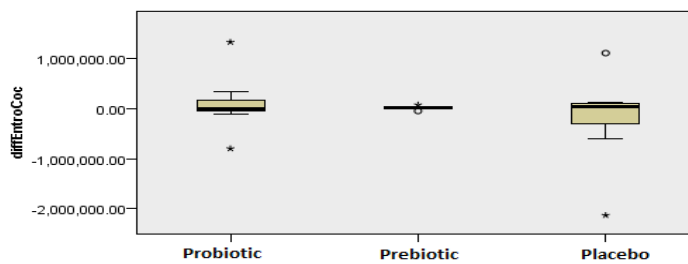
A*

B*
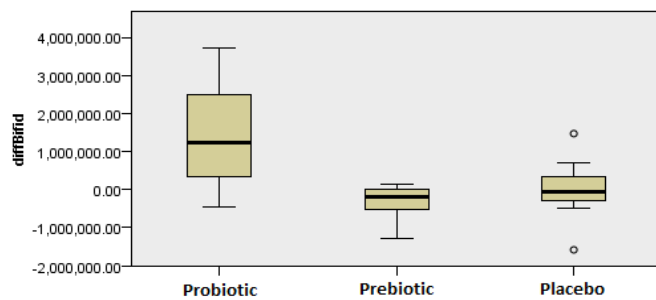
C

D

E
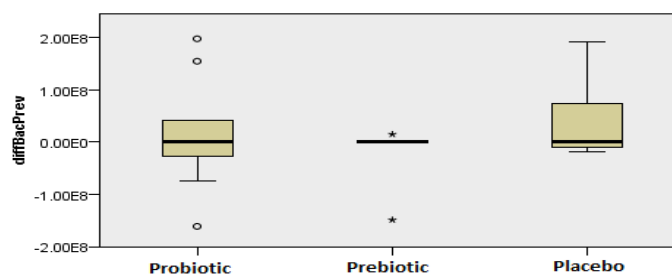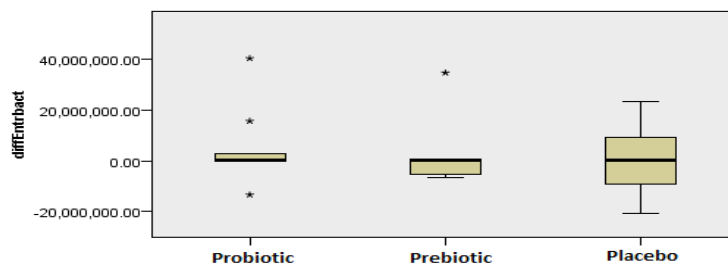

Fig. 4.1- The change of intestinal microbiome A) Lactobacillus group, B) Bifidobacterium spp., C) Enterococcus, D) Bacteroides/Prevotella and E) Enterobacteriaceae (*P<0.05) (Independent sample Kruskal-Wallis test)

Table 4.16. Pairwise comparison of the change of intestinal microbiome

	Probiotic & Prebiotic	Probiotic & Placebo	Prebiotic & Placebo
Δ *Lactobacillus* group	**0.014***	0.330	0.474
Δ *Bifidobacterium spp.*	**0.025***	0.060	1

*P≤0.05 is considered significant. *Post-hoc* Dunn's test for non-parametric Kruskal-Wallis test

4.11. Adverse Events and Compliance Rate

Some adverse events were reported by the participant that may be related to the intervention. The reported adverse events of supplements consumption were listed in Table 4.17. The most common adverse event was increased appetite, which was reported by 5 participants in probiotic group and 1 participant by prebiotic group. Gastrointestinal complaints were the most adverse event in prebiotic group, with 4 reports. Nausea was reported by one in probiotic group and one in prebiotic group. Also one participant in probiotic group reported fever and body aches. There was no adverse event in the placebo group except one report of worse mental state, while it was reported by one in probiotic group as well. It was considered unrelated to the intervention.

Table 4.17. Adverse events related in each group (n)

	Probiotic	Prebiotic	Placebo
Gastrointestinal complaints	2	4	0
Nausea	1	1	0
Increased appetite	5	1	0
Fever and body aches	1	0	0
Worse mental state	1	0	1

Compliance to the study was estimated by counting the returned sachets of supplements at the end of the study. The compliance of participants was strongly acceptable since mean ±SD of consumed intervention products was 91.9% ± 5.53%.

5. DISCUSSION

5.1. Basic characteristics

One hundred and ten eligible depressive patients participated in the study. At baseline, there were no significant differences between three groups in terms of age, gender, education, occupation, duration of depression, duration of antidepressant therapy, weight, BMI, and physical activity (Table 4.1) as hypothesized. The mean age of the participants was 36.5 years, which showed no significant difference among three groups in terms of age. In addition, the higher proportion of women to men in the present study was in agreement with the WHO report of higher prevalence of depression in women than men (36). According to the information obtained from patients, 73.4% were employed which equals to about three-quarters of the participants. Further studies are needed on occupational exposure, stress and environmental factors. The present study in all groups showed that patients with depression had low physical activity, which is itself a risk factor in increasing the likelihood or progression of depression (Table 4.3).

5.2. Body Weight, BMI, and Dietary Intake of the Participants

None of the probiotic and prebiotic supplements in the present study caused a significant change in weight and BMI (Table 4.2). The results of previous studies which examined the effect of probiotics on weight and BMI changes are inconsistent. One possible reason for this inconsistency is the variety of strains used in different studies. According to a meta-analysis, the effect of probiotics on weight varies depending on the bacterial species (241). Concerning the effect of prebiotics on weight changes, in line with our study, five studies found no effect of prebiotics on weight change (242-245) . However, in two studies with overweight and obese individuals, prebiotics showed a weight loss effect (246, 247).

In our study there was increased energy intake in probiotic group while in prebiotic group had lower energy intake, although these changes were not significant

compared to placebo. The findings of the present study showed that there was no significant difference in mean energy, macronutrients, fiber, fatty acids, cholesterol, and micronutrients intakes in three groups at baseline. At the end of the study, the mean fat intake (g) decreased in the prebiotic group compared to the probiotic group. However, there was no significant change in the percentage of energy intake from fat due to the increase in energy intake in the probiotic group and its decrease in the prebiotic group (Table 4.4). Considering that increased appetite was reported by some of the participants in probiotic group (n=5), probiotic group tended to have increased food intake probably of fat, which was not significant in percentage. Unlike to our study probiotic supplementation in an overweight population led to decrease in energy intake compared to baseline (248). In this study probiotic supplements included different probiotic strains (B. bifidum, B. longum, B. infantis, L. acidophilus, L. casei, L. lactis). To our knowledge, the studies on effects of probiotics on eating behaviors in patients with depression are very rare. Sanchez et al. (249) in a study evaluating effects of probiotics on appetite along with restricted energy intake in a population with obesity, found that with probiotics fasting desire to eat increased in women and decreased in men, whereas lunch-time eating desire in both groups was reduced.

Vitamin C, vitamin E, magnesium, and fiber intakes were also lower than recommended level in the study. This might be due to lower consumption of vegetables and fruits which are rich in vitamin C and fiber , as well as whole grains which are rich in fiber, that is in line with various systematic review and meta-analysis findings showing that low intake of vegetables, fruits, seeds and whole grains is associated with higher risk of MDD (250, 251). Our findings are consistent and it seems that recommendations for a healthy eating pattern and increased intake of fruits and vegetables should be considered. Overall, the results of the present study showed that probiotic, prebiotic or placebo did not alter the intake of macronutrients and micronutrients (except for selenium which was lower in probiotic group compared to placebo when adjusted for baseline values) in depressed patients.

5.3.   Beck Depression Inventory Scores

In the present study, reduced Beck depression scores were seen in probiotic group, whereas the reduction in prebiotic group was not significant compared with placebo group (Table 4.10). While 17.5% decrease in BDI scores is considered as clinical significance (252), in our study, this difference was observed only between the probiotic and placebo groups. This result is consistent with most of the studies that have examined the effect of probiotic supplementation on mood-related outcomes, although most of these studies have been conducted in healthy subjects (253). Three studies examined the effects of probiotic versus placebo supplementation on mood in patients with depression (125, 219, 220). One of these studies investigated the effects of probiotics administration along with antidepressants in which the BDI scores were significantly reduced similar to our study (125). The other study showed probiotic (*Bacillus coagulans*) supplementation, which lasted for 90 days, improved depression status in MDD patients with IBS (220). In the third study, administration of bacterial strains (*L. helveticus* and *B. longum*) similar to the bacteria used in our study did not lead to any mood related changes (219). The major difference between this study and our study was that subjects in this study were not taking antidepressant medication and the main treatment agent was only probiotics.

Another RCT compared the effects of probiotic versus synbiotic in hemodialysis patients and found that probiotics, in contrast to synbiotic formulation, did not improve depression status (222). The most important difference of this study with our study was the clinical status of the study population. So that, lack of efficacy of probiotics alone in this study was probably due to intestinal epithelial barrier dysfunction mediated by uremia which is present in ESRD patients (223).

Three main mechanisms have been proposed for the effect of probiotics on treatment of depression, including improvement of inflammation status, normalization of the hypothalamic-pituitary-adrenal axis reactivity to the normal level, and changes in neurotransmitter metabolism (254).

Regarding prebiotics, the results of this study was in line with the results of three studies that examined the effect of prebiotic supplementation in patients with IBS (229) and healthy controls (24, 230). In these studies 12-week supplementation of GOS (7 g) (229), 4-week supplementation of FOS (5 g) (230) and 3-weeks supplementation of GOS or FOS prebiotics (5.5 g) (24) had no effect on depression.

Contrary to our study, two studies showed positive effect of prebiotics on mood and depressive status. However study population and prebiotic type was different from our study. In one study, supplementation of resistant dextrin (10 g) for 8 weeks decreased depression severity in women with T2DM (205). In the other study, 3-week consumption of XOS (alone or in a synbiotic formulation), increased self-reported vitality and happiness in healthy individuals compared to placebo (228). Moreover, acute mood-enhancing effects of prebiotic consumption has been shown in healthy individuals (227). Therefore, more studies are needed to compare the long term effects of different prebiotic compounds at different doses on anxiety and depression.

5.4.   Serum Tryptophan, BCAAs, and Kynurenine

We found a slight but not significant increase of tryptophan levels in probiotic group. While TRP/BCAAs ratios were not different between groups, probiotic supplementation increased TRP/isoleucine ratio compared with placebo. Levels of isoleucine have been found to be positively correlated with depression severity (166, 255). While, it has been previously determined that some bacteria are capable of utilizing or producing BCAAs (256-258), no one has measured BCAAs in probiotic and prebiotic supplementation trials to our knowledge. Since BCAAs compete with TRP for transport system in BBB, measuring BCAA along with TRP in studies evaluating effects of microbiome on depression is suggested (256, 259).

In the present study, probiotic supplementation reduced the ratio of kynurenine to tryptophan compared with placebo, whereas supplementation with prebiotic did not lead to any change. Tryptophan is mainly metabolized in two pathways: either it forms serotonin or it breaks down into kynurenine. Degradation of

tryptophan to kynurenine results in a decrease in serotonin levels. Probiotics can shift the tryptophan metabolism toward the serotonin pathway by deactivating tryptophan-degrading enzymes to kynurenine (22).

Studies on the effect of probiotics and prebiotics on KYN/TRP ratio are very few. Only one study examined the effect of probiotic supplementation for 6 weeks on KYN / TRP ratio in healthy subjects, which showed no change in serum TRP and KYN similar to our study and no decrease in serum KYN to TRP ratio in contrast to our findings (211).

The effects of 8-week supplementation of prebiotic on serum KYN/TRP was investigated in T2DM patients, which, unlike our study, prebiotics significantly reduced the KYN/TRP ratio (260). Further studies are needed to investigate the mechanism of the prebiotics' effect on depression.

5.5.   Serum BDNF Levels

We found that supplementation of probiotic for 8 weeks significantly increased the serum BDNF levels while prebiotic supplements were not effective. Studies of probiotic supplementation in depressive disorders are very rare. A study that examined the effects of probiotics on serum BDNF in MDD patient, demonstrated no significant difference between the probiotic and placebo groups (219) which contrasts with our findings. In this study patients were not receiving any treatment other than probiotic supplementation. Additionally, another study in IBS patients with depression was unable to show any important effect of 6-week supplementation of *Bifidobacterium longum* on serum BDNF levels. Although authors did not ignore the BDNF-modulatory effect of probiotics in some parts of the brain (217).   In Highlight et al. (222)   study which was conducted in hemodialysis patients, BDNF levels increased in synbiotic group and only in depressed subgroup of probiotic group. Inadequate efficacy of probiotics formulation was linked to uremia state of patients (Described above) (223). To our knowledge no study investigated effects of prebiotics in depression. One possible mechanism underlying beneficial effects of probiotics on mental health is neural protection and

growth through up-regulation of BDNF. BDNF promotes neural proliferation, survival, and plasticity via different important neuro-physiological pathways (179).

5.6. The Intestinal Microbiome

We found that the change of *lactobacillus* group (*Lactobacillus / Leuconostoc / Pediococcus*) in probiotic group was significantly higher than prebiotic group. Moreover, probiotic supplementation resulted in a significant increase in *Bifidobacterium spp.* when compared with prebiotic and also a slight increase when compared with placebo (see Table 4.16). The change of other organisms was not important. These changes in intestinal microbiome of the probiotic group are suggesting successful colonization and confirm the compliance of the patients in probiotic group.

The composition of the intestinal microbiome plays a pivotal role in human mental health, which includes mainly *Bacteroides, Firmicutes, Actinobacteria* and *Proteobacteria* (21). Dysbiosis of intestinal microbiota and decreased *Bifidobacterium* and *Lactobacillus* counts have been related with depressive symptoms in humans (17, 117). Comparison of fecal samples of 46 depressed patients with 30 healthy controls revealed specific differences in the intestinal microbiome composition including higher *Bacteroidetes, Proteobacteria* and *Actinobacteria* and lower *Firmicutes* levels in depressed patients compared to healthy controls (17). Evaluation of intestinal microbiome of 1135 subjects from a Dutch cohort study has shown a positive correlation between depression and diversity of the microbiome (alpha diversity) (261). In a study of evaluating the intestinal microbiome of 40 subjects for psychometric risk factors for depression, *Lactobacillus spp.* were associated with positive self-judgment and lower affective empathy (262). Despite all of these findings of microbiome alteration shown in MDD patients, definite difference of microbiome composition is not yet clear (109, 113-115).

Studies evaluating the effects of probiotics on intestinal microbiome composition in psychiatric patients are few and the results are mostly in line with our findings. Haghighat et al. (222) found higher levels of lactobacilli and Bifidobacteria

in intestinal microbiome of hemodialysis patients with depression after 12-week probiotic supplementation period (*L. acidophilus T16, B. bifidum BIA-6, B. lactis BIA-7* and *B. longum BIA-8,*). Similarly, 8-week supplementation of probiotic (*L. casei Shirota*) resulted in higher levels of intestinal *Bifidobacteria spp.* and *Lactobacillus spp.* in patients with CFS (213). In another study in IBS patients, 4-week supplementation of *Lactobacillus paracasei* changed microbiome composition (decreased *Ruminococcus*), meanwhile depressive symptoms were not investigated in this study (218). Similarly, probiotic supplementation (*L. casei Shirota*) in healthy subjects with stress was associated with decreased levels of family *Bacteroidaceae* in probiotic group while phylum Bacteroidetes tended to increase in the placebo group (212). Furthermore, Aizawa *et al.* (117) found higher intestinal Bifidobacterium content in subjects who consumed higher levels of fermented milk. On the other hand, a study evaluating the effects of *B. longum* in depressive IBS patients, did not find any important effect of supplementation on microbiome composition. This contradiction might be due to the shorter supplementation duration which was 6 weeks (217).

Prebiotic supplementation has been considered a microbiota-targeted intervention. However supplementation of sc-FOS (4 weeks) in IBS patients resulted in higher intestinal Bifidobacteria (230). Another study in IBS patients demonstrated that trans-GOS supplementation was associated with dose-dependent efficacy of prebiotics including higher *Bifidobacterium spp.* and altered *Colistridia spp.* and *Bacteroides-Prevotella spp.* levels (229). A study on healthy volunteers showed that XOS consumption (3 weeks) led to increase fecal *Bifidobacterium* (228). Nonetheless, we could not find any important change in intestinal microbiome in prebiotic supplemented patients, which is probably related to relatively low sample numbers in prebiotic group and potential uncontrollable influence factors on fecal microbiota.

5.7. Strength and limitations:

Advantages of this study:

- Working with specific strains of probiotics and a type of prebiotic that their effects on mood have been indicated.

- So far, very few studies have examined the composition of the intestinal microbiome after supplementation of probiotics and prebiotics in patients with depression.

- Measuring the intestinal microbiome which reveals both compliance of the participants and bacterial colonization

Limitations of this study:

- Lack of measurement of sex hormones which may interact with microbiome composition

- Low cooperation of the participant in providing stool samples

6.   CONCLUSION AND SUGGESTIONS

Present study investigated the effects of probiotic and prebiotic supplementation on depression. The results of this study showed that 8-week supplementation with probiotic significantly reduced the symptoms of depression and the serum kynurenine to tryptophan ratio, increased tryptophan to isoleucine ratio and serum BDNF levels compared to placebo. Additionally, the increase in intestinal *Bifidobacterium spp.* and *Lactobacillus* group was significant when compared with prebiotic group. It is also an indicator of compliance of subjects in probiotic group.

Prebiotic supplementation for 8 weeks was not able to change depression status or measured biomarkers except the tryptophan to BCAA ratio which increased significantly compared with placebo. In addition to direct effect on the CNS (local interactions of SCFAs), prebiotics may influence the brain via neuro-immune or neuro-endocrine pathways indirectly. However beneficial effects of prebiotics cannot be independent of the microbiome modulation (263). Therefore the lack of observed effect of prebiotics on psychological outcomes is possibly due to inability to induce microbiota proliferation in addition to the size of the sample, the length of the intervention period, the severity, chronicity or treatment resistance of the sample.

Although the sample size was not sufficient to illustrate some of the investigated mechanisms, overall the results indicate that probiotics are effective in relieving depression and they exert their beneficial effects possibly by: 1) reducing tryptophan degradation to kynurenine and thereby increasing the precursor of serotonin, 2) by increasing serum BDNF levels, and consequently supporting neurogenesis and neuroplasticity and 3) modification of intestinal microbiota by stimulating *Bifidobacteria* and *Lactobacilli* growth in the intestine.

To confirm these underlying mechanisms, further studies with measurement of these parameters in some points between start and end of the study is recommended. This approach may reveal whether the changes in serum or microbiome occurred before the depression symptoms get improved. Present study

estimated the intestinal microbiome using qPCR. Methods such as metagenomics sequencing of the microbiome, is recommended for future studies, which reveals much more about bacterial phylogenies and change of the microbiome.

7. REFERENCES

1. Hlatky MA, Boothroyd D, Vittinghoff E, Sharp P, Whooley MA, Group HR. Quality-of-life and depressive symptoms in postmenopausal women after receiving hormone therapy: results from the Heart and Estrogen/Progestin Replacement Study (HERS) trial. Jama. 2002;287(5):591-7.

2. Solomon DA, Keller MB, Leon AC, Mueller TI, Lavori PW, Shea MT, et al. Multiple recurrences of major depressive disorder. American Journal of Psychiatry. 2000;157(2):229-33.

3. Andrews G. Should depression be managed as a chronic disease? Bmj. 2001;322(7283):419-21.

4. Organization WH. Depression: a global crisis. World Mental Health Day. Geneva: World Health Organization Links. 2012.

5. Organization WH. The global burden of disease: 2004 update. 2008.

6. Sadeghirad B, Haghdoost A-A, Amin-Esmaeili M, Ananloo ES, Ghaeli P, Rahimi-Movaghar A, et al. Epidemiology of major depressive disorder in Iran: a systematic review and meta-analysis. International journal of preventive medicine. 2010;1(2):81.

7. Murrough JW, Charney DS. Is there anything really novel on the antidepressant horizon? Current psychiatry reports. 2012;14(6):643-9.

8. Brunoni AR, Machado-Vieira R, Zarate Jr CA, Vieira EL, Vanderhasselt M-A, Nitsche MA, et al. BDNF plasma levels after antidepressant treatment with sertraline and transcranial direct current stimulation: results from a factorial, randomized, sham-controlled trial. European Neuropsychopharmacology. 2014;24(7):1144-51.

9. Saveanu RV, Nemeroff CB. Etiology of depression: genetic and environmental factors. Psychiatric Clinics. 2012;35(1):51-71.

10. McGrath EE, Keita GPE, Strickland BR, Russo NFE. Women and depression: Risk factors and treatment issues: Final report of the American Psychological Association's National Task Force on Women and Depression: American Psychological Association; 1990.

11. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. The Journal of physiology. 2004;558(1):263-75.

12. Bailey MT, Lubach GR, Coe CL. Prenatal stress alters bacterial colonization of the gut in infant monkeys. Journal of pediatric gastroenterology and nutrition. 2004;38(4):414-21.

13. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Molecular psychiatry. 2011;16(4):383.

14. Guilloux J-P, Douillard-Guilloux G, Kota R, Wang X, Gardier A, Martinowich K, et al. Molecular evidence for BDNF-and GABA-related dysfunctions in the amygdala of female subjects with major depression. Molecular psychiatry. 2012;17(11):1130.

15. Müller N, Schwarz M. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Molecular psychiatry. 2007;12(11):988.

16. Schlösser RG, Wagner G, Koch K, Dahnke R, Reichenbach JR, Sauer H. Fronto-cingulate effective connectivity in major depression: a study with fMRI and dynamic causal modeling. Neuroimage. 2008;43(3):645-55.

17. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain, behavior, and immunity. 2015;48:186-94.

18. Wallace CJ, Milev R. The effects of probiotics on depressive symptoms in humans: a systematic review. Annals of general psychiatry. 2017;16(1):14.

19. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011;474(7351):327.

20. Dutton RJ, Turnbaugh PJ. Taking a metagenomic view of human nutrition. Current Opinion in Clinical Nutrition & Metabolic Care. 2012;15(5):448-54.

21. Burokas A, Moloney RD, Dinan TG, Cryan JF. Microbiota regulation of the mammalian gut–brain axis. Advances in applied microbiology. 91: Elsevier; 2015. p. 1-62.

22. O'Mahony S, Clarke G, Borre Y, Dinan T, Cryan J. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behavioural brain research. 2015;277:32-48.

23.   Wang H, Lee I-S, Braun C, Enck P. Effect of probiotics on central nervous system functions in animals and humans: a systematic review. Journal of neurogastroenterology and motility. 2016;22(4):589.

24.   Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology. 2015;232(10):1793-801.

25.   Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney R, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Molecular psychiatry. 2013;18(6):666.

26.   Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proceedings of the national academy of sciences. 2009;106(10):3698-703.

27.   Luna RA, Foster JA. Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression. Current opinion in biotechnology. 2015;32:35-41.

28.   Butel MJ, Waligora-Dupriet AJ. Probiotics and prebiotics: what are they and what can they do for us. The Human Microbiota and Chronic Disease: Dysbiosis as a Cause of Human Pathology Hoboken, NJ: John Wiley & Sons. 2016:467-78.

29.   Savignac HM, Corona G, Mills H, Chen L, Spencer JP, Tzortzis G, et al. Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-d-aspartate receptor subunits and d-serine. Neurochemistry international. 2013;63(8):756-64.

30.   Messaoudi M, Violle N, Bisson J-F, Desor D, Javelot H, Rougeot C. Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in healthy human volunteers. Gut microbes. 2011;2(4):256-61.

31.   Arseneault-Bréard J, Rondeau I, Gilbert K, Girard S-A, Tompkins TA, Godbout R, et al. Combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 reduces post-myocardial infarction depression symptoms and restores intestinal permeability in a rat model. British Journal of Nutrition. 2012;107(12):1793-9.

32.   Tian P, Zou R, Song L, Zhang X, Jiang B, Wang G, et al. Ingestion of Bifidobacterium longum subspecies infantis strain CCFM687 regulated emotional behavior and the central BDNF pathway in chronic stress-induced depressive mice through reshaping the gut microbiota. Food & function. 2019;10(11):7588-98.

33.   Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. Journal of psychiatric research. 2008;43(2):164-74.

34.   Huang R, Wang K, Hu J. Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. Nutrients. 2016;8(8):483.

35.   Selhub EM, Logan AC, Bested AC. Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry. Journal of physiological anthropology. 2014;33(1):2.

36.   Organization WH. Depression and other common mental disorders: global health estimates. World Health Organization; 2017.

37.   Association AP. Supplement to diagnostic and statistical manual of mental disorders. American Psychiatric Association. Arlington; 2016.

38.   Blier P, Ward HE, Tremblay P, Laberge L, Hébert C, Bergeron R. Combination of antidepressant medications from treatment initiation for major depressive disorder: a double-blind randomized study. American Journal of Psychiatry. 2009;167(3):281-8.

39.   Khin NA, Chen Y-F, Yang Y, Yang P, Laughren TP. Exploratory analyses of efficacy data from major depressive disorder trials submitted to the US Food and Drug Administration in support of new drug applications. The Journal of clinical psychiatry. 2011.

40.   Musso G, Gambino R, Cassader M. Gut microbiota as a regulator of energy homeostasis and ectopic fat deposition: mechanisms and implications for metabolic disorders. Current opinion in lipidology. 2010;21(1):76-83.

41.   Flint HJ. The impact of nutrition on the human microbiome. Nutrition reviews. 2012;70(s1).

42.   Burcusa SL, Iacono WG. Risk for recurrence in depression. Clinical psychology review. 2007;27(8):959-85.

43.   Belsher G, Costello CG. Relapse after recovery from unipolar depression: a critical review. Psychological bulletin. 1988;104(1):84.
44.   Rubio JM, Markowitz JC, Alegría A, Pérez-Fuentes G, Liu SM, Lin KH, et al. Epidemiology of chronic and nonchronic major depressive disorder: results from the national epidemiologic survey on alcohol and related conditions. Depression and anxiety. 2011;28(8):622-31.
45.   Commission E. European pact for mental health and well-being. European Union Brussels; 2008.
46.   Ferrari A, Somerville A, Baxter A, Norman R, Patten S, Vos T, et al. Global variation in the prevalence and incidence of major depressive disorder: a systematic review of the epidemiological literature. Psychological medicine. 2013;43(3):471-81.
47.   Üstün TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. The British journal of psychiatry. 2004;184(5):386-92.
48.   Noorbala AA, Faghihzadeh S, Kamali K, Bagheri Yazdi SA, Hajebi A, Mousavi MT, et al. Mental Health Survey of the Iranian Adult Population in 2015. Archives of Iranian Medicine (AIM). 2017;20(3).
49.   Noorbala AA, Yazdi AB, Faghihzadeh S, Kamali K, Faghihzadeh E, Hajebi A, et al. A survey on mental health status of adult population aged 15 and above in the province of East Azarbaijan, Iran. Archives of Iranian medicine. 2017;20(11):S23.
50.   Noorbala AA, Yazdi SAB, Faghihzadeh S, Kamali K, Faghihzadeh E, Hajebi A, et al. A Survey on Mental Health Status of Adult Population Aged 15 and above in the Province of Gilan, Iran. Archives of Iranian medicine. 2017;20(11):S31.
51.   Noorbala AA, Ahmad Hajebi M, Mansour Shakiba M, MA FS. A Survey on Mental Health Status of Adult Population Aged 15 and above in the Province of Sistan and Bluchestan, Iran. Archives of Iranian medicine. 2017;20(11 Suppl 1):S107.
52.   Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. American Journal of Psychiatry. 2000;157(10):1552-62.
53.   Bagot RC, Labonté B, Peña CJ, Nestler EJ. Epigenetic signaling in psychiatric disorders: stress and depression. Dialogues in clinical neuroscience. 2014;16(3):281.
54.   Coppen A. The biochemistry of affective disorders. The British Journal of Psychiatry. 1967;113(504):1237-64.
55.   Holsboer F. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology. 2000;23(5):477-501.
56.   Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology E-Book: Elsevier Health Sciences; 2014.
57.   Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. Trends in Endocrinology & Metabolism. 1999;10(9):359-68.
58.   Smolen JS, Redlich K, Zwerina J, Aletaha D, Steiner G, Schett G. Pro-inflammatory cytokines in rheumatoid arthritis. Clinical reviews in allergy & immunology. 2005;28(3):239-48.
59.   Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC medicine. 2013;11(1):1-16.
60.   Gillespie C, Garlow S, Schatzberg A, Nemeroff C. Textbook of psychopharmacology. America Psychiatric Publishing, Arlington, VA; 2009.
61.   Zhu C-B, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. Neuropsychopharmacology. 2010;35(13):2510.
62.   Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. Current drug metabolism. 2008;9(7):622-7.
63.   Maes M, Leonard B, Myint A, Kubera M, Verkerk R. The new '5-HT'hypothesis of depression: cell-mediated immune activation induces indoleamine 2, 3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011;35(3):702-21.

64.	Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS. Nuclear factor-κB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proceedings of the National Academy of Sciences. 2010;107(6):2669-74.

65.	Haroon E, Woolwine BJ, Chen X, Pace TW, Parekh S, Spivey JR, et al. IFN-alpha-induced cortical and subcortical glutamate changes assessed by magnetic resonance spectroscopy. Neuropsychopharmacology. 2014;39(7):1777-85.

66.	Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature reviews immunology. 2016;16(1):22.

67.	Khanna S, Tosh PK, editors. A clinician's primer on the role of the microbiome in human health and disease. Mayo Clinic Proceedings; 2014: Elsevier.

68.	Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain–gut–enteric microbiota axis. Nature Reviews Gastroenterology and Hepatology. 2009;6(5):306-14.

69.	Cryan JF, O'mahony S. The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterology & Motility. 2011;23(3):187-92.

70.	Turroni F, Ribbera A, Foroni E, van Sinderen D, Ventura M. Human gut microbiota and bifidobacteria: from composition to functionality. Antonie Van Leeuwenhoek. 2008;94(1):35-50.

71.	Savage DC. Microbial ecology of the gastrointestinal tract. Annual review of microbiology. 1977;31(1):107-33.

72.	Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. nature. 2010;464(7285):59-65.

73.	Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS biology. 2016;14(8):e1002533.

74.	Grenham S, Clarke G, Cryan JF, Dinan TG. Brain–gut–microbe communication in health and disease. Frontiers in physiology. 2011;2.

75.	Gonzalez A, Stombaugh J, Lozupone C, Turnbaugh PJ, Gordon JI, Knight R. The mind-body-microbial continuum. Dialogues in clinical neuroscience. 2011;13(1):55.

76.	Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome medicine. 2016;8(1):1-11.

77.	Kerry RG, Patra JK, Gouda S, Park Y, Shin H-S, Das G. Benefaction of probiotics for human health: A review. Journal of food and drug analysis. 2018;26(3):927-39.

78.	Cebra JJ. Influences of microbiota on intestinal immune system development. The American journal of clinical nutrition. 1999;69(5):1046s-51s.

79.	Lankelma JM, Nieuwdorp M, de Vos WM, Wiersinga WJ. The gut microbiota in internal medicine: implications for health and disease. The Netherlands journal of medicine. 2015;73(2):61-8.

80.	Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. science. 2005;307(5717):1915-20.

81.	Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Frontiers in cellular neuroscience. 2015;9.

82.	Quigley EM. Microbiota-brain-gut axis and neurodegenerative diseases. Current neurology and neuroscience reports. 2017;17(12):94.

83.	Barbara G, Stanghellini V, Brandi G, Cremon C, Di Nardo G, De Giorgio R, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. The American journal of gastroenterology. 2005;100(11):2560-8.

84.	Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour–epigenetic regulation of the gut–brain axis. Genes, Brain and Behavior. 2014;13(1):69-86.

85.	Iyer LM, Aravind L, Coon SL, Klein DC, Koonin EV. Evolution of cell–cell signaling in animals: did late horizontal gene transfer from bacteria have a role? TRENDS in Genetics. 2004;20(7):292-9.

86.	Milligan TW, Doran TI, Straus DC, Mattingly SJ. Growth and amino acid requirements of various strains of group B streptococci. Journal of clinical microbiology. 1978;7(1):28-33.

87.	Li G, Young KD. Indole production by the tryptophanase TnaA in Escherichia coli is determined by the amount of exogenous tryptophan. Microbiology. 2013;159(Pt_2):402-10.

88.   Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. Bioessays. 2011;33(8):574-81.

89.   Jiménez E, Ladero V, Chico I, Maldonado-Barragán A, López M, Martín V, et al. Antibiotic resistance, virulence determinants and production of biogenic amines among enterococci from ovine, feline, canine, porcine and human milk. BMC microbiology. 2013;13(1):288.

90.   Sobko T, Huang L, Midtvedt T, Norin E, Gustafsson LE, Norman M, et al. Generation of NO by probiotic bacteria in the gastrointestinal tract. Free Radical Biology and Medicine. 2006;41(6):985-91.

91.   Abrams GD, Bishop JE. Effect of the Normal Microbial Flora on Gastrointestinal Motility.∗. Proceedings of the society for experimental biology and medicine. 1967;126(1):301-4.

92.   Iwai H, Ishihara Y, Yamanaka J, Ito T. Effects of bacterial flora on cecal size and transit rate of intestinal contents in mice. The Japanese journal of experimental medicine. 1973;43(4):297.

93.   Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain–gut–enteric microbiota axis. Nature reviews Gastroenterology & hepatology. 2009;6(5):306.

94.   Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Annals of gastroenterology: quarterly publication of the Hellenic Society of Gastroenterology. 2015;28(2):203.

95.   Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Comprehensive Physiology. 2011;6(2):603-21.

96.   Mayer EA, Savidge T, Shulman RJ. Brain–gut microbiome interactions and functional bowel disorders. Gastroenterology. 2014;146(6):1500-12.

97.   Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology. 2012;37(9):1369-78.

98.   Meddings JB, Swain M. Environmental stress–induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology. 2000;119(4):1019-28.

99.   Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney R, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Molecular psychiatry. 2013;18(6):666-73.

100.   Heijtz RD, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proceedings of the National Academy of Sciences. 2011;108(7):3047-52.

101.   Marques TM, Cryan JF, Shanahan F, Fitzgerald GF, Ross RP, Dinan TG, et al. Gut microbiota modulation and implications for host health: dietary strategies to influence the gut–brain axis. Innovative Food Science & Emerging Technologies. 2014;22:239-47.

102.   Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience. 2013;246:199-229.

103.   Ait-Belgnaoui A, Colom A, Braniste V, Ramalho L, Marrot A, Cartier C, et al. Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. Neurogastroenterology & Motility. 2014;26(4):510-20.

104.   Roselli M, Finamore A, Britti MS, Mengheri E. Probiotic bacteria Bifidobacterium animalis MB5 and Lactobacillus rhamnosus GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic Escherichia coli K88. British Journal of Nutrition. 2006;95(6):1177-84.

105.   Kunze WA, Mao YK, Wang B, Huizinga JD, Ma X, Forsythe P, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. Journal of cellular and molecular medicine. 2009;13(8b):2261-70.

106.   Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled

receptor 41 (GPR41). Proceedings of the national academy of sciences. 2011;108(19):8030-5.

107. Erny D, de Angelis ALH, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nature neuroscience. 2015;18(7):965.

108. Verdu EF, Bercik P, Verma-Gandhu M, Huang X-X, Blennerhassett P, Jackson W, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut. 2006;55(2):182-90.

109. Liang S, Wu X, Hu X, Wang T, Jin F. Recognizing depression from the microbiota–gut–brain axis. International journal of molecular sciences. 2018;19(6):1592.

110. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho A-M, Quigley EM, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biological psychiatry. 2009;65(3):263-7.

111. Park A, Collins J, Blennerhassett P, Ghia J, Verdu E, Bercik P, et al. Altered colonic function and microbiota profile in a mouse model of chronic depression. Neurogastroenterology & Motility. 2013;25(9):733-e575.

112. Yu M, Jia H, Zhou C, Yang Y, Zhao Y, Yang M, et al. Variations in gut microbiota and fecal metabolic phenotype associated with depression by 16S rRNA gene sequencing and LC/MS-based metabolomics. Journal of pharmaceutical and biomedical analysis. 2017;138:231-9.

113. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linløkken A, Wilson R, et al. Correlation between the human fecal microbiota and depression. Neurogastroenterology & Motility. 2014;26(8):1155-62.

114. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Molecular psychiatry. 2016;21(6):786.

115. Lin P, Ding B, Feng C, Yin S, Zhang T, Qi X, et al. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. Journal of affective disorders. 2017;207:300-4.

116. Liu Y, Zhang L, Wang X, Wang Z, Zhang J, Jiang R, et al. Similar fecal microbiota signatures in patients with diarrhea-predominant irritable bowel syndrome and patients with depression. Clinical Gastroenterology and Hepatology. 2016;14(11):1602-11. e5.

117. Aizawa E, Tsuji H, Asahara T, Takahashi T, Teraishi T, Yoshida S, et al. Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. Journal of affective disorders. 2016;202:254-7.

118. Sanada K, Nakajima S, Kurokawa S, Barceló-Soler A, Ikuse D, Hirata A, et al. Gut Microbiota and Major Depressive Disorder: A Systematic Review and Meta-Analysis. Journal of Affective Disorders. 2020.

119. Belanger PN, Hewlings SJ. The Effects of Probiotic Supplementation on Depressive Symptoms: A Systematic Review. Open Science Journal of Clinical Medicine. 2019;7(1):25.

120. Liu X, Cao S, Zhang X. Modulation of gut microbiota–brain axis by probiotics, prebiotics, and diet. Journal of agricultural and food chemistry. 2015;63(36):7885-95.

121. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology. 2017.

122. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics-a review. Journal of food science and technology. 2015;52(12):7577-87.

123. Chapman C, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? European journal of nutrition. 2011;50(1):1-17.

124. Liang S, Wang T, Hu X, Luo J, Li W, Wu X, et al. Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience. 2015;310:561-77.

125. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, Taghizadeh M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. Nutrition. 2016;32(3):315-20.

126.   Mao Y, Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G, et al. The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology. 1996;111(2):334-44.
127.   Oishi K, Sato T, Yokoi W, Yoshida Y, Ito M, Sawada H. Effect of probiotics, Bifidobacterium breve and Lactobacillus casei, on bisphenol A exposure in rats. Bioscience, biotechnology, and biochemistry. 2008;72(6):1409-15.
128.   Ahrne S, Johansson Hagslatt M-L. Effect of lactobacilli on paracellular permeability in the gut. Nutrients. 2011;3(1):104-17.
129.   Karczewski J, Troost FJ, Konings I, Dekker J KM, Brummer RM, Wells JM. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2010;298(6):G851-G9.
130.   McNaught CE, Woodcock NP, Anderson AD, MacFie J. A prospective randomised trial of probiotics in critically ill patients. Clinical Nutrition. 2005;24(2):211-9.
131.   Phillips JGP. The treatment of melancholia by the lactic acid bacillus. The British Journal of Psychiatry. 1910;56(234):422-NP.
132.   Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biological psychiatry. 2013;74(10):720-6.
133.   Liu W-H, Chuang H-L, Huang Y-T, Wu C-C, Chou G-T, Wang S, et al. Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. Behavioural brain research. 2016;298:202-9.
134.   Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences. 2011;108(38):16050-5.
135.   Bercik P, Park A, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication. Neurogastroenterology & Motility. 2011;23(12):1132-9.
136.   Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. British Journal of Nutrition. 2011;105(5):755-64.
137.   Logan AC, Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. Medical hypotheses. 2005;64(3):533-8.
138.   Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology. 2012;37(11):1885-95.
139.   Kwok L, Wang L, Zhang J, Guo Z, Zhang H. A pilot study on the effect of Lactobacillus casei Zhang on intestinal microbiota parameters in Chinese subjects of different age. Beneficial microbes. 2014;5(3):295-304.
140.   Wang T, Hu X, Liang S, Li W, Wu X, Wang L, et al. Lactobacillus fermentum NS9 restores the antibiotic induced physiological and psychological abnormalities in rats. Beneficial microbes. 2015;6(5):707-17.
141.   Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP. Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. Nutrients. 2016;8(1):56.
142.   Oxenkrug GF. Tryptophan–kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40 years later. The Israel journal of psychiatry and related sciences. 2010;47(1):56.
143.   Lapin IP, Oxenkrug G. Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. The Lancet. 1969;293(7586):132-6.
144.   Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan J, Dinan T. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience. 2010;170(4):1179-88.
145.   Mawe GM, Hoffman JM. Serotonin signalling in the gut—functions, dysfunctions and therapeutic targets. Nature reviews Gastroenterology & hepatology. 2013;10(8):473.

146.   Ruddick JP, Evans AK, Nutt DJ, Lightman SL, Rook GA, Lowry CA. Tryptophan metabolism in the central nervous system: medical implications. Expert reviews in molecular medicine. 2006;8(20):1-27.

147.   Moir A, Eccleston D. The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. Journal of Neurochemistry. 1968;15(10):1093-108.

148.   Fujigaki S, Saito K, Sekikawa K, Tone S, Takikawa O, Fujii H, et al. Lipopolysaccharide induction of indoleamine 2, 3-dioxygenase is mediated dominantly by an IFN-γ-independent mechanism. European journal of immunology. 2001;31(8):2313-8.

149.   PEMBERTON LA, KERR SJ, SMYTHE G, BREW BJ. Quinolinic acid production by macrophages stimulated with IFN-γ, TNF-α, and IFN-α. Journal of interferon & cytokine research. 1997;17(10):589-95.

150.   Ormstad H, Dahl J, Verkerk R, Andreassen OA, Maes M. Increased plasma levels of competing amino acids, rather than lowered plasma tryptophan levels, are associated with a non-response to treatment in major depression. European Neuropsychopharmacology. 2016;26(8):1286-96.

151.   Stone TW, Forrest CM, Darlington LG. Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection. The FEBS journal. 2012;279(8):1386-97.

152.   Zawilska J, Rosiak J, Senderecka M, Nowak J. Suppressive effect of NMDA receptor antagonist MK-801 on nocturnal serotonin N-acetyltransferase activity in the rat pineal gland. Polish journal of pharmacology. 1997;49(6):479-83.

153.   Müller N, Schwarz MJ. A psychoneuroimmunological perspective to Emil Kraepelins dichotomy. European Archives of Psychiatry and clinical neuroscience. 2008;258(2):97-106.

154.   O'Mahony SM, Clarke G, Borre Y, Dinan T, Cryan J. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behavioural brain research. 2015;277:32-48.

155.   Lyons JL, Tovar-y-Romo LB, Thakur KT, McArthur JC, Haughey NJ. Pathobiology of CNS Human Immunodeficiency Virus Infection. Neurobiology of Brain Disorders: Elsevier; 2015. p. 444-66.

156.   Sanacora G, Gueorguieva R, Epperson CN, Wu Y-T, Appel M, Rothman DL, et al. Subtype-specific alterations of γ-aminobutyric acid and glutamatein patients with major depression. Archives of general psychiatry. 2004;61(7):705-13.

157.   Lee L-J, Lo F-S, Erzurumlu RS. NMDA receptor-dependent regulation of axonal and dendritic branching. Journal of Neuroscience. 2005;25(9):2304-11.

158.   Gorman JM, Docherty JP. A hypothesized role for dendritic remodeling in the etiology of mood and anxiety disorders. The Journal of neuropsychiatry and clinical neurosciences. 2010;22(3):256-64.

159.   Fernstrom JD. Role of precursor availability in control of monoamine biosynthesis in brain. Physiological reviews. 1983;63(2):484-546.

160.   Kroenke K, Taylor-Vaisey A, Dietrich AJ, Oxman TE. Interventions to improve provider diagnosis and treatment of mental disorders in primary care: a critical review of the literature. Psychosomatics. 2000;41(1):39-52.

161.   Abelaira HM, Réus GZ, Neotti MV, Quevedo J. The role of mTOR in depression and antidepressant responses. Life sciences. 2014;101(1-2):10-4.

162.   Li N, Lee B, Liu R-J, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64.

163.   Yang C, Zhou Z-q, Gao Z-q, Shi J-y, Yang J-j. Acute increases in plasma mammalian target of rapamycin, glycogen synthase kinase-3β, and eukaryotic elongation factor 2 phosphorylation after ketamine treatment in three depressed patients. Biological psychiatry. 2013;73(12):e35-e6.

164.   Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005;308(5726):1314-8.

165.   De Simone R, Vissicchio F, Mingarelli C, De Nuccio C, Visentin S, Ajmone-Cat MA, et al. Branched-chain amino acids influence the immune properties of microglial cells and their responsiveness to pro-inflammatory signals. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013;1832(5):650-9.

166.   Setoyama D, Kato TA, Hashimoto R, Kunugi H, Hattori K, Hayakawa K, et al. Plasma metabolites predict severity of depression and suicidal ideation in psychiatric patients-a multicenter pilot analysis. PLoS One. 2016;11(12):e0165267.

167.   Dantzer R. Role of the kynurenine metabolism pathway in inflammation-induced depression: preclinical approaches.   Inflammation-Associated Depression: Evidence, Mechanisms and Implications: Springer; 2016. p. 117-38.

168.   Muller N, Myint A-M, J Schwarz M. Immunological treatment options for schizophrenia. Current pharmaceutical biotechnology. 2012;13(8):1606-13.

169.   Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. Journal of affective disorders. 2015;174:432-40.

170.   Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. Archives of general psychiatry. 1997;54(7):597-606.

171.   Sheldrick A, Camara S, Ilieva M, Riederer P, Michel T. Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) levels in post-mortem brain tissue from patients with depression compared to healthy individuals–a proof of concept study. European Psychiatry. 2017;46:65-71.

172.   Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. International Journal of Neuropsychopharmacology. 2008;11(8):1169-80.

173.   Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biological psychiatry. 2008;64(6):527-32.

174.   Kishi T, Yoshimura R, Ikuta T, Iwata N. Brain-derived neurotrophic factor and major depressive disorder: evidence from meta-analyses. Frontiers in psychiatry. 2018;8:308.

175.   Lee B-H, Kim Y-K. The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. Psychiatry investigation. 2010;7(4):231.

176.   Wolkowitz OM, Wolf J, Shelly W, Rosser R, Burke HM, Lerner GK, et al. Serum BDNF levels before treatment predict SSRI response in depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011;35(7):1623-30.

177.   Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Progress in neurobiology. 2005;76(2):99-125.

178.   Greenberg ME, Xu B, Lu B, Hempstead BL. New insights in the biology of BDNF synthesis and release: implications in CNS function. Journal of Neuroscience. 2009;29(41):12764-7.

179.   Groves J. Is it time to reassess the BDNF hypothesis of depression? Molecular psychiatry. 2007;12(12):1079.

180.   Ibáñez CF. Neurotrophic factors: from structure-function studies to designing effective therapeutics. Trends in biotechnology. 1995;13(6):217-27.

181.   Wurzelmann M, Romeika J, Sun D. Therapeutic potential of brain-derived neurotrophic factor (BDNF) and a small molecular mimics of BDNF for traumatic brain injury. Neural regeneration research. 2017;12(1):7.

182.   Hemmings BA, Restuccia DF. Pi3k-pkb/akt pathway. Cold Spring Harbor perspectives in biology. 2012;4(9):a011189.

183.   Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Developmental neurobiology. 2010;70(5):304-22.

184.   Wayman GA, Lee Y-S, Tokumitsu H, Silva A, Soderling TR. Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron. 2008;59(6):914-31.

185.   Pérez-Navarro E, Gavaldà N, Gratacos E, Alberch J. Brain-derived neurotrophic factor prevents changes in Bcl-2 family members and caspase-3 activation induced by excitotoxicity in the striatum. Journal of neurochemistry. 2005;92(3):678-91.

186.   Tamura S, Nagasawa A, Masuda Y, Tsunematsu T, Hayasaka K, Matsuno K, et al. BDNF, produced by a TPO-stimulated megakaryocytic cell line, regulates autocrine proliferation. Biochemical and biophysical research communications. 2012;427(3):542-6.

187.   Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J-i, et al. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thrombosis and haemostasis. 2002;87(04):728-34.

188.   Harker LA, Roskos LK, Marzec UM, Carter RA, Cherry JK, Sundell B, et al. Effects of megakaryocyte growth and development factor on platelet production, platelet life span, and platelet function in healthy human volunteers. Blood. 2000;95(8):2514-22.

189.	Pardridge WM, Kang Y-S, Buciak JL. Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood− brain barrier in vivo using vector-mediated peptide drug delivery. Pharmaceutical research. 1994;11(5):738-46.

190.	De Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Food biotechnology: Springer; 2008. p. 1-66.

191.	Saulnier DM, Gibson GR, Kolida S. In vitro effects of selected synbiotics on the human faecal microbiota composition. FEMS microbiology ecology. 2008;66(3):516-27.

192.	Pokusaeva K, Fitzgerald GF, van Sinderen D. Carbohydrate metabolism in Bifidobacteria. Genes & nutrition. 2011;6(3):285.

193.	Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. Frontiers in microbiology. 2016;7:185.

194.	Evans JM, Morris LS, Marchesi JR. The gut microbiome: the role of a virtual organ in the endocrinology of the host. J Endocrinol. 2013;218(3):R37-47.

195.	Fernandes J, Su W, Rahat-Rozenbloom S, Wolever T, Comelli E. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. Nutrition & diabetes. 2014;4(6):e121-e.

196.	Grider JR, Piland BE. The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2007;292(1):G429-G37.

197.	Burokas A, Arboleya S, Moloney RD, Peterson VL, Murphy K, Clarke G, et al. Targeting the microbiota-gut-brain axis: prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of chronic stress in mice. Biological psychiatry. 2017;82(7):472-87.

198.	Tarr AJ, Galley JD, Fisher SE, Chichlowski M, Berg BM, Bailey MT. The prebiotics 3′ Sialyllactose and 6′ Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. Brain, behavior, and immunity. 2015;50:166-77.

199.	Collins S, Reid G. Distant site effects of ingested prebiotics. Nutrients. 2016;8(9):523.

200.	Aliasgharzadeh A, Dehghan P, Gargari BP, Asghari-Jafarabadi M. Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: a randomised controlled clinical trial. British Journal of Nutrition. 2015;113(2):321-30.

201.	Yarandi SS, Peterson DA, Treisman GJ, Moran TH, Pasricha PJ. Modulatory effects of gut microbiota on the central nervous system: how gut could play a role in neuropsychiatric health and diseases. Journal of neurogastroenterology and motility. 2016;22(2):201.

202.	Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends in immunology. 2006;27(1):24-31.

203.	Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. Journal of gastroenterology. 2017;52(1):1-8.

204.	Savignac HM, Couch Y, Stratford M, Bannerman DM, Tzortzis G, Anthony DC, et al. Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-β levels in male mice. Brain, behavior, and immunity. 2016;52:120-31.

205.	Farhangi MA, Javid AZ, Sarmadi B, Karimi P, Dehghan P. A randomized controlled trial on the efficacy of resistant dextrin, as functional food, in women with type 2 diabetes: Targeting the hypothalamic–pituitary–adrenal axis and immune system. Clinical Nutrition. 2018;37(4):1216-23.

206.	Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013;155(7):1451-63.

207.	Bercik P, Verdu EF, Foster JA, Macri J, Potter M, Huang X, et al. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology. 2010;139(6):2102-12. e1.

208.	Smith CJ, Emge JR, Berzins K, Lung L, Khamishon R, Shah P, et al. Probiotics normalize the gut-brain-microbiota axis in immunodeficient mice. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2014;307(8):G793-G802.

209.   Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. European journal of clinical nutrition. 2007;61(3):355-61.

210.   Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain, behavior, and immunity. 2015;48:258-64.

211.   Mohammadi AA, Jazayeri S, Khosravi-Darani K, Solati Z, Mohammadpour N, Asemi Z, et al. The effects of probiotics on mental health and hypothalamic–pituitary–adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutritional neuroscience. 2016;19(9):387-95.

212.   Kato-Kataoka A, Nishida K, Takada M, Kawai M, Kikuchi-Hayakawa H, Suda K, et al. Fermented milk containing Lactobacillus casei strain Shirota preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress. Appl Environ Microbiol. 2016;82(12):3649-58.

213.   Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, Berardi JM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathogens. 2009;1(1):6.

214.   Simrén M, Öhman L, Olsson J, Svensson U, Ohlson K, Posserud I, et al. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome–a randomized, double-blind, controlled study. Alimentary pharmacology & therapeutics. 2010;31(2):218-27.

215.   Dapoigny M, Piche T, Ducrotte P, Lunaud B, Cardot J-M, Bernalier-Donadille A. Efficacy and safety profile of LCR35 complete freeze-dried culture in irritable bowel syndrome: a randomized, double-blind study. World journal of gastroenterology: WJG. 2012;18(17):2067.

216.   Whitehead WE. Patient subgroups in irritable bowel syndrome that can be defined by symptom evaluation and physical examination. The American journal of medicine. 1999;107(5):33-40.

217.   Pinto-Sanchez MI, Hall GB, Ghajar K, Nardelli A, Bolino C, Lau JT, et al. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. Gastroenterology. 2017;153(2):448-59. e8.

218.   Cremon C, Guglielmetti S, Gargari G, Taverniti V, Castellazzi AM, Valsecchi C, et al. Effect of Lactobacillus paracasei CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: A pilot randomized clinical trial. United European gastroenterology journal. 2018;6(4):604-13.

219.   Romijn AR, Rucklidge JJ, Kuijer RG, Frampton C. A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. Australian & New Zealand Journal of Psychiatry. 2017;51(8):810-21.

220.   Majeed M, Nagabhushanam K, Arumugam S, Majeed S, Ali F. Bacillus coagulans MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-centre, pilot clinical study. Food & nutrition research. 2018;62.

221.   Ghorbani Z, Nazari S, Etesam F, Nourimajd S, Ahmadpanah M, Jahromi SR. The effect of synbiotic as an adjuvant therapy to fluoxetine in moderate depression: a randomized multicenter trial. Archives of Neuroscience. 2018;5(2).

222.   Haghighat N, Rajabi S, Mohammadshahi M. Effect of synbiotic and probiotic supplementation on serum brain-derived neurotrophic factor level, depression and anxiety symptoms in hemodialysis patients: a randomized, double-blinded, clinical trial. Nutritional neuroscience. 2019:1-10.

223.   Vaziri ND, Zhao Y-Y, Pahl MV. Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment. Nephrology Dialysis Transplantation. 2015;31(5):737-46.

224.   Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. Physiological reviews. 2014;94(2):461-518.

225.   Mika A, Day HE, Martinez A, Rumian NL, Greenwood BN, Chichlowski M, et al. Early life diets with prebiotics and bioactive milk fractions attenuate the impact of stress on

learned helplessness behaviours and alter gene expression within neural circuits important for stress resistance. European journal of neuroscience. 2017;45(3):342-57.

226.   Smith AP. The concept of well-being: relevance to nutrition research. British journal of nutrition. 2005;93(S1):S1-S5.

227.   Smith A, Sutherland D, Hewlett P. An investigation of the acute effects of oligofructose-enriched inulin on subjective wellbeing, mood and cognitive performance. Nutrients. 2015;7(11):8887-96.

228.   Childs CE, Röytiö H, Alhoniemi E, Fekete AA, Forssten SD, Hudjec N, et al. Xylo-oligosaccharides alone or in synbiotic combination with Bifidobacterium animalis subsp. lactis induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. British Journal of Nutrition. 2014;111(11):1945-56.

229.   Silk D, Davis A, Vulevic J, Tzortzis G, Gibson G. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Alimentary pharmacology & therapeutics. 2009;29(5):508-18.

230.   Azpiroz F, Dubray C, Bernalier-Donadille A, Cardot JM, Accarino A, Serra J, et al. Effects of sc FOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. Neurogastroenterology & Motility. 2017;29(2):e12911.

231.   Ghassemzadeh H, Mojtabai R, Karamghadiri N, Ebrahimkhani N. Psychometric properties of a Persian-language version of the Beck Depression Inventory-Second edition: BDI-II-PERSIAN. Depression and anxiety. 2005;21(4):185-92.

232.   Strasser B, Geiger D, Schauer M, Gostner J, Gatterer H, Burtscher M, et al. Probiotic supplements beneficially affect tryptophan–kynurenine metabolism and reduce the incidence of upper respiratory tract infections in trained athletes: a randomized, double-blinded, placebo-controlled trial. Nutrients. 2016;8(11):752.

233.   Bartosch S, Woodmansey EJ, Paterson JC, McMurdo ME, Macfarlane GT. Microbiological effects of consuming a synbiotic containing Bifidobacterium bifidum, Bifidobacterium lactis, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria. Clinical Infectious Diseases. 2005;40(1):28-37.

234.   Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. Journal of applied microbiology. 2004;97(6):1166-77.

235.   Furet J-P, Firmesse O, Gourmelon M, Bridonneau C, Tap J, Mondot S, et al. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. FEMS microbiology ecology. 2009;68(3):351-62.

236.   Bartosch S, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol. 2004;70(6):3575-81.

237.   Guo J, Han X, Zhan J, You Y, Huang W. Vanillin Alleviates High Fat Diet-Induced Obesity and Improves the Gut Microbiota Composition. Frontiers in Microbiology. 2018;9(2733).

238.   Staroscik A. dsDNA copy number calculator. URI Genomics & Sequencing Center. 2004.

239.   Songsasen N, Henson L, Tipkantha W, Thongkittidilok C, Henson J, Chatdarong K, et al. Dynamic changes in mitochondrial DNA, distribution and activity within cat oocytes during folliculogenesis. Reproduction in Domestic Animals. 2017;52:71-6.

240.   Taraz M, Khatami M-R, Dashti-Khavidaki S, Akhonzadeh S, Noorbala A-A, Ghaeli P, et al. Sertraline decreases serum level of interleukin-6 (IL-6) in hemodialysis patients with depression: results of a randomized double-blind, placebo-controlled clinical trial. International immunopharmacology. 2013;17(3):917-23.

241.   Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D. Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. Microbial pathogenesis. 2012;53(2):100-8.

242.   Liber A, Szajewska H. Effects of inulin-type fructans on appetite, energy intake, and body weight in children and adults: systematic review of randomized controlled trials. Annals of Nutrition and Metabolism. 2013;63(1-2):42-54.

243.   Verhoef SP, Meyer D, Westerterp KR. Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. British journal of nutrition. 2011;106(11):1757-62.

244.   Jackson KG, Taylor GR, Clohessy AM, Williams CM. The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. British Journal of Nutrition. 1999;82(1):23-30.

245.   Giacco R, Clemente G, Luongo D, Lasorella G, Fiume I, Brouns F, et al. Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. Clinical Nutrition. 2004;23(3):331-40.

246.   Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. The American journal of clinical nutrition. 2009;89(6):1751-9.

247.   Genta S, Cabrera W, Habib N, Pons J, Carillo IM, Grau A, et al. Yacon syrup: beneficial effects on obesity and insulin resistance in humans. Clinical nutrition. 2009;28(2):182-7.

248.   Mahadzir MDA, Shyam S, Barua A, Krishnappa P, Ramamurthy S. Effect of probiotic microbial cell preparation (MCP) on fasting blood glucose, body weight, waist circumference, and faecal short chain fatty acids among overweight Malaysian adults: A pilot randomised controlled trial of 4 weeks. Malaysian Journal of Nutrition. 2017;23(3):329-41.

249.   Sanchez M, Darimont C, Panahi S, Drapeau V, Marette A, Taylor V, et al. Effects of a diet-based weight-reducing program with probiotic supplementation on satiety efficiency, eating behaviour traits, and psychosocial behaviours in obese individuals. Nutrients. 2017;9(3):284.

250.   Lai JS, Hiles S, Bisquera A, Hure AJ, McEvoy M, Attia J. A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. The American journal of clinical nutrition. 2013;99(1):181-97.

251.   Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. Mediterranean diet, stroke, cognitive impairment, and depression: a meta-analysis. Annals of neurology. 2013;74(4):580-91.

252.   Button K, Kounali D, Thomas L, Wiles N, Peters T, Welton N, et al. Minimal clinically important difference on the Beck Depression Inventory-II according to the patient's perspective. Psychological medicine. 2015;45(15):3269-79.

253.   Romijn AR, Rucklidge JJ. Systematic review of evidence to support the theory of psychobiotics. Nutrition reviews. 2015;73(10):675-93.

254.   Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic advances in gastroenterology. 2013;6(1):39-51.

255.   Umehara H, Numata S, Watanabe S-y, Hatakeyama Y, Kinoshita M, Tomioka Y, et al. Altered KYN/TRP, Gln/Glu, and Met/methionine sulfoxide ratios in the blood plasma of medication-free patients with major depressive disorder. Scientific reports. 2017;7(1):4855.

256.   Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376.

257.   Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.

258.   Watson E, MacNeil LT, Ritter AD, Yilmaz LS, Rosebrock AP, Caudy AA, et al. Interspecies systems biology uncovers metabolites affecting C. elegans gene expression and life history traits. Cell. 2014;156(4):759-70.

259.   Baranyi A, Amouzadeh-Ghadikolai O, von Lewinski D, Rothenhäusler H-B, Theokas S, Robier C, et al. Branched-chain amino acids as new biomarkers of major depression-a novel neurobiology of mood disorder. PloS one. 2016;11(8):e0160542.

260.   Perez-Cornago A, Sanchez-Villegas A, Bes-Rastrollo M, Gea A, Molero P, Lahortiga-Ramos F, et al. Intake of high-fat yogurt, but not of low-fat yogurt or prebiotics, is

related to lower risk of depression in women of the SUN cohort study. The Journal of nutrition. 2016;146(9):1731-9.

261.   Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science. 2016;352(6285):565-9.

262.   Heym N, Heasman B, Hunter K, Blanco S, Wang G, Siegert R, et al. The role of microbiota and inflammation in self-judgement and empathy: implications for understanding the brain-gut-microbiome axis in depression. Psychopharmacology. 2019:1-12.

263.   Desmedt O, Broers VJ, Zamariola G, Pachikian B, Delzenne N, Luminet O. Effects of prebiotics on affect and cognition in human intervention studies. Nutrition reviews. 2019;77(2):81-95.