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Journal of Probiotics & Health

Research Article

A Prospective, Randomized, Double Blind, Two Arms, Parallel, Placebo Controlled, Clinical Study to Evaluate the Efficacy of OPTIMEALTH® FOOD P Supplementation in Modulating Gut Microbiome in Healthy Adults

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ABSTRACT

A structured and equilibrated gut microbiome is needed for optimal health status. Factors such as antibiotic use, caesarean-section deliveries, excessive hygiene, stress, and lack of exercise have a major impact on the microbiome which further leads to microbiota shift. This shift may trigger the development of several disorders, such as obesity, metabolic syndrome, cardiovascular diseases, colorectal cancer and Inflammatory Bowel Diseases (IBD). The present study was designed to evaluate the efficacy and safety of the formulation OPTIMEALTH® FOOD P (OF PB-LBF) on gut microbiota population. OPTIMEALTH® FOOD P® is composed of more than 300 metabolites produced by a selected complex of lactic acid bacteria strains through high tech Fermentation Technology. OPTIMEALTH® FOOD P was developed by studying the microbiota of healthy centenarians who have a particular microbiota profile. A total of 59 subjects compliant with inclusion criteria were randomly assigned to one of the two groups, OPTIMEALTH® FOOD P or placebo group using a simple randomization process. Consumption of OPTIMEALTH® FOOD P by healthy men and women with self-selected diets and unchanged physical activity patterns resulted in the group of OPTIMEALTH® FOOD P in an increased of *Bifidobacterium longum* by 27.5%, *Lactobacillus acidophilus* by 24.6%, *Lactobacillus plantarum* by 28.6%, *Roseburia intestinalis* by 14.5%, *Faecalibacterium prausnitzii* by 23.4% and an increase of *Akkermansia muciniphila* by 11.11%. In conclusion, supplementation with OPTIMEALTH® FOOD P resulted in significant gut microbiota modulation. The tolerance was good among subjects. No side effects or adverse events were reported during the study. Hence OPTIMEALTH® FOOD P at 100 mg/day could be recommended as a safe solution to modulate gut microbiota and improve diseases related to microbiota dysbiosis.

Keywords: *Gut microbiome; Lactobacillus acidophilus; Microbiota*

INTRODUCTION

The human Gastrointestinal (GI) tract represents one of the largest interfaces (250–400 m^2) between the host, environmental factors and antigens in the human body. In an average lifetime, around 60 tons of food passes through the human GI tract, along with an abundance of microorganisms from the environment which impose a huge threat on gut integrity [1]. The collection of bacteria, archaea and eukarya colonizing the GI tract is termed the 'gut microbiota' and has co-evolved with the host to form an intricate and mutually beneficial relationship [2,3]. The microbiota offers many benefits to the host, through a range of physiological functions such as strengthening gut integrity or shaping the intestinal epithelium, harvesting energy, protecting against pathogens and regulating host immunity [4-7].

The GI microbiota is one of the densest microbial populations on the planet, and alterations to it have been associated with various diseases for some time. The density and composition of the microbiota are affected by chemical, nutritional and immunological gradients along the gut. In the small intestine, there are typically high levels of acids, oxygen and antimicrobials for a short transit time [8]. These properties limit bacterial growth, such that only rapidly growing, facultative anaerobes with the ability to adhere to epithelial mucus are thought to survive [9].

The microbiome performs many functions, including the production, regulation and break down of small molecules which help the physiological functions of the human body [10]. The important role to maintain the functions of healthy immune system and the integrity of the gut lining by micro biome are production of fatty acids, vitamins, amino acids and the breakdown of dietary

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Received: October 21, 2020; **Accepted:** November 09, 2020; **Published:** November 16, 2020

Citation: Ikeda Y, Nasu M, Klotz C, Bruxer J, Plaisant D (2020) A Prospective, Randomized, Double Blind, Two Arms, Parallel, Placebo Controlled, Clinical Study to Evaluate the Efficacy of OPTIMEALTH® FOOD P Supplementation in Modulating Gut Microbiome in Healthy Adults. J Prob Health. 8:222. DOI: 10.35248/2329-8901.20.8:222.

fiber that has travelled through the digestive tract. The process requires for the breakdown of dietary fiber is the fermentation process that yields a variety of metabolites including butyrate [11].

Butyrate is an organic compound that belongs to the group of "Short-Chain Fatty Acids" (SCFA) [12]. Butyrate is a C-4 fatty acid and is the third most abundant short chain fatty acid in the gut of all mammals [13,14]. Butyrate acts as a major fuel for the colonic epithelial cells and the major regulator of cell proliferation and differentiation [15-18]. This SCFA performs various actions related to cellular homeostasis such as anti-inflammatory, antioxidant and anti-carcinogenic functions [19-21]. Butyrate is produced from carbohydrates by the process of glycolysis. Two molecules of acetyl-CoA combined to form acetoacetyl-CoA, followed by stepwise reduction to butyryl-CoA.

Butyrate also maintains the integrity of the mucosa (gut lining), promotes the growth of villi, finger-like projections that line the intestines and enhances the production of mucin, a gel-like substance that coats the inside of the gut [12]. The mechanism by which it maintains the integrity of bowel wall is called as epithelial defence barrier. The disturbance in butyrate metabolism can cause mucosal damage and inflammatory bowel diseases and Crohn's disease [22]. This short-chain fatty acid has an antioxidant function that helps maintain a healthy gut. Butyrate also regulates colonic motility, natural movements of the gut that move food through it, and increases blood flow in the colon [10].

A structured and equilibrated gut microbiome is needed for optimal health status. Factors such as antibiotic use, caesarean-section deliveries, excessive hygiene, stress, and lack of exercise have a major impact on the microbiome which further leads to microbiota shift [23-25]. This shift may trigger the development of several disorders, such as obesity, metabolic syndrome, cardiovascular diseases, colorectal cancer and Inflammatory Bowel Diseases (IBD) [26]. IBD have been the most studied model of gut microbiota alteration. The alteration of gut microbiome equilibrium is also linked to skin health. A dysbiosis in the gut microbiota promote the weakening of the intestinal barrier, which enable the gut microbiota and their metabolites to quickly enter the bloodstream, accumulate in the skin, and disturb the skin equilibrium. Acne patients exhibited lower gut microbiota diversity and a higher ratio of Bacteroidetes to Firmicutes, and a decreased content in *Lactobacillus*, *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, and *Allobaculum* in acne patients compared with controls.

Thus, manipulation of microbiota has become a promising target for the improvement of host gut and skin health. This can be achieved by consumption of probiotics (live microorganisms) or by consumption of fermented foods which after entering the gut can exert beneficial health effects on the host by improving its intestinal microbial balance [27]. To modulate the microbiome, metabolites are able to impact one of the bacteria main communication system: quorum sensing. To communicate, small molecules are secreted, diffused and detected by bacteria. This enables bacteria to adapt their behaviour on the size of the bacterial population. Indeed, the bigger the size of the bacterial population is, the bigger the quantity of this small molecules is. When the quantity is too important, it triggers the modulation of various genes expression within the bacterial population, as well as the modulation of bacterial proliferation.

OPTIMEALTH® FOOD P (OF PB-LBF) is a supplement containing Lactobacillus metabolites coming from the fermentation of soybeans. With this background, a multicentre, prospective, randomized, double-blind, two-arm, parallel, placebo-controlled study was designed to evaluate the efficacy and safety of OPTIMEALTH® FOOD P (OF PB-LBF), a metabolites supplement, by analysing the modulation of gut microbiota population following supplementation of OPTIMEALTH® FOOD P for 30 consecutive days in healthy adults [28-39].

MATERIALS AND METHODS

Study design

Study concept: This is a double blind, two-arms, parallel group, placebo-controlled, single center randomized clinical trial.

Inclusion criteria: This study recruited 60 women and men between 25 to 60 years with a diagnosis of major depressive disorder. Subjects were recruited according to all the following inclusion criteria. Subjects had to be healthy non-smoker male and female subjects between 25 to 60 years (inclusive) of age, with BMI range of 18-24.9 kg/m^2 (both inclusive). Females of child-bearing potential must agree to use an approved form of birth control and to have a negative pregnancy test result at the screening visit. Female subjects of non-childbearing potential must be amenorrhoeic for at least 1 year or had a hysterectomy and/or bilateral oophorectomy. Subjects had to be willing to give written informed consent, willing to comply with trial protocol ad able to understand the risks/benefits of the protocol. Subject had to be available for the entire study period (1 month).

Exclusion criteria: Subjects representing one or more of the following criteria were excluded from participation in the study. Subjects with diabetes, dyslipidemia, hypertension, cardiovascular diseases, gastrointestinal diseases and any other co-morbidity and considered as not healthy were excluded. The use of antibiotics and/or probiotics three months prior to the study as well as the use of supplementary pro or prebiotics or consumption of large amounts of fermented foods (>500 g/day) in the previous 6 weeks were prohibited. Subject must have had no changes in diet and in physical activity 4 weeks prior the study. Alcoholics and/or drug abusers were excluded as well as subjects having history of psychiatric disorder that may impair the ability of subjects to provide written informed consent. Patients who have completed participation in any other clinical trial during the past 3 months were excluded. Any other condition which the Principal Investigator thinks may jeopardize the study outcome was prohibited.

Subjects were enrolled after signing the written informed consent, having fulfilled all inclusion criteria, presenting none of the exclusion criteria, and have been evaluated at the clinical examination. Of the 120 adults screened, a total of 60 met eligibility criteria and were enrolled in the study.

Study groups: Subjects were randomly assigned using simple randomization process to one of the two treatment groups:

- Group 1: OPTIMEALTH® FOOD P
- Group 2: Placebo

In each study group 30 participants were enrolled, and 59 subjects completed the study. The sample size of 59 subjects was sufficient to know whether the OPTIMEALTH® FOOD P was effective and safe in weight management and satiety levels in overweight subjects.

Study product: The study product contains fermented soybean metabolites. Participants were asked to ingest 100 mg/day before going to sleep. The product was delivered in opaque white bottles containing capsules with 100 mg of the OPTIMEALTH® FOOD P or Placebo 100 mg, with 28 capsules per participant for a period of 4 weeks of study.

Blinding of the study: The product was labelled with an ID number. All products were blinded by the sponsor. An ID number was assigned for each participant, following randomization of participants into groups (allocation ratio1:1). The identity of the specific product was blind to subjects, support staff and investigators. The ID number and the bottle number assign were only known to the sponsor and independent auditing team.

Subject compliance monitoring: A Daily Intake tracking card was distributed to the participants with date and time record. The

participant was required to fill out the time and date on the card indicating daily when they drank the beverage. The participant should record any symptom or reaction observed during the study (information was provided on the consent form of the risks and benefits of being in the study).

The study team made daily phone calling and emailing reminders to track subject compliance with the study regimen and was additionally ask about any reactions or issues. The study coordinator had also registered product intake compliance on a form after calling and talking to volunteer personally.

Study assessments

Microbial composition in stool: Participants were required to collect stool samples at home from 3 days before the study and up to 1 week after end of the study. Stool samples were collected using a flocked swab ESwabTM 480C (COPAN Diagnostics Inc.) which was provided to participants. Participants were required to dip the tip of the flocked swab into a piece of used toilet paper, then place it right back into the tube.

Body weight: Body weight was measured to the nearest 1/10 kg using a calibrated electronic scale, with participants wearing light clothing without shoes.

Physical examination: General and systemic examination was carried out by the investigator at screening, baseline and 4 weeks. Any systemic adverse events during the study period will also be recorded by the investigator in respective patient's CRF after physical examination as considered appropriate by investigator.

Diet: Diet was measured by self-assessment of the participants to measure the estimated calorie intake. Parameters recorded were full description of food, quantity, time of ingestion, any leftovers. Participants were required to not adhere to any specific dietary regimen or exercise regimen in terms of food choices or specific exercises. Participants were required in particular to not take products containing pre or probiotics 6 weeks prior and during the course of the study. Amount of fermented food ingested daily was monitored throughout the study. Diet was measured by self-assessment of the participants to measure the estimated calorie intake.

Physical activity: Physical activity was measured before and after the test period by self-assessment.

16S rRNA sequencing: Total bacterial genomic DNA was extracted from about 220 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). Bacterial species was determined using 16S rRNA sequencing. DNA concentration was determined by a Nanodrop ND-1000 Full-Spectrum UV/Vis Spectrophotometer (Nano Drop Technologies). Quantitative PCR assays were performed and the amplification and detection of DNA were performed with the ABI-PRISM 7500 sequencing detection system (Applied Biosystems). For the determination of DNA, triplicate samples were used and the mean quantity was expressed as log10 genomes per g of sample (wet weight).

Spontaneously reported and observed adverse events: Any adverse event reported by patients or observed by investigator during study period was recorded with type and severity of adverse event. The causality assessment of serious as well as non-serious unexpected adverse events was done and recorded by the investigator.

Study procedures

Safety and adverse events: Study Sponsor Notification by Investigator within the following 48 hours, the investigator or CRA would provide further information on the serious adverse event in the form of a written narrative. This would include a copy of the completed Serious Adverse Event form, and any other diagnostic information that has assisted the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

Study ethic: This study was conducted in compliance with the protocol, Good Clinical Practices Standards, Nuremberg Code, Declaration of Helsinki, Belmont Report and associated regulations, and protocol approved by an IRB or ethic committee.

The identity of subjects/study patients and data generated in the study was handled in strict confidence. Accessibility of the raw data was limited to the authorized personnel of investigator team, ethics committee, sponsor and the regulatory agencies for scheduled monitoring, inspection and audits.

All subjects for this study were provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

Study subjects have not received any stipend or payment other than a free clinical and biochemical examination about their current health status.

Statistical analyses

All statistical analyses were performed with SPSS 16.0 (Japan, IL). Descriptive statistics were used to characterize the sample. Nominal data were analyzed by the use of the chi-square test, whereas continuous data were analyzed by the use of Pearson's correlation analyses, independent sample t-tests, and one-way analysis of variance. The data are presented as mean ± SD. A significance level of 0.05 was determined a priority.

RESULTS AND DISCUSSION

Thirty participants were randomly assigned to the OPTIMEALTH® FOOD P group and thirty participants to the placebo group. There were no significant differences between the two groups in rates of discontinuation. Baseline demographic and clinical characteristics were similar between the two groups. 59 participants completed the study. 1 person in the placebo group got sick and had to use antibiotic treatment (Tables 1 and 2).

Table 1: Subject characteristics at baseline (Mean ± standard error).

	Placebo Group (N=29)	OPTIMEALTH® FOOD P Group (N=30)
Age (years)	39.9 ± 2.2	40.2 ± 2.6
Height (cm)	165.0 ± 4.4	164.5 ± 2.8
Weight (kg)	61.6 ± 3.6	61.7 ± 2.2
BMI (kg/m^2)	22.6 ± 0.8	22.8 ± 0.6
Bifidobacterium longum (log_{10} genomes/g stool sample)	5.3 ± 0.4	5.1 ± 0.5
Lactobacillus acidophilus (log_{10} genomes/g stool sample)	5.9 ± 0.3	5.7 ± 0.6
Lactobacillus plantarum (log_{10} genomes/g stool sample)	6.1 ± 0.4	6.3 ± 0.5

Roseburia intestinalis (log_{10} genomes/g stool sample)	6.8 ± 0.4	6.9 ± 0.3
Faecalibacterium prausnitzii (log_{10} genomes/g stool sample)	6.5 ± 0.7	6.4 ± 0.8
Akkermansia muciniphila (log_{10} genomes/g stool sample)	7.1 ± 0.5	7.2 ± 0.6

Table 2: Microbial composition over 4 weeks in the placebo and OPTIMEALTH® FOOD P group.

	Placebo (N=29)		OF PB-LBF (N=30)	
(log_{10} genomes/g stool sample)	Baseline	4 weeks	Baseline	4 weeks
Bifidobacterium longum	5.3 ± 0.4	5.5 ± 0.2	5.1 ± 0.5	6.5 ± 0.6
Lactobacillus acidophilus	5.9 ± 0.3	6.0 ± 0.2	5.7 ± 0.6	7.1 ± 0.6
Lactobacillus plantarum	6.1 ± 0.4	6.5 ± 0.6	6.3 ± 0.5	8.1 ± 0.6
Roseburia intestinalis	6.8 ± 0.4	6.8 ± 0.5	6.9 ± 0.3	7.9 ± 0.5
Faecalibacterium prausnitzii	6.5 ± 0.7	6.6 ± 0.9	6.4 ± 0.8	7.9 ± 0.6
Akkermansia muciniphila	7.1 ± 0.5	7.1 ± 0.7	7.2 ± 0.6	8.0 ± 0.7

Note: Results are expressed are mean ± SD

There was a significant increase ($p<0.05$) in butyrate producing bacteria: *B. longum, L.acidophilus, L.plantarum, Roseburia intestinalis, Faecalibacterium prausnitzii, Akkermansia muciniphila* with 27.5%, 24.6%, 28.6%, 14.5%, 23.4%, and 11.11% increase respectively in the OPTIMEALTH® FOOD P group, while there was no significant change in the placebo group for the *B. longum, L.acidophilus, Roseburia intestinalis, Faecalibacterium prausnitzii, Akkermansia muciniphila* (Figures 1 and 2).

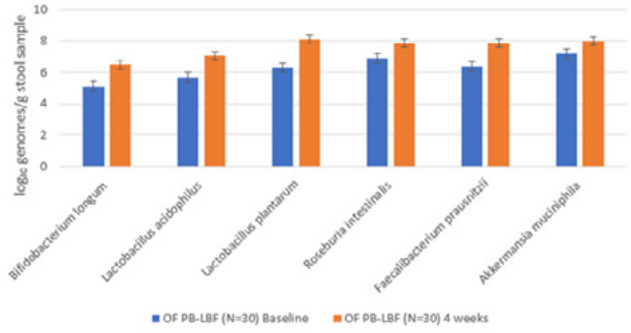

Figure 1: Butyrate producing bacteria population at T0 (baseline) and T4 (4 weeks) in OPTIMEALTH® FOOD P (OF PB-LBF) group.

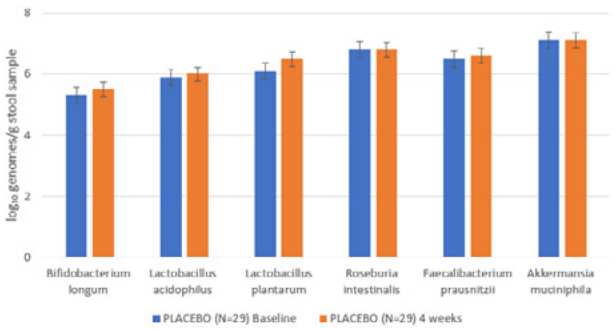

Figure 2: Butyrate producing bacteria population at T0 (baseline) and T4 (4 weeks) in the placebo group.

Participants' diet was monitored during the study to avoid shift in microbiota due to diet changes. In the placebo group, caloric intake was 2196 ± 107.3 kcal/day and diet composition were 28.1% ± 1.2% fat, 52.4% ± 1.5% of carbohydrates and 16.4% ± 1.1% protein. In the test group, caloric intake was 2229 ± 110.5 kcal/day and diet composition were 30.1% ± 1.3% fat, 49.7% ± 1.5% of carbohydrates and 16.5% ± 1.0% protein.

The caloric intake did not change before and during the study.

Physical activity was measured before and after the test period. Participants were asked to self-report work and leisure activity. No significant change was observed during the study compared to before.

Safety

No cases of any other expected and unexpected adverse events were observed/reported during study period. No case of any dropout occurred during study due to any adverse event.

CONCLUSION

The current study aims to demonstrate the positive effects of OPTIMEALTH® FOOD P (OF PB-LBF), containing Lactobacillus metabolites from soybean fermentation, in the microbiota modulation. The double blind, placebo-controlled study resulted in a significant shift in microbiota after 30 days of supplementation of OPTIMEALTH® FOOD P. All the subjects had a good tolerance and none of them manifested any side effect or adverse effect. There were no differences observed at baseline between the OPTIMEALTH® FOOD P group and the placebo group.

Supplementation with OPTIMEALTH® FOOD P for healthy men and women consuming self-selected diets and maintaining usual physical activity patterns resulted in:

- *B. longum*: +27.5%
- *L. acidophilus*: 24.6%
- *L. plantarum*: +28.6
- *Roseburia intestinalis*: +14.5%
- *Faecalibacterium prausnitzii*: +23.4%
- *Akkermansia muciniphila*: +11.11%

Sedentariness contributes to a higher prevalence of health issues and metabolic disorders. Recently, studies have linked dysbiosis in gut microbiota, considered as primary functioning behind various mechanisms, with the development and manifestation of metabolic disorders. For instance, in IBS patients, butyrate producing microbiota is decreased significantly compared to healthy subjects.

Diet-derived substrates such as undigested fibre and starch have major effects upon bacterial community structure and metabolism in the colon. Short-Chain Fatty Acids (SCFA) formed by microbial fermentation have an important effect on colonic health. Butyrate

in particular has an important role in the metabolism and normal development of colonic epithelial cells and has been implicated in protection against cancer and ulcerative colitis. Butyrate is transported by gut epithelial cells, serves as a preferred energy source for colonocytes, and has been shown to exert direct effects upon anti-inflammatory gene expression.

Microbiota modulation is a potential strategy as a health modulator. One study reported that dietary supplementation of acetate, propionate, butyrate or their admixture was shown to influence the bacterial community structure in feces, with a reduction in the proportion of Firmicutes and an increase in the proportion of Bacteroidetes. The present study showed that OPTIMEALTH® FOOD P was efficient to promote a shift in microbiota and to increase the content of butyrate-producing bacteria in the gut.

OPTIMEALTH® FOOD P is composed of more than 300 metabolites produced by a selected complex of lactic acid bacteria strains through high tech Fermentation Technology. OPTIMEALTH® FOOD P was developed by studying the microbiota of healthy centenarians who have a particular microbiota profile. Innovation Labo discovered that the metabolites produced by the microbiota activate gene expression to inhibit and regulate inflammation. Innovation Labo was able to influence gut microbiome by using the science of quorum sensing. OPTIMEALTH® FOOD P metabolites were able to modulate the quorum sensing of bacteria and further modulate their proliferation and their gene expression.

Because of the influence of gut microbiome on skin health, OPTIMEALTH® FOOD P can also be an interesting lead in skin health and more precisely as an acne treatment or prevention solution.

In conclusion, this study's findings indicate OPTIMEALTH® FOOD P as a valuable option for patients and healthcare providers seeking natural alternative approaches for modulating gut microbiome.

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