The effects of probiotics on mental health and hypothalamic–pituitary–adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers

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Objective: The aim of this study was to determine effects of probiotic yogurt and multispecies probiotic capsule supplementation on mental health and hypothalamic–pituitary–adrenal axis in petrochemical workers.

Methods: The present randomized double-blind, placebo-controlled trial was conducted on 70 petrochemical workers. Subjects were randomly divided into three groups to receive 100 g/day probiotic yogurt + one placebo capsule ($n = 25$) or one probiotic capsule daily + 100 g/day conventional yogurt ($n = 25$) or 100 g/day conventional yogurt + one placebo capsule ($n = 20$) for 6 weeks. Mental health parameters including general health questionnaire (GHQ) and depression anxiety and stress scale (DASS) scores were measured. Fasting blood samples were obtained at the beginning and 6 weeks after the intervention to quantify hypothalamic–pituitary–adrenal axis.

Results: After 6 weeks of intervention, a significant improvement of GHQ was observed in the probiotic yogurt (18.0 ± 1.5 vs. 13.5 ± 1.9, $P = 0.007$) and in the probiotic capsule group (16.9 ± 1.8 vs. 9.8 ± 1.9, $P = 0.001$), as well as a significant improvement in DASS scores in the probiotic yogurt (23.3 ± 3.7 vs. 13.0 ± 3.7, $P = 0.02$) and the probiotic capsule group (18.9 ± 3.2 vs. 9.4 ± 4.0, $P = 0.006$). However, there was no significant improvement in the conventional yogurt group ($P = 0.05$ for GHQ and $P = 0.08$ for DASS).

Discussion: The consumption of probiotic yogurt or a multispecies probiotic capsule had beneficial effects on mental health parameters in petrochemical workers.

Keywords: Probiotics, Mental health, Hypothalamic–pituitary–adrenal axis, Petrochemical workers

Introduction

Initial reports indicate a high prevalence of psychological problems such as anxiety and depression among petrochemical workers owing to several factors such as ergonomic and job stress.[1,2] The prevalence of job stress, which is a major risk factor for mental disorders, has reported 19.9% in petrochemical workers.[1] This situation affects between 54.2 and 58.8% of the Iranian petrochemical workers.[3] Previous studies have found that corticotropin-releasing hormone (CRH) in the hypothalamus, adrenocorticotropin hormone (ACTH), and other proopiomelanocortin (POMC)-derived peptides in the anterior pituitary gland, and adrenal corticosteroids in the adrenal gland, are secreted in response to acute stress and chemical exposures.[4,5] In addition, occupational exposure to chemical compounds stimulates the production of some inflammatory cytokines, including interferon gamma (IFN-gamma), tumor necrosis factor alpha (TNF-alpha), and interleukin-6 (IL-6).[6] IFN-gamma induces an enzyme of tryptophan catabolism and indoleamine 2,3-dioxygenase,

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which is responsible for conversion of tryptophan and other indole derivatives such as kynurenine.[7] Elevated kynurenine levels have been found to be involved in the pathogenesis of several brain diseases.[8] Furthermore, earlier studies have shown that occupational exposure to aromatic compounds would result in continuous production of reactive oxygen species and free radicals, which in turn causes oxidative stress, immune suppression, damage of DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, and halogenations in petrochemical workers.[9,10]

In total, various strategies, including having a proper diet pattern,[11] an antioxidant-rich diet such as vitamins,[12] and the administration of antioxidants[13] have been proposed for the management of mood disorders and job stress among workers. Evidences emerging from the intestinal microbiota of the central nervous system performance have shown that oral administration of probiotics may have beneficial effects on mood and psychological problems.[14] However, previous studies have evaluated such favorable effects in healthy subjects,[15] patients with chronic fatigue syndrome,[16] and in children with autism spectrum disorders.[17] In a study by Messaoudi *et al.*,[14] it was observed that depression and anxiety were improved after taking probiotics containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 among healthy individuals for 30 days. However, no beneficial effects of taking probiotic supplements containing *Lactobacillus rhamnosus* strain GG and bifidobacterium in people with schizophrenia after 14 weeks was observed.[18]

Probiotics may improve the mental health through increasing availability of tryptophan and increasing serotonin synthesis as well as reducing serotonin metabolism,[19,20] while decreasing the indoleamine 2,3-dioxygenase (IDO) enzyme activity.[21] Considering that there is evidence that probiotics have a positive impact on mental health and considering that to the best of our knowledge there is no study carried out regarding the beneficial effect of probiotics on the mood of people who have occupational exposure to aromatic compounds. The aim of this study is to determine the effect of probiotics on mental health and its effects on hypothalamic–pituitary–adrenal axis, including kynurenine, tryptophan, neuropeptide Y, cortisol, and ACTH pathway among petrochemical workers.

Methods

Participants

Seventy-five petrochemical workers in a randomized, double-blind, placebo-controlled trial from February 2014 to March 2014 were included in this study. This study was approved by the Institutional Review Board at Tehran University of Medical Sciences (TUMS). Informed written consent was obtained from all workers. This study was done according to the guidelines laid down in the Declaration of Helsinki. The trial was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: IRCT201406222394N11). Petrochemical workers aged 20–60 years old were included in this study. In this study, we excluded those who were using insulin or vitamin supplements, or had chronic kidney disease, lung, and chronic or acute inflammatory disease, hepatic, or thyroid diseases, severe intestinal disease, peptic ulcer, pregnant, allergies, or the use of antibiotics and nutritional supplements. Based on the suggested formula for parallel clinical trials, considering the type one error of 5% and the study power of 80%, we reached a sample size of 21 for each group. However, we recruited 25 workers in each group (total, 75 persons) to compensate for the probable loss to follow up.

Study design

To obtain detailed information about the dietary intakes of study participants, all workers entered into a 2-week run-in period during which all persons had to refrain from taking any other probiotic or synbiotic food. During the run-in period after stratification for BMI (<30 and $\geq 30\ kg/m^2$) and age (<40 and ≥ 40 years), participants were requested to record their dietary intakes for three non-consecutive days (two usual days and one weekend day). At the end of the run-in period, persons were randomly divided into three groups to receive 100 g/day probiotic yogurt + one placebo capsule ($n = 25$: male, $n = 12$; female, $n = 13$) or one probiotic capsule daily + 100 g/day conventional yogurt ($n = 25$: male, $n = 12$; female, $n = 13$) or 100 g/day conventional yogurt + one placebo capsule ($n = 20$: male, $n = 12$; female, $n = 8$) for 6 weeks. A trained nutritionist at the petrochemical technology company did the randomized allocation sequence, enrolled participants, and assigned participants to interventions. At the start of the study, individuals were requested not to change their routine physical activity or usual dietary intakes throughout the study and not to consume any probiotic or synbiotic products other than the one provided to them by the investigators. Probiotic and conventional yogurts or multispecies probiotic supplements were provided for participants every day. All participants provided three dietary records throughout the intervention and three physical activity records to make sure that they maintained their usual diet and physical activity during the study. Both dietary and physical activity records were taken at weeks 1, 3, and 5 of intervention. The dietary records were based on estimated values in

household measurements. To obtain nutrient intakes of participants based on these 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods. In this study, physical activity was described as metabolic equivalents (METs) in hours per day. To compute the METs for each subject, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.[22] On the other hand, we agree that walking or physical activity can have an effect on cortisol levels; therefore, we reminded the subjects not to have any change in their walking or physical activity, before the first blood test (beginning of the study) and at the end of the trial. It is worth mentioning that all subjects commute to petrochemical laboratory every morning by the company's 'bus service'; thus, the physical conditions before each blood test have been the same. In addition, all participants were requested to wake up in an early morning between 6.30 and 7 a.m. They were transferred to the laboratory before 8 a.m. to control for the cortisol awakening response.

Assessment of variables

Weight was measured at baseline study and 6 weeks after the intervention at petrochemical technology company, Tehran, Iran by a trained nutritionist. Body weight was measured in an overnight fasting status without shoes in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated using the height and weight measurements (weight in kg/[height in meters]^2). Fasting blood samples (10 ml) were obtained at the start of the study and end-of-trial at Kavosh medical laboratory in an early morning after an overnight fast. Blood samples were immediately centrifuged (Universal, Germany) at 3500 rpm for 10 minutes to separate serum. Then, the samples were stored at −80°C until analysis at the Kavosh Medical Laboratory.

Outcomes

In this study, the primary outcomes were general health questionnaire (GHQ) and depression anxiety and stress scale (DASS) scores. The GHQ-28 comprises 28-items consisting of four subscales: somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression.[23] The DASS questionnaire consists of three 14-item self-report scales that measure depression, anxiety, and stress.[24]

Secondary outcomes were kynurenine, tryptophan, neuropeptide Y, cortisol, and ACTH. Serum kynurenine (Cusabio Biotech, Wuhan, China) with intra- and interassay CVs 8 and 10%, tryptophan (Labor Diagnostika Nord, Nordhorn, Germany) with intra- and interassay CVs 11 and 15%, neuropeptide Y (Glory Science, Zhejiang, China) with intra- and interassay CVs of 10 and 12%, cortisol (Diametra, Milan, Italy) with intra- and interassay CVs of 8.0 and 15%, and ACTH (Biomerica, Irvine, USA) with intra- and interassay CVs of 3.1 and 6.2% were quantified with ELISA methods.

Characteristics of yogurts and supplements

In this study, the probiotic yogurt contained two strains of *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 with a total of min 1×10^7 CFU. The conventional yogurt contained the starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Both yogurts' PH was in the range of 4.3–4.5 and their fat content was 2.5%. The multispecies probiotic capsule contained seven probiotic bacteria spices *Actobacillus casei* 3×10^3, *L. acidophilus* 3×10^7, *L. rhamnosus* 7×10^9, *L. bulgaricus* 5×10^8, *Bifidobacterium breve* 2×10^{10}, *B. longum* 1×10^9, *S. thermophilus* 3×10^8 CFU/g, and 100 mg fructo-oligosaccharide with lactose as carrier substances. The placebo (the same substance without bacteria) was packed in identical capsules and coded by the producer to guarantee blinding. Both yogurts were provided by Pegah Company, Tehran, Iran. The multispecies probiotic and placebo capsules were produced by ZistTakhmir Co, Tehran, Iran.

Collection of samples

Probiotic and conventional yogurts or multispecies probiotic supplements were provided for participants every day from February 2014 to March 2014. The yogurt samples were purchased from the factory of Pegah Dairy Company and were transferred to petrochemical region. Two packages were refrigerated for transport and deliver to Biotechnology Laboratory of National Nutrition and Food Technology Research Institute within 48 hours for enumeration of bacterial cell. One milliliter of each sample was diluted in 9 ml of sterile ringer solution (Merck, Darmstadt, Germany); subsequently, serial dilutions were prepared, and counts of bacteria were enumerated using the pour plate technique. Differentiative media for complete selection of the colonies of different microorganisms were chosen for each product.[25] Enumeration of *Lactobacillus lactis* was carried out on MRS agar (Merck, Germany) at pH = 5.2 by anaerobic incubation at 45°C for 72 hours,[26] and selective enumeration of *L. acidophilus* was performed on MRS-Bile[27] by anaerobic incubation at 37°C for 72 hours. The selectivity of the growth conditions was confirmed by the microscopic appearance of the cells from single colonies. Enumeration of *Bifidobacterium* was conducted in the same media,

whereas anaerobic condition achieved with GasPak Syetem-Oxoid. Plates containing 30–300 colonies were enumerated, and recorded as colony forming units per milliliter of culture (CFU/ml).

Microbiological analysis

The average of viable total count of probiotic yogurt was 4.03×10^7 with a standard deviation of 0.93. The average total viable count of conventional yogurt was about 4.21 and standard deviation 1.12. There are found no significant variation in total viable count of probiotic and natural yogurt, because defined starter culture is used under proper conditions of fermentation for manufacture of yogurt.

Statistical methods

The normality of the variables was examined by the Kolmogorov–Smirnov test. Log transformation was applied for non-normally distributed variables. To detect differences in general characteristics dietary intakes and to determine the effect of probiotic yogurt, probiotic capsule and conventional yogurt on mental health parameters and markers of hypothalamic–pituitary–adrenal axis among the three groups, one-way independent measures analysis of variance (ANOVA) was used. The changes across three groups were compared using Bonferoni *post hoc* pairwise comparisons. In addition, we have only applied repeated measure ANOVA to detect the differences at study baseline. To identify within-group differences (baseline and end-of-trial), we used paired-samples *t*-tests. Furthermore, to assess if the magnitude of the change in dependent variables depended on the baseline values, we controlled all analyses for baseline values, age, and BMI to avoid the potential bias that might have resulted. These adjustments were done using analysis of covariance (ANCOVA). *P*-values were considered statistically significant at $P < 0.05$. The statistical analyses were carried out using the statistical packages for SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Among subjects in the conventional yogurt group, five persons (withdrawn ($n = 5$)) were excluded. Finally, 70 participants (conventional yogurt group ($n = 20$), probiotic yogurt group ($n = 25$), and multispecies probiotic supplements ($n = 25$)) completed the trial (Fig. 1). Compliance with the consumption of conventional probiotic yogurts and probiotic supplements were monitored once a week through phone interviews and by the use of 3-day dietary records completed at week 1, 3, and 5 of intervention. To increase the compliance, all subjects were receiving short messages on their cell phones to take yogurts and supplement each day. On average, the rate of compliance in our study was high, such that higher than 90% of yogurts

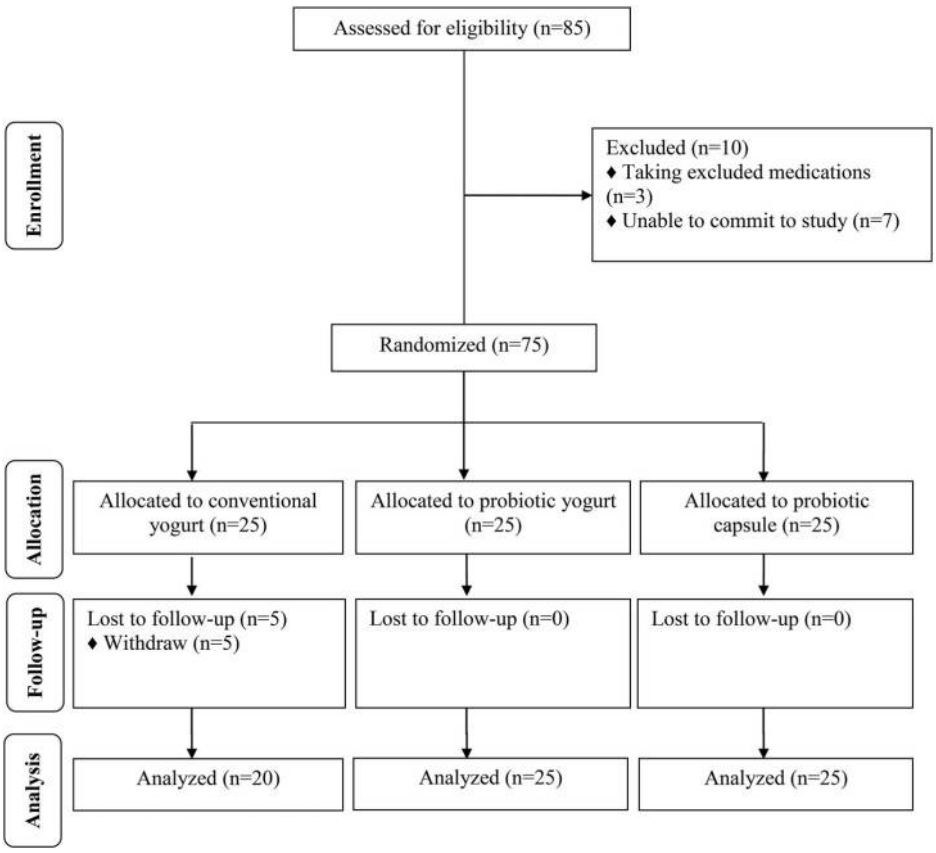

Figure 1 Summary of patient flow diagram.

Table 1 General characteristics of study participants

	Conventional yogurt ($n = 20$)	Probiotic yogurt ($n = 25$)	Probiotic capsule ($n = 25$)	P*
Age (years)	33.1 ± 6.1	33.2 ± 6.4	31.5 ± 5.8	0.77
Height (cm)	169.0 ± 8.7	171.2 ± 7. 2	170.4 ± 10.0	0.53
Weight at study baseline (kg)	75.4 ± 14.03	69.8 ± 15.5	71.2 ± 12.9	0.36
Weight at end-of-trial (kg)	74.7 ± 13.4	72.1 ± 13. 5	69. 9 ± 13.4	0.42
BMI at study baseline (kg/m^2)	24.9 ± 3.3	24.3 ± 3.8	24.6 ± 3.4	0.82
BMI at end-of-trial (kg/m^2)	24.7 ± 3.4	24.5 ± 3.7	24.3 ± 3.7	0.91

Data are mean ± SD.
*Obtained from the ANOVA test.

and capsules were taken throughout the study in the three groups.

No serious adverse reactions or side effects were reported after taking probiotic yogurt and multispecies probiotic supplements in petrochemical workers throughout the study. Mean age, height, weight, and BMI at baseline and 6 weeks after intervention were not significantly different among the three groups (Table 1).

At baseline, no significant differences were found among the three groups in terms of dietary intakes. Comparing the dietary intakes during the run-in period and throughout the study separately in each group, we observed no significant within-group differences in dietary intakes of energy, fat, saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), cholesterol, total dietary fiber (TDF), vitamin C, zinc, magnesium, manganese, and selenium (Table 2).

After 6 weeks of intervention, a significant improvement of GHQ was observed in the probiotic yogurt (18.0 ± 1.5 vs. 13.5 ± 1.9, $P = 0.007$) and in the probiotic capsule group (16.9 ± 1.8 vs. 9.8 ± 1.9, $P = 0.001$), as well as a significant improvement in DASS scores in the probiotic yogurt (23.3 ± 3.7 vs. 13.0 ± 3.7, $P = 0.02$) and the probiotic capsule group (18.9 ± 3.2 vs. 9.4 ± 4.0, $P = 0.006$; Table 3). However, there was no significant improvement in the conventional yogurt group (19.3 ± 1.5 vs. 16.0 ± 1.9, $P = 0.05$ for GHQ and 28.4 ± 4.4 vs. 21.7 ± 4.6, $P = 0.08$ for DASS). We did observe no significant effect of probiotic yogurt consumption and multispecies probiotic capsule supplementation on hypothalamic–pituitary–adrenal axis.

Baseline concentrations of kynurenine and kynurenine/tryptophan ratio differed significantly among conventional, probiotic yogurt groups and multispecies probiotic capsule group ($P = 0.006$); therefore, we controlled for baseline levels in the analyses, and after adjustment no significant changes in our findings occurred. Additional adjustments for age and baseline BMI did not affect our findings (Table 4).

Discussion

This study has shown that the administration of probiotic yogurt and probiotic supplements for 6 weeks among petrochemical workers had a positive effect on their mental health, but did not affect hypothalamic–pituitary–adrenal axis.

The use of petrochemical workers is interesting, as previous probiotic research in humans has generally used community-dwelling samples. To the best of our

Table 2 Dietary intakes and physical activity of study participants throughout the study

	Conventional yogurt ($n = 20$)	Probiotic yogurt ($n = 25$)	Probiotic capsule ($n = 25$)	P*
Energy (kcal/d)	2453 ± 207	2423 ± 184	2399 ± 205	0.67
Carbohydrates (g/d)	331.3 ± 50.7	333.0 ± 35.8	330.8 ± 48.2	0.98
Protein (g/d)	87.7 ± 10.4	86.0 ± 8.4	84.5 ± 20.2	0.76
Fat (g/d)	90.7 ± 13.9	86.8 ± 13.5	85.7 ± 18.7	0.54
SFA (g/d)	26.7 ± 6.3	24.7 ± 4.6	25.2 ± 7.5	0.56
PUFA (g/d)	30.2 ± 8.5	28.7 ± 6.9	26.9 ± 6.9	0.33
MUFA (g/d)	24.4 ± 5.2	22.9 ± 4.8	23.8 ± 8.2	0.70
Cholesterol (mg/d)	220.8 ± 125.4	190.8 ± 83.8	232.4 ± 149.8	0.47
TDF (g/d)	18.9 ± 4.9	17.9 ± 4.8	18.1 ± 4.9	0.75
Vitamin C (mg/d)	83.9 ± 22.8	86.6 ± 17.9	82.2 ± 14.2	0.69
Zinc (mg/d)	11.1 ± 2.7	9.7 ± 2.1	10.3 ± 3.8	0.32
Magnesium (mg/d)	304.7 ± 78.6	283.2 ± 64.8	284.8 ± 85.4	0.59
Manganese (mg/d)	2.3 ± 0.8	2.1 ± 0.7	2.3 ± 0.7	0.55
Selenium (μg/d)	54.6 ± 7.3	54.4 ± 8.2	55.2 ± 9.5	0.94
MET-h/day	35.6 ± 1.3	35.0 ± 1.2	35.1 ± 1.3	0.20

Data are mean ± SD.
*Obtained from the ANOVA test.
METs, metabolic equivalents; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; TDF, total dietary fiber.

Table 3 The effect of daily consumption of probiotic yogurt and probiotic capsule on mental health and hypothalamic–pituitary–adrenal axis

	Conventional yogurt (n = 20)				Probiotic yogurt (n = 25)				Probiotic capsule (n = 25)				
	Wk0	Wk6	Change	P*	Wk0	Wk6	Change	P*	Wk0	Wk6	Change	P*	P**
GHQ scores	19.3 ± 1.5	16.0 ± 1.9	−3.3 ± 1.7	0.05	18.0 ± 1.5	13.5 ± 1.9	−4.5 ± 1.7	0.007	16.9 ± 1.8	9.8 ± 1.9	−7.1 ± 1.7	0.001	0.29
DASS scores	28.4 ± 4.4	21.7 ± 4.6	−6.7 ± 3.3	0.08	23.3 ± 3.7	13.0 ± 3.7	−10.3 ± 3.9	0.02	18.9 ± 3.2	9.4 ± 4.0	−9.5 ± 4.3	0.006	0.80
Kynurenine (pmol/ml)	334.5 ± 73.2	242.6 ± 59.4	−91.9 ± 103.3	0.38	195.2 ± 37.2	166.6 ± 38.4	−28.6 ± 51.7	0.58	122.4 ± 17.6	226.0 ± 49.9	103.6 ± 52.9	0.06	0.13
Tryptophan (μg/ml)	11.9 ± 0.3	11.7 ± 0.6	−0.2 ± 0.7	0.81	12.0 ± 0.3	12.2 ± 0.3	0.2 ± 0.4	0.68	12.1 ± 0.2	12.3 ± 0.3	0.2 ± 0.4	0.63	0.86
Kynurenine/ tryptophan ratio	28.5 ± 6.2	41.8 ± 24.2	13.3 ± 26.5	0.62	16.4 ± 3.2	14.6 ± 3.8	−1.8 ± 4.7	0.71	10.3 ± 1.5	16.5 ± 3.7	6.2 ± 4.0	0.13	0.74
Neuropeptide Y (ng/l)	1565.5 ± 248.4	1364.2 ± 223.7	−201.3 ± 76.7	0.39	1235.2 ± 213.9	1314.2 ± 219.3	79.0 ± 84.1	0.95	1211.2 ± 205.7	1067.8 ± 162.6	−143.4 ± 94.3	0.27	0.46
Cortisol (ng/ml)	116.5 ± 21.9	124.2 ± 16.9	7.7 ± 20.0	0.32	93.0 ± 14.2	104.0 ± 15.1	11.0 ± 14.6	0.57	90.5 ± 15.3	108.5 ± 15.2	18.0 ± 11.1	0.38	0.66
ACTH (pg/ml)	6.3 ± 1.3	6.4 ± 1.1	0.1 ± 1.6	0.09	6.8 ± 1.3	5.4 ± 1.0	−1.4 ± 1.7	0.58	9.6 ± 2.0	6.4 ± 1.2	−3.2 ± 1.5	0.49	0.60

All values are mean ± SE.
*Obtained from the paired samples *t*-test.
**Obtained from ANOVA test.
ACTH, adrenocorticotropic hormone; DASS, depression anxiety and stress scale; GHQ, general health questionnaire.

knowledge, this study is the first that reports the effects of probiotic yogurt and supplements on mental health and hypothalamic–pituitary–adrenal axis among petrochemical workers. Nonetheless, assessment of probiotics administration is interesting especially among people who have occupational exposure to aromatic compounds. It must be taken into account that there are many reports into the research of the interactions of probiotics with either the food matrix or the starter culture in different food matrix, including yogurt, cheese, and dough (a traditional dairy drink in Iran and Turkey). In such fermented foods, influence of several factors must be considered on activity and viability of probiotics in the consumer's gastrointestinal (GI) tract including the physiologic state of the probiotic added; the physicochemical conditions of product storage (e.g., temperature, acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content) and possible interactions of the probiotics with the starter cultures (e.g., bacteriocin production, antagonism, and synergism). Some of these aspects are discussed in our previous research with an emphasis on dairy products such as milk, yogurt, and cheese.[28,29] Also, profile of the bacterial cells during the shelf life of the product was studied.[30] In this study, we found that baseline levels of kynurenine in the conventional yogurt and probiotic capsule were average twofold compared with the probiotic yogurt. Various factors may affect kynurenine levels, including age, leptin levels, and inflammatory markers.[31] Actually, interpretation of these data is difficult. Despite this, we agree that further trials would be needed to explain this observed discrepancy in our study.

As petrochemical staff are subject to occupational stress and some ergonomic issues, they are susceptible to developing different problems including anxiety and depression.[1,2] The findings of this study demonstrated that despite a significant improvement within the GHQ and DASS scores after consumption of 100 g probiotic yogurt and one probiotic capsule among petrochemical workers per day, these changes in GHQ and DASS scores were similar among the three groups. On the whole, few researches have evaluated the effect of probiotics on mental health and hypothalamic–pituitary–adrenal axis. In line with our study, Nishihira *et al.*[32] reported that taking 100 g of yogurt consisting two types of probiotics *Lactobacillus gasseri* $\geq 5 \times 10^8$ CFU and *B. longum* $\geq 1 \times 10^9$ CFU daily for 12 weeks among healthy subjects has resulted in a significant improvement in mental health and decreased stress. In addition, a significant improvement in mental health was observed following the consumption of probiotic sachet containing two strains of *L. helveticus* R0052 and *B. Longum* R0175 (3×10^{12} CFU/1.5 g sachet) for

Table 4 Adjusted changes in mental health and hypothalamic–pituitary–adrenal axis in petrochemical workers

	Conventional yogurt ($n = 20$)	Probiotic yogurt ($n = 25$)	Probiotic capsule ($n = 25$)	P^*
GHQ scores	−3.4 ± 1.8	−4.0 ± 1.6	−7.6 ± 1.7	0.18
DASS scores	−5.5 ± 3.9	−9.0 ± 3.5	−11.4 ± 3.7	0.56
Kynurenine (pmol/ml)	45.0 ± 57.0	−41.6 ± 51.2	1.3 ± 50.8	0.53
Tryptophan (μg/ml)	−0.4 ± 0.4	−0.2 ± 0.4	−0.4 ± 0.4	0.17
Neuropeptide Y (ng/l)	−99.3 ± 101.5	−91.2 ± 96.8	−125.4 ± 86.4	0.96
Cortisol (ng/ml)	4.3 ± 16.3	27.1 ± 15.6	2.7 ± 13.8	0.46
ACTH (pg/ml)	−0.3 ± 1.8	−2.5 ± 1.7	−1.3 ± 1.5	0.65

All values are mean ± SE.
*Obtained from ANCOVA adjusted for baseline values, age, and baseline BMI.
ACTH, adrenocorticotropic hormone; DASS, depression anxiety and stress scale; GHQ, general health questionnaire.

30 days among healthy persons.[15] Similar findings have been reported after 3 weeks of treatment with milk containing *Lactobacillus casei* $\geq 6.5 \times 10^9$ CFU in patients suffering from depression.[33] Furthermore, some animal studies have shown the beneficial effects of probiotic supplementation on anxiety and depression disorders.[34,35] However, few studies did not support such favorable effects after probiotics supplementation on mental health parameters. For instance, supplementation with one tablet containing approximately 10^9 CFU of the probiotic organism *L. rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12 for 14 weeks did not affect the positive and negative syndrome scale (PANSS) in schizophrenia patients.[18] In another study, feeding with *Bifidobacteria infantis* for 14 days did not influence the forced swim test in Sprague–Dawley rats.[19] An accurate mechanism of probiotic supplementation effects on mental health has not been clearly recognized. Beneficial effects of probiotic supplementation may be mediated through its effects on neuronal circuits and central nervous system mediated by microbiota–gut–brain axis[36] and the regulation of gamma-aminobutyric acid GABA receptors by the vagus nerve; known as the major regulator of the interactions between gut microbiota and the brain.[37] In addition, probiotics may improve mental health parameters via inhibition of the pro-inflammatory cytokines including IFN-gamma, TNF-alpha, and interleukin-6 (IL-6).[21]

This study revealed that supplementation with probiotics among petrochemical workers for 6 weeks did not affect markers of hypothalamic–pituitary–adrenal axis, including serum levels of kynurenine, tryptophan, neuropeptide Y, cortisol, and ACTH pathway. However, limited information is available on this issue. In agreement with our study, feeding with *B. longum* could not affect kynurenine levels in mice.[38] However, treatment with yogurt containing two strains of probiotics containing *Lactobacillus gasseri* and *B. longum* has led to a significant reduction of serum ACTH levels among healthy subjects for 12 months. Probiotic yogurt also more effectively inhibited the release of cortisol compared with the group consuming placebo yogurt.[32] In another study, taking *L. helveticus* and *B. longum* in young healthy women for 30 days reduced urine levels of free cortisol.[15] Experimental information has demonstrated that probiotics may influence both the enteric nervous system (ENS) and the central nervous system (CNS) in addition to their effects on the mucosal immune system by modifying the GI tract microbiome.[39,40] In addition, few studies have shown that probiotics improve carbohydrate malabsorption,[41] which in turn are associated with both the early signs of depression[42] and reduced tryptophan levels.[43] The possibility exists that the administration of probiotics may deduce its beneficial effect on mental health through elevating levels of the serotonin precursor, tryptophan, and consequently increasing serotonin availability.[19] Different study designs, the subjects under study, different dosages of used probiotic as well as duration of the intervention might provide some reasons for discrepant findings.

Some limitations must be taken into account in the interpretation of our findings. The short period of supplementation was the main limitation of our study. Long-term interventions might result in greater changes in hypothalamic–pituitary–adrenal axis. Furthermore, owing to budget limitations, we did not assess the rate that short chain fatty acids (SCFA) are produced by probiotics in the gut. Previous studies have reported that anti-inflammatory effects of probiotics might be modulated by gut microbiota–SCFA–hormone axis.[44] Therefore, the evaluation of fecal SCFA may contribute to the interpretation of our findings.

In conclusion, the consumption of probiotic yogurt or a multispecies probiotic capsule had beneficial effects on mental health parameters in petrochemical workers, whereas it did not influence hypothalamic–pituitary–adrenal axis.

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