

RESEARCH ARTICLE

Efficacy and safety of a food supplement with standardized menthol, limonene, and gingerol content in patients with irritable bowel syndrome: A double-blind, randomized, placebo-controlled trial

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Abstract

Background

Irritable bowel syndrome (IBS) affects 9,2% of the global population and places a considerable burden on healthcare systems. Most medications for treating IBS, including spasmolytics, laxatives, and antidiarrheals, have low efficacy. Effective and safe therapeutic treatments have yet to be developed for IBS.

Purpose

This study assessed the efficacy and safety of a food supplement containing standardized menthol, limonene, and gingerol in human participants with IBS or IBS/functional dyspepsia (FD).

Design

A double-blind, randomized, placebo-controlled trial.

Methods

We randomly assigned 56 patients with IBS or IBS/FD to an intervention group (Group 1) or control group (Group 2) that were given supplement or placebo, respectively, in addition to the standard treatment regimen for 30 d. Three outpatient visits were conducted during the study. Symptom severity was measured at each visit using a 7×7 questionnaire. Qualitative and quantitative composition of the intestinal microbiota were assessed at visits 1 and 3 based on 16S rRNA gene sequencing.

Results

At visit 1 (before treatment), the median total 7×7 questionnaire score was in the moderately ill range for both groups, with no difference between the groups ($p = 0.1$). At visit 2, the total 7×7 score decreased to mildly ill, with no difference between the groups ($p = 0.4$). At visit 3, the total score for group 1 indicated borderline illness and for group 2 remained indicated mild illness ($p = 0.009$). Even though we observed some variations in gut microbiota between the groups, we did not find any statistically significant changes.

Conclusion

The food supplement with standardized menthol, limonene, and gingerol content increased the efficacy of standard therapy in IBS and FD patients. The use of the supplement did not cause any obvious side effects.

Registration

ClinicalTrials.gov Identifier: [NCT04484467](https://clinicaltrials.gov/ct2/show/study/NCT04484467)

Introduction

Irritable bowel syndrome (IBS) is the most prevalent functional disorder worldwide and 27.0%–82.6% of patients with IBS exhibit symptoms of functional dyspepsia (FD) [1, 2]. These diseases impair the patient's quality of life, reduce their capacity for social activity, and create significant healthcare expenses [3]. Based on diagnosis by Rome III criteria, the prevalence of IBS is estimated at 10,7% in Bangladesh and 9,2% worldwide [4].

Most medications for IBS, including spasmolytics, laxatives, and antidiarrheals, have relatively low efficacy and optimal treatment approaches for IBS and FD have yet to be developed [5, 6]. Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors have shown superior efficacy in the treatment of IBS and FD in placebo control studies, though adverse effects have a high prevalence, particularly among patients treated with TCAs [7, 8].

Recently, a relationship was established between gut microbiota and a functional gastrointestinal tract disorder [9]. The composition of the intestinal microbiota in patients suffering from IBS is characterized by decreased content of bacterial cells producing short-chain fatty acids (SCFAs) [10], which regulates the expression of tight contact proteins, T-lymphocytes, and cytokines and ensures adequate permeability of the intestinal barrier [11]. Disruption of intestinal barrier permeability leads to inflammatory responses, changes in sensitivity and motility, and the development of disease symptoms in the intestinal wall [12–14]. Therefore, the inclusion of probiotics in the treatment regimen promotes overall therapeutic efficacy [15, 16].

IBS pathogenesis represents a complex interplay among changes in intestinal permeability and microbial dysbiosis within the gut, altered mucosal immune function, visceral hypersensitivity, impaired gut motility [17]. Given the comprehensively described pathology of IBS, we aimed to analyze the efficacy of treatment with a food supplement and how its components affect each pathogenetic stage as well as the composition of intestinal microbiota.

Some herbal ingredients have been reported to relieve IBS symptoms. Menthol has an anti-spasmodic, analgesic effect and suppresses the growth of pathogenic microorganisms; D-limonene participates in esophagus and stomach mucus barrier recovery; and gingerol has an

antispasmodic and prokinetic effect (Table 1). Thus, the proposed treatment consists of these components and should cover almost all known pathophysiological links of IBS disorder.

We designed a double-blind, randomized, placebo-controlled trial to assess the efficacy and safety of a food supplement standardized for menthol, limonene, and gingerol content (hereinafter referred to as “the supplement”) in IBS and IBS/FD patients.

Methods

Trial design

We designed an interventional (clinical trial), randomized, double-blind controlled trial protocol that was approved by the Ethics Committee of the Mental Health Research Center, Moscow, Russian Federation (no. 418, 01/31/2018). Written informed consent was obtained from all participants.

Participants

Participants with IBS or IBS and FD were recruited during their outpatient medical treatment at the Mental Health Research Center and Sechenov University, Moscow, Russia. Symptoms were validated according to the Rome IV criteria. Non-functional causes for the symptoms were excluded after a detailed evaluation of medical history, physical examination, extensive panel of blood tests, stool analysis, and colonoscopy with biopsies [28, 29]. The exclusion criteria were: patients younger than 18 years, older than 59 years, with organic bowel disease, with renal disease, with hepatic insufficiency, or with mental illness significantly impairing self-report (e.g., schizophrenia, bipolar disorder, or epilepsy) (Fig 1).

Interventions

Standard treatment regimen. Diarrhea-predominant IBS patients (IBS-D) and mixed bowel habits IBS patients (IBS-M) were treated with spasmolytics. Constipation-predominant IBS patients (IBS-C) were treated with spasmolytics and laxatives. IBS/FD patients were treated with spasmolytics and proton pump inhibitors.

Additional treatment regimen. At visit 1, all patients were randomly assigned into two groups and were assigned a 30-day standard treatment regimen and 1 capsule, 730 mg, once a day in a double-blind manner. The intervention group (group 1) received the supplement “Standart Zdorovya GASTRO” and the control group (group 2) received a placebo (Table 2). During the study period, three outpatient visits were conducted (visit 1 at day 1, visit 2 at 15 ± 2 d, and visit 3 at 30 ± 2 d).

Outcomes

Primary outcome measures. Changes in symptom severity of IBS and FD (constipation-predominant, diarrhea-predominant, mixed-type IBS and IBS/FD) were assessed at each visit using a 7×7 questionnaire.

Secondary outcome measures. Changes in the number of SCFA producing bacteria as well as the qualitative and quantitative composition of intestinal microbiota were assessed at visits 1 and 3, based on 16S rRNA gene sequencing data.

Sample size

Due to a lack of comparable studies, we were unable to determine an appropriate sample size. Using GPower 3.1 software, a sample size of 38 patients was calculated according to Jacob Cohen’s effect size estimation method: statistical significance $\alpha = 0.05$, power $(1-\beta) = 0.80$, and

Table 1. Components of the supplement and their mechanisms of action.

Component	Antispasmodic effect	Analgesic effect	Effect on the intestinal microbiota composition	Prokinetic effect	Esophagus and stomach mucus barrier recovery
Menthol	+ Calcium channel blocking [18] TRPM8 channel receptor activation [18] Nicotinic cholinergic receptor desensitization [18]	+ Blocking of central [19] and peripheral Na ⁺ channels [20] GABA receptor activation [20]	+ Inhibition of pathogenic microorganisms: <i>H. pylori</i> , <i>S. enteritidis</i> , <i>E. coli</i> O157:H7, and <i>S. aureus</i> [21]	-	-
D-limonene	-	-	-	+ [22]	+ Decrease of NO and prostaglandin E2 expression [23, 24] Decrease of TNF- α and IL-6 levels in plasma [23]
Gingerol	+ Suppression of Ca ²⁺ entry into cells via L-type Ca ²⁺ channels and inhibition of ionotropic 5-HT-3 receptors [25]	-	-	+ Suppression of 5-HT-3 and 5-HT-4 receptors [26] Activation of M3 cholinergic receptors in the stomach [27] Inhibition of M1 and M2 presynaptic cholinergic receptors [27]	-

* The mechanism is not studied, yet.

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expected effect size $d = 0.70$ (moderate to strong) [30]. To increase the statistical power of the study, a total of 56 patients were recruited.

Randomization

According to symptom severity, the patients were assigned to one of six groups, determined by the sum of the 7×7 scores: 0–1, normal (healthy); 2–6, borderline ill; 7–12, mildly ill; 13–18, moderately ill; 19–24, markedly ill; >25, severely ill. Effective therapy of IBS greatly decreased the sum of the 7×7 scores (by more than 6 points) in previous studies [31] and effect size (Cohen's d) can reach 2. The effect size values normally range from 0.3 (low effectiveness of treatment) to 0.5 (moderate effectiveness) and up to 0.8 (high effectiveness). We assumed that the use of food supplements would show a high effect size and reduced the overall 7×7 scores. Thus, clinically significant decreases in the total 7×7 score (5.4 points on average) should have an effect size of 0.07.

Blinding

Being a double-blind trial, both participants and investigators were blinded to the treatment. Placebo and food supplement “Standart Zdorovya GASTRO” were delivered to the center in non-transparent packages. The containers were numbered by the manufacturer and neither the researchers nor the patients knew what number belonged to placebo or food supplement “Standart Zdorovya GASTRO”.

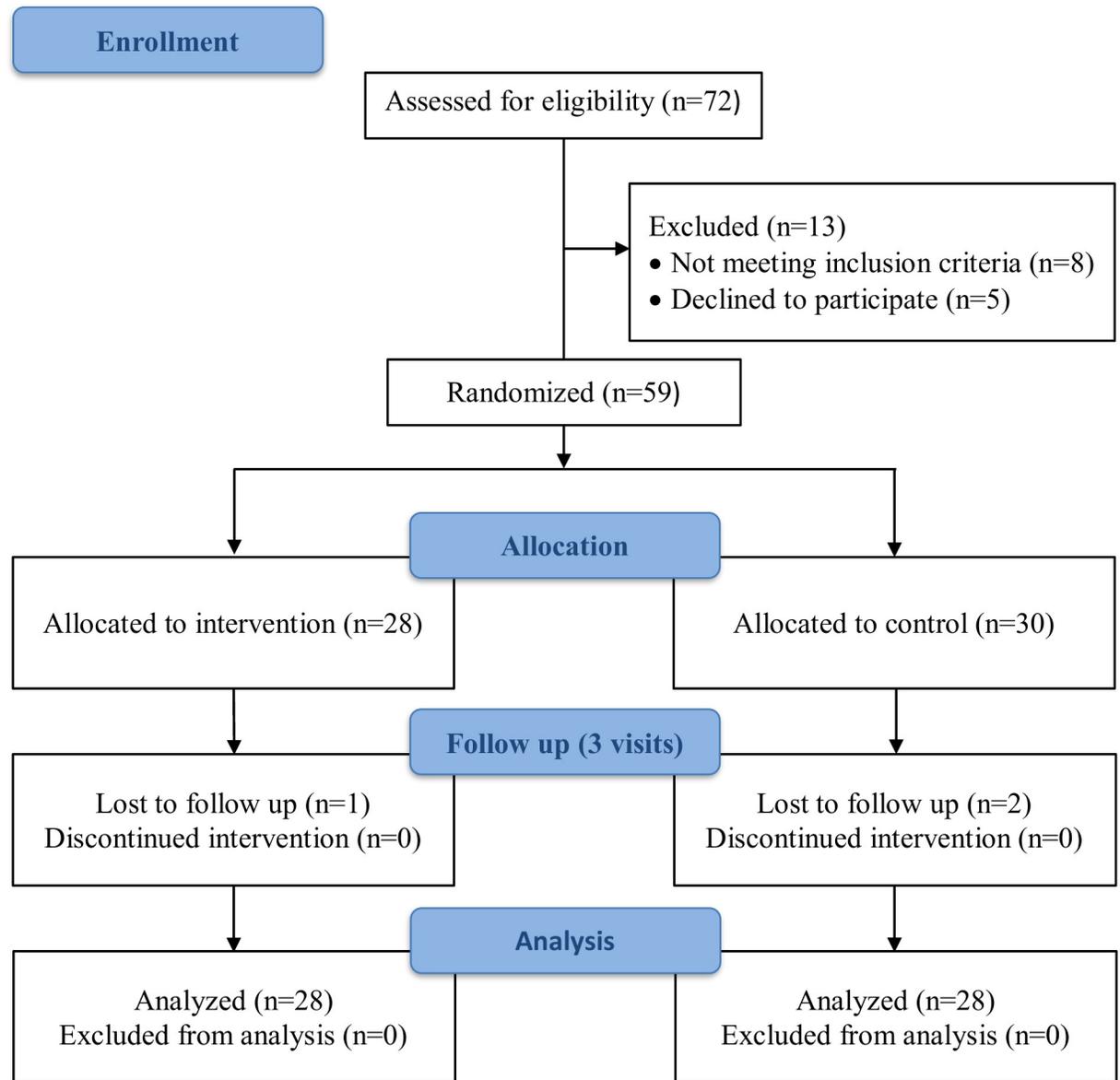


Fig 1. CONSORT flow diagram.

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Table 2. Components in the supplement and placebo capsules.

Drug	Components	Amount of substance (mg)
Supplement “Standart Zdorovya GASTRO”	Peppermint oil (40% menthol, 1.5% limonene)	240
	Ginger oil (14% gingerol)	50
	Olive oil	440
Placebo	Olive oil	730

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Assessment tools

At each visit, patients completed the 7×7 questionnaire [32] as an assessment of symptom severity. At visits 1 and 3, the qualitative and quantitative composition of intestinal microbiota were determined based on 16S rRNA gene sequencing.

7×7 Questionnaire

The 7×7 questionnaire allows patients to record the main symptoms of FD and IBS and the physician to obtain a quantitative description of symptom frequency and intensity for objective evaluation. The following points are assigned according to symptom frequency: none, 0 points; once a week, 1 point; two to three times a week, 2 points; daily, 3 points; and several times a day, 4 points. The intensity is estimated as mild (1 point), moderate (2 points), and severe (3 points). Solid/“nuts” stool once a week and two to three times a week are scored at 5 points and 2 points, respectively. Liquid or mushy stool with other symptoms (pain in the stomach area, a feeling of burning in the stomach area, fullness in the stomach, early satiety, abdominal pain that decreases after a bowel movement, or bloating) is assigned 0 to 4 points. The scores reflecting symptom presence and intensity are summarized, and the scores obtained for each symptom are summed. According to the total score, patients would be assigned to one of six groups: 0–1 points, healthy; 2–6 points, borderline ill; 7–12 points, mildly ill; 13–18 points, moderately ill; 19–24 points, markedly ill; and 25 or more points, severely ill [19].

Safety

The safety of treatment was assessed at Visits 1, 2 and 3 by interviewing the patients about the additional complaints. Blood samples were collected for the routine blood tests (hemoglobin level, red blood cell count, white blood cells count, erythrocyte sedimentation rate, aspartate aminotransferase level, alanine aminotransferase level, alkaline phosphatase level, gamma-glutamyltransferase level, creatinine level, pancreatic alpha-amylase level) at visit 1 and 3.

DNA isolation, 16S library preparation, and qualitative and quantitative composition of intestinal microbiota based on 16S rRNA gene sequencing

Total DNA was isolated with an AmpliPrimeDNA-sorb-AM kit (NextBio, Moscow, Russia) for clinical specimens, according to the manufacturer’s protocol, and stored at -20°C . For qualitative and quantitative assessment of the isolated DNA, we used a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Next, a 16S library was prepared according to the Illumina MiSeq protocol for 16S Metagenomic Sequencing Library Preparation (Illumina, San Diego, CA, USA). The first round of 16S rDNA amplification of the V3–V4 variable regions was performed using the following primers: forward 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′ and reverse 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3′. These primers are aimed at the amplification of bacterial (more than 90% taxonomic coverage) but not archaeal (less than 5%) rRNA genes. The amplification cycle (2720 Thermal Cycler, Applied Biosystems, Waltham, MA, USA) was programmed as: 1) 95°C for 3 min; 2) 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 3) 72°C for 5 min; and 4) 4°C .

The derived amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) according to the manufacturer’s protocol. The second amplification round was used to double-index the samples with a combination of specific primers. The

amplification program was set to: 1) 95°C for 3 min; 2) 8 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 3) 72°C for 5 min; and 4) 4°C.

The products of the second PCR round were purified using Agencourt AMPure XP beads. The concentrations of the 16S rDNA libraries were measured with a Qubit 2.0 fluorimeter (Invitrogen, Waltham, MA, USA) utilizing a Quant-iT dsDNA High-Sensitivity Assay Kit. The purified amplicons were mixed equimolarly according to the concentration values. The quality of the libraries was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and an Agilent DNA 1000 Kit. Sequencing was carried out on a MiSeq system (Illumina) with a MiSeq Reagent Kit v2 (paired-end reads, 2 × 250 nt, Illumina).

The analysis of 16S rRNA gene sequencing data

Since the reads overlap was small (the average amplicon length was about 440–460 bp at sequencing 2 × 250 nt), the reads were pre-merged (before analysis, in DADA2) using the MeFiT tool [33]. For most samples, more than 99.5% of the reads were successfully merged and analyzed by the DADA2 package for R [34]. For the analysis: 1) primer sequences were removed using cutadapt; 2) the reads were filtered by quality; 3) error distribution models were derived based on read quality profiles; 4) sequencing errors were corrected; 5) ribosomal sequence variants (RSV), OTU analogues, were inferred; and 6) chimeric RSVs, accounting for 62% and 4.6% of all RSV and all reads, respectively, were eliminated. Next, taxonomic annotation of the RSVs (7,551 after the removal of chimeras) was carried out using DADA and the 16S RDP reference sequence database [35]. The data obtained were also analyzed utilizing the Piphillin web service [36], which predicts the metabolic potential of a bacterial community based on the analysis of representative 16S rRNA gene sequences (OTU or RSV). Further analysis was carried out using our own R (including the vegan, fossil, ggplot2, and pheatmap packages) and Python scripts. A Mann-Whitney test was used for comparison between groups, a Wilcoxon test for comparison between visits to the same patient, and a Student's *t*-test for comparison of normally distributed data. Spearman correlation coefficients were calculated.

Statistical analysis

Statistical analysis was carried out using standard functions and additional packages in the R environment [37, 38]. To assess differences between groups of patients, analysis of contingency tables (including Fisher's exact test) and a non-parametric Mann-Whitney test were used. The differences between visits in each group of patients was determined using a Friedman Rank test and a post-hoc Wilcoxon signed-rank test for paired samples, with *p*-values adjusted according to Bonferroni multiple testing (FDR). The null hypothesis assumed the absence of a relationship between the type of therapy/stage of the study and the change in the analyzed parameter. Two-sided *p*-values <0.05 were considered statistically significant.

Deviations from the original protocol

There were no deviation from the original protocol.

Study

As this was originally a pilot study, we assumed that the data would not be sufficient for a full-scale clinical trial or publication. Thus, the study was registered only after the initiation of patient recruitment. The authors can confirm that all ongoing and related trials have been registered.

Results

The trial was performed from February 2018 to December 2019 at the Mental Health Research Center, Moscow, Russia. Two groups of 56 patients collectively completed the study (Table 3). There were no significant differences in gender, age, diagnosis, disease duration, and 7×7 questionnaire results between the groups at the first visit.

7×7 Questionnaire

There was no statistically significant difference in the total 7×7 questionnaire scores between the groups at visit 1 and patients in both groups were moderately ill (see Table 2). Throughout all three visits, the treatment effect on the total 7×7 questionnaire scores was tested using the Friedman test (Table 1 in S4 File) followed by the Wilcoxon signed-rank test (Table 2 in S4 File). There was a statistically significant decrease in the total 7×7 questionnaire score in both groups to scores indicating mild illness at visit 2 (mean difference, -4.04; 95% CI, -6.10 to -1.97; $p = 0.001$ in group 1 and mean difference, -4.86; 95% CI, -6.75 to -2.96; $p = 0.0004$ in group 2). There were no significant differences ($p = 0.42$) between groups at visit 2. At visit 3, the total score in group 1 decreased to a score indicating borderline illness (mean difference, -2.68; 95% CI, -4.29 to -1.07; $p = 0.009$). Group 2 remained in the score range indicating mild illness (Fig 2).

The total 7×7 questionnaire score decreased significantly in group 1 from 13 (95% CI 11.4–15.0) at visit 1 to 9.18 (95% CI 7.07–11.3) at visit 2 and 6.5 (95% CI 4.58–8.42) at visit 3. In group 2, the 7×7 questionnaire score decreased significantly between visits 1 and 2 (15.9 [95% CI 13.4–18.5] to 11.1 [95% CI 8.4–13.7]). * The differences between the groups are significant ($p < 0.05$); ns–non-significant.

Between visit 1 to visit 3, there was a decrease in the number of group 1 patients with epigastric pain ($p = 0.029$), solid/"nuts" stool ($p = 0.005$), abdominal pain that decreases after a bowel movement ($p < 0.0001$), and bloating ($p = 0.015$) (S1–S6 Figs in S3 File).

Qualitative and quantitative composition of the intestinal microbiota based on 16S rRNA gene sequencing. At visit 1, we observed some differences in the gut microbiota composition between groups 1 and 2. For several genera, we noticed 1.5–2.5-fold change in the average bacterial content (normalized read counts per taxon) between the groups, some of which passed the Mann-Whitney U-test $p < 0.05$ threshold, but none with statistically significant FDR (e.g., *Blautia*, *Anaerostipes*, and *Paraprevotella*). The 60 bacterial genera with the highest abundance in patients of groups 1 and 2 at visit 1 are presented in Fig 3. A high degree of heterogeneity was observed between patients.

Using the Piphillin web service, we inferred the relative abundance of genes involved in fatty acid biosynthesis (FAB) at visit 1. While the total abundance of all genes involved in FAB was practically identical (differing by less than 1%), significant differences were noted for the *fabH* gene (Fig 4), though it did not pass the FDR threshold.

At visit 3, we observed some differences in the composition of gut microbiota between groups 1 and 2. *Oscillibacter* had higher prevalence in group 1, whereas *Veillonella*, *Collinsella*, and *Gemmiger* were more prevalent in group 2 (Fig 5). However, the overall magnitude of these differences was comparable to that at visit 1. Although p-values (Mann-Whitney U-test) for these events were lower than 0.05, they did not pass the FDR < 0.05 threshold.

The same observations were made for the relative content of genes involved in FAB (Fig 6). In this case, very slight (~2%) differences were noted for individual genes (*fabZ*) in favor of the patients taking dietary supplements. Even though they passed the Mann-Whitney U-test $p < 0.05$ threshold, they did not pass multiple test adjustments (FDR). The magnitude of inter-group differences at visit 3 was comparable to that at visit 1.

Table 3. Comparison of patients in groups 1 and 2 by sex, age, and disease duration.

Sign	Group 1 (intervention) (n = 28)	Group 2 (control) (n = 28)	p-value
Age, years	35.25 (±11.84)	31.8 (±10.76)	0.30
Sex, n (%)			
Men	13 (46%)	10 (36%)	0.79
Women	15 (54%)	18 (64%)	
Diagnosis, n (%)			
IBS	20 (71%)	19 (68%)	0.17
IBS+FD	8 (29%)	9 (32%)	
IBS duration, months*	48 (17.5–111)	48 (24–90)	0.98
Total 7×7 questionnaire score**	13.2 (11.4–15.0)	15.9 (13.4–18.5)	0.1

* - Median (1–3 quartiles, Q25–Q75);

** - 95% Confidence interval (CI).

<https://doi.org/10.1371/journal.pone.0263880.t003>

Correlation analysis between microbiome composition and 7×7 questionnaire scores.

In order to identify the genera and families of bacteria associated with IBS severity, we carried out a correlation analysis between the relative abundance of various bacterial taxa and the sum of the 7×7 questionnaire scores across all visits. Statistically significant associations (that passed the FDR threshold < 0.05) were noticed only for the genus *Fusobacterium* and its

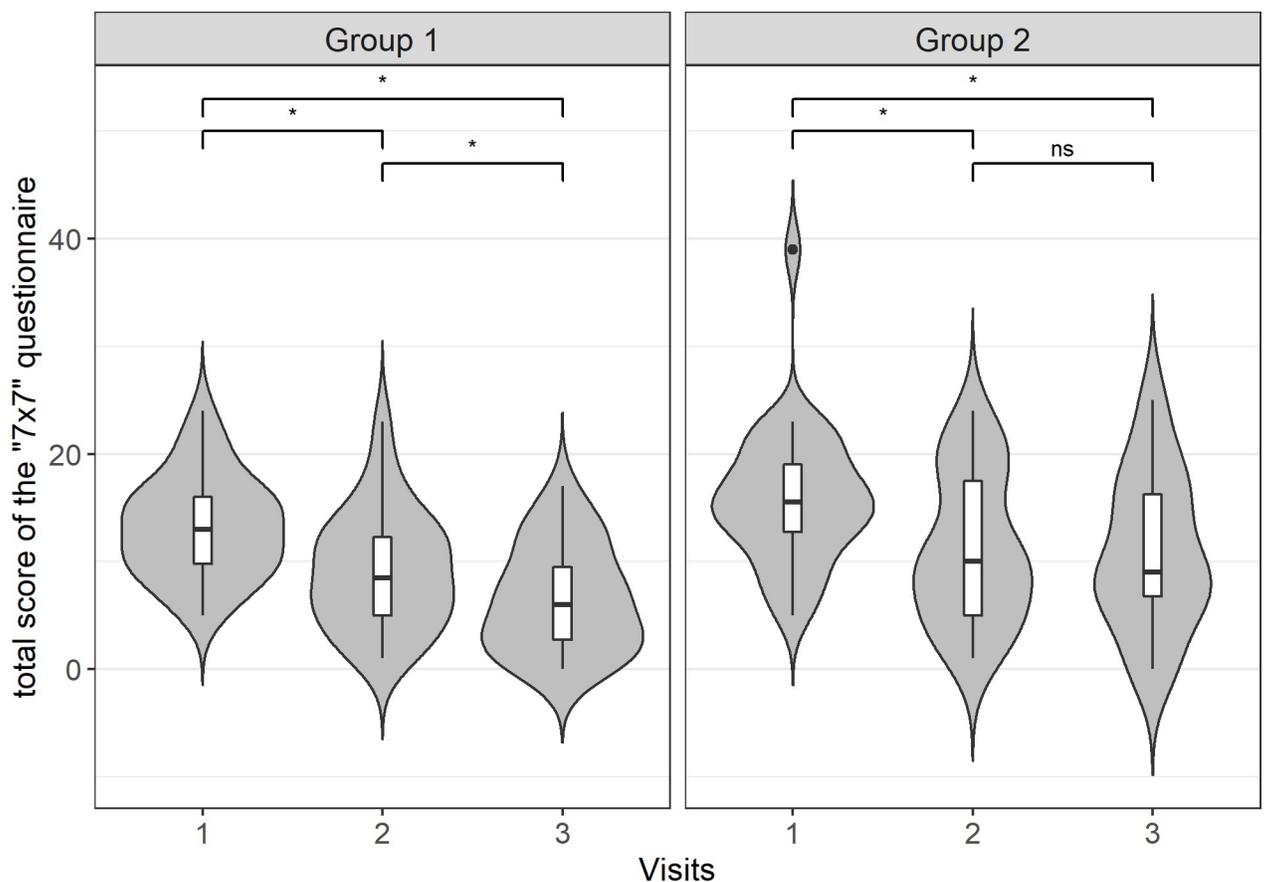


Fig 2. Total 7×7 questionnaire scores in group 1 and group 2 at visits 1, 2, and 3.

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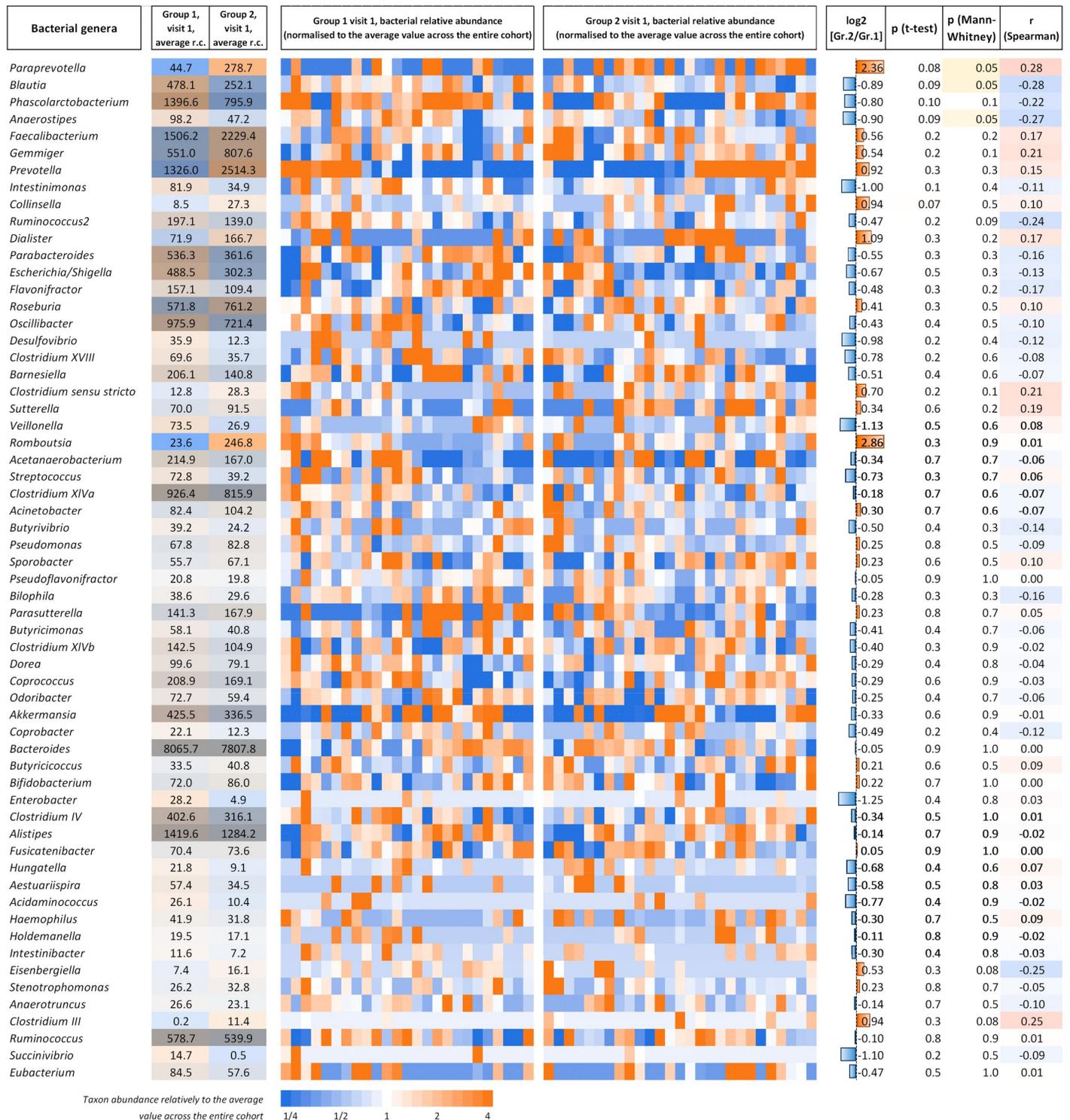


Fig 3. Qualitative and quantitative composition of the intestinal microbiota genera in patients of groups 1 and 2 at visit 1. Columns 2 and 3 present the relative bacterial content of a given genus (r.c., represents the normalized number of reads annotated to the current genus) averaged over all samples of a group. The color brightness reflects the average content, whereas the color hue (blue to orange) indicates the difference between the groups. The heatmaps in columns 4 and 5 show the bacterial content profiles of the genera for individual samples. The genera are sorted by a scoring factor, which reflects the magnitude of differences between groups 1 and 2, statistical significance (based on the Mann-Whitney test), and the average bacterial content across all samples.

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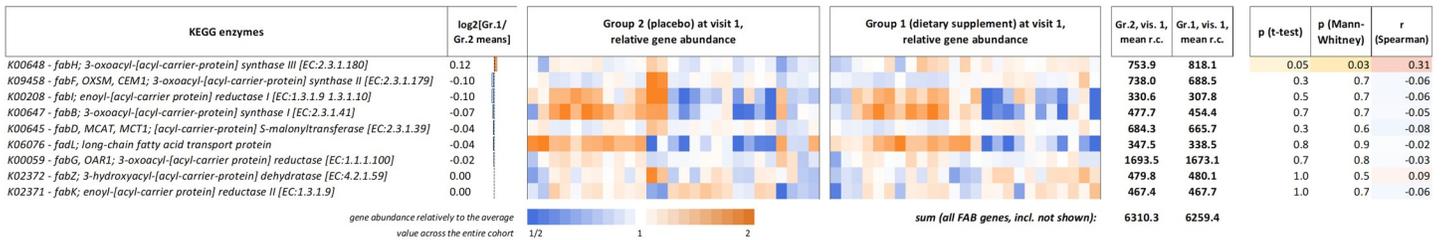


Fig 4. Relative content in the metagenome of genes involved in fatty acid biosynthesis (FAB) in patients of groups 1 and 2 at visit 1 (inferred from taxonomic data). Columns 5 and 6 show the average content of FAB genes in each group. The heatmaps in columns 3 and 4 demonstrate the relative gene abundance per sample. Samples are re-arranged according to the similarity of gene abundance profiles.

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related family *Fusobacteriaceae* ($r = 0.29, p = 0.0002, FDR = 0.02$). However, this family had a relatively low overall abundance (mean of 3 reads per 1 sample, found only in 11% of samples).

Additionally, we identified a number of families statistically associated with IBS severity, though they did not pass the FDR adjustment threshold. Among them were *Acidaminococcaceae* (Spearman’s $r = -0.23, p = 0.005$); *Enterobacteriaceae* ($r = -0.16, p = 0.05$); *Streptococcaceae*, *Coriobacteriaceae*, and *Veillonellaceae* ($r = 0.18 \dots 0.21, p = 0.01 \dots 0.03$). We noted positive correlations between *Butyrivibrio*, *Catenibacterium*, *Akkermansia*, *Alloprevotella*, *Dialister*, and *Prevotella* abundance with age ($r = 0.17 \dots 0.24$); inverse correlations of *Roseburia*, *Megasphaera*, *Bacteroides*, *Odoribacter*, *Parabacteroides*, *Alistipes*, and *Parasutterella* abundance with age ($r = -0.17 \dots -0.28$); and associations of *Roseburia*, *Mitsuokella*, *Coprobacter*, and *Escherichia/Shigella* abundance with gender.

Finally, we assessed the relationship between the metabolic potential of the collective microbiome (inferred from taxonomy) and disease severity (inferred from the 7x7 questionnaire score). Correlations were observed for 360 predicted bacterial genes, though none of them passed the FDR threshold. Interestingly, none of these genes were related to FAB. However, the 26th place (out of 4,405 genes) was marked by the *atoE* gene encoding for a short-chain fatty acid transporter. The correlation coefficient of its relative abundance and 7x7 score was $r = -0.23 (p = 0.004)$. When analyzing associations with age, we noted 1,088 or 186 predicted bacterial genes at a given threshold for $p < 0.05$ or $FDR < 0.05$.

Correlation analysis between microbiome composition and 7x7 questionnaire scores.

The safety of the food supplement “Standart Zdorovya GASTRO” was measured according to additional complaints recorded during each visit. Also, blood tests were performed at visit 1 and 3, with no deviations from the reference values observed. In group 2 some changes were observed in sedimentation rate and red and white blood cell count, but these values did not

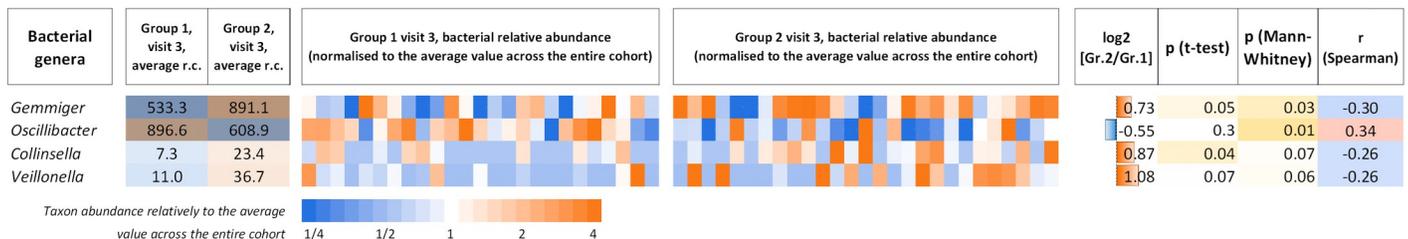


Fig 5. Statistically significant differences in the composition of intestinal microbiota genera between groups 1 and 2 at visit 3. Columns 2 and 3 present the relative content (r.c.) of bacterial genera averaged over all samples in each group. The heatmaps in columns 4 and 5 show the bacterial content profiles for individual samples.

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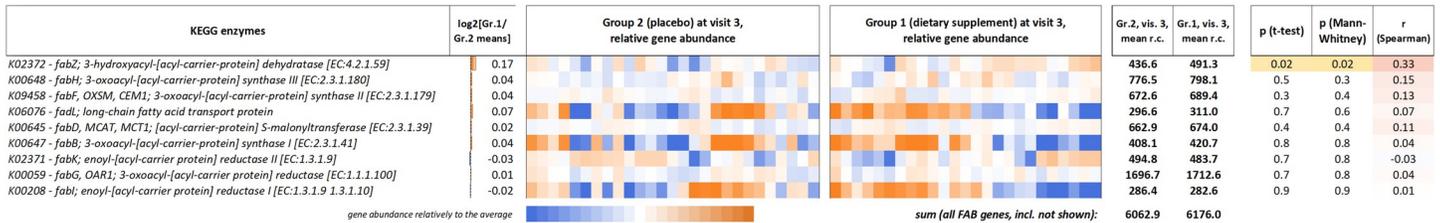


Fig 6. The relative content in the metagenome of genes participating in FAB in patients of groups 1 and 2 at visit 3 (inferred from taxonomic data). Columns 5 and 6 show the average content of FAB genes in each group. The heatmaps in columns 3 and 4 demonstrate the relative gene abundance per sample. The samples are rearranged according to the similarity of gene abundance profiles.

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exceed the reference ranges (S7–S16 Figs in S3 File). No additional complaints were recorded in either of the groups.

Discussion

The development and maintenance of functional gastrointestinal disorders can be attributed to various psychological and biological factors. Changes in the composition of intestinal microbiota and intestinal permeability that decrease tight junction protein expression and reduce the thickness of the mucus layer are key factors impairing gastrointestinal motility and sensitivity [39–41].

Microbial SCFAs are vital to intestinal homeostasis. They suppress the growth of Gram-negative pathogens, function as energy sources for colonocytes, and have anti-inflammatory effects. SCFAs are organic fatty acids with 1–6 carbon atoms and are the principal anions arising from the bacterial fermentation of polysaccharides, oligosaccharides, proteins, peptides, and glycoprotein precursors in the colon [42]. The main SCFAs are produced by bacterial cells using acetyl-CoA by different pathways, such as acrylate and succinate [43].

We used the food supplement “Standart Zdorovya GASTRO” with standardized menthol, limonene, and gingerol content to deliver antispasmodic [18, 25], analgesic [19, 20], anti-inflammatory, and prokinetic [19, 20, 22, 25–27] effects. The supplement has also been shown to contribute to the restoration of intestinal microbiota [21] and gastrointestinal mucosal function [23, 24] (Table 1). Thus, the inclusion of this supplement in IBS or IBS/FD treatment regimens can improve the efficacy of standard therapy. Patients who received the supplement showed significant symptom reduction. A significant decrease in symptom severity to borderline ill was observed in group 1 at visit 3 (7×7 score -6.5, 95% CI 4.58–8.42), whereas group 2 remained mildly ill (total 7×7 score 10.8, 95% CI 8.00–13.6). Furthermore, the number of IBS and FD symptoms decreased in more patients of group 1 than group 2.

The greater treatment efficacy in group 1 (standard treatment regimen and dietary supplement) might be associated with modifications in the intestinal microbiome and metabolome. However, we generally observed heterogeneous taxonomic profiles of microbiome community structure between patients, both at visits 1 and 3. This complicates the interpretation of changes caused by the intake of the dietary supplement. Similarly, we found a very small increase in the content of specific genes involved in the biosynthesis of fatty acids at visit 3. However, here it is also impossible to confirm that the changes were due to the effects of the dietary supplement. Nevertheless, the analysis of clinical indicators (7×7 questionnaire) showed an improvement in the condition of patients who had taken dietary supplements together with conventional therapy.

With regard to microbiome composition, there was no variation observed between groups 1 and 2. Even though several bacterial genera passed the $p < 0.05$ threshold, the number of

these genera was comparable between visit 1 and 3. However, among these genera, we noticed *Oscillibacter* was dominant in group 1 and *Veillonella*, *Gemmiger*, and *Collinsella* were dominant in Group 2. Previously, an animal study showed higher *Oscillibacter* prevalence in healthy controls than in patients with IBS [44, 45] and high *Veillonella* prevalence in IBS-C patients [46, 47]. Although *Oscillibacter* can promote fiber degradation by an array of cellulases and produce butyric acid from glucose, ribose, and xylose, the main metabolite produced is valeric acid [48, 49]. Previously, it was shown that the abundance of *Collinsella* was associated with low a fiber diet and obesity [50], type 2 diabetes [51], and atherosclerosis [52]. Lower content of the *Collinsella* family (Coriobacteriaceae) was associated with a healthier microbiome composition [53]. No data exists for *Gemmiger* prevalence in patients with functional disorders or healthy controls.

We noticed slight changes between group 1 and 2 in the number of bacteria containing genes involved in the synthesis of fatty acids. However, these changes were not statistically significant after correcting for FDR and no correlations were observed with symptom severity. In general, an increased number of bacteria implicated in fatty acid biosynthesis could improve intestinal permeability through mucus layer generation [54].

When analyzing associations with the severity of IBS, we found positive correlations with the abundance of *Fusobacterium*. Moreover, the presence/abundance of these bacteria was strongly associated with increased discomfort and pain. Previous reports found that *Fusobacterium* increased severity of diarrhea-predominant IBS (IBS-D) [55], and *Fusobacterium nucleatum* was involved in the pathogenesis of IBS by causing microbial dysbiosis and exacerbating visceral hypersensitivity [55]. The metabolites of *Fusobacterium* contain butyric acid, which promotes visceral hypersensitivity in an IBS-like model via an enteric glial cell-derived nerve growth factor [56]. However, some studies have shown that *Fusobacterium* was decreased in IBS-D patients [57].

The present study revealed negative correlations between *Acidaminococcaceae* and *Enterobacteriaceae* abundance and positive correlations of *Streptococcaceae*, *Coriobacteriaceae*, and *Veillonellaceae* abundance with IBS severity. Except for *Enterobacteriaceae*, all of these families have been associated with symptom severity in IBS patients (especially IBS-D) compared to healthy controls or patients with other gastrointestinal diseases [58–62].

The current study has several limitations. First, the sample size was relatively small and comprised only 56 patients. Further studies with more participants are needed to validate the trends observed. Moreover, we did not perform follow-up assessments to record the long-term treatment effects. This limitation will also be addressed in future trials.

Conclusion

The components of the food supplement “Standart Zdorovya GASTRO” standardized for menthol, limonene, and gingerol influence the main pathogenetic mechanisms of IBS and FD and increase the efficacy of standard therapy for IBS and FD. The use of the supplement is safe and does not cause side effects.

Supporting information

S1 File.
(DOCX)

S2 File.
(JPEG)

S3 File. Supplementary figures.
(DOCX)

S4 File. Supplementary tables.
(PDF)

S1 Checklist. CONSORT 2010 checklist of information to include when reporting a randomised trial*.
(DOC)

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