

# Effects of a Novel Fortified Dairy Product on the Psychological Status and Sleep Quality of Patients with Polycystic Ovary Syndrome: A Double-Blind Randomized Controlled Trial

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**ABSTRACT:** Polycystic ovary syndrome (PCOS) is a neuroendocrine disorder that commonly causes anovulation and infertility worldwide. Aside from infertility, patients with PCOS suffer from sleep disturbances and mental health issues. Recent studies have shown that functional foods may have a beneficial impact on psychological disorders and sleep quality. Therefore, the present study aimed to investigate the effects of daily intake of a fortified yogurt on the psychological and sleep profiles of women with PCOS. In this 8-week randomized double-blind controlled trial, after a 2-week run-in period, participants in the intervention group (n=45) received yogurt fortified with 10<sup>6</sup> colony-forming units/g of probiotics (*Bifidobacterium animalis* Bb-12 and *Lactobacillus acidophilus* La-5) along with 50 IU of vitamin E and 1,000 IU of vitamin D, and those in the placebo group (n=45) received low-fat yogurt. The psychological status and sleep quality of patients were measured using the Depression, Anxiety, and Stress Scale-21 Items and Pittsburgh Sleep Quality Index before and after the study, respectively. The registration number of this study was IRCT20231210060323N1. The results showed that the intervention group exhibited a significant improvement in depression status compared with the placebo group ( $P=0.01$ ). However, no statistically beneficial impact was observed on sleep quality in patients with PCOS ( $P=0.44$ ). This trial indicated that consuming yogurt with probiotics, vitamin E, and vitamin D may alleviate depression in patients with PCOS and that nutritional interventions could be advantageous, particularly for individuals who prefer not to take antidepressant medications. However, further research is needed to confirm the results.

**Keywords:** depression, functional food, polycystic ovary syndrome, probiotics, vitamins

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a hormonal disorder that affects approximately 10% of women of reproductive age (Singh et al., 2023). According to the Rotterdam criteria, PCOS is characterized by irregular ovulation, elevated androgen levels, and multiple ovarian cysts. It is the primary cause of anovulatory infertility and may also lead to significant cardiovascular events and type 2 diabetes (Ndefo et al., 2013; Norman and Teede, 2018).

PCOS is a complex disorder affecting multiple systems. According to research, various mechanisms, including disruptions in endocrine function and metabolic pathways, contribute to an increased depression risk in women with PCOS. Furthermore, the clinical symptoms of hyperan-

drogenism and menstrual irregularities can exacerbate negative self-perceptions, lower self-esteem, and adverse affect mood. Additionally, many women with PCOS face infertility challenges, and societal and cultural pressures can further worsen depressive symptoms (Gnawali et al., 2021). Women with PCOS reportedly have poorer sleep quality, greater daytime sleepiness, and reduced sleep efficiency compared to healthy controls (Gnawali et al., 2021; Li et al., 2022a). As disrupted sleep patterns are related to metabolic issues [e.g., obesity and insulin resistance (IR)], poor sleep not only affects the quality of life but also has broader health implications (Mesarwi et al., 2013). Therefore, considerable attention should be paid to the role of mental health and sleep patterns in the pathophysiology of PCOS and its related comorbidities

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(Barber et al., 2019).

The primary therapeutic strategy for PCOS management is tailored to the patient's specific symptoms. However, the treatment approach is multifaceted. Although the underlying etiology of PCOS remains unclear, IR is widely considered as a major contributing factor (Rocha et al., 2019). To improve IR, some studies recommend weight reduction, whereas others suggest using insulin sensitizers and/or antiandrogen agents (Li et al., 2022b).

There is increasing interest in the potential health benefits of micronutrients, including vitamins D and E, in managing IR-related disorders (Garcia-Bailo et al., 2011; Tefagh et al., 2022; Younes, 2024). Another important nutritional factor in improving IR is the intake of certain probiotic strains, particularly *Bifidobacterium animalis* Bb-12 and *Lactobacillus acidophilus* La-5 (Salles et al., 2020). In addition to improving IR, some studies have indicated that vitamin D, vitamin E, or probiotic supplementation may have a beneficial impact on depressive disorders and help maintain the circadian rhythm in patients with PCOS (Alzoubi et al., 2012; Ostadmohammadi et al., 2019; Romano et al., 2020; Lee et al., 2022; Sivasankari and Usha, 2022; Jeon, 2024).

Given the high prevalence of PCOS and its notable complications, as well as the importance of nutritional therapies in its management, effective strategies are essential. In this study, a new dairy product fortified with probiotics, vitamin E, and vitamin D was developed considering their beneficial effects on the psychological status, sleep quality, and symptoms of and the limited evidence for multi-component dietary therapies in patients with PCOS.

To the best of our knowledge, no study has investigated the combined effects of vitamins D and E along with probiotics (*B. animalis* Bb-12 and *L. acidophilus* La-5) nor the impact of any fortified dairy product on patients with PCOS. Therefore, the present study aimed to assess the efficacy of yogurt enriched with probiotics, vitamin E, and vitamin D over an 8-week period on the mental health and sleep status of patients with PCOS.

## MATERIALS AND METHODS

### Study design and population

This parallel randomized double-blinded placebo-controlled clinical trial was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines at the infertility clinic of Hazrat Zeinab Hospital affiliated with Shiraz University of Medical Sciences, Shiraz, Iran. The clinical trial protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.SCHEANUT.REC.1402.104) and was also registered with the Iranian Registry of Clinical

Trials (IRCT20231210060323N1).

The participants in this study included patients with PCOS who were referred to the infertility clinic at Hazrat Zeinab Hospital. The inclusion criteria were as follows: women who were aged 18 to 45 years and diagnosed with PCOS in accordance with the Rotterdam criteria (Christ and Cedars, 2023). Patient eligibility was assessed by a gynecologist. The exclusion criteria were as follows: 1) noncompliance with the study protocol; 2) following a specific diet or physical activity program for 3 months before the start of the study or during the study period; 3) using any medications that could affect sleep quality or mental health status from 3 months before the start of the study or during the study period; 4) taking medications or nutritional supplements that could affect the lipid profile, glucose metabolism, body weight, blood pressure, and ovarian function for 3 months before the start of the study or during the study period; 5) pregnant or lactating women; 6) smokers and regular alcohol consumers; 7) allergies to dairy products or probiotics; 8) daily use of probiotic products; and 9) women suffering from medical conditions, including thyroid disorders, Cushing syndrome, congenital adrenal hyperplasia, diabetes mellitus, hypertension, or any other serious medical conditions. Participant screening was conducted from January to March 2024 to reduce the cutaneous synthesis of vitamin D. All participants were explained regarding the study protocol, and they read and signed an informed consent prior to enrolling in the current clinical trial.

### Sample size

The G\*Power software (version 3.1.9.4) was used to determine the sample size based on the observed decrease in the Homeostatic Model Assessment for Insulin Resistance level from a previous study (Shoei et al., 2015). To compute the sample size, we considered an effect size ( $d$ ) of 0.66, a significance level ( $\alpha$ ) of 0.05, a statistical power ( $1-\beta$  error probability) of 0.80, and an equal ratio of participants in the two groups ( $N_2/N_1=1$ ). The resulting sample size was calculated to be 38 participants per group. Finally, 45 subjects were included in each study arm (90 in total) to account for a potential dropout rate of 20%.

### Randomization and blinding

The randomization scheme was provided by an independent statistician who was not involved in data collection. Randomization was generated by applying balanced blocked randomization with a fixed block size of 4 and an allocation ratio of 1:1. The allocation was performed before the start of the study by assigning treatment orders to the participants. The code assignments remained undisclosed until the statistical analyses were

performed. All participants and researchers involved (including care providers, technicians, and dietitians) were blinded to the group allocations.

### Interventions

Two types of yogurt [low-fat yogurt (LY) and fortified yogurt (FY)] were produced and packaged identically by Zarin Ghazal Dairy Industries Co. (DAITY), Shiraz, Iran. Moreover, the color, appearance, smell, and taste of the yogurt were identical. The products were labeled A and B to blind the participants and researchers regarding the interventions and group allocation.

Both groups completed a 2-week run-in period to select subjects for randomization who have a positive clinical response to the trial and prevent confounding effects. The participants were informed to maintain their regular diets and physical activities throughout this period. Afterward, the participants were randomly assigned to receive either fortified or LY for daily consumption over 8 weeks. The participants were randomly allocated into the following groups in an equal 1:1 ratio: (1) intervention group ( $n=45$ ), which consumed 120 g of FY [ $\geq 10^6$  colony-forming units (CFU)/g Bb-12 and La-5, 50 IU of vitamin E, and 1,000 IU of vitamin D] daily for 8 weeks, and (2) the placebo group ( $n=45$ ), which consumed 120 g of low-fat conventional yogurt daily for 8 weeks. The participants were advised to incorporate yogurt into their daily meals, either with lunch or dinner, for the study duration. Moreover, they were instructed to store the products in a refrigerator, maintaining a temperature under 4°C.

This dosage was selected because it effectively increased circulating serum levels of 25-hydroxyvitamin D (25(OH)D) and  $\alpha$ -tocopherol over 8 weeks (Nikooyeh et al., 2011; Hager et al., 2019) and we sought a quantity partly close to the recommended daily amount rather than a high dosage that is routinely prescribed for women with PCOS (Fatemi et al., 2017; Dastorani et al., 2018). Furthermore, yogurt was chosen for fortification because of its favorable acceptance, cost-effectiveness, and appeal (especially among adults) (Bayarri et al., 2010). Additionally, yogurt provides high-quality protein and contains several nutrients that are essential to the participants (El-Abbadi et al., 2014).

LY included the starter cultures of *L. bulgaricus* and *Streptococcus thermophilus*, whereas FY contained the same starter cultures enriched with at least  $10^6$  CFU/g of *B. animalis* Bb-12 and *L. acidophilus* La-5 (Chr. Hansen). During the final stages of yogurt production, once a pH of 4.7 was reached, a powder of vitamin D3 (product number: 5012015; DSM Nutritional Products Ltd.) and vitamin E in the form of  $\alpha$ -tocopherol acetate (product number: 5012740; DSM Nutritional Products Ltd.) was incorporated and distributed throughout the product.

Moreover, the concentration and stability of vitamins E and D in the yogurt were evaluated by high-performance liquid chromatography on the 1st, 3rd, and 7th days of storage. The final quantity of vitamins added to the FY was based on the vitamin loss over the 1-week storage period to maintain the intended amount (50 IU of vitamin E and 1,000 IU of vitamin D per 120 g serving). Furthermore, the results of microbiological analysis showed that the average viable total count of probiotics remained above the recommended minimal limit of  $10^6$  CFU/g for 2 weeks. The participants were advised to maintain their usual physical activities, dietary habits, and lifestyle routines throughout the study.

### Compliance assessment

To check the compliance of participants, the participants completed a self-report intake dairy checklist weekly. Consuming at least 80% of the product was considered acceptable. Any side effects during the study were discussed in our weekly meetings. If any participant experienced significant adverse effects, they were removed from the study. The dairy checklist, which featured unfilled boxes designated for each week, allowed participants to mark their yogurt consumption after each meal. Moreover, yogurt consumption was encouraged by providing daily reminders via short messages, and participants returned empty yogurt packets during their weekly visits for adherence assessment.

### Assessment of participants' characteristics

Demographic evaluations were performed at the start of the study by using a questionnaire containing details regarding age, education, marital status, occupation, health conditions, family history of PCOS, smoking habits, and daily sun exposure.

The height of participants was determined using a stadiometer (Seca<sup>®</sup>, Germany) to the nearest 0.1 cm. The participants stood without shoes, scarves, or hats, ensuring that their shoulders, buttocks, and heels were against the wall, and their head was positioned according to the Frankfurt plane. The body mass index and weight of participants were evaluated using a bioelectrical impedance analysis device (Tanita<sup>®</sup> BC-418, Japan). The participants were measured with minimal clothing, excluding shoes and socks. The waist circumference of participants was recorded with precision (0.1 cm) using an inelastic tape measure. The measurement was taken at the narrowest part between the last rib and the iliac crest, with the participants standing and the tape held parallel to the floor. The hip circumference of participants was measured by determining the maximal protrusion of the buttocks while they stood erect with the feet close together.

Before blood pressure measurements, the participants

were instructed to sit and rest for 5 min. Then, an expert used a sphygmomanometer (Riester Precisa-N) to measure the blood pressure twice from the participant's right arm, and the mean of these measurements was recorded. The participant's right arm was positioned horizontally, stretched out, and aligned with the heart.

An expert dietitian collected information on the dietary intake of participants at the beginning and end of the intervention using a 3-day food recall, which covered two regular days and a weekend. To calculate the energy and nutrient content, the food records were converted to grams using typical household scales for Iranian food. Subsequently, the data were analyzed using Nutritionist IV version 4.1 software (First Databank Inc.), which had been customized for Iranian food.

The physical activity levels of participants before and after the study were evaluated using the seven-question International Physical Activity Questionnaire-Short Form (Lee et al., 2011). The questions cover the intensity of physical activity (light, moderate, and vigorous) and its duration in minutes during a week. To quantify the physical activity, the intensity (MET) was multiplied by the period (min) of activity, resulting in MET minutes per week.

After an overnight fast for 8 to 10 h, 5 mL of venous blood sample was collected from each participant. The serum was obtained by centrifuging the samples at 3,000 g for 5 min. Thereafter, it was stored at  $-75^{\circ}\text{C}$ . The total serum 25(OH)D concentration was determined via enzyme-linked immunosorbent assay (ELISA) using LDS Ltd. kits. Furthermore, the  $\alpha$ -tocopherol concentrations in plasma samples were measured using the Alpha-Tocopherol ELISA Kit (US Biological, 028903).

The following key components related to participants' sleep were evaluated using the validated Pittsburgh Sleep Quality Index (PSQI): sleep latency (the time it takes to fall asleep), sleep quality, sleep efficiency, sleep duration, use of sleep medications, daytime dysfunction, and sleep disturbances. The total PSQI scores ranged from 0 to 21, with a score of 6 or higher indicating the presence of sleep quality disorders (Farrahi Moghaddam et al., 2012).

In this study, the psychological status of participants was evaluated using the validated Depression, Anxiety, and Stress Scale-21 Items (DASS-21) questionnaire (Kakemam et al., 2022). DASS-21 covers depression, anxiety, and psychological distress, and each item comprises seven domains. According to the total score, the participants were further divided into five subclasses for depressive symptoms: very severe ( $>27$  points), severe (21–27 points), moderate (14–20 points), mild (10–13 points), and normal (0–9 points). Similarly, the anxiety scores were grouped into five categories: very severe ( $>20$  points), severe (15–19 points), moderate (10–14 points), mild (8–9 points), and normal (0–7 points). In

addition, the stress scores were categorized as follows: very severe ( $>33$  points), severe (26–33 points), moderate (19–25 points), mild (15–18 points), and normal (0–14 points). Depressive symptoms, anxiety, and stress were defined as having scores of 10, 8, and 15 or higher, respectively.

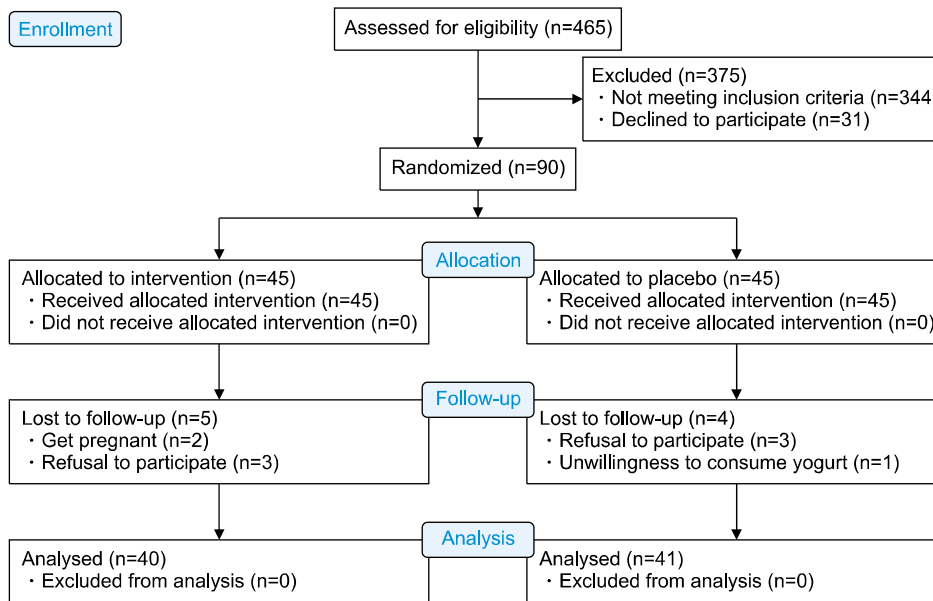
### Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 21 (IBM Corp.), and statistical significance was considered at  $P < 0.05$ . The Kolmogorov-Smirnov test was used to examine the normal distribution of data. Based on normality, continuous and categorical variables are shown as the mean (standard deviation) or median (interquartile range), respectively. To compare the baseline characteristics between the intervention and placebo groups, we used either an independent *t*-test for continuous variables or its non-parametric counterpart (Mann-Whitney U test) along with the chi-square test for categorical variables. For post-intervention characteristics, within-group differences were analyzed by using the paired *t*-test for normally distributed data or the Wilcoxon rank-sum test for non-normally distributed data. Between-group differences were examined using the independent *t*-test for normally distributed data or the Mann-Whitney U test for skewed data.

## RESULTS

Out of the 465 patients who were evaluated for eligibility, 90 patients with PCOS qualified to participate in the study. All 90 participants underwent a 2-week run-in period. During the study, nine participants were removed because of refusal to continue, pregnancy, or unwillingness to consume dairy products. Ultimately, 81 participants completed the trial after the 8-week intervention (Fig. 1). No adverse effects associated with the use of the product were observed among the participants. The composition of both yogurts is shown in Table 1.

No significant differences were observed between the study groups with regard to demographic information, daily sun exposure, anthropometric measurements, blood pressure, physical activity levels, baseline serum levels of vitamins D and E, mental health, or sleep quality scores ( $P > 0.05$ , Table 2). In addition, changes in dietary intake did not significantly differ between the groups during the study ( $P > 0.05$ , Table 3). Moreover, the FY significantly increased serum 25(OH)D<sub>3</sub> levels in the intervention group compared with the placebo group ( $P = 0.003$ ). Similarly, the increase in serum  $\alpha$ -tocopherol levels in the intervention group was sufficient to show a



**Fig. 1.** Flowchart of the study design and participants (CONSORT flow diagram).

**Table 1.** Nutritional composition of fortified yogurt and low-fat yogurt per serving (120 g)

Component	Fortified yogurt	Low-fat yogurt
Energy (kcal)	65	65
Carbohydrate (g)	8.00	8.60
Fat (g)	1.65	1.60
Protein (g)	5.60	5.40
Cholesterol (mg)	2.20	2.20
pH	4.30	4.30
Probiotics (Bb-12 and La-5) (CFU/g)	$\geq 10^6$	0
Vitamin D (IU)	1,000	0
Vitamin E (IU)	50	0

CFU, colony-forming units.

statistically significant difference between the two groups ( $P=0.02$ ).

The intervention (FY) resulted in a significant decrease in the depression ( $P=0.004$ ) and sleep quality scores ( $P=0.003$ ) within the intervention group. However, the difference between the two groups was statistically significant only for depression ( $P=0.01$ ), not for sleep quality ( $P=0.44$ ). Furthermore, the main results showed that within-group and between-group comparisons had no statistically significant differences with regard to stress and anxiety scores (Table 4).

## DISCUSSION

To the best of our knowledge, this study is the first to assess the potential benefits of a novel FY intervention on psychological and sleep profiles among patients with PCOS. The results showed that the daily consumption of yogurt fortified with probiotics (*B. animalis* Bb-12 and *L.*

*acidophilus* La-5), vitamin D, and vitamin E over 8 weeks significantly improved the depression status among women with PCOS. Although the decrease in the sleep quality profile was not statistically significant between the two groups, the approximately 2-unit improvement in the sleep score within the intervention group might be clinically significant. Furthermore, the results indicated no significant difference in stress or anxiety between the intervention and placebo groups over the study duration.

Depression is the leading cause of disability worldwide and is three to eight times more common in patients with PCOS than healthy controls (Cooney et al., 2017). Although the exact mechanisms underlying the high prevalence of depression among patients with PCOS remain unknown, some potential contributing factors have been suggested, including IR [the extensive overproduction of cortisol induced by obesity dysregulates the hypothalamic-pituitary-adrenal axis and decreases the levels of serotonin (an antidepressant neurotransmitter) in the central nervous system], hyperandrogenism (which downregulates brain monoamines and increases hirsutism, acne, and alopecia, altering self-image), inflammation (as an inflammatory condition, PCOS leads to increased cytokines, which decrease the number of serotonin receptors in the brain), and infertility (which can impose high social, family, and economic pressures and is related to immunological imbalance, leading to the dysregulation of cytokines, neuropeptides, and hormones) (Xing et al., 2024).

The findings of the present study revealed for the first time that the combined effect of probiotics, vitamin D, and vitamin E in a low and safe dosage could improve the depression status among women with PCOS. Considering the high prevalence of vitamin D deficiency (67%–85%) among patients with PCOS (Thomson et al., 2012),

**Table 2.** Baseline characteristics and measured parameters of the study participants

Variable	Placebo group (n=45)	Intervention group (n=45)	P-value
Age (years)	33.42 (5.52)	33.46 (5.49)	0.97
Marital status, n (%)			0.99*
Single	2 (4.4)	2 (4.4)	
Married	43 (95.6)	43 (95.6)	
Education status, n (%)			0.78*
Illiterate & elementary	8 (17.8)	9 (20.0)	
Diploma & upper diploma	37 (82.2)	36 (80.0)	
Job-status, n (%)			0.62*
Employed	10 (22.2)	12 (26.7)	
Unemployed	35 (77.8)	33 (73.3)	
Family history of PCOS, n (%)			0.53*
Yes	5 (11.1)	7 (15.6)	
No	40 (88.9)	38 (84.4)	
Sun exposure (min/d)	26 (16)	24 (14)	0.42
Weight (kg)	74.46 (13.08)	74.27 (12.54)	0.94
Body mass index (kg/m <sup>2</sup> )	28.66 (4.83)	28.63 (4.77)	0.97
Waist circumference (cm)	82.38 (12.53)	85.91 (14.37)	0.21
Hip circumference (cm)	92.06 (13.57)	92.46 (13.79)	0.89
Waist/hip ratio	0.91 (0.02)	0.92 (0.05)	0.11
Systolic blood pressure (mmHg)	118.02 (14.63)	120.24 (12.12)	0.43
Diastolic blood pressure (mmHg)	76.20 (12.95)	80.04 (11.54)	0.14
Serum 25(OH)D <sub>3</sub> (ng/mL)	30.54 (16.63)	27.50 (15.28)	0.39
Serum alpha-tocopherol (mg/L)	8.57 (2.25)	8.82 (2.38)	0.63
Physical activity (MET min/week)	555.28 (219.75)	557.15 (202.39)	0.96
Depression score	9.15 (5.16)	7.66 (4.52)	0.14
Anxiety score	8.33 (4.27)	7.31 (4.22)	0.25
Stress score	9.11 (3.94)	7.73 (4.85)	0.14
Sleep quality score	6.82 (3.97)	7.48 (5.25)	0.49

Values are expressed as the number (percentage of participants in each group) or mean (SD).

P-value from independent samples *t*-test (parametric variables with normal distribution); \*chi-square test (non-parametric variables).

PCOS, polycystic ovary syndrome.

the present study found that consuming FY with 1,000 IU of vitamin D and 50 IU of vitamin E for 8 weeks significantly increased serum 25(OH)D and  $\alpha$ -tocopherol levels in the intervention group. This increase in vitamin D and E levels, along with the beneficial interactions of vitamin D, vitamin E, and probiotics, might have contributed to the observed improvement in the depression status. One possible mechanism regarding vitamin D's positive effect on depressive disorders is the upregulation of tyrosine hydroxylase gene expression, which increases dopamine production in the brain. Moreover, vitamin D can enhance the bioavailability of various antidepressant neurotransmitters, including norepinephrine, serotonin, and dopamine.

Consistent with our results, Ostadmohammadi et al. (2019) reported that a 12-week co-supplementation of vitamin D and probiotics had positive effects on the mental health parameters of women with PCOS. Similarly, Moran et al. (2015) observed a reverse association between serum 25(OH)D<sub>3</sub> and depression in overweight women with and without PCOS. The effects of increased serum 25(OH)D<sub>3</sub> levels on ameliorating depressive symp-

toms have been attributed to multiple mechanisms, including the downregulation of inflammatory pathways, such as nuclear factor-kappa B, which has been associated with depression and psychological stress (Ju et al., 2013). With regard to serum  $\alpha$ -tocopherol levels, they have been postulated to balance the dysregulation between lipid metabolism and antioxidant defenses, which is crucial in the etiology of the disease and mental disorders (Lobato et al., 2010).

Inflammation and oxidative stress play an important role in mood disorders. Therefore, reducing inflammation through nutritional intervention may improve depressive disorders directly by suppressing cytokines or indirectly by improving IR, hyperandrogenism, and infertility, which are major contributing factors of depression in PCOS. Consequently, the results of the present study might be attributed to the synergistic anti-inflammatory effects of vitamin E, vitamin D, and probiotics (Jiang, 2014; Almeida Moreira Leal et al., 2020; Virk et al., 2024). Moreover, the effects of probiotics (*L. acidophilus* La-5 and *B. animalis* Bb-12) on the biosynthesis of microflora and the regulation of neurotransmitters, in-

**Table 3.** Comparison of changes in dietary intake and serum vitamin D and E levels during the study period

Component	Placebo group (n=41)			Intervention group (n=40)			P-value <sup>1)</sup>
	Before	After	Change	Before	After	Change	
Energy (kcal/d)	1,750.20 (437.02)	1,672.80 (417.02)	-77.40 (280.48)	1,672.80 (384.23)	1,601.41 (315.47)	-71.39 (306.56)	0.92
Carbohydrate (g/d)	211.37 (54.50)	211.56 (53.55)	0.19 (49.66)	210.72 (60.28)	198.89 (55.67)	-11.83 (45.19)	0.26
Protein (g/d)	74.12 (26.13)	67.11 (29.88)	-7.01 (28.85)	71.53 (18.90)	70.02 (16.99)	-1.51 (27.21)	0.38
Fat (g/d)	73.10 (26.93)	67.09 (25.58)	-6.01 (21.84)	65.18 (19.25)	62.59 (17.77)	-2.59 (20.65)	0.47
Fiber (g/d)	24.68 (12.25)	26.34 (13.11)	1.66 (12.91)	23.75 (10.88)	24.39 (16.41)	0.64 (15.07)	0.74
Cholesterol (mg/d)	169.05 (152.98)	231.60 (169.32)	62.55 (275.23)	184.42 (158.27)	229.76 (199.41)	45.34 (285.26)	0.78
SFA (g/d)	16.30 (7.86)	15.60 (6.98)	-0.70 (9.87)	14.31 (6.05)	14.27 (5.16)	-0.04 (8.73)	0.74
MUFA (g/d)	19.10 (10.39)	16.72 (8.87)	-2.38 (6.76)	16.95 (6.17)	14.86 (5.47)	-2.09 (6.14)	0.83
PUFA (g/d)	26.19 (10.99)	22.85 (8.99)	-3.34 (11.09)	23.08 (8.87)	21.58 (13.47)	-1.50 (12.94)	0.49
Magnesium (mg/d)	286.01 (99.66)	295.29 (96.57)	9.28 (118.45)	284.32 (80.40)	280.84 (111.17)	-3.48 (118.44)	0.62
Zinc (mg/d)	8.33 (3.39)	8.36 (3.44)	0.03 (4.86)	8.56 (3.30)	8.55 (6.47)	-0.01 (5.32)	0.96
Calcium (mg/d)	675.52 (434.80)	671.58 (357.14)	-3.94 (60.87)	697.65 (455.83)	694.15 (414.54)	-3.5 (68.34)	0.98
Vitamin B <sub>1</sub> (mg/d)	1.29 (0.53)	1.31 (0.66)	0.02 (0.61)	1.24 (0.50)	1.21 (0.89)	-0.03 (0.57)	0.66
Vitamin B <sub>3</sub> (mg/d)	17.80 (7.70)	17.45 (7.44)	-0.35 (8.44)	16.74 (7.67)	17.73 (11.64)	0.99 (10.49)	0.52
Vitamin B <sub>6</sub> (mg/d)	1.51 (0.76)	1.68 (0.66)	0.17 (1.22)	1.43 (0.65)	1.67 (1.41)	0.24 (1.14)	0.81
Vitamin B <sub>9</sub> (µg/d)	241.51 (123.91)	349.42 (228.87)	107.91 (440.51)	250.68 (116.11)	311.30 (301.47)	60.62 (444.03)	0.63
Vitamin B <sub>12</sub> (µg/d)	1.68 (1.04)	10.93 (11.87)	9.25 (44.80)	1.75 (1.08)	10.76 (11.47)	9.01 (44.27)	0.98
Vitamin D (µg/d)	8.70 (1.19)	8.74 (1.13)	0.04 (1.90)	0.86 (1.03)	1.26 (1.19)	0.35 (1.68)	0.44
Vitamin E (mg/d)	29.72 (13.49)	26.03 (11.24)	-3.69 (15.99)	26.11 (12.76)	25.83 (19.98)	-0.28 (18.14)	0.37
Serum 25(OH)D <sub>3</sub> (ng/mL)	30.54 (16.68)	29.50 (14.76)	-1.04 (7.78)	27.50 (15.28)	32.05 (13.72)	4.55 (8.34)	0.003*
Serum α-tocopherol (mg/L)	8.57 (2.25)	8.24 (1.58)	-0.33 (2.49)	8.82 (2.38)	9.60 (1.97)	0.78 (1.69)	0.02*

Values are presented as mean (SD).

<sup>1)</sup>Between-group comparison between the mean (SD) changes of the intervention and placebo groups (independent samples *t*-test).\**P*<0.05.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

**Table 4.** Effects of the intervention on mental health status and sleep quality

Variable	Placebo group (n=41)			Intervention group (n=40)			Intervention effect <i>P</i> -value <sup>2)</sup>
	Before	After	Change	Before	After	Change	
Depressive symptoms	9.32 (5.37)	9.37 (5.09)	0.05 (2.64)	7.29 (4.56)	5.73 (4.22)	-1.56 (3.26)	0.01*
Anxiety	8.52 (4.44)	7.86 (4.49)	-0.47 (2.36)	7.09 (4.31)	6.53 (5.17)	-0.78 (3.85)	0.66
Psychological distress	9.12 (4.02)	8.74 (4.24)	-0.37 (2.53)	7.90 (5.03)	6.54 (5.31)	-1.19 (4.11)	0.28
Sleep quality	6.90 (3.73)	5.85 (3.72)	-0.97 (5.52)	7.68 (5.27)	5.63 (5.42)	-1.85 (3.70)	0.44

Values are presented as mean (SD).

The reported findings were derived from participants who completed the study in the placebo (n=41) and intervention groups (n=40).

<sup>1)</sup> *P*-values denote the significance of within-group changes (paired samples *t*-test).

<sup>2)</sup> *P*-values denote the significance of between-group changes (independent samples *t*-test, the intervention group compared with the placebo group).

\**P*<0.05.

cluding serotonin and gamma-aminobutyric acid, are another potential mechanism that can affect mental health (Dziedzic et al., 2024).

On the other hand, this study showed no statistically meaningful improvement in the sleep quality of participants. Recent studies have shown that patients with PCOS are predisposed to sleep disorders, including difficulty sleeping, daytime sleepiness, restless sleep, or obstructive sleep apnea (Fernandez et al., 2018). These disorders are due to metabolic disturbances. Since the neuroendocrine system plays an important role in controlling the sleep-wake cycle by regulating melatonin and cortisol secretion, PCOS patients with alterations in their neurometabolic profile might suffer from arousal and sleep problems (Dziedzic et al., 2024). Contrary to our findings, previous studies have shown a beneficial effect of vitamin D, vitamin E, and probiotic supplementation on sleep quality (Abboud, 2022; Santi et al., 2023; Thongchumnum et al., 2023). These conflicting results might be related to differences in populations, applied questionnaires, or supplementation dosages.

The strengths of this study were as follows: 1) this study used a new functional food, 2) this study demonstrated for the first time the combined effects of vitamin D, vitamin E, and probiotics, 3) this study used FY as a safe adjunctive therapy (no adverse effects were observed among participants) to assess its impact on the mood and sleep quality of patients with PCOS, and 4) the adherence to the study protocol was high among participants in both groups.

The major constraint of this study was its relatively short timeframe. Furthermore, the responses to both questionnaires (PSQI and DASS-21) were dependent on participants' memory, which might have resulted in recall bias. In addition, the multiple ingredients added to the yogurt made it difficult to determine which nutritional ingredients had the most antidepressant effect in the FY. The study also did not measure alterations in the gut microbiota because of budget constraints. Therefore, considering these limitations, the results of this study should not be generalized to all patients with PCOS.

This clinical trial demonstrated that the combination of probiotics and vitamins E and D in a new FY effectively improved depression. The findings suggest that suitable dietary interventions could be considered as an effective adjunctive therapy for mitigating mood disorders among patients with PCOS, independent of antidepressant drugs. However, clinical trials with longer duration of intervention, diverse populations, and larger sample sizes are needed to determine the antidepressant effects of the FY among women with PCOS.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Concept and design: MA, NH, BNJ. Analysis and interpretation: MA, MHE. Data collection: MA, NH. Writing the article: MA. Critical revision of the article: NH. Final approval of the article: All authors. Statistical analysis: MA. Supervision: NH, BNJ, MF, AB. Obtained funding: NH. Overall responsibility: NH.

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