



OPEN Effects of zinc and probiotic co-supplementation on inflammation, gastrointestinal symptoms, quality of life, and mood in irritable bowel syndrome patients

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Zinc supports intestinal integrity, and probiotics restore microbial balance, thereby improving gut health. This study examined the effect of zinc and probiotic co-supplementation on inflammation, gastrointestinal symptoms, quality of life (QoL), and mood in IBS patients. The 84 participants were assigned to three groups and received: (1) a zinc gluconate tablet (20 mg elemental zinc) and a placebo; (2) a zinc gluconate and a probiotic capsule; (3) 2 placebos as a control for 90 days. The serum zinc and TNF- α , IBS severity, mood, and QoL were assessed by validated methods. Supplementation with zinc, without or with probiotics, improved serum zinc (6.36 ± 0.80 and 13.03 ± 3.82 , vs. -1.76 ± 1.82 $\mu\text{g/dL}$; $p = 0.008$), and IBS severity (-182.85 ± 16.27 and -195.71 ± 23.46 , vs. -73.21 ± 17.69 ; $p = 0.003$) compared to placebo. Depression (-3.62 ± 1.46 or -0.78 ± 1.16 vs. 2.07 ± 1.53 , $p = 0.004$) and stress scores (-2.88 ± 1.82 or -2.64 ± 1.49 , vs. 1.85 ± 1.95 , $p = 0.013$) decreased after zinc or placebo supplements compared to zinc plus probiotic. Zinc supplementation, with or without probiotics, improved serum zinc levels and GI symptoms in IBS, whereas zinc alone was more effective than zinc plus probiotics in reducing depression and stress scores. Further research is suggested to confirm our findings.

Keywords Zinc, Probiotic, Inflammation, IBS symptoms, Mood, Quality of life

The disorders of gut-brain axis interactions (DGBI) have a 40% globally prevalence¹. Although DGBI is not considered life-threatening, it exerts a substantial detrimental impact on the quality of life (QoL) and imposes financial burdens on healthcare systems². Irritable bowel syndrome (IBS) is among the most prevalent DGBIs³. The prevalence of IBS in women is more than in men (approximately 12% vs. 9%)⁴. The overlap between IBS and other conditions, such as functional dyspepsia, depressive disorders, somatization disorder, and gastroesophageal reflux disorder, is noteworthy^{5,6}. The pathophysiology of IBS is complex and multifactorial, including gut motility abnormalities, visceral hypersensitivity, increased leaky gut, low-grade inflammation, dysregulation of gut-brain interactions, and psychosocial distress, but is not limited to them. These mechanisms are related to each other and can contribute to the heterogeneity of clinical presentation and symptom severity among patients⁷. Psychosocial distress and the numerous psychiatric comorbidities in IBS contribute to disorder development and symptom exacerbation⁸. Moreover, IBS is related to gut dysbiosis, typically characterized by reduced microbial diversity, altered composition, and depletion of beneficial taxa such as *Bifidobacterium*⁹. Genetic, antibiotic use, infections, stress, and poor diet are factors that can lead to dysbiosis and subsequently increased intestinal permeability (IP)¹⁰. Studies have shown that vitamin D, probiotics, and zinc are related to IP^{11–13}.

Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts¹⁴. Probiotics, through interference with potential pathogens, improvement of gut barrier function, and immunomodulation, exert lucrative effects on the host¹⁵. Studies support the use of probiotics, especially *Bifidobacterium* and *Lactobacillus* genus, to modulate intestinal microbiota in IBS patients^{16,17}. Based on guidelines, probiotics may be an effective treatment for global symptoms and abdominal pain among IBS patients, with the strongest effect for genus *Lactobacillus*¹⁶. Xie et al. revealed that the low FODMAP diet and

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probiotics (especially *Lactobacillus*, *Bifidobacterium*, and *Bacillus*) are two common interventions for patients with IBS before initiation of pharmacological treatments¹⁸. Diet restrictions among IBS patients can cause zinc deficiency, which is the main trace element for improving gut tight junction integrity and reducing inflammation and oxidative stress¹⁹. Beef, poultry, seafood, dairy products, legumes, and whole grains are food sources of zinc²⁰. About 17.3% of the population in the world and approximately 25% of Iranian households are at risk of inadequate zinc intake^{21,22}. Some studies demonstrated that there is a lower intake or serum zinc levels in patients with IBS; however, other studies did not find any association^{23–26}. In our previous study, we showed that serum zinc was negatively correlated with serum zonulin (a marker of leaky gut) in diarrhea-predominant IBS patients¹¹. Zinc and probiotics are two effective components in the reduction of IP, inflammation, and oxidative stress, and improving mood. Some studies have shown that concurrent consumption of zinc with probiotics can help increase zinc absorption and have a synergistic effect in improving antioxidant status and immune system function^{27,28}. Probiotics, through the production of short-chain fatty acids (SCFAs) and the reduction of gut pH, can increase the solubility of certain minerals, like zinc. Also, probiotics produce phytases that break down phytic acid, thereby releasing minerals such as calcium and zinc and making them available for absorption²⁹. Considering that no study has been conducted so far on zinc supplementation and in combination with probiotics in IBS patients, the aim of the present study was to investigate the zinc and probiotic co-supplementation on inflammation, gastrointestinal (GI) symptoms, QoL, and mood states in patients with IBS.

Methods

Trial design

This study was a double-blind parallel randomized placebo-controlled clinical trial stratified based on sex. It was conducted according to the Declaration of Helsinki. The study protocol was approved by the Medical Ethics Committee at the Isfahan University of Medical Sciences (IR.MUI.PHANUT.REC.1402.079). It was also registered at the Iranian Registry of Clinical Trials (<https://www.irct.ir>) under the registration number IRCT20180818040827N5 on 29/01/2024.

Participants

From July 2024, IBS patients who were referred to the subspecialty Clinic of Khorshid Hospital and Al-Zahra Hospital Digestive Health Center and two gastroenterology clinics in Isfahan (Iran) were screened. Men and women aged 20–60 with a body mass index (BMI) between 18.5 and 40 kg/m² and a confirmed diagnosis of IBS by a gastroenterologist based on Rome IV criteria were enrolled³⁰. The exclusion criteria were patients with a history of GI surgery or infection, celiac, inflammatory diseases such as inflammatory bowel diseases, pregnancy, lactation, hospitalization, malignancy, allergic reactions to zinc, probiotic or placebo, supplementation with probiotic products or zinc, following a special diet, smoking and alcohol drinking habit, chronic anemia, being a professional athlete and taking medications including antibiotics, anti-diarrheal or laxative, penicillamine, affecting on GI motility, as well as heparin and warfarin. Written informed consent was obtained from all the participants before enrollment. Medical and demographic characteristics were also collected through interviews.

Sample size

The sample size was calculated using the recommended formula for clinical trials, taking into account type one error of 0.05 and type two errors of 0.20 (power = 80%) based on a previous study³¹. Therefore, 25 IBS patients in each group were required. According to a ~10% drop-out rate, we finally recruited 28 patients with IBS for each group, 84 in total.

Randomization and blinding

The participants were assigned to treatment (zinc or zinc plus probiotic) and control (placebo) groups using stratified (based on sex) permuted block randomization (allocation ratio = 1: 1: 1, block size = 6). We used the website (<https://www.sealedenvelope.com/simple-randomiser/v1/lists>) for the generation of a random allocation sequence. Zinc, probiotic, and placebo tablets were sealed in bottles that were numbered in a specific order. The sequence generation and allocation concealment were implemented by an independent medical expert. The patients, laboratory staff, and researchers were blinded to the randomization. Zinc, zinc placebo, probiotic, and its placebo were similar in size, weight, shape, color, and packaging.

Intervention

Patients were randomized into three parallel groups¹, zinc group: zinc gluconate tablet (20 mg of elemental zinc, Amin Pharmaceutical Company, Isfahan, Iran) and FamiLact placebo capsule (Microcrystalline cellulose, Zist Takhmir, Tehran, Iran)²; zinc and probiotic group: zinc gluconate tablet (20 mg of elemental zinc, Amin Pharmaceutical Company, Isfahan, Iran) and FamiLact capsule (10⁹ colony-forming units (CFUs) of *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*, Zist Takhmir, Tehran, Iran)³; the placebo group: zinc placebo tablet (Microcrystalline cellulose, Amin Pharmaceutical Company, Isfahan, Iran) and FamiLact placebo capsule (Microcrystalline cellulose, Zist Takhmir, Tehran, Iran). The dose and duration of supplementation were chosen based on previous studies^{32–34}. A zinc tablet (supplement or placebo) and a probiotic capsule (supplement or placebo) were prescribed once daily after breakfast with sufficient water for 90 days. All patients in the three groups were instructed with appropriate dietary advice for IBS based on the recommendations of the American Gastroenterological Association guideline³⁵. These recommendations were: having regular meals and taking time to eat, avoiding missing meals or leaving long gaps between eating, drinking at least 8 cups of fluid per day, especially water, restricting caffeine, and reducing intake of alcohol and fizzy drinks. All groups were asked to record their dietary intakes and physical activity

levels during the study. Follow-up phone weekly calls, clinic visits in the middle of the period, and monitoring the number of consumed supplements or placebos were conducted to assess treatment compliance.

General characteristics, anthropometric indices, dietary intake, and physical activity

In the first visit, participants were asked about their general characteristics, including age, sex, marital status, education level, history of diseases, medications, and supplements they consumed. A fixed, non-stretchable tape at 0.1 cm precision was used to measure height in a standing position. Weight was measured in minimal clothing to the nearest 0.1 kg by a digital scale, Omron BF511. Waist circumference was determined at the midpoint between the iliac crest and the last rib. Body mass index was calculated by dividing weight (kg) by height (m²). Food records were made three times during the study (at the beginning and in weeks 5 and 12). Nutritionist IV software customized for Iranian foods was used (First Databank, San Bruno, CA, USA) for the calculation of energy and nutrient intake³⁶. Physical activity levels were estimated using a valid and reliable short form of the International Physical Activity Questionnaire (SF-IPAQ) at the beginning and in weeks 5 and 12³⁷. Also, possible side effects of supplements or placebos, particularly GI complications, were monitored during the trial period.

Outcomes assessment

Venous blood samples were obtained from the participants after 10–12 h of overnight fasting. The serum was separated by centrifugation and then stored at –80 °C until final analysis. Serum zinc level was measured by Cobas integra 400 plus analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The enzyme-linked immunosorbent assay (ELISA) technique using a commercially available kit (Karmania Pars Gene Company, Iran) was used to assess serum TNF- α level.

A validated IBS severity score system (IBS-SSS) was used to assess the IBS severity³⁸. The validated IBS-SSS included five clinical items¹ severity of abdominal pain², frequency of abdominal pain³, severity of abdominal distention⁴, dissatisfaction with bowel habits⁵, interference of IBS with daily activities in general during 10 recent days. Scores less than 75, 75–175, 175–300, and above 300 indicated remission, mild, moderate, and severe IBS, respectively. Participants were evaluated for depression, anxiety, and stress scores using a validated 21-item questionnaire (DASS-21)³⁹. There were four response options, and based on the scores in each question, each parameter of depression, anxiety, and stress was calculated. A self-reported 34-item IBS quality of life (IBS-QoL) questionnaire was provided to IBS patients⁴⁰. The Persian version of this questionnaire was reliable and validated, having high internal consistency⁴¹. The IBS-QoL questionnaire has 8 subscales, including “dysphoria”, “interference with activity”, “body image”, “health worry”, “food avoidance”, “social reaction”, “sexual worries” and “relationship”. A five-point response scale from always¹ to never⁵ was used to measure patients’ QoL. Scores of all items were converted to scales between 0 and 100, in which higher scores showed better QoL.

Statistical analysis

Data analyses were performed according to the intention-to-treat (ITT) approach, using the last-observation-carried-forward (LOCF) method by a statistical software package (SPSS, Inc., Chicago, Illinois, USA), version 23.0. Statistical significance was considered at $P < 0.05$. Normal distribution of quantitative variables was checked using Q–Q plots and skewness. Data with normal distribution were reported as mean $\pm$ standard deviation (SD) or standard error (SE), and categorical data were reported as frequency (percentage). The non-normally distributed data were shown as median (interquartile range). The participants’ baseline characteristics between groups were assessed by one-way ANOVA and Chi-square tests. Within-group comparisons after a 90-day intervention were conducted by a paired-samples t-test. Differences between the three groups after intervention were detected using analysis of covariance (ANCOVA), adjusted for the baseline value of each variable, as well as physical activity, supplementation, and medications (antidepressant, antispasmodic, and antacid) use in the final model. Also, changes in dietary intake components and physical activity over time were assessed using the repeated measure analysis of variance.

Results

The recruitment period and follow-up of participants were from July 2024 to February 2025. Of 84 IBS patients, seven participants were excluded (two patients due to personal reasons from the zinc group, two patients due to personal reasons ($n = 1$), and gastrointestinal complications ($n = 1$) from the zinc plus probiotic group, and three patients due to personal reasons ($n = 2$) and low compliance rate ($n = 1$) from the placebo group). Nevertheless, all 84 participants were included in the final analysis according to the ITT approach (Fig. 1).

Baseline characteristics

The baseline characteristics of participants are presented in Table 1. There were no significant differences between the three groups in terms of all baseline characteristics.

Dietary intake and physical activity level

The dietary intake and physical activity of the subjects throughout the study are summarized in Table 2. The comparison of the three groups did not show any significant differences in energy, macronutrients, and micronutrients intake during the time. As shown in Table 2, physical activity levels, expressed as median (interquartile range), exhibited changes within groups over time; however, repeated measures analysis illustrated that the physical activity levels differed significantly among the three study groups ($p = 0.007$).

Effect of intervention on serum zinc level, inflammation and gastrointestinal symptoms

Within- and between-group comparisons of serum zinc, inflammation and gastrointestinal symptoms at the baseline and 90 days are reported in Table 3. In the zinc supplementation and zinc plus probiotic supplementation

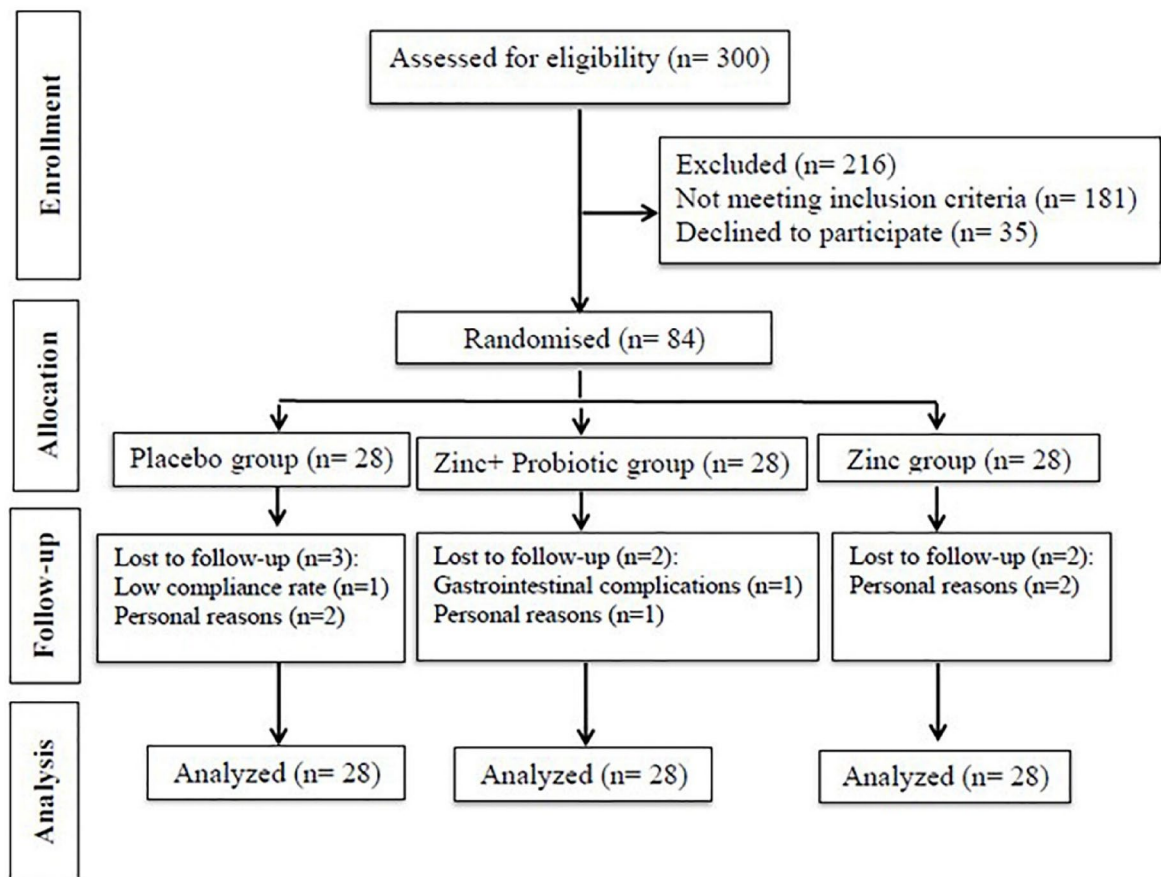


Fig. 1. Study flow diagram based on CONSORT statement.

groups, serum zinc level was significantly increased (mean change: 6.36 ± 0.80 , $p=0.001$, and 13.03 ± 3.82 , $p=0.002$, respectively), and serum TNF- α level (mean change: -116.22 ± 8.16 , and -113.13 ± 12.14 , $p=0.001$, respectively) and severity of IBS symptoms (mean change: -182.85 ± 16.27 , and -195.71 ± 23.46 , $p=0.001$, respectively) were decreased compared with the baseline. Also, in the placebo group, serum TNF- α level (mean change: -110.25 ± 10.51 , $p=0.001$) and IBS severity (mean change: -73.21 ± 17.69 , $p=0.001$) were decreased at the end of the study compared with that of the beginning.

Between-group comparison illustrated that zinc plus probiotic supplementation significantly increased serum zinc level compared to the control in both adjusted models (13.03 ± 3.82 vs. -1.76 ± 1.82 ; model 1: $p=0.004$, final model: $p=0.008$). In the ANCOVA model adjusted for baseline values and other confounders, this significant difference was also revealed in zinc supplementation compared with the placebo group (6.36 ± 0.80 vs. -1.76 ± 1.82 ; $p=0.008$). However, there was no significant effect on TNF- α level between the intervention and the placebo groups.

A significant reduction for IBS severity score were observed in both zinc supplementation separately and plus probiotic in adjusted model for baseline values and only in zinc supplementation in model adjusted for baseline values and other confounders compared to the placebo (-182.85 ± 16.27 or -195.71 ± 23.46 , respectively, vs. -73.21 ± 17.69 ; model 1: $p=0.003$, final model: $p=0.034$).

Effect of intervention on mood status

The effect of the 90-day intervention on depression, anxiety, and stress is shown in Table 3. The within-group comparison revealed a significant reduction of depression and anxiety scores in the zinc supplementation group at the end of the study, compared to the beginning (mean change: -3.62 ± 1.46 , $p=0.02$, and -2.37 ± 1.12 , $p=0.046$, respectively).

In the model adjusted for baseline values, depression score decreased significantly in the zinc group compared to the zinc plus probiotic, not the placebo, group (-3.62 ± 1.46 vs. 2.07 ± 1.53 , $p=0.014$). After adjusting for other confounders and baseline values, in addition to the previously mentioned result, a significant reduction of depression score was found in the placebo group in comparison to the zinc plus probiotic supplementation group (-0.78 ± 1.16 vs. 2.07 ± 1.53 , $p=0.004$). Furthermore, the stress score was diminished significantly in both zinc and placebo groups when compared to the zinc plus probiotic group (-2.88 ± 1.82 or -2.64 ± 1.49 , respectively, vs. 1.85 ± 1.95 , $p=0.013$). We found no significant effect of interventions on anxiety score compared to placebo.

Variable	Zinc group (n = 28)	Zinc + probiotic group (n = 28)	Placebo group (n = 28)	P-value
Sex, n (%)				0.990
Male	7 (25)	7 (25)	7 (25)	
Female	21 (75)	21 (75)	21 (75)	
Age (year)	39.50 ± 12.03	37.07 ± 11.18	39.50 ± 11.34	0.662
Weight (kg)	67.27 ± 12.94	69.08 ± 13.32	71.44 ± 14.81	0.525
BMI (kg/m ²)	25.33 ± 3.85	25.24 ± 4.59	26.31 ± 4.95	0.614
Waist circumference (cm)	89.62 ± 10.64	91.35 ± 12.89	93.46 ± 14.35	0.530
IBS subtype (%)				0.284
IBS-D	6 (21.4)	10 (35.7)	5 (17.9)	
IBS-C	14 (50)	8 (28.6)	17 (60.7)	
IBS-M	8 (28.6)	9 (32.1)	5 (17.9)	
IBS-U	0 (0)	1 (3.6)	1 (3.6)	
IBS severity score (%)				0.063
Mild	1 (3.6)	2 (7.1)	6 (21.4)	
Moderate	12 (42.9)	8 (28.6)	13 (46.4)	
Severe	15 (53.6)	18 (64.3)	9 (32.1)	
Diseases history (%)	10 (35.7)	11 (39.3)	12 (42.9)	0.861
Dietary supplements use (%)	14 (50)	14 (50)	19 (67.9)	0.299
Medications use				
Antidepressants	11 (39.3)	9 (32.1)	14 (50)	0.391
Antispasmodics	5 (17.9)	2 (7.1)	6 (21.4)	0.306
Antacids	8 (28.6)	8 (28.6)	10 (35.7)	0.800
Education level (%)				0.898
Under diploma	5 (17.9)	3 (10.7)	5 (17.9)	
Diploma	5 (17.9)	4 (14.3)	5 (17.9)	
University	18 (64.3)	21 (75)	18 (64.3)	
Marital status (%)				0.173
Single	9 (32.1)	15 (53.6)	7 (25)	
Married	17 (60.7)	11 (39.3)	20 (71.4)	
Divorced	2 (7.1)	2 (7.1)	1 (3.6)	

Table 1. Baseline characteristics of study participants. Values are reported as mean ± SD and frequency n (%) for quantitative and qualitative variables, respectively. P-value obtained from chi-square test for qualitative variables and ANOVA for quantitative variables. BMI: body mass index; IBS-D: diarrhea predominant-irritable bowel syndrome; IBS-C: constipation predominant-irritable bowel syndrome; IBS-M: irritable bowel syndrome with mixed bowel habits; IBS-U: unsubtyped irritable bowel syndrome.

Effect of intervention on quality of life

Within- and between-group comparisons of quality of life and its subscales during the study are indicated in Table 4. After 90 days, social reaction in all three groups (zinc, mean change: 12.26 ± 5.54, $p=0.036$; zinc plus probiotic, mean change: 14.73 ± 6.52, $p=0.032$; placebo, mean change: 12.94 ± 4.86, $p=0.013$) was significantly improved. Also, a significant improvement was observed for dysphoria (mean change: 15.49 ± 6.31, $p=0.021$), interference with activity (mean change: 13.26 ± 5.74, $p=0.029$), health worry (mean change: 15.77 ± 5.80, $p=0.011$), and sexual worries (mean change: 18.30 ± 7.67, $p=0.024$) in zinc plus probiotic group. Moreover, in the placebo group, the relationship (mean change: 9.34 ± 3.68, $p=0.017$) and body image (mean change: 14.95 ± 4.58, $p=0.003$) were significantly ameliorated.

The between-group comparison demonstrated that zinc plus probiotic supplementation had no better effect on body image compared to the placebo supplementation in a fully adjusted model (12.05 ± 6.20 vs. 14.95 ± 4.58, $p=0.031$). There was no significant effect of zinc or zinc plus probiotic supplements on total quality of life and its other subscales in comparison to the placebo.

Side effects

In this trial, just one patient in the zinc-probiotic co-supplementation group reported GI complications.

Discussion

Our study is the first study that investigates the co-supplementation of zinc and probiotics on inflammation, IBS severity symptoms, QoL, and mood in patients with diagnosed IBS. The present study revealed that 20 mg of elemental zinc, separately and in combination with 10⁹ CFUs of probiotic, for 90 days, elevated serum zinc levels and improved the severity of IBS symptoms compared with the placebo. In our study, fiber intake, particularly after intervention with zinc supplement alone and with probiotics for 90 days, was slightly increased, which

Variable	Zinc group (n = 28)	Zinc + probiotic group (n = 28)	Placebo group (n = 28)	P-value
Energy (kcal/day)				0.348
0 week	1749.13 ± 597.10	1959.64 ± 603.89	1939.19 ± 579.60	
5 th week	1803.44 ± 453.33	1937.60 ± 552.30	1867.91 ± 631.28	
12 th week	1715.45 ± 773.85	1934.36 ± 655.32	1989.43 ± 665.27	
Carbohydrate (g/day)				0.405
0 week	246.22 ± 90.24	283.25 ± 103.36	280.01 ± 92.84	
5 th week	255.70 ± 81.05	277.85 ± 92.99	278.59 ± 100.67	
12 th week	253.47 ± 134.72	273.13 ± 92.57	284.10 ± 103.22	
Protein (g/day)				0.819
0 week	64.71 ± 23.48	68.80 ± 16.95	68.32 ± 27.36	
5 th week	68.10 ± 19.87	67.31 ± 19.24	63.22 ± 21.23	
12 th week	62.28 ± 21.78	69.22 ± 24.32	70.68 ± 26.87	
Total fat (g/day)				0.316
0 week	59.07 ± 29.17	65.18 ± 27.76	64.05 ± 22.66	
5 th week	59.83 ± 21.93	66.13 ± 27.13	59.04 ± 22.64	
12 th week	53.70 ± 27.58	66.46 ± 32.59	66.83 ± 30.15	
PUFA (g/day)				0.591
0 week	17.01 ± 11.62	18.72 ± 9.00	18.94 ± 6.79	
5 th week	18.21 ± 9.31	18.28 ± 8.66	17.37 ± 7.68	
12 th week	16.24 ± 10.19	19.04 ± 11.10	20.46 ± 12.29	
Fiber (g/day)				0.544
0 week	12.01 ± 6.71	14.75 ± 8.29	13.30 ± 5.47	
5 th week	13.21 ± 4.34	15.03 ± 7.57	13.79 ± 6.12	
12 th week	15.62 ± 12.15	16.09 ± 9.42	14.54 ± 7.22	
Vitamin C (mg/day)				0.791
0 week	83.58 ± 62.74	95.77 ± 54.09	88.86 ± 48.27	
5 th week	83.13 ± 42.01	87.24 ± 44.87	87.99 ± 59.69	
12 th week	104.61 ± 76.30	112.99 ± 83.47	110.33 ± 77.58	
Zinc (mg/day)				0.956
0 week	7.55 ± 3.45	7.55 ± 2.30	8.09 ± 3.70	
5 th week	7.98 ± 2.54	8.03 ± 3.46	7.21 ± 2.68	
12 th week	7.63 ± 3.60	8.00 ± 3.24	8.55 ± 3.24	
Calcium (mg/day)				0.488
0 week	538.21 ± 250.70	548.83 ± 226.72	614.68 ± 214.73	
5 th week	563.34 ± 193.19	581.51 ± 251.15	580.68 ± 281.12	
12 th week	530.75 ± 312.69	586.01 ± 276.69	632.10 ± 210.95	
Iron (mg/day)				0.640
0 week	11.11 ± 4.68	11.77 ± 4.49	14.67 ± 8.73	
5 th week	11.50 ± 3.72	14.08 ± 6.21	12.28 ± 6.93	
12 th week	14.31 ± 9.13	14.66 ± 9.10	12.99 ± 6.19	
Copper (mg/day)				0.823
0 week	0.99 ± 0.53	1.05 ± 0.45	1.05 ± 0.48	
5 th week	1.17 ± 0.76	1.23 ± 0.43	1.02 ± 0.44	
12 th week	1.08 ± 0.84	1.09 ± 0.66	1.09 ± 0.57	
PAL (MET-min/week)				0.007
0 week	496 (198–1038)	396 (121.50–1479.75)	215.50 (0–675.75)	
5 th week	825 (378–1173)	1435 (470.62–2263.50)	576 (137.25–1739.47)	
12 th week	480 (148.50–834)	1261.50 (275.62–2623.50)	193.25 (0–693)	

Table 2. Dietary intakes and physical activity of the subjects at baseline and 5 and 12 weeks of intervention. Values are reported as mean ± SD or median (interquartile range) for variables with a normal or non-normal distribution, respectively. P-values obtained from repeated-measure. PAL: physical activity level.

may indicate gut tolerance improvement following supplementation. However, this finding was not statistically significant. In the context of mood states, depression and stress scores reduced after zinc or placebo supplement use compared to zinc plus probiotic supplement. Also, zinc plus probiotic supplementation showed no better effect on body image, as one of the quality of life subscales, compared to placebo during the intervention time.

Variable	Zinc group (n = 28)	Zinc + probiotic group (n = 28)	Placebo group (n = 28)	P-value ^b	P-value ^c
Serum zinc level (µg/dL)				0.004 ^{**}	0.008 ^{***}
Baseline	84.68 ± 1.96	79.09 ± 3.24	87.24 ± 1.89		
Endpoint	91.04 ± 2.01	92.12 ± 2.23	85.47 ± 2.23		
Change	6.36 ± 0.80	13.03 ± 3.82	-1.76 ± 1.82		
P-value ^a	0.001	0.002	0.342		
TNF-α (Pg/mL)				0.652	0.526
Baseline	188.67 ± 5.82	194.24 ± 7.77	195.41 ± 7.07		
Endpoint	72.45 ± 6.81	81.11 ± 9.30	85.15 ± 10.15		
Change	-116.22 ± 8.16	-113.13 ± 12.14	-110.25 ± 10.51		
P-value ^a	0.001	0.001	0.001		
IBS severity score				0.003 ^{*,**}	0.034 [*]
Baseline	314.28 ± 14.40	317.50 ± 16.28	257.85 ± 13.76		
Endpoint	131.42 ± 17.30	121.78 ± 16.90	184.64 ± 15.15		
Change	-182.85 ± 16.27	-195.71 ± 23.46	-73.21 ± 17.69		
P-value ^a	0.001	0.001	0.001		
Depression				0.014 ^{***}	0.004 ^{***,***}
Baseline	14.07 ± 2.15	13.14 ± 2.06	10.64 ± 1.46		
Endpoint	10.37 ± 1.74	15.21 ± 2.24	9.85 ± 1.72		
Change	-3.62 ± 1.46	2.07 ± 1.53	-0.78 ± 1.16		
P-value ^a	0.020	0.187	0.506		
Anxiety				0.279	0.159
Baseline	12.87 ± 1.60	13.57 ± 1.68	11.78 ± 1.23		
Endpoint	10.14 ± 1.60	13.78 ± 1.77	10.50 ± 1.76		
Change	-2.37 ± 1.12	0.21 ± 1.54	-1.28 ± 1.21		
P-value ^a	0.046	0.891	0.299		
Stress				0.080	0.013 ^{***,***}
Baseline	20.28 ± 2.12	19.57 ± 1.74	19.21 ± 1.57		
Endpoint	17.18 ± 2.01	21.42 ± 2.28	16.57 ± 1.70		
Change	-2.88 ± 1.82	1.85 ± 1.95	-2.64 ± 1.49		
P-value ^a	0.125	0.350	0.088		

Table 3. Within- and between-group comparisons of serum zinc level, inflammation, gastrointestinal symptoms, and mood status during the 12-week intervention. Values are reported as mean ± SE. ^a Calculated by paired-sample T-test. ^b Calculated by ANCOVA adjusted for the baseline value of each variable. ^c Calculated by ANCOVA adjusted for the baseline value of each variable, physical activity, supplementation and medicine (antidepressant, antispasmodic and antacid) use. * Significant difference between the zinc and the placebo groups using the Bonferroni post-hoc test. ** Significant difference between the zinc + probiotic and the placebo groups using the Bonferroni post-hoc test. *** Significant difference between the zinc and the zinc + probiotic groups using the Bonferroni post-hoc test.

Because of dietary restrictions for the management of GI symptoms in most IBS patients, nutrient deficiencies such as zinc are probable among them¹¹. In our study, co-supplementation of zinc and probiotics could increase serum zinc concentration twice that of zinc supplement alone. Furthermore, more relief of IBS symptoms was observed in the zinc plus probiotics group. There are several possible mechanisms, including improved zinc bioavailability, increased production of SCFAs, reduced leaky gut, anti-inflammatory synergy, and microbiota modulation through probiotics (especially multi-strain formulations)^{42,43}. The review by Wong-Chew et al. highlighted the synergistic potential of zinc and *Alkalihalobacillus clausii* in gut health and immunity promotion. This combination could manage acute diarrhea and antibiotic-associated dysbiosis in children up to adolescence⁴⁴. Moreover, Park et al. found that a blend of probiotics, rosavin, zinc, and prebiotics alleviated acute colitis in mice by modulating the production of pro- and anti-inflammatory cytokines⁴⁵. Other studies have also examined the effect of co-supplementation of vitamins or other trace elements with probiotics on GI problems. A crossover randomized double-blind controlled trial by Bonfrate et al. on 25 IBS patients reported that the novel formulation of *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* HN001 with B6 vitamin can improve symptoms and severity of disease, and restore IP, and gut microbiota⁴⁶. In another study, Zhu et al. demonstrated that selenium-enriched *Bifidobacterium longum* DD98 restored gut microbiota in antibiotic-disrupted mice through boosting antioxidants, increasing *Lactobacillus*, and suppressing pathogens⁴⁷.

In our study, reduction of TNF-α levels after 90 days in all three groups was observed, but the difference between groups was not significant. This can be due to the almost identical significant reduction in TNF-α levels in any three groups, even in the placebo group, but cannot be attributed to dietary modifications, as the

Variable	Zinc group (n = 28)	Zinc + probiotic group (n = 28)	Placebo group (n = 28)	P-value ^b	P-value ^c
Total quality of life				0.681	0.238
Baseline	56.09 ± 3.49	49.57 ± 4.87	64.31 ± 4.58		
Endpoint	60.69 ± 4.48	59.53 ± 5.79	69.03 ± 3.39		
Change	5.32 ± 5.33	9.95 ± 5.88	4.71 ± 4.50		
P-value ^a	0.328	0.102	0.305		
Dysphoria				0.975	0.704
Baseline	50.80 ± 4.69	47.00 ± 5.58	60.59 ± 5.24		
Endpoint	62.18 ± 5.71	62.50 ± 6.58	67.88 ± 4.63		
Change	12.12 ± 6.42	15.49 ± 6.31	7.28 ± 5.56		
P-value ^a	0.070	0.021	0.202		
Interference with activity				0.686	0.209
Baseline	48.21 ± 2.44	44.26 ± 4.41	57.65 ± 4.30		
Endpoint	57.53 ± 4.93	57.52 ± 6.04	66.83 ± 3.23		
Change	9.39 ± 5.12	13.26 ± 5.74	9.18 ± 4.79		
P-value ^a	0.078	0.029	0.066		
Body image				0.080	0.031 [*]
Baseline	56.47 ± 4.77	45.98 ± 5.18	61.83 ± 4.75		
Endpoint	64.58 ± 4.88	58.03 ± 5.56	76.78 ± 3.14		
Change	9.49 ± 6.05	12.05 ± 6.20	14.95 ± 4.58		
P-value ^a	0.129	0.063	0.003		
Health worry				0.817	0.773
Baseline	39.88 ± 5.13	38.39 ± 5.07	52.38 ± 4.77		
Endpoint	50.30 ± 4.90	54.16 ± 5.59	57.44 ± 5.13		
Change	10.49 ± 6.28	15.77 ± 5.80	5.05 ± 5.18		
P-value ^a	0.107	0.011	0.338		
Food avoidance				0.928	0.714
Baseline	47.32 ± 5.72	43.30 ± 5.90	57.58 ± 4.86		
Endpoint	56.48 ± 6.34	54.01 ± 5.96	62.50 ± 4.81		
Change	10.18 ± 7.06	10.71 ± 5.25	4.91 ± 6.53		
P-value ^a	0.161	0.051	0.459		
Social reaction				0.649	0.213
Baseline	54.46 ± 4.85	48.66 ± 4.93	60.49 ± 4.65		
Endpoint	66.43 ± 5.32	63.39 ± 5.94	73.43 ± 3.41		
Change	12.26 ± 5.54	14.73 ± 6.52	12.94 ± 4.86		
P-value ^a	0.036	0.032	0.013		
Sexual worries				0.378	0.361
Baseline	71.87 ± 5.84	62.50 ± 6.52	71.42 ± 6.61		
Endpoint	72.22 ± 6.12	80.80 ± 5.76	81.25 ± 4.24		
Change	1.38 ± 7.79	18.30 ± 7.67	9.82 ± 7.64		
P-value ^a	0.860	0.024	0.210		
Relationship				0.803	0.234
Baseline	62.85 ± 4.04	52.14 ± 5.25	62.85 ± 4.58		
Endpoint	68.67 ± 4.05	63.16 ± 6.18	72.20 ± 3.38		
Change	6.94 ± 4.84	11.02 ± 5.41	9.34 ± 3.68		
P-value ^a	0.164	0.052	0.017		

Table 4. Within- and between-group comparisons of quality of life and its subscales during the 12-week intervention. Values are reported as mean ± SE. ^aCalculated by paired-sample T-test. ^bCalculated by ANCOVA adjusted for the baseline value of each variable. ^cCalculated by ANCOVA adjusted for the baseline value of each variable, physical activity, supplementation and medicine (antidepressant, antispasmodic and antacid) use. ^{*}Significant difference between the zinc + probiotic and the placebo groups using the Bonferroni post-hoc test.

comparison of the three groups did not show any significant differences in dietary intakes during the time. Zinc supplementation may reduce TNF- α levels by inhibiting nuclear factor- κ B (NF- κ B) activation and reducing oxidative stress through cell membrane stabilization and antioxidant defense systems enhancement⁴⁸. Hosseini et al., in a meta-analysis study on 21 clinical trials, showed that zinc supplementation was associated with a significant reduction in TNF- α levels in adults⁴⁹. Also, Jafari et al. in a 90-day randomized trial study on young women with PMS who received 30 mg/day zinc gluconate revealed a reduction of CRP and IL-6 levels as inflammation biomarkers⁵⁰. Probiotic supplementation may reduce TNF- α levels by several mechanisms. Probiotics may enhance the integrity of the intestinal barrier by increasing tight junction proteins expression and limiting LPS translocation. Thereby, it can attenuate Toll-like receptor 4 (TLR 4)-mediated NF- κ B activation and subsequently decrease TNF- α production⁵¹. Moreover, some probiotics can promote anti-inflammatory cytokines and regulate dendritic cell and macrophage responses, leading to TNF- α suppression⁵². In a recent randomized, double-blind, controlled trial in severe traumatic brain injury patients, supplementation with seven-strain probiotic capsules (Zist Takhmir, Iran) a day containing *Lactobacillus (rhamnosus, casei, acidophilus, bulgaricus)* and *Bifidobacterium (breve and longum)* and *Streptococcus thermophilus* significantly decreased serum TNF- α levels compared with placebo after two weeks of treatment⁵³. Another study reported that the combined in ovo (injection into the egg before hatching) use of a multi-strain probiotic and zinc-glycine chelate modulates the immune response and decreases pro-inflammatory cytokines (TNF- α)⁵⁴. A study by Mohammad Malyar, et al. in 2019 found that zinc-enriched probiotics improved growth, antioxidant capacity, and immune function in heat-stressed Wistar rats²⁸. Psychological modulation, stress reduction through diminishing stress hormones, and awareness of being observed, which can lead to lifestyle change, may be underlying mechanisms of the placebo effect⁵⁵.

Depression, stress, and anxiety are prevalent in IBS patients and linked through the gut-brain axis, where psychological distress exacerbates visceral hypersensitivity⁵⁶. The present study showed that supplementation with zinc or a placebo could reduce depression and stress scores compared to zinc plus probiotics. This finding may be due to a conflict of neurotransmitter effects; Zinc boosts serotonin, while some probiotics divert tryptophan to non-mood pathways^{57,58}. Also, it is possible that probiotics supplement in some participants, when introduced for the first time, activate mild mucosal immune responses, causing short-term GI discomfort such as bloating and changes in bowel habits, which may have caused increased stress about GI symptom exacerbation⁵⁹. Although GI symptoms subsequently improved, this early discomfort might have prevented the zinc and probiotics co-supplementation from showing a significant stress-reducing effect compared to the zinc or placebo supplements. Zinc, as an antioxidant, can regulate neurotransmitters and prevent oxidative stress-driven neuronal damage in the enteric nervous system⁶⁰. Also, zinc may reduce stress and depression symptoms by modulating glutamatergic neurotransmission through N-methyl-D-aspartate (NMDA) receptor inhibition and enhancement of brain-derived neurotrophic factor (BDNF) signaling⁶¹. A recent meta-analysis of randomized clinical trials indicated that zinc supplementation can reduce the risk of and alleviate depression⁶². Moreover, this trace element had beneficial effects on physical and psychological symptoms in young women with premenstrual syndrome⁵⁰. In a randomized clinical trial of adults undergoing methadone maintenance treatment, daily zinc supplementation for three months was associated with a significant reduction in depression, anxiety, and stress⁶³. We used microcrystalline cellulose as a placebo, which is a chemically inert, non-digestible excipient that is not absorbed or systemically metabolized in humans and has no direct physiological effect⁶⁴. Placebo administration may trigger dopamine and endogenous opioid release in the brain, improving mood and pain tolerance⁶⁵. Also, placebo-induced relaxation activates the vagus nerve, which inhibits inflammation in the gut mucosa⁶⁶. It can be hypothesized that the use of specific psychobiotic strains may exert more pronounced anti-stress and anti-depressant effects compared with non-specific multi-strain probiotics; however, this hypothesis warrants further investigation in future trials.

According to the studies, IBS patients have lower QoL than the general population⁶⁷. The IBS-QoL questionnaire evaluates QoL across domains that reflect the main burdens of IBS, including emotional distress (dysphoria), limitation of daily activity, food avoidance, health worry, social and relationship impacts, body image, and sexual concerns⁴⁰. Our study revealed that social reaction was improved at the end of the study compared to the baseline in all three groups. This improvement across all groups may partly reflect the well-documented placebo response in IBS, where enhanced patient-provider interaction and regular follow-up can positively affect psychosocial dimensions of QoL independent of intervention. Furthermore, a significant improvement for dysphoria, interference with activity, health worry, and sexual worries was observed after intervention with zinc plus probiotic supplementation. This finding may be partly explained by the role of zinc in reducing neuroinflammation, together with probiotics' capacity to regulate the gut-brain axis through immune modulation, ultimately influencing psychological well-being^{56,68}. We found that although co-supplementation of zinc and probiotics after 90 days could marginally improve body image ($p = 0.063$), relationship ($p = 0.052$), and food avoidance ($p = 0.051$), no better effect on body image was observed compared to the placebo. The zinc and zinc plus probiotic group had a higher number of people with severe IBS at baseline. In comparison, the placebo group included a higher number of IBS patients with moderate symptom severity. This difference was marginally significant ($p = 0.063$). This can be the reason for the lack of significant effect of the intervention groups on the quality of life and its subscales compared to the placebo group. Our previous case-control study showed that dietary zinc intake was positively correlated with psychological health in the healthy controls and with body image in the diarrhea-predominant IBS patients²⁶. A recent study illustrated that three months of supplementation with probiotics (4.5×10^{11} CFUs of a combination of *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve*, and *Streptococcus thermophilus*, 2 times/day), alone or in combination with 25 mg zinc, enhanced health-related quality of life in patients with cirrhosis⁶⁹. Furthermore, zinc supplementation for three months had a positive effect on physical aspects of quality of life in women with premenstrual syndrome⁷⁰.

As physical activity is a potentially important behavioral factor that can affect clinical symptoms, metabolic and gut-brain interactions in IBS, we measured physical activity at baseline, mid-intervention, and the end of the study⁷¹. In our study, physical activity levels were significantly different between groups. Therefore, physical activity was considered a potential confounder and adjusted in the ANCOVA model to control for its possible effect on outcomes. Controlling for this confounder strengthens the internal validity of the results.

To the best of our knowledge, the current study is the first randomized controlled trial investigating the efficacy of zinc supplement alone or in combination with probiotics on inflammation, IBS severity symptoms, QoL, and mood in IBS patients. Also, we used a safe and tolerable dose of zinc and probiotics⁷². However, some limitations of our study should be addressed. Although the sample size was determined a priori and considered sufficient to detect the primary outcome, its relatively modest size may limit the ability to detect smaller effect sizes and reduce the findings generalizability. Randomization based on age and BMI, in addition to sex, could have further balanced the groups, but it was not feasible given practical constraints and the modest sample size. Also, the lack of a probiotics-only intervention group may be considered a limitation; however, the study was specifically designed to evaluate the independent and combined effects of zinc supplementation. Moreover, there were funding limitations; therefore, the gut microbiota composition and IP were not assessed.

Conclusion

A 90-day zinc supplementation, alone or with a probiotic, could improve serum zinc levels and the severity of GI symptoms in IBS patients. Also, zinc supplement compared to the combination of zinc and probiotic, had a favorable effect on depression and stress symptoms. Therefore, supplementation with zinc or zinc plus probiotics may be an effective adjuvant therapy for IBS, whereas the role of probiotics in improving mood-related outcomes remains unclear. It seems that future studies with a higher dose of the aforementioned supplements with other probiotic strains that can be more focused on mental health and a larger sample size are needed to confirm the results of this study.

Data availability

The datasets generated and/or analysed during the current study are not publicly available as per the rules and regulations of the Department of Clinical Nutrition at the Isfahan University of Medical Science, however, they are available from the corresponding author upon a reasonable request.

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References

- Black, C. J., Drossman, D. A., Talley, N. J., Ruddy, J. & Ford, A. C. Functional gastrointestinal disorders: Advances in understanding and management. *Lancet* **396**(10263), 1664–74 (2020).
- Törnblom, H., Goosey, R., Wiseman, G., Baker, S. & Emmanuel, A. Understanding symptom burden and attitudes to irritable bowel syndrome with diarrhoea: Results from patient and healthcare professional surveys. *United Eur. Gastroenterol. J.* **6**(9), 1417–1427 (2018).
- Gwee, K.-A. & Chua, A. S. B. Functional dyspepsia and irritable bowel syndrome, are they different entities and does it matter. *World J. Gastroenterology: WJG*. **12** (17), 2708 (2006).
- Lovell, R. M. & Ford, A. C. Effect of gender on prevalence of irritable bowel syndrome in the community: Systematic review and meta-analysis. *Am. J. Gastroenterol.* **107**(7), 991–1000 (2012).
- De Bortoli, N. et al. Gastroesophageal reflux disease, functional dyspepsia and irritable bowel syndrome: common overlapping gastrointestinal disorders. *Annals Gastroenterol.* **31** (6), 639 (2018).
- Hausteiner-Wiehle, C. & Henningsen, P. Irritable bowel syndrome: Relations with functional, mental, and somatoform disorders. *World J. gastroenterology: WJG* **20**(20), 6024 (2014).
- Drossman, D. A., Camilleri, M., Mayer, E. A. & Whitehead, W. E. AGA technical review on irritable bowel syndrome. *Gastroenterology* **123** (6), 2108–2131 (2002).
- Oświećimska, J., Szymłak, A., Roczniak, W., Girczys-Poledniok, K. & Kwiecień, J. New insights into the pathogenesis and treatment of irritable bowel syndrome. *Adv. Med. Sci.* **62** (1), 17–30 (2017).
- Chong, P. P. et al. The microbiome and irritable bowel syndrome—A review on the pathophysiology, current research and future therapy. *Front. Microbiol.* **10**, 424646 (2019).
- Acevedo-Román, A., Pagán-Zayas, N., Velázquez-Rivera, L. I., Torres-Ventura, A. C. & Godoy-Vitorino, F. Insights into gut dysbiosis: Inflammatory diseases, obesity, and restoration approaches. *Int. J. Mol. Sci.* <https://doi.org/10.3390/ijms25179715> (2024).
- Rezazadegan, M., Soheilipour, M., Tarrahi, M. J. & Amani, R. Correlation between Zinc nutritional status with serum zonulin and gastrointestinal symptoms in diarrhea-predominant irritable bowel syndrome: A case-control study. *Dig. Dis. Sci.* **67**(8), 3632–8 (2022).
- Raftery, T. et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: results from a randomised double-blind placebo-controlled study. *United Eur. Gastroenterol. J.* **3** (3), 294–302 (2015).
- Ahmadi, A. R. et al. The effects of probiotic/synbiotic on serum level of zonulin as a biomarker of intestinal permeability: A systematic review and meta-analysis. *Iran. J. Public Health* **49**(7), 1222 (2020).
- Lopes, C. M., de Jesus Monteiro, C. S., Duarte, A. P. & Dos Santos, J. L. Probiotics and prebiotics for the treatment of irritable bowel syndrome—A narrative review. *J. Clin. Med.* **13**(21), 6337 (2024).
- Sánchez, B. et al. Probiotics, gut microbiota, and their influence on host health and disease. *Mol. Nutr. Food Res.* **61**(1), 1600240 (2017).
- Marlicz, W., Skonieczna-Żydecka, K., Krynicka, P., Łoniewski, I. & Rydzewska, G. Probiotics in irritable bowel syndrome—Is the quest for the right strain over? Rapid review of existing guidelines and recommendations. *Gastroenterol. Rev.* **16**(4), 369–82 (2021).
- Zhang, T., Zhang, C., Zhang, J., Sun, F. & Duan, L. Efficacy of probiotics for irritable bowel syndrome: A systematic review and network meta-analysis. *Front. Cell. Infect. Microbiol.* **12**, 859967 (2022).
- Xie, C.-R. et al. Low FODMAP diet and probiotics in irritable bowel syndrome: A systematic review with network meta-analysis. *Front. Pharmacol.* **13**, 853011 (2022).
- Skrovanek, S. et al. Zinc and gastrointestinal disease. *World J. Gastrointest. pathophysiology.* **5** (4), 496–513 (2014).

20. Sangeetha, V., Dutta, S., Moses, J. & Anandharamakrishnan, C. Zinc nutrition and human health: Overview and implications. *EFood* **3**(5), e17 (2022).
21. Wessells, K. R. & Brown, K. H. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS One* **7**(11), e50568 (2012).
22. Houshiar-Rad, N. et al. Zinc intake pattern in Iranian households and risk of zinc deficiency at national level. (2013).
23. Jin, H. et al. T lymphocyte subgroups and serum levels of trace elements in patients with diarrhea type of irritable bowel syndrome. *Zhejiang Da Xue Xue Bao Yi Xue Ban* **37**(6), 634–7 (2008).
24. Hujoel, I. A. Nutritional status in irritable bowel syndrome: A North American population-based study. *JGH Open* **4**(4), 656–62 (2020).
25. Bahrami, A. et al. The association of trace elements with premenstrual syndrome, dysmenorrhea and irritable bowel syndrome in adolescents. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **233**, 114–9 (2019).
26. Rezazadegan, M., Shahdadian, F., Soheilipour, M., Tarrahi, M. J. & Amani, R. Zinc nutritional status, mood states and quality of life in diarrhea-predominant irritable bowel syndrome: A case–control study. *Sci. Rep.* **12**(1), 11002 (2022).
27. Scholz-Ahrens, K. E. et al. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J. Nutr.* **137**(3), 838S–46S (2007).
28. Mohammad Malyar, R. et al. Zinc-enriched probiotics enhanced growth performance, antioxidant status, immune function, gene expression, and morphological characteristics of Wistar rats raised under high ambient temperature. *3 Biotech.* **9**, 1–12 (2019).
29. Varvara, R.-A. & Vodnar, D. C. Probiotic-driven advancement: Exploring the intricacies of mineral absorption in the human body. *Food Chem. X* **21**, 101067 (2024).
30. Goodoory, V. C., Riggott, C., Khasawneh, M., Black, C. J. & Ford, A. C. Validating simple modifications to the Rome IV criteria for the diagnosis of irritable bowel syndrome in secondary care. *Aliment. Pharmacol. Ther.* **61**(2), 354–362 (2025).
31. Mirzaie, Z. et al. Effects of ellagic acid on oxidative stress index, inflammatory markers and quality of life in patients with irritable bowel syndrome: Randomized double-blind clinical trial. *Clin. Nutr. Res.* **11**(2), 98 (2022).
32. Li, J. et al. Zinc intakes and health outcomes: An umbrella review. *Front. Nutr.* **9**, 798078 (2022).
33. Boonma, P. et al. Probiotic VSL# 3 treatment reduces colonic permeability and abdominal pain symptoms in patients with irritable bowel syndrome. *Front. Pain Res.* **2**, 691689 (2021).
34. Khodadoostan, M., Shavakhi, A., Sherafat, Z. & Shavakhi, A. Effect of probiotic administration immediately and 1 month after colonoscopy in diarrhea-predominant irritable bowel syndrome patients. *Adv. Biomed. Res.* **7**(1), 94 (2018).
35. Chey, W. D., Hashash, J. G., Manning, L. & Chang, L. AGA clinical practice update on the role of diet in irritable bowel syndrome: Expert review. *Gastroenterology* **162**(6), 1737–45. e5 (2022).
36. Ghaffarpour, M., Houshiar-Rad, A. & Kianfar, H. The manual for household measures, cooking yields factors and edible portion of foods. *Tehran: Nashre Olume Keshavarzy.* **7** (213), 42–58 (1999).
37. Moghaddam, M. B. et al. The Iranian version of International Physical Activity Questionnaire (IPAQ) in Iran: Content and construct validity, factor structure, internal consistency and stability. *World Appl. Sci. J.* **18**(8), 1073–80 (2012).
38. Francis, C. Y., Morris, J. & Whorwell, P. J. The irritable bowel severity scoring system: A simple method of monitoring irritable bowel syndrome and its progress. *Aliment. Pharmacol. Ther.* **11**(2), 395–402 (1997).
39. Sahebi, A., Asghari, M. J. & Salari, R. S. Validation of depression anxiety and stress scale (DASS-21) for an Iranian population. (2005).
40. Drossman, D. A. et al. Further validation of the IBS-QOL: A disease-specific quality-of-life questionnaire. *Am. J. Gastroenterol.* **95**(4), 999–1007 (2000).
41. Gholamrezaei, A. et al. Linguistic validation of the irritable bowel syndrome-quality of life questionnaire for Iranian patients. *Acta Med. Iranica* **2011**, 390–395 (2011).
42. Wu, X., Cao, Y., Liu, Y. & Zheng, J. A new strategy for dietary nutrition to improve intestinal homeostasis in diarrheal irritable bowel syndrome: A perspective on intestinal flora and intestinal epithelial interaction. *Nutrients* **16**(18), 3192 (2024).
43. Kwoji, I. D., Aiyegoro, O. A., Okpeku, M. & Adeleke, M. A. Multi-strain probiotics: Synergy among isolates enhances biological activities. *Biology* <https://doi.org/10.3390/biology10040322> (2021).
44. Wong-Chew, R. M., Nguyen, T. V. H., Rogacion, J. M., Herve, M. & Pouteau, E. Potential complementary effect of zinc and *Alkalihalobacillus clausii* on gut health and immunity: A narrative review. *Nutrients* **16**(6), 887 (2024).
45. Park, J.-S. et al. A probiotic complex, rosavin, zinc, and prebiotics ameliorate intestinal inflammation in an acute colitis mouse model. *J. Transl. Med.* **16**(1), 37 (2018).
46. Bonfrate, L. et al. Effects of *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* HN001 in IBS patients. *Eur. J. Clin. Invest.* **50**(3), e13201 (2020).
47. Zhu, H. et al. Preparation and characterization of selenium enriched-*Bifidobacterium longum* DD98, and its repairing effects on antibiotic-induced intestinal dysbacteriosis in mice. *Food Funct.* **10** (8), 4975–4984 (2019).
48. Prasad, A. S. Zinc in human health: Effect of zinc on immune cells. *Mol. Med. (Cambridge Mass)* **14**(5–6), 353–357 (2008).
49. Hosseini, R., Ferns, G. A., Sahebkar, A., Mirshekar, M. A. & Jalali, M. Zinc supplementation is associated with a reduction in serum markers of inflammation and oxidative stress in adults: A systematic review and meta-analysis of randomized controlled trials. *Cytokine* **138**, 155396 (2021).
50. Jafari, F., Amani, R. & Tarrahi, M. J. Effect of zinc supplementation on physical and psychological symptoms, biomarkers of inflammation, oxidative stress, and brain-derived neurotrophic factor in young women with premenstrual syndrome: A randomized, double-blind, placebo-controlled trial. *Biol. Trace Elem. Res.* **194**(1), 89–95 (2020).
51. Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M. & Gil, A. Mechanisms of action of probiotics. *Advances in nutrition (Bethesda, Md)* **10**(suppl_1), S49–s66 (2019).
52. Caliman-Sturdza, O. A. et al. Antioxidant and anti-inflammatory activities of probiotic strains. *Int. J. Mol. Sci.* **27**(2), 1079 (2026).
53. Abbaszadeh, S. H., Yousefi, M., Arefhosseini, S. R., Mahmoodpoor, A. & Mameghani, M. E. Effect of a seven-strain probiotic on dietary intake, inflammatory markers, and T-cells in severe traumatic brain injury patients: A randomized, double-blind, placebo-controlled trial. *Sci. Prog.* **107**(3), 368504241259299 (2024).
54. Ciszewski, A. et al. Influence of a multi-strain probiotic and zinc-glycine chelate, administered in ovo, on immune response in newly hatched chicks. *Front. Physiol.* **16**, 1646143 (2025).
55. Prossin, A. et al. Effects of placebo administration on immune mechanisms and relationships with central endogenous opioid neurotransmission. *Mol. Psychiatry* **27** (2), 831–839 (2022).
56. Dinan, T. G. & Cryan, J. F. The microbiome-gut-brain axis in health and disease. *Gastroenterol. Clin. North Am.* **46**(1), 77–89 (2017).
57. Doboszewska, U. et al. Zinc in the monoaminergic theory of depression: Its relationship to neural plasticity. *Neural Plast.* **2017**, 3682752 (2017).
58. O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G. & Cryan, J. F. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav. Brain Res.* **277**, 32–48 (2015).
59. Doron, S. & Snyderman, D. R. Risk and safety of probiotics. *Clin. Infect. Dis.* **60**(Suppl 2(Suppl 2)), S129–34 (2015).
60. Jarosz, M., Olbert, M., Wyszogrodzka, G., Młyniec, K. & Librowski, T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF- κ B signaling. *Inflammopharmacology* **25** (1), 11–24 (2017).
61. Piao, M., Cong, X., Lu, Y., Feng, C. & Ge, P. The role of zinc in mood disorders. *Neuropsychiatry* **7**(4), 378–86 (2017).
62. Yosae, S. et al. Zinc in depression: From development to treatment: A comparative/ dose response meta-analysis of observational studies and randomized controlled trials. *Gen. Hosp. Psychiatry* **74**, 110–7 (2022).

63. Amini, Z. & HeidariFarsani, E. Investigating the effect of zinc supplementation on probability of relapse and mental health in patients with opioid use disorder undergoing methadone maintenance treatment. *Subst. Abuse Treat. Prev. Policy*. **18** (1), 1 (2023).
64. Janvalkar, M., Kaloji, P. & Shettigar, R. Microcrystalline cellulose in pharmaceutical formulations: A comprehensive review on applications, concentrations, and functional attributes. *Int. J. Pharm. Sci.* **3**, 3098–3112 (2025).
65. Zubieta, J. K. et al. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J. Neurosci.* **25**(34), 7754–62 (2005).
66. Breit, S., Kupferberg, A., Rogler, G. & Hasler, G. Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Front. Psychiatry* **9**, 44 (2018).
67. Cassar, G. E., Youssef, G. J., Knowles, S., Moulding, R. & Austin, D. W. Health-related quality of life in irritable bowel syndrome: A systematic review and meta-analysis. *Gastroenterol. Nurs.* **43**(3), E102–e22 (2020).
68. Swardfager, W. et al. Zinc in depression: A meta-analysis. *Biol. Psychiatry* **74**(12), 872–8 (2013).
69. Amooyi, L., Alizadeh, L., Sarbakhsh, P., Shojaei-Zarghani, S. & Gharekhani, A. The effects of adding probiotic, alone and in combination with zinc, to routine treatment on recurrence of hepatic encephalopathy, quality of life, and sleep quality in patients with cirrhosis: An open-label randomized controlled trial. *Food Sci. Nutr.* **13**(1), e4636 (2025).
70. Jafari, F., Tarrahi, M. J., Farhang, A. & Amani, R. Effect of zinc supplementation on quality of life and sleep quality in young women with premenstrual syndrome: A randomized, double-blind, placebo-controlled trial. *Arch. Gynecol. Obstet.* **302**(3), 657–664 (2020).
71. Li, C. et al. Effects of physical exercise on the microbiota in irritable bowel syndrome. *Nutrients* <https://doi.org/10.3390/nu16162657> (2024).
72. Schoofs, H., Schmit, J. & Rink, L. Zinc toxicity: Understanding the limits. *Molecules* <https://doi.org/10.3390/molecules29133130> (2024).

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Author contributions

MR participated in designing, data collection, performed the statistical analysis and wrote the first draft. RA designed and supervised the study, and edited the manuscript. MS was a gastroenterologist and managed the patients.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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