# Multi-Strain Probiotic Supplement Improves Mood, Anxiety, and Associated Biomarkers

Leah Allen[1], Chad Kerksick[1], Kylie Walden[1], Jessica Moon[1], Anthony Hagele[1], Connor Gaige[1], Joesi Krieger[1], Ralf Jäger[2], Petey Mumford[1], Marco Pane[3], Angela Amoruso[3]

[1]Exercise and Performance Nutrition Laboratory, Lindenwood University, St. Charles, MO USA [2]Increnovo, LLC, Whitefish Bay, WI USA [3]Probiotical Research, Novara, Italy

## Abstract

**PURPOSE:** To examine the efficacy of supplementing with a multi-strain probiotic (MSP) on changes associated with mood, anxiety and neurotransmitter levels.

**METHODS:** In a randomized, double-blind, placebo-controlled fashion, 70 healthy men and women (31.0±9.5 years, 173.0±10.4 cm, 73.9±13.8 kg, 24.6±3.5 kg/m2) supplemented daily with MSP (4 x $10^9$ live cells Limosilactobacillus fermentum LF16, Lacticaseibacillus rhamnosus LR06, Lactiplantibacillus plantarum LP01, and Bifidobacterium longum 04 (Probiotical S.p.A., Novara, Italy) or placebo (PLA). After 0, 2, 4, and 6 weeks of supplementation and 3 weeks after ceasing supplementation, study participants completed the Beck Depression Inventory, State-Trait Anxiety Inventory (STAI), and Leiden Index (LEIDS-R) questionnaires and had plasma concentrations of cortisol, dopamine, and serotonin determined.

**RESULTS:** Beck depression, state and trait anxiety, and total scores in the Leiden Index were reduced from baseline ($p < 0.05$) with MSP supplementation after 4 and 6 weeks of supplementation and 3 weeks after supplementation while no changes ($p > 0.05$) were reported in PLA. When compared to PLA, MSP scores for state anxiety, trait anxiety, and Leiden index (5 out of 6 subscales and total score) were significantly lower ($p < 0.05$) after supplementation. No changes ($p > 0.05$) in dopamine or cortisol were observed between groups. Serotonin levels in MSP were increased from baseline after 6 weeks of supplementation.

**CONCLUSION:** MSP supplementation resulted in widespread improvements in several questionnaires evaluating anxiety and depression in young, healthy men and women. Serotonin increased after 6 weeks of MSP supplementation with no change in dopamine or cortisol.

## Introduction

- Intestinal microbiota are active contributors to a known communicative pathway between the brain and digestive system. This gut-brain axis consists of cellular signaling pathways between the endocrine, nervous and immune systems.
- Limited research has shown the intake of probiotics can influence cognitive function, and may also influence mood, anxiety, and sleep quality.
- The purpose of this study was to examine the efficacy of supplementing with a multi-strain probiotic on changes associated with anxiety, sleep quality, mood and various biomarkers over a six-week study period.

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## Methods

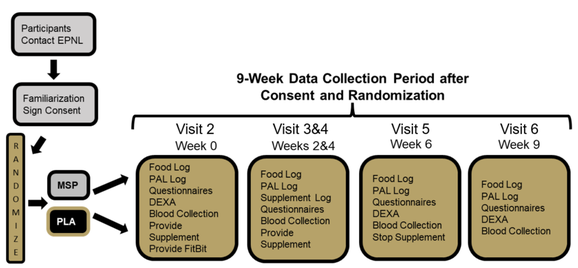
### Participants

- Seventy healthy men and women (31.0±9.5 years, 173.0±10.4 cm, 73.9±13.8 kg, 24.6±3.5 kg/m2 completed this study.

### Experimental Procedure

- In a randomized, double-blind, placebo controlled parallel fashion participants were assigned to consume either multi-strain probiotic MSP (4 x $10^9$ live cells Limosilactobacillus fermentum LF16, Lacticaseibacillus rhamnosus LR06, Lactiplantibacillus plantarum LP01, and Bifidobacterium longum 04 or placebo (PLA).
- Following one baseline visit, participants reported to the laboratory on four additional visits after 2-, 4-, and 6-weeks supplementing, and additionally after a 3-week washout. Participants performed an 8-hour fast and abstained from exercise for 24 h before arrival for visits.
- Daily food and activity logs (recorded via ASA24 and Fitbit, respectively) were completed by the participants throughout the study as well as a supplement log when needed. Logs were checked by research staff at visits.
- At the five full visits, participants completed Beck Depression Inventory, State-Trait Anxiety Inventory (STAI), and Leiden Index (LEIDS-R) questionnaires
- Body composition was analyzed via the DEXA at the baseline visit, the visit at the end of supplementation, and at the visit after the washout.
- Study participants' venous blood was collected at baseline visit and each of the additional four visits and analyzed for serum cortisol, serotonin and dopamine levels.

**Figure 1. Study Design Overview**




## Results

**Figure 2. Leiden Index Total Score**

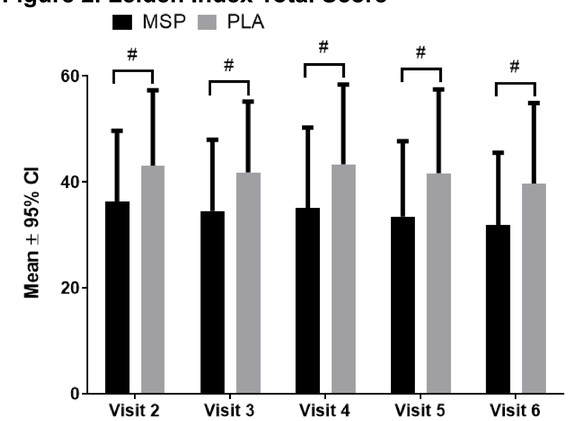



**Figure 3. Trait Anxiety Score**

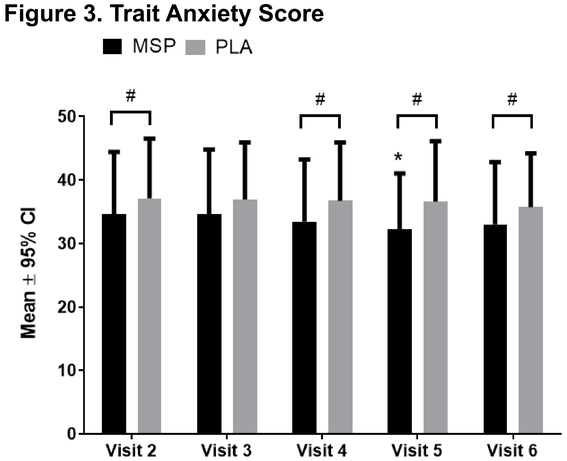



**Figure 4. State Anxiety Score**

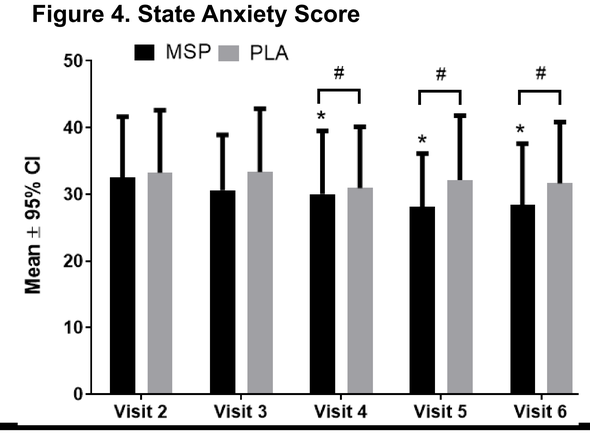
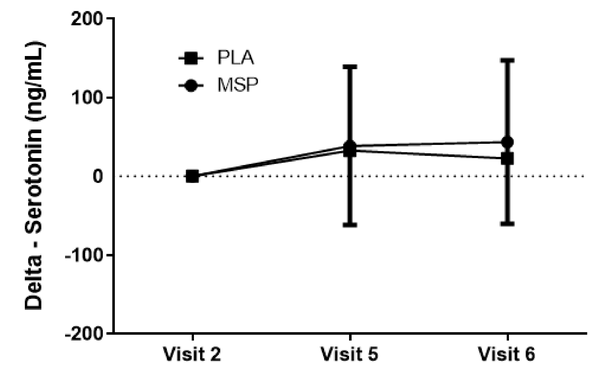



**Table 1. Serotonin Concentrations**

| | MSP mean (±SD) | PLA Mean (±SD) | *p* | |
|---|---|---|---|---|
| Visit 2 | 73 ± 49 | 99 ± 49 | Group | 0.38 |
| Visit 5 | 111 ± 114 * | 131 ± 132 | Time | 0.03 |
| Visit 6 | 116 ± 117 * | 122 ± 129 | G x T | 0.77 |

G x T = Group x Time interaction; Data is presented as mean ± SD

**Figure 5: Serotonin concentration changes from baseline**




## Summary

- MSP supplementation resulted in improvements in the Beck Depression, State and Trait Anxiety, and Leiden Index compared to baseline.
- While neither dopamine or cortisol changed following MSP supplementation, serotonin increased after 6 weeks and the 3-week washout.

### Competing Interests

RJ is a scientific advisor to Ashland, thus only assisted with study design and was not involved in collecting or analyzing data. MP and AA are employed by Probiotical Research srl. MP and AA assisted with study design and data interpretation but had not involvement in collecting or analyzing data.

### Acknowledgements

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