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Research Article

Probiotics Improve Immune and Digestive Health and Augment Quality of Life in Healthy Adults: An Open Label Work-Place Study

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ABSTRACT

Select probiotic strains are widely known to modulate digestive and immune health functions. However, there are very few studies which demonstrate the efficacy of commercialized multi-strain probiotics on digestive and immune function in healthy adults, and even fewer studies have evaluated the impact of such probiotic blends on the overall quality of life. The aim of this study was to evaluate if daily consumption of a commercially available multi-strain probiotic blend could reduce self-assessed respiratory and gastrointestinal symptoms, as well as improve quality of life parameters in healthy adults. Healthy volunteers were recruited from among employees at MeriCal LLC (Orange County, California and Weber County, Utah), and asked to complete a Quality of Life Questionnaire assessing various parameters of digestive health, immune function, and mental and emotional wellbeing. The responses were collected at the beginning of the study period (pre-trial), and at the end of the 90-day study period (post-trial) during which the probiotic blend was ingested daily. Data from this study demonstrates that a three-month probiotic intervention successfully attenuates digestive health issues, reduces the number of sick days, and improves quality of life as indexed by a self-reported questionnaire. In conclusion, daily supplementation with a multi-strain probiotic is effective in increasing work-place health.

Keywords: Probiotics; Immune health; Sick days; Digestive health; Quality of life Probiotics; Immune health; Sick days; Digestive health; Quality of life

INTRODUCTION

Probiotics are naturally occurring bacteria, most commonly from the genera *Bifidobacteria* and *Lactobacillus*, the latter of which, has very recently been reclassified into 25 new genera [1]. They predominantly reside in the gut and provide health benefits to the host. The main health benefits associated with probiotic supplements are modulation of the intestinal microbial balance which leads to improved gastrointestinal health, and augmentation of the host's innate and adaptive immune responses [2,3]. Recent studies have also demonstrated their potential use as a solution for anxiety and depression by impacting the gut-brain axis [4].

Several studies have shown that regular and consistent consumption of specific probiotic strains can provide individuals with digestive and immune health benefits [2,3,5,6]. One of the most widely studied strains for improvement of intestinal health is *Lactobacillus rhamnosus* GG. Multiple studies have demonstrated that use of this strain is associated with reduction in duration of diarrhea and constipation in children [7,8]. Similarly, well-controlled clinical studies with strains such as *Lactobacillus paracasei* Lpc-37 and Bifidobacterium infantis Bi-26 have reported reduction in the incidence of diarrhea in children and infants, pointing to the effectiveness of these strains in supporting intestinal health [9,10].

Modulation of the gut microflora by probiotics not only supports intestinal health, but also prompts an immune response at both the intestinal (local) and a systemic level [11]. This results in priming of the endogenous host defense mechanisms and an improvement in overall immune function. For instance, in a double-blind study Tubelius et al. reported a positive effect of *Lactobacillus reuteri protectis* in reducing both the frequency of total reported sick days, as well as a reduction in the number of shift-workers who reported sick, when compared with placebo [12]. Similarly, strains such as *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La-14, *Lactobacillus paracasei* Lpc-37, and *Bifidobacterium lactis* Bl-04 have been shown to stimulate the immune system and induce a cascade of immunomodulatory effects, which result in reduction in risk of upper respiratory tract infections in human subjects [10,13,14].

As described above, studies on probiotics typically utilize single strains. Very few studies have evaluated the potential health benefits of long-term consumption of a multi-strain probiotic blend containing strains that have been studied individually for pro-digestive or pro-immune health benefits in conjunction with other strains that may not have been evaluated for the same health-related functions. While such combinations of strains are commonly found in many of the most widely consumed commercially available probiotic dietary supplements in the United States, there is a paucity of studies that evaluate the overall efficacy of such blends.

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The widespread commercialization and manufacturing of multi-strain probiotics merits investigation such that it may be possible to establish whether combining multiple probiotic strains has a compounding or diminishing effect on the known health benefits of the individual strains.

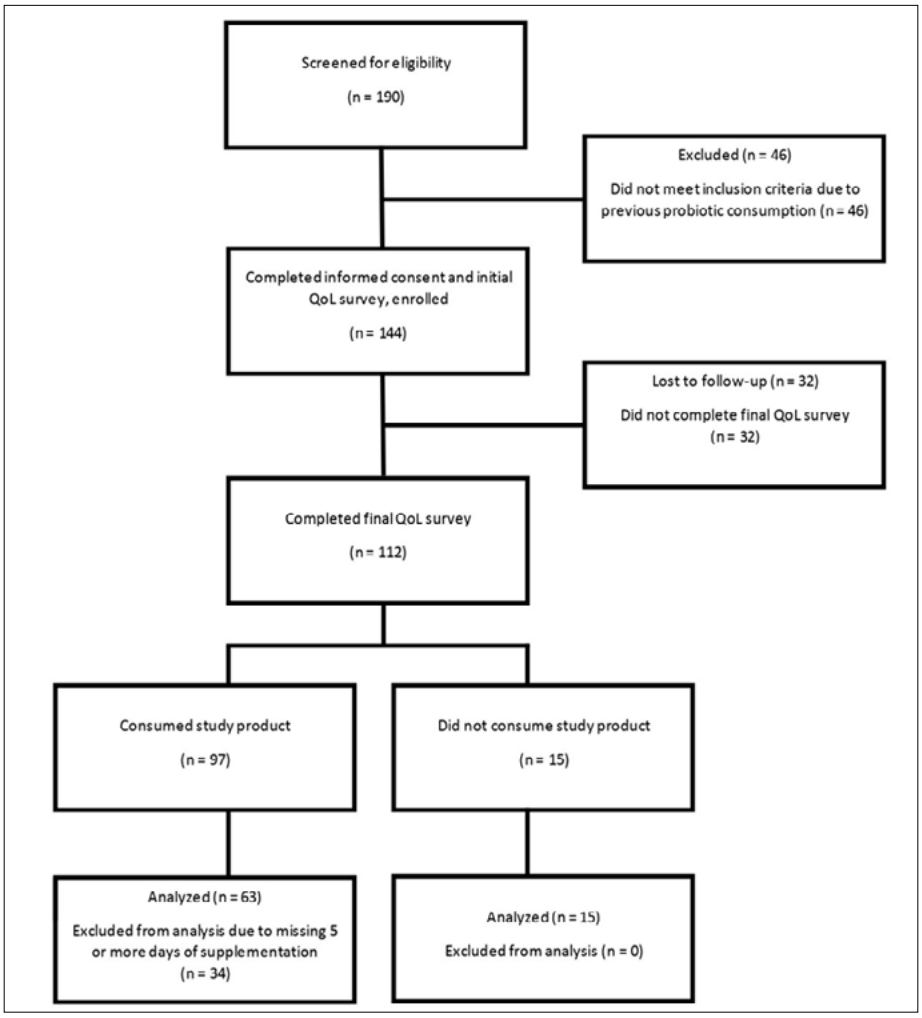
Furthermore, an increasing body of evidence now shows an association of gut microbiota with overall brain function [15,16,17]. There is a bi-directional communication pathway commonly referred to as the microbiota-gut-brain axis that mediates the effects of the gut microbiota on the brain [17]. The pathways implicated in the gut-brain axis span the gastrointestinal tract, Central Nervous System, autonomic nervous system, enteric nervous system, and neuroendocrine system along with the host immune system, acting via microbial neuroactive substances and their precursors such as tryptophan [18-20]. This suggests that gut bacteria may have a role in modulating not just immune response, but also stress responses in the body and that probiotics may be a potential novel approach to positively modulate these effects, leading to improved quality of life in healthy individuals.

Given the above described role of probiotic strains as potent modulators of intestinal and immune function and a regulator of the gut-brain axis, this study aimed to evaluate if daily consumption of a commercially available 12-strain probiotic blend (*Bifidobacterium bifidum* ATCC SD 6576, *Bifidobacterium breve* ATCC SD 5206, *infantis* ATCC SD 6720, *Bifidobacterium lactis* ATCC SD 5220, *Bifidobacterium longum* ATCC SD 5588, *Lactobacillus acidophilus* DSM 32754, *Lactobacillus casei* ATCC SD 5213, *Lactobacillus paracasei* ATCC SD 5275, *Lactobacillus plantarum* ATCC SD 5209, *Lactobacillus reuteri* ATCC SD 6889, *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus salivarius* ATCC SD 5208) in conjunction with the prebiotic-Xylooligosaccharide (XOS) could reduce self-assessed immune and gastrointestinal symptoms and positively impact quality of life parameters in healthy adults.

MATERIALS AND METHODS

Participants

Healthy volunteers were recruited from among employees at MeriCal LLC facilities in Utah and California in the United States. The inclusion criteria for participation were that they should be healthy, non-institutionalized male or female subjects, at least 18 years of age, and willing to comply with the protocol. The study protocol was approved by Aspire IRB, Santee CA (IRB Pr#: 20193122, 11/25/2019). Written informed consent was obtained from all participants (Consort 1).



Consort 1: Consort diagram showing the flow of participants through each stage of a trial.

190 healthy volunteers were screened for participation in the study. 46 volunteers failed screening and were excluded from the study for consuming probiotics prior to the start of the study. 144 volunteers were enrolled in the study. After 90 days of daily probiotic supplementation, 32 volunteers were lost due to follow-up and 34 volunteers were excluded from data analysis for missing 5 or more days of probiotic supplementation during the duration of the study. Upon completion of the study, 63 subjects were in the experimental (P) group, and 15 subjects were in the no-placebo control (C) group.

Study design

The study was designed as an open label trial comparing probiotic to no-treatment controls. The overall study period was set to 90 days, from December 2019 to March 2020. This was done with a view to encompass a period which is generally known to result in many sick days due to flu and infections [6]. Volunteers with one or more of the following criteria were excluded from the study: suffering from any systemic illness; pregnant and/or breastfeeding mothers; have regularly consumed a probiotic dietary supplement or probiotic functional food such as yogurt in the three months before the study trial; or having received antibiotics or non-steroid anti-inflammatory therapy in the three-month period prior to the start of the study. Volunteers were also excluded from statistical analysis if they missed study treatment for more than five (5) days during the intervention period.

Procedures

At the beginning of the study period, volunteers were asked to complete a Quality of Life Questionnaire assessing various parameters of digestive upset, immune function, and mental and emotional wellbeing to establish baseline (pre-trial). The Quality of Life Questionnaire used in this trial contained questions adapted from Patient Assessment Constipation-Quality of Life (PAC-QoL), Short Form 36 (SF-36) Health Survey, and the Beck Depression Inventory, respectively [21-23].

The study product was a commercially available 12-strain probiotic comprising of *Bifidobacterium infantis* ATCC SD 6720, *Bifidobacterium bifidum* ATCC SD 6576, *Bifidobacterium breve* ATCC SD 5206, Bifidobacterium lactis ATCC SD 5220, *Bifidobacterium longum* ATCC SD 5588, *Lactobacillus acidophilus* DSM 32754, *Lactobacillus casei* ATCC SD5213, *Lactobacillus paracasei* ATCC SD 5275, *Lactobacillus plantarum* ATCC SD 5209, *Lactobacillus reuteri* ATCC SD 6889, *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus salivarius* ATCC SD 5208 and 50 mg of the prebiotic XOS. All participants were instructed to take 1 capsule per day for 90 consecutive days at approximately the same time of day. Each capsule consisted of a total 10^{10} CFU of the probiotics and 50 mg of XOS. The control group participants were asked to not take any probiotic product during the duration of the trial. At the end of the trial period, participants were asked to fill out another Quality of Life Questionnaire (post-trial).

Outcome measures

The primary outcome measures in this study were improvements in volunteer self-assessments of digestive upset symptoms, immune health symptoms as measured by total sick days and absent days from work due to sickness, and Quality of Life. The digestive function index was determined by calculating the mean on a 5-point ordinal scale for 4 questions relating to digestive health (constipation, bloating, diarrhea or abdominal discomfort). Sick days were defined as any day that a participant experienced one or more symptoms commonly associated with seasonal respiratory and digestive infection. Absent days due to sickness were defined as any day that an employee did not report to a scheduled work shift due to respiratory or gastrointestinal symptoms. Quality of Life (QoL) was assessed by questions on 5 distinct domains which have been recognized by WHO as denoting an individual's perception of quality of life. These include Psychological, Level of Independence, Social Relationships, and Environment. Individual items were rated on a 5-point scale where 1 indicates low, negative perceptions and 5 indicate high, positive perceptions. Domain score were calculated by computing the mean of each domain, noting that negatively phrased facets are reverse score according to the procedure given below.

Statistical analysis

For each question in the questionnaire, the mean scores were calculated and submitted to an Analysis of Variance (ANOVA) with time (pre-vs. post-intervention) as within-subjects factor and group (placebo vs. probiotics) as between-subjects factor. All alpha levels were set at $p = 0.05$. Post hoc tests were performed to clarify mean differences in case of significant interactions.

RESULTS

Probiotic consumption supports digestive health

In order to evaluate the effect of probiotic supplementation on digestive health, post-trial probiotic and post-trial control groups were compared to the pre-trial baseline group. The post-trial probiotic group was defined as the group that took the 12-strain test probiotic for 3 months during the trial period. The post-trial control group was comprised of participants who signed up for the trial but did not take the probiotics during the study period. Baseline was defined as the data collected for each participant at the beginning of the trial prior to probiotic intervention.

Our results demonstrate that probiotic supplementation improved overall digestive health for subjects during the trial period. This was established by a significantly lower score on the digestive issues dimension of the questionnaire for the probiotic-treated group. Welch's ANOVA testing confirmed an overall effect of supplementation ($F(2,25.5)=17.5$, $p < 0.001$, Figure 1). Multiple comparison t-tests showed significant differences between baseline and post-trial probiotic group ($p < 0.01$) but not between baseline and post-trial control group ($p = 0.24$).

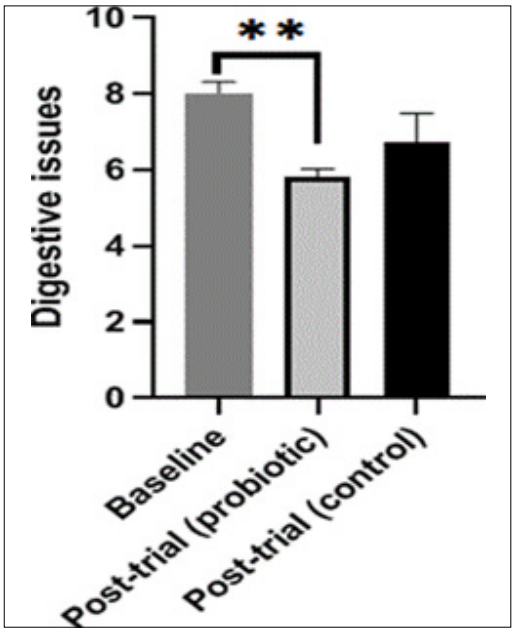


Figure 1: Probiotic supplementation improves digestive health.

Participants completed a Quality of Life survey evaluating the frequency and severity of common digestive health complaints (constipation, bloating, diarrhea or abdominal discomfort) at the start of the trial. Participants were asked to complete the same survey upon completion of the trial. After 3 months of daily supplementation, the post-trial probiotic group showed a significant reduction in frequency and severity of digestive complaints, calculated as the mean derived from a 5-point ordinal scale compared to baseline (**$p < 0.01$, Dunnett's T3 multiple comparisons test following Welch's ANOVA). After 3 months, no significant difference was observed between the post-trial control group and baseline ($p = 0.25$, Dunnett's T3 multiple comparisons test) or between post-trial control and

post-trial probiotic groups (p = 0.34, Mann-Whitney Test).

Probiotic consumption reduces number of sick days and number of days of sick leave from work

Consumption of probiotics during this trial coincided with the traditional United States flu season (December 2019-March 2020). Our findings show that there was a 44.8% reduction in sick days compared to baseline in the probiotic-treated group (Figure 2a). Welch's ANOVA confirmed this overall difference ($F(2,44.3)=4.3$, $p < 0.05$, Figure 2a). Multiple comparison t-tests showed significant differences between baseline vs post-trial probiotic group ($p < 0.05$) and also between post-trial control group vs post-trial probiotic groups ($p < 0.05$) for the total number of sick days.

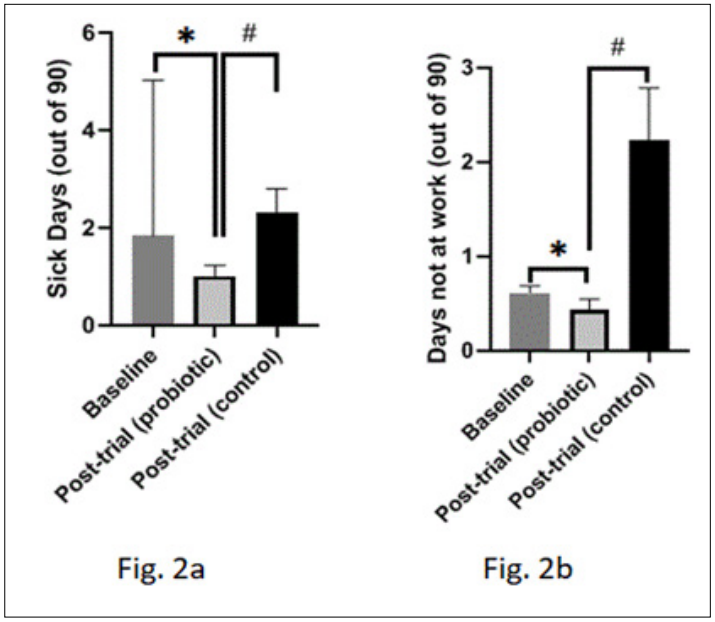


Figure 2: Probiotic supplementation improves immune health.

Participants completed a Quality of Life survey evaluating changes in immune health by assessing total number of days absent from work due to sickness and total number of sick days. Participants were asked to complete the same study upon completion of the trial. a) Participants in the post-trial probiotic group showed a significant decrease in total sick days when compared to baseline (*$p < 0.05$, Dunnett's T3 multiple comparisons test following Welch's ANOVA) and when compared to the post-trial control group (#$p < 0.05$, Dunnett's T3 multiple comparisons test following Welch's ANOVA). b) Participants in the post-trial probiotic group showed a significant decrease in days absent from work due to sickness when compared to baseline (*$p < 0.05$, Dunnett's T3 multiple comparisons test following Welch's ANOVA) and when compared to the post-trial control group (#$p < 0.05$, Dunnett's T3 multiple comparisons test following Welch's ANOVA). The post-trial control group showed an increase in the number of days absent from work due to sickness.

We also found a 27.5% reduction in days taken off work due to sickness. In addition, there was a 271% increase in days taken off work in the control group compared to baseline (Figure 2b). Similar to the total number of sick days, Welch's ANOVA confirmed overall difference between groups ($F(2,44.3) = 5.18$, $p < 0.05$, Figure 2b) for total number of days taken off work. Multiple comparison t-tests showed significant differences between baseline and post-trial probiotic group ($p < 0.05$) and also between post-trial control group and post-trial probiotic groups ($p < 0.05$) for the total number of days taken off work.

Consumption of probiotics improved general health and also perception of overall health compared to 3 months prior

Since probiotic intake is associated with improved physical and mental health and overall well-being, participants were asked to answer a question on their general health compared to 3 months prior. Welch's ANOVA confirmed an overall difference between groups ($F(2,34.2)=12.12$, $p < 0.01$, Figure 3) on the general health measure. Multiple comparison t-tests showed significant difference between the baseline and post-trial probiotic groups ($p < 0.01$) and also between the post-trial control and post-trial probiotic groups ($p < 0.05$).

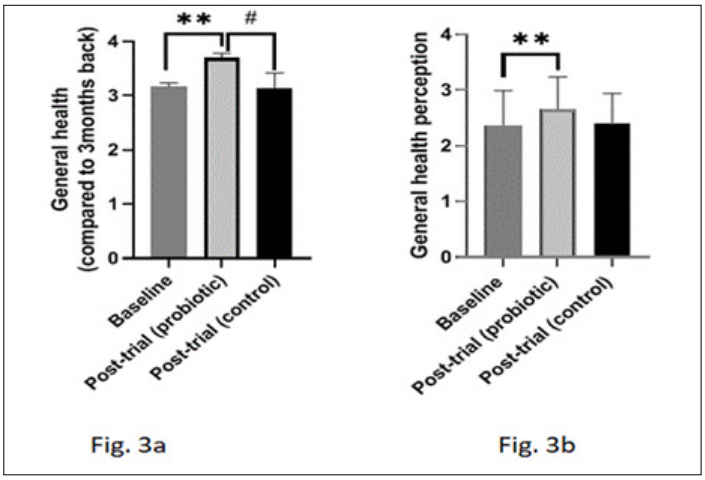


Figure 3: Probiotic supplementation improves general health and wellbeing.

As part of the Quality of Life Survey, participants were asked to evaluate their current general health and overall wellbeing. Participants were asked to complete the same survey upon completion of the trial. a) Participants in the post-trial probiotic group showed a significant increase in the current state of their general health when compared to baseline, 3 months prior (**$p < 0.01$, Dunnett's T3 multiple comparisons test following Welch's ANOVA), and when compared to the post-trial control group (#$p < 0.05$, Dunnett's T3 multiple comparisons test following Welch's ANOVA). Baseline data was captured before participants consumed probiotics and was compared to 3 months prior to the start of the trial. b) Participants in the post-trial probiotic group showed a significant improvement in self-perception of general health compared to baseline (**$p < 0.01$, Dunnett's T3 multiple comparisons test following Welch's ANOVA). There was no significant difference between the post-trial control and baseline (#$p = 0.96$, Dunnett's T3 multiple comparisons test following Welch's ANOVA).

Participant's perception of their overall health compared to the beginning of the trial was also improved following 3 months of probiotic supplementation (Welch's ANOVA; $(F(2,34.6) = 5.39$, $p < 0.01)$. Multiple comparison t-tests showed a significant difference between the baseline and post-trial probiotic groups ($p < .01$) but not between the baseline and post-trial control groups ($p = 0.96$).

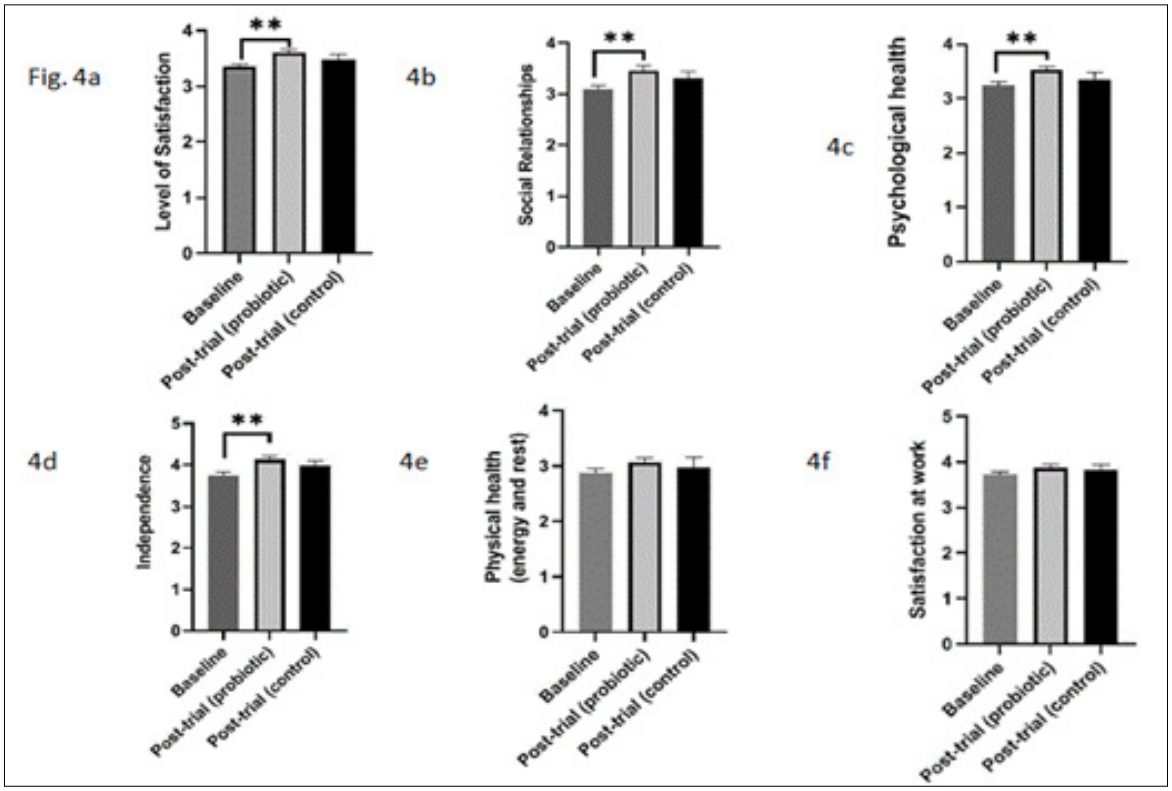


Figure 4: Probiotic supplementation improves overall quality of life.

Participants completed a Quality of Life survey evaluating the current state of their mental health and emotional wellbeing at the start of the trial and were asked to complete the same survey upon completion of the trial. a) After 3 months of daily supplementation, participants in the post-trial probiotic group showed a significant improvement in the Quality of Life Composite Score compared to baseline (**p < 0.01, Dunnett's T3 multiple comparisons test following Welch's ANOVA). An improvement in the Quality of Life Composite Score was observed between the post-trial control group and baseline, but was not significant (**p = 0.45, Dunnett's T3 multiple comparisons test following Welch's ANOVA). b) Participants in the post-trial probiotic group showed a significant improvement in social support and relationships (**p < 0.01, Dunnett's T3 multiple comparisons test following Welch's ANOVA). c) Participants in the post-trial probiotic group showed a significant improvement in positive affect and self-esteem (**p < 0.01, Dunnett's T3 multiple comparisons test following Welch's ANOVA). d) Participants in the post-trial probiotic group showed a significant improvement in their level of independence and productivity when compared with baseline (**p < 0.01, Dunnett's T3 multiple comparisons test following Welch's ANOVA). e) No significant difference was observed for energy and fatigue between baseline and post-trial groups (**p = 0.21, Welch's ANOVA). F) No significant difference was observed for environmental stress and work satisfaction between baseline and post-trial groups (**p = 0.24, Welch's ANOVA).

Consumption of probiotics was associated with improved Quality of Life (QoL)

Ratings on overall Quality of Life (QoL) and the mental component of health-related QoL (i.e., feelings of belonging and social support, positive affect, independence) were improved in the post-trial probiotic group compared to baseline. This was confirmed by Welch's ANOVA ($F(2,43.6) = 6.79$, $p < 0.01$, Figure 4a). Multiple comparison t-tests showed significant differences between the baseline and post-trial probiotic groups ($p < 0.01$) but not between the baseline and post-trial control groups ($p = 0.45$) for overall quality of life.

Significant improvement was observed in the following aspects of QoL: Social Relationship such as feeling of belonging and social support (Welch's ANOVA; $F(2,48.5) = 5.03$ $p < 0.01$, Figure 4b; significant differences between the baseline and post-trial probiotic groups ($p < 0.01$)), Psychological Health such as a positive affect ((Welch's ANOVA; $F(2,43.2) = 4.85$ $p < 0.05$, Figure 4c; significant differences between the baseline and post-trial probiotic groups ($p < 0.01$)), and Level of Independence, such as the ability to carry out daily tasks independently (Welch's ANOVA; $F(2,48.6) = 5.38$ $p < 0.01$, Figure 4d; significant differences between the baseline and post-trial probiotic groups ($p < 0.01$)). Aspects of QoL that remained unimpacted were physical energy and rest (Welch's ANOVA; $F(2,36.) = 1.59$ $p = 0.21$, Figure 4e) 5) and environmental stress affects (Welch's ANOVA; $F(2,43.6) = 1.47$ $p = .24$, Figure 4f).

DISCUSSION

The aim of this study was to examine the effect of a multi-strain probiotic intervention on digestive, immune, and quality of life measures in a healthy population. Data from this study suggests that a three-month multi-strain probiotic intervention successfully attenuates digestive health issues, reduces the number of sick days and number of days taken off work, and improves quality of life as indexed by a self-reported questionnaire. Further analyses of the quality of life data revealed that the strongest positive effects were observed on a feeling of belonging and social support, a positive affect, and the ability to carry out daily tasks independently. Physical energy and the feeling of being well rested remained unimpacted by the intervention.

In a previous study, Hojsak et al. observed a significant reduction in risk for developing gastrointestinal or respiratory infections, as well as vomiting or diarrheal episodes, in hospitalized children supplementing daily with *Lactobacillus rhamnosus* GG during the duration of their stay [13]. Similarly, studies by Hemalatha et al. have also reported improvements in digestive health in children by *Lactabacillus paracasei* and *Lactobacillus rhamnosus* respectively, two species which are part of the multi-species blend used in this study [10]. Findings reported in this study are in line with these previously published reports showing efficacy of select single strains to improve digestive health in children. In addition, the present

data build on and extend previous findings by demonstrating that the pro-digestive effects of strains of probiotic bacteria such as *Lactobacillus rhamnosus* and *Lactobacillus paracasei* remain intact even in a multi-species blend. It should also be noted, that the results from this current study show that the pro-digestive effects of specific probiotic species observed in children maybe be extended to adults. It is plausible that inclusion of additional strains contributed improvement in microflora that resulted in the pro-digestive effects observed here.

A significant finding from this study was the benefit of probiotic consumption on immune health, as measured by a reduction in taken sick days. The number of sick days was reduced by 44.8% in the post-trial probiotic group when compared to baseline. In contrast, there was a 271% increase in absent days due to sickness in the post-trial control group. These finding are consistent with previous reports on effect of individual strains of probiotics in improving immune health. Strains of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei* species, which were all part of this polyblend, have been shown to improve immune health parameters [6,10,14,24]. The data presented here demonstrates that regular supplementation of probiotics in healthy adults can reduce the frequency and duration of sick days, especially during the flu season. To the best of our knowledge, the direct impact of any of the single strains in the blend on sick days has not been reported previously. Further studies will be needed to evaluate the mechanism of action underlying the effects observed in this study; however, probiotics are known to modulate the immune system, in part, by inhibiting the growth of pathogenic bacteria. Several studies have demonstrated the efficacy of *Lactobacilli* species to inhibit growth of the enteropathogenic *Escherichia coli* in the GI tract. Additionally, it has also been shown that probiotic strains can be internalized by the intestinal epithelial cells, which results in initiation of a cascade of signals that stimulate immune cells and activates the innate immune response, including cytokines release [25-27]. While this study did not examine the mechanism of action, it stands to reason that the multispecies probiotic blend may have activated one or more of these mechanisms to elicit the response observed in this study.

In the present study, participants' perception of their overall health was significantly improved after three months of daily supplementation with the 12-strain probiotic combination. When compared to baseline, the post-trial probiotic group showed significant improvements in overall quality of life and specifically in feelings of belonging and social support, positive affect and their ability to perform tasks independently. These results demonstrate that long-term probiotic supplementation in healthy adults may contribute to improved quality of life and positively impact some aspects of mood and stress. While this study did not set out to test specific biological mechanisms that underlie the beneficial effects observed with probiotic consumption, there is much evidence in the literature that points to plausible hypotheses that will be evaluated in future studies. For instance, it has been suggested that intestinal microbiota increase plasma tryptophan levels, and thus facilitate serotonin turnover in the brain [28,29]. Positive affect and a feeling of belonging/social support have been associated with serotonin concentrations, with higher scores correlating with lower serotonin levels [4,30]. Other pathways maybe implicated as well. For instance, it has been suggested that an increased intestinal permeability can induce depressive-like symptoms via modulation of cytokine expression in the gut [31]. Similarly, microbial products have been shown to induce activation of both inflammatory pathways (and) toll-like receptors in glial and neural cells in a manner that results in a direct impact on the HPA axis [32]. It is conceivable that one or more of these mechanisms might account for the beneficial effects of probiotics on overall quality of life in this study.

The present study has a few limitations that should be discussed. First, it is worth noting that our assessment only relied on self-reported QoL questionnaires, and although established as a reliable index of the measures evaluated, it merits consideration as only indirect information on actual digestive, immune and QoL functions. Secondly, we did not include dietary measures and while we did control for consumption of other probiotic products or fermented foods (e.g., yogurt) in the questionnaire, this also relied on self-assessment alone. Hence, we cannot exclude that the consumption of probiotics was accompanied by spontaneous dietary changes that may have indirectly accounted for the effect. Future studies may therefore expand these observations by controlling for these factors.

CONCLUSION

In conclusion, the present study demonstrates that daily supplementation with a multi-strain probiotic combination is effective in increasing work-place health among employees. Participants who consumed the probiotic combination during the study reported reduction in absent days from work due to sickness as well as significant reductions in gastrointestinal symptoms when compared to three months prior. The results in this study also suggest that multi-strain probiotic combinations may have a compounding effect on the known health benefits of an individual probiotic strain. Not much is yet known about the mechanism of action of higher count multi-strain probiotic combinations, which typically make up the majority of commercially sold probiotic products in the United States. Therefore, further studies should be done to understand if there are any additive benefits of combining probiotic strains in probiotic formulations.

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