International Journal of Health, Nutrition and Exercise Science
https://www.gaics.org/Journal/JNES

Daily Ingestion of a Multi-Stain Probiotic Improves Symptoms of Depression, Anxiety, and Stress in College-Aged Individuals

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Abstract

Background: Probiotics may be an efficacious treatment option to manage symptoms of mood disorders, which is the leading cause of disability worldwide. Probiotics may strengthen the intestinal barrier and improve the bidirectional communication between the gut and brain.

Objective: The purpose of this study was to assess the effects of a multi-strain probiotic supplement on symptoms of depression, anxiety, and stress in healthy, college-aged individuals who were not clinically diagnosed with a mood disorder.

Methods: Participants consumed daily either a probiotic (PRO) (n=28) or placebo (PLA) (n=21) supplement for six weeks. Symptoms of depression, anxiety, and stress were measured at baseline, and after 21 and 42 days of supplement ingestion. Total mood and symptoms of depression, anxiety, and stress were compared between probiotic and placebo supplement groups by independent t-tests. To detect within-groups difference over time, 2x3 repeated measures ANOVAs were performed to assess the effects of supplements on total mood and symptoms of depression, anxiety, and stress.

Results: Six weeks of ingestion of a PRO or PLA supplement significantly ($p<0.05$) improved symptoms of depression (PRO post=6.65 ± 1.39 score, PLA post=5.6 ± 5.2 score) anxiety (PRO post=7.70 ± 1.4 score, PLA post=5.53 ± 1.09 score) and stress (PRO post=11.30 ± 1.53 score, PLA post=11.11 ± 1.08 score). A repeated measures ANOVA did not show a significant ($p>0.05$) main effect for supplement.

Conclusion: Daily ingestion of a PRO or PLA supplement for six weeks improved symptoms of mood disorders in college-aged individuals.

Keywords: probiotic, mental health, depression, mood disorders, gut-brain axis

1. BACKGROUND

Mood disorders are the leading cause of disability worldwide[1]. A vulnerable population that is at an increased risk for such disorders are college students. During this time, many different transitions are occurring such as adolescents are growing into young adults, the pace of life is different, and the geographical distance between family and friends has changed[2]. These experiences, among others, place college students at a moderate to high risk for developing symptoms of depression, anxiety, and stress. According to Fernandes and colleagues, the prevalence rate of depression in college-aged students is 30.6% and for anxiety it is between 63%-92%[2]. Another study by Ramon-Arbues and colleagues[1] found that it is estimated that between 12-50% of college students have at least one diagnostic criterion for one or more mental disorders. Depression, anxiety, and stress are critical components of mental health, and if these symptoms are left untreated then negative health effects can occur. For instance, college-aged students with symptoms of depression are at risk for having lower academic performance and physical activity levels and are at an increased risk for dropping out of college[3-5]. Furthermore, depression is linked to dementia and depression in early adulthood may lead to faster cognitive decline in old age[1,6].

Unfortunately, college students rarely seek treatment for mood disorders and related conditions[1]. The stigma associated with mood disorders commonly prevent individuals from seeking treatment[7]. Of those who do seek treatment, 60% of individuals discontinue their medication within 3 months of starting it due to unpleasant side effects such as nausea, vomiting, headache, agitation, and weight gain[7]. Thus, it is important to explore other methods that can be prescribed to help individuals achieve symptom relief. Probiotic supplementation is an emerging approach for improving mental health and mood disorders. Probiotics are live bacteria that colonize the gastrointestinal (GI) tract and exert health benefits to the host[8]. The relationship between the intestinal bacteria, known as the microbiota, and mental health is a growing area of interest. The influence of the intestinal microbiome goes beyond that of the gastrointestinal tract, as the intestinal microbiota has been shown to influence the central nervous system through the bidirectional link between the brain and the gut microbiome, termed the gut-brain axis[9,10]. Dysregulation of the intestinal barrier causes proinflammatory molecules, termed cytokines, to be released from enterocytes. Once proinflammatory cytokines are released, they can cross over the blood-brain barrier and interfere with the production of neurotransmitters and neurotransmitter precursors, as well as impair the hypothalamic-pituitary-adrenal (HPA) axis[11,12]. Previous research studies have found that probiotic administration in individuals who have been clinically diagnosed with mental health illnesses can help alleviate and treat symptoms[13-15]. However, little is known about the effects of probiotic supplementation on individuals who suffer from symptoms of mood disorders, but do not qualify for a clinical mental health diagnosis. One study by Steenbergen and colleagues[16] found that a multi-strain probiotic intervention had positive effects on symptoms of depression in healthy individuals who were not diagnosed with a depressive disorder. Additional research is needed to assess the effects of probiotic

supplementation on mood disorders in healthy individuals, without a clinical diagnosis.

The purpose of this study was to assess the effects of a multi-strain probiotic supplement on symptoms of depression, anxiety, and stress in healthy, college-aged individuals who were not clinically diagnosed with a mood disorder. The findings of this study could potentially add additional information for treatment options for college students experiencing symptoms of depression, anxiety, or stress.

2. METHODS

1.1 Participants

Forty-nine college-aged individuals, between the ages of 18-24 years-old, consuming a standard American diet, defined as total daily calories ingested be composed of 45-65% of total calories in carbohydrates, 20-35% of total calories in fats and 10-35% of total calories in protein (US department of Agriculture, 2014), were recruited to participate in this study. Power analysis (G*power 3.1.9.7) using moderate effect size and power set to 0.80 resulted in a suggested sample size of 30[16,17]. Inclusion criteria included individuals who were not clinically diagnosed with mood disturbance disorders, such as depression, anxiety, and stress. Exclusion criteria included individuals diagnosed with serious psychological disorders that impair normal functioning (bipolar, personality disorder, psychotic disorder or otherwise experiencing psychosis), have physical illness or an illness that compromised the immune system (HIV/AIDS, cancer, Crohn's disease, ulcerative colitis, lactose-intolerance, or gluten intolerance), currently taking probiotic supplements or antibiotics within two weeks of starting the study, on any special diet, such as gluten, ketogenic, vegetarian, paleo, and who were, or within the past 6 months, receiving psychological or pharmacological treatment for mental health issues (including antidepressants and antianxiety medications). All participants were fully informed of any associated risks and discomforts prior to giving their written informed consent to participate. The experimental protocol was approved by the Grove City College Institutional Review Board prior to implementation.

1.2 Experimental Design

This was a six-week long, double-blind, randomized, placebo-controlled, design in which each participant visited the exercise science laboratory four times including a familiarization, pretest, mid-session, and posttest. Participants were randomly assigned, by an online number generator (www.randomizer.org), into either a probiotic (PRO) or placebo (PLA) supplement group and reported to the laboratory every three weeks for data collection. The study design is depicted in Figure 1.

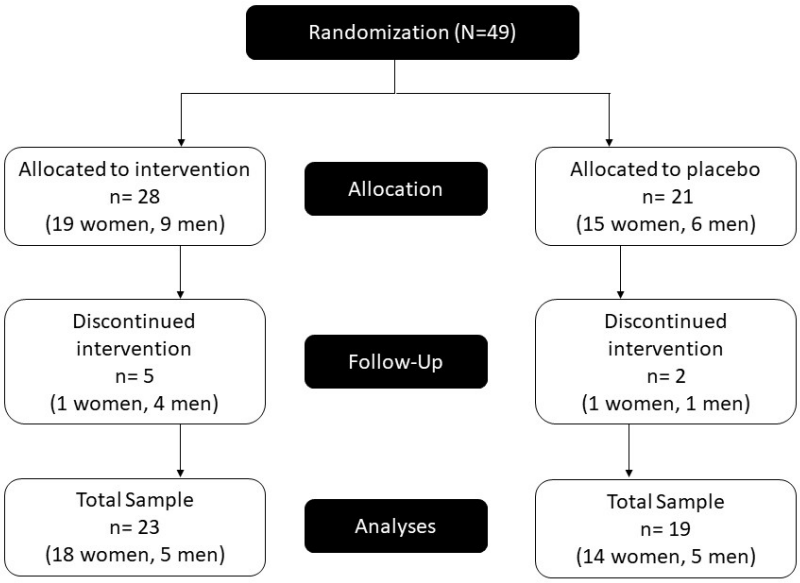

Figure 1. *CONSORT flow diagram of the study design*

Familiarization session

The study began with a familiarization session, which served as an opportunity to record participant's anthropometric measurements and to familiarize the participants to the depression, anxiety, stress score questionnaire (DASS) and testing procedures. Upon arriving to the exercise science laboratory participants' height, weight, and body mass index were recorded. Height was measured on a physician's scale and weight and body mass index were measured on a bioelectrical impedance analyzer (BIA). Researchers explained the DASS questionnaire before participants completed the form to acclimate themselves with the questions. Finally, researchers acquainted participants with the application 'MyFitnessPal' and explained the three-day tracking log participants used to record dietary intake and physical activity.

DASS questionnaire

The DASS scale is a valid and reliable instrument used to assess for symptoms of depression, anxiety, and stress[18]. The DASS scale is a 42-item self-reported computerized instrument designed to measure the three related negative emotional states of depression, anxiety, and stress. Each of the three DASS scales contains 14 items, divided into subscales of 2-5 items with similar content. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal, which includes difficulty relaxing, nervous arousal, being easily upset/agitated, irritable/overreactive, and impatient. Participants were asked to use 4-point severity/frequency (0= did not apply; and 3= applied very much or most of the time) scales to rate the extent to which they have experienced each state over the past week. All the subscales of the DASS questionnaire can be summed

to provide a total DASS score which provides a value for an individual's combined symptoms of depression, anxiety, and stress. To remain in the study, participants' DASS scores were required to be between 0-20 for depression, 0-14 for anxiety, and 0-25 for stress. If at any point participants obtained a scored in the severe to extremely severe range for depression, anxiety, or stress then they were terminated from the study and referred to a psychologist. The results for the DASS questionnaire were calculated electronically. Participants completed the DASS questionnaire at pretest (day 1), three weeks after starting the supplement (day 21), and six weeks (day 42) after starting the supplement.

Multi-Strain PRO supplement

The Winclove Ecologic Barrier supplement has been shown to be effective in alleviating symptoms of depression[16,19]. For the present study, participants were instructed to take the supplement two times daily, once in the morning and evening, without food for the duration of the study. Any missed dosage(s) were not to be compensated for by taking twice the amount at the next designated time to take the supplement. The probiotic multispecies supplement (Ecologic Barrier; 2.5 x 109 CFU/g; daily dosage 2 g) contained nine, freeze-dried strains of bacteria: B. bifidum (W23), B. lactis (W51), B. lactis (W52), L. acidophilus (W22), L. brevis (W63), L. casei (W56), L. salivarius (W24), Lc. Lactis (W19), and Lc. Lactis (W58). The placebo supplement was a sugar, freeze-dried maize starch, maltodextrin dietary supplement that consisted of 200mg of sugar. Both supplements were in powder form and were mixed in a glass of room temperature water. The probiotic and placebo supplements were indistinguishable regarding color, taste, and smell. The probiotic formulation contains probiotic strains, maize starch, and maltodextrin; however, no prebiotics are included. The placebo formulation contains the same ingredients as the probiotic formulation, except for the probiotic strains (Winclove Probiotics, Amsterdam, the Netherlands).

MyFitnessPal and Three-day log

Participants were instructed not to change their dietary or exercise behaviors throughout the duration of the study. An important aspect of this study was to assess the effects of a probiotic supplement on mood disorders in the absence of any changes in dietary intake or physical activity. To ensure participant's dietary intake and physical activity behaviors remained consistent throughout the duration of the study, participants were instructed to record their macronutrient intake and exercise behaviors. Each week participants documented three days, 1 weekend and two weekdays of their macronutrient intake utilizing the application MyFitnessPal (MyFitnessPal Inc., 2005). MyFitnessPal has been shown to be a valid method for recording energy intake[20]. Additionally, participants were instructed to maintain a consistent training intensity and volume throughout the study. Participants were instructed to record their training habits (mode, intensity, and duration) every day for the duration of the study. Every three weeks participant's dietary intake and physical activity logs were analyzed for consistency. Participants were terminated from the study if any inconsistencies occurred in their dietary intake or physical activity

levels.

Pretest session

Between 48-72 hours after the familiarization session, participants reported to the exercise science laboratory for pretesting. Prior to the visit, participants were asked to fast eight hours and refrain from exercise and caffeine for 12 hours. After the participants completed the DASS questionnaire, they were given a three-week supply (42 sachets) of the probiotic or placebo supplement, according to the group that they were randomized too. Lastly, participants were given instructions for taking the supplement and recording their dietary intake and physical activity behaviors.

Midpoint session

Three weeks after the pretest session, participants reported back to the lab for data collection. Participants completed the DASS questionnaire, submitted their dietary intake and physical activity logs, and received their last three weeks supply of the supplement (42 sachets). Lastly, participant's compliance for ingesting the supplement was recorded by counting the number of sachets that was left from the previous three-week supply. To remain in the study, participants had to achieve at least a 70% compliance rate.

Post-test

Three weeks after the midpoint session (a total of six weeks of taking the supplement), participants reported back to the lab for data collection. Prior to the visit, participants were asked to fast eight hours and refrain from exercise and caffeine for 12 hours. Participants completed the DASS questionnaire, submitted their dietary intake and physical activity logs, and had their supplement compliance rate recorded. Lastly, participant's weight and body mass index were recorded.

Statistical Methods

Statistical Package for the Social Sciences (SPSS) version 26.0 was used to analyze the data. Significance was set at $p \leq 0.05$. Descriptive statistics were done on the participant's characteristics. Total mood and symptoms of depression, anxiety, and stress were compared between probiotic and placebo groups by independent t-tests to detect the between groups effects at baseline, three weeks after taking a supplement, and six weeks after taking a supplement. To detect within-groups difference over time, four, 2x3 (supplement x time point) repeated measures ANOVA, was performed to assess the effects of supplements on total mood and symptoms of depression, anxiety, and stress. To test the normality of the data, the sharpiro-wilkes test was performed. For the ANOVA tests, if a violation of sphericity was found using Mauchley's test of sphericity ($p<0.05$), the Greenhouse-Geisser corrected statistical values were reported. All assumptions were met unless otherwise noted. Bonferroni correction was used to measure the differences between time points over groups (post-hoc analysis).

3. RESULTS

Participants

Forty-nine participants (15 males and 34 females) were included in the study and completed at least the pretest study visit (n=28 probiotics, n= 21 placebo). There were no significant differences in participants demographic or anthropometric measurements between the probiotic and placebo supplement group. The participants' baseline values are shown in Table 1. Participants' compliance

rate for ingestion of the supplement throughout the entire duration of the study was 90.8% and 91% for the probiotic and placebo group, respectively. Participants physical activity and dietary behaviors remained similar throughout the study. An independent t-test did not show a significant difference between the probiotic and placebo supplement groups for macronutrient intake (p=0.325) or physical activity (p=0.368) behaviors. A 2x2 (group x time point) repeated measures ANOVA did not show a significant (p=0.973) difference in dietary intake between the placebo and probiotic supplement groups, as well as no significant difference (p=0.411) within groups during the duration of the study. Another 2x2 repeated measures ANOVA did not show a significant (p=0.249) difference in physical activity levels between supplement groups, as well as no significant difference (p=0.134) within groups during the duration of the study. Participants' scores from the DASS questionnaire are presented in Table 2

	Placebo (n=21)	**Probiotic (n=28)**	**p-value**
Age	20.4±.93	20.2±1.16	$p = 4.20$
Height (cm)	170.3±3.97	170.7±4.02	$p = 0.899$
Weight (kg)	92.5±56.5	85.3±44.8	$p = 0.617$
BMI (kg/m^2)	24.9±4.25	24.4±5.25	$p = 0.721$

Table 1. Participants' baseline anthropometric information from the sample (n=42)- Values are presented as mean ± standard deviation.

	Pretesting		**Mid-Session**		**Posttesing**	
	PLA	**PRO**	**PLA**	**PRO**	**PLA**	**PRO**
Total DASS score	42.3±16.1	42.1±15.2	25.9±15.0	31.7±20.7	22.5±11.3	25.7±18.3
Depression score	11.4±8.1	10.8±7.2	6.0±5.6	8.6±8.8	5.6±5.2	6.7±6.7
Anxiety score	12.2±5.6	12.6±6.1	8.3±6.4	8.5±6.3	5.5±4.8	7.7±6.8
Stress score	18.7±7.5	18.7±8.0	11.6±5.1	14.1±8.3	11.1±4.7	11.3±7.4

Table 2. Participants' scores from the DASS questionnaire (n=42). Values are presented mean ± standard deviation.

Total Score of DASS

Maulchy's test of sphericity ($p<0.001$) was violated, and the Greenhouse-Geisser was used to adjust for the lack of sphericity. A between groups repeated measures ANOVA showed no significant ($p=0.331$) supplement * time interaction. There was a significant ($p<0.001$) main effect for time. Bonferroni post-hoc test revealed individual's total DASS score who received the placebo supplement significantly improved from pretest (39.27 ± 4.30) to mid-session (22.53 ± 3.86) and pretest (39.27 ± 4.30) to posttest (21.13 ± 2.82). Interestingly, individual's total DASS score who received the probiotic supplement significantly improved from pretest (37.92 ± 4.38) to mid-session (23.42 ± 4.85), pretest (37.92 ± 4.38) to posttest (17.50 ± 4.38), and mid-session (23.42 ± 4.85) to posttest (17.50 ± 4.38). Thus, total DASS score decreased in participants as a whole from baseline to posttest, irrespective of supplement; however, individuals who received the multi-strain probiotic supplement had a decrease in DASS score from mid-session to posttest (Figure 2). There was no significant ($p >0.05$) main effect for supplement. Participants' symptoms for depression, anxiety, and stress improved by 20.4% and 18.1% from pretest to posttest in the probiotic and placebo supplement groups, respectively.

Independent t-tests did not show a significant difference ($p >0.05$) in individual's total DASS score between a probiotic and placebo supplement at baseline, three weeks after taking a supplement, and six weeks after taking a supplement.

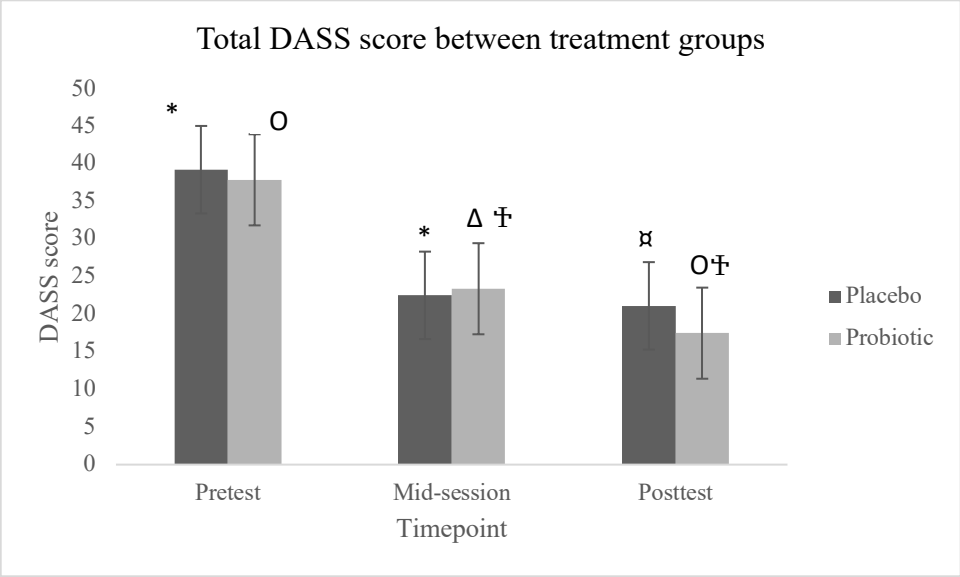

Figure 2. *Comparison of Total DASS score between placebo and probiotic supplement groups at pretest, mid-session, and posttest. Participants that ingested the probiotic supplement significantly improved their total DASS score from pretest to mid-session (p≤0.00; Δ), mid-session to posttest (p≤0.00; Ŧ), and pretest to posttest (p≤0.00; O). The placebo supplement significantly improved participants' total DASS score from pretest to mid-session (p≤.00; *) and pretest to posttest (p≤0.00; ¤).*

Symptoms of Depression

A between groups repeated measures ANOVA showed no significant (p=0.175) interaction between supplement * time. There was a significant (p<0.001) main effect for time. Individual's symptoms for depression who received the placebo supplement significantly improved from pretest (11.37 ± 1.85) to mid-session (6.0 ± 1.3) and pretest (11.37 ± 1.85) to posttest (5.6 ± 1.20). However, individual's symptoms for depression who received the probiotic supplement significantly improved from pretest (10.78 ± 1.50) to posttest (6.65 ± 1.39) (Figure 3). There was no significant (p>0.05) main effect for supplement. Participants' symptoms for depression improved by 4.1% and 5.7% from pretest to posttest in the probiotic and placebo supplement groups, respectively.

Independent t-tests did not show a significant difference (p>0.05) in individual's symptoms for depression between a probiotic and placebo supplement at baseline, three weeks after taking a supplement, and six weeks after taking a supplement.

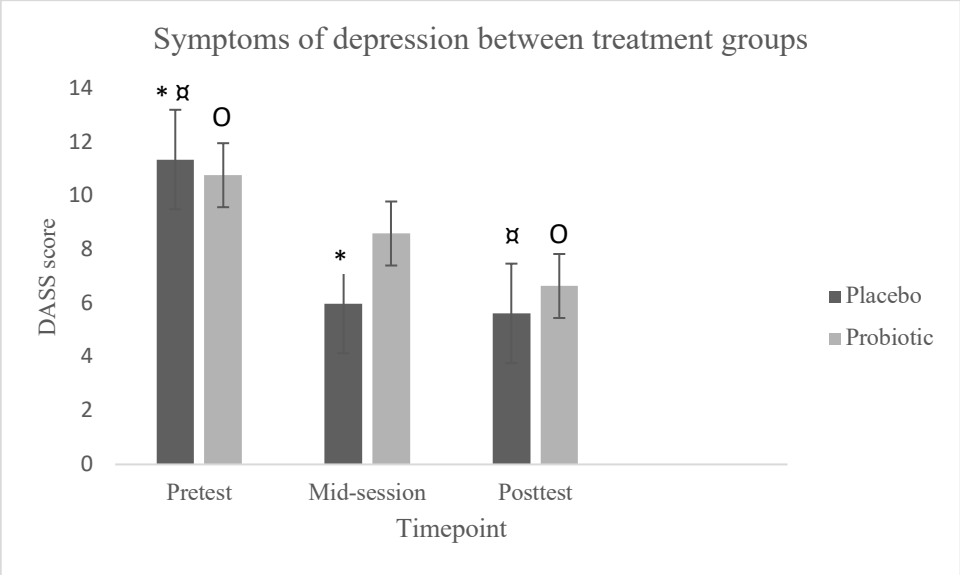

Figure 3. *Symptoms of depression between the probiotic and placebo supplement groups at pretest, mid-session, and posttest. Participants that ingested the probiotic supplement significantly improved their symptoms of depression from pretest to posttest (p=0.009; O). The placebo supplement significantly improved participants' symptoms of depression from pretest to mid-session (p≤0.00; *) and pretest to posttest (p=0.001; ¤).*

Symptoms of Anxiety

A between groups repeated measures ANOVA showed no significant (p=0.479) supplement * time interaction. There was a significant (p<0.001) main effect for time. Individual's symptoms for anxiety who received the placebo supplement significantly improved from pretest (12.16 ± 1.29) to mid-session (8.26 ± 1.47) and pretest (12.16 ± 1.29) to posttest (5.53 ± 1.09). Similarly, individual's symptoms for anxiety who received the pro-

biotic supplement significantly improved from pretest (12.57 ± 1.26) to mid-session (8.52 ± 1.31) and pretest (12.57 ± 1.26) to posttest (7.70 ± 1.4). Results shown in Figure 4. There was no significant (p>0.05) main effect for supplement. Participants' symptoms for anxiety improved by 4.9% and 6.6% from pretest to posttest in the probiotic and placebo supplement groups, respectively.

Independent t-tests did not show a significant difference (p>0.05) in individual's symptoms of anxiety between a probiotic and placebo supplement at baseline, three weeks after taking a supplement, and six weeks after taking a supplement.

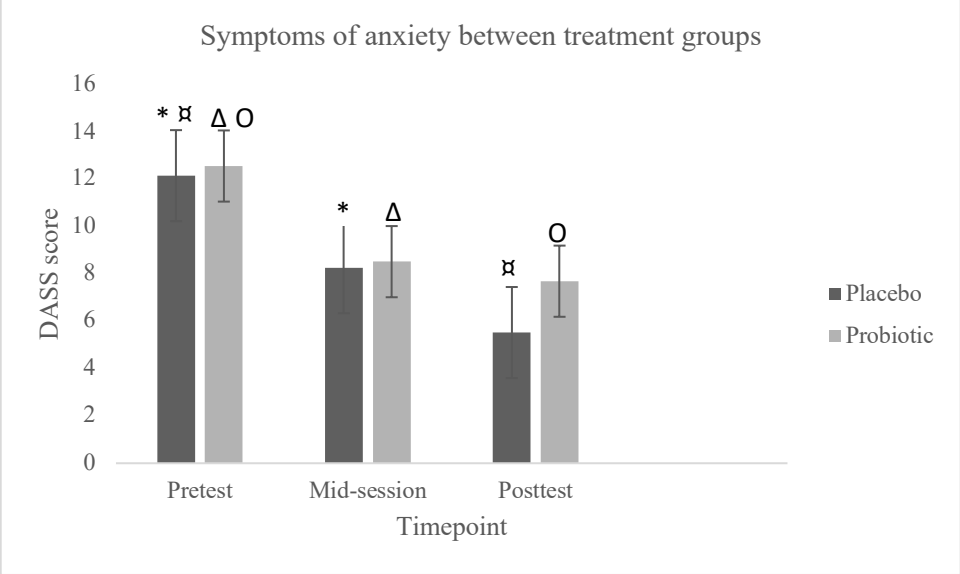

Figure 4. *Symptoms of anxiety between the probiotic and placebo supplement groups at pretest, mid-session, and posttest. Participants that ingested the probiotic supplement significantly improved their symptoms of anxiety from pretest to mid-session (p=.013; Δ) and pretest to posttest (p=.009; O). The placebo supplement significantly improved participants' symptoms of depression from pretest to mid-session (p≤.018; *) and pretest to posttest (p≤.000; ¤).*

Symptoms of Stress

A between groups repeated measures ANOVA showed no significant (p=0.393) supplement * time interaction. There was a significant (p<0.001) main effect for time. Individual's symptoms for stress who received the placebo supplement significantly improved from pretest (18.74 ± 1.72) to mid-session (11.63 ± 1.18) and pretest (18.74 ± 1.72) to posttest (11.11 ± 1.08). Interestingly, individual's symptoms for stress who received the probiotic supplement significantly improved from pretest (18.74 ± 1.66) to mid-session (14.13 ± 1.73), pretest (18.74 ± 1.66) to posttest (11.30 ± 1.53), and mid-session (14.13 ± 1.73) to posttest (11.30 ± 1.53) (Figure 5). There was no significant (p>0.05) main effect for supplement. Participants' symptoms for stress improved by 7.4% and 7.6% from pretest to posttest in the probiotic and placebo supplement groups, respectively.

Independent t-tests did not show a significant

difference (p>0.05) in individual's symptoms for stress between a probiotic and placebo supplement at baseline, three weeks after taking a supplement, and six weeks after taking a supplement. Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

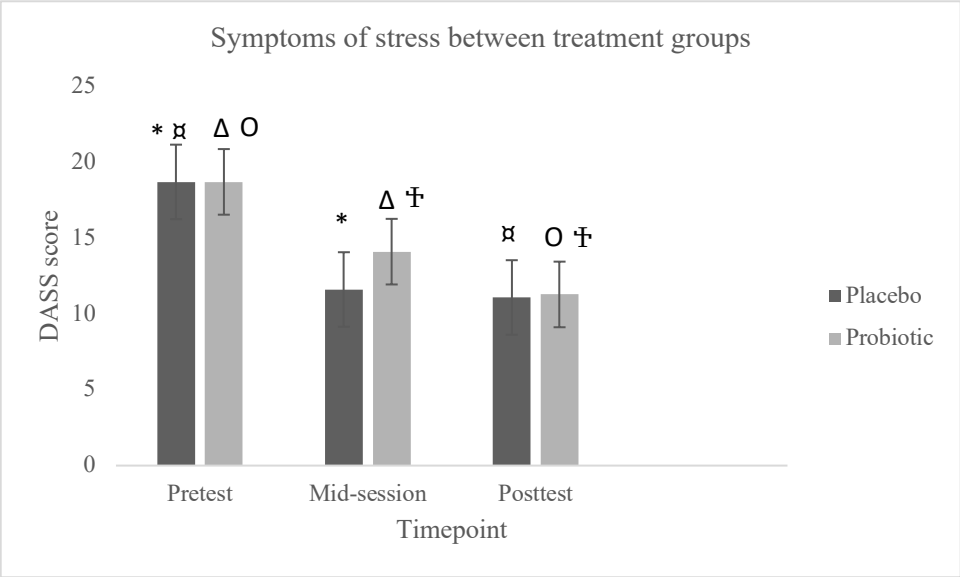

Figure 5. *Symptoms of stress between the probiotic and placebo supplement groups at pretest, mid-session, and posttest. Participants that ingested the probiotic supplement significantly improved their total DASS score from pretest to mid-session (p=.014; Δ), mid-session to posttest (p=.009; Ŧ), and pretest to posttest (p=.001; O). The placebo supplement significantly improved participants' total DASS score from pretest to mid-session (p≤.00; *) and pretest to posttest (p=.001; ¤).*

4. DISCUSSION

Probiotics have been found to strengthen the gastrointestinal barrier and in return improve the gut-brain axis. The present study found that both probiotic and placebo supplementation improved mental health after six weeks. Individuals tend to associate better health and well-being and an increased perceived quality of life with taking a probiotic supplement which may explain participants' decrease in symptoms of depression, anxiety, and stress in both the probiotic and placebo supplement groups after six weeks[21,22]. Thus, even though individuals in the placebo supplement group were not actually ingesting a probiotic supplement the possibility that they were may have led to thoughts of improved vigor and vitality resulting in better mental health scores[23]. Although no significant differences were found between the probiotic and placebo supplement groups at the end of six weeks, within-group differences for total DASS score and symptoms of stress were found to be continuous during the six-week

intervention in the probiotic group compared to the placebo supplement group. Participants that consumed the placebo supplement showed an acute, immediate improvement in total DASS score and symptoms of stress from pretest to three weeks but did not significantly improve from three weeks to six weeks post supplement ingestion. In contrast, participants that consumed the probiotic supplement showed acute, immediate improvement in total DASS score and symptoms of stress during the first three weeks, as well as between three and six weeks of supplement ingestion.

Depression

At the end of six weeks, participant's symptoms of depression were significantly lower after ingesting either a probiotic or placebo supplement. Similarly, a study done by Chahwan and colleagues[19] found that after eight weeks of consuming either a probiotic or placebo supplement depressive symptoms were reduced between pre-intervention and post-intervention assessment. Additionally, a study by Romjin and colleagues[24] did not find a significant difference in symptoms for depression after eight weeks of ingestion of either a probiotic or a placebo supplementation in healthy individuals. Improvement in symptoms of depression for the placebo supplement group may be explained by the placebo-effect, which has commonly been reported in participants undergoing treatment for depression[23]. The placebo-effect regulates the dopaminergic reward mechanism which is controlled by individuals' expectation of receiving treatment[22,23]. In the current study, participants' expectation of receiving the probiotic supplement may have led to the activation of the dopaminergic reward mechanism which then may have improved their symptoms of depression[23]. Due to the bi-directional relationship between the brain and intestinal microbiome, any improvement in the neurological or intestinal environment would result in positive feedback that would lead to further improvements in both areas. That is, improvement in the neurological environment may have positively affected the composition of the gut microbiome which then may have led to improvements in mental health[25-27]. However, participants' dopamine levels nor stool was sampled in the current study, and thus, these specific changes in the neurological and intestinal environments cannot be confirmed.

Nevertheless, Wallace and Milev[8] found a significant reduction in symptoms of depression between baseline, four weeks, and eight weeks in moderately clinically depressed individuals aged between 18-65 years of age. The difference in results may be explained by the current study enrolling healthy, college-aged individuals who were not clinically diagnosed with a mood disorder. Slykermann and colleagues[26] reported a significant improvement in postpartum depressive and anxiety symptoms in women after consuming a daily probiotic supplement for approximately one year. The difference in outcomes between this study and the current study may be explained by the probiotic strains and

length of supplement ingestion. Slykerman and colleagues[26] used Lactobacillus rhamnosis HN001, whereas the current study used a multi-strain probiotic supplement that did not include this strain. The Winclove multi-species probiotic formulation is selected to strengthen the intestinal barrier and reduce low grade inflammation[28]. A study by Akkasheh and colleagues[13] found that probiotic administration in patients with major depressive disorder had beneficial effects on symptoms of depression, measured by the Beck Depressive Inventory total score. The difference in results may be explained by participants in the current study not having a mood disorder clinical diagnosis. Still, a study by Steenbergen and colleagues[16] found that after four weeks of ingesting a multi-strain probiotic, depressive-like thoughts in participants, without mood disorders, when in a sad mood decreased when compared to individuals who consumed a placebo supplement. The difference in results may be explained by this study assessing symptoms of depression through the Leiden index of depression sensitivity scale (LEIDS) and Beck depression inventory II (BDI-II), whereas the current study assessed depression from a subscale of the DASS questionnaire. The LEIDS measures cognitive reactivity to sadness, which is an aspect of cognitive vulnerability to depression, and the BDI-II measures the severity of depression in individuals[29,30]. The DASS questionnaire is different from the LEIDS and BDI-II because it assesses fundamental symptoms of depression, anxiety, and stress as opposed to severity of a condition. Additionally, data collection in the Steenbergen and colleagues[16] study occurred when participants were in a sad mood, whereas in the current study participants' mood was not assessed during data collection. Therefore, it is possible that mood states between participants in the probiotic and placebo groups were not similar which could have made changes in symptoms between groups difficult to compare.

Anxiety

At the end of six weeks participant's symptoms of anxiety were significantly lower after ingesting either a probiotic or placebo supplement. Improvement in symptoms of anxiety in the placebo group may be related to the effect that placebos have on the activation of the dopamine-related reward mechanism[23]. Evidence suggests that dysregulation of neurotransmitters and their receptors can lead to mood disorders, termed 'reward deficiency syndrome'[23,31]. In fact, dopamine plays an important role in anxiety modulation[31]. Few research studies have assessed the effects of a probiotic supplementation on symptoms of anxiety, especially in humans. A study conducted by Tran et al.[11] found that four-weeks of a probiotic supplementation significantly reduced panic anxiety and negative affect in healthy college-aged in-dividuals when compared to a placebo supplement. In the current study anxiety was measured as a subcomponent of the DASS questionnaire whereas Tran and colleagues[11] measured anxiety in a variety of

questionnaires including Beck anxiety inventory, anxiety control questionnaire- revised, and Penn State worry questionnaire. A possible explanation for the benefits of a probiotic supplement in reducing anxiety is through enhancing gene expression of a neurotrophic factor that is part of the HPA axis[32].

Stress

At the end of six weeks participant's symptoms of stress were significantly lower after ingesting either a probiotic or placebo supplement. Improvement in symptoms of stress in the placebo group may be explained by the placebo analgesia which suggests that placebos can induce biochemical changes within the brain that result in the release of endogenous opioids and an increase in cerebral blood flow[23]. The results of this study differ from previous studies. A study by Salleh et al.[32] found that stress and anxiety in healthy, college-aged badminton players was significantly lower after daily consumption of a probiotic supplement for six weeks, but there was no significant difference in stress and anxiety levels in participants who consumed the placebo supplement. Likewise, a study by Papalini et al.[9] found that four weeks of a probiotic supplementation, when compared to a placebo supplement, significantly improved cognitive response to acute stressors in healthy female participants between 18-40 years of age. It is important to note that stress measured in the current study was strictly psychology whereas the stress measured in the study by Papalini and colleagues[9] was both physical and psychology stress which was measured directly in the laboratory during a stress induced test. Additionally, a study conducted by Callaghan et al.[33] found that a probiotic supplementation reversed generational early-life stressors in rats. Changes in stress and anxiety following probiotic supplementation might be explained by the bidirectional communication between the gut microbiota and the brain. The bidirectional interaction can be strengthened through a probiotic supplementation by having the intestinal microbiota interact with the central nervous system, primarily through the vagus nerve[32].

Limitations and Future Direction

The present study had limitations that must be considered. One limitation of the study was the duration of supplementation ingestion. A study by de Roos et al.[34] revealed that symptoms of migraines decreased in participants between 5-8 weeks after probiotic supplementation began, but no improvement in migraine symptoms was observed before that time frame. It may be that the length of supplementation duration for the current study was not long enough to assess maximum benefit of probiotic ingestion, which is supported by the results from the present study that highlight participants continued improvement in mental health symptoms between three and six weeks of probiotic supplement ingestion. Additionally, dietary and physical activity amongst participants was not controlled for. Participants were instructed to not change eating or physical activity behaviors, but there was a lot of

variability in these factors between participants. Dietary intake and exercise have been shown to influence the composition of the gut microbiota[25,35]. Furthermore, participants were not clinically diagnosed with any mood disorders, and were excluded from the study if their symptoms for depression, anxiety, and stress were scored greater than moderate on the DASS questionnaire. Thus, seeing improvements in individuals who are considered healthy and asymptomatic can be challenging. Research evaluating the effects of a multi-strain probiotic supplement on symptoms of anxiety in college-aged individuals is warranted. Additionally, the effects of exogenous ketones and probiotic supplementation on mood disorders has potential to alleviate symptoms through influencing the composition of the gut microbiome which then may beneficial influence the central nervous system[25].

5. CONCLUSION

Probiotic supplementation has been shown to confer cognitive health benefits. The aim of the present study was to assess the effects of a probiotic, and placebo, supplement on symptoms of depression, anxiety, and stress in healthy, college-aged individuals. The results from the present study support the notion that a probiotic supplement improves symptoms of depression, anxiety, and stress in healthy, asymptomatic college-aged individuals. Interestingly, symptoms of depression, anxiety, and stress also improved in individuals' consuming the placebo supplement. The placebo-effect has been commonly found in neurological conditions due to the expected benefits of seeking treatment, regardless of the treatment content[22,23]. The bidirectional communication between the gastrointestinal tract and the brain sugg-ests that the composition of the gut microbiome impacts cognitive activity, and the neurological environment impacts the composition of the gut microbiome[25-27]. Thus, as participants' mood improves, beneficial changes may occur in the intestinal microbiome, which can lead to further improvement in mental health. The findings from the present study support that the daily routine involved with preparation and consumption of a probiotic supplement positively impacts participants' mood and can improve mental health.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

Dana L. Ault- no conflict of interest
Gretchen E. Elsey- no conflict of interest
Kendall W. Brest- no conflict of interest
Paul D. Wilkinson- no conflict of interest
Joseph C. Meola- no conflict of interest
Macey A. Slack- no conflict of interest
Sarah J. Neu- no conflict of interest
Chelsea J. Morris- no conflict of interest
Philip J. Prins- no conflict of interest
Jeffery D. Buxton- no conflict of interest
Annemarieke van Opstal- provided/supplied the multi-strain probiotic and placebo supplement
Maria Stolaki- provided/supplied the multi-strain probiotic and placebo supplement
Hayden D. Gerhart- no conflict of interest

FUNDING/SUPPORT

The multi-strain probiotic and placebo supplements were provided by Winclove Probiotics which is a company located in Amsterdam, Netherlands. Subject compensation was provided by the Swezey grant awarded from Grove City College.

AUTHOR CONTRIBUTIONS

DA contributed to the concept, analysis, and interpretation, drafted the manuscript, and critically revised the manuscript. GE contributed to the concept, analysis, and interpretation. KB contributed to the concept, analysis, and interpretation. PW contributed to the concept, analysis, and interpretation. JM contributed to the concept, analysis, and interpretation. MS contributed to the analysis and interpretation. SN contributed to the analysis and interpretation. CM contributed to the analysis and interpretation. PP, JB, AO, MS, and HG critically revised the manuscript. All authors agree to be accountable for all aspects of the work ensuring integrity and accuracy.