Randomized controlled trial of probiotics and vitamin B_3 on gut microbiome and quality of life in people with Parkinson's disease

by

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M.B., B.S., University of Medicine, Mandalay, 2006
M.P.H., Kansas State University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics, and Health
College of Health and Human Sciences

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Abstract

Evidence suggests that administration of probiotics and vitamin B_3 may improve multiple symptoms and outcomes of Parkinson's Disease through alterations in gut microbiome. Therefore, the purpose of this dissertation is to determine whether a 12-week placebo-controlled randomized clinical trial is able to observe changes in constipation, drug efficacy, neuroendocrine levels, and indicators of quality of life in people with Parkinson's disease.

Methodology: A total of 54 people enrolled for this study, six were either excluded and/or did not meet inclusion criteria. Forty-eight participants were randomly assigned into three groups to receive: 1) probiotics + vitamin B_3; 2) probiotics + vitamin B_3 placebo; or, 3) the placebos for the probiotic and vitamin B_3 for 12 weeks. Constipation, depression, anxiety, quality of life, mood, diet, and nutrition were assessed at the baseline, middle, and end of the supplementation period. Blood and stool samples were collected for blood chemistry and microbiome analyses, respectively. Next-generation sequencing of 16S rRNA genes (Illumina MiSeq) was used for gut microbiota analysis. Within-group and between-group differences were statistically analyzed, with significance set at $p<0.05$.

Results: The results showed improvements in constipation problems, quality-of-life scores, Movement Disorder Society- the Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Parkinson's Disease Questionnaire-39 (PDQ-39), decreased issues with communication via the PDQ-39 in probiotics and vitamin B_3 groups compared to the placebo group. Blood chemistry were within normal reference ranges. Supplementation did not change assessments of anxiety, depression, or mood. Gut microbiome analyses indicated significant differences in alpha and beta diversity, salient gut microbiome composition relating to different interventions, disease status, anxiety, and depression.

Conclusion: Probiotics and vitamin B_3 supplementation was beneficial for constipation symptoms, gut microbiome, and quality of life in these patients. Vitamin B_3 appeared to have a more stabilizing effect on the gut microbiome. Several differences were greater after 12 weeks compared with 6 weeks of the intervention. This appears to support that the duration of supplementation is greater than 6 weeks for most of the assessed outcome measures. For quality of life and mood measures, an increased duration of study and/or larger sample size may be necessary to detect differences.

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Dedication

I dedicate my dissertation work to my parents and sibling, who with unconditional love and support have accompanied me in this journey. A special feeling of gratitude to my family, friends, and those who have been my support in the difficult times.

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Chapter 1 - Gut Microbiota and the Gut-Brain Axis in Parkinson's Disease

Abstract

This review will discuss impacts of gut microbiome on motor, non-motor symptoms and quality of life of people living with Parkinson's disease. The role of the gut-brain axis (GBA) in health and disease, and neurological conditions specifically Parkinson's disease (PD) is explored. PD types, biological and psycho-social determinants are also reviewed. Different types of microbiome diversity, indices, and technologies, phylogenetic differences in those with and without PD, and main phyla and some highlights on specific genera and species are summarized. Probiotic supplement types, effective dosage, and consumer education are also covered.

Introduction

PD is a neurodegenerative disorder that mainly affects dopamine-producing ("dopaminergic") neurons in the brain, substantia nigra (Elkouzi, 2015). People with PD tend to experience: tremors, mainly at rest (pill rolling tremor in hands, other forms of tremor are possible); bradykinesia; limb rigidity; gait issues; and balance problems (Elkouzi, 2015).

One in seven Americans are 65 years old or over, i.e., 16% of the population in 2019, which is expected to reach 22% by 2050. PD is one of the two most common neurodegenerative diseases that cause severe disability and a significant long-term burden on communities and societies, and increased challenges to public health. Globally, disability and death due to Parkinson disease are increasing faster than for any other neurological disorder (World Health Organization, 2022). The incidence of PD increases with age. More than 10 million people

worldwide live with PD. There was a doubling in the number of patients with PD between 1990 and 2016, and the prevalence of PD is projected to increase 20% by 2050.

Ninety-eight percent of people with PD are affected by chronic constipation at some point of the disease (Haug, 2016) which negatively affects the absorption (Jin et al., 2020) of Parkinson's medication to reach maximum concentration, physical, psychological, and/or social aspects of life, and tends to decrease the quality of life (Kaye et al., 2006) (McClurg et al., 2016).

Likewise, research indicates, PD patients suffer from gut microbiome dysbiosis (Parashar & Udayabanu, 2017). Studies demonstrate that gut microbiome/flora and the GBA may impact neurologic, endocrine, and metabolic functions (Appleton, 2018). Beneficial impacts of probiotics were observed previously when studied in gastrointestinal problems, including diarrhea, *Clostridium difficile* infection, and disruption of gastrointestinal flora after prolonged antibiotic use (Harvard, 2020), therefore the author reviewed more on the potentials of probiotics use for the population with PD. According to the Academy of Nutrition and Dietetics (Klemm, 2020) and the Harvard Medical School (Harvard, 2020), probiotics are or resemble live cultures that are naturally found in the human gut. Probiotic supplementation has been observed to balance gut flora, increase immune system variables, and other health outcomes including prevention and treatment of gastrointestinal diseases (Harvard, 2020). Probiotics may ease constipation and improve bowel functions (Barichella et al., 2016) (Cassani et al., 2011) (Corliss, 2019). Bacterial cultures had been used in laboratories to produce levodopa (L-Dopa) (Surwase & Jadhav, 2011), the main medicine to manage PD symptoms. Medication costs an average of $2,500 annually and therapeutic surgery may cost $100,000 per person in the United States (Marras et al., 2018). Globally, there is limited access to L-Dopa in low-income countries, and it is often not subsidized by health-care systems or health insurance (World Health Organization,

2022). Limited accessibility to the most basic treatment with L-Dopa may undermine the quality of life of people living with Parkinson' disease (World Health Organization, 2022).

Potential benefits of probiotics have been seen in the treatment or prevention of diarrhea, irritable bowel syndrome (IBS), ulcerative colitis, Crohn's disease, H. pylori (the cause of ulcers), vaginal infections, urinary tract infections, recurrence of bladder cancer, infection of the digestive tract caused by Clostridium difficile, pouchitis (a possible side effect of surgery that removes the colon), eczema in children" (Harvard, 2018).

Gut microbiota may have effect on neuroendocrine metabolites and dopamine concentration through dopamine-producing enzymes (Aziz et al., 2013). Bacterial cultures had been used in laboratories to produce L-Dopa (Surwase & Jadhav, 2011). Probiotics have been studied in two previous clinical trials to improve constipation (Cassani et al., 2011) (Barichella et al., 2016). However, those studies did not investigate other aspects of gastrointestinal and neuro-endocrine functions, and gut microbiome. Likely, motor function will be impacted via neurological mechanisms that result from changes in nutritional metabolites, short-chain fatty acids (SCFA) and histone deacetylase inhibitor (HDACi) (Westfall et al., 2017). Regarding L-Dopa metabolism, Gut Microbiome affect ghrelin receptor activation, which stimulates tyrosine hydroxylase (Andrews et al., 2009). This is a key step in dopamine synthesis. Additionally, TH needs vitamin B_3 (niacin) for L-Dopa biosynthesis, thereby affecting L-Dopa metabolism (Nakashima et al., 1978).

Recently, the Academy of Nutrition and Dietetics requested controlled trials for the numerous proposed functions of probiotics to develop evidence-based dietetics practice guidelines (Garner et al., 2020) (Marcason, 2013). Dr. Rocca from the Division of Epidemiology, Department of Neurology, Mayo Clinic also resonated with the urgent need for

research focusing on identifying new preventive interventions and new treatments for people with PD (Rocca, 2018). The World Health Organization (WHO) (World Health Organization, 2022) stated that an urgent public health response is necessary to meet the health and social requirements of people with PD and improve functioning, and quality of life and prevent disability as global longevity increases.

Gut-dwelling bacteria keep pathogens (harmful microorganisms) in balanced, aid digestion and nutrient absorption, and contribute to immune function (Harvard, 2018). Probiotics are generally considered safe; they are already present in a normal digestive system (Harvard, 2018). Probiotics do not seem to have any side effects and are generally considered safe (Corliss, 2019).

Parkinson's Disease

PD is a neurodegenerative disorder that affects predominately dopamine-producing ("dopaminergic") neurons in a specific area of the brain called substantia nigra (Elkouzi, 2015). People with PD may experience these symptoms: tremor, mainly at rest (pill rolling tremor in hands, other forms of tremor are possible), bradykinesia, limb rigidity, gait and balance problems (Elkouzi, 2015). Most people living with Parkinson's are older than 65 and about 60% are male (Davis_Phinney_Foundation, 2017) .

Demographics/ Epidemiology of Parkinson's Disease

PD is the second most prevalent neurodegenerative disease worldwide (Bovolenta et al., 2017). It was estimated to have approximately 1 million people living with PD (PD) in the U.S. in 2020 (Marras et al., 2018) . Approximately 60,000 Americans are diagnosed with PD each year (Marras et al., 2018). More than 10 million people worldwide are living with PD (Marras et al., 2018). PD affects the ability to work, earn a salary, support themselves or their family compromising quality of life (Bovolenta et al., 2017).

Parkinson's disease types

There are three main types of PD: idiopathic, early-onset, and familial (Doherty, 2022). There are similar conditions that resemble signs and symptoms of PD but are caused by something else, such as a drug, stroke, or other neurological problems (Doherty, 2022).

Idiopathic, Early-onset or Yong-onset, and Familial types

Idiopathic PD is the most common. Exact cause is unknown. Etiology is likely the complex interaction of environmental and genetic factors (DHHS, 2012). This type occurs in people who apparently have no history of this disorder in their family (DHHS, 2012). The onset is generally between the ages of 55 to 65 and it rarely occurs before the age of 50 (Rizek et al., 2016). About 10% to 20% of people with PD occur symptoms before age 50, which is called young onset Parkinson's disease (YOPD) (The.Michaeal.J.Fox.Foundation, n.d.) . It is a rare type of PD, and studies use varying age cut-offs for YOPD/ Early Onset Parkinson's Disease (EOPD) between the age of 21 and 40 or 50 years (Doherty, 2022). YOPD/EOPD has social, societal, and personal consequences and may progress, with fewer comorbidities than typical, later-onset disease (Camerucci et al., 2021). Familial PD is seen in approximately 15% of people with Parkinson disease. Familial Parkinson disease can be caused by mutations in the LRRK2, PARK7, PINK1, PRKN, or SNCA gene, or in other genes that have not been identified (DHHS, 2012).

Biological and psycho-social factors

Etiology of PD is most likely a complex interaction of environmental and genetic factors (DHHS, 2012). The main risk factor is age, PD is seen in the age older than 60, about 5% to 10% experienced before the age of 50 (NIA, 2022).

Parkinson disease symptoms mainly occur when neurons in the substantia nigra degenerate or die (DHHS, 2012). These neurons produce the neurotransmitter, dopamine, which is required for communication in the brain for smooth physical movements (DHHS, 2012). When these dopamine-producing neurons are lost, the brain eventually becomes unable to

control muscle movement (DHHS, 2012). In Parkinson disease, unwanted proteins deposit in dead or dying dopamine-producing neurons (DHHS, 2012). It is unclear if these proteins kill the nerve cells, or they are part of the cells' response to the PD (DHHS, 2012).

PD is associated with several non-motor or non-movement issues including disorders of mood and affect, anhedonia and depression, cognitive dysfunction and hallucinosis, as well as complex behavioral disorders (Poewe, 2008). Autonomic nervous system (ANS) dysfunctions such as orthostatic hypotension, urogenital dysfunction and constipation are prevalent among majority of Parkinson's patients (Poewe, 2008).

PD also involves psychosocial factors and consequently affects the patient's emotional and communicative changes may elicit major disruptions to social functioning (Prenger et al., 2020). Problems in facial expressions, dysarthria, and recognizing the verbal and nonverbal emotional cues of others may result in severe negative social consequences. These outcomes may evoke feelings of stigma, dehumanization, social isolation, and loneliness, which might affect the patient's quality of life (Prenger et al., 2020).

Symptoms of Parkinson's disease

Table 1.1. Parkinson's disease symptoms*****

Motor symptoms	Non-motor symptoms
Tremor, bradykinesia, rigidity, postural instability	Cognitive impairment, bradyphrenia, tip-of-the-tongue (word finding) phenomenon
Hypomimia, dysarthria, dysphagia, sialorrhoea	Depression, apathy, anhedonia, fatigue, other behavioural and psychiatric problems

Motor symptoms	Non-motor symptoms
Decreased arm swing, shuffling gait, festination difficulty arising from chair, turning in bed	Sensory symptoms: anosmia, ageusia, pain (shoulder, back), paresthesias
Micrographia, cutting food, feeding, hygiene, slow activities of daily living	Dysautonomia (orthostatic hypotension, constipation, urinary and sexual dysfunction, abnormal sweating, seborrhoea), weight loss
Glabellar reflex, blepharospasm, dystonia, striatal deformity, scoliosis, camptocormia	Sleep disorders (REM behaviour disorder, vivid dreams, daytime drowsiness, sleep fragmentation, restless legs syndrome)

*(Jankovic, 2008)

Prognosis and natural history

Data on the short-term rate of progression of cardinal motor features from the placebo-controlled studies suggested aggressive progression of motor dysfunction in early PD with average declines relative to baseline of total UPDRS scores between 30 % and 40 % (Poewe, 2006). With such rates of decline untreated PD would inevitably lead to severe disability after less than 10 years of disease duration. Such projections are indeed consistent with data on the natural history of PD from the pre-levodopa era (Poewe, 2006). Disease progression to marked motor disability with loss of independent ambulation (stage 4) among untreated patients, using the Hoehn and Yahr staging system, was within 7.5 to 9.0 years after disease onset while latencies to stage 5, corresponding to a bedridden state were between 10 and 14 years (Poewe, 2006).

Over a 10-year time span of the drug-naïve Parkinson's patients, 60% of patients remained at the same HY stage and 40% progressed, with rigidity and postural instability scores

increasing significantly (Liu et al., 2015). The mean UPDRS motor score was 39. (Liu et al., 2015).

Healthcare costs related to Parkinson's Disease in the U.S.

The combined direct and indirect cost of Parkinson's, including treatment, social security payments and lost income, was estimated $52 billion per year in the United States (Marras et al., 2018). Medication alone was an average of $2,500 annually and therapeutic surgery was $100,000 per person (Marras et al., 2018).

The cost for a newly diagnosed patient was about $7500, and the cost for an ambulatory assistance device was about $51,000, and for a skilled nursing facility was $102,750 (Kaltenboeck et al., 2012). Hazard rates of mortality were higher among newly diagnosed PD. Medicare beneficiaries with PD have substantially and progressively higher costs and mortality compared with control group (Kaltenboeck et al., 2012).

The cost of early retirement associated with patients with PD was substantial (Johnson et al., 2011). Earnings losses due to changing careers or retiring earlier than expected were $US569 393, $US188 590, $US35 496 and $US2451 for those diagnosed with PD at age 45, 55, 65 and 75 years respectively (Johnson et al., 2011). As the proportion of Americans participating in the labour force into older age groups increases, PD-related early retirement costs are expected to rise (Johnson et al., 2011). Thus, PD not only causes significant economic burden to the patient and caregiver, and the disease significantly reduces the patient's quality of life and those of their families and/or caregivers (Findley, 2007).

Gut-Brain Axis

Roles played in health and disease

The GBA is the physical and chemical networks connecting the gut with the brain. This is a bidirectional communication between the enteric and central nervous systems (CNS) and involves physical and anatomical, neural, endocrine, metabolic, humoral and immune related communications (Appleton, 2018). The ANS, hypothalamic-pituitary-adrenal (HPA) axis, and enteric nervous system (ENS), all link the gut with the brain, which may bidirectionally influence intestinal activities, mood, cognition, and mental health (Appleton, 2018).

Clinical and experimental studies elucidated that gut microbiota have important impacts on the GBA. This interaction is local with intestinal cells and the ENS, and direct interaction with the CNS through neuroendocrine and metabolic pathways (Carabotti et al., 2015). In an animal model study, feeding mice with probiotic bacteria *Lactobacillus. rhamnosus* (JB-1), resulted in reduced stress-induced hormone in blood and reduced anxiety- and depression-related behavior (Bravo et al., 2011). However, when the vagus nerve was cut, previously observed probiotics effects on neurochemical and behavioral effects were not observed, which implied a role of gut microbiome and the communication via GBA (Bravo et al., 2011).

The microbiota- GBA was further depicted in another animal model study. The animals were exposed to probiotic bacteria *Lactobacillus rhamnosus*, which beneficially altered the brain-derived neurotrophic factor (BDNF) and of genes involved in serotonin signaling and metabolism in zebrafish (Borrelli et al., 2016).

Gut microbes produce SCFAs such as butyrate, propionate, and acetoacetate (Ríos-Covián et al., 2016). The SCFAs impact on gut physiological effects, shape the gut environment, and influence the physiology of the colon (Ríos-Covián et al., 2016). In a randomized trial,

consuming propionate significantly reduced high energy food appeal, and reduced energy intake (Byrne et al., 2016). One important mechanism in microbiota, GBA is leaky gut under stressed or other pathological conditions (Hultman et al., 2015). Gut inner layer is lined by epithelial cells connected by tight junctions forming a barrier sealing off the gut contents to the rest of the body (Hultman et al., 2015). Also, an increase in the order Bacteroidales and decrease in Clostridiales have been shown to correlate with gut permeability in mice (Hultman et al., 2015). The gut mucosal barrier contains dendritic cells and macrophages, and its toll-like receptors (TLRs) differentiate between normal flora and pathogenic microorganisms, to initiate inflammatory and immune responses (Hultman et al., 2015). In the presence of psychosocial stressors, tight junctions become leaky to bacteria and bacterial secretions resulting systemic immune response (Hultman et al., 2015). When immune response is initiated, the subsequent release of cytokines, such as interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF)-α, can profoundly impact on brain functions, hippocampus, hypothalamus, prefrontal cortex, and amygdala leading to psychiatric disruption such as major depressive disorder (MDD) (Hultman et al., 2015) . Studies in animal models and humans indicated a persistent imbalance of gut's microbial community, named dysbiosis, relates to CNS disorders, obesity, cancer, diabetes, cardiovascular disease, inflammatory bowel diseases (IBD), and IBS (Belizário & Faintuch, 2018).

Gut Microbiota

Enterotype

The concept of enterotypes was suggested in 2011(Arumugam et al., 2011), that human microbiome can be stratified into three microbiome categories which was mainly associated with their long-term diet. The enterotypes included Bacteroides (enterotype1), Prevotella (enterotype

2), and Ruminococcus (enterotype3) (M. Cheng & Ning, 2019). Various factors influence enterotypes, such as diet, antibiotics, and age (M. Cheng & Ning, 2019). Although later studies questioned the concept of enterotype whether human gut microbiome can be clustered into different types or they are just a continuous gradient, it is actually pragmatic to collapse the microbiome into a few categories given the extremely complexed nature of human gut microbiota (M. Cheng & Ning, 2019). The proper categorization could help to explore the correlations between gut microbiota and diseases to enable precision medicine based on gut microbiota (M. Cheng & Ning, 2019).

Microbiota changes in neurodegenerative diseases

Age related gut microbiota changes in neurodegenerative disease are an increase in Proteobacteria spp. with a decrease in *Bifidobacteria* spp., a reduction in butyrate-producing species (*Ruminococcus* spp., *Faecalibacterium* spp., etc.) and an increase in microbiota that can stimulate an inflammatory response (*Escherichia* spp., *Enterobacteriaceae* spp., *Bacteroides* spp., *Clostridium difficile*, etc.)(Westfall et al., 2017), (Claesson et al., 2011), (Biagi et al., 2010), (J. Cheng et al., 2013). Proteobacteria are known as proinflammatory bacteria (Keshavarzian et al., 2015), so increased abundance of Proteobacteria spp may contribute to inflammatory responses.

Aging is the main unmodifiable risk factor for the development of PD in association with neuroinflammation, which is the breakdown of homeostatic mechanisms that protect against protein misfolding, oxidative stress, decreased mitochondrial function (Santos et al., 2019). Thus, decreasing neuroinflammation during this aging process has been shown to be neuroprotective to PD in animal models (Santos et al., 2019).

The gut is one of the main gateways to environmental exposure to the brain, the microbiome has a protective effect mediating the environmental exposure (Santos et al., 2019). Microbiota dysbiosis is a pivotal risk factor for PD and other neurological disorders (Santos et al., 2019). Maintaining a healthy microbiome throughout the lifetime can potentially decrease the risk of developing PD and other neurodegenerative diseases (Santos et al., 2019). The widespread use of antibiotics can kill gut bacteria indiscriminately, and consequently cause a shift of the microbiome to an alternative stable state with unknown consequences in the long term (Santos et al., 2019).

Specifics to neurological conditions, including Parkinson's disease

The gastrointestinal tract (GIT) connects with the CNS through the GBA for neuronal development and maintenance (Westfall et al., 2017). The main pathways of the GBA are (a) direct neuronal communication (b) endocrine signaling mediators and (c) the immune system (Westfall et al., 2017). The microbiota communicates to the host through its microbial metabolites, biochemical and functional links establishing host homeostasis and health, and gut dysbiosis manifests in neurological disease (Westfall et al., 2017). The nervous, endocrine, and immune systems create a highly integrated molecular communication network along the GBA, that link up systemic imbalances with the development of neurodegeneration including insulin regulation, fat metabolism, oxidative markers and immune signaling (Westfall et al., 2017). Dysregulation of GBA network has been associated with depression, anxiety, autism, and neurodegenerative diseases such as PD, and Alzheimer's disease (Sampson et al., 2016) (T. Zhang et al., 2018), and metabolic syndrome (de Lartigue et al., 2011).

Microbiota-Gut-Brain Axis and Parkinson's Disease

The GBA communicates intestinal cells and the ENS and the CNS through neuroendocrine and metabolic pathways. The gut microbiome can directly communicate the brain through the pathway of vagus nerve which innervates the intestine and proximal colon (Bullich et al., 2019). The gut microbiota influences the ENS function when the microbiota locally produces neurotransmitters such as γ-aminobutyric acid (GABA), serotonin, catecholamines- dopamine and norepinephrine, melatonin, and fatty-acid derivatives acetylcholine in the gut lumen (Ambrosini et al., 2019). The ENS is also affected by bacterial metabolites, SCFAs such as acetic acid, butyric acid, and propionic acid, which can stimulate the sympathetic nervous system (Ambrosini et al., 2019).

The abnormal accumulation of α-synuclein protein is recognized as one of the important etiological factors of PD (Ambrosini et al., 2019). Alpha- synuclein protein can also be detected in intestinal submucosal neuronal structures in apparently neurologically healthy people (Ambrosini et al., 2019). Alpha- synuclein protein is highly soluble and regulates the presynaptic release of dopamine (Ambrosini et al., 2019). Intestinal α-synuclein protein precedes sufficient CNS neurodegeneration to show symptoms of motor dysfunctions (Ambrosini et al., 2019). Increased intestinal permeability (leaky gut) was shown in PD patients compared to healthy controls (Ambrosini et al., 2019). In this case, the metabolite of gut microbiota, butyrate, one of the SCFA, promotes the development of the intestinal barrier (Peng et al., 2009). The spread of α-synuclein protein from ENS to the CNS by transsynaptic transmission in both sympathetic and parasympathetic nervous systems is a foundational concept in PD pathophysiology (Danzer et al., 2012). Clinical gastrointestinal signs and symptoms of ENS pathology such as constipation,

the pre-motor symptom of PD often occur years before the patients present with CNS degeneration, PD, signs and symptoms (X. Gao et al., 2011) (Lesser, 2002).

Depending on the gut microbiome composition, diverse cytokines are released resulting in differing cytokine levels with more pro-inflammatory or more anti-inflammatory characteristics (Bullich et al., 2019). Cytokines released from the enteric mucosal immune cells or microbiome, such as lipopolysaccharide (LPS), can reach the CNS via either the ENS-vagal–mediated route or the bloodstream (Bullich et al., 2019).

Alpha diversity is "Species richness within a fine and homogeneous extent or scope" and beta diversity means "the extent of change in species composition among different communities in a landscape" (Moreno & Rodríguez, 2010). Studies indicated differences in microbiota between established PD and control groups (Tremlett et al., 2017). Gerhardt et. al. stated that the majority of the publications reported significant beta-diversity differences between healthy controls and PD, but alpha-diversity was usually not significantly different when it was reported in the study (Gerhardt & Mohajeri, 2018). Scheperjans et.al. reported changes in the microbiome beta diversity, but not alpha diversity, and decreased *Prevotellaceae* family relative abundance in the feces of patients with PD compared to the control group (Scheperjans et al., 2015). However, significant (or extreme) dietary pattern changes may also alter the microbiota functional and metabolic profiles (David et al., 2014).

Parkinson's disease and human gut microbiota

Human gut microbiota associated with Parkinson's disease

Classically, PD is understood as a neurodegenerative disease and was not considered to be associated with the microbiome because advanced technology and sequencing was discovered

recently. So, there were few classic perspectives of gut microbiome associated with PD. However, research revealed the link of changes in the gut microbiota with neurodegenerative diseases. Studies showed linking pathways of the gut microbiota (neurological, endocrine and immune) to the pathogenesis of neurodegeneration including PD, Amyloid lateral sclerosis, and Alzheimer's disease(Westfall et al., 2017).

Findings from the human microbiome project in 2012 revealed that a given body site has relatively stable functional profiles although there may be interindividual variation of human microbiota (The Human Microbiome, Project Consortium et al., 2012). A human has 100 times more microbial genes than human genes (Qin et al., 2010). The human gut has 10 trillion cells of 1000 different microbial species, that comprises the largest part of humans' microbiota (Sender, Fuchs, & Milo, 2016).

A germ-free mice model was studied to better understand the influence and associations of gut microbiome and PD. Alpha synuclein overexpressing mice (ASOM) raised in a germ-free environment barely developed PD related pathological changes, specifically: alpha synuclein deposition, neuro inflammation, and motor dysfunction (Scheperjans et al., 2015). When feces from wild-type mice are transplanted or bacterial metabolites (short chain fatty acids) are fed to these ASOM, the effect was reversed (Sampson et al., 2016). When ASOM were colonized with feces from human with PD, the motor symptoms deteriorated compared to those colonized with feces from healthy humans (Sampson et al., 2016). After antibiotics were administered to young ASOM depleted microbiota, they apparently were prevented from development of Parkinsonian symptoms and microglia activation (Tremlett et al., 2017).

Sensitivity and specificity of microbiome analysis

Low level of *Prevotellaeceae* was quite sensitive (86.1%) but low specific (38.9%) for PD (Scheperjans et al., 2015). When PD common symptom- the degree of constipation- is combined with the abundance of *Prevotellaceae*, *Lactobacillaceae, Bradyrhyizobiaceae,* and *Clostridiales* IV, the abundance could be able to identify PD cases with increased specificity (90.3%) but lower sensitivity (66.7%) (Scheperjans et al., 2015). The sensitivity of a test is "its ability to determine the patient cases correctly. Sensitivity is the proportion of true positive in-patient cases. Mathematically, Sensitivity=True Positive / (True Positive + False Negative)"(Baratloo, Hosseini, Negida, & El Ashal, 2015). The specificity of a test is its ability to determine the healthy cases correctly. Specificity is the proportion of true negative in healthy cases. Mathematically, Specificity=True Negative /(True Negative + False Positive) (Baratloo et al., 2015). Relative higher abundance of *Enterobacteriaceae* is associated with the postural instability, and gait symptoms (Scheperjans et al., 2015).

Changes in main phylae

Gerhardt et.al reported significant changes in relative abundances within five phylae: Firmicutes, Actinobacteria, Bacteroides, Verrucomicrobia and Proteobacteria (Gerhardt & Mohajeri, 2018). An increase in *Lactobacillus, Bifidobacterium,* Verrucomicrobiaceae and *Akkermansia,* and a decrease in *Faecalibacterium* spp., *Coprococcus* spp., *Blautia* spp., *Prevotella* spp. and *Prevoteallaceae* were observed in PD (Gerhardt & Mohajeri, 2018).

Bifidobacterium

Bifidobacteriaceae was found consistently increased in the patients with PD and so similar changes were observed in any other neurodegenerative diseases (Gerhardt & Mohajeri, 2018). However, in the two-year follow up study, decreased abundance of *Bifidobacterium* was observed, and it also correlated with the Unified PD rating scale I (UPDRS I) score (Gerhardt & Mohajeri, 2018). So *Bifidobacteriaceae* in PD was not considered as harmful to the patients and more likely to be helpful against the progress in neurodegeneration (Gerhardt & Mohajeri, 2018). Therefore, *Bifidobacterium* was considered for a probiotic intervention to prevent the progress of the PD into severe stages (Gerhardt & Mohajeri, 2018).

Akkermansia

Akkermansia muciniphila has beneficial barrier function of the intestinal mucosal layer (Bedarf et al., 2017). Contrarily, *Akkermansia* consumes intestinal mucus as energy source, degrades the mucus layer, exposes microbial antigens to immune cells leading to the inflammatory responses (Forsyth et al., 2011). Colonic abundance of *Akkermansia* and PD negatively correlated to body mass index (BMI) (Derrien et al., 2008), i.e. weight loss in people with PD. Weight loss in PD correlates with worsened health related quality of life(Akbar et al., 2015), and has negative impact on the disease severity and mortality indicative of disease progression (Sharma & Lewis, 2017).

F/B ratio

The Firmicutes:Bacteroidetes ratio of the human microbiota changes with age, infants, adults and elderly individuals have Firmicute: Bacteroidetes ratios of 0.4, 10.9 and 0.6, respectively (Mariat et al., 2009), and decreased ratio of Firmicutes: Bacteroidetes with age in

older adults was considered to associate with neurodegeneration (Gerhardt & Mohajeri, 2018). Firmicutes: Bacteroidetes ratio was found to associate with obesity as well (Gerhardt & Mohajeri, 2018).

The increased F/B ratio was observed in IBS, liver cirrhosis patients than in healthy controls; and decreased F/B ratio with reduced microbial diversity in patients with heart failure (HF) (S. Wei et al., 2021).

Prevotella

Within the phylum of Bacteroidetes, *Prevotella* is a well-known genus. Increased relative abundances of *Prevotellaceae, Prevotella copri* was observed more in healthy control compared to patients with PD (Gerhardt & Mohajeri, 2018). *Prevotella* species (*Prevotella copri*) were decreased in all neurodegenerative diseases, except Alzheimer disease (Gerhardt & Mohajeri, 2018). Reduction in *Prevotellaceae* is important because they are one of the main producers of mucin, a highly glycosylated protein that protects against invading pathogens by building a mucin barrier along the epithelial wall (Westfall et al., 2017).

In majority of PD cases, decreased in *Prevotellaceae* was seen with other symptoms such as idiopathic rapid eye movement behavioral sleep disorder that occured before PD was diagnosed. Therefore, hypothesis from the studies was *Prevotella* might only be changed in the early stages of PD, and a decrease of *Prevotella* might contribute to the onset of PD symptoms (Heintz-Buschart et al., 2018).

Prevotella grows best on carbohydrates, dietary fiber provides a competitive advantage to *Bifidobacteria*, and Bacteroidetes has a substrate preference for certain fats (Alcock, Maley, & Aktipis, 2014). Some microbes have specialized function, for instance *Akkermansia mucinophila*

degrades mucin lives on carbohydrates, and butyrate producing microbes, *Roseburia* spp., thrive on polysaccharide in the diet (Alcock et al., 2014).

Prevotella is more common in people who eat plant-rich diet (Ley, 2016). In Western populations *Prevotella* has also been associated with vegetarian or Mediterranean diets rich in fruits and vegetables (Ley, 2016). *Prevotella copri* is deficient in the ability to degrade host glycans and is more genetically equipped to degrade plant glycan (Ley, 2016).

Table 1.2. Definitions

Microbial Functional Diversity	"Functional diversity is a component of biodiversity that generally concerns the range of things that organisms do in communities and ecosystems" (Petchey & Gaston, 2006).
Microbial Functional redundancy	"The ability of one microbial taxon to carry out a process at the same rate as another under the same environmental conditions" (Allison & Martiny, 2008). The state that members of the microbial community have similar functional niches and can substitute for one another (Lozupone, 2012).
Microbial Functional similarity	"The ability of two microbial communities to carry out a functional process at a similar rate, regardless of differences in composition" (Allison & Martiny, 2008).
Functional group	"All organisms that directly contribute to the rate of a particular functional process in an ecosystem" (Allison & Martiny, 2008).
Microbial Phylotypic diversity	It is the diversity of microbial phylotypes. "In microbiology, a phylotype is an environmental DNA sequence or group of sequences sharing more than an arbitrarily chosen level of similarity of a particular gene marker. The most widely used phylogenetic marker is the small subunit ribosomal RNA gene. Two prokaryotic sequences are generally considered as belonging to the same phylotype when they are more than 97–98 % identical (for eukaryotes, the values generally used are in the 98–99 % nucleotide identity range). In prokaryotic microbiology, phylotypes, often referred to as Operational Taxonomic Units (OTUs), are a proxy for species" (Moreira & Lopez-Garcia, 2014).

The measurement of each of the aspects of microbial diversity

Table 3.1.3. The measurement of each of the aspects of microbial diversity†

Measurement technology/ technique	Method (-Omics)	Measurement aspects
16S rRNA genes • Pyrotagged sequencing for community composition	Microbiome analysis	Microbial community composition
Extract DNA • Shotgun metagenome sequencing for genetic potential	Metagenome	Microbial Gene composition (including functional genes) - Metagenomic data shows microbiome functional potential
Extract RNA • RNA Seq for transcripts	Meta-transcriptome	Microbial expressed genes
Extract Protein • Shotgun proteomics	Meta-proteome	Proteins produced
Extract metabolites • Measurement of metabolites or activities • System biology to integrate omics data	Metabolome	Metabolites produced

† (Zeglin, 2018)

Measuring microbial phylotypic diversity, functional diversity

Community fingerprint analyses, clone libraries, qPCR, 454 pyrosequencing, and phylogenetic and diversity statistics are some measurement methods of modern or post PCR techniques (Zeglin, 2018). Some pre-PCR techniques are biomass, CLPP (Caseinolytic mitochondrial matrix peptidase proteolytic subunit), extracellular enzyme, Phospholipid Fatty Acid Analysis (PLFA), and culture-based methods (Zeglin, 2018).

Quantification of molecules from microbial habitat

DNA is useful to look into the presence or abundance of organisms – population size, diversity, RNA is the first step in gene expression, and proteins are used as functional molecules in quantification of molecules from microbial habitat.

Analysis

Microscopy or fluorescence in situ hybridization (FISH) methods are used for analysis within the sample matrix; extraction and PCR amplification is conducted to analyze diversity and community composition (Zeglin, 2018). For direct analysis after extraction from sample Phospholipid Fatty Acid Analysis (PLFA), enzyme assays, microarrays, meta-omics methods are used (Zeglin, 2018).

Measuring population phylotypic diversity

The following methods are used to measure population phylotypic diversity (a) sequence abundance or relative abundance (b) sample richness (c) within sample alpha diversity – Shannon Index (d) between sample beta diversity - principal component analysis (PCoA), Bray-Curtis, Unifrac, Jaccard index, and the Aitchison distance.

For alpha diversity, the number (richness) and distribution (evenness) of taxa expected within a single population— the Chao1, Abundance-based Coverage Estimator (ACE), and Jackknife measures are calculated (X. C. Morgan & Huttenhower, 2012). Collector's or rarefaction curves— increasing numbers of sequenced taxa allow increasingly precise estimates of total population diversity (X. C. Morgan & Huttenhower, 2012). Beta diversity compares multiple populations, absolute or relative overlapping and how many taxa are shared between

them (X. C. Morgan & Huttenhower, 2012). An alpha diversity is a summary statistic of a single population, while a beta diversity is a similarity score between populations, analysis by sample clustering or by dimensionality reductions such as PCA (X. C. Morgan & Huttenhower, 2012). Alpha diversity is often quantified by the Shannon Index, or the Simpson Index (X. C. Morgan & Huttenhower, 2012). Beta diversity can be measured by simple taxa overlap or quantified by the Bray-Curtis dissimilarity (X. C. Morgan & Huttenhower, 2012).

Principal Component Analysis (PCA) is clustering and finding patterns without reference to prior knowledge about whether the samples come from different treatment groups or have phenotypic differences (Lever et al., 2017). Principal components (PCs) are considered geometrically orthogonal (Lever et al., 2017). The PC selection process has the effect of maximizing the correlation (r^2) between data and their projection and is equivalent to carrying out multiple linear regression on the projected data against each variable of the original data (Lever et al., 2017).

Summary of measurement of microbial phylotypic diversity, microbial functional diversity, and microbial functional redundancy†

- Small subunit (SSU) ribosomal RNA targeted gene sequencing [16S rRNA]
- Cultured isolates from fecal samples undergo genome sequencing (Genome sequences relate functions to species)
- Shotgun metagenomic sequencing

Targeted sequencing of phylogenetically informative genes (SSU ribosomal RNA) or random sequencing of all genes (shotgun metagenomic sequencing) can be used to assess DNA extracted from fecal samples. Genome sequences from cultured isolates link the two data sets by

indicating which species contain which genes, and therefore functions †(Lozupone, 2012). Small subunit ribosomal RNA (SSU rRNA) gene sequences are related to each other in the form of phylogenetic trees because related phylotypes (clusters of similar sequences defined by sequence similarity) generally have more similar functional attributes (Lozupone, 2012). Genes that encode proteins that perform known enzymatic reactions are related using metabolic networks, because genes that participate in the same metabolic pathway can work together to produce a phenotype (Lozupone, 2012).

Combining analysis results from targeted and shotgun sequencing data from the same samples, and sometimes using genome sequences to relate the two, can provide the interpretation on whether there is functional redundancy, or whether functional genes have considerable phylogenetic signal (Lozupone, 2012). If additional taxa (compositional diversity) do not increase the number of functions (functional diversity), there is "functional redundancy" (Lozupone, 2012). Combining targeted sequencing with mRNA, protein and metabolite level analyses will enable direct measurements of expressed community properties, OTU, operational taxonomic unit (Lozupone, 2012).

Measurement of functional diversity/ redundancy

Metabolomics technologies enabled the deeper characterization of the molecular mechanisms and pathways underlying the ecological assembly and their associated host and microbial phenotypes (Shafquat et al., 2014).

Measuring functional diversity (Petchey & Gaston, 2006) requires each of the following:

(a) Appropriate functional information (traits) about organisms to be included in the measure, and irrelevant information to be excluded (what functional traits should be included?).

(b) Traits to be weighted according to their relative functional importance

(c) The statistical measure of trait diversity to have desirable mathematical characteristics. (Discontinuous vs. continuous measures of diversity)

(d) The measure to be able to explain and predict variation in ecosystem level processes.

Microbial functional diversity

Non-Parkinson's condition

Microbial functional diversity across different body sites involves in the biosynthesis of compounds for the human host, such as essential amino acids (histidine, tyrosine, phenylalanine, methionine, arginine, and lysine); vitamins such as biotin and thiamine; and other families of bioactives such as isoprenoids to contribute to the host health (Shafquat et al., 2014). The microbiome also involves in the uptake of several compounds including metals (nickel and cobalt), polyamines (spermidine and putrescine), and the production of LPS (Shafquat et al., 2014). When the functional diversity of microbiome is disrupted (due to dybiosis), the ecological changes impact the corresponding overall functions of the host that may lead to the diseased states.

In each environment, groups of functionally similar microbes associate with enriched metabolic pathways (Shafquat et al., 2014). They can be within but sometimes spanning phylogenetic groups (Shafquat et al., 2014). As our understanding of uncharacterized microbial genes and pathways in the human microbiome improves, the lists of typical microbial processes within each habitat is expanding (Shafquat et al., 2014).

A few had been discovered about the phylogeny of human-associated microbes linked to their functional roles, and many things remained to study in this area (Shafquat et al., 2014).

Metabolomics technologies enabled the deeper characterization of the molecular mechanisms and pathways underlying the ecological assembly and their associated host and microbial phenotypes (Shafquat et al., 2014).

Parkinson's disease condition

Studies indicated the pathogenesis of PD may have originated in the gastrointestinal tract in association with microbiota. Deposition of alpha-synuclein within the nervous system of gastrointestinal tract and olfactory bulb may be the origin of PD (Braak et al., 2003). The GBA may involve in alpha synuclein-mediated pathogenesis of PD that spreads from the gut to the brain(Holmqvist et al., 2014). GBA concept was supported by further evidence that truncal vagotomy decreased the risk of PD (Liu et al., 2017). The gut or olfactory bulb are very closely located to the mucosal surfaces which have high populations of microbes (Braak et al., 2003). Premotor symptoms such as hyposmia, anosmia, or constipation, may occur many years before the motor symptoms develop in patients with PD (Goldman & Postuma, 2014). Healthy human intestinal barrier function is required to keep neurological health (Clairembault et al., 2015).

Germ-free mice model was studied to better understand the influence and associations of gut microbiome and PD. Alpha synuclein overexpressing mice (ASOM) raised in a germ-free environment barely developed PD related pathological changes: alpha synuclein deposition, neuro inflammation, and motor dysfunction (Scheperjans et al., 2015). When feces from wild-type mice were transplanted or bacterial metabolites (short chain fatty acids) were fed to these ASOM, the effect was reversed (Sampson et al., 2016). When ASOM were colonized with feces from human with PD, the motor symptoms got worse compared to those colonized with feces

from healthy humans. After antibiotics were administered to young ASOM depleted microbiota, they did not develop Parkinsonian symptoms and microglia activation (Tremlett et al., 2017).

Human gut microbiome communicates to the CNS through "gut-brain-axis" by using neuronal factor, endocrine pathways and immune (inflammatory) functions. PD disease duration and abundance of Firmicutes were negatively correlated (Keshavarzian et al., 2015). PD medication with Catechol-O-Methyl-Transferase inhibitors (COMTi) such as entacapone was inversely correlated with abundance of Firmicutes (Unger et al., 2016). It was considered that medication and disease duration were important factors for the gut microbiota composition. And patients with PD who lost their body weights were associated with negative prognosis. Firmicutes is the main bacterial phylum that includes *Lactobacillus, Streptococcus, Mycoplasma, and Clostridium* which can produce SCFAs such as butyrate (Marciano & Vajro, 2017).

Fecal SCFA concentrations were significantly reduced in PD patients compared to controls (Unger et al., 2016). Theoretically, the reduction in SCFA may bring alterations in the ENS and contribute to gastrointestinal dysmotility in PD (Unger et al., 2016). Reduction in butyrate (SCFA) is important for PD patients because sodium butyrate is a HDACi that protects dopaminergic neurons from degeneration by upregulating neurotrophic factors including BDNF and glial cell line-derived neurotrophic factor (GDNF) (Westfall et al., 2017). These findings elucidated potential benefits of rebalancing of *Firmicutes:Bacteroides* ratio in elderly or individuals with PD and having low *Firmicute: Bacteroides* ratio.

The gut microbiota was found to have significant impact on the brain and to affect behavior (anxiety, depression, learning and memory, sociability), microglial activity, BBB integrity, neurogenesis, and neurotransmitter production (Westfall et al., 2017), (Luczynski et al., 2016).

Ghrelin hormone has a protective effect in normal nigrostriatal dopamine function (Andrews et al., 2009). People with PD who had altered ghrelin secretion or decreased ghrelin level were found to have increased abundance of *Lactobacillaceae* and decreased abundance of *Prevotella* (Unger et al., 2016). Unger et.al 2016 reported the bacterial phylum Bacteroidetes and the bacterial family *Prevotellaceae* were reduced, *Enterobacteriaceae* were more abundant in fecal samples from PD patients compared to matched controls (Unger et al., 2016). Constipation was very common among the patients with PD and *Lactobacillus* was positively correlated with constipation type of IBS, and negatively correlated with diarrhea-type IBS (Calkwood et al., 2016). Increased abundance of *Lactobacillus* was seen in 3 out of 10 PD studies (Gerhardt & Mohajeri, 2018). Linking with previous finding on *Firmicutes:Bacterioides* ratio, it looks as if Lactobacillus (Phylum=Firmicutes) may be desirable however too much increased relative abundance of *Lactobacillaceae* over *Prevotella* may result untoward impact on constipation.

Gut microbiota consists of a diverse community of bacterial species, but there are two main phyla – *Firmicutes* (51%) and *Bacteroidetes* (48%) and the rest (1%) includes less populous phyla- *Proteobacteria, Actinobacteria, Fusobacteria, Spirochaetes, Verrucomicrobia* and *Lentisphaerae* (Westfall et al., 2017). *Firmicutes* includes the well-known genus *Lactobacillus, Bacteroidetes* includes well-known genera *Bacteroides* and *Prevotella*, and *Actinobacteria* includes the well-known genus *Bifidobacteria* (Westfall et al., 2017).

Despite high inter-individual variations of gut microbiota, the core gut microbiota can conserve overall physiological functionality (Westfall et al., 2017). Corrective interventions of disrupted microbiota may provide safe and effective treatments to slow or halt the progression of often debilitating motor symptoms of PD (Sampson et al., 2016). Studies indicated that the effect of certain probiotics with *Bifidobacterium spp.*, *Bacillus* spp., *Lactobacillus* spp., *Escherichia*

spp., *Streptococcus* spp., and *Clostridium* spp. are modulatory on dopamine level (Westfall et al., 2017). Dopamine and its precursor L-Dopa levels affect the motor symptoms of the patients with PD.

The probiotic study found absence of adverse events in young and elderly subjects in the study who ingested probiotic supplementations containing *Lactobacillus rhamnosus* GG (LGG) alone, or LGG in combination with *Lactobacillus rhamnosus* Lc705, *Propionibacterium freudenreichii* JS, *Bifidobacterium breve* 99, or *Bifidobacterium lactis* BB12 (Tapiovaara et al., 2016).

Microbial functional redundancy

Functional response diversity is the degree of the microbial species' sensitivity variation in response of the changes in the ecosystem to the same ecosystem function (Lozupone, 2012). High functional response diversity may allow a species that is relatively rare but functionally similar to fill a niche when an abundant species is compromised by an environmental disturbance (Lozupone, 2012).

Due to antibiotic treatment, a previously rare human gut microbe may increase in abundance to fill the position of dominant gut microbe that have higher antibiotic sensitivity (Lozupone, 2012). So, as a whole ecosystem, the gut microbiome appears the same stable state but there is a decreased resilience due to the decrease in functional redundancy (Lozupone, 2012).

Human gut microbes are likely to have high functional response diversity because phylogenetically disparate microbes often perform similar metabolic functions (Lozupone, 2012). Methanogenic Archaea, sulphate-reducing bacteria and phylogenetically diverse

acetogens in humans and mice can all consume hydrogen made by other microbes during fermentation (Lozupone, 2012). Clostridiales order, butyrate producers, have different ecological strategies, such as adaptation to different stages of community succession, oxygen tolerance and substrate preference (Lozupone, 2012). Infants have highest abundance of *Anaerostipes caccae*, whereas *Eubacterium hallii* and *R. intestinalis* are more abundant in adults(Lozupone, 2012). *A. caccae* survives 10–60-minute periods in the air better than *E. hallii* or *R. intestinalis*. *E. hallii* but not *F. prausnitzii* can use lactate as a substrate(Lozupone, 2012). Thus, butyrate production in the human gut can continue despite different successional and metabolic states (Lozupone, 2012).

Regarding functional redundancy, Bradley et. al. hypothesized that healthy human microbiome appears functionally redundant due to factors that obscure differences in gene abundance between individuals (Bradley & Pollard, 2017). Study result showed that housekeeping pathways contained a mix of variable and invariable genes, though most highly conserved genes were significantly invariable. Interestingly, variable genes tended to be associated with *Proteobacteria*, as opposed to taxa used to define enterotypes or the dominant phyla Bacteroidetes and Firmicutes (Bradley & Pollard, 2017). The researchers concluded the results indicated the limits on functional redundancy and predicted specific genes and taxa that could explain physiological differences between human gut microbiome (Bradley & Pollard, 2017). Studies or reviews on the microbial functional redundancy specific to PD does not prevail currently.

Microbial phylotypic diversity

In those with and without Parkinson's disease conditions

Studies have documented differences in microbiota between established PD and control groups (Tremlett et al., 2017). Gerhardt et. al. stated that the majority of the publications reported significant beta-diversity differences between healthy control and PD, but alpha-diversity was usually not significantly different (Gerhardt & Mohajeri, 2018). Scheperjans et.al. reported changes in the microbiome beta diversity, but not alpha diversity, and decreased *Prevotellaceae* family relative abundance in the feces of patients with PD compared to the control group (Scheperjans et al., 2015). Alpha diversity is "Species richness within a fine and homogeneous extent or scope" and beta diversity means "the extent of change in species composition among different communities in a landscape" (Moreno & Rodríguez, 2010). However, significant or extreme dietary pattern changes may also alter the microbiota functional and metabolic profiles (David et al., 2014).

Gerhardt et.al reported significant changes in relative abundances within five phylae: Firmicutes, Actinobacteria, Bacteroides, Verrucomicrobia and Proteobacteria (Gerhardt & Mohajeri, 2018). An increase in *Lactobacillus*, *Bifidobacterium*, Verrucomicrobiaceae and *Akkermansia*, and a decrease in *Faecalibacterium* spp., *Coprococcus* spp., *Blautia* spp., *Prevotella* spp. and *Prevoteallaceae* are observed in PD (Gerhardt & Mohajeri, 2018).

Firmicutes

Studies found most changes in relative abundance of colonic microbial taxa within Firmicutes phylum. Firmicutes represents majority (approximately 51%) of human gut microbiome. Gerhardt's report is re-summarized in the below table to illustrate (a) the only

microbiota taxa that had congruent findings among the 10 reported studies for PD, and (b) those have changes on the genus and species levels of the stated taxa in PD compared to Healthy control (HC). *Roseburia, Coprococcus eutactus, Blautia glucerasea*, *Faecalibacterium prausnitzi* and *Clostridium coccoides* were most found as higher abundance in PD.

Table 1.4. *Firmicute* - significant changes in healthy control (HC) Vs. Parkinson's disease (PD)

#	Taxa	Congruent # of reported # studies out of all 10 studies	Higher abundance in healthy control (HC)	Lower abundance in healthy control (HC)	+ indicates a change in Parkinson's Disease (PD) compared to HC on the genus and species levels
1	*Lactobacillus mucosae, gassero, caseo, fermentum, reuteri, ruminis*	3		X	+
2	*Roseburia*	2	X		+
3	*Coprococcus eutactus*	3	X		+
4	*Blautia glucerasea*	4	X		+
5	*Dorea longicatea*	1	X		+
6	*Catabacteriaceae Catabacter, honkongenesis*	1		X	+
7	*Christensenella minuta*	1		X	+

Unlike table 1.4, table 1.5 is re-summarized to illustrate (a) the only microbiota taxa that had congruent findings among the reported studies out of all 10 studies reviewed by Gerhardt et. al, 2018, however (b) there were no reports (shown by '-' sign in the table) on changes on the genus and species levels of these taxa in PD compared to HC.

Table 1.5. *Firmicutes* changes in healthy control (HC) Vs. Parkinson's disease (not up to the genus and species level)

#	Taxa	Congruent # of reported # studies out of all 10 studies	Higher abundance in healthy control (HC)	Lower abundance in healthy control (HC)	+ indicates a change in Parkinson's Disease (PD) compared to HC on the genus and species levels
1	Unclassified Firmicutes	1		X	-
2	*Enterococcus*	1		X	-
3	*Ruminococcaceae* unclassified	1		X	-
4	*Papillibacter cinnamivorans*	1		X	-
5	*Clostridiaceae Anaerotruncu*	1		X	-
6	*Erysipeltrichoceae* unspec.	1		X	-
7	*Christensenellaceae* unclass	1		X	-
8	*Christensenellaceae* unspec	1		X	-
9	*Oscillospiraceae, oscillospira*	2		X	-
10	*Streptococcaceae* unspec	1		X	-
11	*Streptococcus*	1		X	-
12	*Acidaminococcaceae, Acidaminococcus*	1		X	-
13	*Veillonellaceae* unspec	1		X	-
14	*Veillonellaceae Megamonas*	1		X	-
15	*Veillonellaceae Megasphera*	1		X	-
16	[*Tissierellaceae*] unspec	1		X	-
17	*Firmicutes unspecified* (unspec.)	1	X		-
18	*Faecalibacterium prausnitzi*	4	X		-
19	*Lachnospiraceae* unclassified	1	X		-
20	*Lachnospiraceae* unspec	1	X		-
21	*Clostridium coccoides, C. saccharolyticum*	2	X		-

22	*Eubacteriaceae,* [*candidatus stoquefichus massiliensis*]	1	X	-
23	*Eubacterium biforme*	1	X	-

Actinobacteria

There were no contradictory findings of relative abundance of Actinobacteria, all studies that found relative abundances within the phylum of Actinobacteria were congruent. Relatively higher abundance of Bifidobacteriaceae, Bifidobacterium were found in PD patients compared to HC in 3 out of 3 studies, and Bifidobacteriaceae (in 1 study), unspecified, and Coriobacteriaceae, unspecified (in 1 study) accordingly (Gerhardt & Mohajeri, 2018).

Verrucomicrobia

Like Actinobacteria, all reported relative abundances of taxa within the phylum of Verrucomicrobia were seen among the patients with PD. And there was a high overlap of findings for Verrucomicrobiaceae, unspecified, and Akkermansiaceae, Akkermansia- all 4 out of 4 studies that reported relative abundances in PD compared to HC (Gerhardt & Mohajeri, 2018).

Bacteroidetes

There were various findings of relative taxa abundances within the phylum of Bacteriodetes in each single study. Decreased relative abundances of *Porphyromonadaceae*, *Parabacteroides*, *Porphyromonadaceae*, *Barnesiella* (*Barnesiellaceae*), *Rickenellaceae*, *Alistipes shahii* were reported in HC compared to PD. Increased relative abundance of *Prevotellaceae,* Unspec was seen in HC compared to patients with PD in 1 out of 10 studies. Increased relative abundances of *Bacteroidetes* (Unspec), *Bacteroidaceae*, *Bacteroides coprocola*, *Bacteroides dorei*, *Bacteroides frgailis*, *Bacteroides phlebeus*, *Bacteroides*

massiliensis were observed in HC compared to patients with PD in (2 out of 10 studies) however reverse (decreased relative abundances) was observed in 1 out of 10 studies.

Within the phylum of Bacteroidetes, *Prevotella* is a well-known genus. Increased relative abundances of *Prevotellaceae*, *Prevotella copri* was observed in HC compared to patients with PD in 2 out of 10 studies, however, decreased relative abundance was observed in HC compared to patients with PD in 1 out of 10 studies (Gerhardt & Mohajeri, 2018).

Contribution to the maintenance of human gastrointestinal tract microbial community function under "normal" and PD conditions

Microbial diversity is important for maintaining human gastrointestinal tract microbial function because diverse gut microbiota helps digestion and generating nutrients and/or energy from dietary substrates, promote host tissues differentiation, stimulate immune system, and protect the host from invasion by pathogens (Costello et al., 2012). Human health is now considered as the net effect of the collective ecosystem: human body and microbiome. e.g. Decreased *Faecalibacterium prausnitzii* was associated with IBD, and *Faecalibacterium prausnitzii* functioned as an ecosystem service provider in gut for human health (Costello et al., 2012).

In the management of plants and animal communities, the “adaptive management of the ecosystem” includes managing biodiversity in a variety of habitats, including communities in highly disturbed environments, e.g. ecosystem affected by overfishing and climate change (Costello et al., 2012). Likewise, the adaptive management of the human body involves monitoring of the microbiome during health, establishing as the healthy baseline, and monitoring of their changes during diseased states and treatments (Costello et al., 2012). This adaptive

management approach to clinical medicine will enable the treatments corresponding to the basis of diagnostic changes in people's microbiome and continually adjusting through regular monitoring for the maintenance of human gastrointestinal tract microbial community functions (Costello et al., 2012).

Relating to the efforts on the maintenance of human gastrointestinal microbial community functions, recent studies shown that microbial therapy, probiotics and fecal microbial transplantation (FMT) may be a useful and novel approach for treatment of PD (X. Fang, 2019). Probiotics improve the symptoms associated with constipation in PD patients (X. Fang, 2019). In addition, fecal microbiota transplantation (FMT) was recently shown to provide a protective effect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced neurotoxicity in mice (X. Fang, 2019).

Symbiosis: Research showed that gut flora existence is not merely as commensal in human, but mutualistic, symbiotic relationship, the microorganisms perform many functions for human hosts such as fermentation for energy generation, producing SCFAs, manufacturing vitamins – biotin, vitamin K etc. Sears stated humans enjoy health through a productive collaboration with their colonizing flora, the majority of whom reside in the colon (Sears, 2005). To maintain human gastrointestinal tract microbial community functions and homeostasis state, under normal conditions, the host (human) help gastrointestinal tract limit the exposure of host immune system to the microbiota by recruitment of a multifactorial and dynamic intestinal barrier (Thursby & Juge, 2017). The barrier comprises several integrated components including physical (the epithelial and mucus layers), biochemical (enzymes and antimicrobial proteins) and immunological (IgA and epithelia-associated immune cells) factors (Thursby & Juge, 2017).

Human gut microbiota are crucial in maintaining immune and metabolic homeostasis and protecting against pathogens (Thursby & Juge, 2017). Altered gut bacterial composition (dysbiosis) was associated with the pathogenesis of many inflammatory diseases and infection (Thursby & Juge, 2017).

Human (gut) microbiome has some resistance to perturbation, but changes in diet, drugs, prebiotics or probiotics use can overcome that this resistance (Lozupone, 2012). Continued dietary changes may alter the gut microbiome flora over long time periods (Lozupone, 2012). Microbial treatment for Clostridium difficile associated disease in human with whole community transplants was successful, it shows that exogenous therapeutic microbes (fecal transplant) can colonize the gut despite resistance from the rooted microbiota initially (Lozupone, 2012). However, it is challenging to instantly know which microbes the best colonizers will be, and how a microbia configuration and its functional attributes will change in response to dietary changes or exogenous microbes (Lozupone, 2012). Studies are needed to determine and better understand conditions that promote the desired species for health and exclude the undesirable ones, in the same way as a gardener would do in gardening (Lozupone, 2012).

Factor(s) more or less likely to impact each of the three facets of microbial diversity under contrasting host health and environmental conditions

The following factors may impact each of the three facets of the microbial diversity under contrasting host health and environmental conditions (Shafquat et al., 2014), (Yatsunenko et al., 2012), (Alcock et al., 2014):

Age

Diet

Geography/ location (habitats)

Culture/tradition

Early life exposures

Pregnancy

Diseases

Medication/Drugs

Antibiotics use

Probiotics, prebiotics, synbiotics use

Genetics

genetic variation between closely related microbes to microbiome community function

the extent to which the phylogeny of human-associated microbes is linked to their functional roles

the extent of contribution of low-abundance taxa (the ‘rare biosphere’) to microbiome function

Age is an important factor that may impact the facets of the microbial diversity under host health and environmental conditions. Microbial phylotypic diversity is more common in adults compared to the children however interpersonal differences are more common among children (Yatsunenko et al., 2012). Regarding microbial functional diversity, microbiomes in adults have more involved in fermentation, metabolism of glutamate, aspartate, arginine and lysine whereas, microbiomes in the infants are mainly concerned with cysteine metabolism and fermentation (Yatsunenko et al., 2012).

Diets have selective influences on the gut microbiota, *Prevotella* grows best on carbohydrates, dietary fiber provides a competitive advantage to *Bifidobacteria*, and Bacteroidetes has a substrate preference for certain dietary fats (Alcock et al., 2014). Some microbes have specialized function. For instance *Akkermansia mucinophila* degrades mucin lives on carbohydrates, and butyrate producing microbes, *Roseburia* spp., thrives on polysaccharide in the diet (Alcock et al., 2014). Diet culture and environment may impact on the maintenance of microbial functional and phylotypic diversity, for instance, specialist microbes that are able to digest seaweed have been isolated from people in Japan, and specialist microbes that can digest cellulose have been found in African children whose diet includes sorghum (Alcock et al., 2014). Even microbiota with no specialized digestive functions tend to grow better on some combinations of nutrients than others, and competition determines which microbiota survive (Alcock et al., 2014). People who mainly eat meat and animal products have higher abundances of *Alistipes*, *Bilophila*, and Bacteroides which are bile resistant and can enhance inflammation in the gut (David et al., 2014). People who mainly consume plant-based foods have more diverse gut microbiota with higher abundances of *Prevotella* which was observed to help long-term digestion of fibers rather than bile resistant species. To change the microbiome composition to delay the onset of certain diseases, people need the perseverance in long-term dietary changes (David et al., 2014).

Geography and/or cultural traditions can also impact the three facets of microbial diversity. Yatsunenko et. al. examined how gut microbiomes differ between human populations (n=531 individuals) representing healthy Amerindians from the Amazonas of Venezuela, residents of rural Malawian communities, and inhabitants of USA metropolitan areas (Lesser, 2002). Enzyme classifications involved in the degradation of glutamine and other amino acids,

are observed higher proportion in in the USA fecal microbiome, and these findings are consistent as seen in carnivorous versus herbivorous mammals (Yatsunenko et al., 2012). Glutamate synthase is identified in the higher proportion of the fecal microbiome from the Malawians and Amerindians, glutamate synthase is also highly represented in herbivorous mammalian microbiomes (Yatsunenko et al., 2012).

Many other enzyme classifications that are required for degrading other amino acids—aspartate, proline, ornithine and lysine; for simple sugars—glucose-6-phosphate dehydrogenase, 6-phosphofructokinase; and for sugar substitutes—Liditol 2-dehydrogenase, which degrades sorbitol, as well as for host glycans –alphamannosidase, beta-mannosidase, alpha-fucosidase were overrepresented in adult USA fecal microbiomes (Yatsunenko et al., 2012). In contrast, alpha-amylase, which is required for digesting starch, was overrepresented in the Malawian and Amerindian microbiomes, reflecting their corn-rich diet (Yatsunenko et al., 2012). USA microbiomes also had significant overrepresentation of enzyme classifications involved in the vitamin biosynthesis: cobalamin, biotin and lipoic acid; in the metabolism of xenobiotics—phenylacetate CoA ligase, that is important for metabolizing aromatic compounds, and mercury reductase, and bile-salt-metabolizing enzyme cholylglycine hydrolase, likely due to their high fat meals (Yatsunenko et al., 2012).

Diet, age, stress and diseases cause increases or decreases in relative abundance and diversity bacterial specie of gastrointestinal and other body sites (Belizário & Faintuch, 2018). Both human and animal studies have shown that microbiota dysbiosis, a persistent imbalance of gut's microbial community, is related to IBD, IBS, diabetes, obesity, cancer, cardiovascular and CNS disorders (Belizário & Faintuch, 2018).

Fields et al. (Fields et al., 2018) indicated the diversity of study reports on the microbiota changes in PD: some studies found increases in specific *Clostridial* species or species within the Firmicutes phylum (Bedarf et al., 2017) (Hill-Burns et al., 2017) (Heintz-Buschart & Wilmes, 2018) (Qian et al., 2018), but others reported decreases in clusters of *Clostridial* species (Hasegawa et al., 2015) (Scheperjans et al., 2015) (Hill-Burns et al., 2017) (Li et al., 2017). Clostridia from phylum Firmicutes includes several spore forming species that enhance serotonin production in the body (Yano et al., 2015), which may potentiate systemic inflammation (Patrick & Ames, 2015). Increased *Lactobacillus* in PD patients was found in other studies (Minato et al., 2017) (Petrov et al., 2017), which is a genus of the Firmicutes phylum that contains species associated with antiinflammation in a number of autoimmune disease models (Plaza-Díaz et al., 2017). Potential confounding effects across study cohorts, such as differences in drug treatment and dietary habits, may have caused these discrepancies. However, they may also reflect specific changes within the genus level that may go undetected by the bacterial sequencing methods (e.g., 16S rRNA sequencing) used in these studies, and large-scale metagenomic analysis might provide a clearer indication of dysbiosis in PD (Poretsky et al., 2014)" (Fields et al., 2018).

Gut microbiome produce significant varieties of hormone/ neurochemicals(Alcock et al., 2014). Half of the neurotransmitter dopamine and the vast majority of the body's serotonin are the intestinal source (Alcock et al., 2014). Many transient and persistent inhabitants of the gut, including *Escherichia coli, Bacillus cereus, B. mycoides, B. subtilis, Proteus vulgaris, Serratia marcescens, and Staphylococcus aureus* can produce dopamine (Alcock et al., 2014). Concentrations of dopamine in culture of these bacteria were reported to be 10–100 times higher than the typical concentration in human blood. *B. subtilis* appears to secrete both dopamine and norepinephrine into their environment (Alcock et al., 2014). Certain probiotic strains alter the

plasma levels of other neurochemicals. *B. infantis* 35624 raises tryptophan levels in plasma, a precursor to serotonin (Alcock et al., 2014). The lactic acid producing bacteria found in breast milk and yogurt also produce the neurochemicals histamine and GABA (Alcock et al., 2014). GABA activates the same neuroreceptors that are targeted by anti-anxiety drugs such as valium and other benzodiazepines (Alcock et al., 2014).

Discussion

Humans have rich microbial phylotypic diversity especially in the gut, which depends on several factors: acquisition of microbiota since birth/infants, age, diet, geographical location, culture and tradition, environment, diseases, drugs taken, antibiotics use, probiotics, prebiotics and synbiotics use, microbial phylotypic diversity is altered in "(previously) normal conditions" and in "diseased states (PD)". Diseases may be either "the cause" or "the effect" in the alteration of microbial diversity. Microbial functional diversity often corresponds to the microbial phylotypic diversity and all modifying factors stated above. However, microbial functional redundancy might not be influenced by all of the factors stated above. Therefore, I hypothesize that a few factors such as microbial genetics, genetic mutations like single nucleotide polymorphism (SNPs), disease states or drugs impact microbial functional redundancy and their sensitivity to ecological and environmental changes. Human (gut) microbial communities are enormously diverse, and it is incredible how the coexistence of such diverse microbes collectively perform metabolic functions, the reason is some of the microbes have redundant/similar functionality, microbial functional diversity. I consider functional redundancy is very important particularly when the human (gut) microbiome deviates from normal homeostatic states, i.e. microbial dysbiosis. Even among people with dysbiosis in human (gut)

microbiome, the degree of impact on their health and functions (including the diseased states/ PD) may be different, I hypothesize this relates to their microbial functional redundancy, and which taxa are knocked out and which taxa replace the disappearing taxa providing that some taxa with redundant functions are weak while some taxa are equally strong compared to the disappearing taxa. It is an formidable task to discover all redundant functions of incredibly diverse microbial taxa, however we need to study what conditions promote the desired species that resemble to the microbial composition of the people in health, and try to recover to the homeostatic state by reasonably manipulating the human (gut) microbiome in the same way as a gardener would do in gardening.

Pre and Probiotics to purposefully alter gut microbiome

Probiotic biotherapies are known to make a healthy gut environment by balancing bacterial populations and supporting their favorable metabolic action (Westfall et al., 2017).

Definitions and examples

Probiotics

According to Harvard Medical School (Harvard, 2020), probiotics are like live cultures that are naturally found in the human gut, probiotics help to balance gut flora, boost immunity, and overall health; prevent and treat gastrointestinal diseases. The U.S. Food and Drug Administration (FDA) and WHO joint-definition of probiotics is “Live microorganisms which when administered in adequate amounts confer a health benefit to the host” (Hill et al., 2014). Probiotics can be defined as a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or

colonization) in a compartment of the host and by that exert beneficial health effects in this host (Schrezenmeir & De Vrese, 2001).

Probiotics has a cytoprotective effect in the intestinal mucosa and strengthen the epithelial tight junctions and preserve mucosal barrier function (Krishna Rao & Samak, 2013) . Probiotics can also prevent disruption of tight junctions by injurious factors (Krishna Rao & Samak, 2013).

Prebiotics

Prebiotics are nondigestible food components that selectively stimulate the growth or activity of desirable microorganisms (NCCIH et al., 2019). Prebiotics definition is similar to dietary fiber except of prebiotics' selectivity for certain species. Selectively, *Bifidobacteria* spp. may be promoted by consumption of fructooligosaccharides, inulin, transgalactosylated oligosaccharides, and soybean oligosaccharides (Schrezenmeir & De Vrese, 2001).

Synbiotic

Synbiotic is defined as a product that contains both probiotics and prebiotics that selectively favors the probiotic compound, e.g., a product containing oligofructose and probiotic *Bifidobacteria* , but a product containing oligofructose and a probiotic *Lactobacillus casei* would not meet the criteria of synbiotic (Schrezenmeir & De Vrese, 2001). The International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the definition of a synbiotic as a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host (Swanson et al., 2020). The ISAPP further defined a complementary synbiotic must be composed of a probiotic plus a prebiotic, but

a synergistic synbiotic did not need to be so (Swanson et al., 2020). A synergistic synbiotic is a synbiotic for which the substrate is designed to be selectively utilized by the co-administered microorganisms (Swanson et al., 2020).

Supplementary type (dairy, capsule, fermented foods)

Probiotics supplements can come in variety of forms including capsules or pills, powders, liquids, foods, and drinks. The seven core genera of microbial organisms most often used in probiotic products are *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia,* and *Bacillus* (NIH, 2022).

The live microorganisms used to make many fermented foods and yogurt usually do not survive transit through the stomach, due to hydrolytic enzymes, and bile salts and, therefore, might not reach the distal gut (NIH, 2022). Some processed fermented foods such as sourdough bread and most commercial pickles do not contain live cultures in the form in which they are consumed; many commercial yogurts claim to contain probiotic microorganisms such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (NIH, 2022).

Many cheeses, kimchi, kombucha, sauerkraut, miso, pickles, and raw unfiltered apple cider vinegar made from fermented apple sugars contain live cultures but do not typically contain proven probiotic microorganisms (NIH, 2022).

Probiotics supplements in capsules, powders, liquids, and other forms claim to contain a wide variety of strains and doses, and some organizations have systematically reviewed the available evidence for recommendation on appropriate specific probiotics product, dose, and formulation to use for preventing or treating various health conditions (NIH, 2022).

Effective dose

Health Canada accepted the following bacterial species, when delivered in food at a level of 1 × 10^9 colony forming units (CFUs) per serving, as probiotics for which nonstrain-specific claims might be made: *Bifidobacterium (adolescentis, animalis, bifidum, breve and longum)* and *Lactobacillus (acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus and salivarius)* (Hill et al., 2014). Probiotics minimum 5.2 billion CFUs/capsule was based on the current evidence of effective probiotic dosages. Association of American Family Physicians stated, based on a Cochrane review, that a dosage of 5 x 10^9 colony-forming units or greater per day was significantly more effective than a lower dosage (Wilkins & Sequoia, 2017). In order to obtain the clinical effectiveness of probiotics products, number of probiotic capsules taken needs to increase to obtain adequate dosages of colony-forming units (Wilkins & Sequoia, 2017). Doses of the effective probiotics interventions ranged from 10^7 to 3.63 × 10^10 CFUs (Wang, Lee, Braun, & Enck, 2016). Doses around 10^9 and 10^10 were used most often(Wang et al., 2016). Doses between 10^9 and 10^10 CFUs have shown sufficient effects (Wang et al., 2016).

Consumer education and protection

Some unfermented foods and drinks such as smoothies, juices, milks, nutrition bars, cereals, and infant and toddler formulas claim to include added microorganisms (NIH, 2022). However, truly getting probiotics from these foods and drinks depends on the quantity of microorganism, survival of the organisms through gastrointestinal tract and presence of specific species and strains that have health effects (NIH, 2022). Probiotics must be consumed alive to have health benefits and these microorganisms can die during their shelf life, consumers should

check the label for the number of CFUs at the end of the product's shelf life, not at the time of manufacture (NIH, 2022).

Some probiotics have been scientifically studied in people with gastrointestinal problems, some studies found the probiotics helpful and other studies could not say for sure, therefore, more scientific studies are needed (Brenner & Chen, 2011). Probiotics are safe for most people and people have been using probiotics for more than 100 years without much trouble (Brenner & Chen, 2011).

Based on a probiotic product's intended use, the U.S. Food and Drug Administration (FDA) might regulate it as a dietary supplement, a food ingredient, or a drug (NCCIH et al., 2019). Many probiotics are sold as dietary supplements, which don't require FDA approval before they are marketed (NCCIH et al., 2019).

Parkinson's disease, constipation, and clinical, neuro-endocrinal, and biomedical factors

Constipation: Day-to-day overburden in the Parkinson's Disease

Dr. Aaron Haug, on the Davis Phinney Foundation for Parkinson's educational page, stated "98% of people with Parkinson's are affected by constipation at some point of the disease" (Haug, 2016). The excess burden of constipation is affecting physical, psychological, social distress, and negatively impacting **q**uality of life (Kaye et al., 2006).

Regarding the proposal pertaining to the use of probiotics to relieve constipation, recently, on 25th June 2019, Corliss, Executive Editor, Harvard Health Publishing, Harvard Medical School stated, "Probiotics may ease constipation"(Corliss, 2019). In that, Dr. Allan Walker, Director of the Division of Nutrition at Harvard Medical School and a world-renowned expert in the probiotics field said his opinion (Corliss, 2019)—Probiotics may be very helpful in the future as a way of dealing with constipation and other health problems, however there's still not enough evidence to recommend a *specific* probiotic for constipation; what's needed is a large, multicenter trial, with standardized outcomes to determine which probiotic species and strains are *most effective*, how much to take, and for how long (Corliss, 2019). Until that happens, experimenting on your own with probiotics for constipation relief is probably a safe bet (Corliss, 2019). Probiotic supplements do not seem to have reported side effects and are generally considered safe (Corliss, 2019).

Mainstay of treatment for Parkinson's Disease: L-Dopa

L-Dopa is the mainstay of drug therapy for PD (NIH, 2013) (NIH, 2020). There are patients with PD who need to take L-Dopa dose 6 or more times/day (immediate release-Carbidopa-L-Dopa) and 7 or more times/day with (Carbidopa, L-Dopa, Entacapone) (J. C. Morgan et al., 2018) (Morgan, Dhall, Rubens, Khanna, & Gupta, 2018). If they are on extended release, PD patients may still need to take Carbidopa-L-Dopa 4 times/day (J. C. Morgan et al., 2018). Dopaminergic drug therapies have on-off phenomena, serious side effects, and decreased efficacy over time (Jenner, 2008), probably due to the intermittent, non-physiological stimulation of striatal dopamine receptors (by drugs) which destabilizes an already unstable system in the brain of PD patients (Chaudhuri et al., 2013). Therefore, recent major therapeutic goals of PD treatment have been to attain a continuous dopaminergic stimulation, and a continuous low-level drug delivery (Chaudhuri et al., 2013). This is where the role of gut microbiota may be useful.

Min, Park, Park, & Yoo (2015) stated, "nowadays, so many elderly people suffer from the symptoms of PD such as rigidity, akinesia, and rest tumor" (Min et al., 2015). Therefore, the demand for the medicine, L-Dopa, is substantial: the world market of L-Dopa is about 250 ton per year and the total market volume is about 101 billion per year in 2005 (Min et al., 2015). Since Monsanto developed and commercially produced L-Dopa by asymmetric hydrogenation, most L-Dopa were supplied by chemical synthesis (Min et al., 2015). However, the chemical synthesis for L-Dopa has critical limitations such as a poor conversion rate and a low enantioselectivity: the chemical synthesis usually adapts a complicated reaction procedure and requires expensive metal catalysts (e.g., Rb-complex) that work under harsh operational conditions with a low substrate specificity (Min et al., 2015). Therefore, the commercial industry is exploring the biotechnological approaches to use microbial fermentation and enzymatic

methods to produce L-Dopa not only for improving the conversion rate and the enantioselectivity, and economizing the process (Min et al., 2015).

Probiotics and constipation

Probiotics were shown in previous clinical trials to cause improvements in constipation, in patients with PD (Cassani et al., 2011), (Barichella et al., 2016). However, previous studies have not yet investigated the possible impacts of probiotics on the modulation of disrupted gut microbiota, changes of serum L-Dopa level, and consequent impact on the motor functions and well-being of people with PD. Dr. Mary L. Wagner et. al, on the American Parkinson Disease Association (APDA) educational page, stated probiotics can provide a health benefit to the host when given in adequate amounts and may improve constipation as well (Wagner et al., 2015). Probiotics, prebiotics and symbiotic may impact on the initiation and progression of PD by decreasing pro-inflammatory responses, improving intestinal integrity (Perez-Pardo et al., 2017). Probiotics can be used as a potent neuroprotective nutraceutical for neurological disorders including PD, Alzheimer's disease, multiple sclerosis and autism (Lim et al., 2015). Patients with PD have non-motor symptoms and sometimes the symptoms appear in the initial stages before motor symptoms appear (Goldman & Postuma, 2014). The sign, constipation, could show up 20 years before an individual is diagnosed with PD (Savica et al., 2009). Constipation is the most common non-motor symptom among the patients with PD and negatively affects the quality of life (Frazzitta et al., 2019). Previous studies (Cassani et al., 2011), (Barichella et al., 2016) indicated that daily consumption of fermented milk containing *Lactobacillus casei* Shirota improved stool consistency, sensation of incomplete emptying, and bowel habits among PD patients.

Gut microbiota in relationship with Levodopa (L-Dopa), Dopamine, and SCFA

Gut microbiota (GM) may control dopamine levels in the brain through their DA producing enzymes (Aziz, Doré, Emmanuel, Guarner, & Quigley, 2013). Thus, probiotics, prebiotics and symbiotic may impact the initiation and progression of PD (Perez-Pardo et al., 2017). Probiotics can be used as potent neuroprotective nutraceuticals for neurological disorders including PD (Lim et al., 2015). Bacterial cultures are used in laboratories to produce L-Dopa (Surwase & Jadhav, 2011) (Bizzarri et al., 2015) (T. Wei et al., 2016). Gut microbiota produce the SCFA, butyrate, which is a HDACi, and protects dopaminergic neurons from degeneration (Westfall et al., 2017). Broad HDACi salvage α-synuclein-dependent cytotoxicity both in cellular and fly models of PD (Didonna & Opal, 2015), and alleviate motor deficits and mitigate striatal dopaminergic neurons depletion in PD models (Didonna & Opal, 2015).

Nicotinic acid

A low dietary niacin intake was found to associate with the reduced alpha-diversity and *Bacteroidetes* abundance in the microbiome of people with obesity (Fangmann et al., 2018). Slow-release niacin may help increase in the abundance of *Bacteroidetes* and improved metabolic inflammation (Fangmann et al., 2018).

Niacin (Vitamin B_3 or Nicotinic acid) is a precursor for NAD–NADH which is needed for dopamine production (Wakade & Chong, 2014). Consequently, reduced niacin levels in PD may reduce striatal dopamine production (Wakade & Chong, 2014). Moreover, studies found that nicotinic acid mononucleotide, NAD+ precursor, protects against axonal degeneration after

axotomy (Wakade & Chong, 2014). Nicotinic acid deficiency is the well-known etiology of pellagra, that damage CNS, leading to dementia (Nakashima, Sanada, Utsuki, & Kawada, 1978). People with PD also have impact in the CNS and dementia. Antagonistic agents of nicotinic acid caused mental deficiency (Nakashima et al., 1978). L-Dopa (L-Dopa is metabolized to the neurotransmitter, dopamine in later steps) is synthesized from tyrosine by tyrosine hydroxylase enzyme, the rate limiting enzyme (Mittal et al., 2017), which needs nicotinic acid in the biosynthetic pathway (Nakashima et al., 1978). Therefore, nicotinic acid deficiency can affect the activity of tyrosine hydroxylase and metabolism of catecholamines in the brain and nervous systems (Nakashima et al., 1978). Additionally, nicotinic acid could be used to inhibit (Kang et al., 2018) tyrosine decarboxylase enzyme that can metabolize L-Dopa as a substrate.

Probiotics therapy for the Parkinson's Disease

Probiotics were also shown in previous clinical trials to cause improvements in constipation, in patients with PD (Cassani et al., 2011), (Barichella et al., 2016). Recent review study (X. Fang, 2019) also concluded microbial therapy may be a useful and novel approach for treatment of PD. However, previous studies have not yet investigated the possible impacts of probiotics on the modulation of disrupted gut microbiota, changes of serum L-Dopa level, and consequent impact on the motor functions and well-being of people with PD.

The preservation of gut barrier integrity and an increased ability to fight infections are the main reported immune benefits of probiotics for healthier aging (Landete et al., 2017). *Lactobacillus plantarum* has been shown to have the capacity to enhance the production and secretion of mucins esp. MUC2 and MUC3 from the human intestinal epithelial cells, which improves the epithelial barrier function (Ganesh & Versalovic, 2015).

Helicobacter pylori infection, effects on L-Dopa, and Benefits of H. pylori eradication and co-administration with Probiotics

Parkinson patients have an increased risk of peptic ulceration which disease is known to be caused primarily by *Helicobacter pylori* that induce damage to the mucosa of the stomach and duodenum (Carmody & Turnbaugh, 2014). Studies observe *H. pylori* eradicating antibiotics increases L-Dopa bioavailability in Parkinson patients, with a single antibiotic dose improving motor symptoms for 3 months or more (Carmody & Turnbaugh, 2014). *H. pylori* may affect L-Dopa bioavailability by disrupting absorption at the duodenal mucosa, by producing ROS that inactivate the drug, and via direct metabolism of L-Dopa or by direct binding to the drug (Carmody & Turnbaugh, 2014).

These findings indicate *H. pylori* infection cause leaky gastrointestinal tract, in which probiotics are considered to protect gut from leaky state. *H. pylori* infection increases gastric acid secretion in patient with duodenal ulcers (Calam et al., 1997). Certain cytokines released in *H. pylori* gastritis, such as tumor necrosis factor alpha (TNFα) (Calam et al., 1997), ammonia cause gastric G-cells to release gastrin (Calam et al., 1997) which increases gastric acid pH. That's why, H. pylori eradication mostly include gastric acid reduction. Rekdal et. al (Rekdal et al., 2019) depicted bacterial tyrosine decarboxylase worked the best in acidic pH. So, reducing gastric acid appears to contribute to the benefits of *H. pylori* eradication in PD. Probiotics mix survives the best in the less acidic pH. So, these findings match up with reducing gastric acid and *H. pylori* eradication benefits People with PD and increase bioavailability of L-Dopa.

Magistrelli et. al (May 2019) (Magistrelli et al., 2019) indicated that probiotics have beneficial effects in PD, probiotics were able to inhibit inflammatory cytokines and ROS

production in both patients with PD and controls. Moreover, most strains of probiotics determined restoration of membrane integrity and inhibition of pathogenic bacteria, *E. coli* and *K. pneumoniae,* and do not carry bacterial tyrosine decarboxylase gene, which is considered to decrease L-Dopa bioavailability in PD patients.

Recently published (in January, 2019) meta-analysis of randomized controlled trials showed improved efficacy of probiotics Lactobacillus-supplemented triple therapy for eradication of *H. pylori* infection and reduce side-effects in children (H. R. Fang et al., 2019). A higher dose and longer duration of supplementation may conduce to the positive impact of *Lactobacillus* on *H. pylori* eradication (H. R. Fang et al., 2019).

In addition, another meta-analysis (2017) showed the similar result that the combination of the bismuth containing quadruple therapy (BCQT) and probiotics may improve the eradication rate of *H. pylori* especially in patients receiving front-line eradication regimen or failed from triple therapy (Si et al., 2017). Probiotics may reduce the adverse reactions when combined with other eradication agents (Si et al., 2017).

Feng et. al. (2017) conducted systematic review and meta-analysis to assess the efficacy and safety of probiotic-supplemented triple therapy for eradication of *Helicobacter pylori* in children, and the results recommended to use probiotics to supplement triple therapy in pediatrics (Feng et al., 2017). Probiotics multi-strain of *Bifidobacterium infantis, Bifidobacterium longum, L. acidophilus, L. casei, Lactobacillus plantarum, Lactobacillus reuteri, L. rhamnosus, Lactobacillus salivarius, Lactobacillus sporogenes, and Streptococcus thermophilus* was the best to reduce the incidence of diarrhea; multi-strain of *Bacillus mesentericus, Clostridium butyricum, and Streptococcus faecalis* for loss of appetite; multi-strain of *B. longum, Lactobacillus bulgaricus, and S. thermophilus* for constipation; multi-strain of *Bifidobacterium bifidum, B.*

infantis, L. acidophilus, L. bulgaricus, L. casei, L. reuteri, and Streptococcus for taste disturbance; *Saccharomyces boulardii* for bloating; and multi-strain of *Bifidobacterium breve, B. infantis, L. acidophilus, L. bulgaricus, L. casei, L. rhamnosus, and S. thermophilus* for nausea/vomiting (Feng et al., 2017).

The researchers from Saint Barnabas Medical Center, Saint George's University School of Medicine, and New Jersey Medical School conducted a meta-analysis (2016) of RCTs assessing the use of probiotics in addition to triple therapy for the treatment of *H. pylori*, and the results indicated the addition of probiotics improves *H. pylori* eradication rates in both children and adults (Lau et al., 2016). *Lactobacillus, Bifidobacterium, Saccharomyces*, and mixtures of probiotics are beneficial in *H. pylori* eradication (Lau et al., 2016). Another meta-analysis (2015) showed that probiotic treatment for longer than 2 week-time and including *Lactobacillus* or multiple probiotic strains significantly enhanced the efficacy (Lv et al., 2015). Probiotics are effective for *H. pylori* eradication in increasing eradication rates and decreasing therapy-related side effects (Lv et al., 2015). Probiotic administration prior or subsequent to therapy and for a duration of >2 weeks may increase the eradication efficacy (Lv et al., 2015). A systematic review and meta-analysis indicated *Lactobacillus*-containing and *Bifidobacterium*-containing probiotic compound preparation during initial *H. pylori* eradication therapy in the adult may have beneficial effects on eradication rate and incidence of total side effects (Z. H. Wang et al., 2013), and another systematic review indicated fermented milk-based probiotic preparations improve *H. pylori* eradication rates by approximately 5-15% (Sachdeva & Nagpal, 2009). Gutiérrez-Zamorano et. al. found increased anti-*Helicobacter pylori* effect of the probiotic *Lactobacillus fermentum* encapsulated in carrageenan (Gutiérrez-Zamorano et al., 2019). The researchers

suggested to consume low pH resistant probiotics for *H. pylori* eradication (Gutiérrez-Zamorano et al., 2019).

Asgari et. al found that honey-derived *Lactobacillus rhamnosus* probiotics provided similar results as clarithromycin in treatment of *H. pylori* infection and gastritis in mice model, without antibiotic resistance (Asgari et al., 2018). Probiotics were reported to inhibit *Helicobacter pylori* not only in vitro, but also in vivo studies (Kamiya et al., 2019).

Emara et. al. ‘s histopathologic findings support the promising role of probiotics in eradication of *H. pylori* infection: Probiotics lowered *H. pyloric* density at the luminal side of epithelium, improved histological inflammatory and activity scores both in the gastric corpus and antrum (Emara et al., 2016). This effect persists for long period of time after discontinuation of probiotic supplementation and this is probably through an immune mechanism (Emara et al., 2016).

Chen et. al. described the paradigm from germ theory to germ therapy. Chen recalled the germ theory of disease and Koch's postulates about the role of microbes in human health since 19th century (Chen et al., 2019). The discovery of *Helicobacter pylori* (*H. pylori*) and *H. pylori* associated diseases as per the concept and framework of Koch's postulates, 19th century, and eradication of *H. pylori* to prevent peptic ulcers recurrence and gastric cancer had been the triumph of this microbiology paradigm (Chen et al., 2019). Advances of next generation sequencing in the past decade provided a great insight into the unculturable microbes, followed by better understanding of dysbiosis, and symbiosis of microbiome and host human, manipulation of the microbiota, either by restoring missing functions or by eliminating harmful functions (Chen et al., 2019). Current evidences of two common germ therapies are fecal microbiota transplantation and probiotics, in treating diseases (Chen et al., 2019). The

researchers from the Department of Medicine, Division of Gastroenterology, McGill University Health Center, Montreal, Québec, Canada reported the reconciliation of Recent *Helicobacter pylori* Treatment Guidelines due to increasing antibiotic resistance and reduced effectiveness of standard therapies to eradicate *Helicobacter pylori* infection (Fallone et al., 2019). Options under investigation for *H. pylori* eradication treatment guideline include substituting vonoprazan for proton pump inhibitors, adding probiotics, and vaccine development (Fallone et al., 2019).

Thus, all this evidence align with my dissertation research pertaining to the use of probiotics to enhance the quality of life of People Living with PD including L-Dopa bioavailability.

Ghrelin

Gut bacteria stimulates ghrelin production, and ghrelin receptors activation may stimulate tyrosine hydroxylase which acts as the key step in dopamine synthesis (Andrews et al., 2009). Ghrelin has neuroprotective, anti-apoptotic abilities (Westfall et al., 2017), help mitochondrial integrity, and may decrease dopaminergic cell loss (Andrews et al., 2009).

Short-chain fatty acids (SCFAs)

SCFAs are main metabolic products of gut bacteria (Unger et al., 2016) and have potent anti-inflammatory effects (Westfall et al., 2017). Butyrate, a HDACi, protects dopaminergic neurons from degeneration (Westfall et al., 2017). SCFAs (mainly butyrate and propionate) regulate tyrosine hydroxylase gene expression (DeCastro et al., 2005) in addition to dopamine synthesis, degradation and transport genes (Nankova et al., 2014). Tyrosine hydroxylase is required to synthesize dopamine and is usually decreased in PD patients (Westfall et al., 2017).

Faecalibacterium prausnitzii known as butyrate producing sigmoidal bacteria are decreased in patients with PD (Keshavarzian et al., 2015). A clinical trial reported that fecal SCFA concentrations were significantly reduced in PD patients compared to controls (Unger et al., 2016). *Bacteroidetes*, *Prevotellaceae* phylae were reduced, *Enterobacteriaceae* were more abundant in the fecal samples from PD patients compared to their controls (Unger et al., 2016).

To date, there is a dearth of data pertaining to the benefits of nutritional factors on the quality of life of those with PD and their impact on the underlying pathophysiologic functions. Since dietary and nutritional factors are daily occurrences, it is imperative to learn more about which dietary and nutritional innovative approaches to elicit the best benefits to those with PD, as these changes will significantly benefit the patient and their caregiver(s). This study new work will investigate the impacts of probiotics, vitamin B_3 via the modulation of gut microbiome, ghrelin, SCFA, and L-Dopa. This study will add to the field of knowledge with the data on new alternative therapeutic options and additional benefits for those with PD, including neuroprotective benefits to further delay the rate of progression of the disease to allow improved function, life enjoyment and QoL.

MDS-UPDRS

1. Part 1: Intellectual function, mood, behavior
    1.1. Forgetfulness, disorientation in time and space
    1.2. Vivid dreaming
    1.3. Hallucinations
    1.4. Delusions and paranoia
    1.5. Depressed mood
    1.6. Anxious mood

1.7. Apathy

1.8. Features of dopamine dysregulation syndrome

1.9. Nighttime sleep problems

1.10. Daytime sleepiness

1.11. Pain and other sensations

1.12. Urinary problems

1.13. Constipation problems

1.14. Lightheadedness on standing

1.15. Fatigue

2. Part 2: Activities of daily living

2.1. Speech: difficulty being understood

2.2. Salivation and drooling

2.3. Chewing and swallowing

2.4. Cutting food

2.5. Small handwriting

2.6. Needing help with getting dressed, buttons, arms in sleeves

2.7. Requires assistance with bathing, brushing teeth

2.8. Trouble doing hobbies and other activities

2.9. Difficulties with turning in bed

2.10. Tremor impact on activities

2.11. Getting in and out of bed

2.12. Walking, balance, falling

2.13. Freezing

3. Part 3: Motor examination
    3.1. Speech – volume, diction
    3.2. Reduced facial expressions
    3.3. Rigidity
    3.4. Finger tapping
    3.5. Slowed hand movements
    3.6. Rapid alternating movements of hands (pronation-supination)
    3.7. Toe tapping
    3.8. Leg agility – when tapping heel on the ground, is it slowed, early fatiguing
    3.9. Arising from a chair – degree of difficulty
    3.10. Gait – shuffling, walking with difficulty
    3.11. Freezing of gait
    3.12. Postural stability – difficulty recovering balance
    3.13. Posture – stooped
    3.14. Global spontaneity of movement (body bradykinesia) – slowness of movement, lack of movement
    3.15. Tremor at rest
4. Part 4: Motor complications
    4.1. Dyskinesia, including time spent with dyskinesia, functional impact of dyskinesia, and painful off-state dystonia
    4.2. Motor fluctuations, including time spent in the off state, functional impact of fluctuations, and complexity of motor fluctuations

Conclusion

Based on the state of knowledge of human gut microbiome and PD, the key questions are- do human gut microbiome composition associate with PD progress or even contribute to the pathogenesis (etiology) of the disease? As studies found some potentials and associations between human gut microbiome and PD (neurodegenerative disease), my key questions are: can we intervene by manipulating human gut microbiome to slow down the disease progress, improve the quality of life, or even prevent the disease occurrence as a primary or secondary prevention (public health) and how? Can some of the human gut microbiome changes be used for biomarkers of PD (to assess the risk way before any symptoms or to assess the prognosis and response to the treatment)? Human gut microbiome- Firmicutes, Bacterioides, Lactobacillaceae- are the good potential for further study to better understand their functions/interactions with PD and medication as the current knowledge proves the association with constipation, medication (Entacapone), Ghrelin etc. I also believe if we could examine up to the higher levels of phylogenetic tree, we would be in better position to address the correlation or causative factors of human gut microbiome with PD. The advanced technology may enable to do that; however, it seems quite challenging to identify which technical methods are the best to use as a standard for all studies so that more valid comparisons and conclusions can be made.

With this review and evidence from scientific studies, the author of this review was designing a randomized controlled clinical trial of probiotics, vitamin B_3 and impacts on the motor, non-motor symptoms, constipation, neuroendocrinal parameters, and quality of life for the population with PD. This novel study will have significance for PD research in a way of "hitting multiple targets with one arrow": (1) assessment on an immediate impact on Quality-of-Life People living with PD and (2) layout the new therapeutic targets for long-term

neuroprotection and delaying the disease progression (2) layout the status of dietary, nutrients and vitaminology, and associated physical, mental and general wellbeing statuses in the study population with PD. The resulting publication of this study may contribute to advancing the efforts in various therapeutics for living well with PD and improving quality of life.

To our knowledge, at the time of conceptualizing (2017) and grant submission (2018-2019) for my proposed research of probiotics RCT for the people with PD, we did not find other probiotics RCTs in Parknson's disease conducted in the United States. A few references are updated in this review.

Chapter 2 - Research Project Proposal

The research long-term objectives

The long-term objectives are to layout the possible neuroprotective benefits to further delay the progression of the disease to allow improved function, life enjoyment and QoL by developing steps on new alternative therapeutic options for those with PD.

Specific Aims

With specific aims of this novel study to contribute to the significance of current Parkinson's research, and immediate & long-term therapeutic benefits for the lives of People living with Parkinson's disease, this study will determine modulatory effects of probiotics and vitamin B_3 in people living with PD on (a) levels of L-Dopa, SCFA(Butyrate), and Ghrelin, (b) modulation of healthy gut microbiome, (c) motor and non-motor function, constipation, and quality of life.

Research Design

This three-armed randomized control trial will recruit forty-two people living with PD. They will be randomly assigned to receive: 1) probiotics; 2) probiotics and vitamin-B3; or 3) a placebo therapy for 12 weeks. Gut microbiota, serum L-Dopa, SCFA, ghrelin, QoL indices, PDQ-39, MDS-UPDRS scores, depression and anxiety, Profile of Mood States (POMS), ROME-IV, and dietary assessments will be assessed at the beginning, week-6 and week-12 (end). Bowel and medication diaries will be recorded throughout. Next-generation sequencing of 16S rRNA genes will be used for gut microbiota analysis. Within group differences and between-group observations will be statistically analyzed.

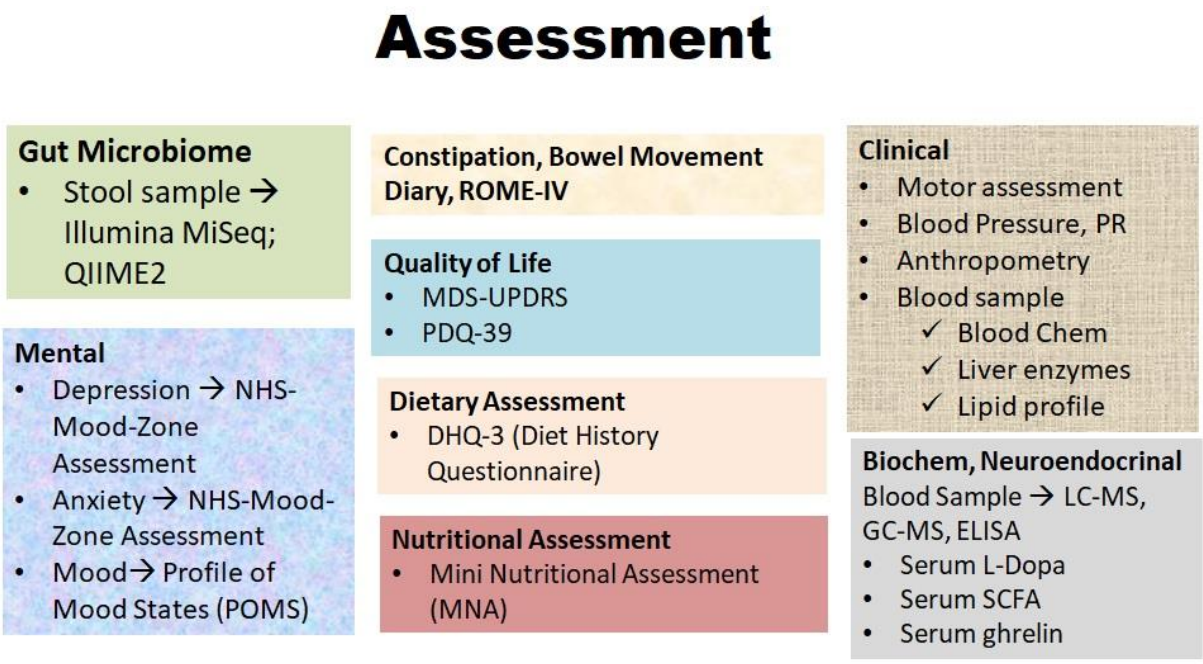

Figure 2.1. Assessment

Significance of the study

This novel study will have significance for PD research in a way of "hitting two targets with one arrow": (1) an immediate impact on QoL of people living with PD and (2) layout the new therapeutic targets for long-term neuroprotection and delaying the disease progression. Significance of the project: This study will have a significant immediate significant impact in the lives of people living with PD on their day-to-day overburden of constipation, social distress, and better understanding of the intervention effect on serum L-Dopa, an important determinant for daily activities, and living well with PD. Dr. Aaron Haug, on the Davis Phinney Foundation for Parkinson's educational page, stated "98% of people with Parkinson's are affected by constipation at some point of the disease". The excess burden of constipation is affecting physical, psychological, social distress, and negatively impacting quality of life (Kaye et al.,

2006) (McClurg et al., 2016). So, this study will have a significant immediate significant impact in the lives of people living with PD on their day-to-day overburden of constipation, social distress, and better understanding of the therapy effect on serum L-Dopa, which is the mainstay of drug therapy for PD (NIH, 2013) (NIH, 2020), the important determinant for daily activities, and living well with PD.

This study's new work

This study's new work will specifically assess the benefits of probiotics-mix and nicotinic acid (niacin) supplementation on motor and non-motor experiences of daily living (by assessing with MDS-UPDRS, PDQ-39), depression, and anxiety, Profile of Mood States (POMS), microbiome-gut-brain connection: L-Dopa, butyrate, ghrelin levels through the modulation in the gut microbiome ecology. This study will add to the field of knowledge with the novel data on the efforts for new alternative options and additional benefits for those with PD, including neuroprotective benefits to further delay the rate of progression of the disease to allow improved function, life enjoyment and QoL.

Innovation

This novel nutritional therapeutic study innovatively designs PD research in a way of "hitting two targets with one arrow" by - Intervention and assessment on the effects of probiotics and nicotinic acid supplementation for (1) an immediate impact on QoL of people living with PD and (2) layout the new therapeutic targets for long-term neuroprotection and delaying the disease progression.

There is a dearth of data pertaining to the benefits of nutritional factors on the quality of life of those with PD and their impact on the underlying pathophysiologic functions. Since dietary and nutritional factors are daily occurrences, it is imperative to learn more about which dietary and nutritional innovative approaches to elicit the best benefits to those with PD, as these changes will significantly benefit the patient and their caregiver(s). This study new work will investigate the impacts of probiotics, vitamin B_3 via the modulation of gut microbiome, ghrelin, SCFAs, and L-Dopa.

Probiotics

Probiotics were also shown in previous clinical trials to cause improvements in the non-motor symptom, constipation, in patients with PD (Cassani et al., 2011) ,(Barichella et al., 2016). However, previous studies have not yet investigated the possible impacts of probiotics on the modulation of disrupted gut microbiota, changes of serum L-Dopa level, and consequent impact on the motor functions and well-being of people with PD.

Ghrelin

Ghrelin: Gut bacteria stimulates ghrelin production, and ghrelin receptors activation may stimulate tyrosine hydrolase which acts as the key step in dopamine synthesis (Andrews et al., 2009). Ghrelin has neuroprotective, anti-apoptotic abilities (Westfall et al., 2017), help mitochondrial integrity, and may decrease dopaminergic cell loss (Andrews et al., 2009).

Short chain fatty acids (SCFAs)

SCFAs are main metabolic products of gut bacteria (Unger et al., 2016) and have potent anti-inflammatory effects (Westfall et al., 2017). Butyrate, a HDACi, protects dopaminergic neurons from degeneration (Westfall et al., 2017). SCFAs (mainly butyrate and propionate) regulate tyrosine hydrolase gene expression (DeCastro et al., 2005) in addition to dopamine synthesis, degradation and transport genes (Nankova et al., 2014). Tyrosine hydroxylase is required to synthesize dopamine and is usually decreased in PD patients (Westfall et al., 2017). *Faecalibacterium prausnitzii* known as butyrate producing sigmoidal bacteria are decreased in patients with PD (Keshavarzian et al., 2015). A clinical trial reported that fecal SCFA concentrations were significantly reduced in PD patients compared to controls (Unger et al., 2016). Bacteroidetes, Prevotellaceae phylum were reduced, Enterobacteriaceae were more abundant in the fecal samples from PD patients compared to their controls (Unger et al., 2016). Gut bacteria stimulates ghrelin production, and ghrelin receptors activation may stimulate tyrosine hydrolase which acts as the key step in dopamine synthesis (Andrews et al., 2009). Ghrelin has neuroprotective, anti-apoptotic abilities (Westfall et al., 2017), help mitochondrial integrity, and may decrease dopaminergic cell loss (Andrews et al., 2009).

Method and procedures

Approach

Forty-two patients with Parkinson disease will be randomly assigned into 3 groups. The interventions would include Probiotics mix, Vitamin B_3 (Nicotinic acid), and Placebo-Control. Each group will receive either (a) I1: probiotics mix: “Probiotic Formula (5.2 Billion CFUs/ capsule) (Labdoor, 2017)” - that contains 40.0 million CFUs of Bacillus, 6.5 million CFUs of

Bifidobacterium, 2.4 billion CFUs of Lactobacillus, 2.8 billion CFUs of Saccharomyces) (Labdoor, 2017)" minimum 6.5 x 10^9 CFUs (n=14), or (b) I2: Probiotic mix + nicotinic acid (niacin) 250 mg (n=14), or (c) C: a placebo (n=14) every day for 12 weeks. Probiotics minimum 5.2 billion CFUs/capsule was based on the current evidence of effective probiotic dosages. Association of American Family Physicians stated, based on a Cochrane review, that a dosage of 5 x 10^9 colony-forming units or greater per day was significantly more effective than a lower dosage (Wilkins & Sequoia, 2017). In order to obtain the clinical effectiveness of probiotics products, number of probiotic capsules taken needs to increase to obtain adequate dosages of colony-forming units (Wilkins & Sequoia, 2017). Doses of the effective probiotics interventions ranged from 10^7 to 3.63 × 10^10 CFUs (Wang, Lee, Braun, & Enck, 2016). Doses around 10^9 and 10^10 were used most often (Wang et al., 2016). Doses between 10^9 and 10^10 CFUs have shown sufficient effects (Wang et al., 2016). The intervention period is determined based on the clinical trials that evaluated effects of probiotics intervention and major non-motor symptom, constipation. Cassani et al.'s (Cassani et al., 2011) and Barichella et al.'s (Barichella et al., 2016) studies indicated that 4-6 weeks of probiotics intervention was sufficient to see the effects. The placebo will be an inert substance (sugar or starch powder) that resembles to the active probiotics product.

Biological samples

Blood samples are drawn from the antecubital fossa of the participants' arms, immediately centrifuged and serum samples are frozen at -80°C. Participants are provided with sterile containers and instructed how to collect stool samples at the residence. Then stool samples are transported to the laboratory at Kansas State University and frozen at -35°C until further

analysis. Blood samples will be stored in the deep freezer (-80°C) till they are ready to run LC-MS, GC-MS, ELISA analyzers in batches, the processed stool sample will be stored in the refrigerator and/or freezer until they are ready for DNA extraction, PCR, libraries preparation, and sequencing in batches. Human serum/blood, and other bodily fluid will be (disinfected and) placed in a biohazard bag or medical waste container for disposal at the Environmental Health and Safety (EH&S) department facility as outlined. We will make sure the bag or container is closed. Sharps, medical (syringes, needles, vacutainer, phlebotomy sets) will be placed in an approved sharps container. Liquid biological waste will be collected in containers for autoclaving or chemical disinfection (10% bleach). Following autoclaving or chemical disinfection, category 1 liquid wastes may be disposed via sanitary sewer (e.g., laboratory sink). Environmental Health and Safety (EH&S) will provide biohazard bags.

Analyses

Gut microbiota analysis of the participant's stool samples, serum L-Dopa, SCFA (butyrate), ghrelin levels, quality of life indices: PDQ-39 scores and MDS-UPDRS scores - measuring mentation, behavior, mood, activities of daily living and motor manifestations, in the intervention groups compared to the control group will be assessed at the beginning, 6 weeks-time (half-way of the study time), and 12 weeks-time (end of study). Next-generation sequencing of 16S rRNA genes (Illumina MiSeq) (Caporaso et al., 2012), (Warne et al., 2017) will be used for gut microbiota analysis, to quantify the relative abundance of all bacterial and archaeal populations. R and/or SPSS software will be used to calculate ANOVA assessing the differences of these parameters within-group and between groups.

Survey, assessment, and questionnaires

MDS-UPDRS, PDQ-39, mood self-assessment, POMS, height and weight measurements, dietary assessments, bowel habit and bowel movement history, Rome IV questionnaire; Biosample/Lab. data collection - stool, blood for gut microbiome data, L-Dopa, Dopamine, Butyrate/SCFA, Ghrelin levels; lipid profile, CMP (comprehensive metabolic panel)

Instruments and laboratory

Stadiometer/ tape, weighing scale (height and weight measurement), sphygmomanometer (blood pressure), glucometer (blood glucose), Cholestec Multi-analyte analyzer (lipid profile), Phlebotomy kits, PCR machine, (Refrigerated) centrifuge, pipets, LC-MS/GC-MS, ELISA, Stool sample kit, Refrigerator, Freezer, and Cold-chain container/carriers will be used. Microbiome and chemistry laboratory facilities and technical expertise are shared by two K-State professors, Dr. Lydia Zeglin and Dr. Christopher Culbertson, as the interdisciplinarity collaborative effort for this research project. Dr. Lydia Zeglin's laboratory will assist in processing microbiome samples, then Illumina MiSeq Sequencing at the Kansas State Integrated Genomics Facility (IGF), and Dr. Christopher Culbertson's laboratory (LC-MS, GC-MS), will assist in measuring serum L-Dopa, Dopamine, SCFAs, and Ghrelin levels.

Recruitment

Prospective participants will be recruited in the areas of Parkinson's Programs of the Flint Hills, Meadowlark Hills, Riley County, Kansas and surrounding Regions and States.

Inclusion criteria

People living with Parkinson's disease including those who have constipation, with or without medical treatment, are not taking antibiotic treatment by the time of the study, are not taking long-term (> 3 continuous months) high doses of supplements: Probiotics and Vitamin B_3 or special foods that may impact gut microbiota composition.

Exclusion criteria

The exclusion criteria are patients with PD who:

- are clinically unstable (vital signs),
- presents with immunosuppression including HIV or with other severe immune disorders,
- have hepatitis B virus or hepatitis C virus infection, symptomatic liver injury (e.g., jaundice, itchiness, edema), and significantly higher than the normal range of liver enzymes (AST >> 40 IU/L, ALT >> 59 IU/L for men and >> 41 IU/L for women, and/or AST:ALT ratio of >1),
- have active severe inflammatory bowel disease (IBD) or ulcerative colitis (UC), Crohn’s disease, or indeterminate colitis, irritable bowel syndrome (IBS),
- have uncontrolled hypertension of hypertension stage > 3 / high blood pressure (>160/100),
- have fever (temperature >100 °F) within the past 48 hours of registration, we may admit them in the following week if the fever is no longer present.
- recently have had large doses of probiotic use (> 10^10 CFUs or organisms /day) for > 12 continuous weeks,

- are known to be intolerant to probiotics,
- are currently going through deep brain stimulation (DBS) surgery for PD and may need to take long-term and repeated doses of antibiotic treatment within 3 months before or during this study.

Chapter 3 - Randomized controlled trial of probiotics and vitamin B3 on gut microbiome and quality of life in people with Parkinson's disease

Abstract

Evidence suggests that administration of probiotics and vitamin B_3 may improve multiple symptoms and outcomes of Parkinson's Disease through alterations in gut microbiome. Therefore, the purpose of this study was to determine whether a 12-week placebo-controlled randomized clinical trial elicited changes in gut health (constipation and gut microbiome), drug efficacy, neuroendocrine and blood chemistry levels, and indicators of quality of life in people with Parkinson's disease.

Methodology: A total of 54 people enrolled for this study, six were either excluded and/or did not meet inclusion criteria. Forty-eight participants were randomly assigned into three groups to receive: 1) probiotics + vitamin B_3; 2) probiotics + vitamin B_3 placebo; or, 3) the placebos for the probiotic and vitamin B_3 over 12 weeks. Gut health, blood chemistry, depression, anxiety, quality of life, mood, diet, and nutrition were assessed at the baseline, middle (Week 6), and end (Week 12) of the supplementation period. Blood and stool samples were collected for blood chemistry and microbiome analyses, respectively. Next-generation sequencing of 16S rRNA genes (Illumina MiSeq) was used for gut microbiota analysis. Within-group and between-group differences were statistically analyzed, with significance set at $p<0.05$.

Results: The results showed improvements in constipation problems, quality-of-life scores, Movement Disorder Society- the Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Parkinson's Disease Questionnaire-39 (PDQ-39), decreased issues with

communication via the PDQ-39 in probiotics and vitamin B_3 groups compared to the placebo group. Blood chemistry were within normal reference ranges. Supplementation did not change assessments of anxiety, depression, or mood. Gut microbiome analyses indicated significant differences in alpha and beta diversity, salient gut microbiome composition relating to different interventions, disease status, anxiety, and depression.

Conclusion: Probiotics and vitamin B_3 supplementation was beneficial for constipation symptoms, gut microbiome, and quality of life in these patients. Vitamin B_3 appeared to have a more stabilizing effect on the gut microbiome. Several differences were greater after 12 weeks compared with 6 weeks of the intervention. This appears to support that the duration of supplementation is greater than 6 weeks for most of the assessed outcome measures. For quality of life and mood measures, an increased duration of study and/or larger sample size may be necessary to detect differences.

Background

One in seven Americans are 65 years old or over, i.e., 16% of the population in 2019, which is expected to reach 22% by 2050. PD is one of the two most common neurodegenerative diseases that cause severe disability and a significant long-term burden on communities and societies, and increased challenges to public health. Globally, disability and death due to Parkinson disease are increasing faster than for any other neurological disorder (World Health Organization, 2022). The incidence of PD increases with age. More than 10 million people worldwide live with PD. There was a doubling in the number of patients with PD between 1990 and 2016, and the prevalence of PD is projected to increase 20% by 2050.

Ninety-eight percent of people with PD are affected by chronic constipation at some point of the disease (Haug, 2016) which negatively affects the absorption (Jin et al., 2020) of Parkinson's medication to reach maximum concentration, physical, psychological, and/or social aspects of life, and tends to decrease the quality of life (Kaye et al., 2006) (McClurg et al., 2016). According to the Academy of Nutrition and Dietetics (Klemm, 2020) and the Harvard Medical School (Harvard, 2020), probiotics are or resemble live cultures that are naturally found in the human gut. Probiotic supplementation has been observed to balance gut flora, increase immune system variables, and other health outcomes including prevention and treatment of gastrointestinal diseases (Harvard, 2020). Probiotics may ease constipation and improve bowel functions (Barichella et al., 2016) (Cassani et al., 2011) (Corliss, 2019). Bacterial cultures had been used in laboratories to produce levodopa (L-Dopa) (Surwase & Jadhav, 2011), the main medicine to manage PD symptoms. Likely, the motor function may be impacted via neurological mechanisms that result from changes in nutritional metabolites facilitated by the probiotics and gut flora. Studies have elucidated that gut microbiome/flora and GBA may impact neurologic,

endocrine, and metabolic functions (Appleton, 2018). Metabolic, neurological, and endocrine diseases, such as Diabetes, Alzheimer's, PD, and Mood disorders are widespread in the United States (Feigin et al., 2021) (CDC, n.d.) (NIMH, n.d.). There isn't a cure for these diseases yet, and drugs alone are not always the answer, where dietetics and nutritional approaches with probiotics and vitamin B_3 may complement the well-being and quality of life of those with PD. However, controlled trials for probiotics that examine the concept of the GBA and their neuroendocrinal and metabolic effects on PD and the aging population are scarce.

Significance of this study: This is a novel, interdisciplinary RCT at the cutting edge of public health, nutrition, dietetics, molecular biology, humanity, clinical and neuroendocrinal fields.

Contributions: This study is aimed to significantly contribute to the body of science with novel data. Academy of Nutrition and Dietetics has called for controlled trials for the numerous proposed functions of probiotics to develop evidence-based dietetics practice guidelines (Marcason, 2013) (Garner et al., 2020). Dr. Rocca from the Division of Epidemiology, Department of Neurology, Mayo Clinic also resonated with the urgent need for research focusing on identifying new preventive interventions and new treatments for people with PD (Rocca, 2018). The World Health Organization (WHO) (World Health Organization, 2022) stated that an urgent public health response is necessary to meet the health and social requirements of people with PD and improve functioning, and quality of life and prevent disability as global longevity increases.

Research Design

A total of 54 people enrolled for this study, six were either excluded and/or did not meet inclusion criteria. Forty-eight participants were randomly assigned into three groups to receive: 1) probiotics + vitamin B_3; 2) probiotics + vitamin B_3 placebo; or, 3) the placebos for the probiotic and vitamin B_3 for 12 weeks. Constipation, depression, anxiety, quality of life, mood, diet, and nutrition were assessed at the baseline, middle, and end of the supplementation period. Blood and stool samples were collected for blood chemistry and microbiome analyses, respectively. Next-generation sequencing of 16S rRNA genes (Illumina MiSeq) was used for gut microbiota analysis. Within-group and between-group differences and were statistically analyzed, with significance set at $p<0.05$. The microbiome analysis software QIIME2 and statistical software IBM SPSS 28.0.1.1 were used.

This study's new work

This study's new work was implemented to specifically assess the benefits of probiotics-mix and nicotinic acid (niacin) supplementation on: gut health, motor and non-motor experiences of daily living (by assessing with MDS-UPDRS, PDQ-39), depression, and anxiety, Profile of Mood States (POMS), microbiome-gut-brain connection: L-Dopa, butyrate, ghrelin levels through the modulation in the gut microbiome ecology. This study was implemented to add to the field of knowledge with the novel data on the efforts for new alternative options and additional benefits for those with PD, including neuroprotective benefits to further delay the rate of progression of the disease to allow improved function, life enjoyment and QoL.

There was a dearth of data pertaining to the benefits of nutritional factors on the quality of life of those with PD and their impact on the underlying pathophysiologic functions. Since dietary and nutritional factors are daily occurrences, it is imperative to learn more about which

dietary and nutritional innovative approaches to elicit the best benefits to those with PD, as these changes will significantly benefit the patient and their caregiver(s). This study new work was implemented to investigate the impacts of probiotics, vitamin B_3 via the modulation of gut microbiome, ghrelin, SCFA, and L-Dopa.

Probiotics

Probiotics were also shown in previous clinical trials to cause improvements in the non-motor symptom, constipation, in patients with PD (Cassani et al., 2011), (Barichella et al., 2016). However, previous studies have not yet investigated the possible impacts of probiotics on the modulation of disrupted gut microbiota, changes of serum L-Dopa level, and consequent impact on the motor functions and well-being of people with PD.

Niacin

Niacin (Vitamin B_3 or Nicotinic acid) is a precursor for NAD–NADH which is needed for dopamine production (Wakade & Chong, 2014). Consequently, reduced niacin levels in PD may reduce striatal dopamine production (Wakade & Chong, 2014). Moreover, studies found that nicotinic acid mononucleotide, NAD+ precursor, protects against axonal degeneration after axotomy (Wakade & Chong, 2014). Nicotinic acid deficiency is the well-known etiology of pellagra, that damage CNS, leading to dementia (Nakashima, Sanada, Utsuki, & Kawada, 1978). People with PD also have impact in the CNS and dementia. Antagonistic agents of nicotinic acid caused mental deficiency (Nakashima et al., 1978). L-Dopa (L-Dopa is metabolized to the neurotransmitter, dopamine in later steps) is synthesized from tyrosine by tyrosine hydroxylase enzyme, the rate limiting enzyme (Mittal et al., 2017), which needs nicotinic acid in the

biosynthetic pathway (Nakashima et al., 1978). Therefore, nicotinic acid deficiency can affect the activity of tyrosine hydroxylase and metabolism of catecholamines in the brain and nervous systems (Nakashima et al., 1978). Additionally, nicotinic acid could be used to inhibit (Kang et al., 2018) tyrosine decarboxylase enzyme that can metabolize L-Dopa as a substrate.

Method and procedures

Approach

A total of 54 people enrolled for this study, 6 were ineligible. This Randomized Controlled Trial (RCT) randomly assigned eligible participants with PD (n=48) into three groups. The interventions included Probiotics mix, Vitamin B_3 (Nicotinic acid 250 mg), and Placebo-Control. Each group will receive either (a) Treatment1: probiotics mix: "Probiotic Formula (5.2 Billion CFUs[1]/ capsule) (Labdoor, 2017)" - that contained[2] 40.0 million CFUs of Bacillus, 6.5 million CFUs of Bifidobacterium, 2.4 billion CFUs of Lactobacillus, 2.8 billion CFUs of Saccharomyces (Labdoor, 2017)" minimum 5.2×10^9 CFUs (n=17) + nicotinic acid (niacin) 250 mg, or (b) Treatment2: Probiotic mix + a placebo niacin (n=17), or (c) Treatment3: a placebo probiotics + a placebo niacin (n=14) every day for 12 weeks.

The recruitment was halted before the placebo group reached a total of 17 participants due to financial constraints. A total of 5 participants- two from probiotics+B_3 group, two from

[1] Labdoor is the independent third party that assesses and reports probiotics and many other supplements. Labdoor reported "Labdoor's label accuracy score looks at the total amount of tested viable bacteria compared to claimed, amount of specific genera compared to claimed, and whether the label lists the minimum viable numbers for each probiotic strain at the end of shelf-life, per the World Health Organization's labelling recommendations."

[2] Labdoor's report February 2017 analysis of this product (Lot # 50083507)

probiotics+ placebo B_3 group, and one from placebo probiotics+ placebo B3 group- dropped out due to the family issues, COVID-19, and unable or unwilling to complete the survey questionnaires, diet history questionnaires and bowel movement diary which need to keep recording throughout the study. Probiotics minimum 5.2 billion CFUs/capsule was based on the current evidence of effective probiotic dosages. Association of American Family Physicians stated, based on a Cochrane review, that a dosage of 5 x 10^9 colony-forming units or greater per day was significantly more effective than a lower dosage (Wilkins & Sequoia, 2017). In order to obtain the clinical effectiveness of probiotics products, number of probiotic capsules should be increased to obtain adequate dosages of colony-forming units (Wilkins & Sequoia, 2017). Doses of the effective probiotics interventions ranged from 10^7 to 3.63 × 10^10 CFUs (H. Wang et al., 2016). Doses around 10^9 and 10^10 were used most often (H. Wang et al., 2016). Doses between 10^9 and 10^10 CFUs had shown sufficient effects (H. Wang et al., 2016). The intervention period was determined based on the clinical trials that evaluated effects of probiotics intervention and major non-motor symptom, constipation. Cassani et al.'s (Cassani et al., 2011) and Barichella et al.'s (Barichella et al., 2016) studies indicated that 4-6 weeks of probiotics intervention was sufficient to see the effects. The placebo was an inert substance (sugar powder) that resembled to the active product. Participants were not encouraged to change their existing diet habit and pattern over the study period.

Biological samples

Blood and stool samples were collected for gut microbiome and metabolic panel, blood chemistry, liver enzymes, lipid profile. Blood samples (15 ml) were drawn from the antecubital fossa of the participants' arms, immediately centrifuged and serum samples are frozen at -80°C.

Participants were provided with sample collection kits (DNAgenotek-Omnigene.GUT for microbiome) and instructed how to collect stool samples at the residence. Then stool samples were transported to the Kansas State University, processed, and stored in the freezer at for further analysis.

Analyses

Gut microbiota analysis of the participant's stool samples, quality of life indices: PDQ-39 scores and MDS-UPDRS scores - measuring mentation, behavior, mood, activities of daily living and motor manifestations, in the intervention groups compared to the control group were assessed at the beginning, 6 weeks-time (half-way of the study time), and 12 weeks-time (end of study). Next-generation sequencing of 16S rRNA genes (Illumina MiSeq) (Caporaso et al., 2012), (Warne et al., 2017) was used for gut microbiota analysis, to quantify the relative abundance of all bacterial and archaeal populations. The next-generation microbiome bioinformatics platform, QIIME2 software (Bolyen et al., 2019), was used for the microbiome analyses, and statistical software SPSS 28.0.1.1 was used in assessing the differences of these parameters within-group and between groups. For mental aspect, NHS-Mood Zone questionnaire (NHS, 2020) was used for depression and anxiety assessment, and Profile of Mood State (POMS-2, MHS) (Heuchert et al., n.d.) was administered, and results were statistically analyzed. For diet and nutritional assessment, the survey results from diet history questionnaires (DHQIII) (NCI, 2018) such as energy intake, macro and micronutrients, dietary fibers, water, and HEI-scores, and data gathered by Mini nutritional assessment (MNA) were statistically analyzed. Clinical motor exam, anthropometric findings, blood pressure, pulse rate, and metabolic panel, blood chem, liver enzymes, lipid profiles from blood samples were statistically analyzed. Serum

samples for metabolic panel, blood chem, liver enzymes, and lipid profile were assessed at the Kansas State University, Lafene Health Laboratory.

Profile of Mood States (POMS 2) (Heuchert et al., n.d.) instrument assessed the transient, fluctuating feelings and enduring affect states. Individual Profile of Mood States assessment scores were assessed to generate percentile results of elevations at the scale and subscale levels, total mood disturbance (TMD) and anger-hostility, confusion-bewilderment, dejection-depression, fatigue-inertia, tension-anxiety, vigor-activity, friendliness, compared to those in the normative sample representative of the general U.S. population in terms of ethnicity/race. Total Mood Disturbance was computed as sum of 'Anger-Hostility + Confusion-Bewilderment + Dejection-Depression + Fatigue-Inertia + Tension-Anxiety' deducted by 'Vigor-Activity'.

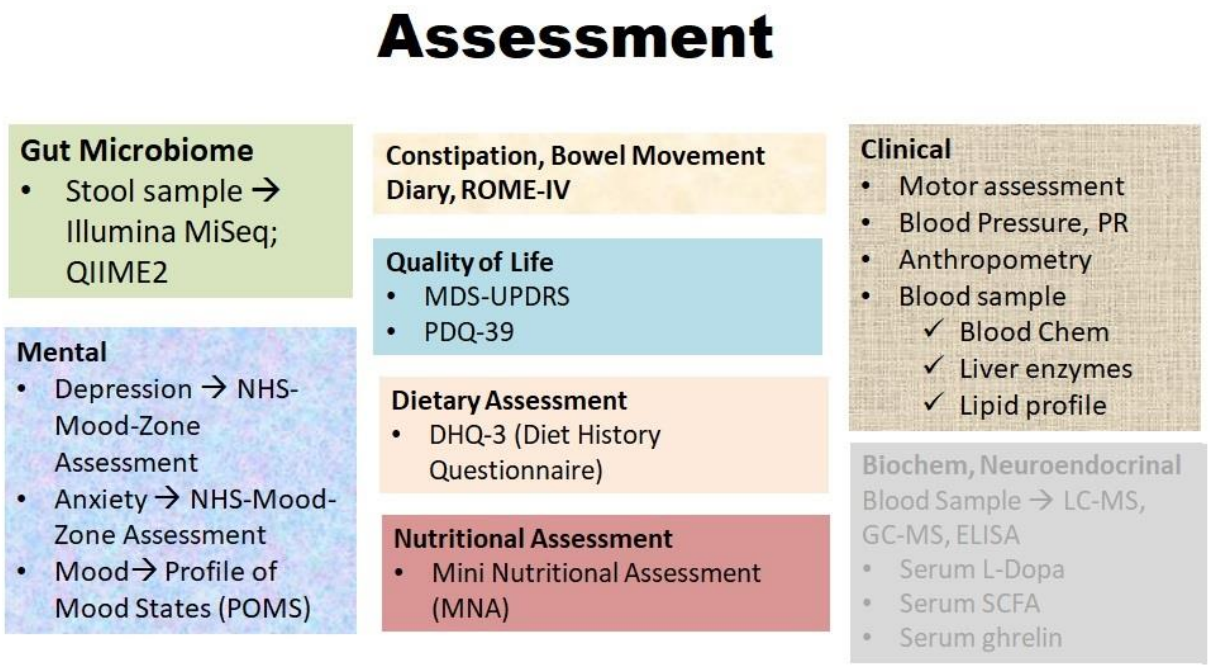

Figure 3.1. Assessment

Survey, assessment, and questionnaires included MDS-UPDRS, PDQ-39, mood self-assessment, POMS, height and weight measurements, dietary assessments, bowel habit and bowel movement history, Rome IV questionnaire. Bio sample/Lab. data collection included stool for gut microbiome data, blood for L-Dopa, Dopamine, Butyrate/SCFA, Ghrelin levels; lipid profile, CMP (comprehensive metabolic panel).

Recruitment

Prospective participants were recruited in the areas of Parkinson's Programs of the Flint Hills, Meadowlark Hills, Riley County, Kansas and surrounding Regions and States.

Results

Demographics, clinical, and lifestyle

The demographics, medication, fiber supplements, laxative use, average water intake were described in detail in the following tables. The mean age for treatment1 (probiotics+vitamin B_3) group was 71±9 years, treatment2 (probiotics only) group was 71±9 years, and treatment3 (placebo) group was 74±6 years. youngest 47 and eldest 85 years old; and the youngest was 47 years old and the eldest was 87 years old for the whole cohort.

Table 3.1. Demographics

	Treatment1 (Probiotics+B_3)			Treatment2 (Probiotics)			Treatment3 (Placebo)			F	p-value
	Mean	SD	N	Mean	SD	N	Mean	SD	N		
Age (Years)	71	9	15	71	9	15	74	6	13	0.5	0.6
Disease duration (months)	55	29	15	55	53	15	62	48	13	0.1	0.9
Average water intake (Oz/day)	42	19	14	38	19	14	43	23	13	0.2	0.8

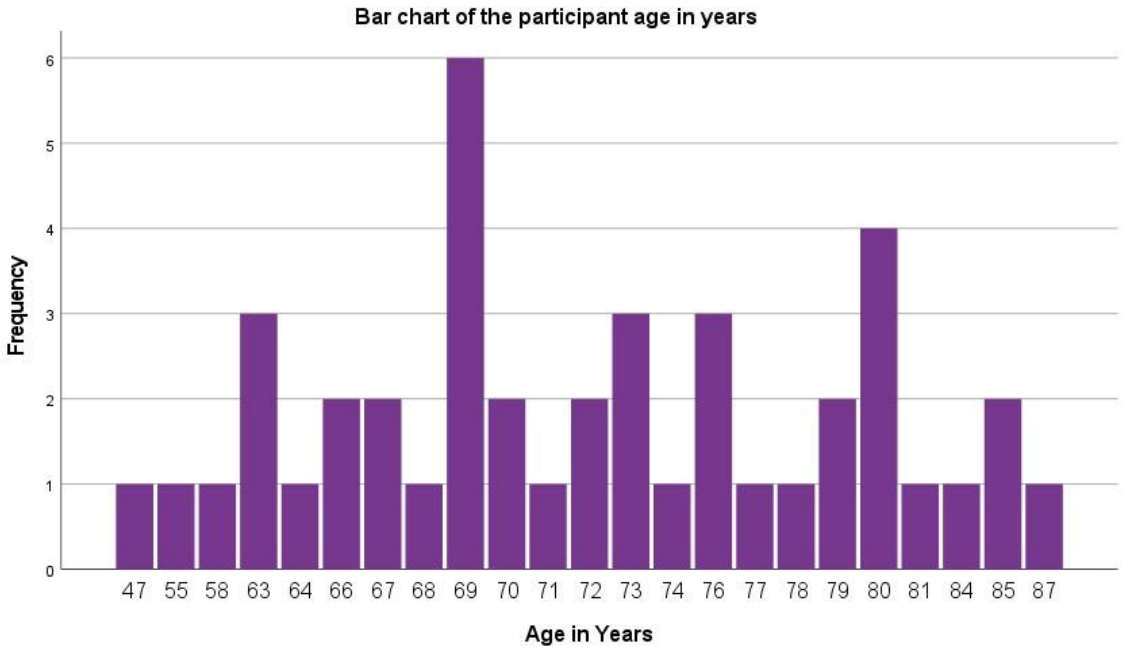

Figure 3.2. Bar chart of the participant age in years

Table 3.2. Demographics, lifestyle, and medication

	Treatment1 (Probiotics+B_3)	%	Treatment2 (Probiotics)	%	Treatment3 (Placebo)	%	Total	%	H	p-value
Gender										
Male	10	67	9	60	8	62	27	63		
Female	5	33	6	40	5	38	16	37		
	15	100	15	100	13	100	43	100	0.2	0.9
History of pesticide exposure										
Yes	5	33	4	27	6	46	15	35		
No	9	60	11	73	6	46	26	61		
Not reported	1	7	0	0	1	8	2	4		
Total	15	100	15	100	13	100	43	100	1.5	0.5
Currently smoking										
Yes	1	7	0	0	0	0	1	2		
No	13	87	15	100	13	100	41	96		
Not reported	1	6	0	0	0	0	1	2		
Total	15	100	15	100	13	100	43	100	3.8	0.1
Deep brain stimulation										
Yes	1	7	2	13	1	8	4	9		
No	14	93	13	87	12	92	39	91		
Not reported	0	0	0	0	0	0	0	0		
Total	15	100	15	100	13	100	43	100	0.4	0.8
DOPA decarboxylase inhibitor and dopamine precursor (L-Dopa)										
Yes	15	100	14	93	13	100	42	98		
No	0	0	1	7	0	0	1	2		
Total	15	100	15	100	13	100	43	100	1.9	0.4
COMT inhibitor										
Yes	0	0	0	0	0	0	0	0		
No	15	100	15	100	13	100	43	100		
Total	15	100	15	100	13	100	43	100	0.0	1.0

Dopamine agonist										
Yes	6	40	4	27	7	54	17	40		
No	9	60	11	73	6	46	26	60		
Total	15	100	15	100	13	100	43	100	2.1	0.3
Mono amine oxidase inhibitor										
Yes	0	0	0	0	0	0	0	0		
No	15	100	15	100	13	100	43	100		
Total	15	100	15	100	13	100	43	100	0.0	1.0
N-methyl-D-aspartate antagonist										
Yes	2	13	1	7	1	8	4	9		
No	13	87	14	93	12	92	39	91		
Total	15	100	15	100	13	100	43	100	0.4	0.8
Anticholinergic										
Yes	0	0	1	7	1	8	2	5		
No	15	100	14	93	12	92	41	95		
Total	15	100	15	100	13	100	43	100	1.1	0.6
Antidepressant										
Yes	10	67	7	47	4	31	21	49		
No	5	33	8	53	9	69	22	51		
Total	15	100	15	100	13	100	43	100	3.6	0.2
Laxative or stool softener										
Yes	8	53	5	33	8	62	21	49		
No	7	47	10	67	5	38	22	51		
Total	15	100	15	100	13	100	43	100	2.3	0.3
Fiber supplements										
Yes	8	53	4	27	5	39	17	40		
No	7	47	11	73	8	61	26	60		
Total	15	100	15	100	13	100	43	100	2.2	0.3

Constipation problems over the past week of each of three assessment times

The participants provided their perceived constipation problems over the past week with the following scale: No constipation 0-point, Slight constipation 1-point, Mild constipation 2 points, Moderate constipation 3 points, Severe constipation 4 points.

Table 3.3. Constipation problems category

Term	Definition
Normal	No Constipation
Slight	I have been constipated. I use extra effort to move my bowels. However, this problem does not disturb my activities or my being comfortable.
Mild	Constipation causes me to have some troubles doing things or being comfortable.
Moderate	Constipation causes me to have a lot of trouble doing things or being comfortable. However, it does not stop me from doing anything.
Severe	I usually need physical help from someone else to empty my bowels.

Table 3.4. Constipation problems descriptives

	Treatment	Mean	Std. Deviation	N
Constipation problems over the past week, Time 1	1	1.57	0.94	14
	2	0.86	0.53	14
	3	1.42	0.90	12
	Total	1.28	0.85	40
Constipation problems over the past week, Time 2	1	0.93	1.00	14
	2	0.86	0.53	14
	3	1.42	1.16	12
	Total	1.05	0.93	40
Constipation problems over the past week, Time 3	1	0.93	0.83	14
	2	0.64	0.50	14
	3	1.50	1.00	12
	Total	1.00	0.85	40

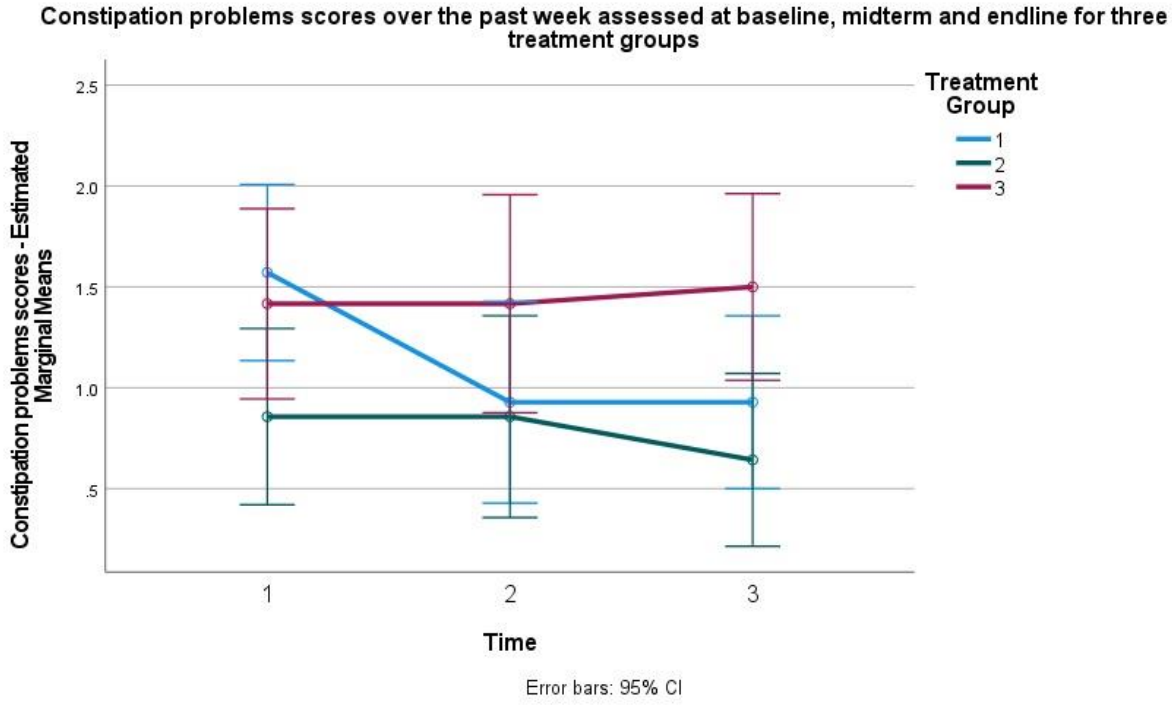

Figure 3.3. Constipation problem scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

The participants' reported constipation problems over the past week at each of three assessment time points (baseline, midterm, and endline) showed statistically significant differences between three times (F=5.94, p-value=0.02, Eta^2=0.14), and effect of interaction of time*treatment (F=3.91, p-value=0.03, df=2, Eta^2=0.18).

At the beginning, there was no statistically significant difference between three treatment group. Then, treatment1(probiotics+B_3 group) started showing decreasing trend (slope) towards the midterm (time2) and leveled-off until the end of the study (time3). Treatment2 (probiotics alone group) did not show much changing until midterm (time2), then it showed decreasing trend up to the end of the study (time3). Treatment3 (placebo group) also did not show much changing until midterm (time2), then it showed increasing trend up to the end of the study (time3). Overall, the decreased constipation problems in treatment2 (probiotics alone group) reached to the statistically significance compared to increased constipation problems in treatment3 (placebo group) after they consumed the probiotics or placebo for 12 weeks (mean difference=-0.857, Bonferroni adjusted p-value=0.027, SE=0.311). Changes over time of constipation problems in treatment1 and treatment2 groups were not statistically significant.

ROME-IV Questionnaires

ROME-IV diagnostic questionnaires for adult covered last 3 months duration. ROME-IV assessment questionnaires were analyzed for two assessment time points, baseline and endline.

Stool types – type 1 or 2 (hard or lumpy stools)

There was no statistically significant difference in stool type 1 or 2 (hard or lumpy stools) among the three treatment groups (F=0.037, df=2, p=0.964, N=38).

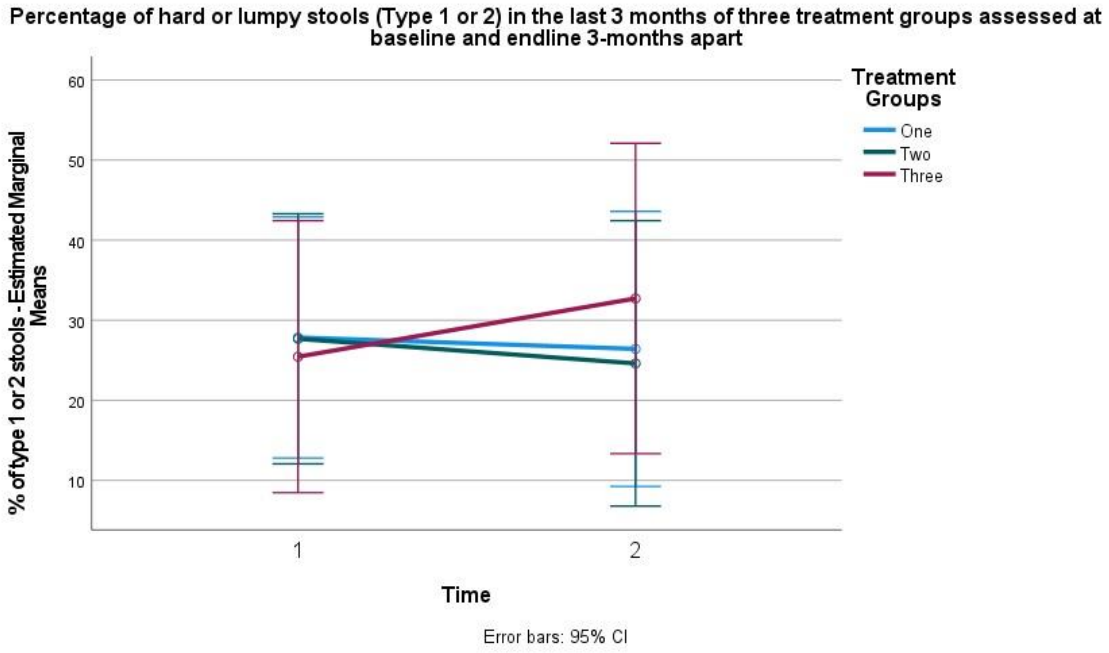

Figure 3.4. Stool type 1 or 2 in the last 3 months of three treatment groups assessed at baseline and endline

Stool types – type 6 or 7 (mushy or watery stools)

There was not statistically significant difference in stool type 6 or 7 (mushy or watery stools) among the three treatment groups (F=0.284, df=2, p=0.754, N=37).

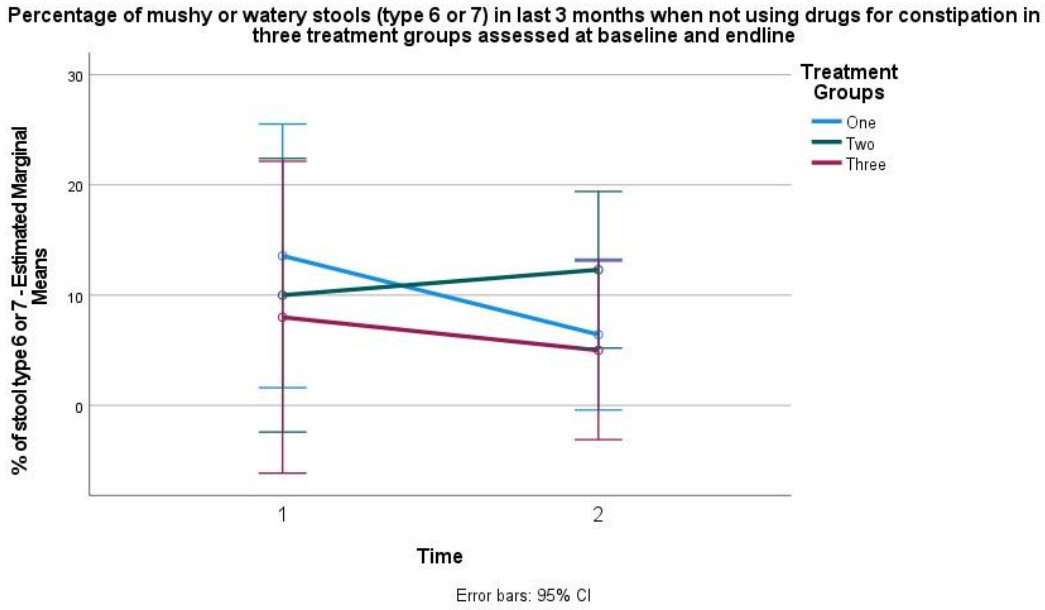

Figure 3.5. Percentage of mushy or watery stools (type 6 or 7) in last 3 months when not using drugs for constipation in three treatment groups assessed at baseline and endline

Percentage of fewer than 3 bowel movements a week without taking a laxative

There was not statistically significant difference in % of fewer than 3 bowel movements a week without taking a laxative or enema (F=0.356, df=2, p=0.703, N=38) among three treatment groups assessed at baseline and endline.

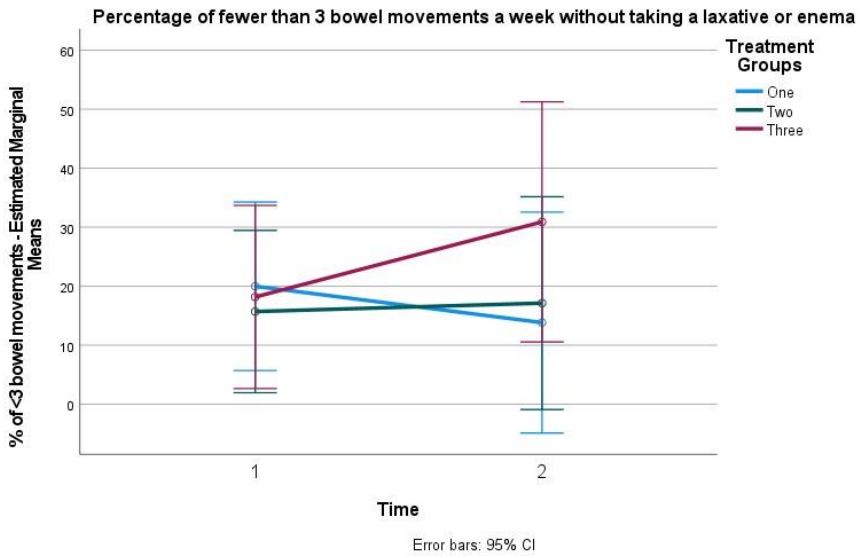

Figure 3.6. Percentage of fewer than 3 bowel movements a week without taking a laxative or enema in three treatment groups assessed at baseline and endline

Percentage of straining bowel movements in the last 3 months

There were not statistically significant difference in % straining bowel movements in the last 3 months from each assessment time (F=0.811, df=2, p=0.453, N=38) among three treatment groups assessed at baseline and endline.

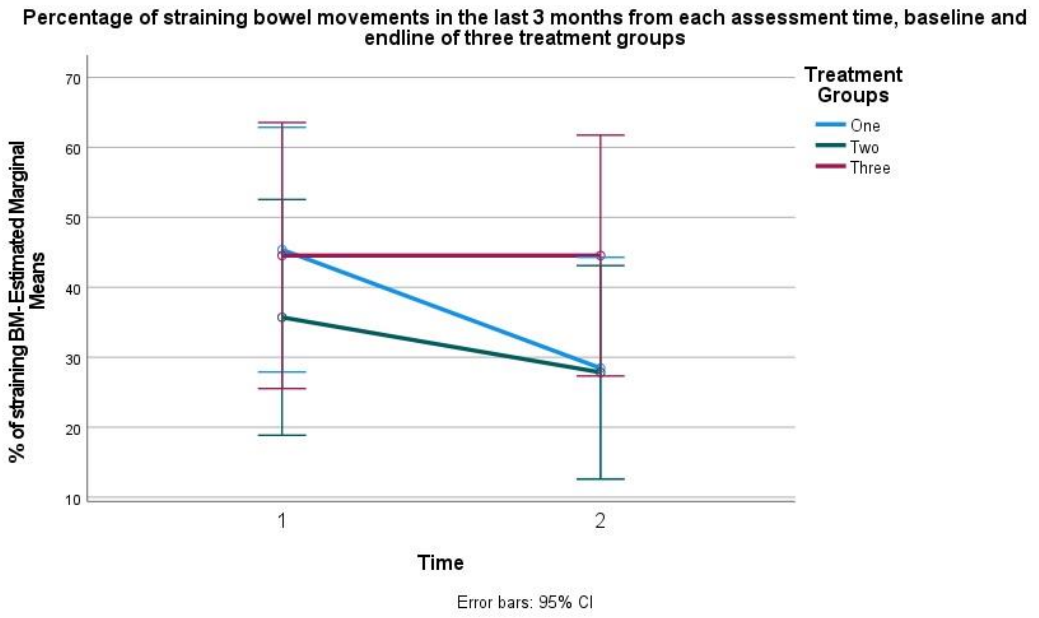

Figure 3.7. Percentage of straining bowel movements in the last 3 months from each assessment time, baseline and endline of three treatment groups

Percentage of feeling of incomplete emptying bowel movements in the last 3 months

There was not statistically significant difference in % incomplete emptying bowel movements in the last 3 months from each assessment time of baseline and endline (F=0.021, df=2, p=0.979, N=38) among three treatment groups.

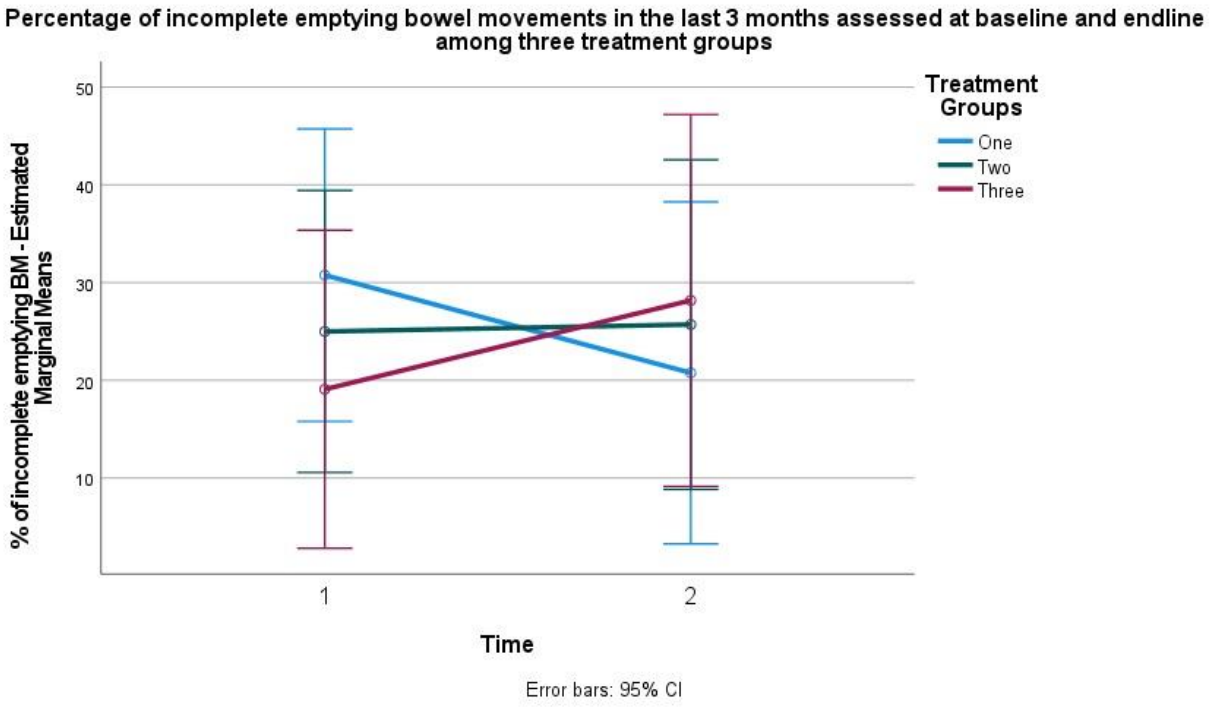

Figure 3.8. Percentage of incomplete emptying bowel movements in the last 3 months assessed at baseline and endline among three treatment groups

Percentage of sensation of stool blockage/ anorectal obstruction in the last 3 months

There was not statistically significant difference in % sensation of stool blockage/ anorectal obstruction in the last 3 months from each assessment time of baseline and endline (F=1.218, df=2, p=0.308, N=37) among three treatment groups.

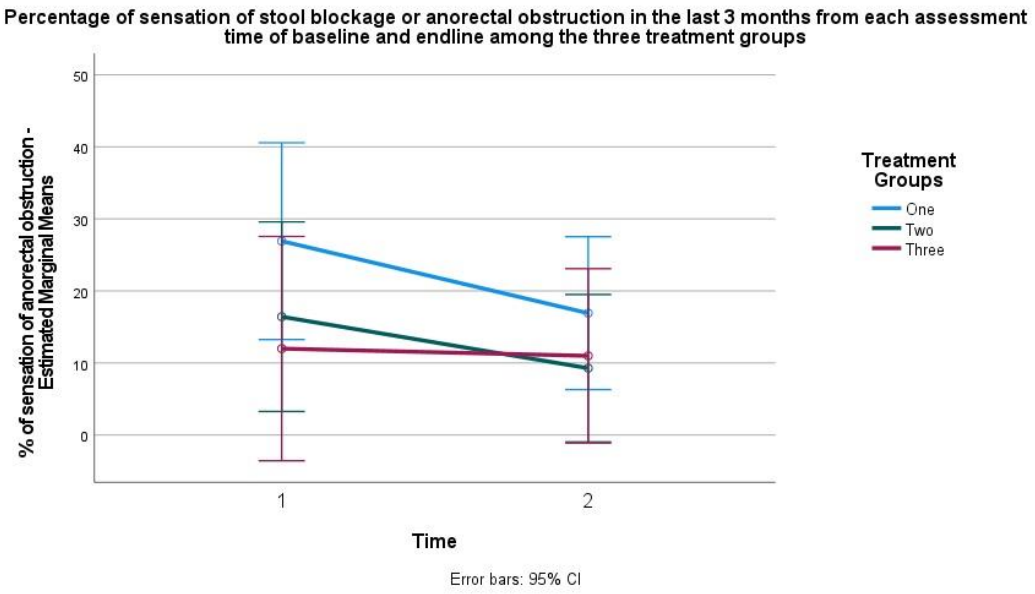

Figure 3.9. Percentage of sensation of stool blockage or anorectal obstruction in the last 3 months assessed at baseline and endline among the three treatment groups

Percentage of manual maneuver required in the last 3 months

There was not statistically significant difference in % manual maneuver required in the last 3 months from each assessment time of baseline and endline (F=0.024, df=2, p=0.976, N=38) among three treatment groups.

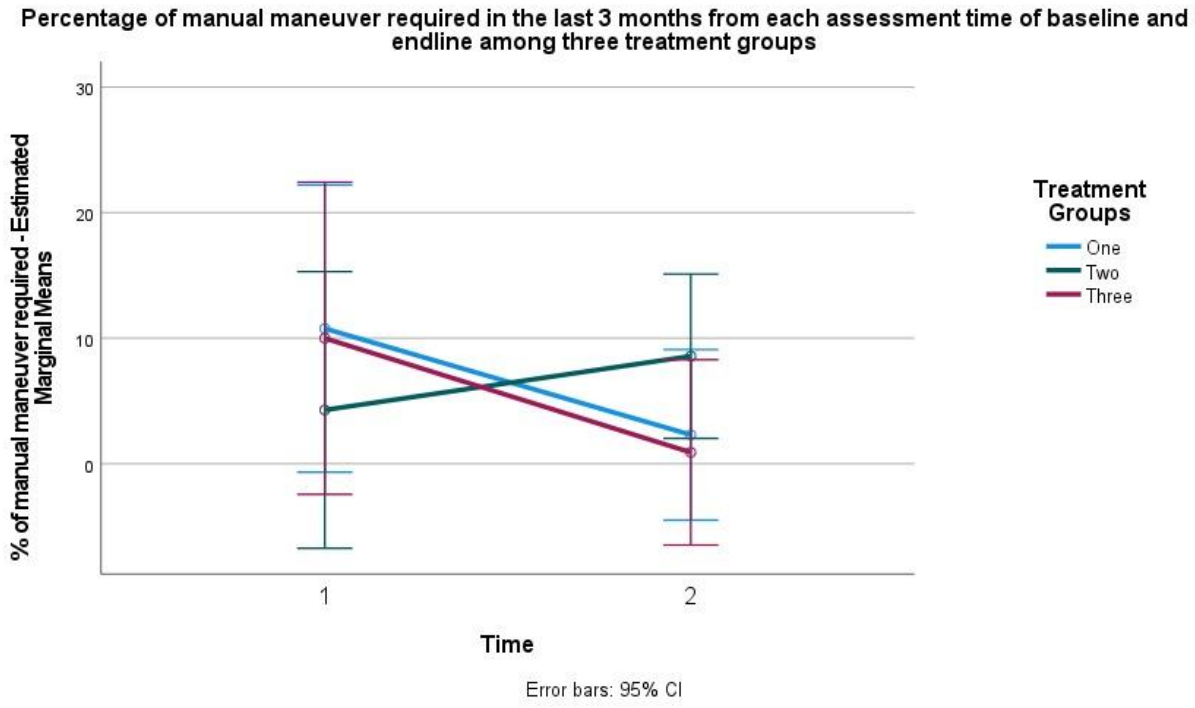

Figure 3.10. Percentage of manual maneuver required in the last 3 months assessed at baseline and endline in three treatment groups

Abnormal stools in the last three months

There was not statistically significant difference in having abnormal stools in the last 3 months from each assessment time of baseline and endline (Friedman test statistics=0.818, df=1, p=0.366, N=36) among three treatment groups.

Bowel Movement Diary – 12 weeks record analyses

Stool type 1 or 2 or 0 (hard or lumpy stools)

There was not statistically significant difference in stool type 1 or 2 or 0 in three treatment groups over the 12-week intervention period (F=0.202, df=2, p=0.818, N=33).

Stool type 3 or 4 or 5 (normal stools)

Percentage of stool types (3 or 4 or 5) report was statistically significantly increased between the beginning and mid-term in placebo group, treatment3, (mean difference=-24.3, Bonferroni adjusted p-value=0.032, N=33). There were no other statistically significant differences for normal stools between treatment groups.

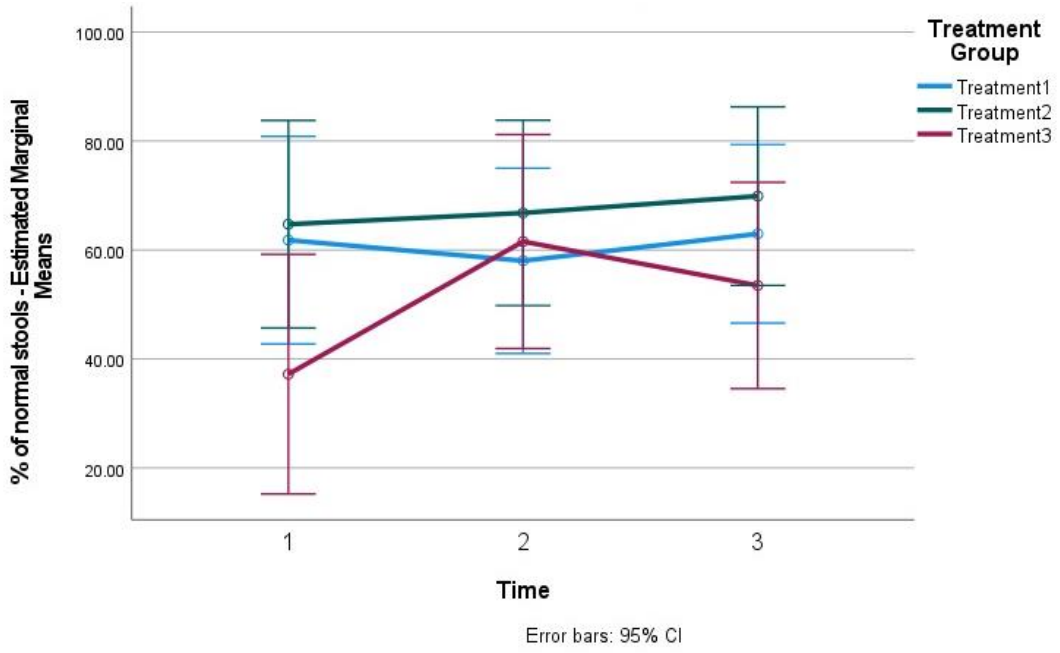

Figure 3.11. Percentage of stool types 3 or 4 or 5 reported in bowel movement diary over the 12-week intervention period

Stool type 6 or 7 (mushy or watery stools)

There were not statistically significant differences in stool type 6 or 7 in three treatment groups over the 12-week intervention period (Friedman test statistics=0.851, df=2, p=0.654, N=33).

Thee were no statistical differences for percentages of no bowel movement (p=0.520), straining bowel movements (p=0.416), incomplete evacuation (p=0.365), anorectal obstruction

(p=0.535), manual maneuver needed (p=0.06), spontaneou defecation (p=0.844), laxative use (p=0.426) over the 12-week period.

Table 3.5. Descriptives of bowel movement diary recorded for 12 weeks

Time	Bowel Movement Diary Report	Treatment1			Treatment2			Treatment3		
		Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N
Time1	% of stool type 1 or 2 or 0	32.4	39.8	12	30.1	32.1	12	32.2	29.8	9
	% of stool type 3 or 4 or 5	61.8	37.6	12	64.7	28.9	12	37.2	28.7	9
	% of stool type 6 or 7	5.7	10.8	12	5.2	12.1	12	30.6	35.8	9
	% of straining abdominal pain (Part1)	18.8	23.7	12	24.2	21.0	12	22.4	18.8	9
	% of incomplete evacuation (Part1)	19.0	27.3	12	17.0	20.0	12	37.6	29.4	9
	% of anorectal obstruction (Part1)	2.1	7.2	12	3.0	7.6	12	0.0	0.0	9
	% of manual maneuvers required (Part1)	0.0	0.0	12	4.5	12.5	12	0.0	0.0	9
	% of spontaneous defecations (Part1)	44.9	48.5	12	47.5	42.5	12	43.3	44.8	9
	% of laxative use (Part1)	43.3	47.4	12	13.3	30.0	12	37.8	48.4	9
Time2	% of Stool type 1 or 2 or 0	36.3	35.8	12	27.3	26.8	12	26.4	25.0	9
	% of Stool type 3 or 4 or 5	58.0	34.4	12	68.8	26.7	12	61.6	22.6	9
	% of Stool type 6 or 7	5.7	10.6	12	5.9	8.1	12	12.1	16.8	9
	%of straining abdominal pain (Part2)	16.2	17.7	12	24.3	19.4	12	29.5	28.2	9
	% of incomplete evacuation (Part2)	14.5	17.2	12	26.7	22.6	12	25.6	23.7	9
	% of anorectal obstruction (Part2)	3.3	10.6	12	2.9	8.6	12	0.4	1.2	9
	% of manual maneuvers required (Part2)	0.0	0.0	12	7.2	14.3	12	0.0	0.0	9
	% of spontaneous defecations (Part2)	46.4	43.8	12	45.3	38.8	12	40.8	36.5	9
	% of laxative use (Part2)	40.5	48.6	12	14.9	31.0	12	36.9	46.8	9
Time3	% of Stool type 1 or 2 or 0	31.5	34.4	12	22.6	29.5	12	39.7	19.4	9
	% of Stool type 3 or 4 or 5	63.0	31.8	12	69.9	28.6	12	53.5	19.6	9
	% of Stool type 6 or 7	5.5	7.4	12	7.4	10.1	12	6.8	10.5	9
	% of straining abdominal pain (Part3)	14.7	20.2	12	20.2	18.2	12	32.6	31.3	9
	% of incomplete evacuation (Part3)	11.7	16.2	12	23.7	29.0	12	22.1	30.0	9
	% of anorectal obstruction (Part3)	2.8	9.5	12	3.0	7.3	12	0.7	1.7	9
	% of manual maneuvers required (Part3)	0.3	0.6	12	5.0	7.6	12	0.2	0.6	9
	% of spontaneous defecations (Part3)	47.6	41.6	12	46.7	44.6	12	28.2	34.7	9
	% of laxative use (Part3)	40.5	39.3	12	20.7	37.8	12	37.4	47.5	9

Microbiome Analyses Results

Alpha Diversity

Faith's Phylogenetic Diversity

The Faith's phylogenetic diversity showed there were statistically significant differences (Kruskal-Wallis, H=6.24, p-value=0.04) among all groups of Times: Time 1 (n=40), Time 2 (n=41), Time 3 (n=40); and the phylogenetic diversity richness difference was detected between the pre-intervention, Time1 (baseline or Week 0) and Post-intervention, Time 3 (week-12), (Kruskal-Wallis pairwise, H=6.45, p-value=0.01, q-value=0.03). There were no statistically significant differences of the Faith's phylogenetic diversity between Time1/Week0 and Time2/Week6 (Kruskal-Wallis pairwise, H=0.70, p-value=0.40, q-value=0.40), and between Time 2/Week6 and Time 3/Week12 (Kruskal-Wallis pairwise, H=2.23, p-value=0.13, q-value=0.20). Stool consistency was negatively correlated with species richness (Vandeputte et al., 2016).

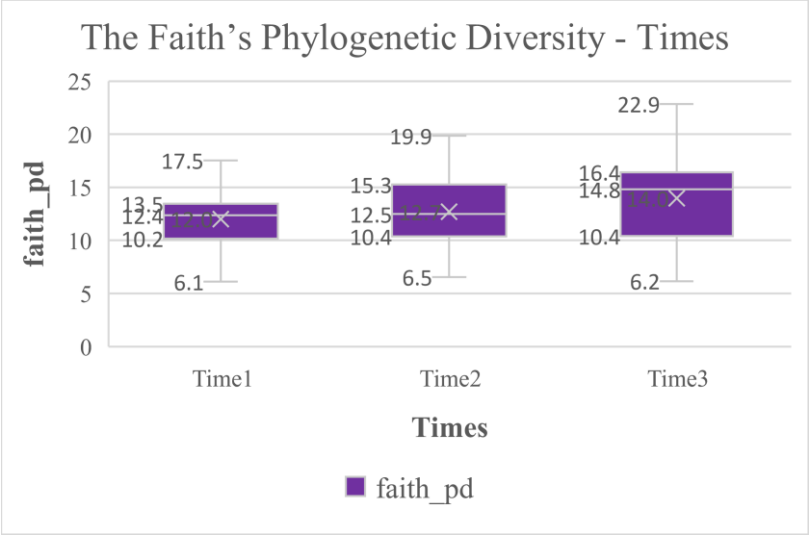

Figure 3.12. The Faith Phylogenetic Diversity of all assessed at three times- Time1: Week 0, Time2: Week 6, Time3: Week 12

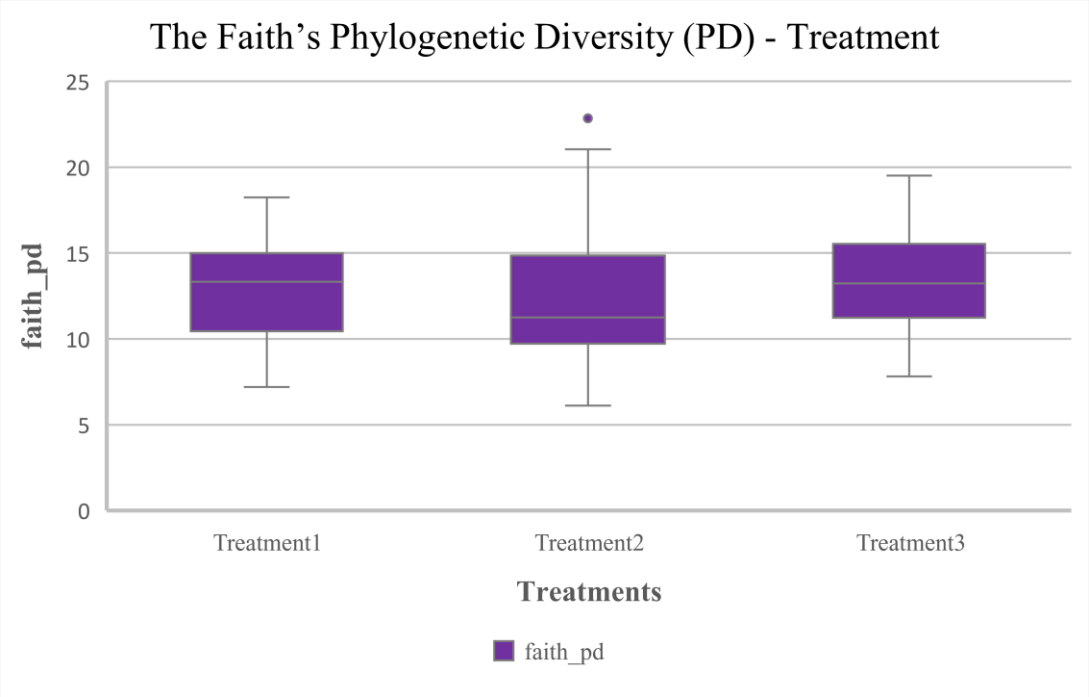

Figure 3.13. The Faith Phylogenetic Diversity of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

The Faith's phylogenetic diversity showed there was not statistically significant differences (Kruskal-Wallis, H=4.07, p-value=0.13) between treatment groups: Treatment 1 (n=43), Treatment 2 (n=41), Treatment 3(n=37).

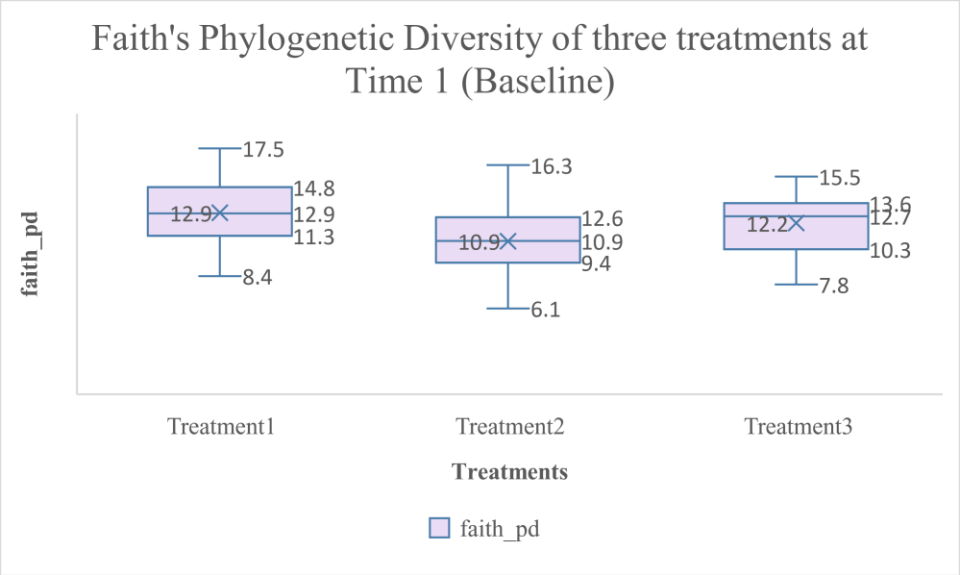

Figure 3.14. The Faith's Phylogenetic Diversity of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0

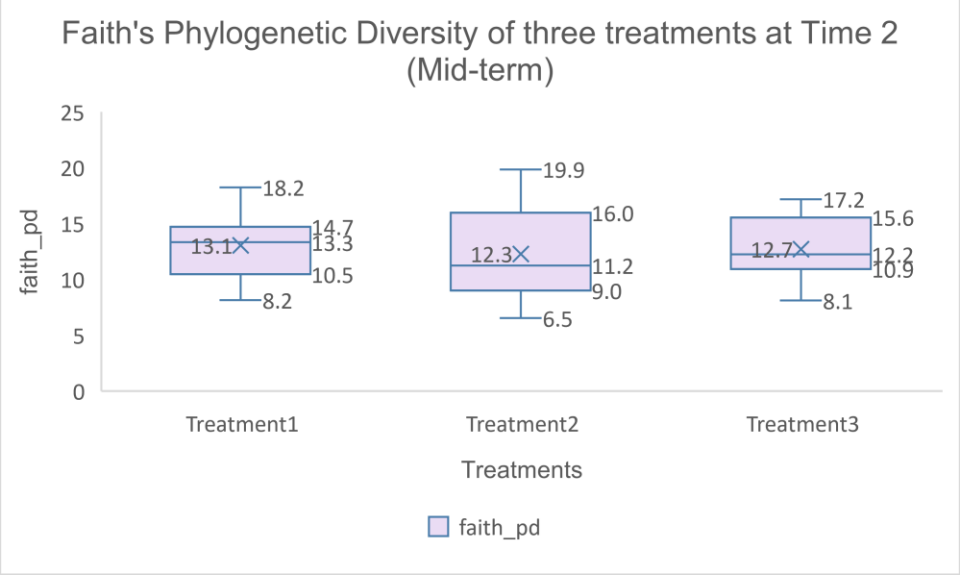

Figure 3.15. The Faith's Phylogenetic Diversity of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time2: Week 6

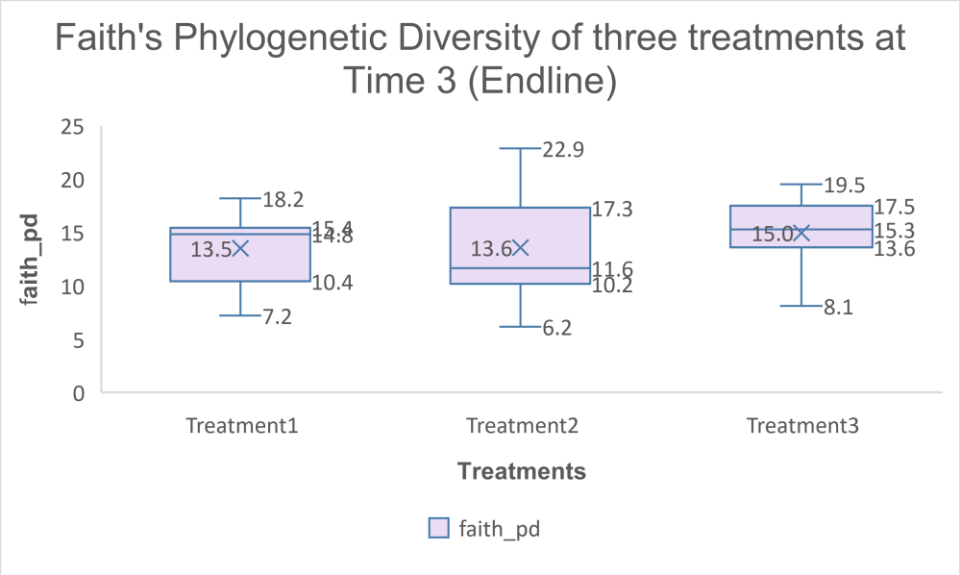

Figure 3.16. The Faith's Phylogenetic Diversity of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time3: Week 12

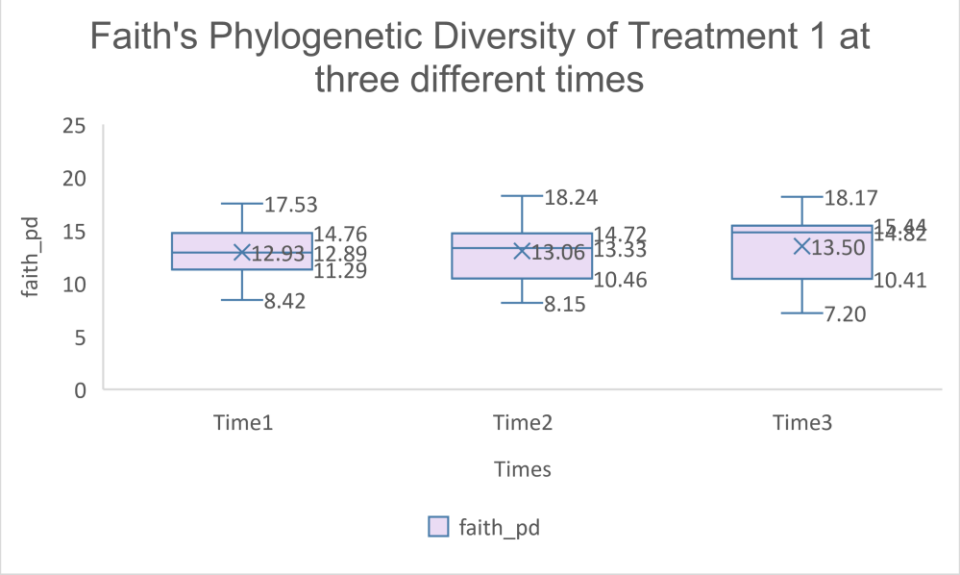

Figure 3.17. The Faith's Phylogenetic Diversity of Treatment1: Probiotics+B_3 group assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

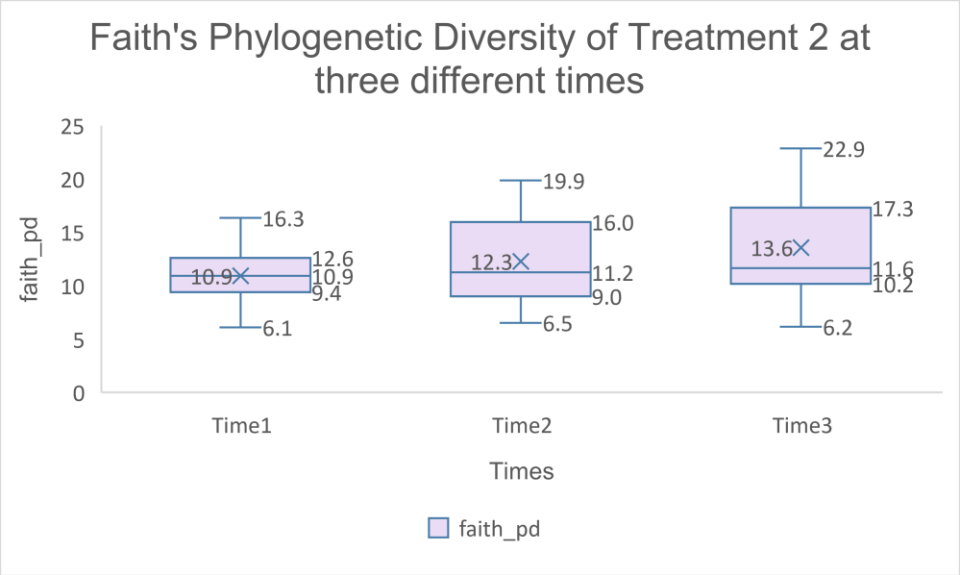

Figure 3.18. The Faith's Phylogenetic Diversity of Treatment2: Probiotics group assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

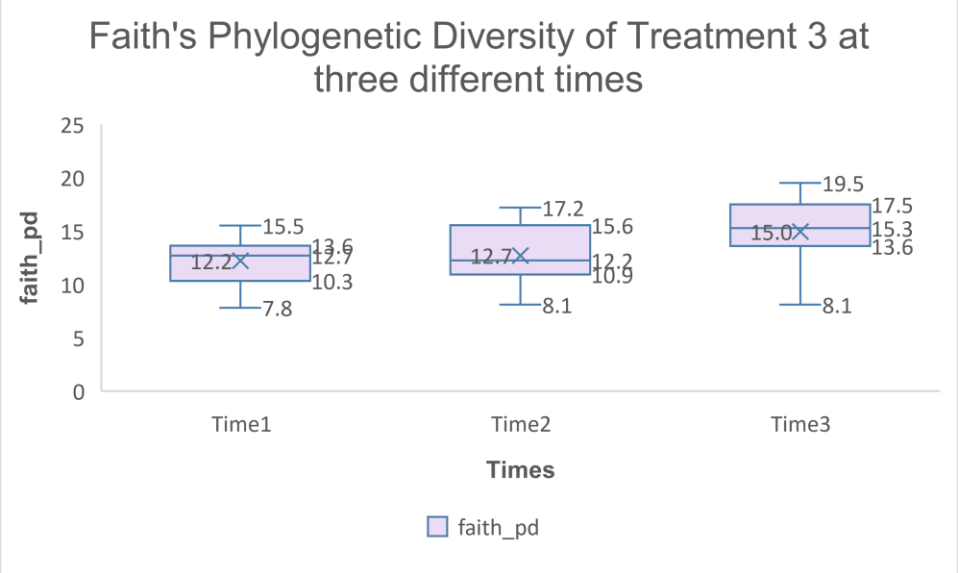

Figure 3.19. The Faith's Phylogenetic Diversity of Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

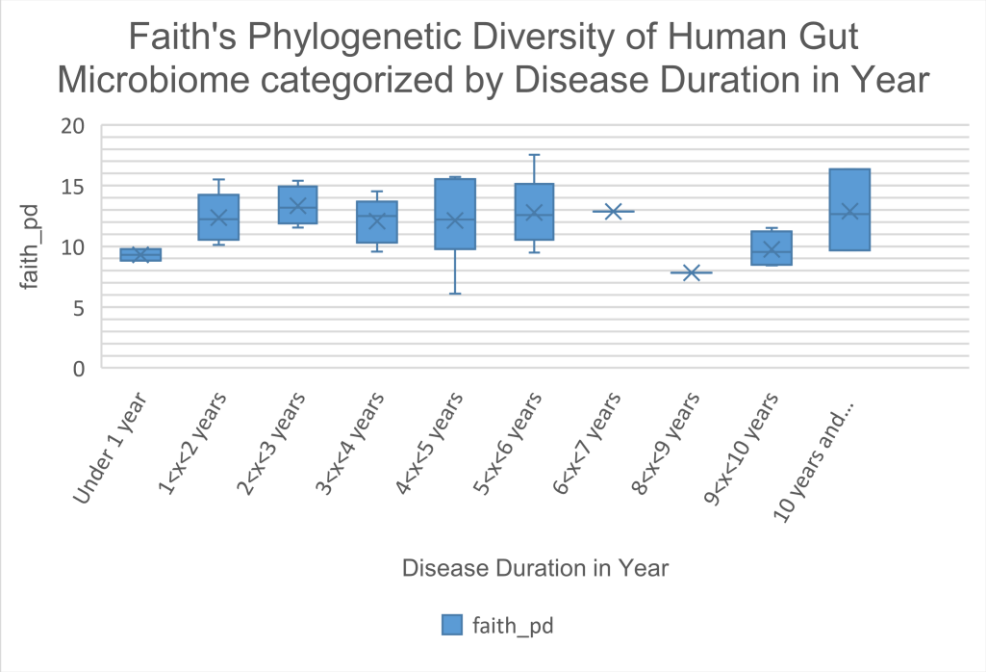

Figure 3.20: Faith's Phylogenetic Diversity of human gut microbiome samples from participants categorized by their disease duration from diagnosis (in years)

In this study, the alpha diversity index (Shannon-group-significance) indicated no statistically significant differences (H=4.4, p-value=0.11) among samples collected from treatment group 1 (n=43), treatment group 2 (n=41), and treatment group 3 (n=37) (Figure 3.13).

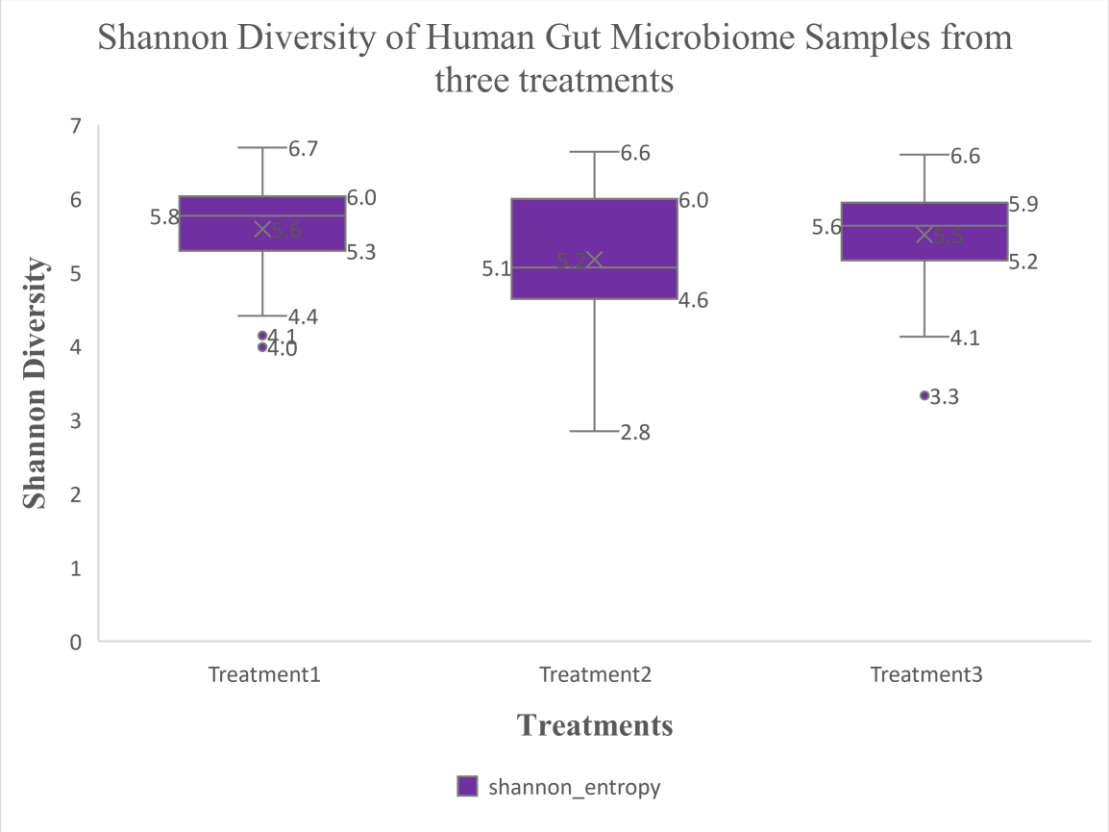

Figure 3.21. Shannon Diversity of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

Table 3.6. Shannon Diversity, Kruskal-Wallis (pairwise) of human gut microbiome samples from three treatments groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

Group 1	Group 2	H	p-value	q-value
Treatment1 (n=43)	Treatment2 (n=41)	3.75	0.05	0.16
Treatment1 (n=43)	Treatment3 (n=37)	0.31	0.58	0.58
Treatment2 (n=41)	Treatment3 (n=37)	2.48	0.12	0.17

Evenness Group Significance

In terms of alpha-diversity evenness (pielou_e) among gut microbiome samples, there was statistically significant difference of evenness (H=9.94, p-value=0.01) between the disease condition groups by MDS-UPDRS scores (Martínez-Martín et al., 2015): Mild (n=79), Moderate

(n=41), and Advance (n=1), and the statistically significant differences of evenness (H=9.76, p-value=0.00, q-value=0.01, Kruskal-Wallis Pairwise) was seen between 'Mild Condition Group' and 'Moderate Condition Group'. Mild condition group has more evenness of phylogenetic diversity compared to moderate condition group (Table 3.5, Figure 3.14). There were no statistically significant differences of evenness (H=3.83, p-value=0.14) among treatment groups: Treatment 1 (n=43), Treatment 2 (n=41) and Treatment 3 (n=37).

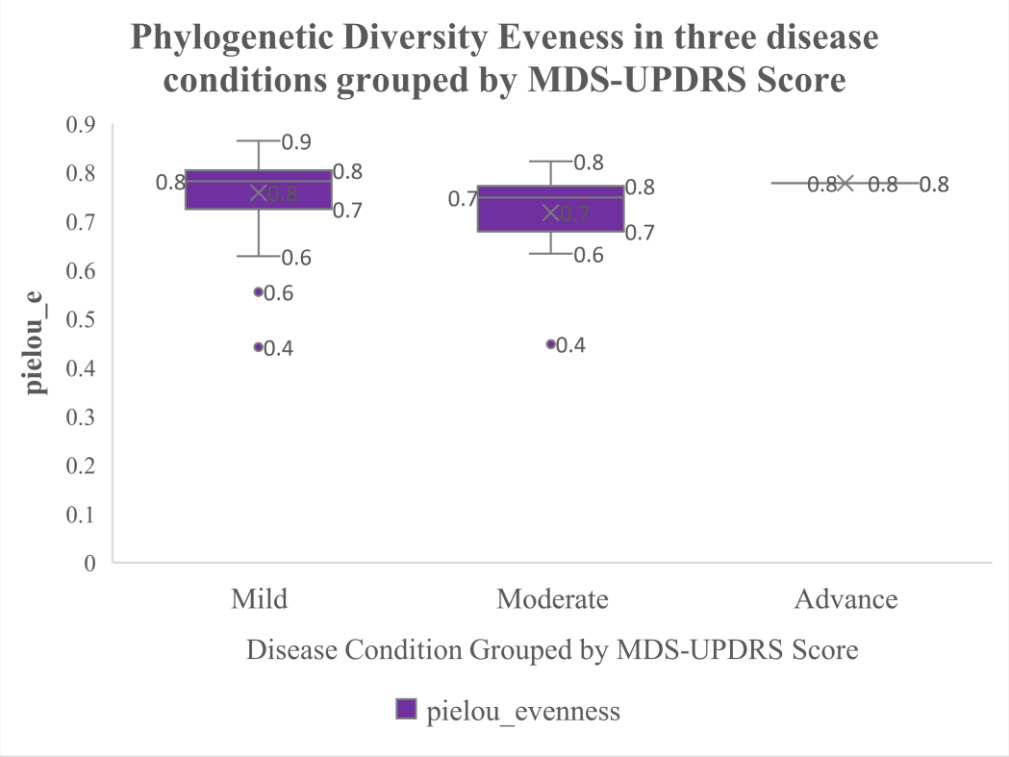

Figure 3.22. Phylogenetic Diversity Evenness of gut microbiome samples from three disease condition groups categorized by MDS-UPDRS Score

Table 3.7. Phylogenetic Diversity Evenness Among Gut Microbiome Samples from Three Disease Condition by MDS-UPDRS Score

Group 1	Group 2	H	p-value	q-value
Advance (n=1)	Mild (n=79)	0.04	0.85	0.85
Advance (n=1)	Moderate (n=41)	0.90	0.34	0.51
Mild (n=79)	Moderate (n=41)	9.76	**0.00***	0.01

Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) was performed to test whether multiple effects significantly impacted alpha diversity. Treatment alone did not show statistically significant difference on alpha diversity (F=1.15, p-value=0.32). Time (i.e. Baseline or Week-0, Midterm or Week-6, and Endline or Week-12) showed statistically significant difference (F=3.80, p-value=0.025), and the pairwise analyses indicated the statistically significant difference was between ‘Time3 or Endline’ Vs ‘Time1 or Baseline’ (test statistics=2.72, p-value=0.008, FDR p-value=0.022). When time and treatment were accounted, ‘Time’ showed statistically significant difference (F=3.78, p-value=0.026), ‘Treatment’ (Treatment1, Treatment2, Treatment3) did not show statistically significant difference (F=1.12, p-value=0.301), and ‘Treatment:Time’ effect did not show statistically significant difference (F=0.59, p-value=0.672) in alpha diversity, Faith phylogenetic diversity.

Table 3.8. ANOVA model results of Faith's Phylogenetic Diversity for Treatment, Time, Treatment:Time

	SS	df	F	p-value
Treatment	25.33	2	1.21	0.302
Time	79.09	2	3.78	0.026
Treatment:Time	24.61	4	0.59	0.672
Residual	1171.88	112		

When age was accounted, there was statistically significant impact of Age-Treatment interaction on alpha diversity (F=4.21, p-value=0.00), and Age became more significant than Treatment for alpha diversity, Age had statistically significant impact (F=2.44, p-value=0.04) but Treatment was not statistically significant (F=0.0, p-value=0.9).

When age, disease duration, disease status by MDS-UPDRS score (Martínez-Martín et al., 2015), and fiber supplements were accounted for in the model, Age:Disease duration interaction showed statistically significant (F=3.15, p-value=0.04) impact on alpha diversity, Age: Disease duration: Disease status by MDS-UPDRS score interaction showed statistically significant (F=6.35, p-value=0.00), Age: Treatment: Disease duration: Disease status by MDS-UPDRS score interaction showed statistically significant (F=7.41, p-value=0.00), Age: Disease duration: Disease status by MDS-UPDRS score: Fiber supplements interaction showed statistically significant (F=3.61, p-value=0.03) impact on alpha diversity, Treatment: Disease duration: Disease status as per MDS-UPDRS score: Fiber supplements interaction showed statistically significant (F=23.07, p-value=0.00) impact on alpha diversity, and Age: Treatment: Disease duration: Disease status by MDS-UPDRS score: Fiber supplements interaction showed statistically significant (F=9.52, p-value=0.00) impact on alpha diversity.

Beta Diversity

Beta Diversity analysis comparing dissimilarity of microbial communities in samples collected from three treatment groups

Some dissimilarity of microbial communities in samples from treatment 1, 2 and 3 were investigated with beta diversity analysis performed using QIIME2 based on unweighted unifrac metric, visualized as Principal Coordinate Analysis (PCOA) plot (Figure 3.15). Based on the PCoA plot, the sample clusters had a maximum variation in 15.56% in Axis 1 and 7.18% in Axis 2 and 5.98% in Axis 3 with presumably separate clustering of samples from treatment 1, 2 and 3. Samples collected from treatment 2 grouped a little more distinctly from treatment 1 and 3. The

variation in clustering patterns indicated some differences in microbial community composition among three treatments.

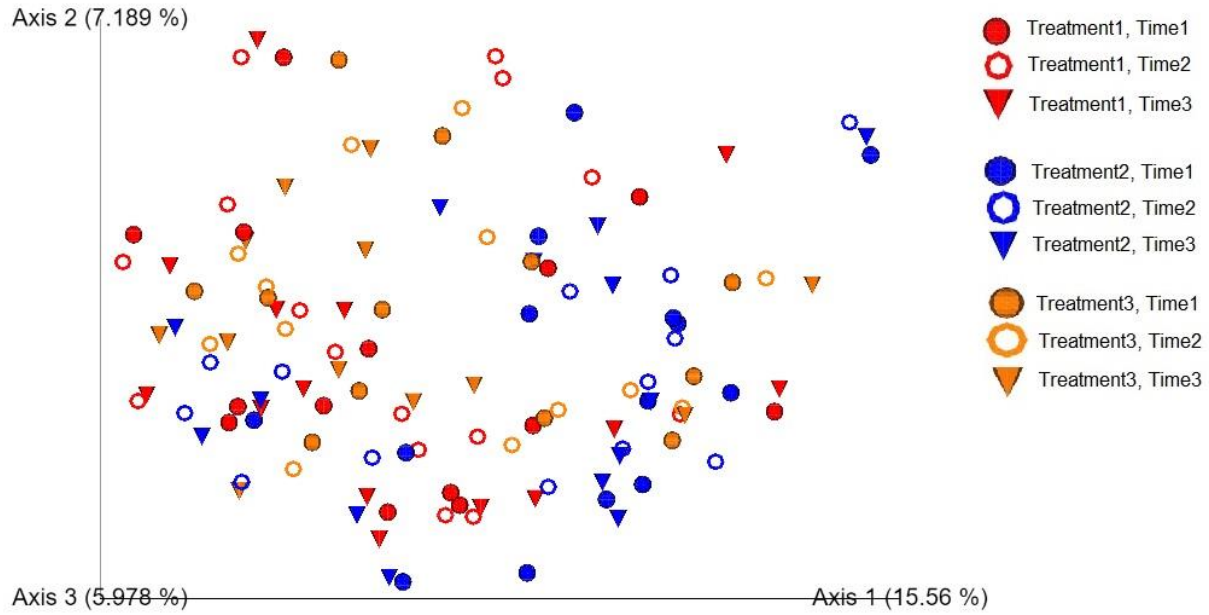

Figure 3.23. Unweighted Unifrac Emperor of Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed for three times- Time1: Week 0, Time2: Week 6, and Time3: Week 12

The dissimilarity of microbial communities in the samples from People living with PD receiving three different treatment groups: treatment 1, 2 and 3 were investigated with beta diversity analysis performed using QIIME2 based on weighted unifrac metric, visualized as Principal Coordinate Analysis (PCOA) plot (Figure 3.16). Based on the PCoA plot, the sample clusters had a maximum variation in 45.46% in Axis 1 and 8.83% in Axis 2 and 7.84% in Axis 3 with presumably separate clustering of samples from treatment 1, 2 and 3. Samples collected from treatment 2 spread more distinctly from clusters of treatment 1 and 3.

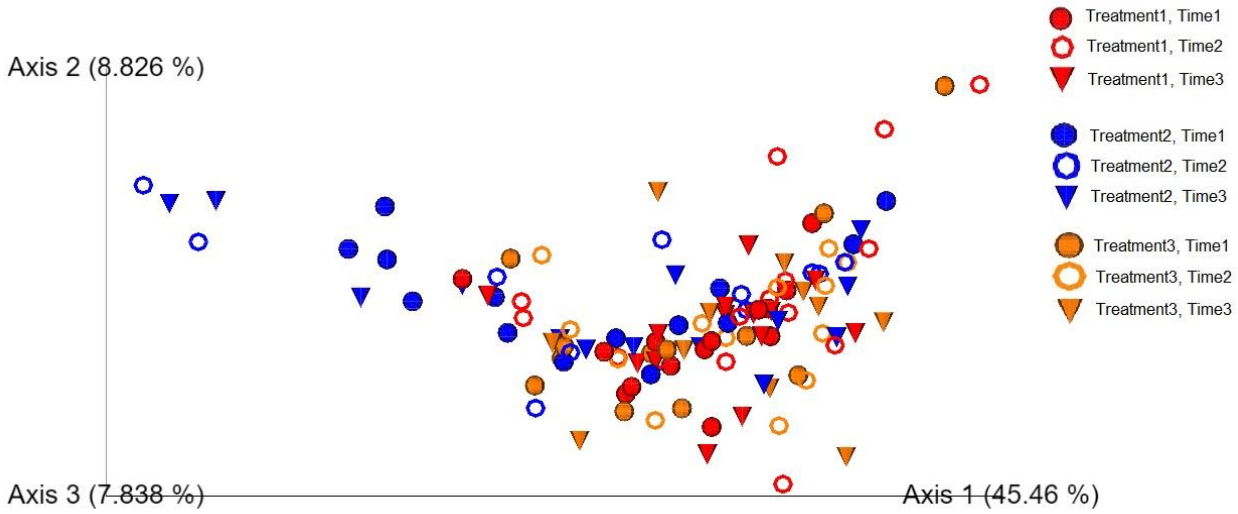

Figure 3.24. Weighted Unifrac Emperor PCoA plot showing microbial community dissimilarity among three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at three times- Time1: Week 0, Time2: Week 6, and Time3: Week 12

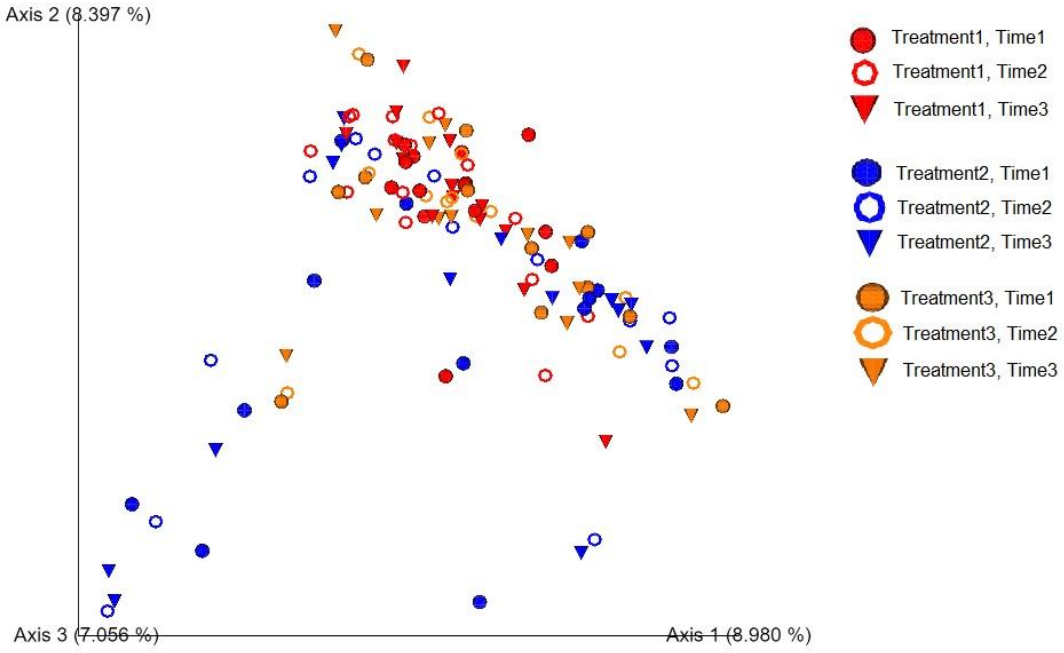

Figure 3.25. Bray Curtis Emperor PCoA plot showing microbial community dissimilarity among three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at three times- Time1: Week 0, Time2: Week 6, and Time3: Week 12

Permutational Multivariate Analysis of Variance (PERMANOVA) and Multivariate Analyses

The significance of the treatment group differences on human gut bacterial ecology was evaluated by performing a permutation based PERMANOVA (Permutational Multivariate Analysis of Variance). In unweighted UniFrac distance, statistically significant difference (test statistic=3.1, p-value=0.001, N=121, number of groups=3), and the statistically significant differences were found in Treatment1 Vs Treatment2 [test statistics=3.63, adjusted p-value (q-value)=0.002, n=84], Treatment1 Vs Treatment3 [test statistics=2.48, adjusted p-value (q-value)=0.002], and Treatment2 Vs Treatment3 [test statistics=3.15, adjusted p-value (q-value)=0.002]. Also, in weighted UniFrac distance, statistically significant difference (test statistic=5.27, p-value=0.001, N=121, number of groups=3) was observed. The statistically significant differences were found in Treatment1 Vs Treatment2 [test statistics=7.36, adjusted p-value (q-value) =0.006, n=84], and Treatment2 Vs Treatment3 [test statistics=5.63, adjusted p-value (q-value)=0.008], but in Treatment1 Vs Treatment3 [test statistics=1.90, adjusted p-value (q-value)=0.078].

Age also had statistically significant difference in human gut bacterial ecology (test statistics=2.37, p-value=0.001, N=121, number of groups=8). Age groups are 45-49 years, 50-54 years, 55-59 years, 60-64 years, 65-69 years, 70-74 years, 75-79 years, 80-84 years, 85-89 years, 90 years and above. Disease duration had statistically significant differences in human gut bacterial composition (test statistics=2.6, p-value=0.001, N=121, number of groups=10). Disease duration groups were under 1 year, 1-2 years, 2-3 years, 3-4 years, 4-5 years, 5-6 years, 6-7 years, 7-8 years, 8-9 years, 9-10years, and 10 years and above.

There were no statistically significant differences of beta phylogenetic diversity between times (time1 or week-0, time2 or week-6, and time3 or week 9), test statistics=0.72, p-value=0.94, N=121, number of groups=3).

The Adonis multivariate analysis results indicated that treatments (Probiotics+Vitamin B_3, Probiotics+Placebo, and Placebo) (p-value= 0.001), Age (p value=0.001), Disease Duration (p-value=0.001), Disease Status by MDS-UPDRS Score (p-value=0.02), and Fiber supplements (p-value=0.001) had a statistically significant association or influence over the differences in human gut bacterial community in people with PD who participated in this study. In this case, treatment explains 4.9% (R^2=0.049), age explains 13.5% (R^2=0.135), gender explains 2.4% (R^2=0.024), disease duration explained 16.9% (R^2=0.169), disease status based on MDS-UPDRS Score explained 1.8% (R^2=0.018), and fiber supplements explained 2% (R^2=0.020) of the overall diversity of human gut bacteria composition in this study.

Analysis of Composition of Microbiome (ANCOM)

"The W statistic is the number of ANCOM hypotheses that have passed for each individual taxon, indicating that the ratios of that taxon's relative abundance to the relative abundances of W other taxa were detected to be significantly different (typically FDR-adjusted $p < 0.05$). Because differential abundance in ANCOM is based on the ratio between tests, it does not produce a traditional p-value"(QIIME2)(Bolyen et al., 2019).

Center log ratio (CLR) indicated the importance of the Amplicon Sequence Variant (ASV) and the W statistics showed the impact of differential abundance of the ASV.

For the volcano plot, the W statistic was placed on the y-axis, and the F-score on the x-axis. The x-axis represented the effect size difference of the given taxa/species between the treatment groups, and the y-axis was the strength of the ANCOM test statistic. In the plot, the

ASVs with a high F-score and a high W-statistic were close to the top right corner. That indicated these ASVs were truly different across the treatment groups.

Veillonellaceae Dialister sp. was statistically significant in relative abundance composition of microbiome among different treatment groups (W=2619).

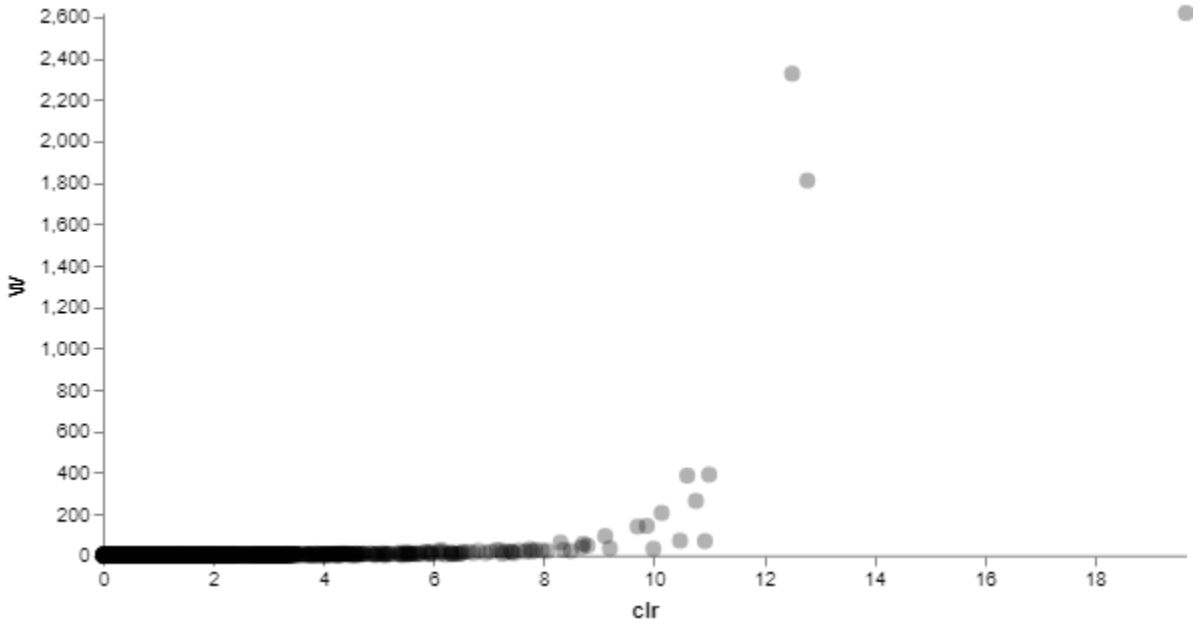

Figure 3.26. ANCOM Volcano Plot of significant abundance of *Dialister* sp.

Table 3.9. Percentile abundances of *Dialister* sp. by group

Percentile	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Treatment Group	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Dialister;s__	1	1	1	1	63	1	1	33	235	922	1	1	1	1	1374

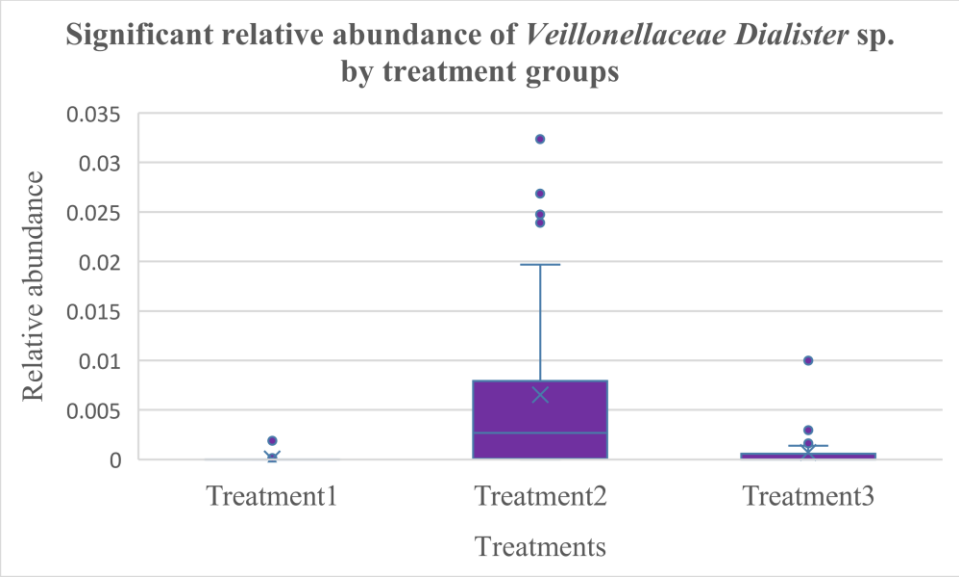

Figure 3.27. Significant relative abundance of microbiome among three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

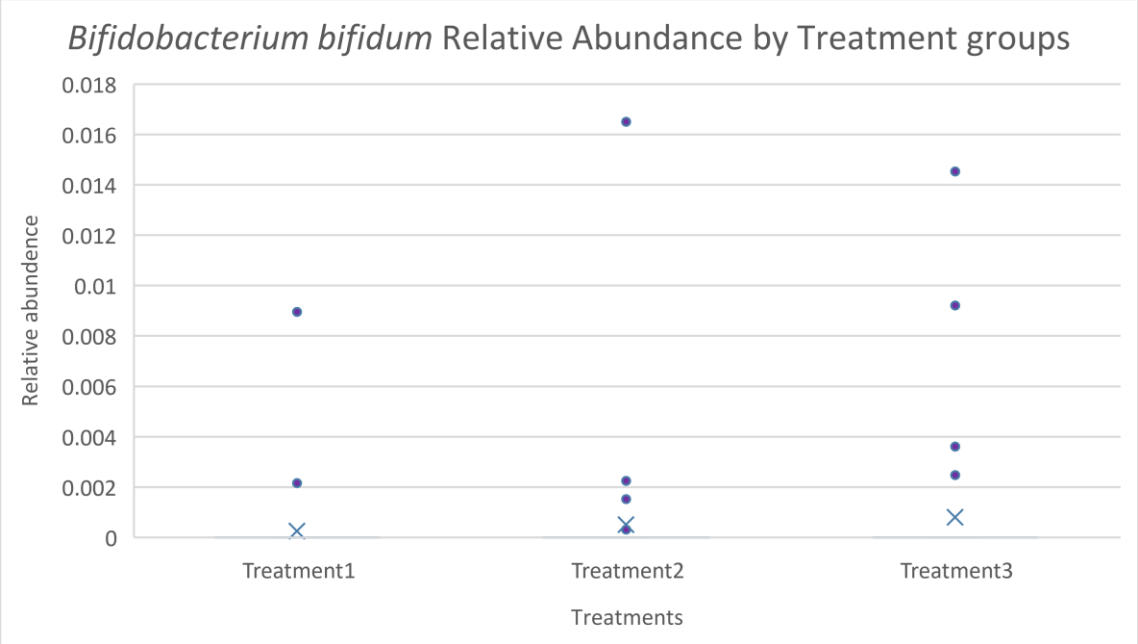

Figure 3.28. Differences in *Bifidobacterium bifidum* relative abundance among three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

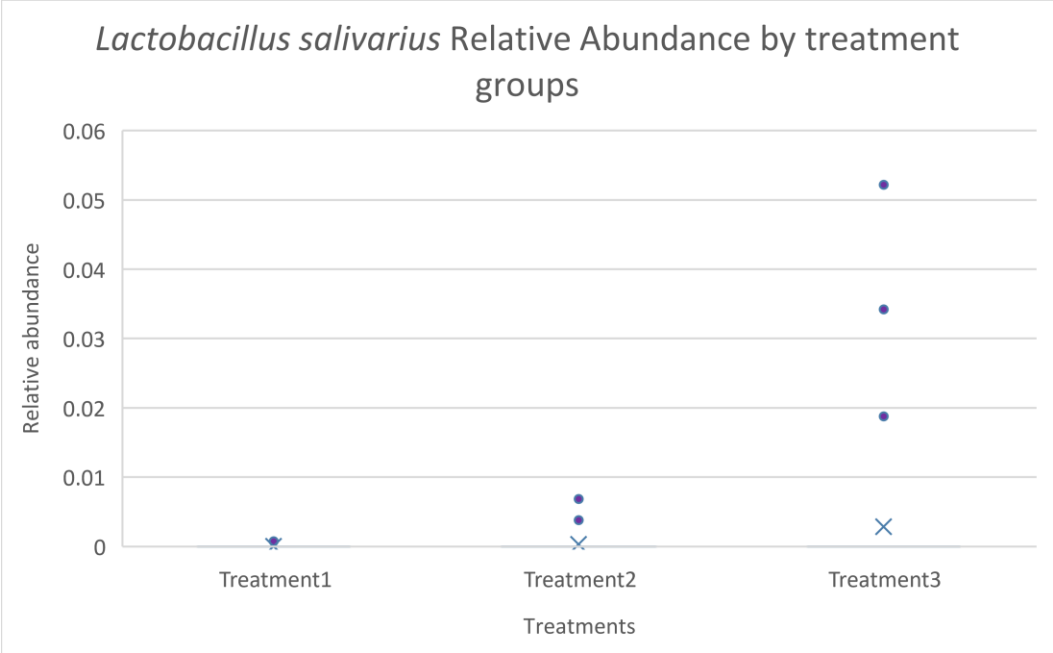

Figure 3.29. Differences in *Lactobacillus salivarius* relative abundance among three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo

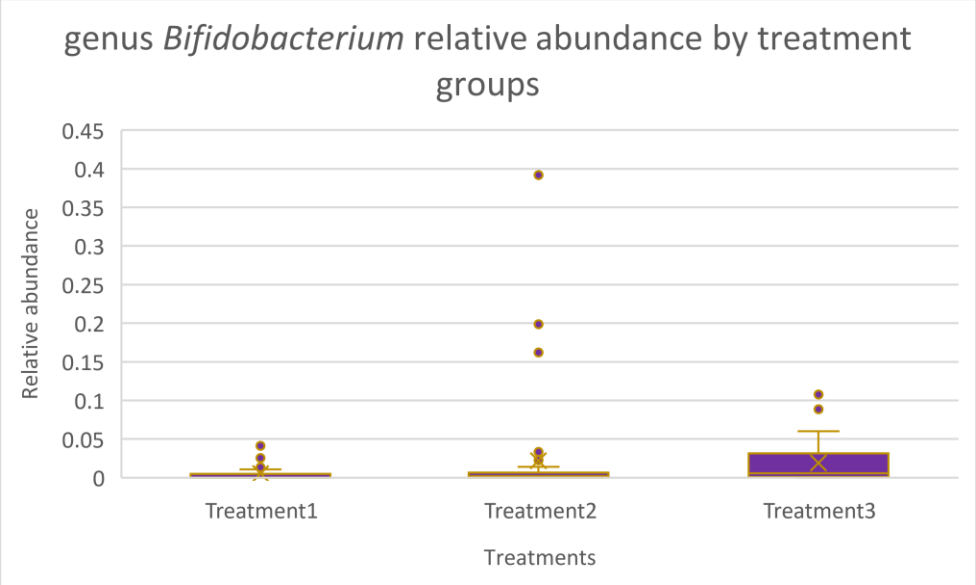

Figure 3.30. Differences in *Bifidobacterium* spp. relative abundance by treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

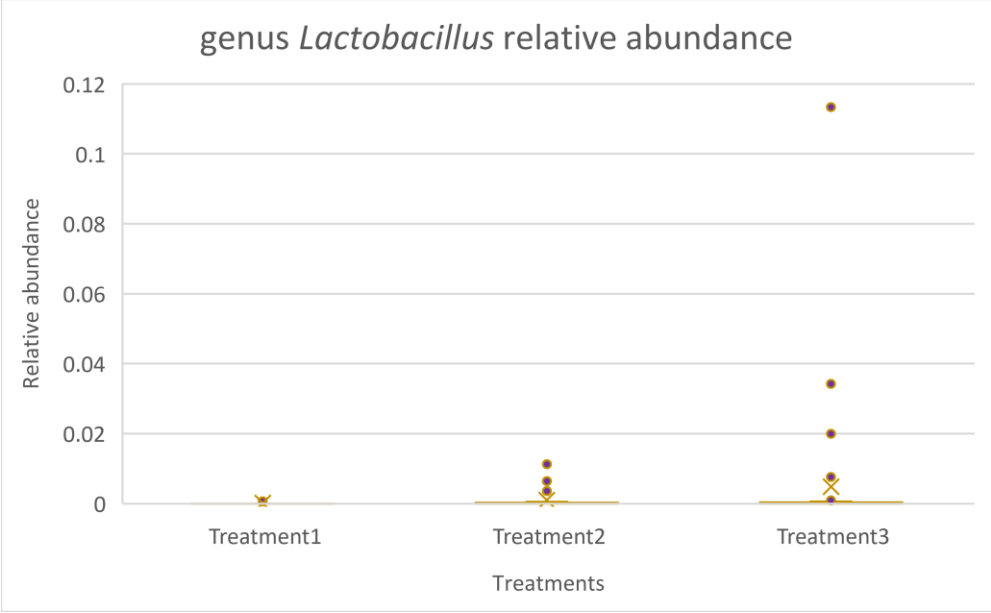

Figure 3.31. Differences in *Lactobacillus* spp. relative abundance by treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

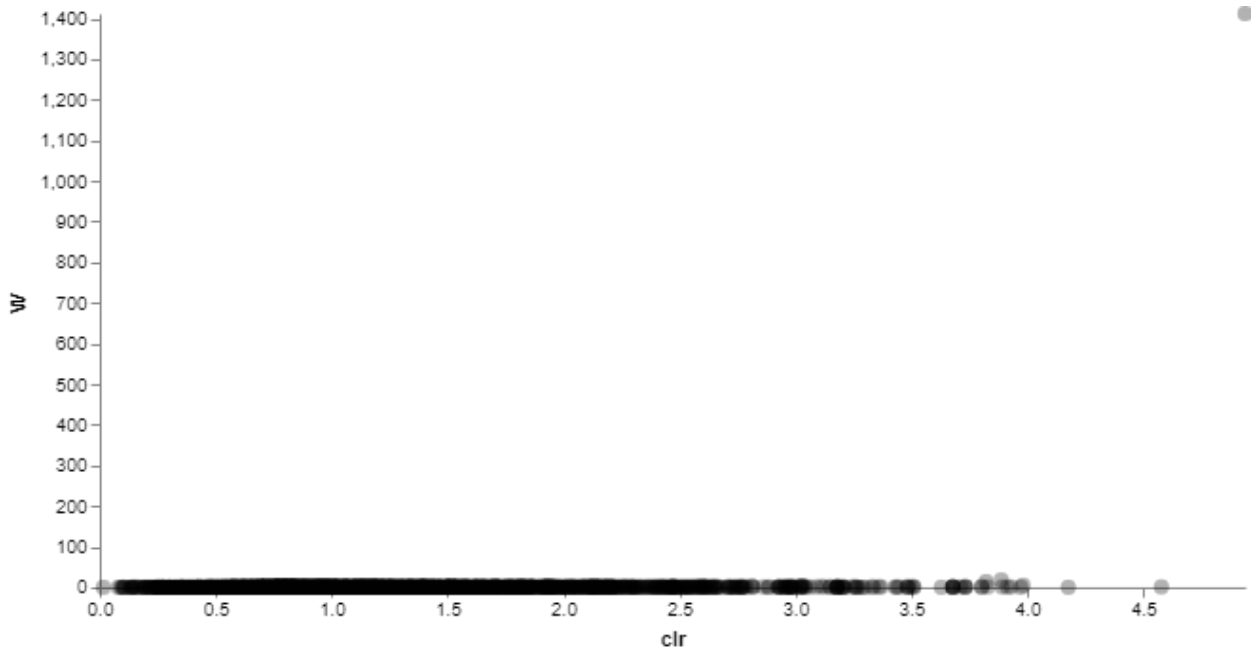

Figure 3.32. ANCOM Volcano Plot of significant abundance of human gut microbiome by Treatment and Time (W= W statistics, clr= Center Log Ratio)

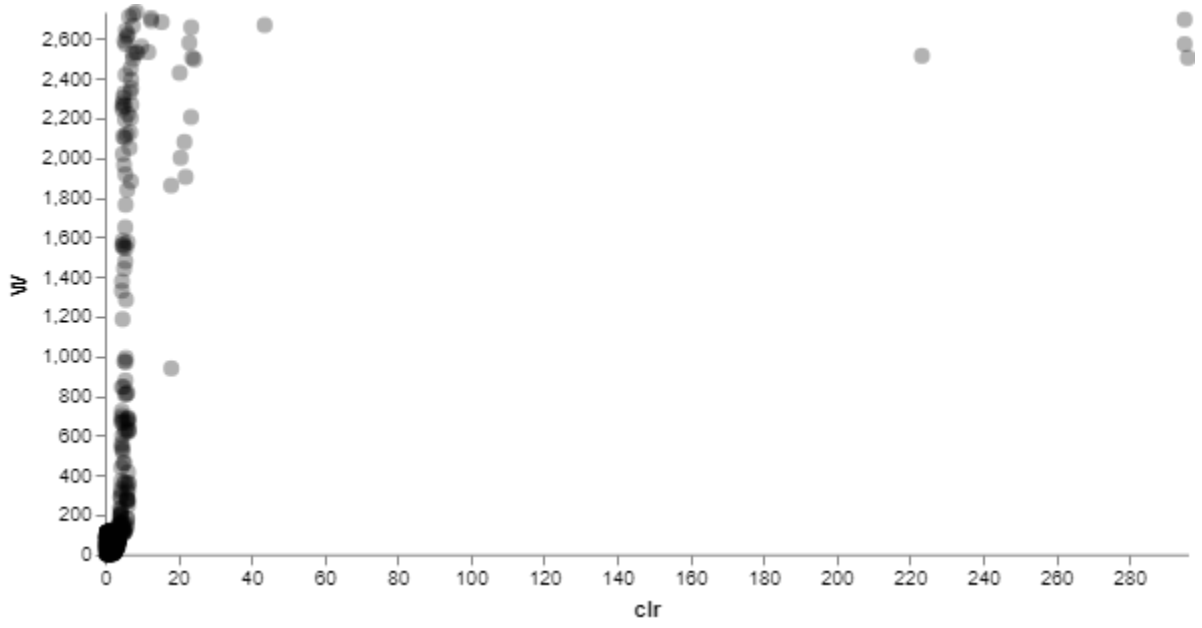

Figure 3.33. ANCOM Volcano Plot of significant abundance of human gut bacteria by Parkinson's disease durations (W= W statistics, clr= Center Log Ratio)

Table 3.10. Human gut bacteria that have statistically significant impact on the composition of human gut microbiome of People with Parkinson's disease by the Disease Durations

ANCOM statistical results	W
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uniformis	2735
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii	2723
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae	2711
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	2705
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__obeum	2697
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uniformis	2690
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	2684
k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	2669
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__ovatus	2665
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__hiranonis	2658
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	2641
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__	2622
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	2609
k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Akkermansia; s__muciniphila	2590
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__hiranonis	2580
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__	2573
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	2573
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2561
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2533
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	2530

k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__	2529
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides	2526
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	2514
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__	2505
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2504
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__	2497
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__	2495

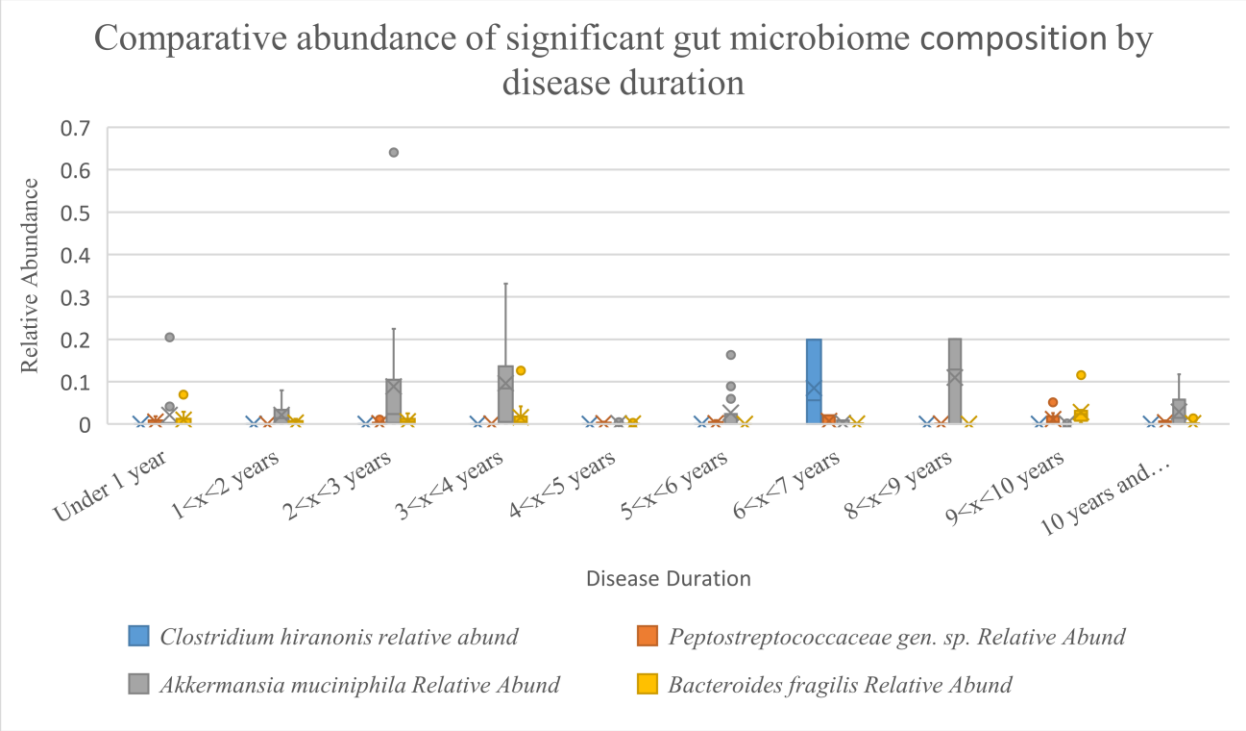

Figure 3.34. Comparative abundance of significant gut microbiome composition by the participants' Parkinson's disease durations from their diagnosis

Clostridium hiranonis appeared scarce in the gut microbiome of this study population with PD. *Phascolarctobacterium* was differentially present in longer disease duration group (6-7 years) of this study. *Bacteroides uniforms* was almost lacking in longer disease duration groups (8-9 years, 10 years and above) in this study. Bacteria family *Ruminococcaceae* was statistically significantly different in the microbiome composition between the samples from the groups that took fiber supplementation and that did not. Regarding time (Time1 or Week0; Time2 or Week6; Time 3 or Week12), they showed no statistically significant different taxa in gut microbiome composition.

The disease status by MDS-UPDRS score showed statistically significant impact in the microbiome composition of the samples collected from people with PD participated in this study. The gut microbiome composition with bacteria family *Ruminococcaceae*, bacteria genera *Coprococcus*, *Blautia*, *Oscillospira* were founded much higher in moderate and advance disease status as categorized by the MDS-UPDRS Score. *Bacteroides fragilis* was statistically significantly present in gut microbiome composition of moderate and advance disease status but unfounded in those with mild disease status.

In this RCT, *Desulfovibrio* was statistically significantly present in gut microbiome composition of Parkinson's patients with 10 year or longer disease duration but almost unfounded in other Parkinson's patient groups with shorter disease durations.

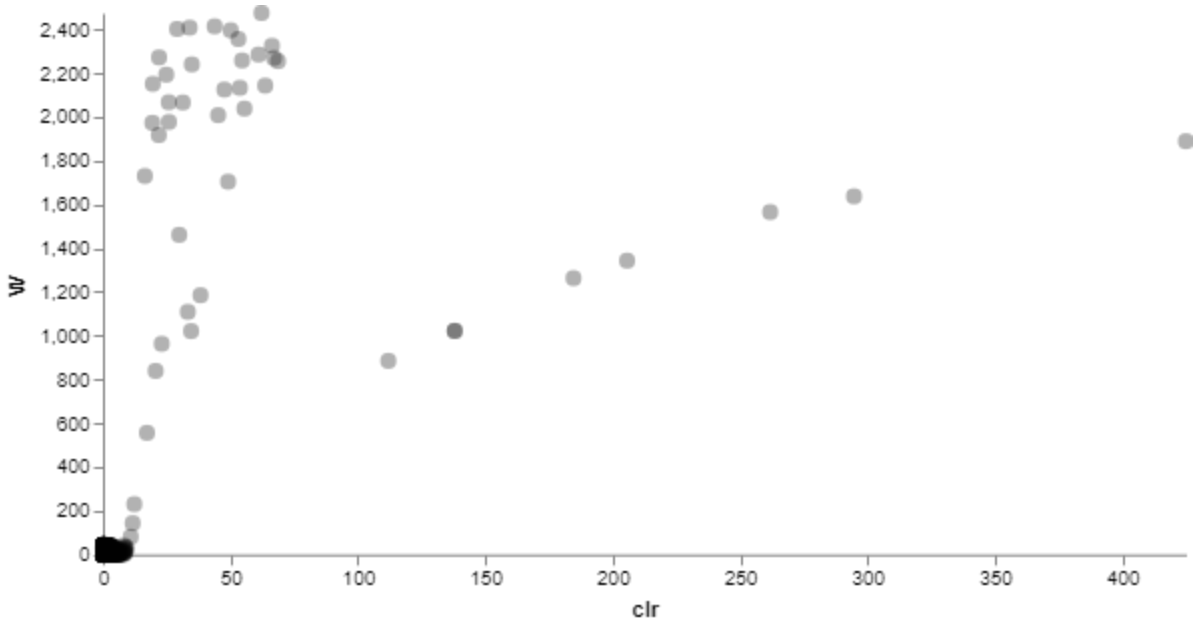

Figure 3.35. ANCOM Volcano Plot indicating the significant impact in the composition of human gut bacteria by Parkinson's disease status as categorized by MDS-UPDRS Score (W= W statistics, clr= Center Log Ratio)

Table 3.11. Human gut bacteria that have statistically significant impact on the composition of human gut microbiome of People with Parkinson's disease by disease status as categorized by MDS-UPDRS Score

ANCOM statistical results	W
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2475
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	2414
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__	2408
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2402
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis	2396
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	2356
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	2325
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2285
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	2272
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	2269
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	2258
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	2255
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	2240

Table 3.12. Percentile abundances of specific gut bacteria in the different disease status groups as categorized by MDS-UPDRS Score

Percentile	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Group	Mild	Mild	Mild	Mild	Mild	Moderate	Moderate	Moderate	Moderate	Moderate	Advance	Advance	Advance	Advance	Advance
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	1	1	1	1	1	1	1	1	1	609	760	760	760	760	760
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	1	1	1	1	26	1	1	1	1	783	637	637	637	637	637
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__	1	1	1	1	93	1	1	1	1	320	901	901	901	901	901
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	1	1	1	1	43	1	1	1	1	1036	886	886	886	886	886
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis	1	1	1	1	1	1	1	1	1	662	411	411	411	411	411
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	1	1	1	1	1	1	1	1	1	390	302	302	302	302	302
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	1	1	1	1	1	1	1	1	1	147	220	220	220	220	220
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	1	1	1	1	1	1	1	1	1	152	182	182	182	182	182

k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	1	1	1	1	29	1	1	1	1	719	596	596	596	596	596
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	1	1	1	1	1	1	1	1	1	40	158	158	158	158	158
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	1	1	1	1	1	1	1	1	1	167	149	149	149	149	149
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	1	1	1	1	1	1	1	1	1	81	131	131	131	131	131
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	1	1	1	1	79	1	1	1	1	46	212	212	212	212	212

Different anxiety levels showed statistically significant differences in the microbiome composition of the samples collected from people with PD participated in this study. Bacteria family Ruminococcaceae and *Akkermansia muciniphila* stood out in the human gut microbiome composition of participants with PD in relationship with anxiety levels. Significantly higher relative abundance of *Akkermansia muciniphila* and bacteria family Ruminococcaceae were found in severe anxiety groups compared to mild, moderate, moderately severe group.

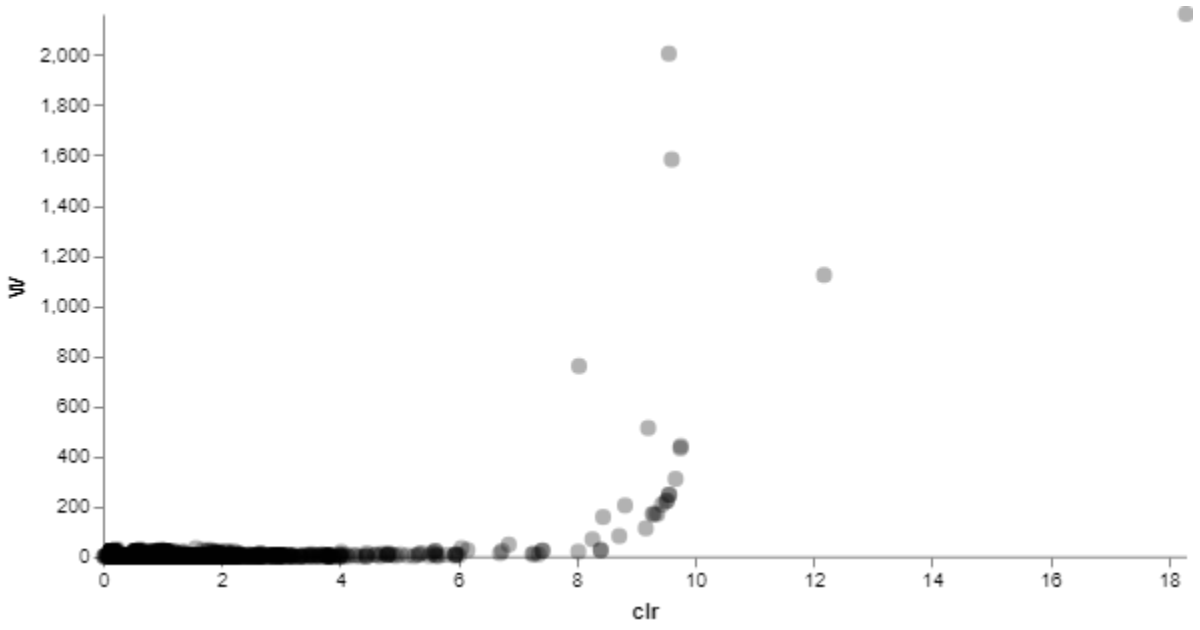

Figure 3.36. ANCOM Volcano Plot indicating the significant difference in human gut microbiome composition relating to the anxiety levels (W= W statistics, clr= Center Log Ratio)

Genus *Odoribacter* and *Clostridium* showed statistically significant differences in human gut microbiome composition of participants with PD relating to their depression levels. The higher relative abundance of genus *Clostridium* was detected in those with no depression, and the lower relative abundance of genus *Odoribacter* was detected in those with higher levels of depression.

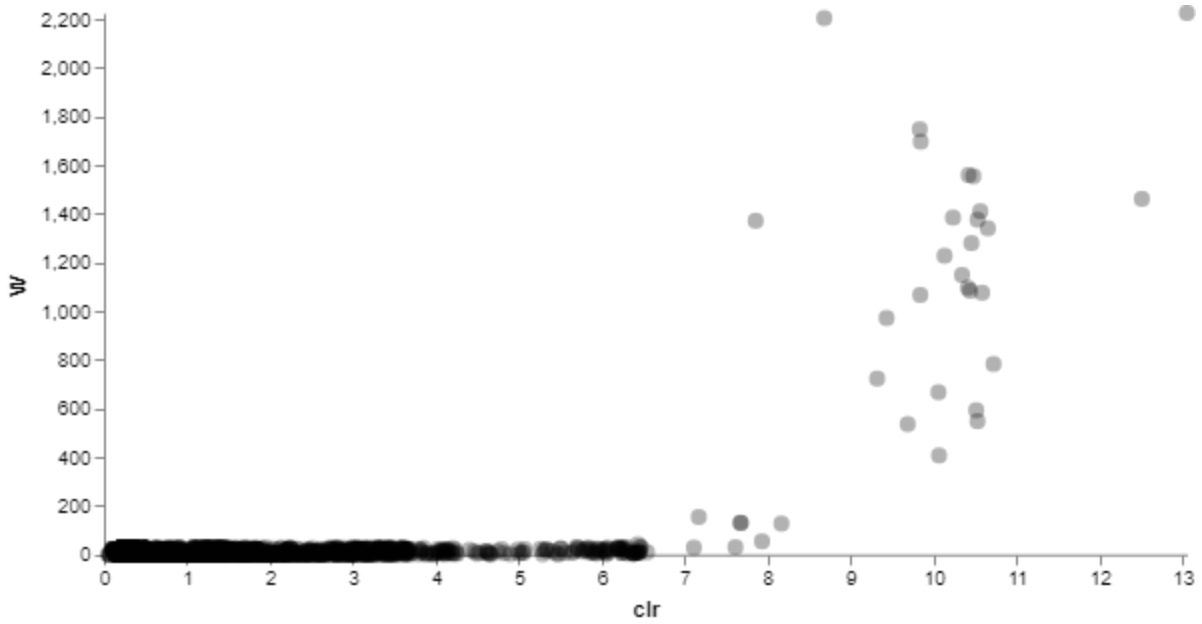

Figure 3.37. ANCOM Volcano Plot indicating the significant difference in human gut microbiome composition relating to the depression levels (W= W statistics, clr= Center Log Ratio)

Firmicute:Bacteroidetes Ratio

Firmicute:Bacteroidetes ratio was known to decrease with age and associated with neurodegenerative disease. The *Firmicute:Bacteroidetes* ratio was assessed for treatment groups, and there was no statistically significant difference between treatment groups and times (Kruskal-Wallis test statistic=8.92, N=122, df=8, p-value=0.35).

Table 3.13. *Firmicute:Bacteroidetes* Ratio Kruskal-Wallis Test Result for different treatments and times

Independent-Samples Kruskal-Wallis Test Summary	
Total N	122
Test Statistic	8.920
Degree Of Freedom	8
Asymptotic Sig.(2-sided test)	.349

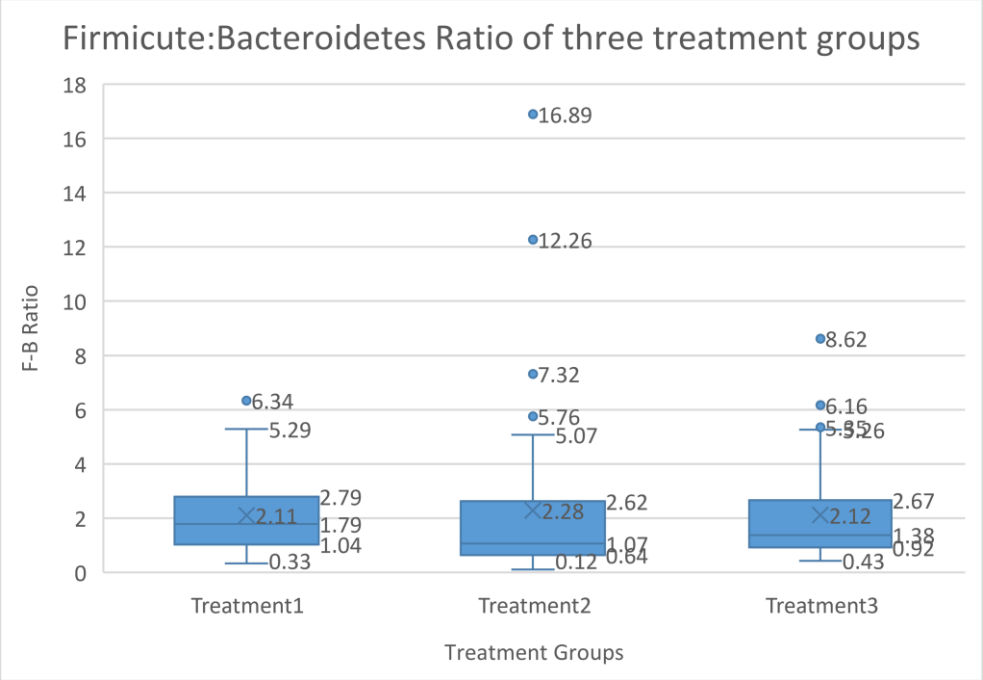

Figure 3.38: Firmicute:Bacteroidetes Ratio of three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

Longitudinal Study

Longitudinal Paired Differences

Shannon – Alpha diversity – Richness & Evenness

Shannon vector of paired samples from three treatment groups at baseline and endline showed not statistically significant pairwise differences in Wilcoxon signed-rank test: Treatment 1 (W=26, p-value=0.19, FDR-adjusted p-value=0.29), Treatment 2 (W=45, p-value=1.00, FDR-adjusted p-value=1.00), Treatment 3 (W=7.0, p-value=0.02, FDR-adjusted p-value=0.055). Treatment 3 was significantly different by p-value=0.02, but only marginally significant or not significant after FDR-adjusted p-value=0.055.

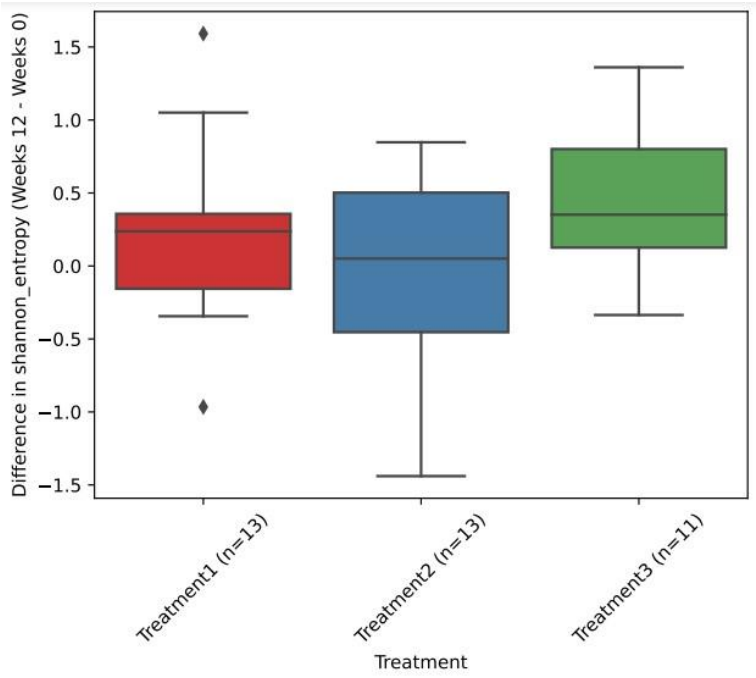

Figure 3.39. Pairwise difference boxplot of Shannon vector in three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week 0) and endline (Week 12)

Observed-features – Alpha diversity - Richness

The longitudinal pairwise difference tests of observed-features changes (Alpha-diversity, richness) showed marginally statistically significant differences between treatment groups (H=5.93, p-value=0.05), and the statistically significant change difference was between treatment 1 and treatment 3 (Mann-Whitney U test statistics=29.0, p-value=0.01, FDR adjusted p-value=0.04). There were no statistically significant change differences of observed-features (i.e., Alpha-diversity, richness) between treatment 1 and treatment 2 (Mann-Whitney U test statistics=58.0, p-value=0.18, FDR adjusted p-value=0.27), nor between treatment 2 and treatment 3 (Mann-Whitney U test statistics=54.5, p-value=0.34, FDR adjusted p-value=0.34). This finding appeared to support the investigator's hypothesis that the one of the components of treatment 1 could have a positive balancing or controlling effect on untoward bacterial overgrowth.

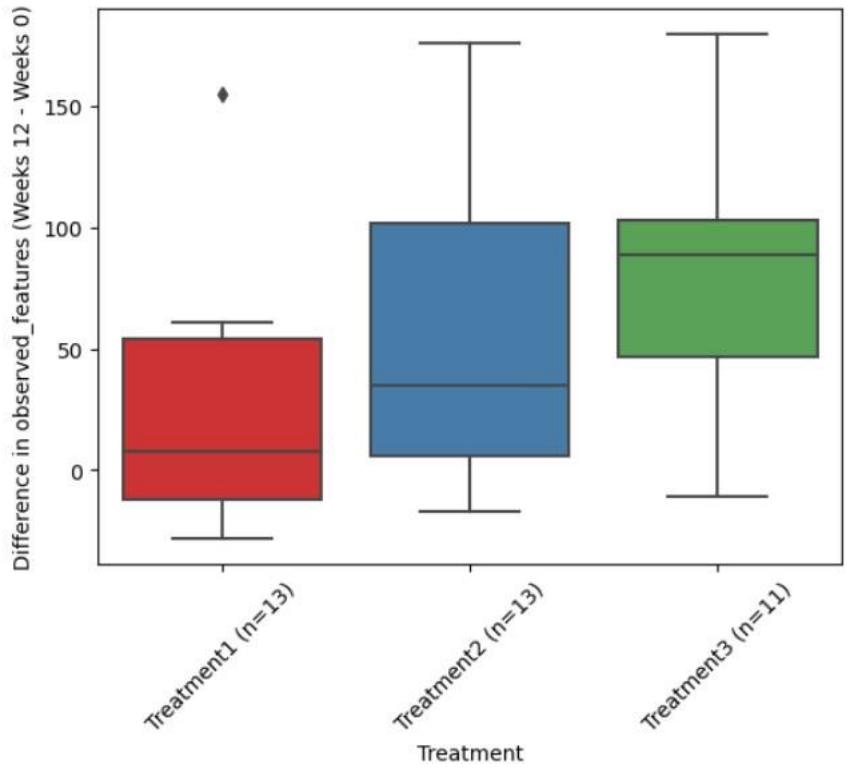

Figure 3.40. Pairwise difference boxplot of observed-features (Alpha diversity- richness) in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week 0) and endline (Week 12)

Firmicute:Bacteroidetes Ratio

Over the 12-week intervention study, the longitudinal pairwise difference tests of Firmicute:Bacteroidetes ratio changes showed not statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics=49.0, FDR p-value=0.893), Treatment2 group (Wilcoxon signed-rank test statistics=43, FDR p-value=0.893), and Treatment3 group (Wilcoxon signed-rank test statistics=9.0, FDR p-value=0.193). Also, the Firmicute:Bacteroidetes ratio changes did not either show statistically significant differences between three treatment groups over the 12-week study period (Kruskal Wallis test statistics H=4.50, p-value=0.105).

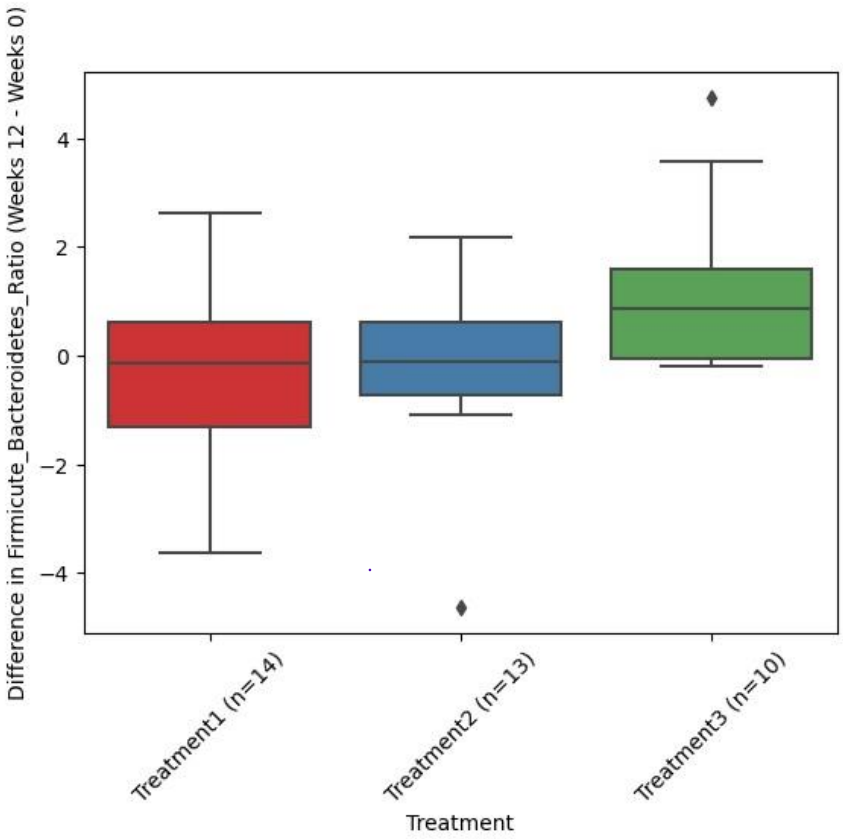

Figure 3.41. Pairwise difference boxplot of Firmicute:Bacteroidetes Ratio changes in three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week-0) and endline (Week-12)

Between baseline (Time1) and midterm (Time2), the longitudinal pairwise difference tests of Firmicute:Bacteroidetes ratio changes showed not statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=30.0, FDR p-value=0.305), Treatment2 group (Wilcoxon signed-rank test statistics W=23, FDR p-value=0.305), and Treatment3 group (Wilcoxon signed-rank test statistics W=10.0, FDR p-value=0.126). The Firmicute:Bacteroidetes ratio changes did not show either statistically significant differences between three treatment groups over the first half of 12-week study period (Kruskal Wallis test statistics H=0.079, p-value=0.961).

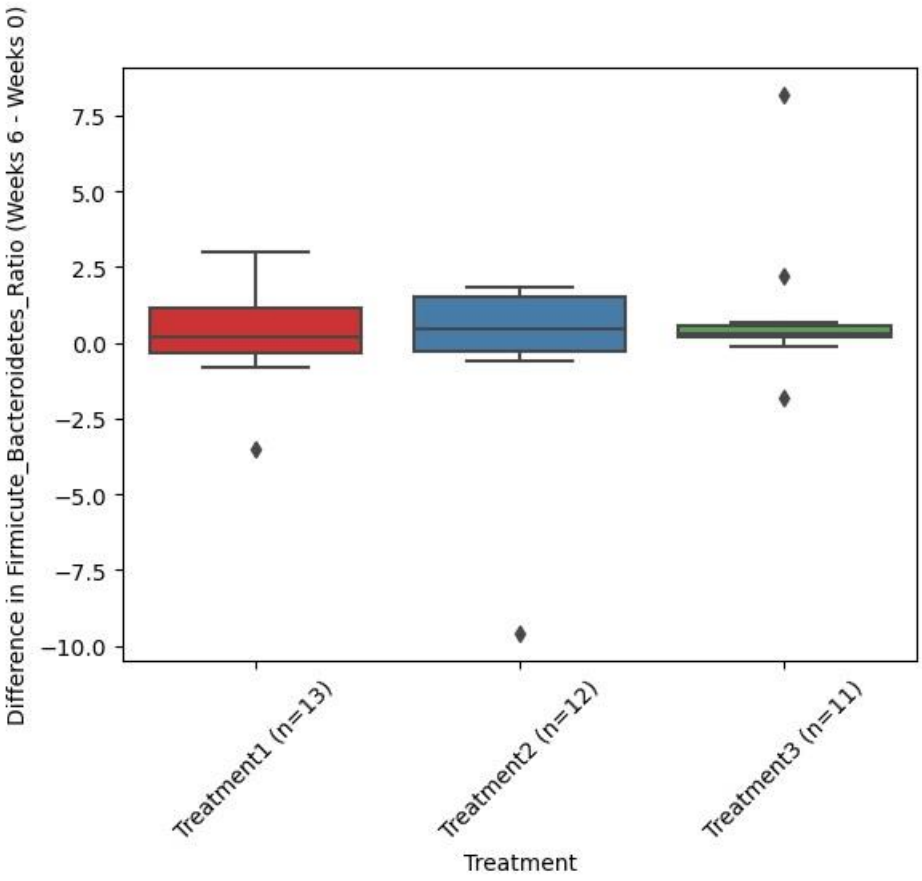

Figure 3.42. Pairwise difference boxplot of Firmicute:Bacteroidetes Ratio changes in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week-0) and endline (Week-6)

During the second-half of the study, between midterm (6-week) and endline (12-week), the longitudinal pairwise difference tests of Firmicute:Bacteroidetes ratio changes showed not statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=28.0, FDR p-value=0.519), Treatment2 group (Wilcoxon signed-rank test statistics W=30, FDR p-value=0.519), and Treatment3 group (Wilcoxon signed-rank test statistics W=30.0, FDR p-value=0.519). The Firmicute:Bacteroidetes ratio changes did not show either statistically significant differences between three treatment groups over the second half of 12-week study period (Kruskal Wallis test statistics H=1.257, p-value=0.533).

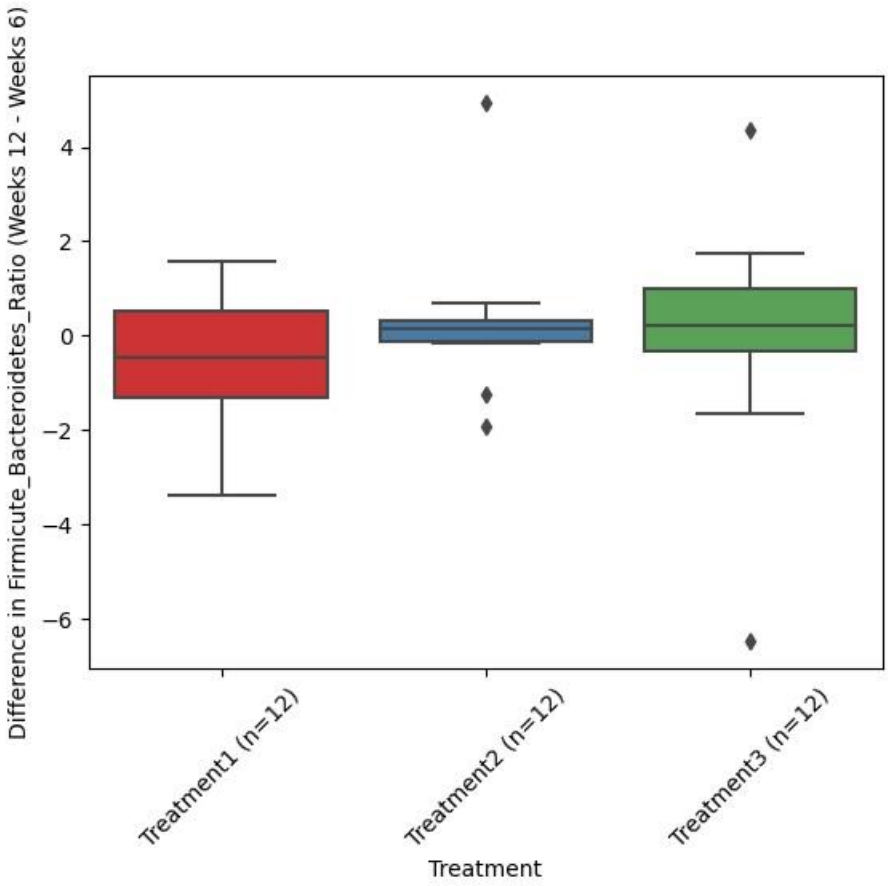

Figure 3.43. Pairwise difference boxplot of Firmicute:Bacteroidetes Ratio changes in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week-6) and endline (Week-12)

Firmicute Relative Abundance Changes

During the 12-week intervention study, the longitudinal pairwise difference tests of Firmicute relative abundance changes did not show statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=47.0, FDR p-value=0.893), Treatment2 group (Wilcoxon signed-rank test statistics W=43, FDR p-value=0.893), and Treatment3 group (Wilcoxon signed-rank test statistics W=14.0, FDR p-value=0.580). Also, the Firmicute relative abundance changes did not show statistically significant differences between

three treatment groups over the 12-week study period (Kruskal Wallis test statistics H=1.821, p-value=0.402).

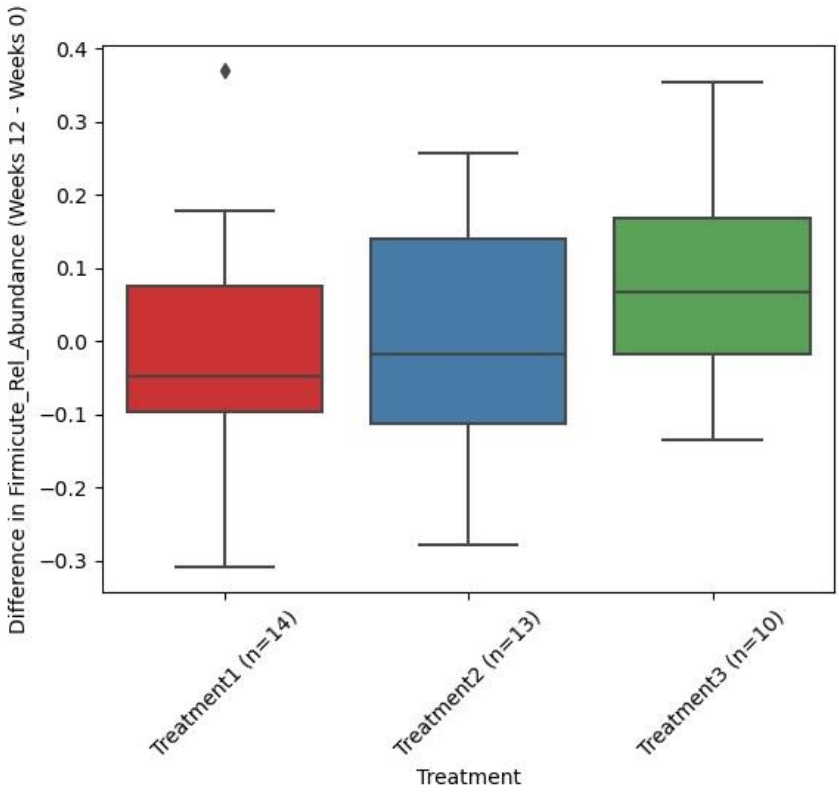

Figure 3.44. Pairwise difference boxplot of changes in Firmicute relative abundance in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week 0) and endline (Week 12)

During the first-half of the 12-week intervention study, i.e. between baseline (week-0) and midterm (week-6), the longitudinal pairwise difference tests of Firmicute relative abundance changes did not show statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=38.0, FDR p-value=0.635), Treatment2 group (Wilcoxon signed-rank test statistics W=26, FDR p-value=0.509), and Treatment3 group (Wilcoxon signed-rank test

statistics W=16.0, FDR p-value=0.442). The Firmicute relative abundance changes did not either show statistically significant differences between three treatment groups over the first half of 12-week study period (Kruskal Wallis test statistics H=0.733, p-value=0.693).

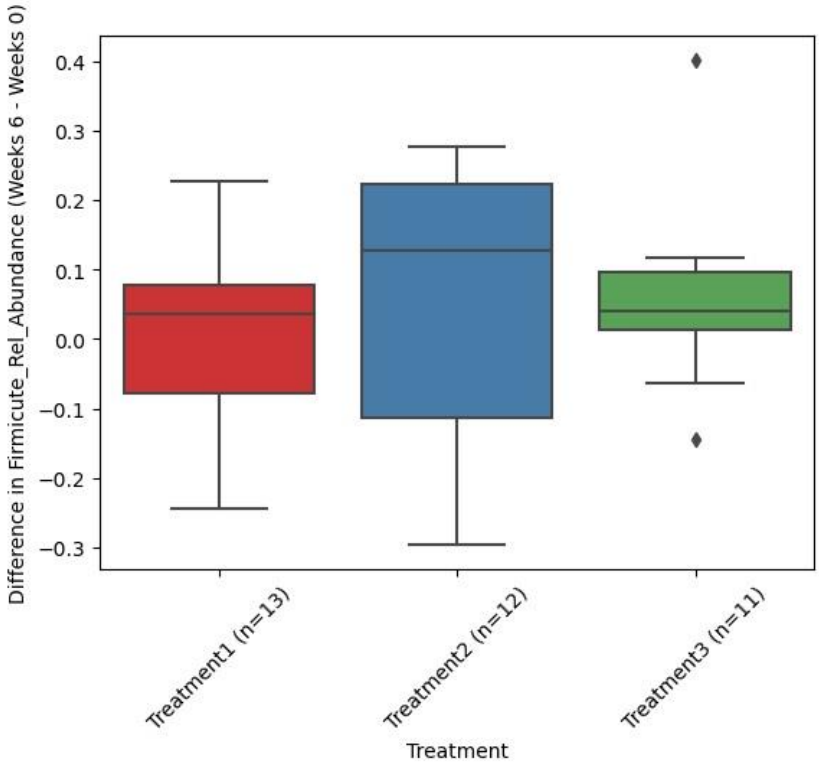

Figure 3.45. Pairwise difference boxplot showing the changes in Firmicute relative abundance in the first 6-week study period of the three treatment groups between baseline (Week 0) and midterm (Week 6)

During the second-half of the study, between midterm (week-6) and endline (week-12), the longitudinal pairwise difference tests of Firmicute relative abundance changes did not show statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=28.0, FDR p-value=0.910), Treatment2 group (Wilcoxon signed-rank test statistics W=35, FDR p-value=0.910), and Treatment3 group (Wilcoxon signed-rank test statistics W=37.0, FDR p-value=0.910). The Firmicute relative abundance changes did not either show statistically

significant differences between three treatment groups over the second half of 12-week study period (Kruskal Wallis test statistics H=0.234, p-value=0.889).

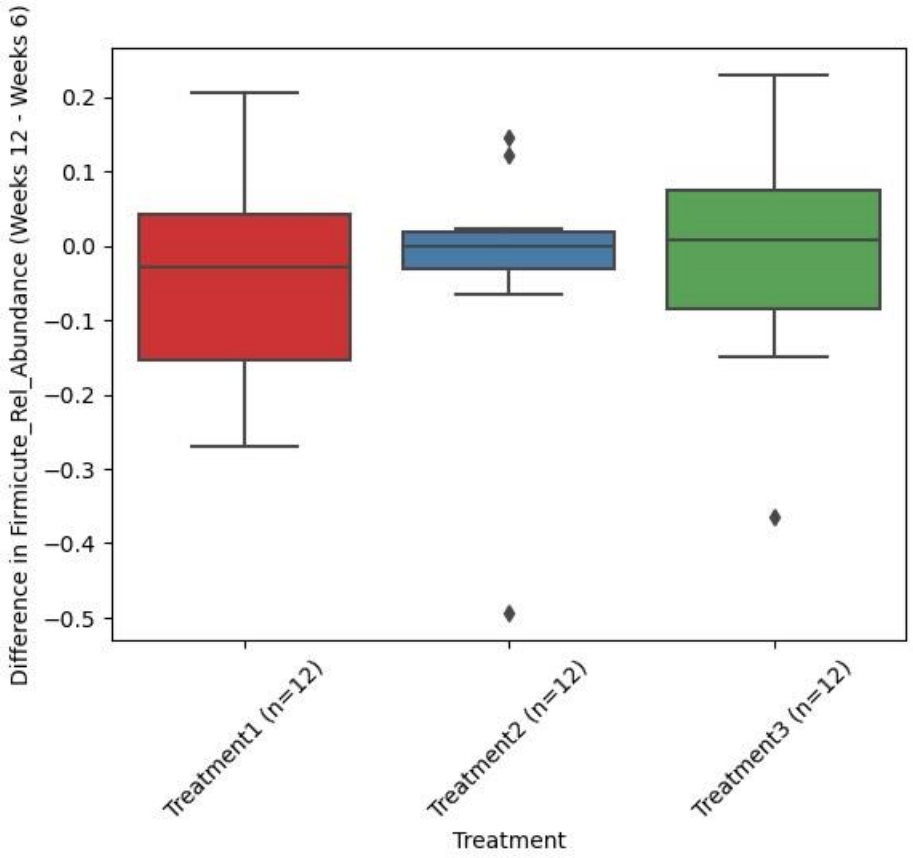

Figure 3.46. Pairwise difference boxplot showing the changes in Firmicute relative abundance in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between midterm (Week 6) and endline (Week 12)

Bacteroidetes Relative Abundance Changes

During the 12-week intervention study, the longitudinal pairwise difference tests of Bacteroidetes relative abundance changes showed statistically significant differences in Treatment3 group (Wilcoxon signed-rank test statistics W=2.0, FDR p-value=0.018), but in the Treatment1 group (Wilcoxon signed-rank test statistics W=45, FDR p-value=0.893). and Treatment2 group (Wilcoxon signed-rank test statistics W=43.0, FDR p-value=0.893).

Bacteroidetes relative abundance changes did not show statistically significant differences between three treatment groups over the 12-week study period (Kruskal Wallis test statistics H=4.075, p-value=0.130).

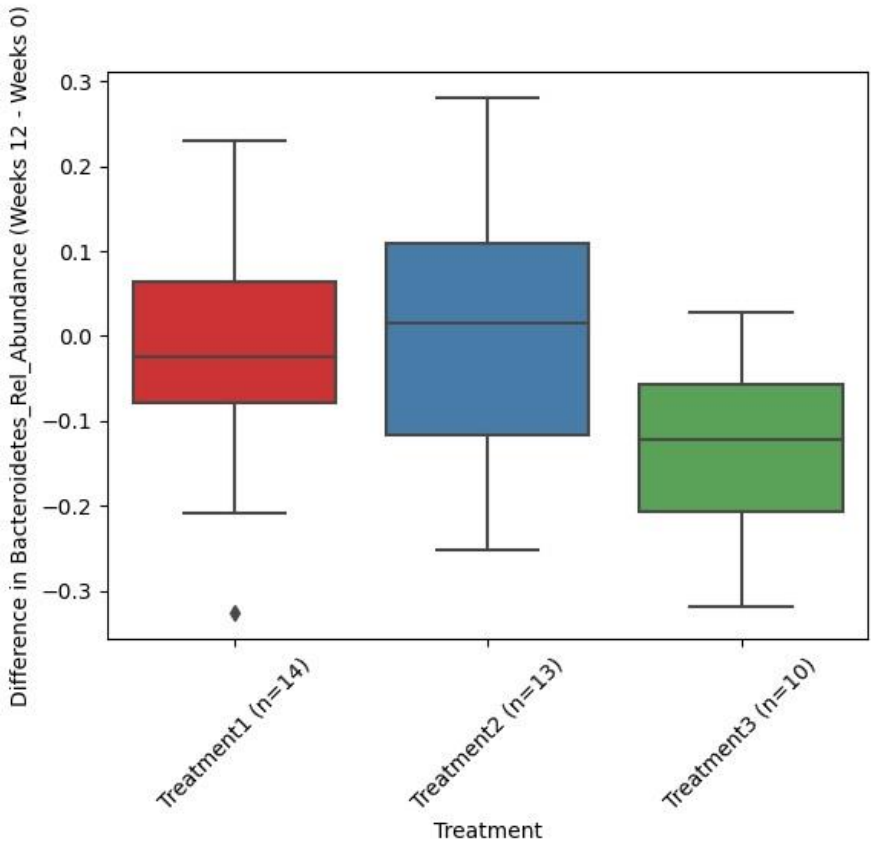

Figure 3.47. Pairwise difference boxplot of changes in Bacteroidetes relative abundance in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week 0) and endline (Week 12)

During the first half of 12-week intervention study, i.e. between baseline (week-0) and midterm (week-6), the longitudinal pairwise difference tests of Bacteroidetes relative abundance changes showed statistically significant differences in Treatment3 group (Wilcoxon signed-rank test statistics W=5.0, FDR p-value=0.029), but in the Treatment1 group (Wilcoxon signed-rank test statistics W=26, FDR p-value=0.233). and Treatment2 group (Wilcoxon signed-rank test statistics W=23.0, FDR p-value=0.233). Bacteroidetes relative abundance changes did not show

statistically significant differences between three treatment groups over the first 6-week study period (Kruskal Wallis test statistics H=0.150, p-value=0.928).

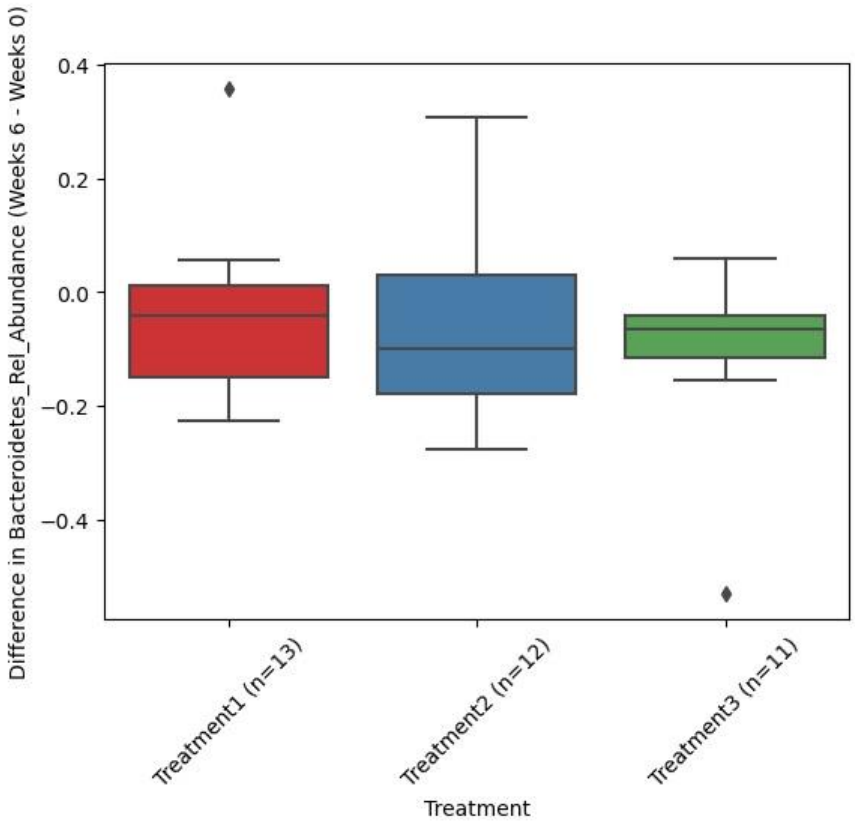

Figure 3.48. Pairwise difference boxplot showing the changes in Bacteroidetes relative abundance in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between baseline (Week 0) and midterm (Week 6)

During the second-half of the study, between midterm (week-6) and endline (week-12), the longitudinal pairwise difference tests of Bacteroidetes relative abundance changes showed not statistically significant differences in Treatment1 group (Wilcoxon signed-rank test statistics W=30.0, FDR p-value=0.622), Treatment2 group (Wilcoxon signed-rank test statistics W=32, FDR p-value=0.622), and Treatment3 group (Wilcoxon signed-rank test statistics W=29.0, FDR p-value=0.622). The Bacteroidetes relative abundance changes did not show either statistically

significant differences between three treatment groups over the second half of 12-week study period (Kruskal Wallis test statistics H=1.146, p-value=0.570).

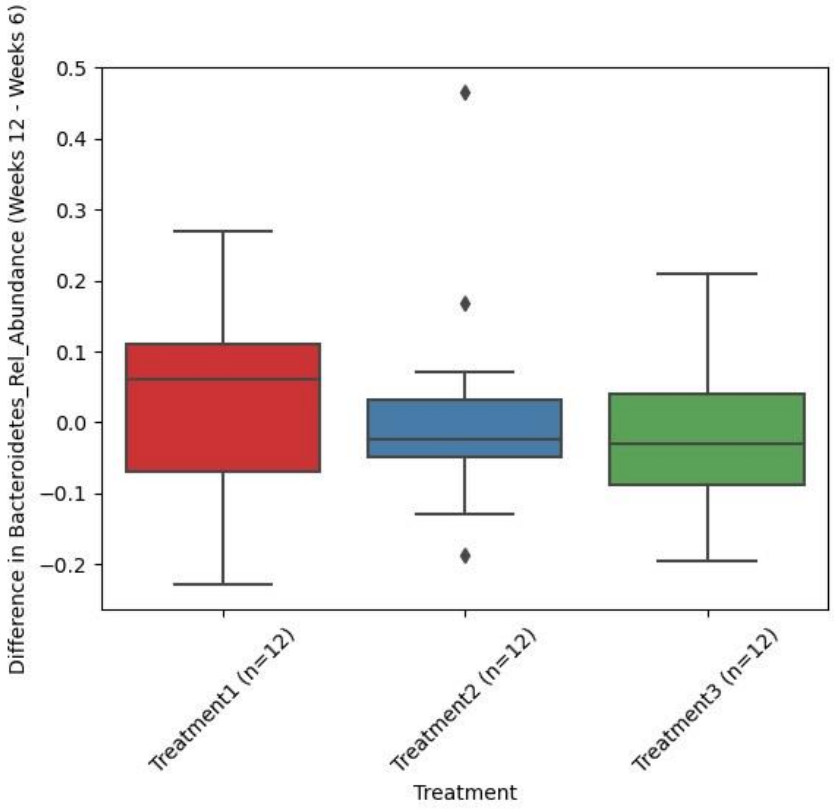

Figure 3.49. Pairwise difference boxplot showing the changes in Bacteroidetes relative abundance in three treatment groups- Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo between midterm (Week 6) and endline (Week 12)

Longitudinal Mixed Effect Model

Tracking rate of change of beta diversity (Unifrac), first differencing method

The linear mixed model of treatment * time (weeks) did not show statistically significant difference in the rate of change of distances (Unifrac) at different time points (Time2 and Time3) and interactions of treatment and time.

The reference group in this model was Treatment 1 at Time 1(Week 0). On average, there's a within-group distance of 0.279 [95% CI 0.200, 0.359]. When everything else was held constant, Treatment 2 increased the distance by 0.025 [95% CI -0.092, 0.142, p-value=0.677], which was not significant; and Treatment 3 decreased the distance by -0.066 [95% CI -0.182, 0.051, p-value=0.272], which was not significant.

The interaction term Weeks: Treatment[T.Treatment2] indicated the slope changes by -0.006 [95% CI -0.017, 0.005, p-value=0.309], which was not significant, compared to the reference group Time 1 (Week0) of Treatment 1, when everything else was held constant. The interaction term Weeks: Treatment[T.Treatment3] indicated the slope changes by 0.009 [95% CI -0.003, 0.020, p-value=0.134], which was not significant, compared to the reference group Time 1 (Week0) of Treatment 1, when everything else was held constant.

Table 3.14. Linear Mixed Effect Model (Treatment x Time) Results

	Coef.	Std.Err.	z	P>|z|	[0.025	0.975]
Intercept	0.279	0.041	6.899	0.000	0.200	0.359
Treatment[T.Treatment2]	0.025	0.060	0.416	0.677	-0.092	0.142
Treatment[T.Treatment3]	-0.066	0.060	-1.098	0.272	-0.182	0.051
Weeks	0.002	0.004	0.509	0.611	-0.006	0.010
Weeks:Treatment[T.Treatment2]	-0.006	0.006	-1.017	0.309	-0.017	0.005
Weeks:Treatment[T.Treatment3]	0.009	0.006	1.497	0.134	-0.003	0.020
Group Var	0.004	0.030				

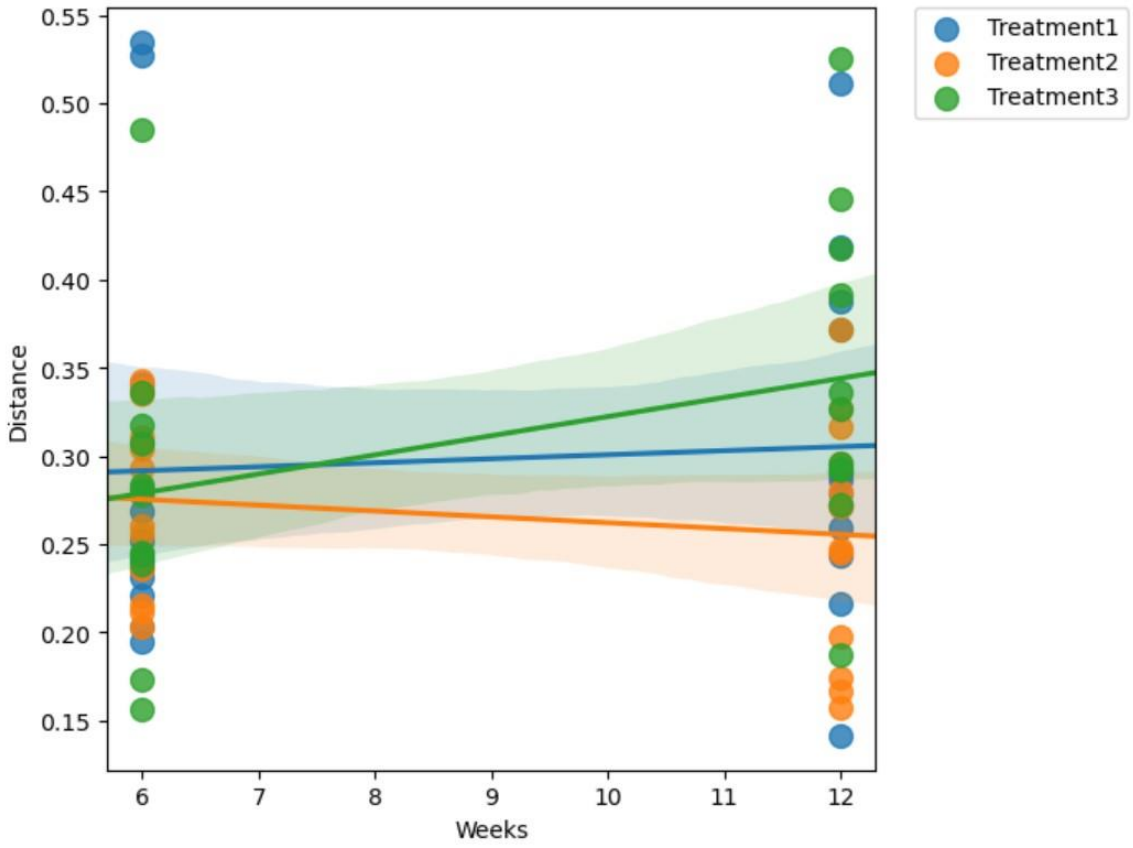

Figure 3.50. Regression scatterplot showing the linear mixed effects of changes in distances (Beta Diversity) of Treatment and Time (Weeks)| Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

Tracking longitudinal changes of alpha diversity, Shannon

The linear mixed model of treatment * time (weeks) did not show statistically significant difference in the rate of change of Shannon_entropy at different time points (Time2 and Time3) and interactions of treatment and time.

The reference group in this model was Treatment 1 at Time 1(Week 0). On average, there's a within-group distance of 5.456 [95% CI 5.060, 5.853, p-value=0.000]. When everything else was held constant, Treatment 2 decreased the alpha diversity, Shannon_entropy, by -0.244 [95% CI -0.806, 0.318, p-value=0.395], which was not significant; and Treatment 3 decreased the distance by -0.114 [95% CI -0.697, 0.469, p-value=0.700], which was not significant.

The interaction term Weeks: Treatment[T.Treatment2] indicated the slope changes by -0.026 [95% CI -0.071, 0.019, p-value=0.260], which was not significant, compared to the reference group Time 1 (Week0) of Treatment 1, when everything else was held constant. The interaction term Weeks: Treatment[T.Treatment3] indicated the slope changes by 0.003 [95% CI -0.044, 0.050, p-value=0.909], which was not significant, compared to the reference group Time 1 (Week0) of Treatment 1, when everything else was held constant.

Table 3.15. Linear Mixed Effect of changes of alpha diversity – richness and evenness by (Treatment x Time)

	Coef.	Std.Err.	z	P>|z|	[0.025	0.975]
Intercept	5.456	0.202	26.958	0.000	5.060	5.853
Treatment[T.Treatment2]	-0.244	0.287	-0.850	0.395	-0.806	0.318
Treatment[T.Treatment3]	-0.114	0.297	-0.385	0.700	-0.697	0.469
Weeks	0.022	0.016	1.328	0.184	-0.010	0.054
Weeks:Treatment[T.Treatment2]	-0.026	0.023	-1.128	0.260	-0.071	0.019
Weeks:Treatment[T.Treatment3]	0.003	0.024	0.114	0.909	-0.044	0.050
Group Var	0.378	0.256				

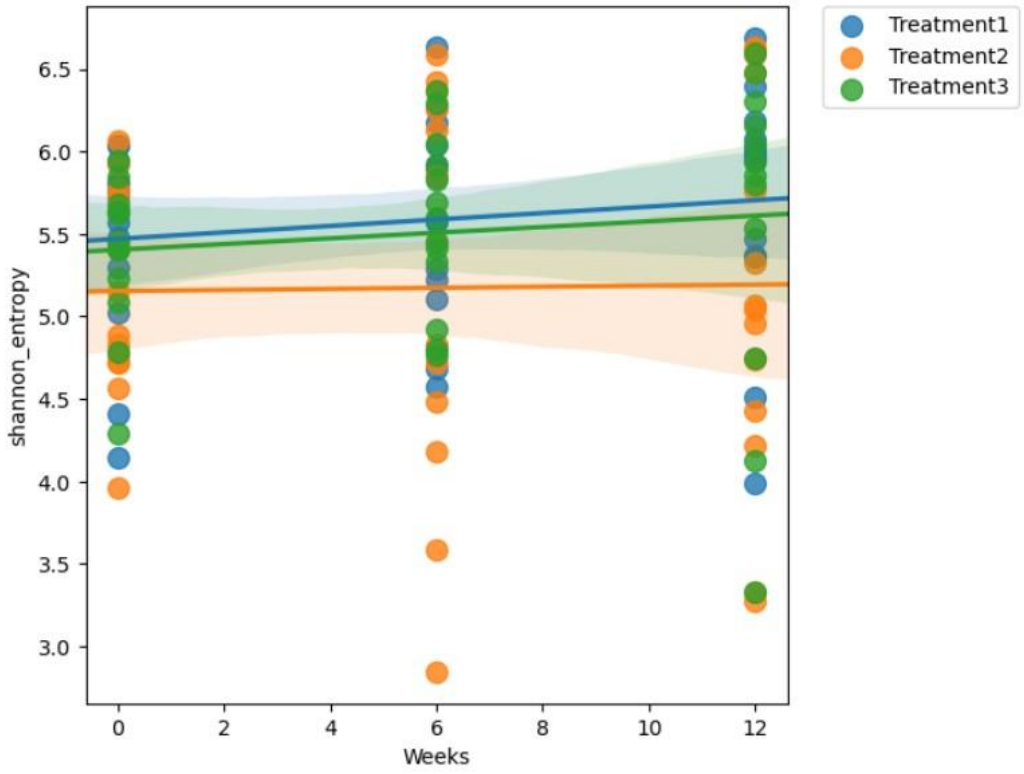

Figure 3.51. Regression scatterplot showing the linear mixed effects in changes of alpha diversity, Shannon entropy, the richness and evenness of species, by Treatment and Time (Weeks)| Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

Identifying Important Features and Corresponding Gut Microbiome

By Treatment Groups

The top twenty important features across three different treatments were shown in the heatmap labelled with their corresponding taxonomic classification. They were *Veillonellaceae Dialister* sp., *Erysipelotrichaceae* gen. sp., *Oscillospira* sp., *Prevotella copri*, *Blautia* sp., *Coprococcus* sp., *Ruminococcus* sp., *Ruminococcaceae* gen. sp., *Methanobrevibacter* sp., *Anaerotruncus* sp., *Bacteroides* sp., *Adlercreutzia* sp., *Streptococus* sp., *Sutterella* sp.. They were all bacteria except one of them belongs to Archaea, *Methanobacteriaceae Methanobrevibacter* sp. Most of them are Firmicutes, followed by Bacteroidetes, Proteobacteria, Actinobacteria and Euryarchaeota. *Veillonellaceae Dialister* sp. was the one which showed statistically significant difference across the three treatment groups. *Dialister* sp. was almost absent in treatment 1 but significantly present in treatment 2 followed by treatment 3. Apparently *Erysipelotrichaceae* gen. sp., *Oscillospira* sp. were relatively more abundant in treatment 1 and 2 groups which had a common treatment component while much lower relative abundance or frequency was seen in treatment 3. *Prevotella copri* and *Blautia* sp. were relatively the highest abundance or frequency in treatment 2, followed by treatment 3, then treatment 1. *Methanobrevibacter* sp. and *Anaerotruncus* sp. were relatively much lower abundance in treatment 2 groups compared to treatment 3 and 1. *Oscillospira* was relatively much more abundant in treatment 1 and 2 groups, and much lesser in treatment 3 group. *Anaerotruncus* sp. was relatively more abundant in treatment1 and treatment 3 than treatment2.

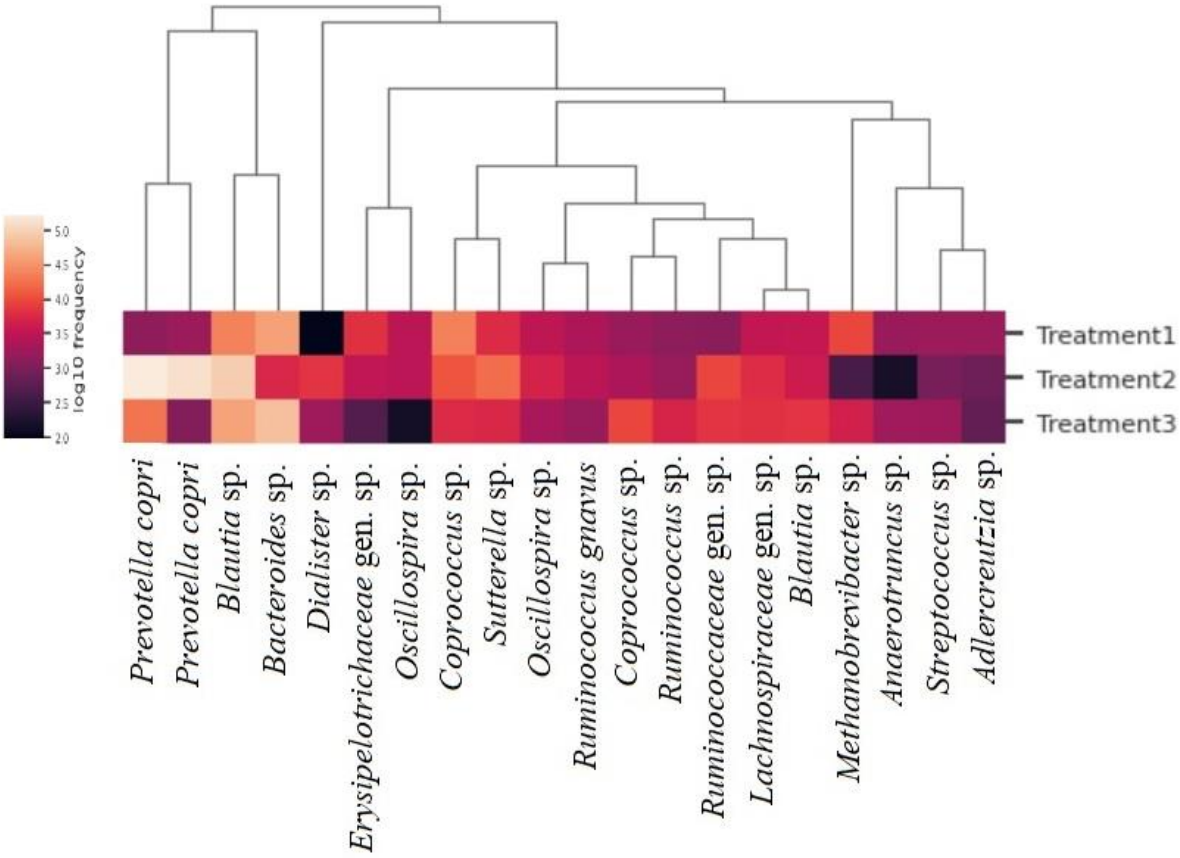

Figure 3.52. Heatmap showing the important (features) gut microbiome in three treatment - Treatment1: Probiotics+B_3, Treatment2: Probiotics, and Treatment3: Placebo

By Treatment_and_Time Groups

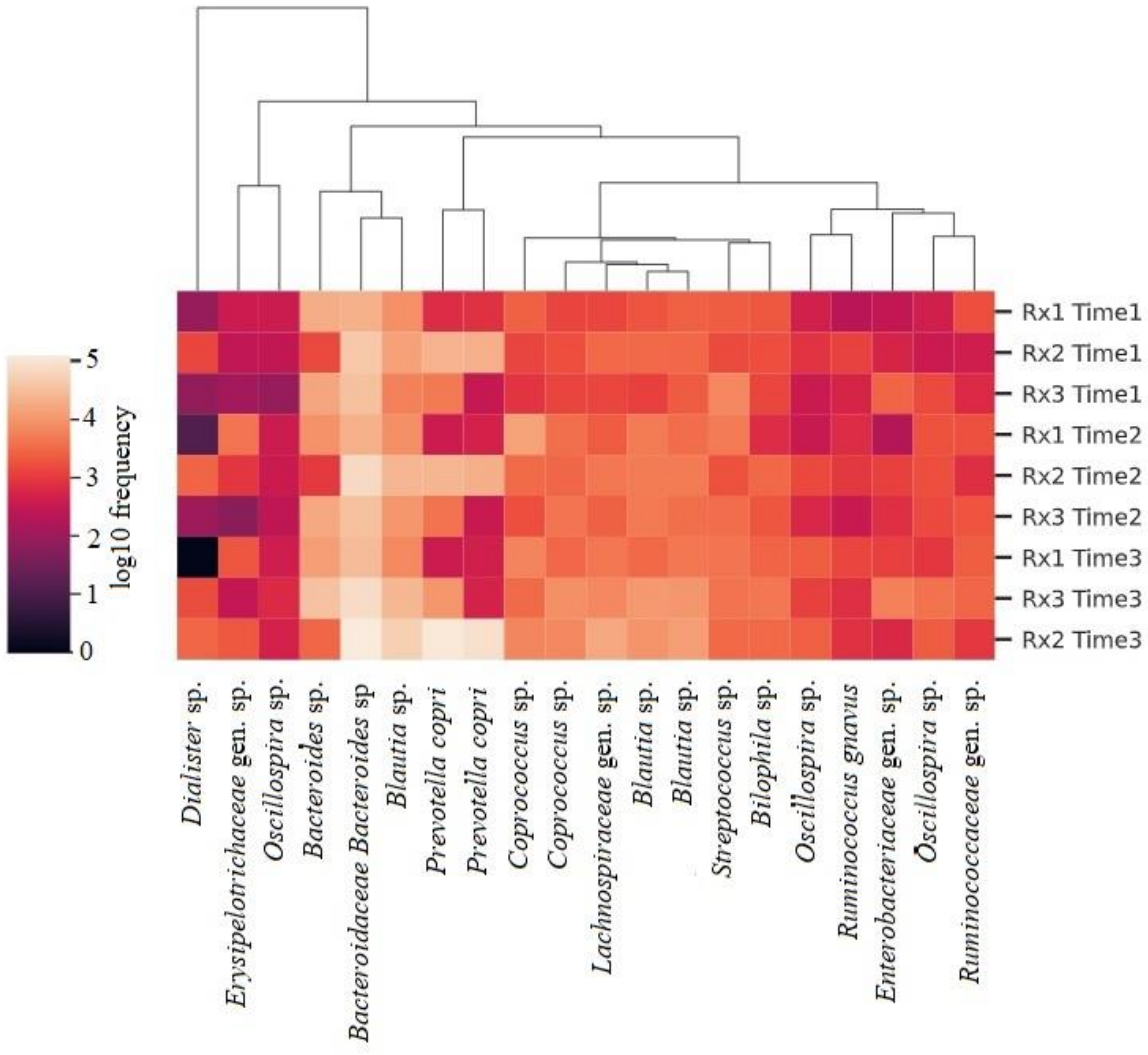

Figure 3.53. Heatmap showing the important (features) gut microbiome in nine 'treatment & time' groups [Rx means Treatment, e.g., Rx1 is Treatment1: Probiotics+B_3, Rx2 is Treatment2: Probiotics, Rx3 is Treatment3: Placebo | Time1=Week 0, Time2= Week 6, Time3= Week 12]]

Dialister sp. was significantly lower and *Erysipelotrichaceae* gen. sp was higher in treatment1 at time 2 and time 3 compared to time 1. *Prevotella copri* was relatively higher abundance in treatment 2 at time 3 compared to time 1 and 2, and more prominently higher abundance than those in treatment 1 at all 3 times, and treatment 3 at all three times.

Correlations of beta diversity with some salient variables

Parkinson's drugs, antidepressants, fiber supplements, laxative, or stool softener use

There were statistically significant negative correlations between beta-diversity of gut microbiome (The principal coordinates, PCoA, of Bray-Curtis) and laxative or stool softener use (r=-0.181, p=0.05, axis1; r=-0.257, p=0.00, axis2), and fiber supplementation use (r=-0.183, p=0.04, axis2), and antidepressants medication (r=-0.192, p=0.03, axis2). Those who did not use laxative or stool softener, or fiber supplements, or antidepressants medication would have greater levels of beta-diversity coordinates. Also, there were fewer number of positive correlations between gut microbiome beta-diversity and laxative or stool softener use (r=0.229, p=0.01, axis3) and antidepressant medications (r=0.199, p=0.03, axis3). These findings made to conclude that laxative or stool softener use or antidepressant medications could have negative effect on some taxa of gut microbiome and positive effect on some taxa of gut microbiome on the other hand. The study population did not take COMTi and Monoamine Oxidase Inhibitors (MAOI).

Constipation problems, stool types, profile of mood states, body mass index, and number of different types of Parkinson's medication taken

There were statistically significant negative correlations between gut microbiome beta diversity, principal coordinates (Bray-Curtis) and frequency % of having mushy or watery stools (stool type 6 or 7) (r=-0.21, p=0.00, axis1; r=-0.39, p=0.00, axis3), and BMI, (r=-0.33, p=0.00, axis3). Constipation problems, frequency % of having hard or lumpy stool (stool type1 or 2), profile of mood states (POMS) and total number of different Parkinson's medication taken did not show statistically significant correlations. These findings made to conclude that individuals

with greater beta-diversity may have lesser frequency % of watery or mushy (diarrhea) stools, and leaner or lower BMI.

Table 3.16. Correlation between gut microbiome beta diversity principal coordinates and Parkinson's drugs, fiber supplements, and laxative or stool softener use

		Fiber supplements (nu)	Laxative or stool softener use	Carbidopa-L-Dopa	COMTi	Dopamine agonist	MAOI	Amantadine	Anticholinergics	Antidepressants
PCoA Axis1	r	-.183*	-.181*	0.045	.b	-0.139	.b	0.075	0.077	-0.123
	p-value	**0.04**	**0.05**	0.63		0.13		0.41	0.40	0.18
PCoA Axis2	r	0.003	-.257**	-0.004	.b	0.021	.b	-0.007	0.110	-.192*
	p-value	0.97	**0.00**	0.96		0.82		0.94	0.23	**0.03**
PCoA Axis3	r	-0.037	.229*	0.076	.b	0.035	.b	0.110	0.046	.199*
	p-value	0.68	**0.01**	0.41		0.71		0.23	0.62	**0.03**

*. Correlation was significant at the 0.05 level (2-tailed).

**. Correlation was significant at the 0.01 level (2-tailed).

b. correlation was not computed as none of the participants took COMT inhibitor or MAOI

r=Pearson correlation, N=121

Bray-Curtis PCoA Axes

Carbidopa-L-Dopa:Dopa decarboxylase inhibitor and dopamine precursor (L-Dopa)

COMTi: Catechol-O-Methyltransferase Inhibitor; MAOI: Monoamine oxidase inhibitors

Amantadine: Amantadine, MNDA receptor antagonists

Table 3.17. Correlation between gut microbiome beta diversity principal coordinates (Bray-Curtis) and constipation problems, stool types, profile of mood states, body mass index, and number of different types of Parkinson's medication taken

		Constipation problems	Stool type 1 or 2 (%)	Stool type 6 or 7 (%)	POMS- TMD	BMI	# of PD drug types
PCoA Bray-Curtis Axis1	r	-0.01	0.18	-.209**	-0.08	-0.10	-0.03
	p-value	0.88	0.06	**0.00**	0.45	0.29	0.75
	N	120	109	108	98	120	121
PCoA Bray-Curtis Axis2	r	-0.17	0.03	0.09	-0.11	0.15	0.04
	p-value	0.07	0.72	0.34	0.28	0.10	0.64
	N	120	109	108	98	120	121
PCoA Bray-Curtis Axis3	r	0.11	-0.07	-0.39**	-0.02	-0.33**	0.10
	p-value	0.22	0.45	**0.00**	0.82	**0.00**	0.29
	N	120	109	108	98	120	121

POMS-TMD: Profile of Mood States - Total Mood Disturbance Percentile

BMI: Body Mass Index

# of PD drugs: Total number of different Parkinson's drug types taken

r=Pearson correlation

**. Correlation is significant at the 0.01 level (2-tailed).

MDS-UPDRS

The MDS-UPDRS scores of the participants receiving a treatment from three different treatments were assessed at the baseline (time1), midterm (time2), and endline (time3). The results indicated that there was a statistically significant difference of MDS-UPDRS scores over the period of 12-week intervention (F=13.85, df=2, p-value <0.001), and in terms of 'treatment*time' (F=5.134, df=4, p-value=0.001). The statistically significant differences were between time2 and time1 (mean difference=-12.62, SE=2.36, Bonferroni adjusted p-value<0.001), and between time3 and time1 (mean difference=-11.01, SE=2.98, Bonferroni adjusted p-value=0.003); and between treatment1 and treatment3 at time3 (mean difference=-28.36, SE=9.86, Bonferroni adjusted p-value=0.022), and between treament2 and treatment3 at time3, i.e. by the end of the study 12-week study period (mean difference=-29.22, SE=9.98, Bonferroni adjusted p-value=0.019).

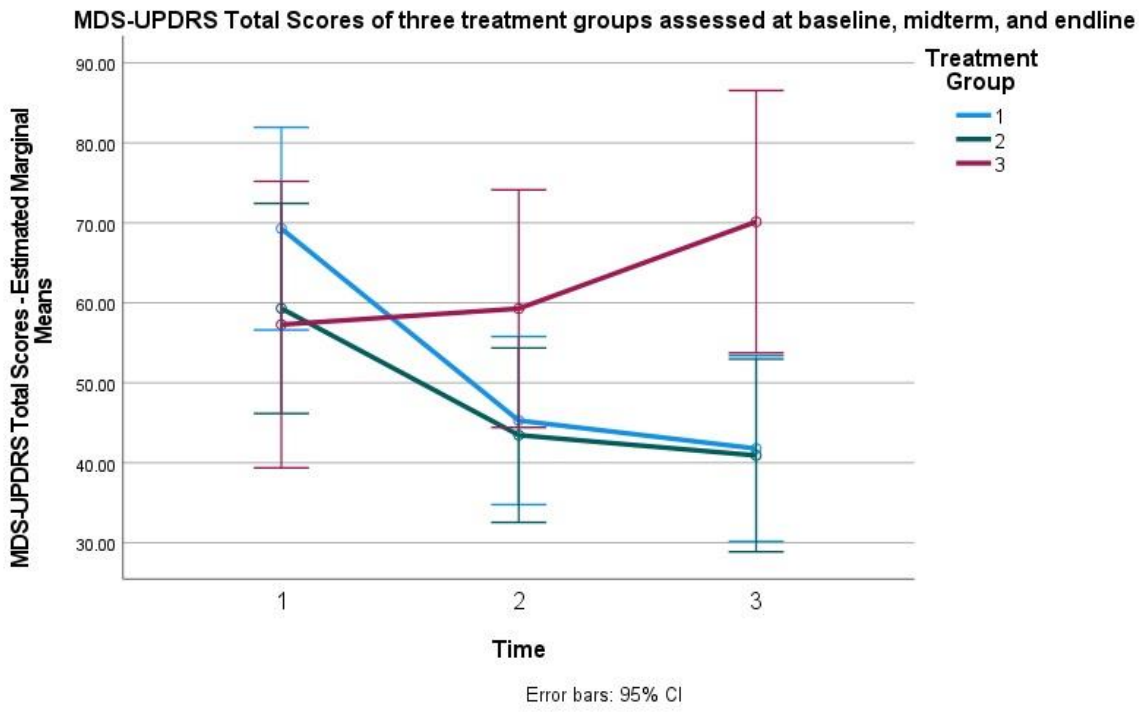

Figure 3.54. MDS-UPDRS Total Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

MDS-UPDRS Part I – nM_EDL (non-motor experience of daily living)

MDS-UPDRS Part I covered intellectual function, mood, and behavior dimension. The MDS-UPDRS Part I sub-scores analyses showed statistically significant differences between the over the 12-week intervention period (Friedman test statistics=8.47, df=2, p-value=0.014). The post-hoc test indicated that the statistically significant differences were between time1 and time3 (Wilcoxon Signed Ranks Test Statistics= -2.67, Bonferroni adjusted p-value=0.024). Treatment2 showed statistically significant differences of MDS-UPDRS Part I sub-scores over the 12-week intervention period, and it was between time1 and time3 (Wilcoxon Signed Ranks Test statistics= -2.575, Bonferroni adjusted p-value=0.030). Treatment1 showed marginally

statistically significant difference of MDS-UPDRS Part I sub-scores over the 12-week intervention period.

Table 3.18. MDS-UPDRS Part I Score Test Results of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

	Group1	Group2	Test Statistics	p-value	N
MDS-UPDRS Part I Score			8.47	0.014	37
	Time1	Time2	-2.237	0.075	37
	Time1	Time3	-2.67	0.024	37
	Time2	Time3	-0.049	1.000	37
MDS-UPDRS Part I Scores at Time 1			0.317	0.854	37
MDS-UPDRS Part I Scores at Time 2			1.465	0.481	37
MDS-UPDRS Part I Scores at Time 3			2.163	0.339	37
MDS-UPDRS Part I Score - Treatment 1			6.26	0.044	14
	Time1	Time2	-1.58	0.342	14
	Time1	Time3	-2.046	0.123	14
	Time2	Time3	-0.951	1.000	14
MDS-UPDRS Part I Score - Treatment 2			9.042	0.011	13
	Time1	Time2	-2.087	1.000	13
	Time1	Time3	-2.575	0.030	13
	Time2	Time3	0.000	1.000	13
MDS-UPDRS Part I Score - Treatment 3			0.378	0.828	10
	Time1	Time2	-0.119	1.000	10
	Time1	Time3	0.000	1.000	10
	Time2	Time3	-0.701	1.000	10

MDS-UPDRS Part II- M-EDL (motor aspects of experiences of daily living)

MDS-UPDRS Part II covered activities of daily living. MDS-UPDRS Part II score showed decreased trend in treatment1, and decreased trend first then went back to baseline level in treatment2, and increased trend in treatment3. However, the differences in changes of MDS-UPDRS Part II score did not reach a statistical significance (F=2.081, df=2, p-value=0.139, N=40)

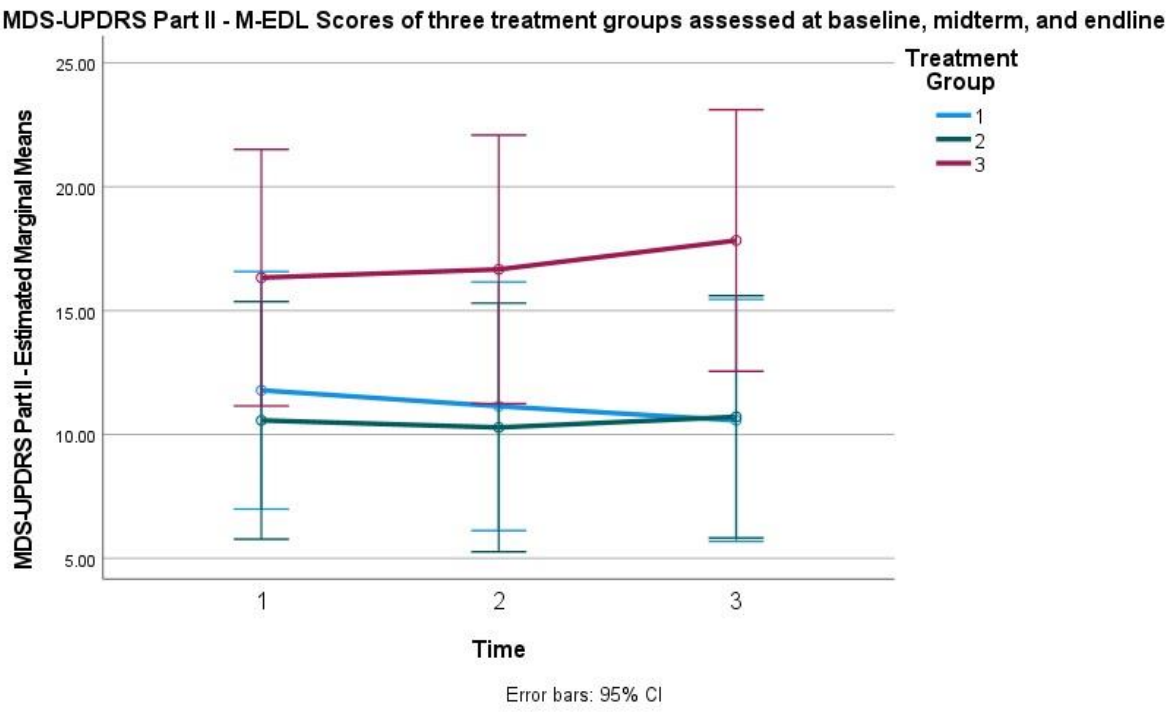

Figure 3.55. MDS-UPDRS Part II - M-EDL Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

MDS-UPDRS Part III – Motor Examination

MDS-UPDRS Part III Scores showed statistically significant differences in changes over time (F=35.77, df=1.48, Greenhouse-Geisser adjusted p-value<0.001, Eta^2=0.528, N=35), and in their combined effect of time*treatment (F=15.27, df=2.96, Greenhouse-Geisser adjusted p-value<0.001, Eta^2=0.488, N=35). At baseline, i.e. at time1, the MDS-UPDRS Part III mean scores of three different treatments (treatment1, treament2, and treament3) were almost the same (F=1.93, df=2, p-value=0.16, Eta^2=0.11, N=35). At mid-term (time2), their scores changed, and these changes showed statistically significant difference between three treatment groups (F=4.92, df=2, p-value=0.014, Eta^2=0.235, N=35). At the endline (time3), the MDS-UPDRS Part III

scores of three treatment groups showed more pronounced statistically significant difference ($F=11.07$, $df=2$, p-value<0.001, $Eta^2=0.409$, $N=35$).

The statistically significant differences were between treatment1 and treatment3 at time2 (mean difference -11.518, p-value=0.015), and between treatment2 and treatment3 at time2 (mean difference=-9.99, p-value=0.044); between treatment1 and treatment3 at time3 (mean difference=-17.196, p-value<0.001), and between treatment2 and treatment3 at time3 (mean difference=-16.587, p-value<0.001).

Table 3.19. MDS-UPDRS Part III - Motor Examination Score Test Results of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

	Group1	Group2	Test Statistics	p-value	N	df	Eta^2	SE
MDS-UPDRS Part III Score (Time)			35.770	<0.001	35	1.479	0.528	
	Time1	Time2	10.898	<0.001				1.530
	Time1	Time3	11.239	<0.001				1.852
	Time2	Time3	0.342	1.000				1.041
			Test Statistics					
MDS-UPDRS Part III Score (Treatment)			1.750	0.190	35	2.000	0.099	
			Mean difference					
	Treatment 1	Treatment2	2.244	1.000				3.774
	Treatment1	Treatment3	-5.917	0.548				4.343
	Treatment2	Treatment3	-8.16	0.219				4.403
			Test Statistics					
MDS-UPDRS Part III Score (Treatment x Time)			15.270	<0.001	35	2.958	0.488	
			Mean difference					
	Treatment1,Time1	Treatment2,Time1	8.868	0.363				5.569
	Treatment1,Time1	Treatment3,Time1	10.964	0.290				6.408
	Treatment2,Time1	Treatment3,Time1	2.096	1.000				6.497
	Treatment1,Time2	Treatment2,Time2	-1.527	1.000				3.324
	Treatment1,Time2	Treatment3,Time2	-11.518	0.015				3.824
	Treatment2,Time2	Treatment3,Time2	-9.99	0.044				3.877
	Treatment1,Time3	Treatment2,Time3	-0.610	1.000				3.44
	Treatment1,Time3	Treatment3,Time3	-17.196	<0.001				3.959

	Treatment2,Time3	Treatment3,Time3	-16.587	<0.001	4.014

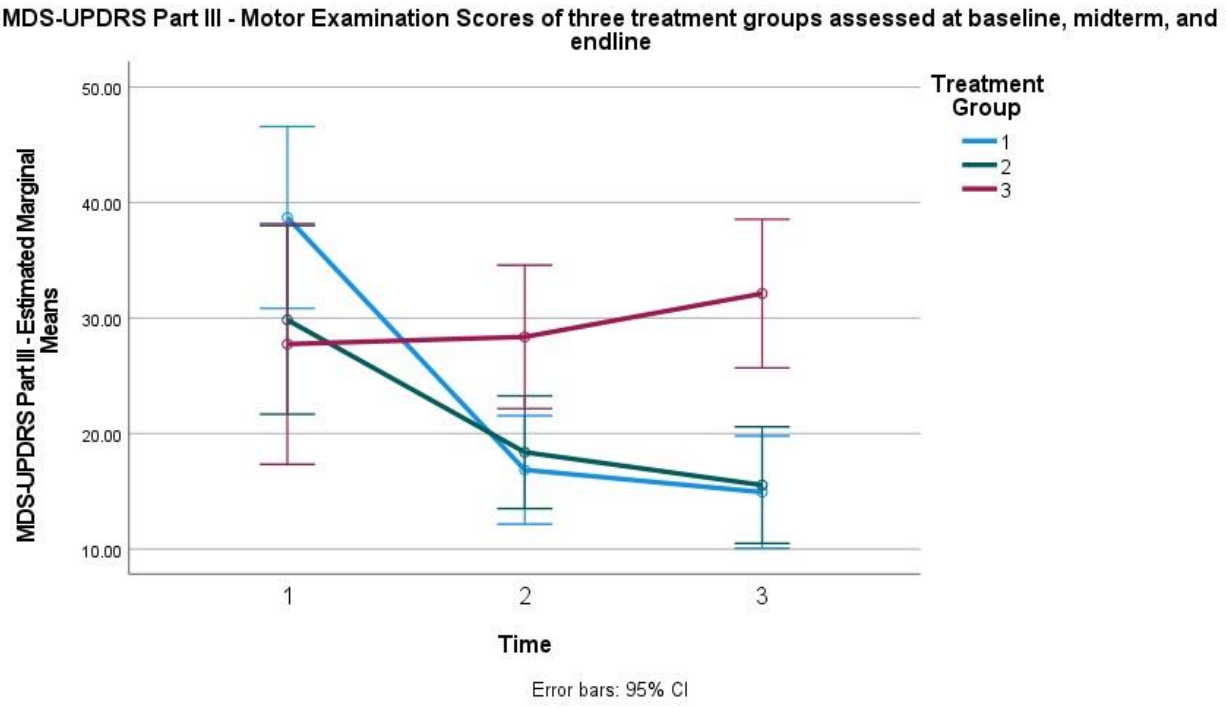

Figure 3.56. MDS-UPDRS Part III - Motor Examination Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

MDS-UPDRS Part IV – Motor Complications

MDS-UPDRS Part IV covered motor complications such as dyskinesia: irregular jerking, wiggling, twitching, and motor fluctuations that may impact on the patients' daily activities and social interactions. When the participants completed the study, their MDS-UPDRS Part IV Motor Complications Scores indicated statistically significant differences in effects of three different treatments ($F=5.25$, $df=2$, p-value=0.01, $Eta^2=0.231$), and that difference was found between treatment2 and treatment3 (mean difference=-3.371, Bonferroni adjusted p-value=0.009). The effects of three different treatments on motor complications (MDS-UPDRS Part IV) were not different at the baseline (time1) and mid-term (time2).

Table 3.20. MDS-UPDRS Part IV - Motor Complications Scores Test Results of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

	Group1	Group2	Test Statistics	p-value	N	df	Eta^2	SE
MDS-UPDRS Part IV Score (Effect of Treatments)								
	At the base line (time1)		0.215	0.808	38	2	0.012	
	At the mid-term (time2)		0.77	0.47	38	2	0.042	
	At the endline (time3)		5.251	0.01	38	2	0.231	
MDS-UPDRS Part IV Score (Treatment x Time)								
			Mean difference					
	Treatment1,Time1	Treatment2,Time1	-0.813	1.000				1.481
	Treatment1,Time1	Treatment3,Time1	-0.883	1.000				1.549
	Treatment2,Time1	Treatment3,Time1	-0.07	1.000				1.575
	Treatment1,Time2	Treatment2,Time2	1.022	1.000				1.228
	Treatment1,Time2	Treatment3,Time2	-0.558	1.000				1.285
	Treatment2,Time2	Treatment3,Time2	-1.58	0.703				1.306
	Treatment1,Time3	Treatment2,Time3	0.962	1.000				0.999
	Treatment1,Time3	Treatment3,Time3	-2.409	0.082				1.045
	Treatment2,Time3	Treatment3,Time3	-3.371	0.009				1.063

*Pair-wise comparison p-values are Bonferroni adjusted p-values.

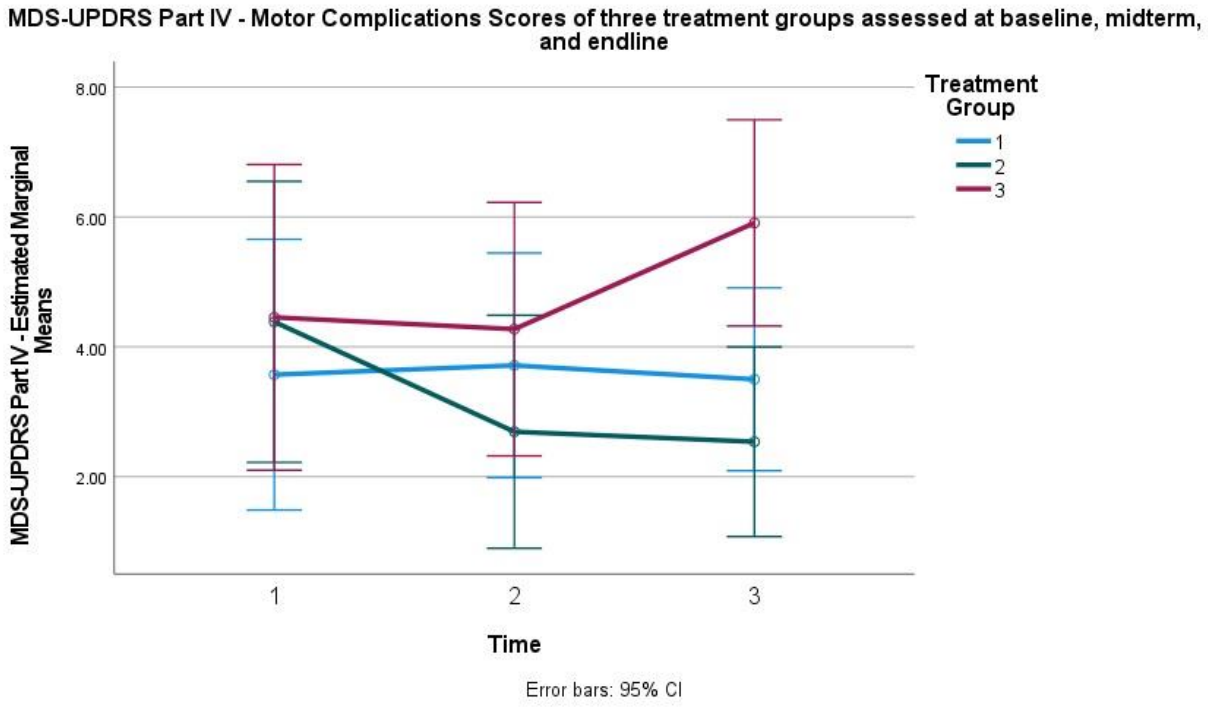

Figure 3.57. MDS-UPDRS Part IV - Motor Complications Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

PDQ-39

Overall

The PD Questionnaire-39 (PDQ-39) total scores showed statistically significant differences in three assessments over the 12-week intervention period (Friedman test statistics=6.795, df=2, p-value=0.033, N=38). The statistically significant difference was between time 1 and time 3 (Test Statistics=2.581, p-value=0.010, Bonferroni adjusted p-value=0.030, n=38); there was not statistically significant difference between time 1 and time 2 (Test Statistics=-1.032, p-value=0.0.302, Bonferroni adjusted p-value=0.906, n=38); and not statistically significant difference between time 2 and time 3 (Wilcoxon Signed Rank Test Statistics=1.549, p-value=0.121, Bonferroni adjusted p-value=0.364, n=38).

Treatment 1 did not show statistically significant difference of PDQ-39 overall scores over the 12-week invention period (Friedman test statistics=2.873, df=2, p-value=0.238, n=14). Treatment 2 did not show statistically significant difference of PDQ-39 overall scores over the 12-week period (Friedman test statistics=4.667, df=2, p-vale=0.097, n=11). Treatment 3 did not show statistically significant difference of PDQ-39 overall scores over the 12-week intervention period (Friedman test statistics=3.5, df=2, p-value=0.174, n=12).

Table 3.21. PDQ-39 Overall score test results of three treatment groups - Treatment 1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

	Group1	Group2	Test Statistics	p-value*	N
PDQ-39 Total Score			6.795	0.033	38
	Time1	Time2	1.032	0.906	
	Time1	Time3	2.581	0.030	
	Time2	Time3	1.549	0.364	
PDQ-39 Total Score at Time 1			3.278	0.194	42
	Treatment 1	Treatment2	-3.667	1.000	
	Treatment1	Treatment3	-8.600	0.211	
	Treatment2	Treatment3	-4.933	0.897	
PDQ-39 Total Score at Time 2			1.304	0.521	40
	Treatment 1	Treatment2	-0.279	1.000	
	Treatment1	Treatment3	-4.475	0.969	
	Treatment2	Treatment3	-4.196	1.000	
PDQ-39 Total Score at Time 3			2.145	0.342	40
	Treatment 1	Treatment2	-2.429	1.000	
	Treatment1	Treatment3	-6.690	0.437	
	Treatment2	Treatment3	-4.262	1.000	
PDQ-39 Total Score - Treatment 1			2.873	0.238	14
	Time1	Time2	-1.161	0.735	
	Time1	Time3	-1.224	0.663	
	Time2	Time3	-1.664	0.288	

PDQ-39 Total Score - Treatment 2			4.667	0.097	12
	Time1	Time2	-1.050	1.000	
	Time1	Time3	-1.224	0.663	
	Time2	Time3	-1.687	0.276	
PDQ-39 Total Score - Treatment 3			3.5	0.174	12
	Time1	Time2	-1.569	0.351	
	Time1	Time3	-0.549	1.000	
	Time2	Time3	-1.099	0.816	

* Post hoc pairwise comparison p-values are Bonferroni-corrected.

Mobility Scores

There appeared to have some lesser degree of mobility score changes (mobility issue progression) in treatment 1 and 2, compared to treatment 3 in which higher degree of mobility score change (mobility issues progression) observed. However, the differences of changes did not reach to the statistical significance (Friedman test statistics= 1.717, df=2, p-value=0.424, N=39).

Activities of Daily Living Scores

The mean scores of difficulties in activities of daily living in the treatment 1 and 2 groups were decreased and apparently getting better while that of treatment 3 (control) group was increased. However, these changes did not reach to the statistically significant level (Friedman's test statistics=4.8, df=2, p-value=0.090, N=39).

The activities of daily living (ADL) scores showed statistically significant difference between time 1 and time 2 (Friedman test statistics=4.84, p-value=0.028, n=40) but not statistically significant difference between time 1 and time 3 (Friedman test statistics=3.13, p-value=0.077, n=41) and time 2 and time 3 (Friedman test statistics=0.571, p-value=0.45, n=39).

Emotional Wellbeing Scores

The emotional wellbeing scores were decreased (improvements) in treatment1 &3 and increased at midterm and decreased at endline in treatment2. Overall, the differences in the (difficulties/issues of) emotional wellbeing changes did not show statistically significance between three treatment groups over the 12-week intervention period- time 1, 2 and 3 (Friedman test statistics=5.349, p-value=0.069, n=39).

Stigma Scores

Stigma scores in the three treatment groups varied in three assessments. However, the changes in stigma scores over the 12-week period did not show statistical significance among three treatment groups (Friedman test statistics=0.467, df=2, p-value=0.792, N=39).

Social Support Scores

The social support scores were decreased (decreased problems) in treatmet1 and 2 but increased in treatment3, placebo group. However, the changes in social support scores did not show statistically significant differences among three treatment groups over the 12-week intervention period (Friedman's test statistics=0.325, df=2, p-value=0.85, N=39).

Cognition Scores

The cognition dimension of PDQ-39 scores were slightly increased in treatment1 and treatment2 and decreased in treatment3 group. However, the differences of changes in cognition scores were not statistically significant among three different groups over the 12-week intervention period (Friedman test statistics=2.855, df=2, p-value=0.240, N=38).

Communication Scores

The differences in changes of communication scores showed statistically significant difference between times (F=4.76, df=1, p-value=0.036), but in within subjects effects (F=3.18, df=2, p-value=0.047, Eta^2=0.083), and in between subjects effects (treatment groups) (F=2.90, df=2, p-value=0.068). At post hoc test, the marginally statistically significant difference was between treatment1 and treatment3 at time3 (Bonferroni adjusted p-value=0.045).

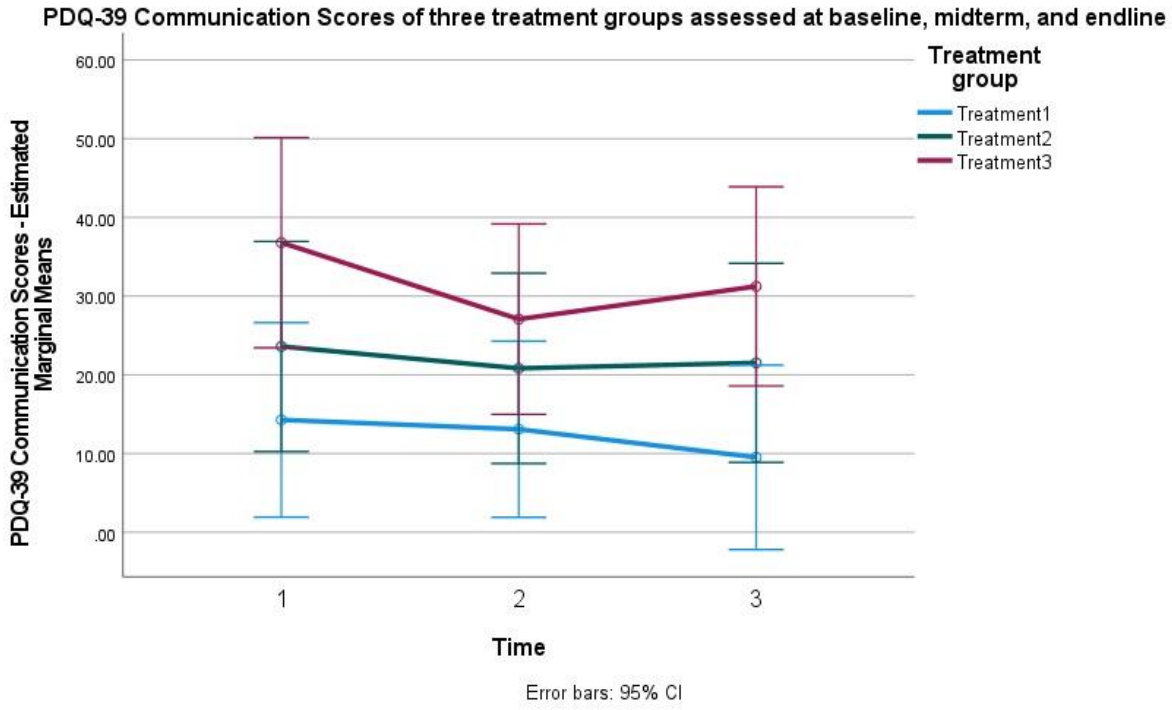

Figure 3.58. PDQ-39 Communication Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

Bodily Discomfort Scores

The changes of bodily discomfort scores were decreased in treatment2 and treatment1 and increased in treatment3. However, these changes in bodily discomfort scores did not reach to

the statistically significant levels between three treatment groups (F=1.34, df=2, p-value=0.275), over the 12-week period of time (F=0.812, df=2, p-value=0.453).

Table 3.22. PDQ-39 scores: Total Score and 8 Dimensional Scores of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

		Time 1			Time 2			Time 3		
PDQ-39 Score		Mean	SD	n	Mean	SD	n	Mean	SD	n
Total	Treatment1	143.2	103.9	14	155.0	109.0	14	133.7	98.2	14
	Treatment2	161.4	99.9	12	165.6	130.9	12	145.6	138.1	12
	Treatment3	255.3	157.5	12	219.6	175.7	12	248.9	204.1	12
Mobility										
	Treatment1	20.5	28.3	14	25.7	26.3	14	22.5	26.1	14
	Treatment2	15.6	21.1	13	18.1	24.9	13	18.2	26.7	13
	Treatment3	31.7	24.5	12	30.2	35.0	12	36.2	36.5	12
ADL										
	Treatment1	14.9	17.0	14	14.9	15.4	14	12.8	15.1	14
	Treatment2	21.8	18.0	13	17.0	17.6	13	19.4	23.6	13
	Treatment3	31.0	32.0	12	27.8	33.9	12	33.0	36.0	12
Emotional Wellbeing										
	Treatment1	22.3	21.3	14	21.1	24.0	14	21.1	20.5	14
	Treatment2	19.2	16.0	13	27.0	23.8	13	18.6	17.0	13
	Treatment3	33.0	28.8	12	27.8	28.0	12	28.5	26.0	12
Stigma										
	Treatment1	8.0	10.2	14	14.3	14.6	14	6.7	10.0	14
	Treatment2	13.5	15.3	13	14.9	16.2	13	15.9	19.3	13
	Treatment3	31.8	28.6	12	22.9	30.0	12	27.6	32.4	12
Social Support										
	Treatment1	14.3	21.5	14	14.3	19.7	14	12.5	19.5	14
	Treatment2	10.3	15.3	13	14.1	20.2	13	10.3	13.7	13
	Treatment3	16.7	16.7	12	17.0	19.8	12	20.1	30.7	12
Cognition										
	Treatment1	17.9	14.7	14	18.3	16.0	14	18.8	14.7	14
	Treatment2	19.8	14.3	12	21.4	17.8	12	20.3	24.4	12
	Treatment3	30.7	20	12	26.6	18.3	12	27.1	21.9	12

Communication										
	Treatment1	14.3	13.2	14	13.1	12.1	14	9.5	13.0	14
	Treatment2	23.6	21.9	12	20.8	19.6	12	21.5	22.0	12
	Treatment3	36.8	31.1	12	27.1	28.2	12	31.3	28.2	12
Bodily Discomfort										
	Treatment1	30.9	24.1	14	33.3	22.2	14	30.9	22.5	14
	Treatment2	36.1	25.5	12	29.2	28.8	12	25.0	21.3	12
	Treatment3	43.8	13.8	12	40.3	25.6	12	45.1	25.5	12

Depression

Depression causes feelings of sadness, hopelessness, and reduced energy, anxiety causes feelings of nervousness, worry, or dread (Watson & Bhandari, 2021). Participants were asked about having depression and anxiety symptoms for some day, several days, most of the days, almost every day, over the past 2 weeks. NHS-Mood Zone questionnaire (NHS, 2020) was applied for depression and anxiety assessment.

Analysis of Variance (ANOVA) of depression scores for treatment 1, 2, and 3 measured at three different times (time 1, 2 and 3 or pre-test, mid-term, and post-test) indicated statistically significant difference (F=3.98, df=2, N=39, p-value=0.028) in terms of time but the interaction of Time*Treatment.

The post-hoc test shows statistically significant difference (*p=0.016)* was from the treatment 3 between time 1 (pre-test) and time 2 (mid-term) (mean difference=2.75, Bonferroni adjusted p-value=0.016, SE=0.925).

There were vulnerable factors that may impact depression and anxiety, and about 9 factors were accounted during the assessments at three times. The number of vulnerable factors each person had showed significant differences in treatment groups at each assessment time: at

time1 (F=22.61, p-value<0.001), at mid-term (time2) (F=11.44, p-value=0.002), at the end-line (time3) (F=10.64, p-value=0.002).

Table 3.23. Post-hoc test, pairwise comparisons of depression score of participants received three different treatments and measured at three different times

Depression

	Group1	Group2	Test Statistics	p-value	N	df	Eta^2	SE
Depression Scores								
	Time effect		3.98	0.028	39	2	0.185	
	Time* Treatment		0.77	0.266	39	4	0.069	
	Treatment effect		0.99	0.381	39	2	0.052	
Depression Scores								
			Mean difference					
	Treatment1,Time1	Treatment2,Time1	-0.214	1.000				0.856
	Treatment1,Time1	Treatment3,Time1	0.429	1.000				0.922
	Treatment2,Time1	Treatment3,Time1	0.643	1.000				0.975
	Treatment1,Time2	Treatment2,Time2	1.077	0.700				0.889
	Treatment1,Time2	Treatment3,Time2	1.462	0.406				0.957
	Treatment2,Time2	Treatment3,Time2	0.385	1.000				1.012
	Treatment1,Time3	Treatment2,Time3	2.750	0.016				0.925
	Treatment1,Time3	Treatment3,Time3	2.167	0.109				0.996
	Treatment2,Time3	Treatment3,Time3	-0.583	1.000				1.053

Table 3.24. Depression Scores Statistics (3 treatments, at 3 different times)

	Treatment	Mean	Std. Deviation	N
Depression Score Time 1	1	6.50	5.20	14
	2	6.85	3.72	13
	3	10.00	5.56	12
	Total	7.69	5.00	39
Depression Score Time 2	1	6.71	5.76	14
	2	5.77	4.51	13
	3	7.25	4.18	12
	Total	6.56	4.82	39
Depression Score Time 3	1	6.07	4.55	14
	2	5.38	4.01	13
	3	7.83	5.86	12
	Total	6.38	4.81	39

Table 3.25. Anxiety Scores Statistics (3 treatments, at 3 different times)

	Treatment	Mean	Std. Deviation	N
Anxiety Score Time 1	1	4.93	4.70	14
	2	4.77	4.19	13
	3	7.08	6.97	12
	Total	5.54	5.32	39
Anxiety Score Time 2	1	5.43	5.61	14
	2	3.92	3.86	13
	3	5.08	4.29	12
	Total	4.82	4.61	39
Anxiety Score Time 3	1	4.00	4.40	14
	2	3.77	3.92	13
	3	5.50	5.70	12
	Total	4.38	4.63	39

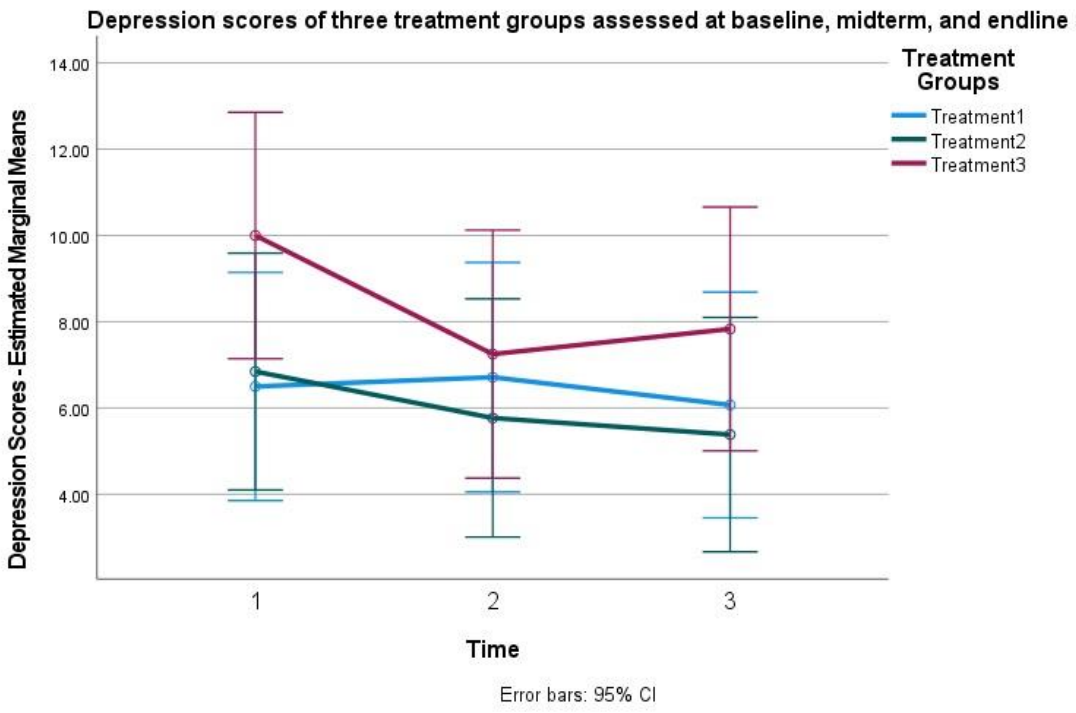

Figure 3.59. Depression Scores of three treatment groups - Treatment 1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

Anxiety Scores

Anxiety Scores did not show statistically significant difference between three treatment groups at three times.

There were vulnerable factors that may impact depression and anxiety, and about 9 factors were asked during the assessments at three times. The 9 vulnerable factors asked for depression and anxiety assessment were: 'Financial problems or worries', 'Something bad that happened recently', 'Difficulties with partner', 'Having no one to turn to', 'Worrying about weight or look', 'Stress at work, or outside home', 'Stress of taking care of family members', 'Worrying about health', and 'Little or no sexual desire or pleasure during sex' as outlined in the mood zone assessment, NHS, UK (NHS, 2020). By univariate analyses for each assessment

time, the number of vulnerable factors reported by each person at each assessment time showed statistically significant differences (impact) in treatment groups at each assessment time: at time1 (F=31.34, p-value<0.001), at mid-term (time2) (F=20.22, p-value<0.001), at the end-line (time3) (F=15.17, p-value<0.001).

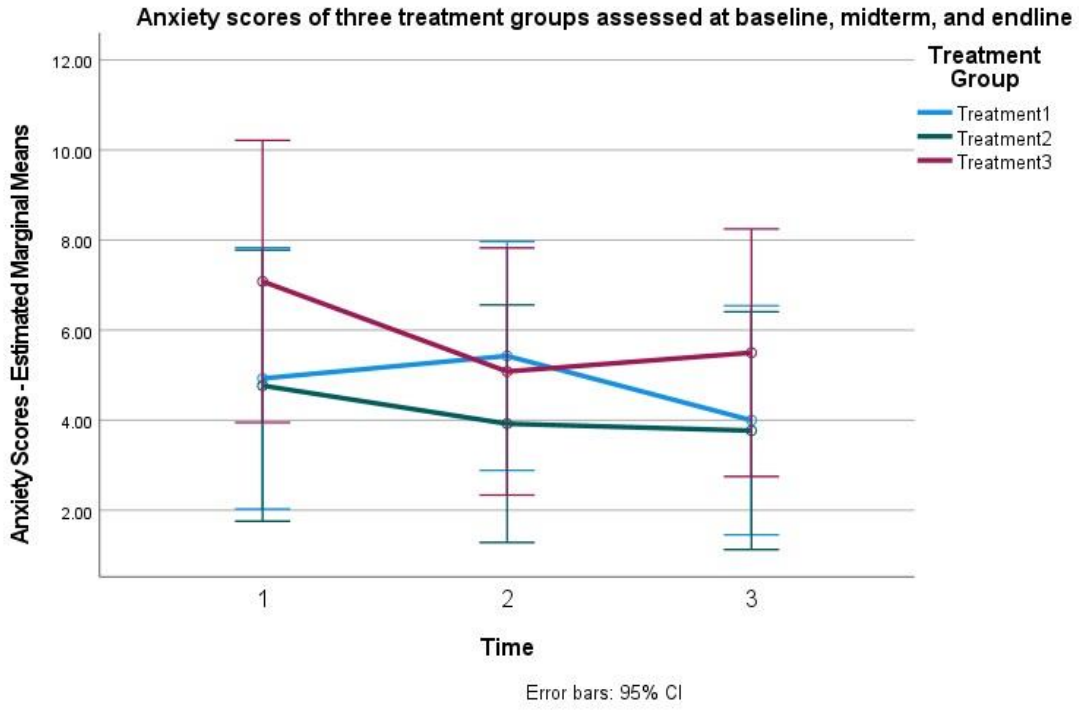

Figure 3.60. Anxiety Scores of three treatment groups - Treatment 1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

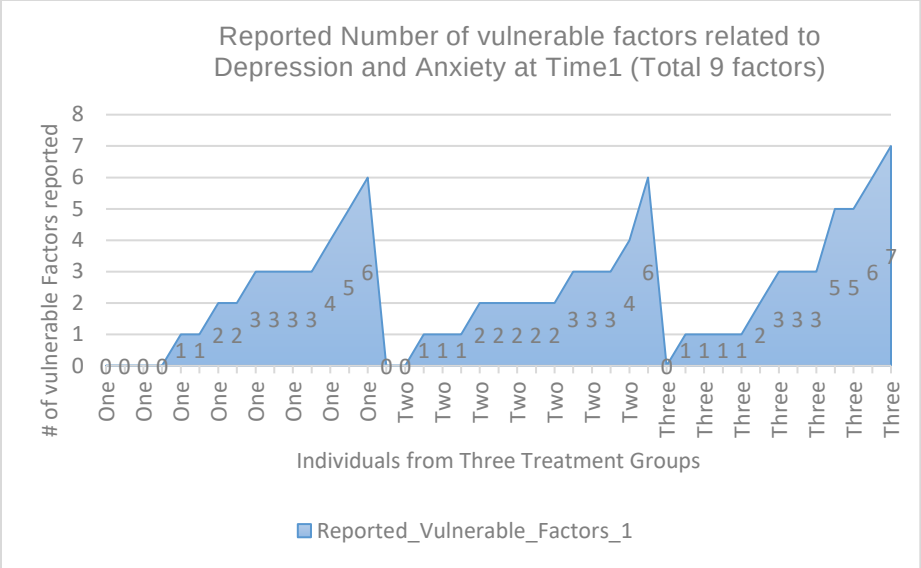

Figure 3.61. Reported number of vulnerable factors related to depression and anxiety at Time1 among three treatment groups- Treatment-One: Probiotics+B_3, Treatment-Two: Probiotics, Treatment-Three: Placebo

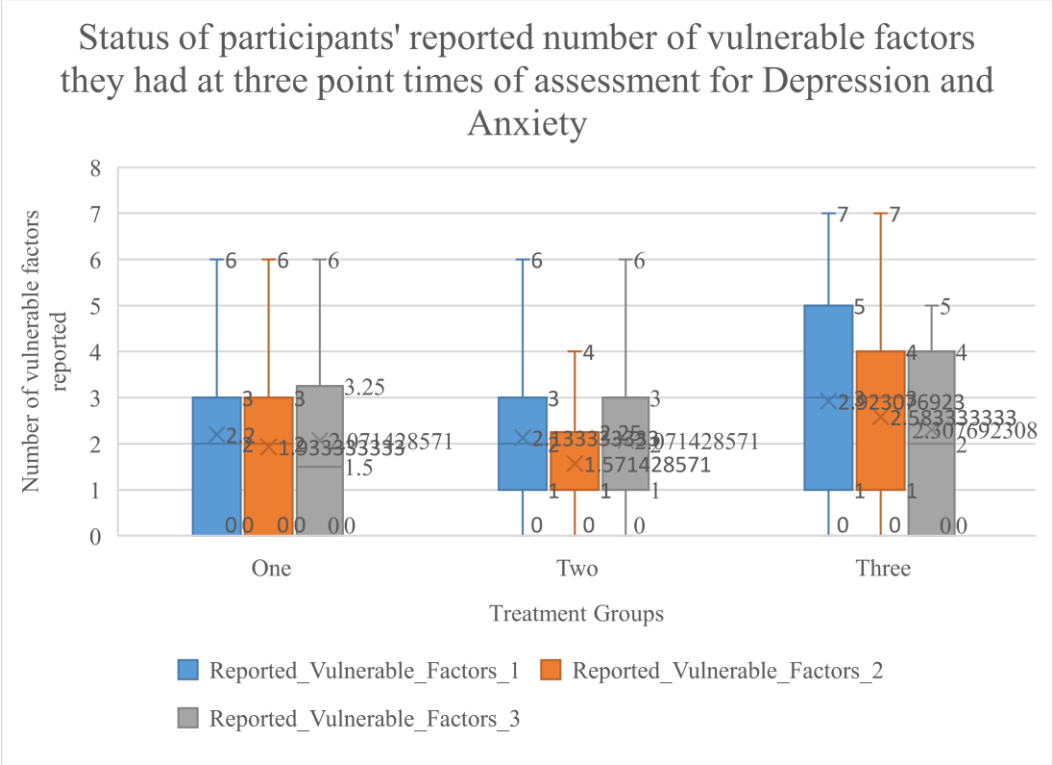

Figure 3.62. Reported number of vulnerable factors (total 9 factors) among three treatment groups - Treatment 1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

Correlation between Profile of Mood States (POMS), constipation problems, frequency % of hard or loose stool types, BMI, and total number of different Parkinson's drugs taken

There were statistically significant strong positive correlations between mood disturbances (Profile of Mood States, POMS- Total Mood Disturbance (TMD)) and constipation problems (r=0.046, p=0.00), and frequency of having hard and lumpy stools (stool type 1 or 2) (r=0.38, p=0.00). These findings made to conclude that having hard and lumpy stools had greater influence on mood disturbances compared to mushy or watery stools, and constipation problems had significant influence on mood disturbances in this study. BMI showed statistically significant positive correlation with mushy or watery stools among study population (r=0.23, p=0.02), and negative correlation with mood disturbances (POMS-TMD) (r=-0.24, p=0.02). These finding made to conclude that individuals with heavier or higher BMI could have more frequent mushy or watery stools (type 6 or 7) and their counter parts with PD who had thinner or leaner or lighter BMI, likely at risk malnutrition, could experience greater mood disturbances.

Table 3.26. Correlation between Profile of Mood States (POMS), constipation problems, frequency % of hard or loose stool types, and BMI

		Stool type 1 or 2 (%)	Stool type 6 or 7 (%)	POMS-TMD	BMI	# of PD drugs
Constipation problems	r	0.40**	0.11	0.46**	-0.04	-0.01
	p-value	0.00	0.27	0.00	0.69	0.94
	N	109	108	98	119	120
Stool type 1 or 2 (hard or lumpy stools) %	r		-0.05	0.38**	-0.02	-0.08
	p-value		0.59	0.00	0.83	0.40
	N		106	90	108	109
Stool type 6 or 7 (mushy or watery stools) %	r			0.08	0.23*	-0.07
	p-value			0.47	0.02	0.45
	N			88	107	108
Profile of Mood States - Total Mood Disturbance Percentile	r				-0.24*	0.02
	p-value				0.02	0.81
	N				97	98
BMI	r					0.00
	p-value					0.96
	N					120

POMS-TMD: Profile of Mood States - Total Mood Disturbance Percentile

BMI: Body Mass Index

# of PD drugs: Total number of different Parkinson's drug types taken

r=Pearson correlation

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Profile of Mood States Analyses

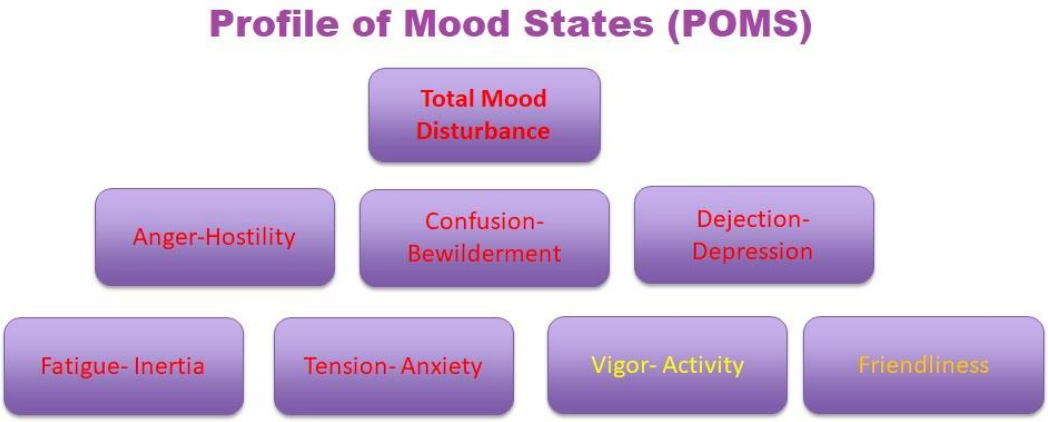

Figure 3.63. Dimensions of Profile of Mood States (POMS)

Total Mood Disturbance (TMD) Percentile

The total mood disturbance (TMD) percentiles of the participants from three treatment groups who completed all mood assessments in all of three assessment times- baseline, midterm and endline were analyzed. The result did not show statistically significant difference in three treatment groups over the 12-week intervention period ($F= 0.698$, p-value=0.51, $Eta^2= 0.72$, N=21)

Anger – Hostility Percentile

Anger and hostility score decreased at midterm assessment in treatment1 and 2, then increased back to the original level. Anger-hostility score was increased in treatment3 (placebo group). Overall, the result did not show statistically significant difference among three treatment groups over the 12-week intervention period ($F= 1.987$, p-value=0.166, $Eta^2= 0.181$, N=21).

Confusion-Bewilderment Percentile

Confusion- Bewilderment increased in placebo group, did not change in probiotics groups till midterm. Confusion- bewilderment of placebo group decreased slightly at the endline which was slightly lower than baseline level. From midterm to endline, confusion-bewilderment of probiotics+vitaminB_3 group increased about 3 percentiles in average, and that of probiotics alone group decreased. Overall, the result did not show statistically significant difference in three treatment groups over the 12-week intervention period (F= 0.432, p-value=0.656, Eta^2= 0.046, N=21).

Dejection-Depression Percentile

Depression- Dejection percentiles of the participants from three treatment groups who completed all mood assessments in all of three assessment times- baseline, midterm and endline, did not show statistically significant difference in three treatment groups over the 12-week intervention period (F=0.162, p-value=0.852, Eta^2= 0.018, N=21).

From baseline to midterm, depression, dejection mood cluster percentiles of probiotics alone group and placebo group were decreased by 10 percentiles on average. From midterm to endline, both groups' scores increased back to the near-baseline levels. Dejection-depression score of treatment1 (probiotics+B_3) group increased by 4 percentiles on average between baseline and midterm, and between midterm-endline.

Tension-Anxiety Percentile

Tension-Anxiety percentiles of the participants from three treatment groups who completed all mood assessments in all of three assessment times- baseline, midterm and endline,

showed no statistically significant difference among three treatment groups over the 12-week intervention period (F=1.31, p-value=0.294, Eta^2= 0.127, N=21).

Fatigue-Inertia Percentile

The positive aspect: Fatigue and inertia decreased, but that occurred in all treatment groups. Overall, the result did not show statistically significant difference in three treatment groups over the 12-week intervention period (F=0.162, p-value=0.852, Eta^2= 0.018, N=21).

Vigor-Activity Percentile

Vigor-Activity percentiles of the participants from three treatment groups did not show statistically significant difference over the 12-week intervention period (Friedman test statistics=1.726, p-value=0.422, N=21).

Friendliness Percentile

Friendliness percentiles of the participants from three treatment groups did not show statistically significant difference over the 12-week intervention period (Friedman test statistics=1.30, p-value=0.522, N=21).

Nutritional Assessment

Body Weight

There was no statistically significant difference of body weight in three treatment groups over the 12-week intervention period (F=0.489, df=2, p=0.617, Eta^2=0.26, N=40).

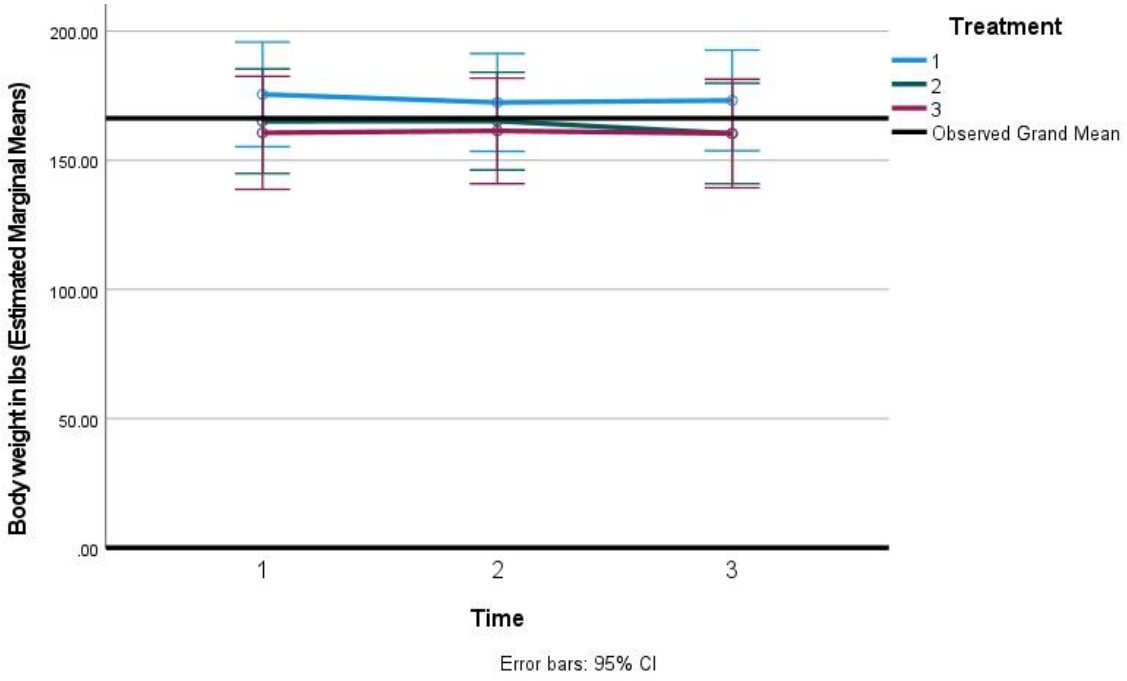

Figure 3.64. Body weight in lbs of three treatment groups over the 12-week intervention period

Body Mass Index (BMI)

BMI of treatment1 and treatment2 showed decreased trend while treatment3 showed slight increased trend until midterm, then each of them appeared to change to the opposite directions, i.e., BMI of treatment1 and treatment2 showed increased trend, and treatment3 showed decreased trend between midterm and endline. Overall, there were always no statistically significant differences between the three treatment groups at any point in time (F=0.508, df=2, p-value=0.606).

Table 3.27. Bodyweight (kg) and Body Mass Index of three treatment groups assessed at baseline (Week 0), midterm (Week 6) and endline (Week 12)

Time		Treatment1			Treatment2			Treatment3		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
Baseline	Bodyweight in kg	80	18	14	75	16	14	73	18	12
Midterm	Bodyweight in kg	78	14	14	75	16	14	73	18	12
Endline	Bodyweight in kg	79	14	14	73	17	14	73	19	12
Baseline	Body Mass Index	26	5	14	27	4	14	25	5	12
Midterm	Body Mass Index	26	4	14	26	5	14	25	5	12
Endline	Body Mass Index	26	4	14	27	5	14	25	5	12

Vitals

Systolic Blood Pressure

Blood pressure was taken twice using the sphygmomanometer and average the readings. Systolic blood pressure was insignificantly increased in probiotics alone group, and significantly increased in placebo group at midterm, and stable or slightly decreased trend in probiotics+vitaminB_3 group at midterm. From midterm to endline, probiotics+vitaminB_3 group and placebo group changed to opposite trends.

There was a statistically significant difference in average systolic blood pressure over the 12-week intervention period ($F=5.164$, $df=2$, p-value=0.014, $Eta^2=0.319$, $N=26$). The statistically significant differences were observed at midterm between treatment1 (probiotics+vitaminB_3) group and treatment3 (placebo+placebo) group (mean difference=-21.27, Bonferroni adjusted p-value=0.046), and in treatment3 (placebo+placebo) between baseline and midterm (mean difference=-25.78, Bonferroni adjusted p-value<0.001).

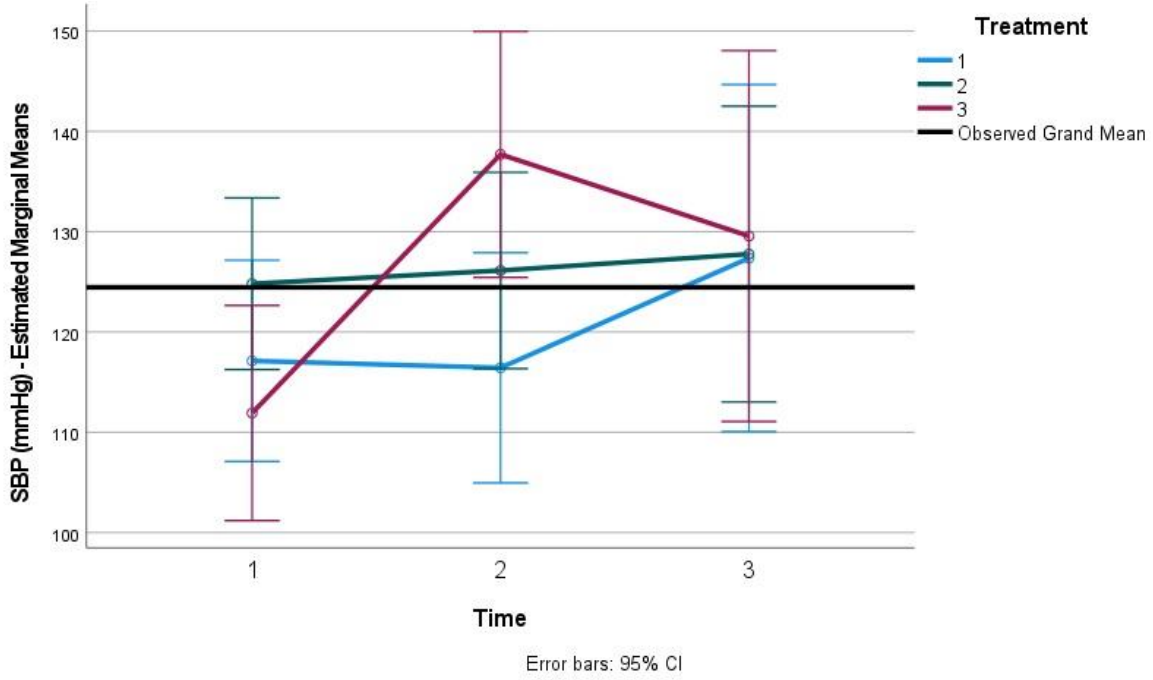

Figure 3.65. Average systolic blood pressure of three treatment groups over the 12-week intervetnion period

Diastolic Blood Pressure

There was a statistically significant difference in the treatment groups over the 12-week intervention period (F=3.547, df=2, p-value=0.037, N=26), and the difference was observed in treatment1 (probiotics+vitaminB_3) between midterm and endline (mean difference=-8.81, Bonferroni adjusted p-value=0.02).

Pulse Rate

There was not statistically significant difference in three treatment groups over the 12-week intervention period (F=0.947, df=2, p-value=0.385, Eta^2=0.041, N=26).

Blood Chemistry

Serum Electrolytes

Serum Sodium (mmol/L)

Serum sodium levels (mmol/L) was increased in treatment3 from baseline to midterm, and leveled off at endline, and decreased in treatment1 throughout, and initially slightly increased at midterm then decreased at endline to the baseline level in treatment2. There was marginally statistically significant difference of serum sodium level in terms of treatment*time (F=4.193, df=4, p=0.05), and the difference was between treatment2 and treatment3 at time3 (mean difference=-2.5, Bonferroni adjusted p-value=0.039, N=29, n=6).

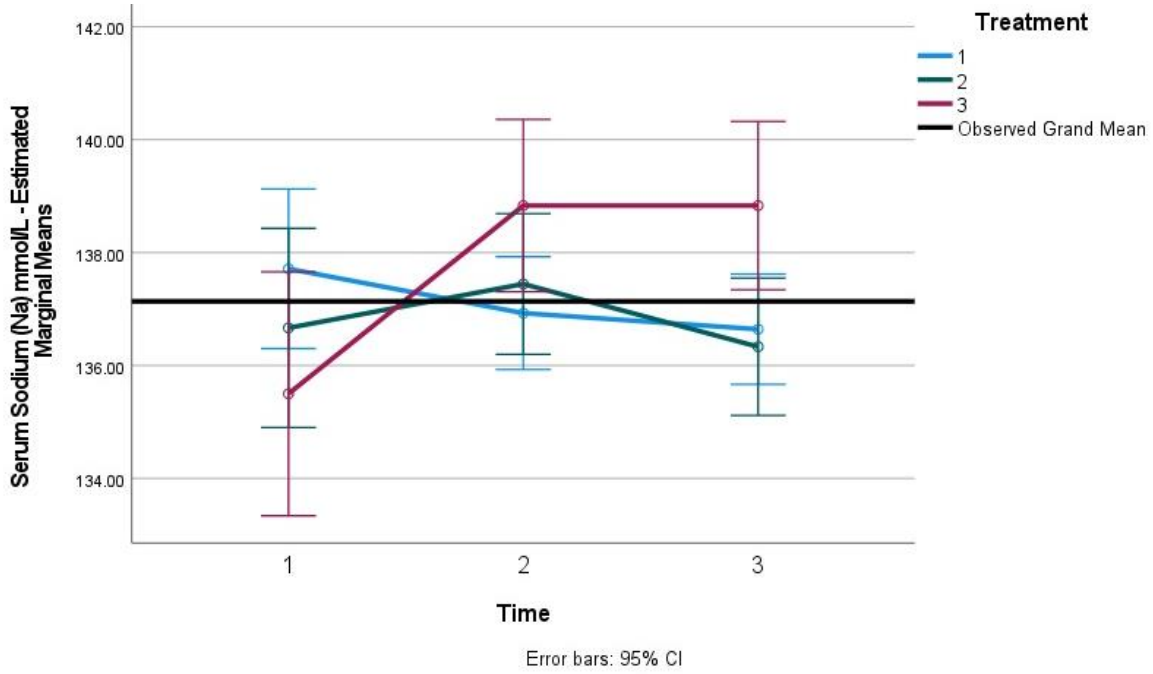

Figure 3.66. Serum sodium levels of three treatment groups over the 12-week intervention period

Serum Carbon dioxide (CO_2) mmol/L

There tests showed there was a statistically significant difference within subjects over time (F=4.182, df=2, p=0.021, N=29), and that difference was between midterm and endline (mean difference=1.384, p=0.037), however, when further navigated, that difference in terms of treatment group, the Bonferroni adjusted p-value became statistically insignificant (p-value=0.127).

Serum Chloride (mmol/L)

Serum chloride level showed there was no statistically significant differences in all treatment groups (F=0.095, df=2, p-value=0.910, Eta^2=0.007, N=29).

Serum Glucose levels (mg/dL)

Serum glucose levels was apparently increased in all treatment groups. The probiotics alone group and placebo showed slightly decreased at midterm, then increased at endline.

There was statistically significant difference in within group changes of serum glucose level (F=8.87, df=2, p-value=0.001, N=29), and it was in treatment3, placebo group, between time2, midterm, and time3, endline (mean difference=-12.33, p-value=0.011). However, all the mean serum glucose levels were within the normal reference range of 73-118 mg/dL.

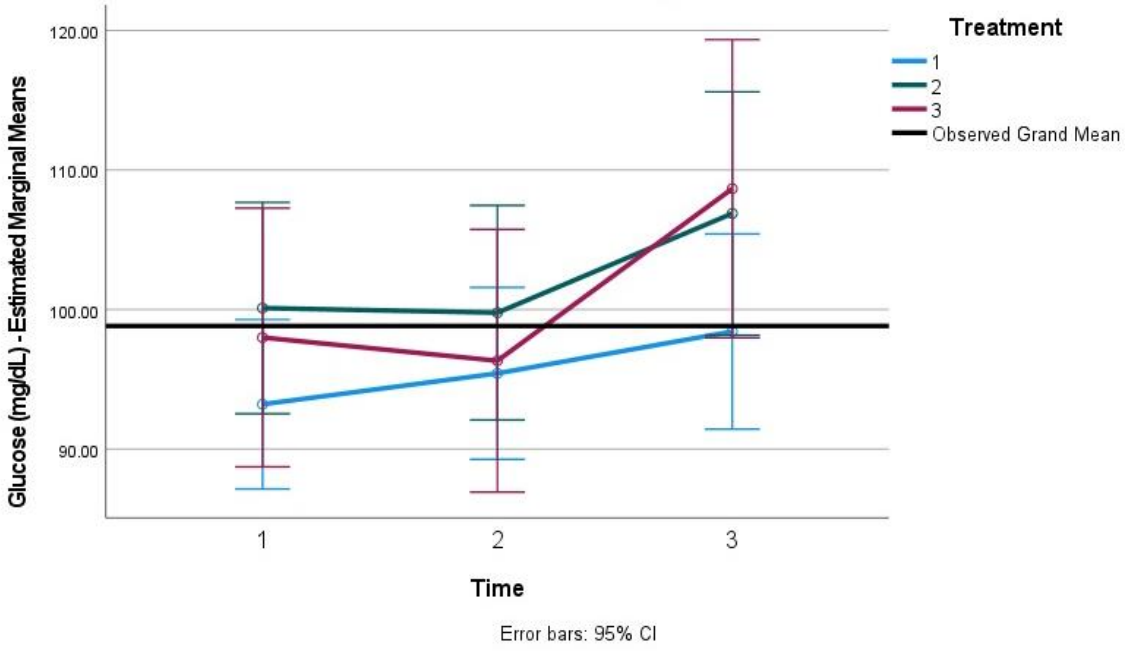

Figure 3.67. Serum glucose levels (mg/dL) of three treatment groups over the 12-week intervention period | Normal reference range 73-118 mg/dL

Serum potassium level (mmol/L)

Serum potassium level was constantly increased trend from baseline till endline in probiotic+B_3 group and placebo group, and initial upward trend from baseline to mid-term and downward trend till endline in treatment2. There was statistically significant difference within subjects in terms of time (F=7.128, df=2, p-value=0.02, N=29), and treatment*time (F=2.862, df=4, p-value=0.032, N=29). The difference in treatment1 was between baseline and endline (mean difference=-2.57, Bonferroni adjusted p-value=0.018, N=29, n=14), the statistically significant difference in treatment2 was between baseline and midterm (mean difference= -0.278, Bonferroni adjusted p-value=0.045, N=29, n=9), and between midterm and endline (mean difference=0.244, Bonferroni adjusted p-value=0.033, N=29, n=9). However, all mean levels of Potassium in three treatment groups were within the normal reference range of 3.6-5.1 mmol/L.

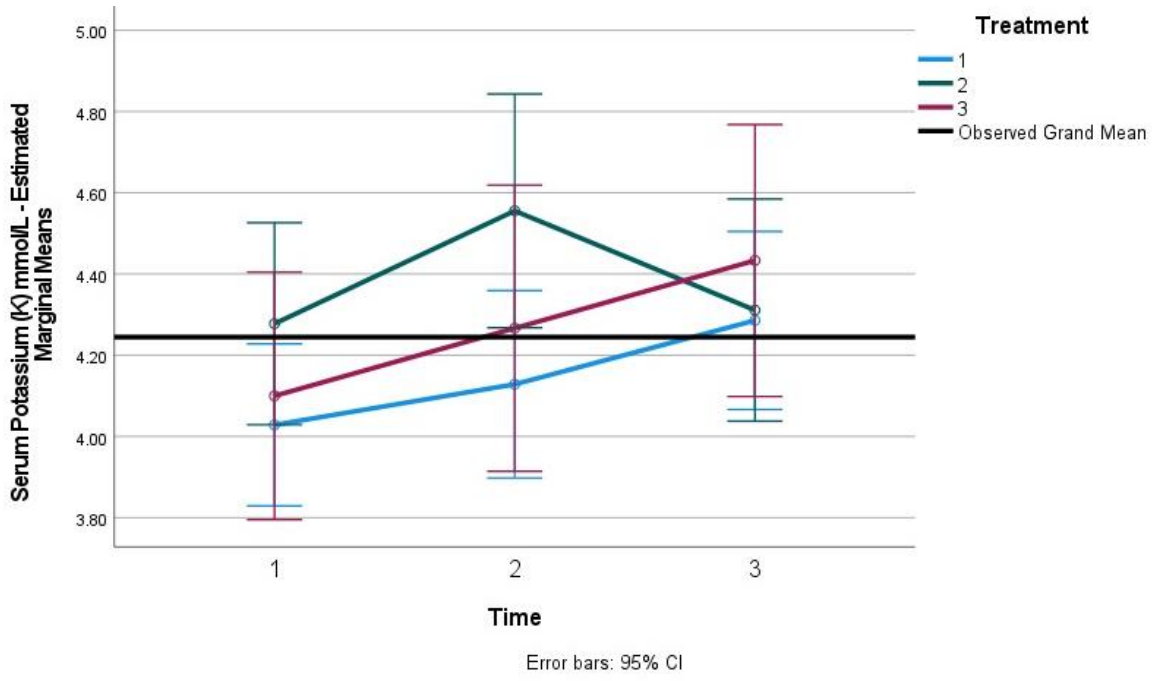

Figure 3.68. Serum potassium (K) levels (mmol/L) of three treatment groups over the 12-week intervention period

Serum calcium levels (mg/dL)

There was a statistically significant difference in serum calcium level in terms of treatment*time (F=2.741, df=4, p-value=0.38, N=29), and it was at the midterm between treatment1 (probiotics+vitaminB_3) group and treatment3 (placebo+placebo) group (mean difference=0.457, Bonferroni adjusted p-value=0.006) and between treatment2 (probiotics+placeboB3) group and treatment3 (placebo+placebo) group (mean difference=0.389, Bonferroni adjusted p-value=0.035); and in treatment 3 (placebo+placebo) group there was a statistically significant difference between time2 (midterm) and time3 (endline) (mean difference=-0.433, Bonferroni adjusted p-value=0.018). However, all mean values of three treatment groups were within the normal reference range of 8.0-10.3 mg/dL.

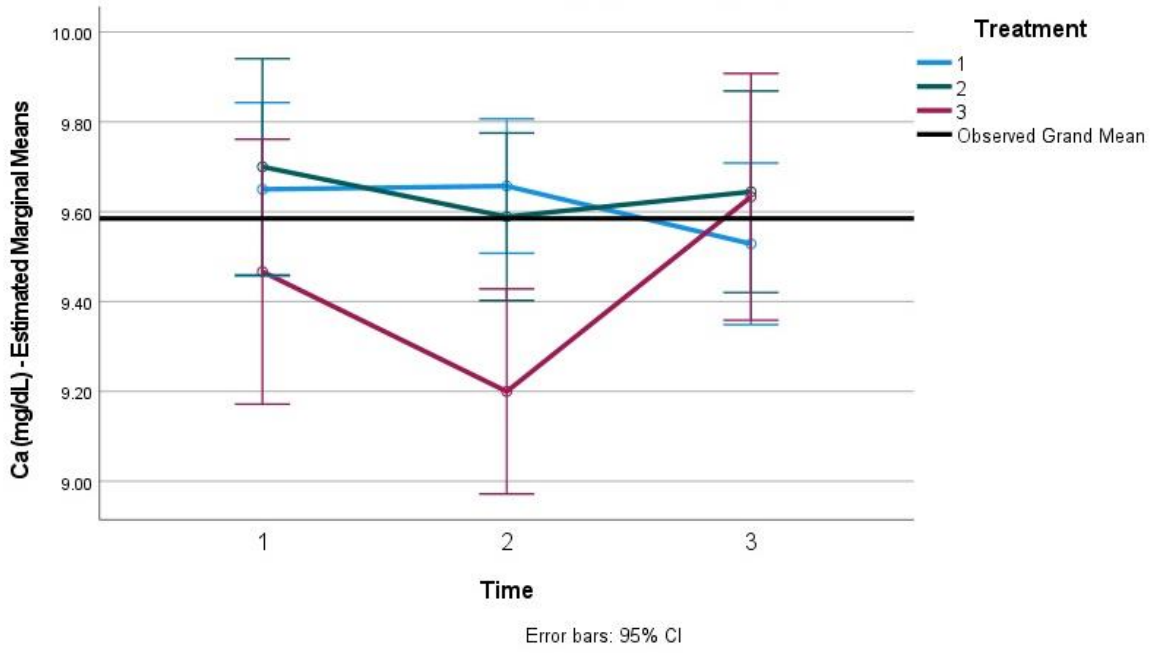

Figure 3.69. Serum calcium levels (mg/dL) of three treatment groups over the 12-week intervention period | Normal reference range 8.0-10.3 mg/dL

Blood Urea Nitrogen (BUN) mg/dL

There was no statistically significant difference of BUN (mg/dL) among three treatment groups (F=0.861, df=2, p-value=0.434, Eta^2= 0.062, N=29).

Serum creatinine (mg/dL)

There were statistically significant differences of serum creatinine levels (mg/dL) over the 12-week intervention period (F=3.558, df=2, p-value=0.036, N=29), and the difference was in treatment2 (probiotics+placeboB3) between time1, baseline, and time2, midterm (mean difference=-0.178, Bonferroni adjusted p-value=0.048) and between time2, midterm, and time3, endline (mean difference=0.222, Bonferroni adjusted p-value=0.018). The mean serum creatinine levels were within normal reference range 0.6-1.2 mg/dL.

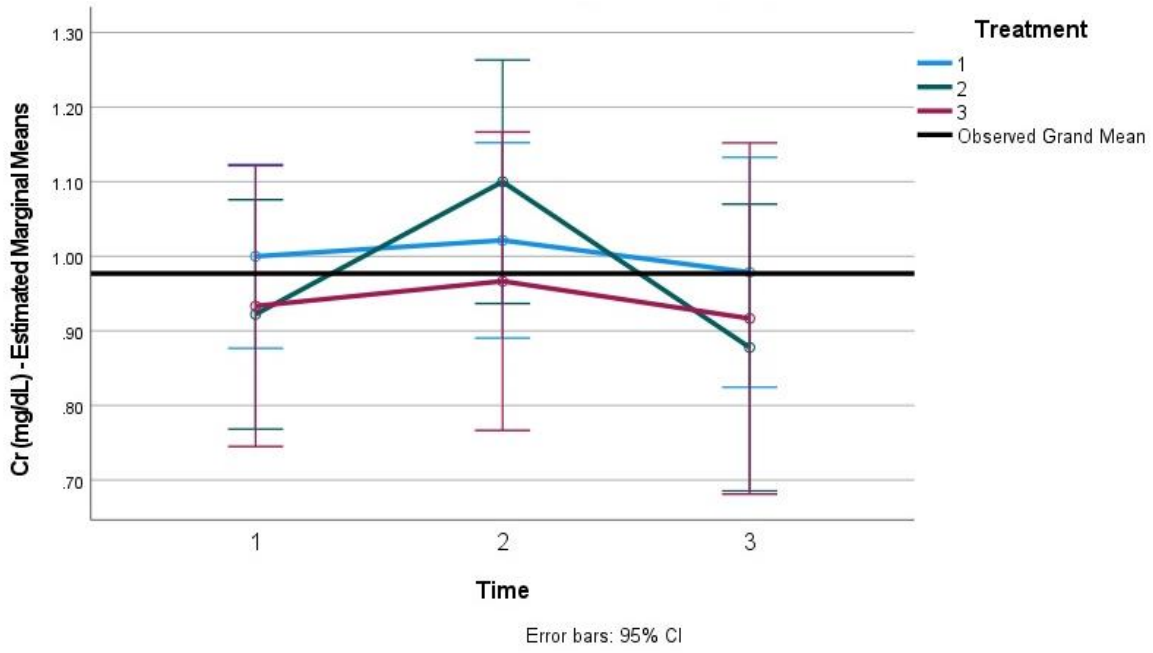

Figure 3.70. Serum creatinine levels (mg/dL) of three treatment groups over the 12-week intervention period | Normal reference range 0.6-1.2 mg/dL

Serum alanine aminotransferase (ALT) levels (unit/L)

There was no statistically significant difference in serum aminotransferase levels among three treatment groups (F=0.214, df=2, p-value=0.809, Eta^2=0.017, N=29). Alanine aminotransferase mean levels were within the normal reference range 10-47 unit/L.

Serum aspartate aminotransferase (AST) levels (unit/L)

There was not statistically significant difference in serum aspartate aminotransferase levels over the time in treatment groups (F=1.582, df=2, p-value=0.225, Eta^2=0.112, N=29). The AST mean levels were within the normal reference range 11-38 unit/L.

Serum alkaline phosphatase (ALP) (unit/L)

Serum ALP level was constantly increased trend to the endline in placebo group, initially decreased trend till midterm, then slight went up till the endline in treatment1, and increased trend half-way through midterm then decline back to almost the baseline level in probiotics only group. There was statistically significant difference within subject in terms of time*treatment ($F=2.861$, df=4, p-value=0.032, $Eta^2=0.18$, N=29), the differences were marginally significant or not significant in treatment1 (Probiotics+B_3 group) (mean difference=7.071, p-value=0.052, N=29, n=14), and the difference also became statistically insignificant or marginally significant in treatment2 (Probiotics+Placebo group) between baseline and midterm (mean difference=3.468, Bonferroni adjusted p-value=0.057, N=29, n=9). However, the ALP mean levels were within the normal reference range 42-141 unit/L.

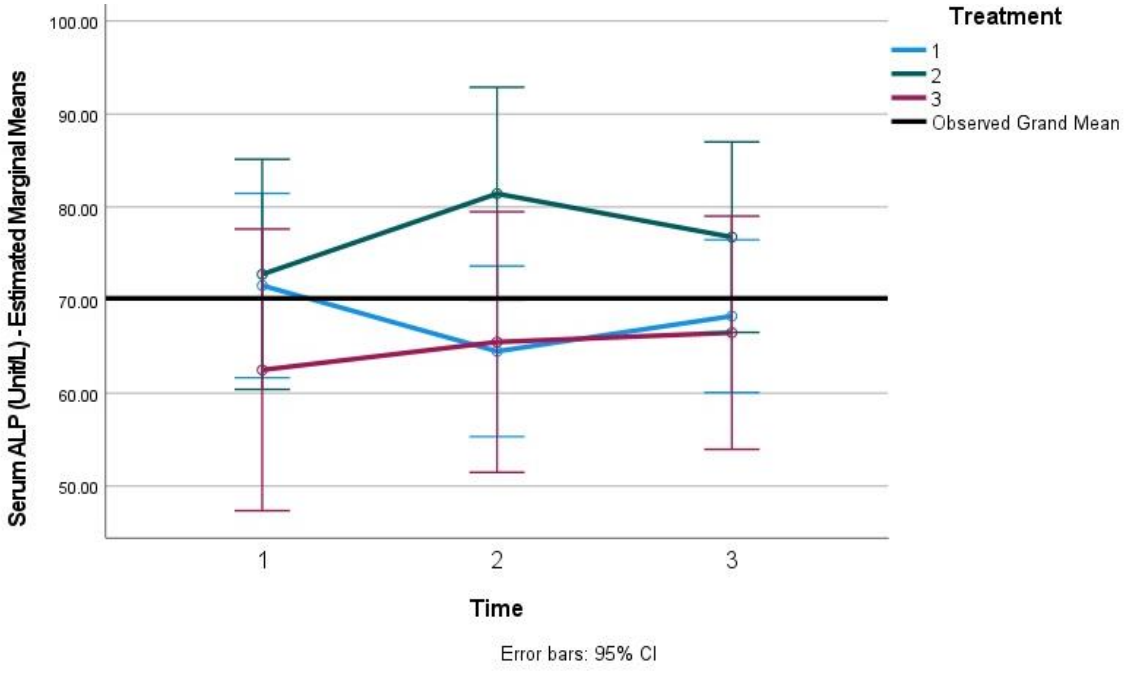

Figure 3.71. Serum Alkaline Phosphatase (ALP) unit/L of three treatment groups over the 12-week intervention period

Serum total bilirubin levels (mg/dL), Reference range 0.2-1.6 mg/dL

There was not statistically significant difference in serum total bilirubin levels in the treatment groups over the 12-week intervention period (F=1.912, df=2, p-value=0.169, Eta^2=0.133, N=29). The mean total bilirubin levels were within normal reference range 0.2-1.6 mg/dL.

Serum albumin level (G/dL), Reference range 3.3-5.5 G/dL

There was not statistically significant difference in serum albumin levels among the three treatment groups over the 12-week intervention period (F=1.315, df=1.769, Huynh-Feldt p-value=0.276, Eta^2=0.048, N=29). Serum albumin mean levels were within normal reference range 3.3-5.5 G/dL.

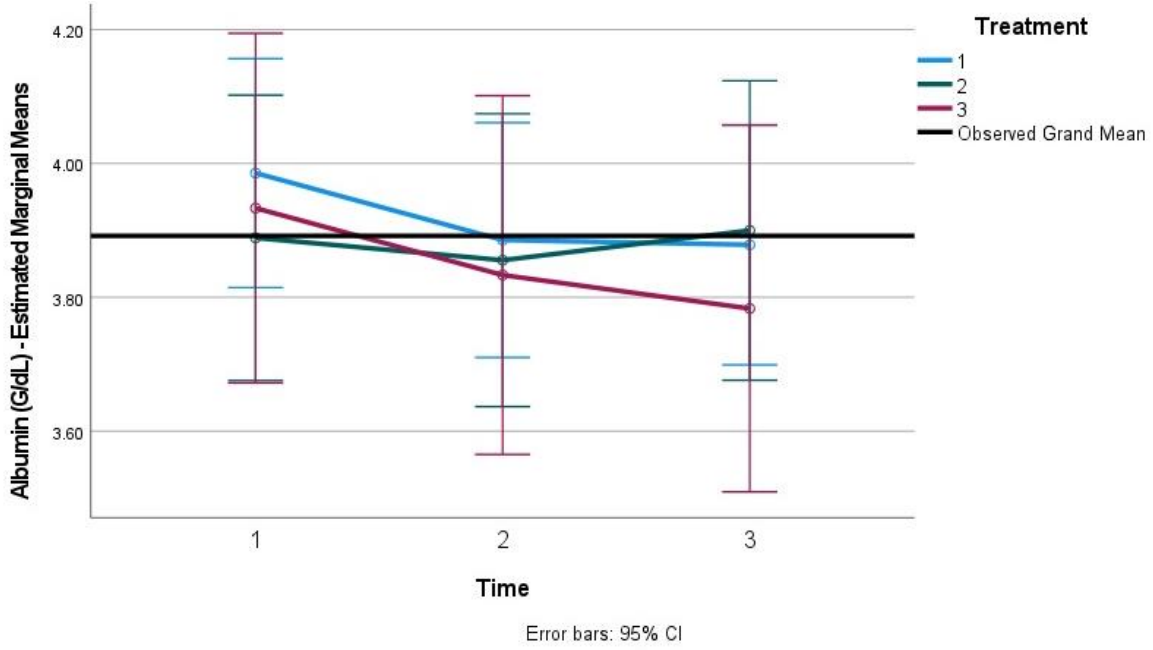

Figure 3.72. Serum albumin levels (G/dL) of three treatment groups over the 12-week intervention period | Normal reference range 3.3-5.5 G/dL

Serum total protein level (G/dL), Normal reference range 6.4-8.0 G/dL

Total protein levels (G/dL) were increased trend all the half-way through till midterm in probitoics+placeboB3 group and placebo+placebo group, while probiotics+vitaminB_3 group had decreased trend until midterm, in the second half of the 12-week intervention, the previous trends of three treatments flipped opposite directions. There was marginally statistically significant difference in serum total protein level between treatment groups (F=3.189, df=2, p-value=0.058, Eta^2=0.197, N=29), and the difference was between treatment2 (probiotics+placeboB3) group and treatmet3 (placebo+placebo) group at time3, at the end of the study (mean difference=0.622, Bonferroni adjusted p-value=0.024). Some individuals from placebo+placebo group had slightly lower serum total protein levels compared to the normal reference range 6.4-8.0 G/dL at the

baseline and endline but in the midterm. Apart from that, total serum protein mean levels were within normal reference range 6.4-8.0 G/dL.

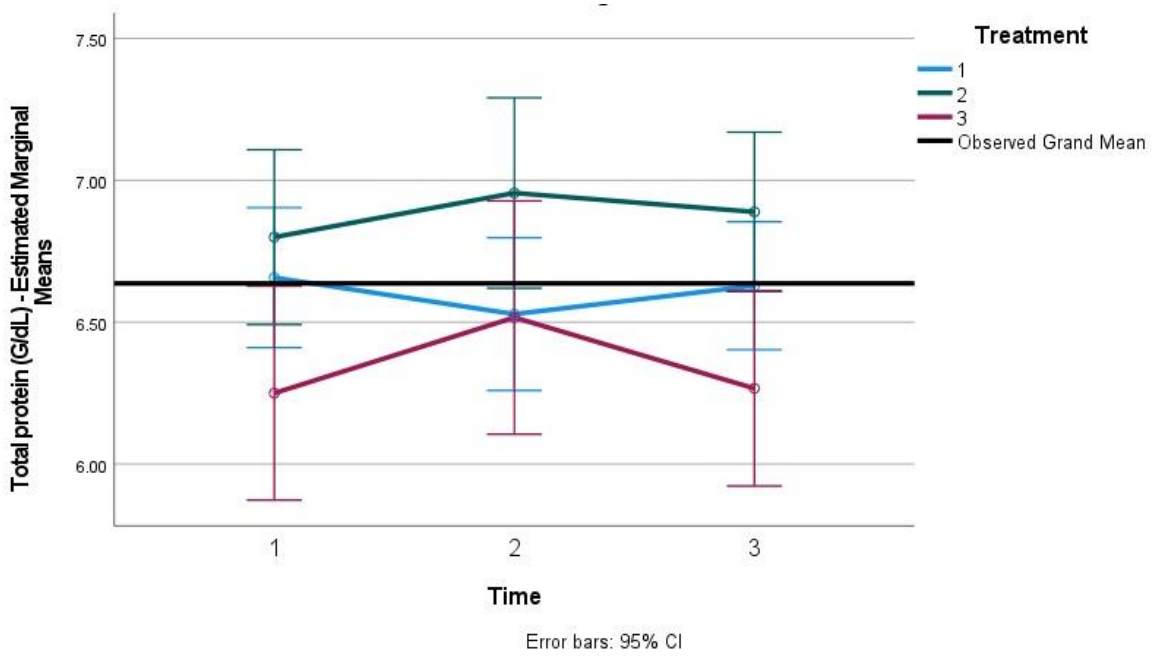

Figure 3.73. Serum protein levels (G/dL) of three treatments over the 12-week intervention period | Normal reference range 6.4-8.0 G/dL

Serum cholesterol levels (mg/dL), normal reference range <200 mg/dL

There was not statistically significant difference in serum cholesterol levels among the three treatment groups over the 12-week intervention period (F=0.079, df=1.769, p-value=0.924, Eta^2=0.006, N=29). Mean serum cholesterol levels were within normal reference range <200 mg/dL except a few individuals from the placebo+placebo group reached a little higher than 200 mg/dL at midterm.

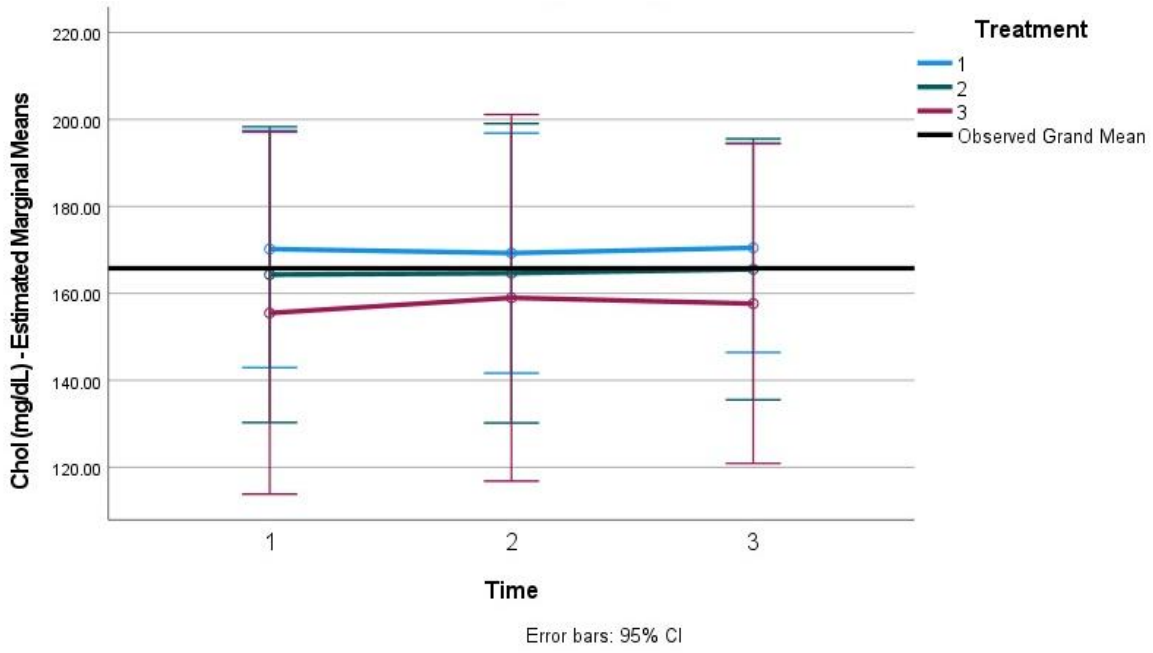

Figure 3.74. Serum cholesterol levels (mg/dL) of three treatments over the 12-week intervention period | Normal reference range <200 mg/dL

Serum HDL, levels (mg/dL), normal reference range >59 mg/dL

There was a marginally statistically significant difference in serum high density lipoprotein, HDL, levels at time1, week 0, between treatment1 (probiotics+vitaminB_3) group and treatment3 (placebo+placebo) group. However, that significance no longer existed over the 12-week intervention period ($F=3.446$, $df=2$, p-value=0.047, $Eta^2=0.210$, $N=29$). Almost all of the mean HDL levels from three treatment groups were lower than normal reference range, which is not favorable, except the mean HDL level of treatment1 (probiotics+vitaminB_3) at mid-term was in line with the recommended reference range level >59 mg/dL.

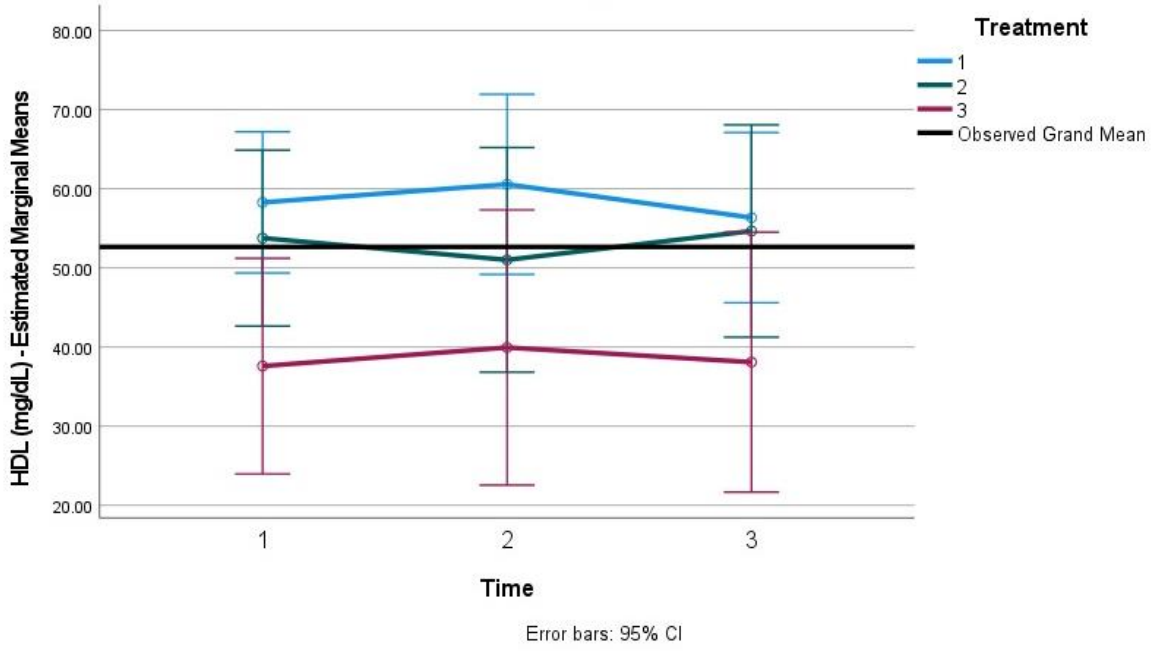

Figure 3.75. Serum HDL levels (mg/dL) of three treatments over 12-week intervention period | Normal reference range >59

Serum triglyceride levels (mg/dL), Normal reference range <150 mg/dL

There was not statistically significant difference in serum triglyceride levels among the three treatment groups over the 12-week intervention period (F=0.580, df=1.505, Huynh-Feldt p-value=0.518, Eta^2=0.022, N=29). Mean serum triglyceride levels were within normal reference range <150 mg/dL except a few individuals from treatment2 (probiotics+placeboB3) group reached higher than 150 mg/dL at midterm.

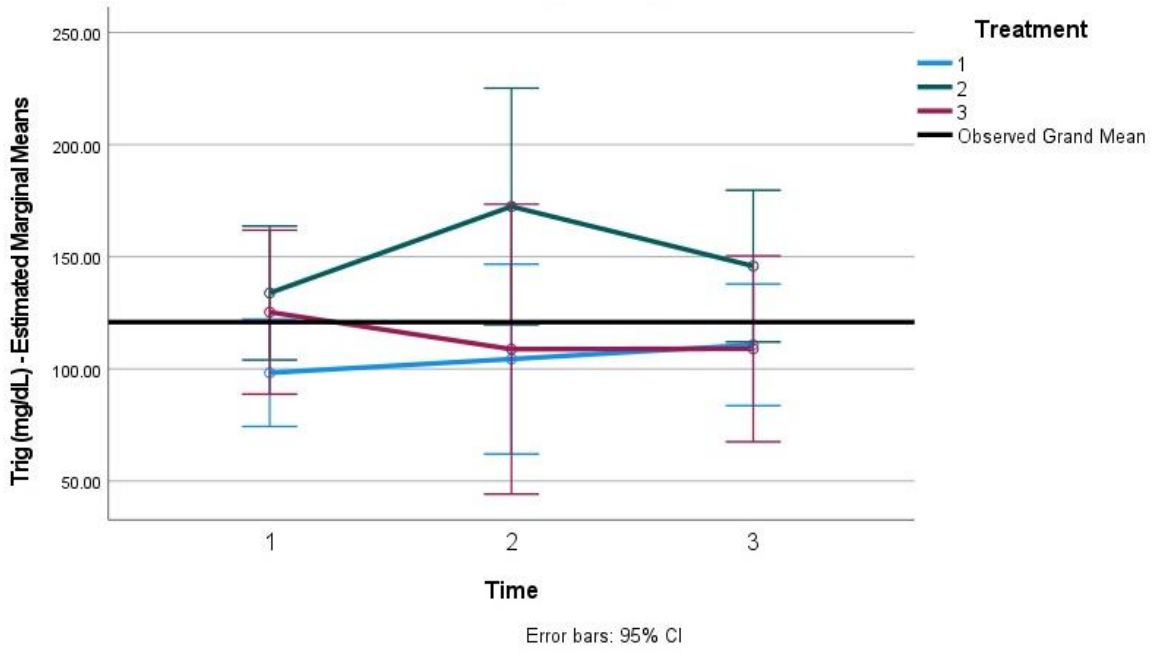

Figure 3.76. Serum triglyceride levels (mg/dL) of three treatments over 12-week intervention period | Normal reference range <150 mg/dL

Cholesterol:HDL ratio, Normal reference range <5.0

Cholesterol:HDL ratio was not statistically significantly different in three treatment groups over the 12-week intervention period (F=0.279, df=1.456, Greenhouse-Geisser p-value=0.688, Eta^2=0.011, N=29). The cholesterol:HDL ratio was quite high in placebo+placebo group due to their lower levels of HDL (denominator in the ratio) but the ratio remained stable throughout the 12-week intervention period.

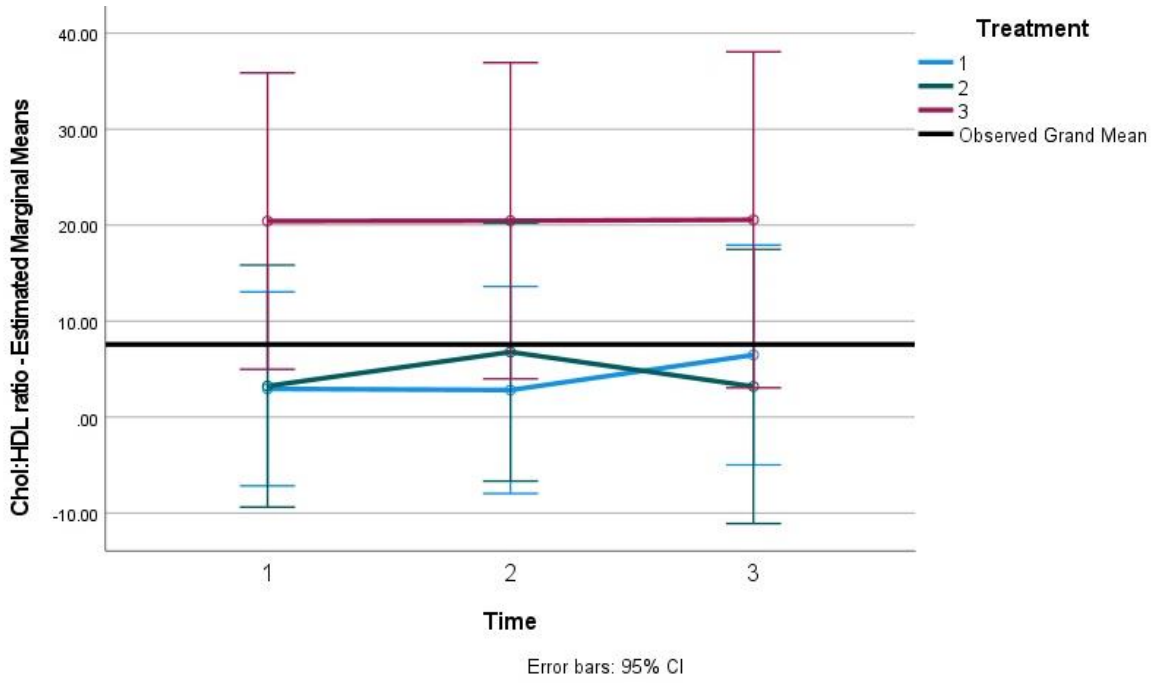

Figure 3.77. Chol:HDL ratio of three treatments over 12-week intervention period | Normal reference range <5.0

LDL cacl (mg/dL), Normal reference range <99 mg/dL

There was no statistically significant difference of low-density lipoprotein, LDL cal (mg/dL), levels among three treatment groups over the 12-week intervention period (F=0.592, df=2, p-value=0.561, Eta^2=0.045, N=29).

VLDL calc (mg/dL), Normal reference range <30 mg/dL

There was no statistically significant difference of very low density lipoprotein, LDL cal (mg/dL), levels among three treatment groups over the 12-week intervention period (F=0.294, df=1.541, Greenhouse-Geisser p-value=0.689, Eta^2=0.011, N=29).

Diet and Nutritional Assessment

Diet History Results

The statistical analyses showed the participants from all treatment groups showed no statistically significant difference from each other and did not change their energy intake (kcal/day) over the study period (Friedman test statistics =0.636, df=2, p-value=0.727). Over the past month from each assessment time (baseline, midterm, and endline), the followings did not show statistically significant change in all three treatment groups during the study period: carbohydrate consumption (g/day) (F=1.115, p-value=0.349), total dietary fiber (g/day) (F=2.43, p-value=0.12), added sugar (g/day) (F=1.707, p-value=0.208), supplementary dietary fiber (g/day) (Friedman test statistics=1.00, p-value=0.61), total protein intake (g/day) (F=0.881, p-value=0.431), total fat intake (g/day) (F=0.157, p-value=0.855), saturated fat (g/day) (F=0.595, p-value=0.562), monounsaturated fatty acid (g/day) (F=0.176, p-value=0.84), polyunsaturated fatty acids (g/day) (F=0.860, p-value=0.439), cholesterol (mg/day) (F=0.806, p-value=0.461), niacin (mg/day) (F=0.664, p-value=0.526), soy products protein foods (oz) (Friedman test statistics=0.824, p-value=0.662), yogurt (cups/day) (Friedman test statistics=5.51, p-value=0.06), cheese (cups/day) (F=0.84, p-value=0.45), water (g/day) (F=1.64, p-value=0.22), Omega3 fatty acids (g/day) (F=2.56, p-value=0.10).

Total HEI 2015 Score showed marginally statistically significant difference between treatment1 group and treatment2 group at the baseline (mean difference=17.22, Bonferroni adjusted p-value=0.047, SE=6.50), however, all groups appeared to show no significant changes over the study period, and that difference between treatment1 and treament2 group no longer existed as throughout the study, no differences at mid-term till the completion of the study.

Table 3.28: Nutrient intake over the past month of three treatment groups - Treatment1: Probiotics+B_3, Treatment2: Probiotics, Treatment3: Placebo assessed at Time1: Week 0, Time2: Week 6, and Time3: Week 12

Research Participants Nutrient Intake

	Treatment	Mean	SD	N
Energy (kcal) Time 1	1	1745	649	12
	2	1671	572	14
	3	1844	437	11
	Total	1746	553	37
Energy (kcal) Time 2	1	1665	639	10
	2	1699	554	11
	3	2107	860	10
	Total	1820	698	31
Energy (kcal) Time 3	1	1485	519	11
	2	1680	615	13
	3	1810	1017	10
	Total	1655	720	34
Total carbohydrates (g), Time 1	1	216	82	12
	2	195	66	14
	3	233	69	11
	Total	213	73	37
Total carbohydrates (g), Time 2	1	201	83	10
	2	217	96	11
	3	260	138	10
	Total	226	107	31
Total carbohydrates (g), Time 3	1	174	69	11
	2	204	83	13
	3	243	177	10
	Total	206	115	34
Added sugar (g), Time 1	1	40	18	12
	2	54	41	14
	3	66	33	11
	Total	53	33	37
Added sugar (g), Time 2	1	41	23	10
	2	53	29	11
	3	74	48	10
	Total	56	36	31
Added sugar (g), Time 3	1	33	16	11
	2	50	25	13
	3	93	139	10
	Total	57	79	34
Total dietary fiber (g), Time 1	1	23	9	12
	2	17	5	14

	3	20	6	11
	Total	20	7	37
Total dietary fiber (g), Time 2	1	21	8	10
	2	18	7	11
	3	23	12	10
	Total	21	9	31
Total dietary fiber (g), Time 3	1	18	7	11
	2	19	8	13
	3	18	6	10
	Total	18	7	34
Total protein (g), Time 1	1	66	26	12
	2	61	19	14
	3	64	15	11
	Total	63	20	37
Total protein (g), Time 2	1	66	27	10
	2	60	21	11
	3	77	29	10
	Total	67	26	31
Total protein (g), Time 3	1	60	19	11
	2	63	21	13
	3	61	22	10
	Total	61	20	34
Total fat (g), Time 1	1	64	25	12
	2	69	29	14
	3	72	23	11
	Total	68	25	37
Total fat (g), Time 2	1	63	25	10
	2	67	26	11
	3	85	35	10
	Total	72	30	31
Total fat (g), Time 3	1	57	18	11
	2	64	22	13
	3	65	32	10
	Total	62	24	34
Saturated fat (g), Time 1	1	18	8	12
	2	22	11	14
	3	25	13	11
	Total	22	11	37
Saturated fat (g), Time 2	1	20	9	10
	2	21	9	11
	3	30	15	10
	Total	23	12	31
Saturated fat (g), Time 3	1	17	5	11
	2	21	9	13
	3	22	14	10

	Total	20	10	34
Monounsaturated fat (g), Time 1	1	23	9	12
	2	25	10	14
	3	26	7	11
	Total	25	9	37
Monounsaturated fat (g), Time 2	1	23	9	10
	2	25	9	11
	3	30	11	10
	Total	26	10	31
Monounsaturated fat (g), Time 3	1	20	7	11
	2	23	7	13
	3	24	11	10
	Total	24	8	34
Polyunsaturated fat (g), Time 1	1	17	7	12
	2	15	5	14
	3	15	3	11
	Total	16	5	37
Polyunsaturated fat (g), Time 2	1	16	6	10
	2	16	6	11
	3	18	6	10
	Total	16	6	31
Polyunsaturated fat (g), Time 3	1	15	6	11
	2	15	5	13
	3	14	5	10
	Total	14	5	34
Cholesterol (mg), Time 1	1	174	81	12
	2	215	133	14
	3	244	133	11
	Total	210	119	37
Cholesterol (mg), Time 2	1	193	70	10
	2	196	102	11
	3	254	125	10
	Total	214	102	31
Cholesterol (mg), Time 3	1	202	59	11
	2	196	67	13
	3	225	194	10
	Total	206	114	34
Water (g), Time 1	1	3118	1783	12
	2	3293	604	14
	3	3410	1644	11
	Total	3271	1366	37
Water (g), Time 2	1	3372	1169	10
	2	3123	1056	11
	3	2751	1549	10
	Total	3083	1252	31

Water (g), Time 3	1	3046	928	11
	2	2985	1017	13
	3	3051	1697	10
	Total	3024	1193	34
Total HEI 2015 Score, Time 1	1	71	10	12
	2	66	14	14
	3	66	12	11
	Total	68	12	37
Total HEI 2015 Score, Time 2	1	70	12	10
	2	65	11	11
	3	64	11	10
	Total	66	11	31
Total HEI 2015 Score,Time 3	1	70	12	11
	2	66	11	13
	3	65	14	10
	Total	67	12	34

Discussion

In a randomized controlled trial from Malaysia, Ibrahim et. al. provided participants with Parkinsn's disease with a probiotic (Hexbio®) containing *Lactobacillus* sp and *Bifidobacterium* sp at 30 x 10^9 CFUs with fructo-oligosaccaride weighing 3 grams in sealed envelopes or placebo twice daily for 8 weeks (Ibrahim et al., 2020). Ibrahim et. al.'s study probiotic dosage (30 x 10^9 CFUs twice a day) is 12 times higher than this RCT (5x10^9 CFUs twice a day). According to Hexbio® label on their webpage 'HEXBIO® MCP® 3g x 45's (Working Adults)', each sachet comes with 30 billion cells/g consisting of *Lactobacillus acidophilus* BCMC® 12130, *Lactobacillus casei* subsp BCMC® 12313, *Lactobacillus lactis* BCMC® 12451, *Bifidobacterium bifidum* BCMC® 02290, *Bifidobacterium longum* BCMC® 02120, *Bifidobacterium infantis* BCMC® 02129 (HEXBIO, n.d.). If the label of 'HEXBIO® MCP® 3g x 45's (Working Adults)' is applied, the probiotic total dosage might be 90 x 10^9 CFUs twice a day given that 30 billion cell/g x 3 grams. If so, Ibrahim et al.'s study total probiotic dosage per day could be 180 billion per day, which is 18 times higher than this RCT, 10 billion per day. Ibrahim's study showed that consumption of the probiotic over 8 weeks improved bowel opening frequency and whole gut transit time in PD patients with constipation but there were no statistically significant differences in stool types, hard stools, straining abdominal pain, sensation of incomplete bowel movement, sensations of anorectal obstruction (feel blockage in anus), the need to use manual maneuver (need to press around anus/vagina), frequency of laxative use, frequency of enema use, BMI, PDQ-39, Non motor symptoms score, MDS-UPDRS II, and MDS-UPDRS III between the probiotic and placebo groups, while motor examination score (UPDRS Part III) showed statistically significant improvements in probiotic group ($p<0.001$), motor aspects of experiences of daily living (UPDRS Part II) showed statistically significant

improvement in probitoic group (p=0.040) and no differences in placebo group; PDQ-39 score showed statistically significant improvement in in probiotic group (p=0.013) and no difference in placebo group, and no statistically significant improvements of non-motor symptoms score in both groups (Ibrahim et al., 2020), which appeared congruent with several findings of this RCT.

Another randomized trial from Malaysia, which used a multistrain probiotics capsule 10 × 10^9 CFUs consisted of *Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus gasseri, and Lactobacillus rhamnosus, Bifidobacterium bifidum and Bifidobacterium longum, Enterococcus faecalis, and Enterococcus faecium* for 4 weeks in Parkinson's patients, reported significant improvement in quality of life related to constipation, and stool consistency, increased spontaneous bowel movement by 1 time per week on average after treatment with probiotics and decreased by 0.3 time per week on average in the placebo group (Tan et al., 2021). The probiotic dosage of Tan et al.'s study was the same dosage used in this RCT, and it is interesting to see Tan et al.'s study probiotic group had changes in stool consistency with the same dosage of probiotics.

A 4-week randomized controlled trial from Italy, which used fermented milk containing multiple probiotic 250 × 10^9 CFUs combined with 125 gram of prebiotic fiber, consisting of *Streptococcus salivarius, Streptococcus thermophilus, Enterococcus faecium, Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii, Lactobacillus bulgaricus,* and *Bifidobacterium breve, Bifidobacterium animalis, Bifidobacterium lactis* or a placebo for Parkinson's patients, reported a higher number of patients that reported 3 or more complete bowel movements during week 3 and 4, and higher number of increase in stool consistency, and higher number of decrease in laxative use in probiotic group compared to placebo group (Barichella et al., 2016). The probiotic dosage of

Barichella et al.'s study (250 billion CFUs per day) appeared to be 25 times higher than the dosage used in this RCT (10 billion CFUs per day), this was likely a reason to use much higher dose of probiotics to see changes in stool consistency, which this RCT did not see in the study population.

Another trial from Italy, which used diet therapy with fermented milk containing *Lactobacillus casei Shirota* (6.5 × 10^9 CFUs) for 6 weeks in Parkinson's patients, reported significant improvement of constipation including stool consistency, and reduction in feeling bloated, abdominal pain, and sensation of incomplete emptying (Cassani et al., 2011). This RCT's findings appeared to be congruent with Cassani et al.'s study finding but not necessarily in all of other measures such as stool consistency.

This randomized controlled trial did not find statistically significant differences in depression and anxiety scores in three treatment groups. These findings appeared to be congruent with the recent meta-analysis evidence from the randomized controlled trials on the effects of probiotics on depression or anxiety in people with depression and anxiety diagnosis or healthy participants (Chao et al., 2020). The meta-analysis result indicated that even though probiotics significantly reduced the depression score of patients with anxiety and depression, and healthy participants under stress, there was no significant difference between the probiotics and placebo groups in the reduction of patient anxiety scores, even if they are depressive or anxious patients or healthy participants under stress (Chao et al., 2020). Probiotics showed significant effect on depressive symptoms only in patients with depression, and there was not significant change in anxiety score in patients, and no improvement in healthy participant under stress (Chao et al., 2020).

This study did not find statistically significant difference of profile of mood states (POMS) assessment among PD participants from three treatment groups (probiotics+B_3, probiotics alone, and placebo). These findings appeared to be in line with recent meta-analysis finding on effect of probiotics on mood, psychiatric symptoms, and CNS functions in human (Le Morvan de Sequeira et al., 2022). The meta-analysis result indicated that the probiotics mostly exerted effects on CNS function (Le Morvan de Sequeira et al., 2022). However, most probiotics did not show effects on mood, stress, anxiety, depression and psychiatric distress compared to placebo at the qualitative level (Le Morvan de Sequeira et al., 2022). In quantitative analysis, depression and psychiatric distress improved slightly in the probiotic groups (Le Morvan de Sequeira et al., 2022).

In this RCT, the probiotics+B_3 group and probiotics alone group showed significant improvements in UPDRS scores, which appeared to be congruent with the findings from other studies. The results from Chong et al.'s clinical trial, which provided 16 Parkinson's patients with niacin 100 mg, 15 Parkinson's patients with niacin 250 mg, and 16 Parkinson's patients with placebo for 3 months as the first part of the study, indicated significant improvements in UPDRS III motor examination scores in niacin 100 mg group (Chong et al., 2021). After 3 months, when all participants were provided with open-label niacin 250 mg for next 12 months, Chong et al. found the statistically significant improvements in UPDRS III motor examination scores ($p=0.0009$). A randomized controlled trial of niacin 250 mg (vitamin B_3), indicated supplementation for 47 Parkinson's patients showed no significant changes in UPDRS III motor examination scores after 6 months (Wakade et al., 2021). Then, at the end of 6-month, Wakade et al.'s study provided all participants from the placebo and niacin groups with open-label niacin for the next 6 months. Wakade et al. indicated that the UPDRS III motor examination scores

from 6-month to 12-month significantly decreased. There was a significant decrease occurred across both groups over a treatment period of one year ($p = 0.012$), However, there was no significant differences between groups at any time point. A trial of niacin for PD reported UPDRS scores at 3 months were variable as expected, since niacin is not a drug. Only the 100-mg group was detected to have improved with the supplementation, $p = 0.0076$. Summarily, this RCT's findings were congruent with findings from several other studies, all these discussion points put forward that probiotics supplementation appeared to work well in people living with PD, and could improve Parkinson's symptoms and/or delay deteriorations. Likewise, vitamin B_3 supplementation seemed to work well in this study population as in line with findings from other studies, and vitamin B_3 might have some h armonious effects when combined with probiotics supplementation.

The RCT found significant differences in alpha and beta diversity among treatment groups over 12-week intervention period. Despite all treatment groups consisted of Parkinson's patients, they might not show uniformed gut microbiome composition, and so it is possible to find differences in alpha and beta diversity in the study population given that differences in their treatments, possible impacts from age, disease duration, disease category, medication, and gender. This RCT finding of differences in beta-diversity appeared to be congruent with recent review study of PD gut microbiome which confirmed again that the gut microbiome of PD patients significantly differed from those of controls (Romano et al., 2021). In this RCT, the alpha diversity changes was significant in placebo group likely this group lacked balancing effect of probiotics supplementation. This finding appeared to be congruent with recent review finding of PD gut microbiome which stated that bacterial alpha-diversity could be explained by a

decrease in the abundances of the most abundant species and an increase in the rare ones in Parkinson's patients (Romano et al., 2021).

This RCT checked the Firmicutes to Bacteroidetes ratio (F/B ratio) because F/B ratio is commonly used to assess gut health (Romano et al., 2021) and F/B ratio was known to change with age (Mariat et al., 2009), and decreased F/B ratio with age in older adults was considered to associate with neurodegeneration (Gerhardt & Mohajeri, 2018). This RCT did not find changes among three treatment groups (probitoics+B_3, probiotics alone, and placebo group) of Parkinson's patients in this study population, which appeared to be congruent with the finding from a recent review by Romano et. al., which indicated that when F/B ratio was assessed in PD studies, only one study (Keshavarzian et al., 2015) showed significant difference in the F/B ratio between PD and control and overall difference was not significant (Romano et al., 2021).

Regarding the gut microbiome composition analyses findings from this RCT, *Clostridium hiranonis* appeared scarce in the gut microbiome of this study population with PD. *Clostridium hiranonis* was found in the group with disease duration between 6 years and 7 years but in other groups. This finding was congruent with Takáčová et al.'s study that stated that disruption of proper microbiome function due to lack of commensal bacteria, *Clostridium hiranonis* may lead to bacterial overgrowth (*Clostridium difficile, Clostridium perfringens, Escherichia coli*) and lower anti-inflammatory microbial-derived metabolites (Takáčová et al., 2022). In the animal study, an increase in fecal bacterial diversity of beneficial bacteria such as *Clostridium. hiranonis* and *Faecalibacterium* and a decrease of *Escherichia coli* was found in the group treated with Fecal Microbiota Transplantation (FMT) (Takáčová et al., 2022). *Clostridium hiranonis* is the main bile acid converting bacterial species converting bile acids to secondary bile acids in the colon and involves in maintaining normal glucose concentration through the

farnesoid X receptor (Takáčová et al., 2022). Lack of *Clostridium hiranonis* e.g. in chronic enteropathies may cause secretory diarrhea, and represent a high risk of colon cancer in humans eating diet rich in fat due to increased secondary bile acids (Takáčová et al., 2022). The fasting blood glucose levels of the participants from this RCT did not show statistically significant differences though.

Phascolarctobacterium was differentially present in longer disease duration group (6-7 years) of this study. This finding appeared to be congruent with Aho et al.'s and Baert et al.'s studies. Aho et. al's study stated that lower abundance of *Phascolarctobacterium* was correlated with increasing short chain fatty acid concentration (Aho et al., 2021). The abundances of *Butyricicoccus, Clostridium* sensu stricto, and *Roseburia* were positively correlated with levels of SCFAs while the abundances of *Akkermansia*, *Escherichia/Shigella*, *Flavonifractor*, *Intestinimonas*, *Phascolarctobacterium*, and *Sporobacter* decreased with increasing SCFA concentrations (Aho et al., 2021). Butyrate production was lower in Parkinson's patients compared to healthy controls (Baert et al., 2021). These connections made to hypothesize that the longer disease duration group (6-7 years) of this RCT who had higher comparative abundance of *Phascolarctobacterium* might have lower butyrate level, SCFAs levels. SCFAs, particularly butyrate was deemed favorable for PD. Butyrate reduced motor symptoms in PD animal model study (Baert et al., 2021). PD diagnosis limited short-chain fatty acid production and was negatively associated with butyrate producers (Baert et al., 2021).

Bacteroides uniforms was almost lacking in longer disease duration groups (8-9 years, 10 years and above) in this study which seems congruent with Hartstra et. al.'s study in humans with metabolic syndrome. Hartstra et. al. found that *Bacteroides uniformis* upregulates brain dopamine transporter (DAT) / Dopamine binding efficiency. These connections from this RCT

made to hypothesize that lesser abundance of *Bacteroides uniforms* in Parkinson's patient with longer disease duration might be part of the reasons of needing higher dosages, increased frequencies and different types of Parkinson's drugs in the Parkinson's patients with longer disease duration.

Prevotella copri is relatively higher abundance in treatment 2 at time 3 compared to time 1 and 2, and more prominently higher abundance than those in treatment 1 at all 3 times, and treatment 3 at all three times as seen in the heatmap. *Prevotella copri* was observed more in healthy control compared to patients with PD (Gerhardt & Mohajeri, 2018). *Prevotella* species (*Prevotella copri*) were decreased in all neurodegenerative diseases, except Alzheimer disease (Gerhardt & Mohajeri, 2018). Vandeputte et. al. indicated that *Prevotella* had stronger adherence to host tissues, and stool consistency and water activity in individuals were independent of accelerated transit, and mainly reflected increased fecal water-binding capacity (Vandeputte et al., 2016). Therefore, the relatively higher abundance of *Prevotella* in treatment 2 (probiotics group) appeared to be a favorable change in microbiota composition. *Prevotella* (P) enterotype was more abundant in the study group with loose stool while the Ruminococcaceae-*Bacteroides* (RB) enterotype with the harder stool samples (Vandeputte et al., 2016). Therefore, the relatively higher abundance of *Prevotella* in treatment 2 in this RCT appeared to be a favorable change in microbiota composition. One thing that might be seen as unfavorable regarding *Prevotella copri* is that there was a report that *Prevotella copri* might downregulate DAT binding efficiency (Hartstra et al., 2020).

Bacteria family *Ruminococcaceae* was statistically significantly different in the microbiome composition between the participants who took fiber supplementation and those did not take it. This finding appeard relevant to De Wolfe et. al.'s study which indicated multi-strain

probiotic showed significant representation of *Ruminococcus* during probiotic treatment, and was significantly more abundant in a stable microbiota (De Wolfe et al., 2018). Wang et al. indicated that an engineered probiotic promoted the growth of *Ruminococcaceae* and enhanced the butyrate production, which decreased inflammation and clinical activity of the colitis in the animal model IBD study (L. Wang et al., 2021). Therefore, finding from this RCT appeared congruent with other studies supporting probiotic supplementation could modulate relative abundance of *Ruminococcaceae*.

Ruminococcus gnavus was not statistically significantly different between treatment groups or treatment-and-time groups but it was included as one of the top 20 features found in Parkinson's patients of this study. It appears *Ruminococcus gnavus* may be associated with neurological conditions involve dopamine, dopaminergic neurons, and receptors as congruently reported in Xi et. al.'s study, in which fecal samples from children with a tic disorder showed higher abundance of *Ruminococcus lactaris* and *Ruminococcus gnavus* compared to healthy control group (Xi et al., 2021).

The disease status by MDS-UPDRS score showed statistically significant impact in the microbiome composition of the samples collected from people with PD participated in this study. The gut microbiome composition with bacteria family *Ruminococcaceae*, bacteria genera *Coprococcus*, *Blautia*, *Oscillospira* were found much higher in moderate and advance disease status as categorized by the MDS-UPDRS Score. The finding of higher abundance of *Ruminococcaceae* in moderate and advance PD status in this study appeared to support Hamamah et. al.'s hypothesis that the abundance of *Ruminococcacae* seen in patients with PD caused downregulation of dopamine receptor (D_2 receptor) expression, manifesting bradykinesia (Hamamah et al., 2022).

Bacteroides fragilis was statistically significantly present in gut microbiome composition of moderate and advance disease status but unfounded in those with mild disease status group in this RCT. This finding appeared to be congruent with Lukiw's and Zhao et al's finding that *Bacteroides fragilis* could release LPS as neurotoxins and pro-inflammatory LPS, which was associated with inflammatory signaling in Alzheimer's disease brain (Lukiw, 2016) (Zhao & Lukiw, 2018). The detection of *Bacteroides fragilis* appeared to be relevant and its inflammatory metabolites become more important for older population witn neurodegenerative disease such as PD because the gastrointestinal tract and blood-brain barriers alter or increase their permeability with aging and disease (Lukiw, 2016).

In this RCT, *Desulfovibrio* was statistically significantly present in gut microbiome composition of Parkinson's patients with 10 year or longer disease duration but unfounded in other Parkinson's patient groups with shorter disease durations. This finding is congruent with reports from other studies. *Desulfovibrio* was found in the gut microbiota in patients with PD in southern China (Lin et al., 2018). Fan et. al. stated that *Desulfovibrio* was associated with PD (Fan et al., 2022). Desulfovibrio produces extracellular magnetite and hydrogen sulfide and induce aggregation of α-synuclein (Fan et al., 2022).

Regarding the gut microbiome composition analyses in association with anxiety and depression, bacteria family *Ruminococcaceae* and *Akkermansia muciniphila* stood out in the human gut microbiome composition of participants with PD in relationship with anxiety levels. Significantly higher relative abundance of *Akkermansia muciniphila* and bacteria family *Ruminococcaceae* are found in severe anxiety groups compared to mild, moderate, moderately severe group. Genus *Odoribacter* and *Clostridium* showed statistically significant differences in human gut microbiome composition of participants with PD relating to their depression levels.

The higher relative abundance of genus *Clostridium* was detected in those with no depression, and the lower relative abundance of genus *Odoribacter* was detected in those with higher levels of depression.

Zhang et.al, stated that *Odoribacter* appeared to be specifically correlated with chronic unpredictable mild stress, depression-like behaviors and depression-related indicators (M. Zhang et al., 2021). Interesting finding from this study was that *Odoribacter* was relatively higher abundance in lower degree of depression or no depression in human with PD, this finding appeared to be satisfied by some positive findings from Jiang et al.'s study which described that BDNF levels were significantly positively correlated with *Odoribacter* (Jiang et al., 2020). BDNF is a key player in antidepressant action, it serves as a transducer and impacts the neuroplastic changes that result in the improvement of the depressive symptoms (Björkholm & Monteggia, 2016). BDNF is considered essential in adults for creating new neurons from stem cells (Numakawa et al., 2017).

Regarding identifying important features and corresponding gut microbiome taxa as shown in the heatmap in the result section, Vogt et. al. reported that *Dialister*, cc115 family *Erysipelotrichaceae* were less abundant in Alzheimer's disease participants (Vogt et al., 2017). *Erysipelotrichaceae* has capabilities for butyrate production but for propionate among dominant bacterial species detected in faecal samples of human subjects (Louis & Flint, 2017). So, the higher relative abundance of *Erysipelotrichaceae* in treatment 1 (probiotics+B_3) and treatment 2 (probioitcs alone) group may be deemed as a positive aspect of probiotic supplmentation.

In this RCT, *Oscillospira* was relatively much more abundant in treatment 1 and 2 groups, and much lesser in treatment 3 group, this could be another positive aspect of probiotic supplemented treatment groups as Yang et. al. reported that *Oscillospira* directly or indirectly

exhibited positive regulatory effects in areas related to obesity and chronic inflammation (Yang et al., 2021). *Oscillospira* can produce butyrate and may play an important role in various aspects of human bodily functions and health(Yang et al., 2021). Considering the positive effects of Oscillospira in some specific diseases, such as obesity-related metabolic diseases, *Oscillospira* had already been characterized as one of the next-generation probiotic candidates (Yang et al., 2021). It is worth to note that Yang et al. suggested a high abundance of *Oscillospira* positively correlated with constipation because *Oscillospira* was difficult to culture and slow to grow, and thus benefiting from a slow transit time in the gut (Yang et al., 2021). Fast colonic transit times favor fast-growing microbes, so, in the same way, slower colonic transit times allowed slower microbes to remain in the lumen and avoid being eluted (Yang et al., 2021). It was an interesting question whether *Oscillospira* caused constipation or higher abundance of *Oscillospira* was considered as the marker or the effect of constipation due to slow colonic transit. However, the referenced article apparently did not directly correlate *Oscillospira* with constipation and stated that *Oscillospira* showed significance in terms of correlation between stool microbiota and breath methane concentration; after adjusting for age, BMI, diet, and colonic transit, the fecal microbiota was not associated with constipation status (Parthasarathy et al., 2016).

Anaerotruncus sp. was relatively more abundant in treatment1 and treatment 3 than treatment2. In a study on probiotic supplementation following antibiotic treatment, Grazul et. al. found that the probiotic likely enhanced the growth of the *Anaerotruncus* from the resident flora during the recovery process from antibiotics and prevented the overgrowth of opportunistic pathogen Enterobacteriaceae (*Escherichia. coli* and *Shigella*) (Grazul et al., 2016). In a microbiota features and high-fat, low fiber diet study, Bailén et. al. found that *Anaerotruncus* was positively associated with Saturated Fatty Acids (SFA), whereas *Dialister* and *Anaerostipes*

were negatively associated with Saturated Fatty Acids, the abundance of *Blautia* was partly due to consumption of Poly Unsaturated Fatty Acids (PUFAs) (Bailén et al., 2020). Gao et. al. found that *Anaerotruncus* was related to intestinal permeability indices, LPS, and tight junction proteins in rats on high-fat diet, which may involve in loss of barrier function and LPS production (Z. Gao et al., 2020). Gao et. al. found negative correlations between tight junction proteins and the genera *Alloprevotella, Roseburia, Anaerotruncus, Blautia, Ruminococcus_1, Ruminiclostridium_6, Oscillibacter,* and *Ruminococcaceae*_UCG-004 (Z. Gao et al., 2020). These facts and observation from this RCT are relevant as probiotics are considered to protect gut from leaky state.

Helicobacter pylori, Enterococcus faecalis, and *Desulfovibrio,* might be involved in PD pathogenesis or interfere with therapy (Fan et al., 2022). In this RCT, *Desulfovibrio* was detected in patients with 10 year or longer disease duration, however, *Helicobacter pylori, Enterococcus faecalis* were not detected in the study population.

This RCT had strengths and limitations. The strengths of this study were probiotics mix contained Lactobacillus sp. and Bifidobacterium sp. wich were known as beneficial probiotics species, and used in other studies including Parkinson's disease. The probiotics mix was selected based on the top ranking and assessment results evaluated by an independent third party (Labdoor, 2017). The study outcome assessments covered quality of life measures, motor and non-motor symptoms, mental health variables, biological parameters such as gut microbiome and blood chemistry, liver enzymes, and lipid profile. The limitations were we had to omit some participants follow-up assessments due to financial and other constraints to travel. Serum level assessments of L-dopa, SCFAs, and ghrelin were delayed due to the mechanical issues of the chromatography laboratory, and could not be integrated into the analyses at the moment. These

serum samples were stored in the deep freezer (-80°C) and scheduled to measure in the fall semester. The study was single-blinded due to the limitations in dispensing, however, all the assessments were conducted objectively resembling to the objective assessments conducted in the surgery/surgical method randomized controlled trials where it was difficult to be double-blinded. Moreover, most assessments were solely on the sigle-blinded patients' evaluations, and many assessments were biological samples and laboratory results. The COVID-19 pandemic halted the study for more than one year. The study had to be implemented aftermath of COVID-19 pandemic. Most of the limitations would be taken care by the randomization.

Conclusion

The probiotics and vitamin B_3 provision showed improvements in constipation problems, motor and non-motor symptoms n those with PD in this study. Motor symptoms appeared to have quicker response to see the changes and improvements. Probiotics and vitamin B_3 or probiotics groups showed improvements in quality of life. Overall, the changes were more pronounced in 12-week consumption compared to 6-week consumption of probiotics and vitamin B_3. This appears to support the duration of probiotics consumption in sufficient dosages to begin to observe the impacts is about 12 weeks. *Dialister* sp. is significantly decreased and *Erysipelotrichaceae* gen. sp was increased in probiotics+vitamin B_3 group at midterm and endline compared to the baseline. In association with reported butyrate production function of *Erysipelotrichaceae* (Louis & Flint, 2017), the higher relative abundance of *Erysipelotrichaceae* in probiotics+vitaminB_3 group and probiotics group may be deemed as a positive aspect of probiotic supplementation.

Increased number of more different bacterial features (alpha diversity) in control group compared to probiotics and vitamin B_3 group prompted to conclude that the nicotinic acid combination may have a more balancing or stabilizing effect on untoward bacterial overgrowth, which deemed favorable particularly if the individual with PD was susceptible to dysbiosis and untoward bacterial overgrowth. The relatively higher abundance of *Prevotella* in probiotics alone group appeared to be a favorable change in microbiota composition in connection with its known function of fecal water-binding activity and loosening stool. Probiotics may have helped ease constipation problems in several different ways including changes in neurotransmitters, and SCFAs levels and impacts on neuroendocrinal communication, autonomic and physiological responses. When the blood levels of L-dopa, catecholamines, SCFAs and ghrelin levels are

available, additional pieces of the whole picture of this randomized controlled clinical trial can be more comprehensively captured.

From some notorious potential pathogens correlating to the PD such as *Helicobacter pylori*, *Enterococcus faecalis,* and *Desulfovibrio* (Fan et al., 2022), this RCT detected *Desulfovibrio* in patients with 10 year or longer disease duration. However, *Helicobacter pylori, Enterococcus faecalis* were not detected in the study population.

The findings from gut microbiome beta diversity principal coordinates correlation analyses made to conclude that using laxative or stool softener or antidepressant medications appeared to suppress the diversity of some gut microbiome taxa but also enhanced on the diversity of other taxa conversely. The study population did not take COMTi and Monoamine Oxidase Inhibitors (MAOI).

The findings from correlation analyses of clinical, mental, nutritional status, body weight, constipation problems and stool types made to conclude that having hard and lumpy stools (stool types 1 or 2) had greater impact on mood disturbances compared to mushy or watery stools (stool types 6 or 7), and the greater the constipation problems, the worse the mood disturbance levels in this study. Individuals with higher BMI tended to have more frequent mushy or watery stools (type 6 or 7) and their counterparts who had lesser BMI, likely malnutrition at-risk, experienced greater mood disturbances in the study population. Nicotinic acid, niacin, might have enhanced ghrelin hormone and its satiety effect as the probiotics with vitamin B_3 group decreased energy intake over the course of study. The metabolic parameters including liver enzymes and blood chem overall stable results made to conclude that probiotics and vitamin B_3 was well tolerated. Vitamin B_3 might have some harmonious effects when combined with probiotics supplementation.

Chapter 4 - Reflection

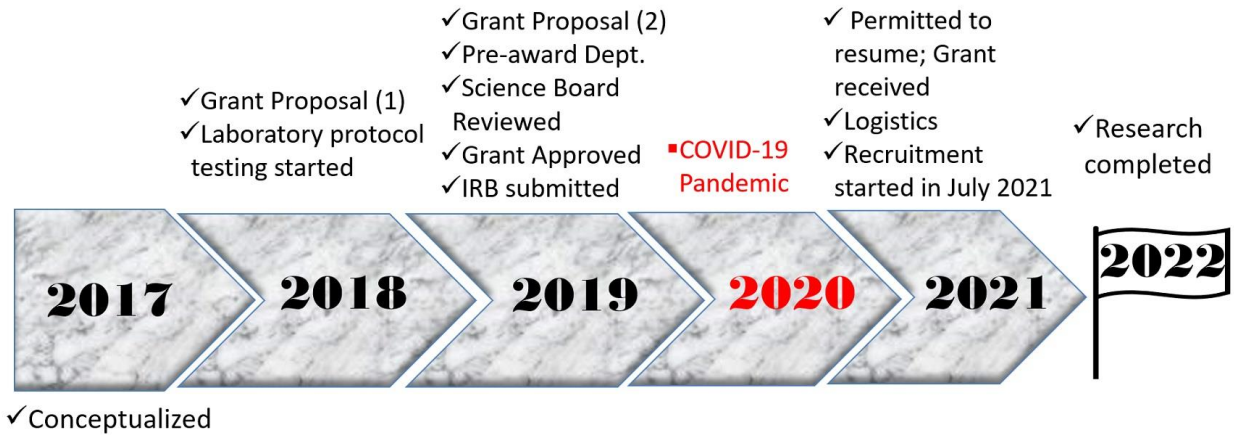

Figure 4.1. Timeline or landmarks of this randomized controlled trial (2017 ~ 2022)

I had the great opportunity to learn and grow in the entire process from the inception time, 2017 through 2022. I was motivated to design and apply my dissertation research to offer the science body with some novel data, that could contribute in the efforts to fill the gaps or needs in science to some extent to improve the quality of life and humanity. Keeping that goal alive in mind, I committed to learn new skills and invest time, money, efforts and what it takes to reach the goal. I learned some new applications, software, biotechnology, information technology, such as QIIME-2 software, application of Beocat in big data computing, statistical software, DNA extraction, Polymerase Chain Reaction (PCR), library preparation techniques, genomics and bioinformatic applications and others. I got the opportunity to learn and develop knowledge and skills not only in technical or academic areas but also soft skills in several aspects including research project management from the beginning to the completion: conceptualization, grant writing, working with pre-award department, going through review process for approvals from local and up levels, going through and surviving pandemic COVID-19 for more than one year, paperwork and getting permissions to resume the research project,

logistics, procurement, perseverance and resilience, research implementation in multiple sites both in State and out of the State, coordination, communication, and collaboration to achieve the goals of this research.

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Appendix A - Biological samples and procedures

Blood samples (15 ml) were drawn from the antecubital fossa of the participants' arms, immediately centrifuged and serum samples are frozen at -80°C. Participants were provided with sample collection kits (DNAgenotek-Omnigene.GUT for microbiome) and instructed how to collect stool samples at the residence. Then stool samples were transported to the Kansas State University, processed, and stored in the freezer at -35°C for further analysis. Blood samples were be stored in the deep freezer (-80°C) till they were ready to run LC-MS, GC-MS, ELISA analyzers in batches, the processed stool sample will be stored in the refrigerator and/or freezer until they are ready for DNA extraction, PCR, libraries preparation, and sequencing in batches. Human serum/blood, and other bodily fluid were (disinfected and) placed in a biohazard bag or medical waste container for disposal at the Environmental Health and Safety (EH&S) department facility as outlined. The bags or containers were securely closed. Sharps, medical (syringes, needles, vacutainer, phlebotomy sets) were placed in an approved sharps container. Liquid biological waste were collected in containers for autoclaving or chemical disinfection (10% bleach).

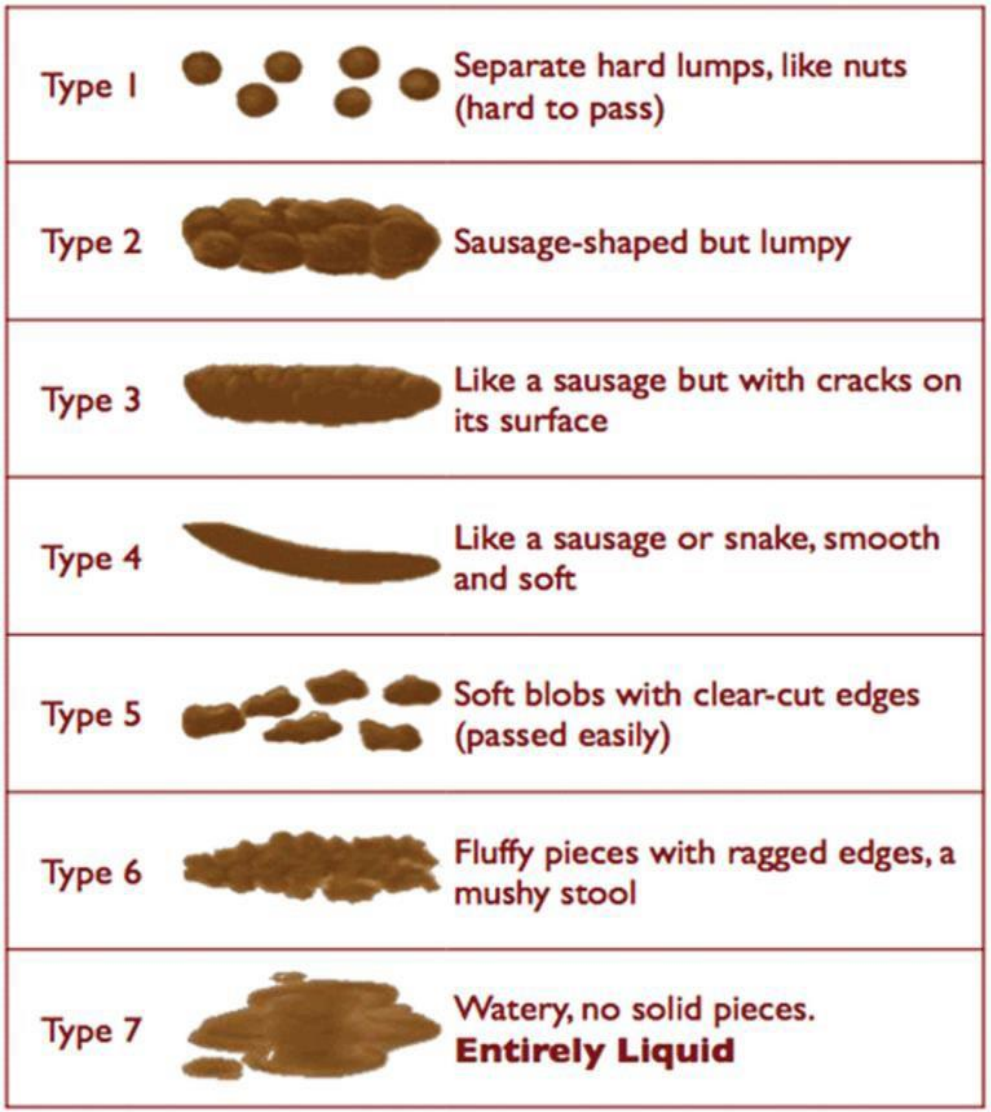
Appendix B - Instruments and laboratory

Stadiometer/ tape, weighing scale (height and weight measurement), sphygmomanometer (blood pressure), Phlebotomy kits, PCR machine, centrifuge, pipets, LC-MS/GC-MS, ELISA, Stool sample kit (DNAgenotek-Omnigene.GUT for microbiome), Refrigerators, Deep freezers, and Cold-chain containers/carriers were be used. Microbiome and chemistry laboratory facilities and technical expertise were shared by two K-State professors, Dr. Lydia Zeglin and Dr. Christopher Culbertson, as the interdisciplinarity collaborative effort for this research project. Dr. Lydia Zeglin's laboratory assisted in processing microbiome samples, then Illumina MiSeq Sequencing at the Kansas State Integrated Genomics Facility (IGF), and Dr. Christopher Culbertson's laboratory (LC-MS, GC-MS), assisted in measuring serum L-Dopa, Dopamine, SCFAs, and Ghrelin levels. However, at this point, due to the technical difficulties and issues of mass spectrometer in the chemistry lab, serum levels of neuroendocrinal and nutritional metabolites, L-Dopa, Dopamine, SCFAs and Ghrelin are unavailable until the issues are fixed. All the serum samples collected from the research participants are stored in the deep freezer at -80°C while waiting for the time to start testing. Due to the financial constraints, these serum level measurements could not be outsourced at this time.

Appendix C - Bowel movement diary.

Bowel Movement Diary was recorded over the 12-week intervention period. (Chumpitazi et al., 2016)

Bowel movement Diary		1	2	3	4	5	6	7	8	9	10	11	12	13	14
#	**Instruction: ‡For stool type, please look at the picture and read the description of the stool form, and note down which type conforms with your stool, at each time. In the other Yes/No questions, note + for "Yes", or – for "No".**														
1	Date														
2	Time														
3	**Stool type (Type 1 to 7) ‡ Please look at the picture.**														
4	Number of bowel movements														
5	Straining or abdominal pain [Yes (+) /No (-)]														
6	Sensation of incomplete evacuation [Yes (+) /No (-)]														
7	Sensation of anorectal obstruction or blockage [Yes (+) /No (-)]														
8	Manual maneuvers required [Yes (+) /No (-)]														
9	Spontaneous defecations [Yes (+) /No (-)]														
10	Laxatives use [Yes (+) /No (-)]														
11	Name of Laxative														
12	Dosage of Laxative														



Chumpitazi et. al. 2016 BSF, Rome Foundation, 2000

Manual maneuvers include digital evacuation, or support of the pelvic floor etc.

Figure: Stool Types Picture

Instruction for question 3: ‡For stool type, please look at the picture and read the description of the stool form, and note down which type conforms with your stool, at each time

Appendix D - Rome IV questionnaire & Bristol stool from scale.

Rome IV questionnaires and Bristol stool form scale were applied in the assessment of bowel movements and stool types. The use of ROME-IV questionnaires and Bristol Stool Form Scale for this research was licensed at the ROME Foundation. The ROME-IV questionnaires and Bristol Stool Form Scale are available at below URL:

- https://theromefoundation.org/rome-iv/rome-iv-questionnaire/
- https://theromefoundation.org/products/copyright-and-licensing/

Appendix E - MDS-UPDRS – The movement disorder society-unified Parkinson's disease rating scale.

The use of MDS-UPDRS Scale for this randomized controlled trial (#9924) was licensed at the International Parkinson and Movement Disorder Society, Product Code: MDSUPDRS-LIC, License ID#2011, Account#32200-001.

- The Movement Disorder Society- Unified Parkinson's Disease Rating Scale (MDS-UPDRS) can be viewed in the below link**: https://www.movementdisorders.org/MDS-Files1/PDFs/Rating-Scales/MDS-UPDRS_English_FINAL_Updated_August2019.pdf
- Standard use of an MDS-owned Rating Scale is granted on a per study, per protocol, per language basis. MDS Rating Scales Permission Request Form is available in the below link: https://mds.movementdisorders.org/publications/rating_scales/request_form.php

The participants' MDS-UPDRS assessment was conducted in the dimensions as summarized below. Complete assessment tool is referred to the MDS-UPDRS URL provided above**.

Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

1.1 Cognitive Impairment

0: Normal: No cognitive impairment.

1: Slight: Impairment appreciated by patient or caregiver with no concrete interference with the patient's ability to carry out normal activities and social interactions.

2: Mild: Clinically evident cognitive dysfunction, but only minimal interference with the patient's ability to carry out normal activities and social interactions.

3: Moderate: Cognitive deficits interfere with but do not preclude the patient's ability to carry out normal activities and social interactions.

4: Severe: Cognitive dysfunction precludes the patient's ability to carry out normal activities and social interactions.

1.2 Hallucinations and psychosis

0: Normal: No hallucinations or psychotic behavior.

1: Slight: Illusions or non-formed hallucinations, but patient recognizes them without loss of insight.

2: Mild: Formed hallucinations independent of environmental stimuli. No loss of insight.

3: Moderate: Formed hallucinations with loss of insight. 4: Severe: Patient has delusions or paranoia.

4: Severe: Patient has delusions or paranoia.

1.3 Depressed Mood

0: Normal: No depressed mood.

1: Slight: Episodes of depressed mood that are not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions.

2: Mild: Depressed mood that is sustained over days, but without interference with normal activities and social interactions.

3: Moderate: Depressed mood that interferes with, but does not preclude the patient's ability to carry out normal activities and social interactions.

4: Severe: Depressed mood precludes patient's ability to carry out normal activities and social interactions.

1.4 Anxious mood

0: Normal: No anxious feelings.

1: Slight: Anxious feelings present but not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions.

2: Mild: Anxious feelings are sustained over more than one day at a time, but without interference with patient's ability to carry out normal activities and social interactions.

3: Moderate: Anxious feelings interfere with, but do not preclude, the patient's ability to carry out normal activities and social interactions.

4: Severe: Anxious feelings preclude patient's ability to carry out normal activities and social interactions.

1.5 Apathy

0: Normal: No apathy.

1: Slight: Apathy appreciated by patient and/or caregiver, but no interference with daily activities and social interactions.

2: Mild: Apathy interferes with isolated activities and social interactions. 3: Moderate: Apathy interferes with most activities and social interactions.

4: Severe: Passive and withdrawn, complete loss of initiative.

1.6 Features of dopamine dysregulation syndrome

0: Normal: No problems present.

1: Slight: Problems are present but usually do not cause any difficulties for the patient or family/caregiver.

2: Mild: Problems are present and usually cause a few difficulties in the patient's personal and family life.

3: Moderate: Problems are present and usually cause a lot of difficulties in the patient's personal and family life.

4: Severe: Problems are present and preclude the patient's ability to carry out normal activities or social interactions or to maintain previous standards in personal and family life.

1.7 Sleep problems

0: Normal: No problems.

1: Slight: Sleep problems are present but usually do not cause trouble getting a full night of sleep.

2: Mild: Sleep problems usually cause some difficulties getting a full night of sleep.

3: Moderate: Sleep problems cause a lot of difficulties getting a full night of sleep, but I still usually sleep for more than half the night.

4: Severe: I usually do not sleep for most of the night.

1.8 Daytime sleepiness

0: Normal: No daytime sleepiness.

1: Slight: Daytime sleepiness occurs, but I can resist and I stay awake.

2: Mild: Sometimes I fall asleep when alone and relaxing. For example,
while reading or watching TV.

3: Moderate: I sometimes fall asleep when I should not. For example, while eating or talking with other people.

4: Severe: I often fall asleep when I should not. For example, while eating or talking with other people.

1.9 Pain and other sensations

0: Normal: No uncomfortable feelings.

1: Slight: I have these feelings. However, I can do things and be with other people without difficulty.

2: Mild: These feelings cause some problems when I do things or am with other people.

3: Moderate: These feelings cause a lot of problems, but they do not stop me from doing things or being with other people.

4: Severe: These feelings stop me from doing things or being with other people.

1.10 Urinary problems

0: Normal: No urine control problems.

1: Slight: I need to urinate often or urgently. However, these problems do not cause difficulties with my daily activities.

2: Mild: Urine problems cause some difficulties with my daily activities. However, I do not have urine accidents.

3: Moderate: Urine problems cause a lot of difficulties with my daily activities, including urine accidents.

4: Severe: I cannot control my urine and use a protective garment or have a bladder tube.

1.11 Constipation problems

0: Normal: No constipation.

1: Slight: I have been constipated. I use extra effort to move my bowels.

However, this problem does not disturb my activities or my being comfortable.

2: Mild: Constipation causes me to have some troubles doing things or being comfortable.

3: Moderate: Constipation causes me to have a lot of trouble doing things or being comfortable.

However, it does not stop me from doing anything.

4: Severe: I usually need physical help from someone else to empty my bowels.

1.12 Light headedness on standing

0: Normal: No dizzy or foggy feelings.

1: Slight: Dizzy or foggy feelings occur. However, they do not cause me troubles doing things.

2: Mild: Dizzy or foggy feelings cause me to hold on to something, but I do not need to sit or lie back down.

3: Moderate: Dizzy or foggy feelings cause me to sit or lie down to avoid fainting or falling.

4: Severe: Dizzy or foggy feelings cause me to fall or faint.

1.13 Fatigue

0: Normal: No fatigue.

1: Slight: Fatigue occurs. However, it does not cause me troubles doing things or being with people.

2: Mild: Fatigue causes me some troubles doing things or being with people.

3: Moderate: Fatigue causes me a lot of troubles doing things or being with people. However, it does not stop me from doing anything.

4: Severe: Fatigue stops me from doing things or being with people.

Part II: Motor Aspects of Experiences of Daily Living (M-EDL)

2.1 Speech

0: Normal: Not at all (no problems).

1: Slight: My speech is soft, slurred or uneven, but it does not cause others to ask me to repeat myself.

2: Mild: My speech causes people to ask me to occasionally repeat myself, but not every day.

3: Moderate: My speech is unclear enough that others ask me to repeat myself every day even though most of my speech is understood.

4: Severe: Most or all of my speech cannot be understood.

2.2 Saliva and drooling

0: Normal: Not at all (no problems).

1: Slight: I have too much saliva, but do not drool.

2: Mild: I have some drooling during sleep, but none when I am awake.

3: Moderate: I have some drooling when I am awake, but I usually do not need tissues or a handkerchief.

4: Severe: I have so much drooling that I regularly need to use tissues or a handkerchief to protect my clothes.

2.3 Chewing and swallowing

0: Normal: No problems.

1: Slight: I am aware of slowness in my chewing or increased effort at swallowing, but I do not choke or need to have my food specially prepared.

2: Mild: I need to have my pills cut or my food specially prepared because of chewing or swallowing problems, but I have not choked over the past week.

3: Moderate. I choked at least once in the past week.

4: Severe: Because of chewing and swallowing problems, I need a feeding tube.

2.4 Eating tasks

0: Normal: Not at all (no problems).

1: Slight: I am slow, but I do not need any help handling my food and have not had food spills while eating.

2: Mild: I am slow with my eating and have occasional food spills. I may need help with a few tasks such as cutting meat.

3: Moderate: I need help with many eating tasks but can manage some alone.

4: Severe: I need help for most or all eating tasks.

2.5 Dressing

0: Normal: Not at all (no problems).

1: Slight: I am slow, but I do not need help.

2: Mild: I am slow and need help for a few dressing tasks (buttons, bracelets).

3: Moderate: I need help for many dressing tasks.

4: Severe: I need help for most or all dressing tasks.

2.6 Hygiene

0: Normal: Not at all (no problems).

1: Slight: I am slow, but I do not need any help.

2: Mild: I need someone else to help me with some hygiene tasks.

3: Moderate: I need help for many hygiene tasks.

4: Severe: I need help for most or all of my hygiene tasks.

2.7 Handwriting

0: Normal: Not at all (no problems).

1: Slight: My writing is slow, clumsy or uneven, but all words are clear.

2: Mild: Some words are unclear and difficult to read.

3: Moderate: Many words are unclear and difficult to read.

4: Severe: Most or all words cannot be read.

2.8 Doing hobbies and other activities

0: Normal: Not at all (no problems).

1: Slight: I am a bit slow but do these activities easily.

2: Mild: I have some difficulty doing these activities.

3: Moderate: I have major problems doing these activities, but still do most.

4: Severe: I am unable to do most or all of these activities.

2.9 Turning in bed

0: Normal: Not at all (no problems).

1: Slight: I have a bit of trouble turning, but I do not need any help.

2: MildI have a lot of trouble turning and need occasional help from someone else.

3: Moderate: To turn over I often need help from someone else.

4: Severe: I am unable to turn over without help from someone else.

2.10 Tremor

0: Normal: Not at all. I have no shaking or tremor.

1: Slight: Shaking or tremor occurs but does not cause problems with any activities.

2: Mild: Shaking or tremor causes problems with only a few activities. 3: Moderate: Shaking or tremor causes problems with many of my daily activities.

4: Severe: Shaking or tremor causes problems with most or all activities.

2.11 Getting out of bed, a car, or a deep chair

0: Normal: Not at all (no problems).

1: Slight: I am slow or awkward, but I usually can do it on my first try.

2: Mild: I need more than one try to get up or need occasional help.

3: Moderate: I sometimes need help to get up, but most times I can still do it on my own.

4: Severe: I need help most or all of the time.

2.12 Walking and balance

0: Normal: Not at all (no problems).

1: Slight: I am slightly slow or may drag a leg. I never use a walking aid.

2: Mild: I occasionally use a walking aid, but I do not need any help from another person.

3: Moderate: I usually use a walking aid (cane, walker) to walk safely without falling. However, I do not usually need the support of another person.

4: Severe: I usually use the support of another person to walk safely without falling.

2.13 Freezing

0: Normal: Not at all (no problems).

1: Slight: I briefly freeze, but I can easily start walking again. I do not need help from someone else or a walking aid (cane or walker) because of freezing.

2: Mild: I freeze and have trouble starting to walk again, but I do not need someone's help or a walking aid (cane or walker) because of freezing.

3: Moderate: When I freeze I have a lot of trouble starting to walk again and, because of freezing, I sometimes need to use a walking aid or need someone else's help.

4: Severe: Because of freezing, most or all of the time, I need to use a walking aid or someone's help.

Part III: Motor Examination

3.1 Speech

0: Normal: No speech problems.

1: Slight: Loss of modulation, diction, or volume, but still all words easy to understand.

2: Mild: Loss of modulation, diction, or volume, with a few words unclear, but the overall sentences easy to follow.

3: Moderate: Speech is difficult to understand to the point that some, but not most, sentences are poorly understood.

4: Severe: Most speech is difficult to understand or unintelligible.

3.2 Facial expression

0: Normal: Normal facial expression.

1: Slight: Minimal masked facies manifested only by decreased frequency of blinking.

2: Mild: In addition to decreased eye-blink frequency, masked facies present in the lower face as well, namely fewer movements around the mouth, such as less spontaneous smiling, but lips not parted.

3: Moderate: Masked facies with lips parted some of the time when the mouth is at rest.

4: Severe: Masked facies with lips parted most of the time when the mouth is at rest.

3.3 Rigidity

0: Normal: No rigidity.

1: Slight: Rigidity only detected with activation maneuver.

2: Mild: Rigidity detected without the activation maneuver, but full range of motion is easily achieved.

3: Moderate: Rigidity detected without the activation maneuver; full range of motion is achieved with effort.

4: Severe: Rigidity detected without the activation maneuver and full range of motion not achieved.

3.4 FINGER TAPPING

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps.

2: Mild: Any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence.

3: Moderate: Any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st tap.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.

3.5 Hand movements

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the task.

2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the task.

3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st open-and-close sequence.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.

3.6 Pronation-supination movements of hands

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the sequence.

2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the sequence.

3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st supination-pronation sequence.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.

3.7 Toe tapping

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) amplitude decrements near the end of the ten taps.

2: Mild: Any of the following: a) 3 to 5 interruptions during the tapping movements; b) mild slowing; c) amplitude decrements midway in the task.

3: Moderate: Any of the following: a) more than 5 interruptions during the tapping movements or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) amplitude decrements after the 1st tap.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.

3.8 Leg agility

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) amplitude decrements near the end of the task.

2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowness; c) amplitude decrements midway in the task.

3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing in speed; c) amplitude decrements after the 1st tap.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.

3.9 Arising from chair

0: Normal: No problems. Able to arise quickly without hesitation.

1: Slight: Arising is slower than normal; or may need more than one attempt; or may need to move forward in the chair to arise. No need to use the arms of the chair.

2: Mild: Pushes self up from the arms of the chair without difficulty.

3: Moderate: Needs to push off, but tends to fall back; or may have to try more than one time using the arms of the chair, but can get up without help.

4: Severe: Unable to arise without help.

3.10 Gait

0: Normal: No problems.

1: Slight: Independent walking with minor gait impairment.

2: Mild: Independent walking but with substantial gait impairment.

3: Moderate: Requires an assistance device for safe walking (walking stick, walker) but not a person.

4: Severe: Cannot walk at all or only with another person's assistance.

3.11 Freezing of gait

0: Normal: No freezing.

1: Slight: Freezes on starting, turning, or walking through doorway with a single halt during any of these events, but then continues smoothly without freezing during straight walking.

2: Mild: Freezes on starting, turning, or walking through doorway with more than one halt during any of these activities, but continues smoothly without freezing during straight walking.

3: Moderate: Freezes once during straight walking.

4: Severe: Freezes multiple times during straight walking.

3.12 Postural stability

0: Normal: No problems. Recovers with one or two steps.

1: Slight: 3-5 steps, but subject recovers unaided.

2: Mild: More than 5 steps, but subject recovers unaided.

3: Moderate: Stands safely, but with absence of postural response; falls if not caught by examiner.

4: Severe: Very unstable, tends to lose balance spontaneously or with just a gentle pull on the shoulders.

3.13 Posture

0: Normal: No problems.

1: Slight: Not quite erect, but posture could be normal for older person.

2: Mild: Definite flexion, scoliosis or leaning to one side, but patient can correct posture to normal posture when asked to do so.

3: Moderate:Stooped posture, scoliosis or leaning to one side that cannot be corrected volitionally to a normal posture by the patient.

4: Severe:Flexion, scoliosis or leaning with extreme abnormality of posture.

3.14 Global spontaneity of movement (body bradykinesia)

0: Normal: No problems.

1: Slight: Slight global slowness and poverty of spontaneous movements.

2: Mild: Mild global slowness and poverty of spontaneous movements.

3: Moderate: Moderate global slowness and poverty of spontaneous movements.

4: Severe: Severe global slowness and poverty of spontaneous movements.

3.15 Postural tremor of the hands

0: Normal: No tremor.

1: Slight: Tremor is present but less than 1 cm in amplitude.

2: Mild: Tremor is at least 1 but less than 3 cm in amplitude.

3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude.

4: Severe: Tremor is at least 10 cm in amplitude.

3.16 Kinetic tremor of the hands

0: Normal: No tremor.

1: Slight: Tremor is present but less than 1 cm in amplitude.

2: Mild: Tremor is at least 1 but less than 3 cm in amplitude.

3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude.

4: Severe: Tremor is at least 10 cm in amplitude.

3.17 Rest tremor amplitude

Extremity ratings

0: Normal: No tremor.

1: Slight: < 1 cm in maximal amplitude.

2: Mild: ≥ 1 cm but < 3 cm in maximal amplitude.

3: Moderate: ≥ 3 cm but < 10 cm in maximal amplitude.

4: Severe: ≥ 10 cm in maximal amplitude.

Lip/Jaw ratings

0: Normal: No tremor.

1: Slight: < 1 cm in maximal amplitude.

2: Mild: ≥ 1 cm but < 2 cm in maximal amplitude.

3: Moderate: ≥ 2 cm but < 3 cm in maximal amplitude.

4: Severe: ≥ 3 cm in maximal amplitude.

3.18 Constancy of rest tremor

0: Normal: No tremor.

1: Slight: Tremor at rest is present ≤ 25% of the entire examination period.

2: Mild: Tremor at rest is present 26-50% of the entire examination period.

3: Moderate: Tremor at rest is present 51-75% of the entire examination period.

4: Severe: Tremor at rest is present > 75% of the entire examination period.

Dyskinesia impact on part III ratings

A. Were dyskinesias (chorea or dystonia) present during examination? No/Yes

B. If yes, did these movements interfere with your ratings? No/Yes

Hoehn and Yahr stage

0: Asymptomatic.

1: Unilateral involvement only.

2: Bilateral involvement without impairment of balance.

3: Mild to moderate involvement; some postural instability but physically independent; needs assistance to recover from pull test.

4: Severe disability; still able to walk or stand unassisted.

5: Wheelchair bound or bedridden unless aided.

Part IV: Motor complications

A. Dyskinesias [exclusive of off-state dystonia]

4.1 Time spent with dyskinesias

0: Normal: No dyskinesias.

1: Slight: ≤ 25% of waking day.

2: Mild: 26 - 50% of waking day.

3: Moderate: 51 - 75% of waking day.

4: Severe: > 75% of waking day.

4.2 Functional impact of dyskinesias

0: Normal: No dyskinesias or no impact by dyskinesias on activities or social interactions. 1: Slight: Dyskinesias impact on a few activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods.

2: Mild: Dyskinesias impact on many activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods.

3: Moderate: Dyskinesias impact on activities to the point that the patient usually does not perform some activities or does not usually participate in some social activities during dyskinetic episodes.

4: Severe: Dyskinesias impact on function to the point that the patient usually does not perform most activities or participate in most social interactions during dyskinetic episodes.

B. Motor fluctuations

4.3 Time spent in the off state

0: Normal: No OFF time.

1: Slight: ≤ 25% of waking day.

2: Mild: 26 - 50% of waking day.

3: Moderate: 51 - 75% of waking day.

4: Severe: > 75% of waking day.

4.4 Functional impact of fluctuations

0: Normal: No fluctuations or no impact by fluctuations on performance of activities or social interactions.

1: Slight: Fluctuations impact on a few activities, but during OFF, the patient usually performs all activities and participates in all social interactions that typically occur during the ON state.

2: Mild: Fluctuations impact many activities, but during OFF, the patient still usually performs all activities and participates in all social interactions that typically occur during the ON state.

3: Moderate: Fluctuations impact on the performance of activities during OFF to the point that the patient usually does not perform some activities or participate in some social interactions that are performed during ON periods.

4: Severe: Fluctuations impact on function to the point that, during OFF, the patient usually does not perform most activities or participate in most social interactions that are performed during ON periods.

4.5 Complexity of motor fluctuations

0: Normal: No motor fluctuations.

1: Slight: OFF times are predictable all or almost all of the time (> 75%).

2: Mild: OFF times are predictable most of the time (51-75%).

3: Moderate: OFF times are predictable some of the time (26-50%).

4: Severe:OFF episodes are rarely predictable (≤ 25%).

C. "Off" dystonia

4.6 Painful off-state dystonia

0: Normal: No dystonia OR NO OFF TIME.

1: Slight: ≤ 25% of time in OFF state.

2: Mild: 26-50% of time in OFF state.

3: Moderate: 51-75% of time in OFF state.

4: Severe: > 75% of time in OFF state.

Appendix F - Parkinson's disease questionnaire-39 (PDQ-39).

The use of PDQ-39 questionnaires for this research #9924 was licensed at Oxford University Innovation (license# or Order # PDQ-3-729137).

A license can be requested at https://innovation.ox.ac.uk/outcome-measures/parkinsons-disease-questionnaire-pdq-39-pdq-8/

https://process.innovation.ox.ac.uk/clinical

The questionnaires could be previewed at https://vsymca.org/wp-content/uploads/2016/09/PDQ-39-Form-A.pdf

Appendix G - Depression and anxiety assessment.

For mental aspect, NHS-Mood Zone questionnaire (NHS, 2020) was used for depression and anxiety assessment.

- Depression and anxiety assessment questionnaires can be viewed in the below URL: https://www.nhs.uk/mental-health/self-help/guides-tools-and-activities/depression-anxiety-self-assessment-quiz/

Appendix H - Profile of mood states.

Profile of Mood States (POMS 2) instrument from the Multi Health System Inc., MHS, was used (Heuchert et al., n.d.) for the profile of mood states assessment.

The POMS assessment asked how the respondent or participant felt in the past week, including assessment day (today). The POMS assessment contained 65 words or statements that described the feelings people have. The POMS 2 assessment questionnaire is available at https://storefront.mhs.com/collections/poms-2 .

Appendix I - Diet History Questionnaire (DHQIII)

Diet history questionnaire (DHQIII) can be seen in the link below:

• Username: I-002

• Password: WZwvsVDb

• Questionnaire Login URL

https://www.dhq3.org/study/study_id=562/view-questionnaire/

(or)

https://www.dhq3.org/study/demo/?language=en

- Questionnaire Login URL http://www.dhq3.org/respondent-login/?uuid=74a90245-4ee1-4d98- a0c3-ce11a9d396d0

Appendix J - Mini nutritional assessment (MNA).

Mini Nutritional Assessment (MNA) was used for the nutritional assessment in this study. MNA is available at the below URL:

https://www.mna-elderly.com/

https://www.mna-elderly.com/sites/default/files/2021-10/mna-mini-english.pdf