

ORIGINAL RESEARCH ARTICLE

Effects of targeted colonic synbiotic supplementation with *Bacillus subtilis* DSM 32315 and L-alanyl-L-glutamine on mental well-being: An open-label pilot study

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Abstract

The gut–brain axis plays a pivotal role in mental health, with synbiotic supplements emerging as promising interventions for stress and anxiety management. This observational open-label pilot study aims to evaluate the effects of a synbiotic supplement containing *Bacillus subtilis* DSM 32315 and L-alanyl-L-glutamine, delivered via a targeted colonic-release capsule, on stress, anxiety, and mental well-being. Conducted as a non-placebo-controlled real-world case study, the study recruited 100 adults with elevated perceived stress and followed them for over eight weeks. Validated self-reporting tools, including the Depression Anxiety Stress Scales-21 items (DASS-21), Perceived Stress Scale-10 items (PSS-10), Athens Insomnia Scale (AIS), Short Form Health Survey-12 items, and Gastrointestinal Symptom Rating Scale questionnaires, were used to assess outcomes. Statistical analyses, primarily using linear mixed models, indicated reductions in DASS-21, PSS-10, and AIS scores, along with self-reported improvements in gastrointestinal symptoms and mental well-being. These findings should be interpreted cautiously due to the exploratory, uncontrolled study design. Notably, DASS-21 total scores declined within one week, with reported benefits sustained at four and eight weeks. However, these rapid changes may partly reflect expectancy effects or regression to the mean. These observations provide valuable insights for further hypothesis generation, notably that the synbiotic supplement could support stress management and mental health by modulating the gut–brain axis. However, further placebo-controlled trials including measurement of relevant biomarkers are warranted to confirm these observations and explore underlying mechanisms.

Keywords: Synbiotic; *Bacillus subtilis*; L-alanyl-L-glutamine; Colonic delivery; Gut–brain axis; Mental well-being; Short-chain fatty acids; Real-world study

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1. Introduction

Mental health is increasingly acknowledged as a fundamental pillar of overall well-being. The World Health Organization emphasizes that mental well-being empowers

individuals to manage stress, recognize their potential, and make meaningful contributions to society.¹ Among the most widespread mental health challenges globally are stress-induced mood disorders, such as anxiety and depression, which collectively affect over 300 million people and significantly diminish quality of life while contributing to disability.² Chronic stress, a pervasive concern in modern society, is linked to both emotional distress and physiological dysregulation, particularly in relation to intestinal health, as highlighted by Person and Keefer.³ Chronic stress is known to disrupt the composition of the gut microbiome, as summarized by Leigh *et al.*⁴ Conversely, the intestinal microbiota influences mental health disorders, including depression, through mechanisms involving neurotransmitter production, hormonal regulation, neurogenesis, neuroplasticity, and inflammatory pathways.⁵ This bidirectional communication system connects the gastrointestinal tract with the central nervous system and is called the gut–brain axis. It is increasingly recognized as a crucial factor in the pathophysiology of mood and mental health disorders. Therefore, actively modulating the gut microbiome to address issues such as stress, depression, and anxiety, through neurochemical, immunological, and endocrine pathways, may offer promising therapeutic avenues.⁶

One of the most compelling mechanisms by which the gut microbiota influences brain function is through the production of short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate. These microbial metabolites are generated via fermentation of dietary fibers and have been shown to modulate neuroinflammation, neurotransmitter synthesis, and blood–brain barrier integrity.⁷ However, adequate fiber intake remains a challenge in various developed countries. For example, a recent umbrella review reports inadequate fiber intake in >90% of Americans, and in the United Kingdom, average daily fiber consumption is well below the recommended 30 g.⁸

Butyrate, in particular, has demonstrated neuroprotective properties, including histone deacetylase inhibition and anti-inflammatory effects, which may mimic or enhance the action of conventional antidepressants. It can cross the blood–brain barrier and influence gene expression and neuroplasticity, thereby improving mood regulation and stress resilience.⁹ Butyrate is increasingly recognized as an important mediator in gut–brain communication.¹⁰ In turn, stress can alter gut microbiota composition and reduce the availability of SCFAs, particularly butyrate, which plays a key role in maintaining intestinal barrier integrity and modulating systemic inflammation.^{11,12} Conversely, modulation of

the gut microbiota through dietary interventions may positively influence mental health outcomes. Recent studies have shown that individuals with depression and anxiety often exhibit gut dysbiosis, characterized by reduced abundance of SCFA-producing bacteria and increased pro-inflammatory strains.⁶ Experimental models have confirmed that fecal microbiota transplants from depressed individuals can induce depression-like behaviors in rodents, suggesting a causal role for microbial composition in mood regulation.^{13,14}

Probiotic and synbiotic interventions have shown promise in modulating the gut microbiota to restore microbial balance and enhance SCFA production, with positive effects on mood and mental well-being.⁷ In contrast, a recent study showed that a prebiotic diet reduced mood disorder symptoms relative to placebo after eight weeks, while probiotic and synbiotic treatments had no effect.¹⁵

While previous research has explored dietary interventions, probiotics, and prebiotics in controlled settings, with varying results, there is limited evidence on real-world applications of targeted synbiotic formulations designed to enhance butyrate production *in vivo*. While adequate dietary fiber intake remains a prerequisite for optimal SCFA synthesis, current consumption levels frequently fall below established recommendations.⁸ To address this gap, a targeted synbiotic approach has been developed to stimulate intrinsic SCFA production substantially. This strategy offers the potential of improved practicality, as achieving sufficient prebiotic fiber intake typically necessitates high consumption volumes. Our previous study introduced a novel approach by evaluating an advanced synbiotic supplement combining *Bacillus subtilis* DSM 32315 and the dipeptide L-alanyl-L-glutamine, delivered via a colonic-release capsule, to optimize gut microbial fermentation and SCFA generation. We have shown that butyrate-producing taxa and butyrate levels were enhanced in feces upon intake of this supplement. Unlike traditional synbiotics, which primarily combine prebiotic fibers with probiotics, this novel combination integrates two distinct microbiome-modulating components that act synergistically to promote a microbiota composition favoring butyrate production.¹⁶

The present study investigates the effect of the same synbiotic combination on mental well-being in an open-label, uncontrolled observational design. The results are intended to generate hypotheses for future controlled trials. In this pilot study, 100 adults with elevated perceived stress were recruited and observed over eight weeks using validated psychometric and gastrointestinal assessment tools (Depression Anxiety Stress Scales-21

items [DASS-21], Perceived Stress Scale-10 items [PSS-10], Athens Insomnia Scale [AIS], Short Form Health Survey-12 items [SF-12], and Gastrointestinal Symptom Rating Scale [GSRS]). Results demonstrated rapid and sustained improvements in stress, anxiety, and mental well-being, along with enhanced gastrointestinal comfort, suggesting that targeted modulation of the gut microbiome to promote butyrate synthesis may represent a promising strategy for stress management. This is an exploratory study providing preliminary real-world data on the potential association between a butyrate-focused synbiotic intervention and self-reported mental health outcomes. Although the findings suggest promising benefits, further placebo-controlled trials are needed to confirm efficacy and elucidate underlying mechanisms.

2. Materials and methods

2.1. Study design and participants

The study was conducted as an open-label, single-center, consumer experience study from Oct 7, 2024, to Dec 18, 2024, with an independent nutritional contract research organization, analyze and realize, in Germany. This post-launch, single-arm, real-world study was not placebo-controlled and was conducted over eight weeks to evaluate the effects of a synbiotic supplement on stress, anxiety, and mental well-being. A total of 101 adult participants (aged 21–65 years) were recruited via digital outreach and screened for eligibility. Inclusion criteria required participants to be in generally good health, to report elevated perceived stress accompanied by stress-related complaints for at least two months, and to score ≥ 12 on the stress subscale of the DASS-21 and between 15–30 on the PSS-10 at baseline, with access to a smartphone, tablet, or computer and familiarity with digital tools. Exclusion criteria included diagnosed psychiatric disorders (e.g., depression, anxiety), sleep disorders, chronic pain or fatigue, regular psychological treatment, recent antibiotic use (within one month), night-shift work, and substance abuse. Participants were also excluded if they were using psychotropic drugs, adaptogens, or sleep- or psychological-well-being products, or if any changes in medication, supplementation, or lifestyle were expected during the study period. All participants provided informed consent prior to enrollment and were introduced to the digital patient-reported experience tool (PRE-tool).

After enrollment, the test product was mailed to the participant for use over the subsequent eight weeks. In the PRE-tool, the participant was asked to document the start date of using the test product. One week after the start, participants completed the DASS-21 questionnaire and

questions about test product use. The latter was repeated weekly until the end of the intervention period. After four weeks, the participants were asked to complete the questionnaires, including the DASS-21, PSS-10, AIS, SF-12, and GSRS. Eight weeks after the start, the participants were asked to complete the five questionnaires again. In addition, participants were asked to answer questions about the benefits, satisfaction, and potential future use of the test product.

2.1.1. Compliance

The evaluation of adherence to the protocol use was based on the information retrieved from the PRE-tool. The overall test product adherence (%) was defined as the total number of capsules taken divided by the total number of capsules to be taken multiplied by 100. The total number of capsules to be taken was calculated for each participant, based on the actual length of participation, as reported in the PRE-tool. Overall adherence (%) was assessed on an individual level. Participants who consumed more than 80% of the indicated daily amount of the test product were considered fully compliant.

One participant was excluded from the full analysis set (FAS) population as the participant did not take the product. Furthermore, 12 participants were excluded from the per protocol (PP) population due to major deviations, but remained in the FAS (Table 1).

Table 1. Overview of study subjects and analysis populations

Study subjects	<i>n</i>	Percentage of screened/ included subjects
Screened subjects	296	100.0%
Included subjects	101	34.1%
Full analysis set population	100	99.0%
Per protocol population	88	87.1%

The FAS population included all participants with no relevant deviation (as judged during the data review) from preconditions for participation who have used the test product at least once and whose evaluation parameters are available. The PP population consisted of all participants in the FAS population who had no relevant deviations from the project plan (as documented in the data review report) and who showed test product intake exceeding 80% of the planned daily rate.

2.2. Intervention

Participants received a daily oral synbiotic supplement formulated to release the components in the colon. The supplement (consumer product “FML-01 Butyrate Booster”, Fun Ingredients GmbH, Germany, based on synbiotic concept IN VIVO BIOTICS™ butyrate basic, Evonik, Germany) consisted of 2×10^9 CFU *B. subtilis* DSM 32315 (B4U™ 21, Evonik, Germany), a spore-forming probiotic strain, and 290 mg dipeptide L-alanyl-L-glutamine. The combination was encapsulated in a size 0 hydroxypropyl methylcellulose capsule with a functional coating (EUDRAGUARD® biotic, Evonik, Germany) with pH-dependent solubility to enable targeted colon delivery of the capsule, ensuring that the ingredients reach the distal gut, the SCFA production site. The formulation aims to enhance *in vivo* butyrate synthesis through microbiota modulation, thereby potentially influencing systemic inflammation and neurochemical pathways involved in stress regulation. One capsule was taken daily with water for eight weeks.

2.3. Outcome measures

Participants completed the DASS-21 at baseline and at one, four, and eight weeks of supplementation. Other questionnaires (the PSS-10, the AIS, the SF-12, and the GSRS) were completed at baseline and at four and eight weeks of supplementation. All data were collected via a secure electronic data capture system, ensuring standardized administration and minimizing reporting bias.

2.3.1. Depression, anxiety, and stress scale-21 items

The DASS-21 is a self-report questionnaire designed to measure the emotional states of depression, anxiety, and stress. It has demonstrated good reliability and validity across various populations.¹⁷ It consists of 21 items, with seven items dedicated to each of the three constructs. Respondents rate the severity of their experiences over the past week on a four-point Likert scale, ranging from 0 (did not apply to me at all) to 3 (highly applicable, or most of the time). The questionnaire assessed difficulty relaxing, nervous arousal, being easily upset/agitated, being irritable/overreactive, being impatient, dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia and inertia, autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. Higher values indicated higher symptom levels. Subscale scores range from 0 to 21.

For comparability with interpretation guidelines originally developed for the DASS-42, each subscale

score in the DASS-21 is multiplied by two to generate the total score. The resulting total score ranges from 0 to 126. Symptom severity categories (normal, mild, moderate, severe, and very severe) are presented in [Table 2](#).

Table 2. Score ranges for the Depression Anxiety Stress Scales-21 total score, according to the manual

Severity	Score range
Normal	0–30
Mild	31–40
Moderate	41–60
Severe	61–80
Extremely severe	>81

2.3.2. Perceived stress scale-10 items

The PSS-10 is a widely used psychological instrument for measuring perceived stress. It consists of 10 items that assess how unpredictable, uncontrollable, and overloaded respondents find their lives to be. Items are rated on a five-point scale, from 0 (never) to 4 (very often). The PSS-10 has shown strong psychometric properties and is effective in various populations.¹⁸

2.3.3. Athens Insomnia Scale

The AIS is a self-reported questionnaire that assesses the severity of insomnia symptoms. It consists of eight items, each rated on a 0 to 3 scale, reflecting the frequency of insomnia symptoms over the past month. The total score ranges from 0 to 24, with higher scores indicating more severe insomnia. The AIS has been validated in various populations and is recognized for its reliability.¹⁹

2.3.4. Short Form Health Survey-12 items

The SF-12 is a 12-item questionnaire that measures health-related quality of life. It includes two summary measures: the physical component summary (PCS) and the mental component summary (MCS). Respondents rate their health status over the past month, and the scores are standardized to a mean of 50 and a standard deviation of 10. The SF-12 has been validated in diverse populations and is widely used in health research.²⁰

2.3.5. Gastrointestinal Symptom Rating Scale

The GSRS is a self-administered questionnaire designed to assess gastrointestinal symptoms. It consists of 15 items that cover five dimensions: abdominal pain, reflux,

indigestion, diarrhea, and constipation. Respondents rate the severity of their symptoms on a seven-point scale, from 1 (no symptoms) to 7 (very severe symptoms). The GSRS has been shown to have good reliability and validity in clinical and non-clinical populations.²¹

2.4. Data capture, data management, and statistical analysis

DataCapt, an electronic data capture platform (version 4.15.1); including an electronic diary and questionnaires), was used to collect the patient-reported experience data. The entries in the PRE-tool were recorded by the participants. Project data were stored in an appropriate format in accordance with applicable data protection regulations.

Descriptive statistics were used to summarize baseline characteristics and outcome measures. All parameters received an explorative examination. For the evaluation items, the following statistical characteristics were shown in figures by week: mean, median, extremes, and quartiles. Primary and secondary endpoints were analyzed using a linear mixed model (LMM) with week and baseline score as fixed effects and the participant as a random effect.

The means (least squares means) and 95% confidence intervals for the change from start were calculated at weeks one, four, and eight. Assumptions of normality and homoscedasticity in linear models were examined using graphical representations of residuals produced by statistical models. For substantial deviations from normality and/or homoscedasticity, a Wilcoxon test for paired data was used at weeks one, four, and eight.

Analyses were performed on the FAS population. Changes of DASS-21 values from project start to weeks one, four, and eight were also analyzed on the PP population. Clinically relevant improvements were defined using thresholds for each scale. Statistical analyses were performed using standard R software packages (version 4.3.3, R Foundation for Statistical Computing, Austria), and significance was set at $p < 0.05$ for all statistical tests.

3. Results

3.1. Subject characteristics

This section presents the outcomes of the eight-week consumer experience study evaluating the effects of the test product on stress, mental well-being, sleep quality, gastrointestinal symptoms, and overall satisfaction. The results are structured by validated assessment tools and participant-reported outcomes. Subjects' characteristics at the beginning of the study were as follows: The mean age of participants was 38.93 ± 11.16 years. Among the

participants included, 72.0% were female, and 28.0% were male. The mean weight of participants was 75.54 ± 14.96 kg, and the mean height of participants was 172.50 ± 7.79 cm. All results presented here refer to the FAS population of this study.

3.2. Missing data

The proportion of missing data in this study was extremely low. For the DASS-21 questionnaires, two participants provided no responses—one at week one and another at week eight—constituting “fully missing” cases. These observations were excluded from all statistical analyses. At week four, a single-item response was missing; this value was imputed using the item's mean score for that visit. A comparable pattern of adherence was observed for the remaining questionnaires, with at most one fully missing case per visit and no additional item-level missingness. Given the small fraction of missing data, its impact on the statistical results is negligible.

3.3. Synbiotic supplementation significantly improved psychological evaluation items

3.3.1. Depression Anxiety Stress Scales-21 items

The DASS-21 total score showed reductions from baseline at all time points (weeks one, four, and eight). These findings suggest potential improvements in self-reported emotional distress, though they should be interpreted cautiously given the study's open-label design (Figure 1).

There were statistical differences between baseline DASS-21 total score and total score after one, four, and eight weeks of synbiotic supplement intake in the LMM (Table 3). These results were supported by the Wilcoxon test.

The DASS-21 showed a clear shift in total score categories, indicating physiological significance, with a marked reduction in the “severe” and “extremely severe” categories (Figure 2).

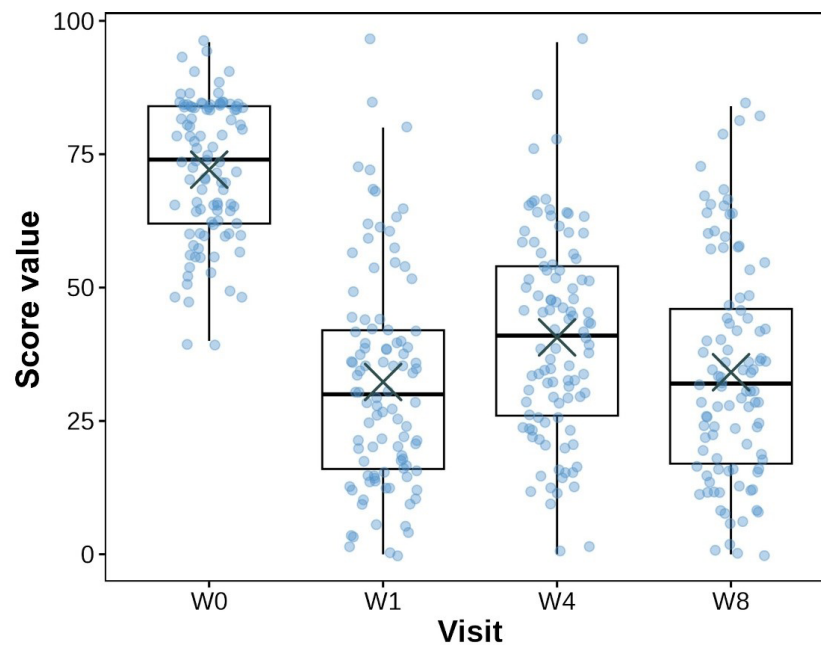
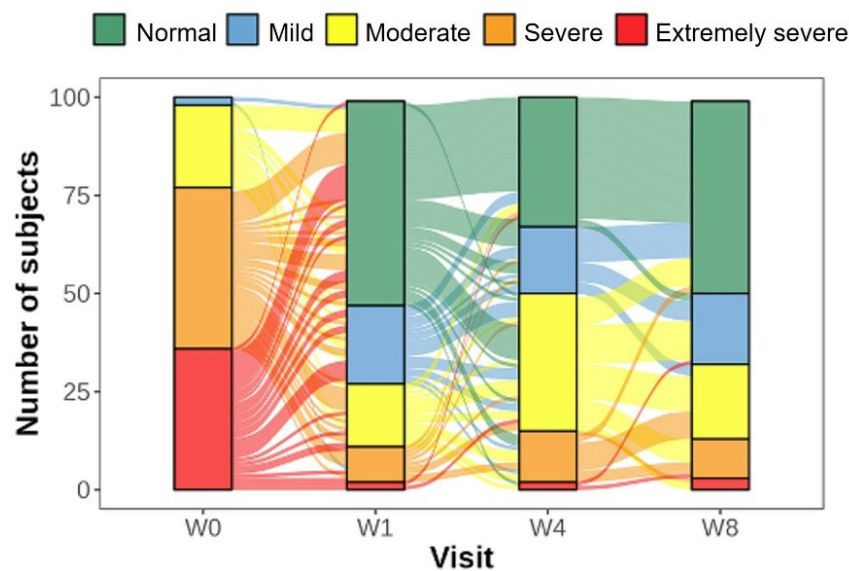
The DASS-21 stress subscores were decreased compared to baseline in 87.0% and 93.9% of participants after four and eight weeks, respectively; DASS-21 depression subscores were reduced compared to baseline in 90.0% and 89.9% of participants after four and eight weeks, respectively; DASS-21 anxiety subscores were reduced compared to baseline in 85.0% and 88.9% of participants after four and eight weeks, respectively.

The DASS-21 stress subscores decreased by at least 5 points from baseline in 54.0% and 69.7% of participants after four and eight weeks, respectively. Such a reduction represents a reliable improvement, corresponding to a shift to an adjacent, less severe range. For depression and

Table 3. Depression Anxiety Stress Scale-21 items total score (variations Wn–W0): Linear mixed model (full analysis set)

Comparison	Mean change estimate	Standard error	95% CI	p-value
W1–W0	–39.48	2.01	(–43.45, –35.51)	<0.0001
W4–W0	–31.39	2.01	(–35.34, –27.43)	<0.0001
W8–W0	–37.96	2.01	(–41.93, –33.99)	<0.0001

Abbreviation: CI: Confidence interval.

**Figure 1.** Depression Anxiety Stress Scale-21 items total score in the full analysis set population at study start (W0), week 1 (W1), week 4 (W4), and week 8 (W8)**Figure 2.** Transition between the doubled Depression Anxiety Stress Scale-21 total scores categories across the weeks, at study start (W0), after week 1 (W1), week 4 (W4), and week 8 (W8)

anxiety subscores, an improvement of at least 4 points represents a reliable improvement (i.e., moved into an adjacent and less severe range) according to Ronk *et al.*²² DASS-21 depression and anxiety subscores were reduced by at least 4 points from baseline in 69.0% and 73.7% and in 58.0% and 70.7% of participants after four and eight weeks, respectively. These findings suggest that the synbiotic product may be associated with self-reported improvements in emotional well-being. However, the lack

of a control group limits the ability to draw conclusions about clinical relevance or causality.

3.3.2. Perceived Stress Scale-10 items

The PSS-10 total score decreased from baseline at weeks four and eight, indicating a rapid and sustained improvement in emotional distress (Figure 3).

The PSS-10 total score decreased significantly after four and eight weeks in the LMM (Table 4). These results were

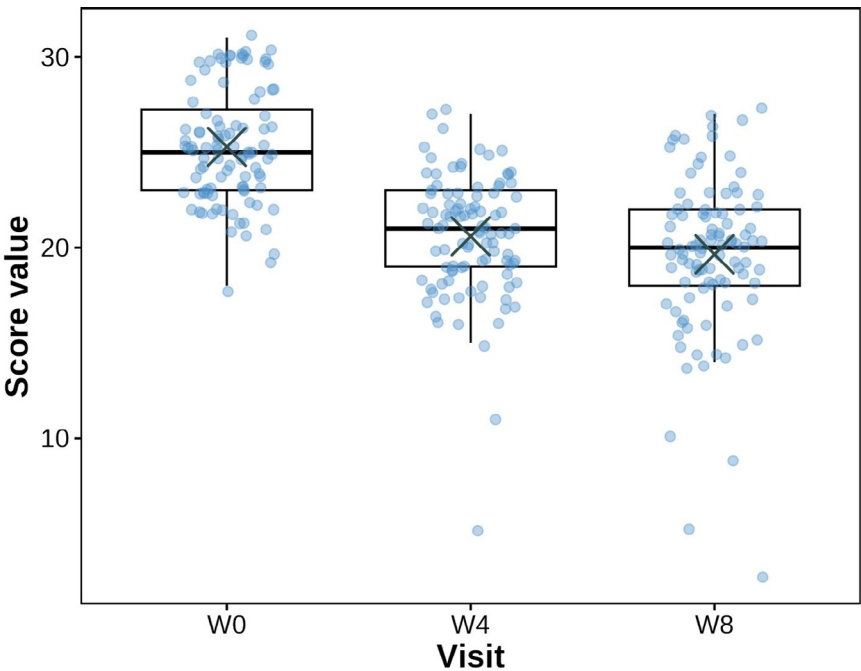


Figure 3. Perceived Stress Scale-10 items total score in the full analysis set population at study start (W0), after week 4 (W4), and week 8 (W8)

Table 4. Perceived Stress Scale-10 items total score (variations Wn–W0): Linear mixed model (full analysis set)

Comparison	Mean change estimate	Standard error	95% CI	p-value
W4–W0	–4.69	0.37	(–5.43, –3.95)	<0.0001
W8–W0	–5.62	0.38	(–6.36, –4.88)	<0.0001

Abbreviation: CI: Confidence interval.

supported by the Wilcoxon test.

3.3.3. Athens Insomnia Scale

The AIS total score decreased from baseline at weeks four and eight, indicating a rapid and sustained improvement in emotional distress (Figure 4).

The AIS total score reduced significantly at four and

eight weeks in the LMM (Table 5). These results were supported by the Wilcoxon test.

3.3.4. Short Form Health Survey-12 items mental component summary and physical component summary

The MCS-12 of the SF-12 increased from baseline at weeks four and eight, suggesting potential improvements in self-

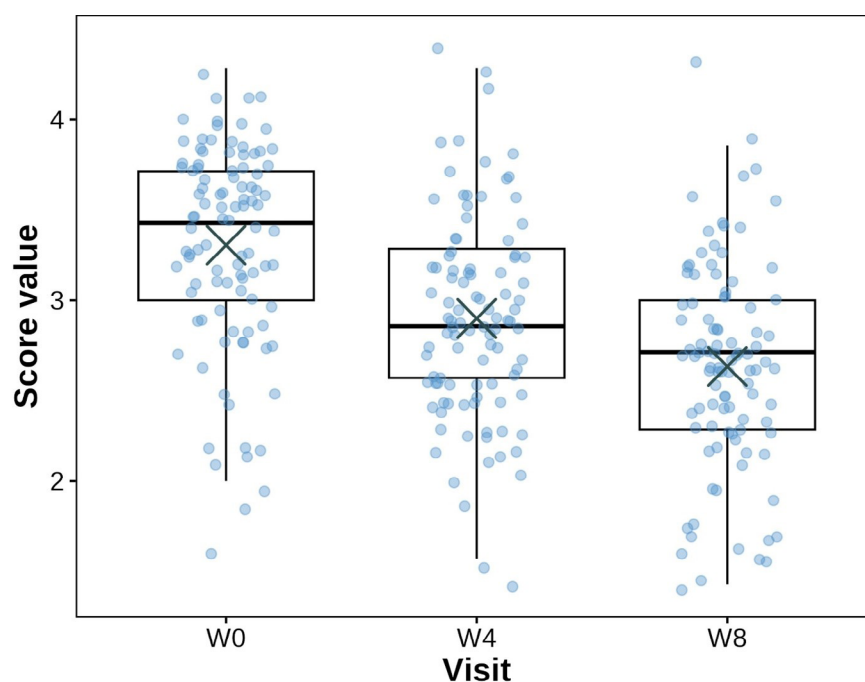


Figure 4. Athens Insomnia Scale total score in the full analysis set population at study start (W0), after week 4 (W4), and week 8 (W8)

Table 5. Athens Insomnia Scale total score (variations Wn–W0): Linear mixed model (full analysis set)

Comparison	Mean change estimate	Standard error	95% CI	p-value
W4–W0	–0.41	0.05	(–0.51, –0.31)	<0.0001
W8–W0	–0.68	0.05	(–0.78, –0.58)	<0.0001

Abbreviation: CI: Confidence interval.

reported mental distress, while the PCS-12 of the SF-12 remained stable throughout the study (Figure 5).

The MCS of the SF-12 increased from baseline after four and eight weeks in the LMM. There was no statistically significant change in the SF-12 PCS from baseline, after four and eight weeks in the LMM (Table 6). These results were supported by the Wilcoxon test.

3.4. Gastrointestinal outcomes

3.4.1. Gastrointestinal symptom rating scale total score

The GSRS total score showed reductions from baseline at weeks four and eight, suggesting improvement in self-reported gastrointestinal symptoms (Figure 6).

The GSRS total score reduced significantly after four and eight weeks in the LMM (Table 7). These results were supported by the Wilcoxon test. This improvement

is reflected in the subscores for reflux, abdominal pain, indigestion, diarrhea, and constipation.

3.4.2. Gastrointestinal symptom rating scale subscores

All five domains—reflux, abdominal pain, indigestion, diarrhea, and constipation—showed significant reductions as shown in Figure 7 and Table 8, respectively.

3.5. Participant feedback

3.5.1. Global evaluation of benefit

At the end of the study, 76% of participants rated the product's benefit either as “good” or “very good.” The satisfaction was rated either as “very satisfied” or “satisfied” in 71% of participants, whereas 29% of participants were either “moderately satisfied” or “not satisfied.” The willingness to use the product in the future was rated “yes” by 49% and “maybe” by 36% of participants.

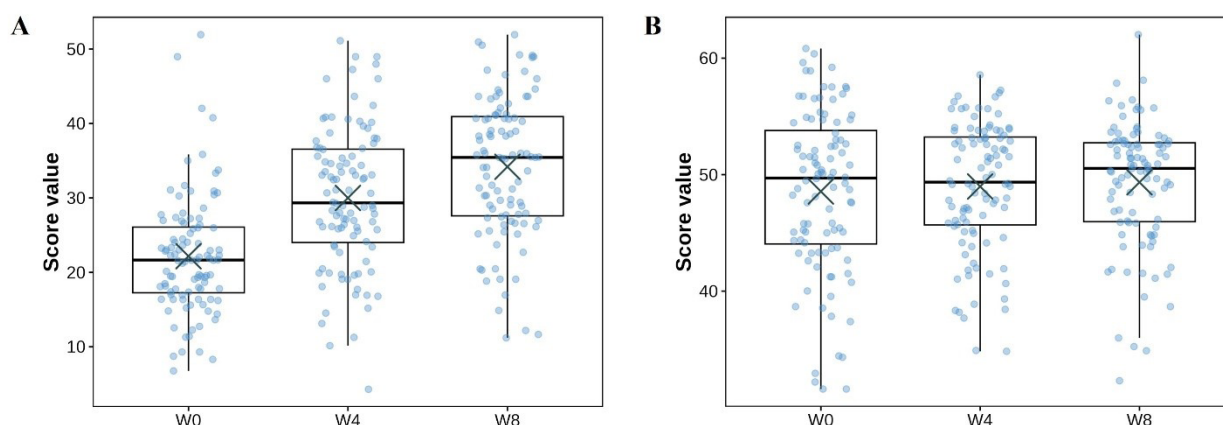


Figure 5. Short Form Health Survey-12 items score. (A) Mental component summary-12 and (B) Physical component summary-12 score in the full analysis set population at study start (W0), after week 4 (W4), and week 8 (W8).

Table 6. MCS-12 and PCS-12 score (Variations Wn–W0): Linear mixed model (full analysis set)

Comparison	Subscore of SF-12	Mean change estimate	Standard error	95% CI	p-value
W4–W0	MCS-12	7.78	0.90	(6.00, 9.57)	<0.0001
W8–W0	MCS-12	11.87	0.90	(10.08, 13.66)	<0.0001
W4–W0	PCS-12	0.45	0.49	(–0.53, 1.42)	0.3638
W8–W0	PCS-12	0.83	0.50	(–0.15, 1.81)	0.0971

Abbreviations: CI: Confidence interval; MCS: Mental component summary; PCS: Physical component summary; SF: Short Form Health Survey.

3.5.2. Compliance

The average compliance with the foreseen product intake was 90.8%. Overall, the results demonstrate that the use of synbiotic supplements was associated with significant improvements in stress, anxiety, depression, perceived stress, sleep quality, and gastrointestinal symptoms. These effects were observed as early as one week after initiation and sustained throughout the eight-week study period. The high compliance and positive user feedback further support the product's acceptability and potential utility in stress management and mental well-being.

4. Discussion

The present open-label observational pilot study provides valuable insights for generating a hypothesis. The results suggest that a consumer product, based on a synbiotic combination of *B. subtilis* DSM 32315 and L-alanyl-L-glutamine, delivered via a targeted colonic-release capsule, may be associated with self-reported improvements in stress-related psychological and physiological outcomes. The intervention showed improvements in

self-reported measures of mental well-being, including stress, anxiety, depression, perceived stress, sleep quality, and gastrointestinal symptoms. The most pronounced effects were observed in the DASS-21 total score, which decreased after one week of product use (-39.48 ± 2.01), with sustained reduced values at four and eight weeks. Subscores for stress, anxiety, and depression also showed consistent reductions that may be physiologically relevant. However, as this trial was conducted as an open-label observational pilot study without a placebo-controlled group, the interpretation of the results is limited. Given the high susceptibility of subjective psychological scales (particularly DASS-21 and PSS-10) to expectancy effects, the observed improvements may also be attributable to a placebo response, regression to the mean, or natural fluctuation in symptoms. To rule this out and substantiate a hypothesis of a beneficial effect on mood, stress, and sleep, it is indispensable to conduct additional placebo-controlled studies.

Furthermore, the participants in this study were predominantly female (72%) with a mean age of

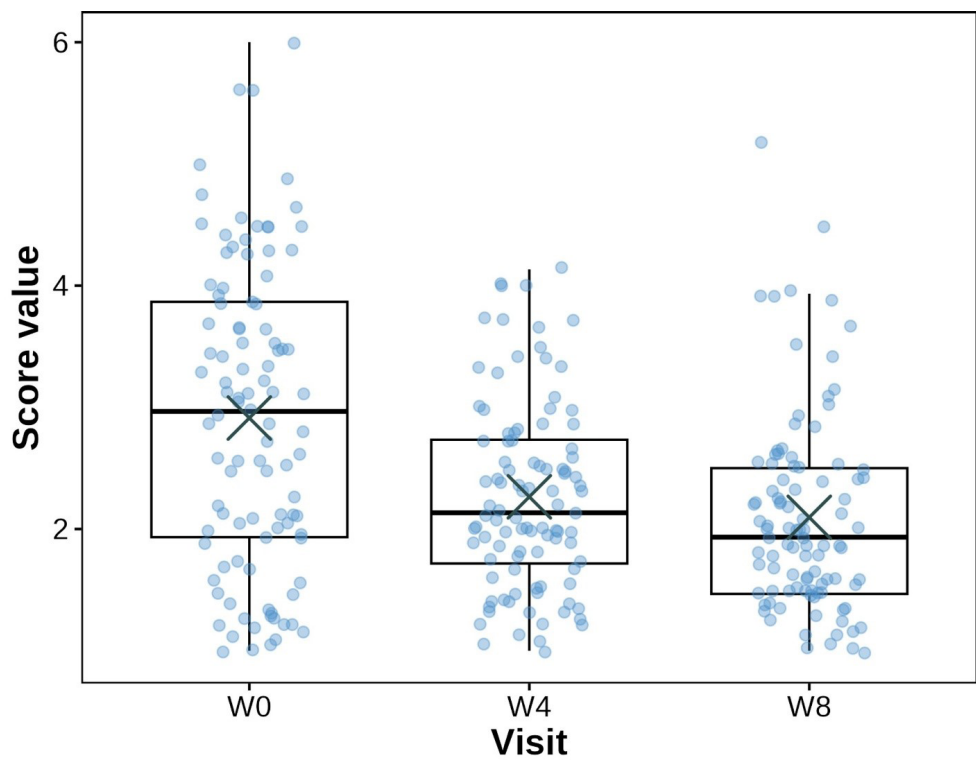


Figure 6. Gastrointestinal symptom rating scale total score in the full analysis set population at study start (W0), after week 4 (W4), and week 8 (W8)

Table 7. Gastrointestinal symptom rating scale total score (Variations Wn–W0): Linear mixed model (full analysis set)

Comparison	Mean change estimate	Standard error	95% CI	p-value
W4–W0	–0.65	0.07	(–0.78, –0.51)	<0.0001
W8–W0	–0.82	0.07	(–0.95, –0.68)	<0.0001

Abbreviation: CI: Confidence interval.

38.93 ± 11.16 years, which restricts generalizability to males and older adults. To address this, a broader, more balanced study population must be investigated in future studies.

It is worth noting that the effect sizes observed in this study are unusually large—particularly regarding the reduction in DASS-21 scores—raising questions about possible analytical weaknesses. However, the extremely low proportion of missing data underscores the statistical validity and subsequent interpretability of the outcomes. Nevertheless, as the study lacks a control group, as previously mentioned, these results may partly reflect a significant placebo effect, which cannot be ruled out.

Previous studies have shown that the synbiotic supplement used in the current study—comprising *B.*

subtilis DSM 32315 and L-alanyl-L-glutamine in a targeted colonic delivery capsule—promotes butyrate production *in vivo* through microbiota modulation via potential cross-feeding mechanisms.¹⁶

Emerging evidence suggests that butyrate, an SCFA produced by gut microbiota, may exert neuroactive effects through multiple pathways, including histone deacetylase inhibition,²³ modulation of neuroinflammation,²⁴ and enhancement of neuroplasticity.²⁵ Butyrate has been shown to modulate microglial activity²⁶ and reduce neuroinflammation,²⁷ which may improve mood and cognitive function. Recent meta-analyses have demonstrated that pre- and probiotic interventions can increase butyrate levels and improve depression scores, particularly when measured using tools such as DASS-21.⁹ Although direct correlations between butyrate

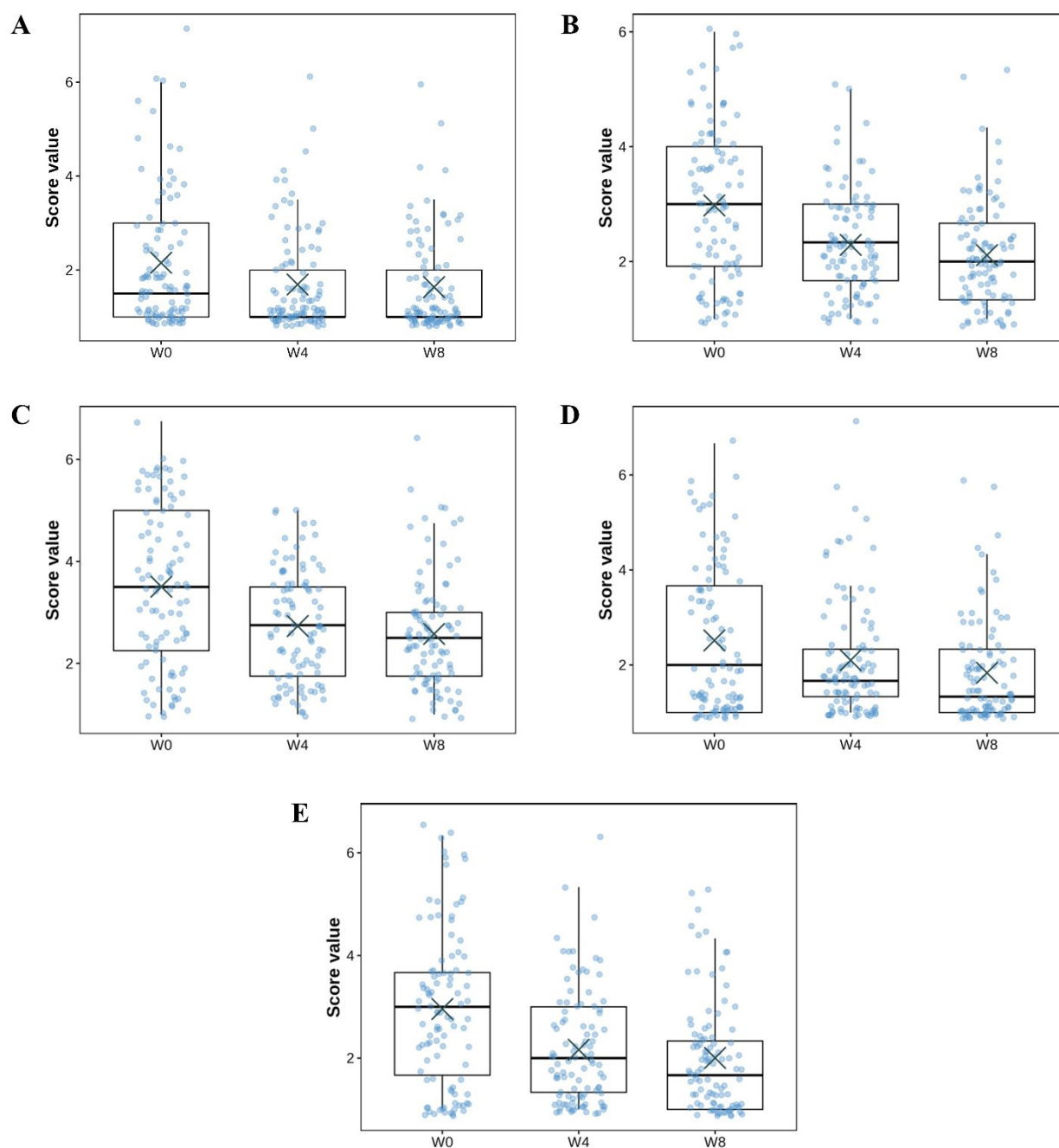


Figure 7. Gastrointestinal symptom rating scale subscores in the full analysis set population at study start (W0), after week 4 (W4), and week 8 (W8). (A) Reflux. (B) Abdominal pain. (C) Indigestion. (D) Diarrhea. (E) Constipation.

concentrations and depression scores remain inconclusive, the biological plausibility of the role of butyrate in mood regulation is supported by its ability to cross the blood–brain barrier and influence hippocampal function.²⁸

The reduction in perceived stress (PSS–10) and insomnia symptoms (AIS) in this study could support the hypothesis that modulation of the gut microbiota and

SCFA production can positively affect the hypothalamic–pituitary–adrenal axis and sleep regulation. SCFAs, including butyrate, are known to interact with free fatty acid receptors 2/3 and G protein-coupled receptors in neural and immune cells, thereby influencing systemic and central stress responses.¹¹

The SF-12 MCS in this study improved after the

Table 8. Gastrointestinal symptom rating scale subscores (Variations Wn–W0): Linear mixed model (full analysis set)

Subscores	Comparison	Mean change estimate	Standard error	95% CI	p-value
Reflux	W4–W0	–0.47	0.08	(–0.63, –0.31)	<0.0001
	W8–W0	–0.53	0.08	(–0.69, –0.37)	<0.0001
Abdominal pain	W4–W0	–0.69	0.08	(–0.85, –0.53)	<0.0001
	W8–W0	–0.87	0.08	(–1.03, –0.71)	<0.0001
Indigestion	W4–W0	–0.78	0.10	(–0.98, –0.58)	<0.0001
	W8–W0	–0.93	0.10	(–1.13, –0.73)	<0.0001
Diarrhea	W4–W0	–0.41	0.10	(–0.61, –0.21)	<0.0001
	W8–W0	–0.67	0.10	(–0.87, –0.47)	<0.0001
Constipation	W4–W0	–0.79	0.10	(–0.99, –0.59)	<0.0001
	W8–W0	–0.95	0.10	(–1.15, –0.75)	<0.0001

Abbreviation: CI: Confidence interval.

intervention, while the physical component score remained stable. This could suggest that the intervention primarily targeted psychological domains rather than physical functioning.

Improvements in GSRS total and subdomain scores (reflux, abdominal pain, indigestion, diarrhea, and constipation) may indicate a beneficial effect on gastrointestinal well-being, which would be consistent with the known anti-inflammatory and barrier-supporting properties of butyrate as summarized by Blaak *et al.*²⁹

The observed self-reported improvements in mental health may hypothetically be linked to a modulation of the gut–brain axis, potentially mediated by enhanced butyrate production. However, given that the present study was non-invasive and did not measure any biomarker levels, the mechanism of action remains speculative. Additionally, placebo-controlled studies measuring actual biomarkers, such as butyrate, are necessary to support this hypothesis.

Aside from the limitations mentioned, the findings of this study are consistent with recent randomized, placebo-controlled trials investigating synbiotic or probiotic interventions with regard to *in situ* butyrate production, the latter targeting the gut–brain axis. For instance, Napier *et al.*³⁰ demonstrated that a multi-species synbiotic (DS-01) significantly increased microbial diversity and butyrate production, while Guan *et al.*³¹ reported improvements in DASS-21 scores, sleep quality, and serum serotonin, as well as microbial metabolites, especially butyrate, following supplementation with *Lactiacaseibacillus*

paracasei K56 in stressed students. Although our study did not include biological sampling, previous *in vitro* and *in vivo* studies on the same synbiotic combination have shown *in vivo* promotion of butyrate production via microbiota modulation and cross-feeding mechanisms.¹⁶ This may support the biological plausibility of the observed psychological improvements, which would align our findings with the broader evidence base. However, the lack of biological or mechanistic measures, such as butyrate levels or microbiome-related markers, limits the ability to substantiate the proposed gut–brain mechanisms. Future studies should include such measures to strengthen the biological plausibility of the observed effects.

High levels of satisfaction (71%) and willingness for future use (85% combined “yes” and “maybe”) reflect the acceptability and perceived benefit of the product. Compliance was also high (90.8%), supporting the feasibility of long-term use in real-world settings.

While the results are promising, the study was open-label and observational, and it lacked a placebo control. Additionally, due to its exploratory nature, the study was non-invasive and did not measure actual butyrate levels; hence, the mechanism of action remains hypothetical. Future randomized controlled trials are needed to confirm causality and explore dose-response relationships. Additionally, mechanistic studies measuring actual butyrate levels in plasma or feces, along with concurrent microbiome and metabolome analyses, could strengthen the link between microbiota modulation and psychological outcomes. Taken together, the present results

are informative, suggesting that the synbiotic formulation of *B. subtilis* DSM 32315 and L-alanyl-L-glutamine may improve psychological well-being through microbiome-mediated mechanisms.

5. Conclusion

The findings of this exploratory study suggest that the synbiotic supplement containing *B. subtilis* DSM 32315 and L-alanyl-L-glutamine may be associated with self-reported improvements in stress and mental well-being. However, given the uncontrolled, open-label design, these results should be interpreted with caution and regarded primarily as hypothesis-generating. While the findings are consistent with current hypotheses regarding the role of the microbiota gut–brain axis, their interpretation is limited by the exploratory nature of the study and the absence of mechanistic data. Future placebo-controlled trials that measure relevant biomarkers are essential to validate these observations and clarify the underlying mechanisms.

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Conflict of interest

Heike tom Dieck and Ellen Ehring declare competing interests as employees of Evonik Operations GmbH. All other authors declare no conflict of interest.

Author contributions

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Writing–review & editing: All authors

Ethics approval and consent to participate

Ethical review and approval were waived for this study due to the observational pilot study product test design with volunteer participation in a post-launch consumer experience study, outside the ethical committee's area of responsibility. Written informed consent to participate was obtained from all study participants (e-consent).

Consent for publication

Consent for publication of data in anonymous format was obtained from all subjects involved in the study in electronic

form/writing (e-consent) as agreement to “Publication of the results in scientific journals in anonymized form, e.g., as statistical or aggregated information.”

Availability of data

The datasets generated and analyzed during this study are not publicly available due to proprietary restrictions. However, they can be made available upon reasonable request by contacting the corresponding author at ellen.ehring@evonik.com via email. Requests will be evaluated in line with the confidentiality policies and data sharing guidelines of Evonik Operations GmbH.

References

1. World Health Organization. Mental health. Published 2025. Available from: <https://www.who.int/news-room/fact-sheets/detail/mental-health-strengthening-our-response> [Last accessed on 2026 Feb 15].
2. World Health Organization. Depression and other common mental disorders: global health estimates. Published 2017. Available from: <https://iris.who.int/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf> [Last accessed on 2026 Sep 11].
3. Person H, Keefer L. Psychological comorbidity in gastrointestinal diseases: Update on the brain–gut–microbiome axis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;107:110209.
doi: 10.1016/j.pnpbp.2020.110209
4. Leigh SJ, Uhlig F, Wilmes L, *et al.* The impact of acute and chronic stress on gastrointestinal physiology and function: a microbiota-gut-brain axis perspective. *J Physiol*. 2023;601(20):4491–4538.
doi: 10.1113/JP281951
5. Nazir MM, Ghaffar W, Mustafa G, Saeed S, Ijaz MU, Ashraf A. Modulating depression through the gut–brain axis: the role of gut microbiota in therapeutic interventions. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2025;398(12):16893–16911.
doi: 10.1007/s00210-025-04464-6
6. Zhou X, Wang S, Wang X, *et al.* Mechanisms of the effect of gut microbes on depression through the microbiota–gut–brain axis. *Front Nutr*. 2025;12:1634548.
doi: 10.3389/fnut.2025.1634548
7. Cheng J, Hu H, Ju Y, *et al.* Gut microbiota–derived short-chain fatty acids and depression: deep insight into biological mechanisms and potential applications. *Gen Psychiatry*. 2024;37(1):e101374.
doi: 10.1136/gpsych-2023-101374
8. Veronese N, Gianfredi V, Solmi M, *et al.* The impact of dietary fiber consumption on human health: An umbrella review of evidence from 17,155,277 individuals. *Clin Nutr*.

- 2025;51:325-333.
doi: 10.1016/j.clnu.2025.06.021
9. Breuling M, Tomeva E, Ivanovic N, Haslberger A. Butyrate- and Beta-Hydroxybutyrate-Mediated Effects of Interventions with Pro- and Prebiotics, Fasting, and Caloric Restrictions on Depression: A Systematic Review and Meta-Analysis. *Life (Basel)*. 2024;14(7):787.
doi: 10.3390/life14070787
 10. Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front Endocrinol (Lausanne)*. 2020;11:25.
doi: 10.3389/fendo.2020.00025
 11. van de Wouw M, Boehme M, Lyte JM, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. *J Physiol*. 2018;596(20):4923-4944.
doi: 10.1113/JP276431
 12. Tian P, Zhu H, Qian X, et al. Consumption of Butylated Starch Alleviates the Chronic Restraint Stress-Induced Neurobehavioral and Gut Barrier Deficits Through Reshaping the Gut Microbiota. *Front Immunol*. 2021;12:755481.
doi: 10.3389/fimmu.2021.755481
 13. Kelly JR, Borre Y, O' Brien C, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res*. 2016;82:109-118.
doi: 10.1016/j.jpsychires.2016.07.019
 14. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry*. 2016;21(6):786-796.
doi: 10.1038/mp.2016.44
 15. Freijy TM, Cribb L, Oliver G, et al. Effects of a high-prebiotic diet versus probiotic supplements versus synbiotics on adult mental health: The "Gut Feelings" randomised controlled trial. *Front Neurosci*. 2023;16:1097278.
doi: 10.3389/fnins.2022.1097278
 16. tom Dieck H, Schön C, Wagner T, Pankoke HC, Fluegel M, Speckmann B. A Synbiotic Formulation Comprising *Bacillus subtilis* DSM 32315 and L-Alanyl-L-Glutamine Improves Intestinal Butyrate Levels and Lipid Metabolism in Healthy Humans. *Nutrients*. 2021;14(1):143.
doi: 10.3390/nu14010143
 17. Lovibond PF, Lovibond SH. *Manual for the Depression Anxiety Stress Scales*. 2nd ed. Sydney: Psychology Foundation of Australia; 1995.
 18. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24(4):385-396.
doi: 10.2307/2136404
 19. Soldatos CR, Dikeos DG, Paparrigopoulos TJ. Athens Insomnia Scale: validation of an instrument based on ICD-10 criteria. *J Psychosom Res*. 2000;48(6):555-560.
doi: 10.1016/s0022-3999(00)00095-7
 20. Ware J Jr, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care*. 1996;34(3):220-233.
doi: 10.1097/00005650-199603000-00003
 21. Svedlund J, Sjödin I, Dotevall G. GSRS? A clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Digest Dis Sci*. 1988;33(2):129-134.
doi: 10.1007/bf01535722
 22. Ronk FR, Korman JR, Hooke GR, Page AC. Assessing clinical significance of treatment outcomes using the DASS-21. *Psychol Assess*. 2013;25(4):1103-1110.
doi: 10.1037/a0033100
 23. Cao T, Zhou X, Zheng X, et al. Histone Deacetylase Inhibitor Alleviates the Neurodegenerative Phenotypes and Histone Dysregulation in Presenilins-Deficient Mice. *Front Aging Neurosci*. 2018;10:137.
doi: 10.3389/fnagi.2018.00137
 24. Damiani F, Cornuti S, Tognini P. The gut-brain connection: Exploring the influence of the gut microbiota on neuroplasticity and neurodevelopmental disorders. *Neuropharmacology*. 2023;231:109491.
doi: 10.1016/j.neuropharm.2023.109491
 25. Fang Y, Li Y, Liao X, et al. *Corydalis yanhusuo* Polysaccharides Ameliorate Chronic Stress-Induced Depression in Mice through Gut Microbiota-Derived Short-Chain Fatty Acid Activation of 5-Hydroxytryptamine Signaling. *J Med Food*. 2023;26(12):890-901.
doi: 10.1089/jmf.2023.K.0050
 26. Duan C, Huang L, Zhang C, et al. Gut commensal-derived butyrate reverses obesity-induced social deficits and anxiety-like behaviors via regulation of microglial homeostasis. *Eur J Pharmacol*. 2021;908:174338.
doi: 10.1016/j.ejphar.2021.174338
 27. Cavaliere G, Catapano A, Trinchese G, et al. Butyrate Improves Neuroinflammation and Mitochondrial Impairment in Cerebral Cortex and Synaptic Fraction in an Animal Model of Diet-Induced Obesity. *Antioxidants (Basel)*. 2022;12(1):4.
doi: 10.3390/antiox12010004
 28. Ma X, Shi W, Wang Z, et al. Butyric acid and valeric acid attenuate stress-induced ferroptosis and depressive-like behaviors by suppressing hippocampal neuroinflammation. *J*

Transl Med. 2025;23(1):974.

doi: 10.1186/s12967-025-06950-0

29. Blaak EE, Canfora EE, Theis S, *et al.* Short-chain fatty acids in human gut and metabolic health. *Benef Microbes.* 2020;11(5):411-455.

doi: 10.3920/BM2020.0057

30. Napier BA, Allegretti JR, Feuerstadt P, *et al.* Multi-Species Synbiotic Supplementation Enhances Gut Microbial

Diversity, Increases Urolithin A and Butyrate Production, and Reduces Inflammation in Healthy Adults: A Randomized, Placebo-Controlled Trial. *Nutrients.* 2025;17(17):2734.

doi: 10.3390/nu17172734

31. Guan Y, Zhu R, Zhao W, *et al.* Effects of *Lactocaseibacillus paracasei* K56 on perceived stress among pregraduate students: a double-blind, randomized, placebo-controlled trial. *Front Nutr.* 2025;12:1544713.

doi: 10.3389/fnut.2025.1544713