Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2024, **15**, 10732

Lacticaseibacillus paracasei 207-27 alters the microbiota–gut–brain axis to improve wearable device-measured sleep duration in healthy adults: a randomized, double-blind, placebo-controlled trial†

Jinxing Li, ‡[a] Jincheng Zhao,‡[a] Xiaolei Ze,[b] Liang Li,[b] Yapeng Li,[a] Zhimo Zhou,[a] Simou Wu,[a] Wen Jia,[a] Meixun Liu,[a] Yun Li,[a] Xi Shen,[a] Fang He*[a] and Ruyue Cheng *[a]

Objective: Probiotics have been reported to exert beneficial effects on sleep through the gut–brain axis. Therefore, this randomized, double-blind, placebo-controlled trial assessed the effects of *Lacticaseibacillus paracasei* 207-27 supplementation on sleep quality and its safety and potential mechanisms. *Method and study design*: Healthy adults under mild stress aged 18–35 years consumed low or high doses of *L. paracasei* 207-27 or a placebo for 28 days. Fecal samples, blood samples, and questionnaires were collected at the baseline and the end of the intervention. Sleep quality was measured using wearable devices and Pittsburgh sleep quality index (PSQI) questionnaire. Serum inflammatory markers, corticotropin-releasing hormone, adrenocorticotropic hormone (ACTH), cortisol (COR), γ-aminobutyric acid, and 5-hydroxytryptamine levels were detected using enzyme-linked immunosorbent assay. The gut microbiota was analyzed using 16S rRNA sequencing and bioinformatics. Short-chain fatty acids levels were detected using gas chromatography–mass spectrometry. *Results*: Both the low-dose and high-dose groups exhibited significant improvements in wearable device- measured sleep duration compared to the placebo group. The global scores of PSQI in three groups significantly decreased after intervention without statistical difference between groups. At the phylum level, the low-dose group exhibited a higher relative abundance of *Bacteroidota* and a lower *Firmicutes*-to-*Bacteroidetes* (*F*/*B*) ratio. At the genus level, two treatment groups had higher relative abundance of *Bacteroides* and *Megamonas*, alongside lower levels of *Escherichia-Shigella*. Furthermore, the low-dose group exhibited significant increases in acetic acid, propionic acid, butyric acid, and valeric acid levels, while two treatment groups exhibited a significant decrease in COR levels. Correlation analysis revealed that the increased levels of acetic acid and butyric acid in the low-dose group may be associated with decreased ACTH. *Conclusion*: *L. paracasei* 207-27 administration in healthy adults resulted in improvements in gut microbiota community and sleep duration. The mechanisms might involve modulation of the gut microbiota structure to regulate the function of the gut–brain axis, including increases in SCFA levels and decreases in hypothalamic–pituitary–adrenal axis activity. The Chinese clinical trial registry number is ChiCTR2300069453 (**https://www.chictr.org.cn/showproj.html?proj=191193**, registered 16 May 2023 - retrospectively registered).

Received 11th April 2024,
Accepted 27th September 2024

DOI: 10.1039/d4fo01684j

rsc.li/food-function

Introduction

The human digestive tract contains trillions of extremely diverse microorganisms.[1] The gut microbiota serves as part of the intestinal barrier, and it can modulate the brain.[2] The microbiota–gut–brain axis consists of the gut microbiota, metabolites, the enteric nervous system, the sympathetic and parasympathetic branches of the autonomic nervous system, the neural–immune system, the neuroendocrine system, and

[a]*Department of Nutrition and Food Hygiene, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu 610041, China. E-mail: hf18602880124@163.com, ruyuecheng1993@163.com; Tel: +86-186-0288-0124, +86-18011462163*
[b]*BYHEALTH Institute of Nutrition & Health, Guangzhou 510663, China*

†Electronic supplementary information (ESI) available. See DOI: **https://doi.org/10.1039/d4fo01684j**

‡These authors have contributed equally to this work.

the central nervous system.[3,4] There are five communication pathways between the gut microbiota and the brain, including the gut–brain neural network, the hypothalamic–pituitary–adrenal (HPA) axis, the gut immune system, some neurotransmitters synthesized by the gut microbiota, and barrier pathways including the intestinal mucosal barrier and blood–brain barrier.[5,6] Sleep is widely acknowledged as a fundamental physiological process intricately regulated by complex neurobiological mechanisms. Recent scientific investigations have shed light on the potential role of the gut microbiota as a key modulator of the intricate regulatory pathways underlying sleep.[7,8]

The World Health Organization defines probiotics as "live microorganisms that confer a health benefit on the host when administered in adequate quantities".[9] Probiotics have gained increasing attention for their ability to modulate brain health *via* the microbiota–gut–brain axis.[10,11] Indeed, probiotic strains can regulate the gut microbiota and HPA axis hormones in humans, both of which are associated with sleep disorders.[12] Numerous investigations have demonstrated that *Lactobacillus* species can improve sleep quality by influencing the gut–brain axis.[13,14] However, data regarding the sleep-improving effect of *Lacticaseibacillus paracasei* are insufficient and heterogeneous because of the lack of microbiome analyses and the limited sample size and strain-specific effects of probiotics.

L. paracasei 207-27 was originally isolated from healthy infant feces in China and it is now deposited at the Guangdong Microbial Culture Collection Center (GDMCC) under the Budapest Treaty, with deposit code GDMCC 60960. *L. paracasei* 207-27 is also named Lacticaseibacillus paracasei LPB27 in its commercialized product. In our previous *in vitro* and *in vivo* studies, we found that *L. paracasei* 207-27 has good probiotic properties, and it has been considered a candidate probiotic strain. Furthermore, *L. paracasei* 207-27 can modulate gut microbiota composition and the levels of its metabolites short-chain fatty acids (SCFAs), and it exhibited an anti-allergy effect in OVA-induced allergic mice.[15–17] However, the safety and efficacy of *L. paracasei* 207-27 in humans remain unstudied. To determine the safety of *L. paracasei* 207-27 in humans and its effects on the physiological activities of the gut–brain axis such as sleep, we conducted a randomized, double-blind, placebo-controlled trial. In addition, we aimed to elucidate the potential mechanisms of the effects of *L. paracasei* 207-27 by identifying the alterations and associations among gut microbiota, metabolites, and relevant hormones.

Materials and methods

Study design

We conducted a randomized, double-blind, placebo-controlled trial examining the effects of *L. paracasei* 207-27 consumption on sleep quality in adults under mild stress aged 18–35. Study researchers who were not involved in the intervention process generated a random sequence using Excel, and the random numbers were stratified by sex with a 1 : 3 allocation. The allocation sequence was concealed from the researchers, and details of the allocated groups were given on number code containing the sequential numbers prepared by the product providers. All participants and researchers were blinded to study allocation throughout the entire study. The study was unblinded after all statistical analyses were completed. Subjects were randomly distributed into three groups (n = 40 per group): placebo group, low-dose *L. paracasei* group (1×10^{10} colony-forming units [CFU]) and high-dose *L. paracasei* group (5×10^{10} CFU) group.

The study consisted of an intervention and a post-observation, as presented in Fig. 1. Participants were asked to wear a wearable device (Huawei B6, Huawei, Guangdong, China) and make no changes to their exercise and eating habits during the study. Participants consumed one sachet of the study product per day after having lunch for 28 consecutive days. The wearable device data, blood samples, fecal samples, and subjects' questionnaires were collected on days 0 and 28.

Sample size calculation

The sample size was determined using the superiority design method (the sample size was calculated when the outcome measure was a continuous variable).[18] We estimated the sample size based on the total PSQI scores of differences after probiotic treatment in published papers (pre: 11.6 ± 3.1, post: 7.75 ± 3.6, P = 0.0007).[19] The minimal required sample size was calculated as 96 subjects (n = 32 per group) using the two-sample, continuous outcome, two-tailed, independent t-test approach with a significance level of 5% and power of 90%. Considering a withdrawal rate of 20%, we planned to enroll 120 subjects (n = 40 per group).

Subjects

The test subjects were 120 healthy participants (30 men and 90 women). Participants were excluded if they had any known disease or were taking medicine for them, including metabolic arthritis, heart/cardiopulmonary disease, diabetes, hypertension, and constipation. All participants were prohibited from taking probiotics, prebiotics, fermented products (yogurt or foods), antibiotics, and anti-inflammatory medications to

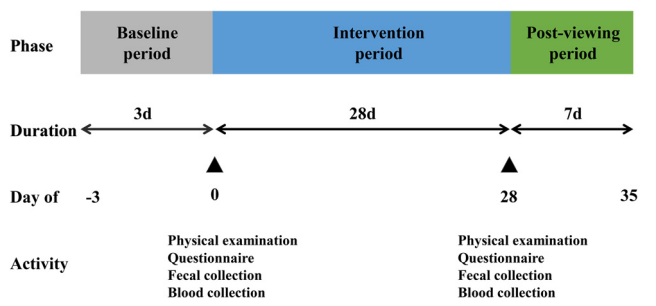

Fig. 1 Study design.

avoid unnecessary interference during the experimental period.

Study products

L. paracasei 207-27, a human-derived *Lactobacillus* strain, was cultivated and produced by BY-HEALTH (Guangdong, China). The product of this study consisted of 1×10^{10} or 5×10^{10} CFU of *L. paracasei* 207-27, and the content was standardized using maltodextrin in sachet packing. The placebo powder contained maltodextrin similarly as the *L. paracasei* 207-27 powder without the addition of the probiotic. Study products could not be distinguished by package, color, taste, and smell to maintain treatment allocation concealment from participants and study staff. To take the product, participants mixed one sachet with cold water.

Data and sample collection

Questionnaire. The questionnaires included questions regarding Pittsburgh sleep quality index (PSQI), dietary habits, physical activity,[20] product intake (only during the intervention period), the frequency of bowel movements, fecal quality (consistency and color),[21,22] any medications received, and any symptom of discomfort, such as diarrhea, constipation, vomiting, gas, and sensations of illness.

Participants recorded a food diary for 2 days (one workday and one weekend day) each week using the Huawei health application, which automatically accessed energy and macronutrient consumption. The wearable device was used daily to track physical activity. Participants were required to uploaded a week-period energy intake, and energy consumption data to the investigator before the beginning and the end of the intervention.

Physical examination. Anthropometric measurements were performed using standardized examination procedures and calibrated equipment on days 0 and 28. All patients fasted for 8 h prior to the examination. Body weight and body fat rate were detected using InBody H20N (Biospace Coltd, Seoul, South Korea). Participants were weighed while wearing lightweight clothing and no shoes. Waist circumference was measured at the midpoint between the lowest margin of the last rib and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks. Systolic and diastolic blood pressure was measured twice after 2–5 min of rest in the seated position using an automatic sphygmomanometer (YE-680B1; Yuwell, Jiangsu, China), and the measurements were separated by a 1 min interval.

Feces collection. The materials and instructions for fecal sample collection were provided to the subjects prior to the time of fecal collection. The subjects were required to immediately obtain a sample by scraping the feces with the scooper and capping the tube tightly. Each fecal sample was collected into a sterile tube using a scoop built inside the lid by the subjects at home, and the sample was immediately transported to the laboratory in a cold storage container (−80 °C) by the researchers.

Blood collection. Venous blood samples were obtained after 8 h of fasting. Blood lipid profiles, serum biochemistry, and complete blood counts were determined in the West China Medical Detection Institute of Sichuan University. Additionally, other blood samples were stored at 4 °C and centrifuged for 15 min at 1000*g* (or 3000 rpm) at 2–8 °C within 30 min. Then, the serum was transferred into pyrogen-free tubes using pyrogen-free pipette tips and stored at −80 °C until further analysis.

Outcome assessments

Sleep quality. Sleep records, electroencephalography (EEG), polysomnography (PSG), and wearable devices are all available for measuring sleep. EEG and PSG remain the gold standard for accurately reflecting sleep patterns. However, they necessitate overnight stays in sleep laboratories, which limits their practical usage.[23] Except PSQI questionnaire, the present study further adopted an innovative approach to assist PSQI by utilizing wearable devices to collect accurate and quantitative data on objective sleep attributes. Wearable devices have immense potential in health monitoring, particularly for tracking sleep patterns.[24]

In Deng's investigation, the Huawei smartwatch was employed to assess the sleep quality of the subjects.[26] Xie's study performed a comparative examination of several prominent smartwatch models, and the outcomes demonstrated the commendable precision of the Huawei smartwatch in quantifying sleep duration.[25] Moreover, the results reported by Liang[27] provided additional substantiation regarding the accuracy of the Huawei smartwatch in measuring sleep parameters.

Therefore, the Huawei band 6 was utilized as a non-invasive monitoring tool to obtain objective measurements of sleep attributes, including several key parameters such as the durations of sleep, deep sleep, and rapid eye movement and the number of awakenings. The Huawei band 6 extracted the normal sinus rhythm intervals and respiratory signals from the heart rate signal. It analyzed the coherence and cross-spectral power of these signals using Hilbert-Huang transform (HHT) technology. This process generated a cardiopulmonary coupling (CPC) dynamic spectrum during sleep, which accurately reflected various sleep stages: deep sleep, light sleep, rapid eye movement sleep, and wakefulness.[28] The application of this technology in wearable devices enables more convenient, accurate and continuous monitoring of sleep, leading to its increasingly widespread adoption.[29–31]

Using a wearable device, we collected precise and quantitative sleep data. This objective approach enhanced the accuracy and reliability of our findings, contributing to a more comprehensive understanding of sleep patterns and their potential implications.

After enrollment, all subjects were provided with the Huawei band 6 and instructed to wear it continuously to monitor their sleep from enrollment onwards. The average data collected during the one-week period between enrollment and the start of the intervention was defined as baseline data. Similarly, the average data collected during the week preceding

the end of the intervention was defined as outcome data. The sleep duration denoted the total amount of time an individual spent asleep, providing an objective measure of sleep quantity. The number of awakenings indicated the frequency of transitions from sleep to wakefulness throughout the sleep period, reflecting sleep fragmentation and disturbances.

Serum biochemical markers. Safety parameters including blood lipid profiles, blood glucose levels, serum biochemistry parameters, and complete blood count variables were determined in the West China Medical Detection Institute of Sichuan University.

Serum inflammatory factors. Inflammatory markers, including TNF-α, IL-1β, and IL-6, were measured using Luminex 200 according to the manufacturer's instructions (R&D Systems China, Shanghai, China). Cytokine levels were estimated using a four-parameter polynomial curve (LINCOplex3.5.5.0).

Serum hormones and neurotransmitters. Serum hormone and neurotransmitter levels were measured using enzyme-linked immunosorbent assay (ELISA). ELISA kits for corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and 5-hydroxytryptamine (5-HT) were obtained from Elabscience Biotechnology Co. Ltd (Wuhan, China). The ELISA kit for cortisol (COR) was obtained from R&D Systems China. The ELISA kit for γ-aminobutyric acid (GABA) was purchased from CLOUD-CLONE Corp (Wuhan, China).

16S rRNA gene sequencing and bioinformatics analysis. Total bacterial DNA was extracted from stool samples using a TIANamp Stool DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) according to the manufacturer's instructions. We used specific primers to amplify the 16S rRNA genes of V3–V4 (forward primer: 338F ACTCCTACGGGAGGCAGCAG, reverse primer: 806R GGACTACHVGGGTWTCTAAT). For PCR (Polymerase chain reaction), the 25 μL reaction mixture consisted of 12.5 μL of Phusion Hot Start Flex 2× Master Mix, 1 μmol L^{-1} of the forward and reverse primers, approximately 50 ng of template DNA, and double-distilled water. The cycling conditions were 98 °C for 1 min followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. A DNA PCR-Free Sample Preparation Kit (Illumina. Inc., USA) was used to generate sequencing libraries. A Qubit@ 2.0 Fluorometer (Thermo Scientific. Co., Ltd, USA) was used to assess the quality of the libraries.

The subsequent data analysis involved a series of rigorous procedures. Initially, the raw sequencing data were demultiplexed according to the barcode sequences and PCR primer sequences, and subsequently, the barcode and primer sequences were trimmed. FLASH software (version 1.2.11)[32] was then utilized to merge the reads, resulting in the generation of raw tags. Then, fastp software (version 0.20.0) was employed for quality control to obtain a collection of high-quality clean tags. Subsequently, the clean tags were aligned against a reference database using Vsearch (version 2.9.0) to identify and remove chimeric sequences,[33] thereby yielding a set of effective tags representing the valid data. For the obtained effective tags, the DADA2 module within QIIME2 software (Qiime2-202006) was employed for denoising accompanied by the removal of sequences with an abundance smaller than five. Consequently, the amplicon sequence variants (ASVs) and a corresponding feature table were generated. Furthermore, the classify-sklearn module within QIIME2 was utilized to assign species information to each ASV by performing a comparative analysis against a reference database. Furthermore, SVG software (version Perl-5.18.2) was employed to generate a heatmap representing the relative abundance distribution of the species. Alpha diversity indices, including the Chao1, observed_asvs, Shannon, and Simpson indices, were calculated using QIIME2 based on the simplified ASV table. QIIME2 was employed to compute Unifrac distances, and R software (version 2.15.3) was utilized to generate a principal coordinate analysis (PCoA) plot.

SCFAs. Feces (100 mg) were collected into an Eppendorf tube, and 100 μL of 15% phosphoric acid, 20 μL of 375 μg mL^{-1} 4-methylvaleric acid solution, and 280 μL of ether were added to fix the content. Finally, the mixture was centrifuged at 12 000 rpm and 4 °C for 10 min after vortexing for 1 min, and the supernatant was transferred into the vial prior to GC-MS analysis (Agilent 7890A).[34]

Statistical analysis. All blood indicators and SCFAs data were presented and analyzed based on changes before and after the intervention. Additionally, demographic characteristics and gut microbiota data on day 0 and day 28 were also included for comparison.

The categorical variables were presented as absolute numbers and percentages, while continuous variables were expressed as the mean ± standard deviation. Multiple comparisons among the three groups were conducted using one-way analysis of variance or the Kruskal–Wallis H-test, with *P* values adjusted using the Benjamin–Krieger–Yekutieli method for pairwise comparisons. This adjustment method was also applied to *P* values in the comparison of multiple taxa in the gut microbiota analysis.

Self-comparison within the groups were conducted using the paired T-test or the Wilcoxon signed-rank test. Spearman correlation analysis was utlized to assess associations between variables.

All statistical analyses were performed using Prism 9.0 (GraphPad, San Diego, CA, USA), and a two-tailed adjusted *P*-value of less than 0.05 was considered statistically significant.

Results

Participant flow and demographics

As presented in Fig. 2, 120 participants were enrolled in the study, and 104 individuals completed the study (completion rate, 86.67%). No clinically relevant adverse events were reported during the intervention. In all analyses, we included data from participants who completed the intervention. The demographic and clinical characteristics of the participants at

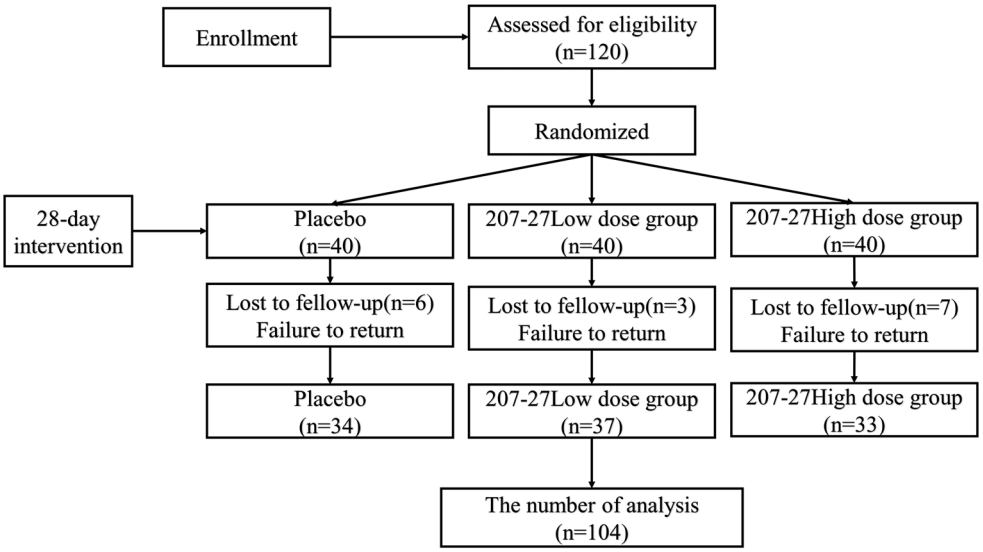

Fig. 2 Consort flow diagram.

baseline and the end of the intervention are summarized in Table 1. The male-to-female ratio did not significantly differ among the groups. The mean ages were 25.02, 25.17, and 24.78 years in the placebo, low-dose, and high-dose groups, respectively, and no significant difference was detected. At baseline and the end of the intervention, no significant differences were observed among the groups in terms of weight, body fat, waistline, hipline, blood pressure, calorie intake, carbohydrates intake, protein intake, fat intake, and energy consumption during exercise. In addition, no significant difference was observed in these variables in the three groups between baseline and the end of the intervention. Therefore, we confirmed that randomization was successful.

Data expressed as mean ± standard deviations. P_{Baseline} were analyzed among three groups at baseline, $P_{4\ \text{weeks}}$ were analyzed among three groups at the end of intervention.

Safety assessment

Over the entire course of the study, no participant exhibited any untoward gastrointestinal symptoms, such as diarrhea, constipation, vomiting, and regurgitation. Moreover, there were no reported instances of subjective illness perceptions or any form of discomfort. Furthermore, blood lipid profiles, blood glucose levels, serum biochemistry parameters, and complete blood counts were evaluated at baseline and after the intervention to assess any potential changes. The statistical analysis revealed no significant differences in these indices among the three experimental groups (ESI Tables 1 and 2†). The lack of significant differences suggests that the intervention did not significantly affect physiological markers among the study participants.

Changes in sleep quality following the probiotic intervention

Significant differences were observed in sleep duration among the three groups. Sleep duration was significantly increased in the low-dose and high-dose groups compared to that in the placebo group (Fig. 3A). Specifically, the mean sleep duration was increased by 1.07 h in the low-dose group and 1.04 h in the high-dose group. However, there were no statistically significant differences observed in the number of awakenings among the three groups (Fig. 3B). Furthermore, although the changes before and after intervention were used for statistical analysis, the original results of pre - and post-intervention comparisons between groups were also accurately presented (ESI Fig. 1†). The PSQI results showed that scores of sleep duration, sleep disturbances in the low-dose group, and global scores in three groups significantly decreased after intervention. However, no significant differences were found in the changes of scores of seven "component" scores and global score between three groups (ESI Table 3†).

Changes in serum HPA axis hormone levels following the probiotic intervention

The HPA axis, a fundamental component of the gut–brain axis, plays a pivotal role in orchestrating the primary biological response to stressful stimuli. In this study, we quantified the levels of CRH, ACTH and COR to assess the activity of the HPA axis. Significant differences were observed in the changes of CRH levels among the low-dose, high-dose, and placebo groups (Fig. 4A). CRH levels slightly increased in the high-dose group, whereas they decreased in the low-dose and placebo groups. Furthermore, ACTH levels were significantly lower in the low-dose group than in the placebo group (Fig. 4B). In addition, COR levels decreased in the low-dose and high-dose groups but increased in the placebo group (Fig. 4C).

Changes in serum neurotransmitter levels following the probiotic intervention

The changes in 5-HT and GABA levels during the intervention did not differ among the three groups (Fig. 5A and B).

Table 1 Demographic characteristics of the participants

Variable	Placebo group (n = 34)		Low-dose group (n = 37)		High-dose group (n = 33)			
	Baseline	4 weeks	Baseline	4 weeks	Baseline	4 weeks	P_{Baseline}	$P_{4\text{ weeks}}$
Sex								
Male	9(25.7%)		8(21.6%)		7(21.2%)		0.77	
Female	26(74.3%)		29(78.4%)		26(78.8%)		0.86	
Age (years)	25.02 ± 4.32		25.17 ± 2.78		24.78 ± 3.02		0.65	
Weight (kg)	59.96 ± 12.18	59.67 ± 12.34	57.60 ± 9.10	57.24 ± 9.33	58.12 ± 11.26	57.88 ± 10.93	0.63	0.62
Body fat (%)	26.43 ± 6.17	25.67 ± 6.2	26.41 ± 6.1	25.75 ± 6.00	27.06 ± 7.50	26.41 ± 7.32	0.90	0.88
Waistline (cm)	72.91 ± 8.78	72.81 ± 9.27	71.78 ± 7.67	70.85 ± 7.5	72.10 ± 9.54	71.85 ± 9.25	0.86	0.62
Hipline (cm)	94.29 ± 6.97	94.03 ± 6.99	94.07 ± 5.52	93.72 ± 5.70	93.76 ± 8.07	94.43 ± 7.24	0.95	0.90
Systolic blood pressure (mmHg)	114.04 ± 12.06	115.50 ± 11.21	116.20 ± 10.08	115.90 ± 11.73	115.50 ± 9.15	114.80 ± 8.89	0.79	0.90
Diastolic blood pressure (mmHg)	71.21 ± 7.31	71.85 ± 8.68	73.05 ± 8.16	72.05 ± 7.78	73.73 ± 6.91	72.52 ± 5.73	0.36	0.93
Calorie intake (kcal)	1485 ± 446.20	1522 ± 514.80	1524 ± 386.00	1469 ± 418.80	1409 ± 392.90	1376 ± 489.70	0.49	0.45
Carbohydrate intake (g)	194.30 ± 100.40	193.30 ± 80.43	199.00 ± 65.97	179.90 ± 65.76	177.80 ± 60.25	177.80 ± 85.24	0.49	0.67
Protein intake (g)	69.16 ± 29.53	75.51 ± 29.31	67.85 ± 22.67	73.67 ± 35.93	65.52 ± 23.62	62.19 ± 25.52	0.83	0.15
Fat intake (g)	53.94 ± 25.66	52.49 ± 22.79	54.57 ± 19.24	55.31 ± 24.49	50.37 ± 17.53	48.80 ± 23.81	0.68	0.52
Energy consumption of exercise (kcal)	368 ± 190.60	366 ± 125.80	368.8 ± 130.70	359.6 ± 132.20	351.1 ± 176.50	348.7 ± 109.40	0.81	0.34

Fig. 3 The changes of sleep duration (A) and awakening times (B) were compared among three groups. **adjusted P < 0.01.

Gut microbiota composition

The relative abundance of specific bacterial strains at both the phylum and genus levels on day 0 and 28 is presented in Fig. 6A and B, as well as in ESI Tables 4 and 5.†

At the phylum level, the low-dose group exhibited a significantly higher relative abundance of Bacteroidota than the placebo group (Fig. 6C), whereas the *Firmicutes*-to-*Bacteroidetes* (*F*/*B*) ratio was significantly lower in the low-dose group than in the placebo group (Fig. 6D).

At the genus level, the relative abundance of *Blautia* was significantly lower in the low-dose group than in the placebo group (Fig. 6E). Moreover, the relative abundances of *Escherichia-Shigella* and *Megamonas* were significantly higher in both the low-dose and high-dose groups than in the placebo group (Fig. 6F and G), whereas *Bacteroides* was significantly lower (Fig. 6H).

We further analyzed the changes of relative abundance of gut microbiota among groups. Change of relative abundance of *Blautia* in the low-dose group (−2.951% ± 3.821%) was significantly lower than in the placebo group (1.610% ± 5.297%). The relative abundance of *Lactobacillus* was increased in the low-dose (0.166% ± 0.784%) and high-dose groups (0.136% ± 0.317%), while decreased in the placebo group (−0.886% ± 1.659%) after intervention. Compared with the placebo group, changes of relative abundance of *Lactobacillus* in the low-dose and high-dose groups were statistically significant. The relative abundance of *Clostridia*_UCG_014 decreased in the high-dose groups (−0.110% ± 3.246%), while increased in the placebo (2.146% ± 5.284%) and low-dose groups (0.474% ± 1.444%). The difference between the placebo and high-dose groups was statistically significant. No significant changes were observed in *Enterococcus*, *Enterobacter*, *etc.*

Regarding alpha diversity indices of gut microbiota, no significant differences were observed among the groups (ESI Fig. 2†). Similarly, based on the unweighted Unifrac distance, the PCoA plot revealed that the gut microbiota composition did not exhibit significant dispersion among the groups at the end of the intervention (ESI Fig. 3†).

SCFAs

SCFAs play a crucial role in the bidirectional communication of the gut–brain axis, both directly and indirectly. In this

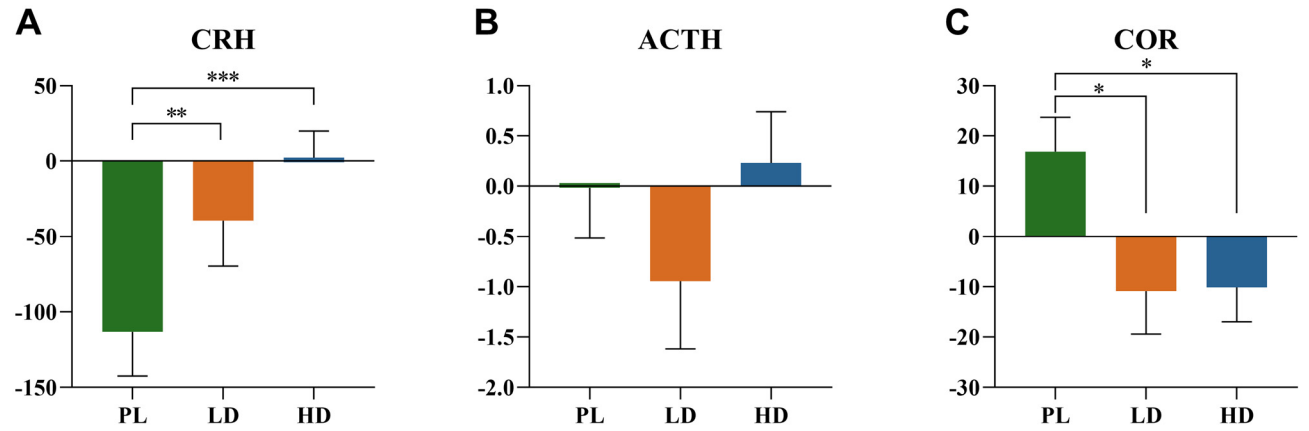

Fig. 4 The changes of serum levels of CRH (A), ACTH (B) and COR (C) were compared among three groups. *adjusted $P < 0.05$, **adjusted $P < 0.01$, ***adjusted $P < 0.001$.

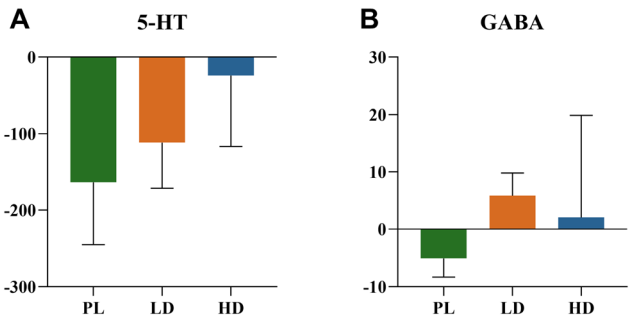

Fig. 5 The changes of serum levels of 5-HT (A) and GABA (B) were compared among three groups.

study, we quantified the levels of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid in fecal samples obtained from 104 subjects.

Significant differences were observed in the changes of acetic acid, propionic acid, butyric acid, and valeric acid levels among the groups. Specifically, the low-dose group exhibited significant increases in the levels of acetic acid, propionic acid, butyric acid, and valeric acid (Fig. 7A–C and E). However, no significant differences were observed in the changes of isobutyric acid and isovaleric acid levels among the three groups (Fig. 7D and F).

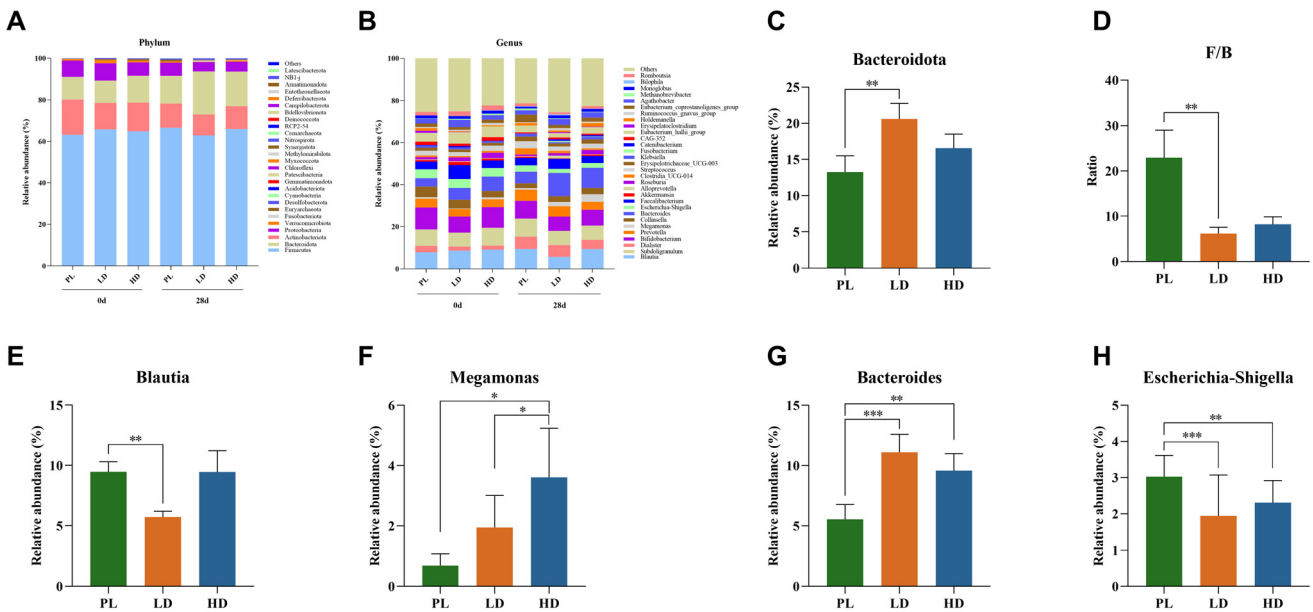

Fig. 6 Top 30 species according to relative abundance at the phylum and genus levels among the three sets of feces samples (A and B). The relative abundance of *Bacteroidota* (C), the ratio of *Firmicutes*/*Bacteroidota* (*F*/*B*) (D), *Blautia* (E), *Megamonas* (F), *Bacteroides* (G), and *Escherichia-Shigella* (H) were compared among three groups at the end of intervention. *adjusted $P < 0.05$, **adjusted $P < 0.01$, ***adjusted $P < 0.001$.

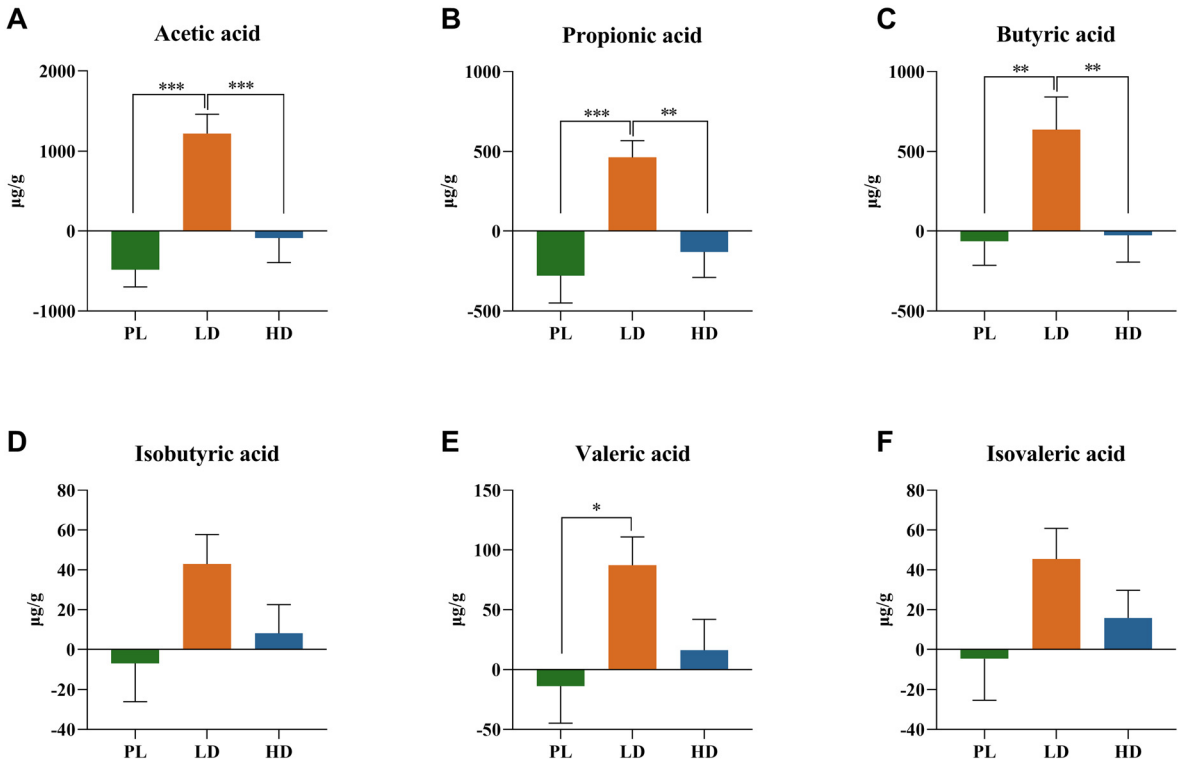

Fig. 7 The changes of the levels of acetic acid (A), propionic acid (B), butyric acid (C), isobutyric acid (D), valeric acid (E), and isovaleric acid (F) were compared among three groups. *adjusted $P < 0.05$; **adjusted $P < 0.01$, ***adjusted $P < 0.001$.

Associations among the gut microbiota, sleep quality, and metabolites

To investigate the potential correlations among sleep quality, HPA axis hormones, SCFAs, and gut microbiota, Spearman correlation analysis was performed.

At the phylum level, no significant correlations were found between gut microbiota and other parameters after adjusting P values (Fig. 8A–C).

At the genus level, the relative abundance of *Escherichia-Shigella* was positively correlated with changes in valeric acid

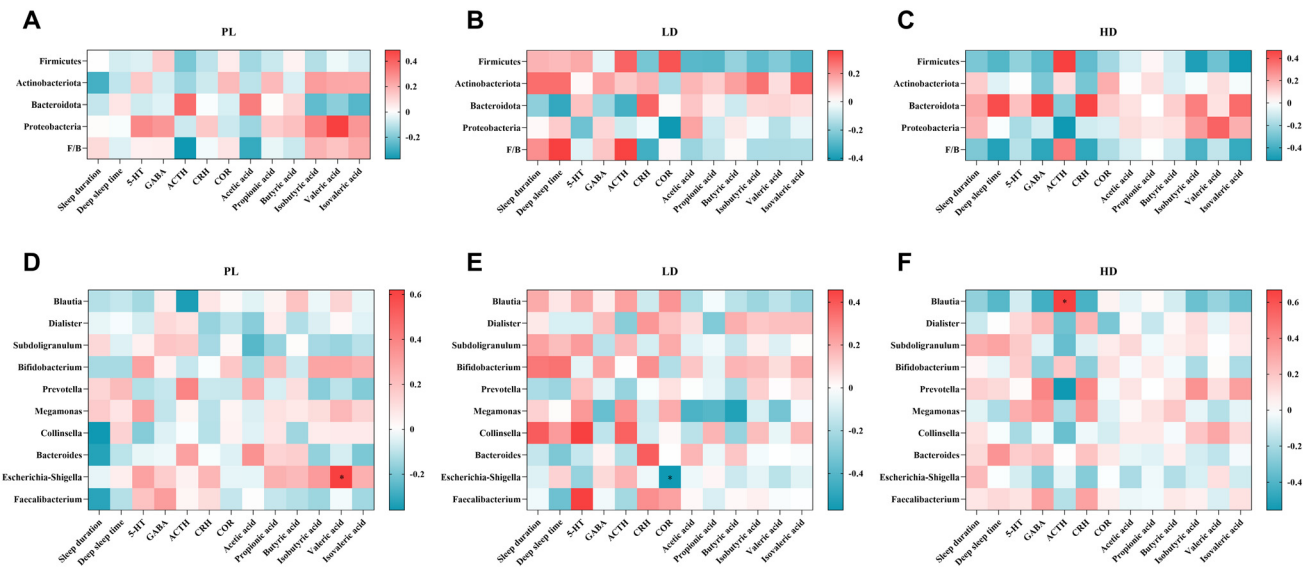

Fig. 8 Association between the relative abundance of gut microbiota at the phylum (A–C) and genus levels (D–F) and the changes of sleep duration and metabolites. The heatmap represents the correlation coefficient from Spearman correlation analysis. *adjusted $P < 0.05$.

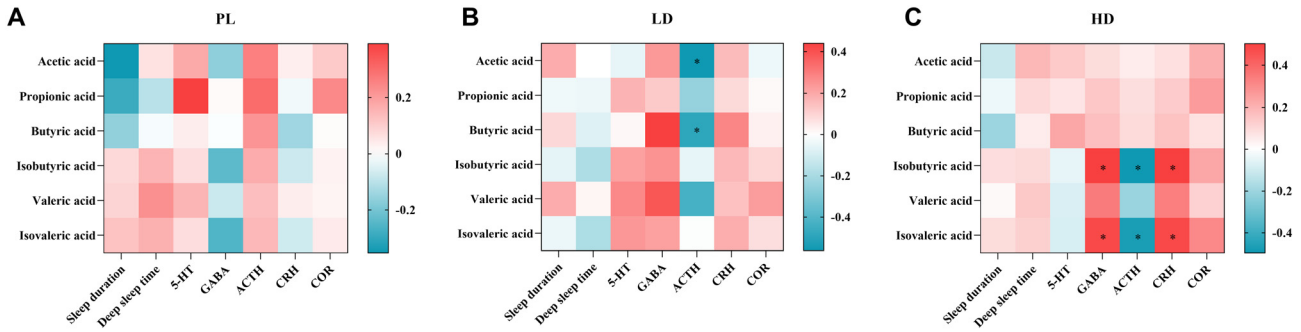

Fig. 9 Association between the changes of SCFAs and the changes of sleep duration, serum hormones and neurotransmitters in the placebo (A), low-dose (B), and high-dose groups (C). The heatmap represents the correlation coefficient from Spearman correlation analysis. * adjusted $P < 0.05$.

levels ($r = 0.619$, $P < 0.05$) in the placebo group (Fig. 8D). Regarding HPA axis hormones, changes in COR levels were negatively correlated with the abundance of *Escherichia-Shigella* ($r = 0.-0.569$, $P < 0.05$) in the low-dose group (Fig. 8E). Changes in ACTH levels were positively correlated with the abundance of *Blautia* ($r = 0.667$, $P < 0.05$) in the high-dose group (Fig. 8F)

Associations among SCFAs, sleep quality, serum hormones, and neurotransmitters

In the placebo group, there were no significant correlations observed among sleep quality, HPA axis hormones, neurotransmitters, and SCFAs (Fig. 9A).

In the low-dose group, changes in ACTH levels were negatively correlated with acetic acid ($r = -0.562$, $P < 0.05$) and butyric acid ($r = -0.491$, $P < 0.05$) levels (Fig. 9B).

In the high-dose group, elevated levels of isobutyric acid and isovaleric acid were positively correlated with GABA ($r = 0.502$, $P < 0.05$; $r = 0.476$, $P < 0.05$) and CRH ($r = 0.501$, $P < 0.05$; $r = 0.476$, $P < 0.05$) levels. However, isobutyric acid ($r = -0.495$, $P < 0.05$) and isovaleric acid levels (($r = -0.473$, $P < 0.05$)) were negatively correlated with ACTH levels (Fig. 9C).

Discussion

A variety of gut health benefits have been reported for probiotics, and recent evidence suggests that probiotics play a novel role in mental health *via* the microbiota–gut–brain axis.[35] Clinical investigations have demonstrated that probiotic administration can reduce anxiety-like and depressive-like behavior and normalize associated physiological outputs, including corticosterone and neurotransmitter levels and immune function.[36,37] However, strain specificity is the key factor. For example, our previous study found that *L. paracasei* 207-27 and *Bifidobacterium breve* 207-1, both derived from healthy infant's feces, have similar effects in regulating gut microbiota (for example, they both increase the relative abundance of *Alistipes*), they show great differences in emotional regulation,[16] which actually reflects the specificity of the strains. It reveals that the correlation between probiotics and mental health needs further research. Although studies have demonstrated the health-promoting effects of probiotics on the brain, few studies have examined their effects on mental health, particularly their ameliorative effects on sleep in healthy adults. In the present randomized, double-blind, placebo-controlled trial, we examined the potential effects of different doses of *L. paracasei* 207-27 on sleep quality, as well as the underlying mechanisms, in young healthy adults.

The present study demonstrated that daily consumption of the *L. paracasei* 207-27 supplement caused no adverse effects or clinical symptoms, such as gastrointestinal symptoms and excessive inflammation. Furthermore, comprehensive analysis of blood lipid profiles, blood glucose, serum biochemistry, and the complete blood count revealed no significant adverse signs. Although self-comparison within the groups showed significant changes in some blood indicators, all values remained within the normal range. Based on self-reported adverse event records from the subjects, there were no significant adverse events attributed to the short-term administration of *L. paracasei* 207-27. These findings collectively indicated that the regular intake of *L. paracasei* 207-27 is safe for healthy adults. Furthermore, no statistically significant differences were found in calorie intake, dietary composition (the ratios of carbohydrate, protein, and fat), or exercise expenditure before and after the intervention. These results suggested that the potential effects of probiotic treatment on outcomes were not confounded by variations in these factors (exercise and diet).

Most previous studies employed the PSQI as a measure to assess the sleep quality of participants. However, it should be noted that PSQI relies on subjective responses and only captures a snapshot of sleep quality, failing to provide a comprehensive representation of individuals' habitual sleep patterns.[38] Furthermore, empirical evidence indicates that the PSQI questionnaire does not adequately capture the nuances of sleep–wake behavior disparities between weekdays and non-weekdays.[39] Tian *et al.* found that supplemented *Bifidobacterium breve* CCFM1025 for four weeks decreased scores of sleep quality, sleep disturbance and PSQI.[40] However, there is no significant difference between placebo and treatment groups. Similar results were found in this study, again

highlighting the importance of objective measures of sleep quality.

Previous research established the potential of modulating the gut microbiota through live probiotic administration to improve sleep quality *via* intricate gut–brain axis interactions.[10] The findings of Lee's study revealed a statistically significant enhancement in sleep quality following an 8-week intervention of combined *L. reuteri* NK33 and *B. adolescentis* NK98 supplementation.[41] Furthermore, Ho *et al.* corroborated these results, demonstrating the effectiveness of *L. plantarum* PS128 supplementation in prolonging the duration of deep sleep among the study participants.[14] Consistently, our findings underscored the remarkable efficacy of different doses of 207-27 in significantly improving sleep quality as indicated by increased sleep duration.

Alterations in the gut microbiota structure might potentially contribute to improvements in sleep quality.[42] Several studies observed significant changes in the gut microbiota of patients with sleep disorders.[43,44] In this study, the low-dose group had a lower *F*/*B* ratio, and they also had lower counts of *Blautia* compared to the placebo and high-dose groups after the intervention. Compared with the control group, the changes of *Lactobacillus* in low-dose and high-dose groups were significantly increased, *Clostridia*_UCG-014 was significantly decreased in high-dose group, while *Enterococcus* and *Enterobacter* had no significant differences, indicating the regulatory roles of the tested *L. paracasei* 207-27 in the gut microbiota. An increased abundance of *Firmicutes* and decreased abundance of *Bacteroidota*, resulting in an increased *F*/*B* ratio, were detected in the microbiomes of patients with sleep disorders.[45] Similarly, a study by Grosicki *et al.*[46] observed a significantly negative association between sleep quality and the *F*/*B* ratio in adults. Additionally, Liu *et al.*[47] reported a lower *F*/*B* ratio in subjects diagnosed with insomnia. Furthermore, Haimov *et al.*[48] illustrated that a higher relative abundance of *Blautia* was associated with lower cognitive performance. Consistent with these findings, a previous study detected a higher abundance of *Blautia* in adults with insomnia.[49] Our findings provide evidence supporting the hypothesis that the improvement in sleep quality following *L. paracasei* 207-27 administration was mediated by targeted alterations in the relative abundance of specific microbial taxa. These results are consistent with prior investigations, which postulated that probiotics, such as *Lactobacillus*, can exert regulatory effects on the intricate composition of the gut microbiota, thereby potentially enhancing sleep quality.[50,51]

In addition to differences in the relative abundance of gut microbes, alterations in microbial metabolites play a crucial role in the regulation of sleep quality by probiotics. In our study, the low-dose group exhibited greater changes in acetic acid, propionic acid, butyric acid, and valeric acid levels than the placebo group. The production of SCFAs involves a variety of bacteria and multiple pathways.[52–54] Although this study did not identify a relationship between SCFAs and gut microbiota at the top 10 genus level, the increase in SCFAs content may be associated with changes in the abundance of multiple bacteria induced by the low dose of *L. paracasei* 207-27, particularly some low-abundance bacteria. However, the specific identification of these key bacteria was not conducted in this study. Some previous studies demonstrated that *Bacteroides* was a predominant producer of acetic acid, butyric acid, and propionic acid within the gastrointestinal tract.[55,56] In animal models, SCFAs produced by the gut microbiota regulate the expression of clock genes in the host.[57] Furthermore, in a mouse model of vascular dementia, *Clostridium butyricum* administration resulted in increased levels of butyrate in both the brain and feces.[58] In addition, decreased consumption of dietary fiber, which serves as a substrate to produce SCFAs by the gut microbiota in healthy individuals, has been associated with decreases in the total sleep duration. A study conducted by Magzal *et al.*[59] revealed that individuals with insomnia and shorter sleep durations exhibited decreased SCFA levels. Similarly, Heath *et al.*[60] detected a positive correlation between increased fecal propionic acid levels and a longer sleep duration in infants. These findings consistently suggest a potential association between SCFAs and sleep duration in different populations. The available evidence indicates that *L. paracasei* 207-27 administration potentially enhances sleep quality through the modulation of SCFA levels.

An additional plausible pathway establishing a connection between the gut microbiota and sleep quality involves the potential inhibition of HPA axis activity. Stress triggers pro-inflammatory cytokines, activating the HPA axis and leading to CRH and ACTH release, resulting in COR secretion.[61] Existing evidence has established that excessive activation of the HPA axis can exert adverse effects on sleep, characterized by sleep fragmentation, attenuated deep slow-wave sleep, and a shortened overall sleep duration.[62] In this study, the high-dose group exhibited a significant reduction in COR levels accompanied by a relative increase in CRH levels, reflecting the activation of negative feedback regulation. Correlation analysis revealed a negative association between the relative abundance of *Escherichia-Shigella* and COR levels and a positive correlation between the relative abundance of *Blautia* and ACTH levels. These findings suggested potential interactions between specific gut microbial taxa and the neuroendocrine regulation of the HPA axis. The results of human studies suggest that probiotics can inhibit HPA axis activation. Rudzki *et al.*[63] found that supplementation with *L. plantarum* 299v decreased COR levels, thereby improving cognitive function in patients with major depression. *Lactobacillus* supplementation has displayed promising activity in reducing stress-induced COR levels and improving sleep quality.[64] Overall, daily consumption of *L. paracasei* 207-27 could inhibit HPA axis activation. These findings supported the notion that probiotics can reduce HPA activity consequently enhance sleep quality.

Furthermore, this study identified a significant positive correlation between SCFA levels and GABA content in the high-dose group. GABA has been implicated in the development of various psychiatric disorders, including anxiety, depression, and sleep disorders.[65] Recent research by Yu *et al.*[66] demonstrated the potential beneficial effects of GABA-rich fermented

milk on sleep quality in mice. Moreover, Strandwitz *et al.*[67] demonstrated that certain probiotic strains, such as *Lactobacillus*, could produce GABA, thereby improving sleep quality. The present study revealed a non-significant trend toward increased GABA levels in both the low- and high-dose groups, whereas a decrease in GABA levels was observed in the placebo group following the intervention. However, the lack of significant differences among the groups might be attributable to the relatively short duration of the intervention. Further investigations with extended treatment durations are warranted to elucidate the potential interplay between probiotic administration and GABA modulation and its implications for sleep quality. Additionally, no significant differences in inflammatory factor levels were observed among the three groups in this study, likely because of the inclusion of healthy adult participants with initially low levels of inflammation (ESI Table 6†).

This study had several limitations that should be addressed in future research. Firstly, participants were instructed to maintain their usual lifestyle habits, which does not completely rule out the potential influence of factors like caffeinated drinks or sleep aids on the results. Secondly, the study was unable to definitively determine whether participants had severe sleep disorders or other medical conditions, relying solely on self-report for inclusion and exclusion criteria. The subject recruitment and screening were important and irreversible. Researchers need to pay more attention to this period to avoid introducing confounding. Thirdly, although research staff reminded all subjects to take the probiotics every day with text messages, it was difficult to supervise the subjects' actual adherence to the treatment regimen. Developing a strain-specific primer to determine the relative abundance of the probiotic in the feces and calculate the actual adherence rate could help address this issue. Fourthly, factors that could affect sleep quality, such as women's menstrual cycles, were not adequately considered and adjusted for in the study. Finally, due to operability issues, the study did not use objective sleep tests such as EEG and respiratory events to identify the type of sleep disorders. Although wearable devices were used to obtain sleep parameters indirectly, the accuracy of these measurements warrants further evaluated. Issues such as wearing norms and adaptation periods also need careful considered. Further studies with larger sample sizes and longer follow-up durations are warranted to establish a detailed assessment of the therapeutic effects of *L. paracasei* 207-27 and unravel the underlying mechanisms. Addressing the issues would provide precise guidance for future probiotic use in managing sleep disorders.

Conclusion

The administration of *L. paracasei* 207-27 regulated gut microbiota community and improved sleep duration in healthy adults with no obvious side effects. These findings support the health-promoting properties of *L. paracasei* 207-27 as part of a healthy diet for adults. The mechanisms involve modulation of the gut microbiota structure to regulate the function of the gut–brain axis, including increases in SCFA levels and decreases in HPA axis activity.

Author contributions

Conceptualization, R.C., X.S. and F.H.; methodology, R.C., L.L. and X.Z.; formal analysis, Z.Z., S.W. and W.J.; investigation, J. L., J.Z. and P.L.; writing – original draft preparation J.L., and J. Z.; writing – review and editing, R.C. and F.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was performed from May 2022 to June 2022 at Sichuan University (Sichuan, China) in accordance with the guidelines of the Declaration of Helsinki. The protocol was approved by the Medical Ethics Committee of the West China School of Public Health, Sichuan University (Gwll2022003). Information about the clinical trial was registered at the Chinese Clinical Trial Registry (Registration number: ChiCTR2300069453). The purpose and protocol of the study and foreseeable risks were explained to the participating subjects. All subjects have signed written informed consent.

Data availability

Data for this article, including types of excel and graph pad prism are available at OSF database at **https://osf.io/q93mb/?view_only=c9e558e6bc504a1fbb988286af3b6f3b**.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to express their sincere appreciation for the invaluable support extended by the Public Health and Preventive Medicine Provincial Experiment Teaching Center at Sichuan University and the Food Safety Monitoring and Risk Assessment Key Laboratory of Sichuan Province. The substantial contributions and resources provided by these esteemed institutions have played an indispensable role in facilitating the seamless execution and successful completion of this scholarly investigation.

References

1 A. Adak and M. R. Khan, An insight into gut microbiota and its functionalities, *Cell. Mol. Life Sci.*, 2019, **76**(3), 473–493.
2 S. M. Jandhyala, R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala and D. Nageshwar Reddy, Role of the normal gut microbiota, *World J. Gastroenterol.*, 2015, **21**(29), 8787–8803.
3 H. X. Wang and Y. P. Wang, Gut Microbiota-brain Axis, *China Med. J.*, 2016, **129**(19), 2373–2380.
4 M. Ding, Y. Lang, H. Shu, J. Shao and L. Cui, Microbiota-Gut-Brain Axis and Epilepsy: A Review on Mechanisms and Potential Therapeutics, *Front. Immunol*, 2021, **12**, 742449.
5 L. Dumitrescu, I. Popescu-Olaru, L. Cozma, D. Tulbă, M. E. Hinescu, L. C. Ceafalan, M. Gherghiceanu and B. O. Popescu, Oxidative Stress and the Microbiota-Gut-Brain Axis, *Oxid. Med. Cell. Longevity*, 2018, **2018**, 2406594.
6 P. Strandwitz, Neurotransmitter modulation by the gut microbiota, *Brain Res.*, 2018, **1693**(Pt B), 128–133.
7 A. Galbiati, M. Sforza, M. Poletti, L. Verga, M. Zucconi, L. Ferini-Strambi and V. Castronovo, Insomnia Patients With Subjective Short Total Sleep Time Have a Boosted Response to Cognitive Behavioral Therapy for Insomnia Despite Residual Symptoms, *Behav. Sleep Med.*, 2020, **18**(1), 58–67.
8 X. Wang, Z. Wang, J. Cao, Y. Dong and Y. Chen, Gut microbiota-derived metabolites mediate the neuroprotective effect of melatonin in cognitive impairment induced by sleep deprivation, *Microbiome*, 2023, **11**(1), 17.
9 N. T. Williams, Probiotics, *Am. J. Health Syst. Pharm*, 2010, **67**(6), 449–458.
10 C. Irwin, D. McCartney, B. Desbrow and S. Khalesi, Effects of probiotics and paraprobiotics on subjective and objective sleep metrics: a systematic review and meta-analysis, *Eur. J. Clin. Nutr.*, 2020, **74**(11), 1536–1549.
11 M. Shafie, A. Homayouni Rad, S. Mohammad-Alizadeh-Charandabi and M. Mirghafourvand, The effect of probiotics on mood and sleep quality in postmenopausal women: A triple-blind randomized controlled trial, *Clin. Nutr. ESPEN*, 2022, **50**, 15–23.
12 M. L. Jackson, H. Butt, M. Ball, D. P. Lewis and D. Bruck, Sleep quality and the treatment of intestinal microbiota imbalance in Chronic Fatigue Syndrome: A pilot study, *Sleep Sci*, 2015, **8**(3), 124–133.
13 M. Takada, K. Nishida, Y. Gondo, H. Kikuchi-Hayakawa, H. Ishikawa, K. Suda, M. Kawai, R. Hoshi, Y. Kuwano, K. Miyazaki and K. Rokutan, Beneficial effects of Lactobacillus casei strain Shirota on academic stress-induced sleep disturbance in healthy adults: a double-blind, randomised, placebo-controlled trial, *Benefic. Microbes*, 2017, **8**(2), 153–162.
14 Y. T. Ho, Y. C. Tsai, T. B. J. Kuo and C. C. H. Yang, Effects of Lactobacillus plantarum PS128 on Depressive Symptoms and Sleep Quality in Self-Reported Insomniacs: A Randomized, Double-Blind, Placebo-Controlled Pilot Trial, *Nutrients*, 2021, **13**(8), 2820.
15 H. Liang, Z. Luo, Z. Miao, X. Shen, M. Li, X. Zhang, J. Chen, X. Ze, Q. Chen and F. He, Lactobacilli and bifidobacteria derived from infant intestines may activate macrophages and lead to different IL-10 secretion, *Biosci., Biotechnol., Biochem.*, 2020, **84**(12), 2558–2568.
16 H. Liang, X. Ze, S. Wang, Y. Wang, C. Peng, R. Cheng, F. Jiang, S. Wu, R. He, F. He, X. Zhang and X. Shen, Potential Health-Promoting Effects of Two Candidate Probiotics Isolated from Infant Feces Using an Immune-Based Screening Strategy, *Nutrients*, 2022, **14**(17), 3651.
17 H. Liang, Y. Zhang, Z. Miao, R. Cheng, F. Jiang, X. Ze, X. Shen and F. He, Anti-allergic effects of two potential probiotic strains isolated from infant feces in China, *J. Funct. Foods*, 2022, **92**, 105070.
18 B. Zhong, How to calculate sample size in randomized controlled trial?, *J. Thorac. Dis.*, 2009, **1**(1), 51–54.
19 Y. Lan, J. Lu, G. Qiao, X. Mao, J. Zhao, G. Wang, P. Tian and W. Chen, Bifidobacterium breve CCFM1025 Improves Sleep Quality via Regulating the Activity of the HPA Axis: A Randomized Clinical Trial, *Nutrients*, 2023, **15**(21), 4700.
20 X. Zhang, M. Chen, R. Duan, H. Xue, J. Luo, X. Lv, H. Jia, F. He, L. Zhang and G. Cheng, The Nutrition and Health in Southwest China (NHSC) study: design, implementation, and major findings, *Eur. J. Clin. Nutr.*, 2021, **75**(2), 299–306.
21 K. W. Heaton, J. Radvan, H. Cripps, R. A. Mountford, F. E. Braddon and A. O. Hughes, Defecation frequency and timing, and stool form in the general population: a prospective study, *Gut*, 1992, **33**(6), 818–824.
22 L. Wang, L. Wang, P. Tian, B. Wang, S. Cui, J. Zhao, H. Zhang, L. Qian, Q. Wang, W. Chen and G. Wang, A randomised, double-blind, placebo-controlled trial of Bifidobacterium bifidum CCFM16 for manipulation of the gut microbiota and relief from chronic constipation, *Food Funct.*, 2022, **13**(3), 1628–1640.
23 S. Miyata, K. Iwamoto and N. Ozaki, Evaluating sleep-wake rhythm using a wearable device in patients with mental disorders, *Psychiatry Clin. Neurosci.*, 2023, **77**(8), 419.
24 S. Y. Tobin, P. G. Williams, K. G. Baron, T. M. Halliday and C. M. Depner, Challenges and Opportunities for Applying Wearable Technology to Sleep, *Sleep Med. Clin*, 2021, **16**(4), 607–618.
25 J. Xie, D. Wen, L. Liang, Y. Jia, L. Gao and J. Lei, Evaluating the Validity of Current Mainstream Wearable Devices in Fitness Tracking Under Various Physical Activities: Comparative Study, *JMIR Mhealth Uhealth*, 2018, **6**(4), e94.
26 S. Deng, Q. Wang, J. Fan, X. Yang, J. Mei, J. Lu, G. Chen, Y. Yang, W. Liu, R. Wang, Y. Han, R. Sheng, W. Wang, L. Ba and F. Ding, Correlation of Circadian Rhythms of Heart Rate Variability Indices with Stress, Mood, and Sleep Status in Female Medical Workers with Night Shifts, *Nat. Sci. Sleep*, 2022, **14**, 1769–1781.
27 J. Liang, D. Xian, X. Liu, J. Fu, X. Zhang, B. Tang and J. Lei, Usability Study of Mainstream Wearable Fitness Devices:

Feature Analysis and System Usability Scale Evaluation, *JMIR Mhealth Uhealth*, 2018, **6**(11), e11066.

28 R. J. Thomas, J. E. Mietus, C. K. Peng, A. L. Goldberger, L. J. Crofford and R. D. Chervin, Impaired sleep quality in fibromyalgia: Detection and quantification with ECG-based cardiopulmonary coupling spectrograms, *Sleep Med*, 2010, **11**(5), 497–498.

29 D. Liu, X. Yang, G. Wang, J. Ma, Y. Liu, C. K. Peng, J. Zhang and J. Fang, HHT based cardiopulmonary coupling analysis for sleep apnea detection, *Sleep Med*, 2012, **13**(5), 503–509.

30 H. S. Al Ashry, Y. Ni and R. J. Thomas, Cardiopulmonary Sleep Spectrograms Open a Novel Window Into Sleep Biology-Implications for Health and Disease, *Front. Neurosci.*, 2021, **15**, 755464.

31 R. J. Thomas, Dynamic Tracking of Brain Health via Interrogation of Sleep Networks With Cardiopulmonary Coupling Sleep Spectrograms, *Neurology*, 2024, **102**(10), e209517.

32 T. Magoč and S. L. Salzberg, FLASH: fast length adjustment of short reads to improve genome assemblies, *Bioinformatics*, 2011, **27**(21), 2957–2963.

33 B. J. Haas, D. Gevers, A. M. Earl, M. Feldgarden, D. V. Ward, G. Giannoukos, D. Ciulla, D. Tabbaa, S. K. Highlander, E. Sodergren, B. Methé, T. Z. DeSantis, J. F. Petrosino, R. Knight and B. W. Birren, Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons, *Genome Res.*, 2011, **21**(3), 494–504.

34 X. Han, J. Guo, Y. You, M. Yin, C. Ren, J. Zhan and W. Huang, A fast and accurate way to determine short chain fatty acids in mouse feces based on GC-MS, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2018, **1099**, 73–82.

35 S. Morkl, M. I. Butler, A. Holl, J. F. Cryan and T. G. Dinan, Probiotics and the Microbiota-Gut-Brain Axis: Focus on Psychiatry, *Curr. Nutr. Rep*, 2020, **9**(3), 171–182.

36 E. Navarro-Tapia, L. Almeida-Toledano, G. Sebastiani, M. Serra-Delgado, O. Garcia-Algar and V. Andreu-Fernandez, Effects of Microbiota Imbalance in Anxiety and Eating Disorders: Probiotics as Novel Therapeutic Approaches, *Int. J. Mol. Sci.*, 2021, **22**(5), 2351.

37 M. Eslami, A. Bahar, M. Keikha, M. Karbalaei, N. M. Kobyliak and B. Yousefi, Probiotics function and modulation of the immune system in allergic diseases, *Allergol. Immunopathol.*, 2020, **48**(6), 771–788.

38 T. Mollayeva, P. Thurairajah, K. Burton, S. Mollayeva, C. M. Shapiro and A. Colantonio, The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis, *Sleep Med. Rev.*, 2016, **25**, 52–73.

39 L. K. Pilz, L. K. Keller, D. Lenssen and T. Roenneberg, Time to rethink sleep quality: PSQI scores reflect sleep quality on workdays, *Sleep*, 2018, **41**(5), zsy029.

40 P. Tian, Y. Chen, H. Zhu, L. Wang, X. Qian, R. Zou, J. Zhao, H. Zhang, L. Qian, Q. Wang, G. Wang and W. Chen, Bifidobacterium breve CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: A randomized clinical trial, *Brain, Behav., Immun.*, 2022, **100**, 233–241.

41 H. J. Lee, J. K. Hong, J. K. Kim, D. H. Kim, S. W. Jang, S. W. Han and I. Y. Yoon, Effects of Probiotic NVP-1704 on Mental Health and Sleep in Healthy Adults: An 8-Week Randomized, Double-Blind, Placebo-Controlled Trial, *Nutrients*, 2021, **13**(8), 2660.

42 B. A. Matenchuk, P. J. Mandhane and A. L. Kozyrskyj, Sleep, circadian rhythm, and gut microbiota, *Sleep Med. Rev*, 2020, **53**, 101340.

43 Z. Wang, Z. Wang, T. Lu, W. Chen, W. Yan, K. Yuan, L. Shi, X. Liu, X. Zhou, J. Shi, M. V. Vitiello, Y. Han and L. Lu, The microbiota-gut-brain axis in sleep disorders, *Sleep Med. Rev*, 2022, **65**, 101691.

44 Z. Wang, W. H. Chen, S. X. Li, Z. M. He, W. L. Zhu, Y. B. Ji, Z. Wang, X. M. Zhu, K. Yuan, Y. P. Bao, L. Shi, S. Q. Meng, Y. X. Xue, W. Xie, J. Shi, W. Yan, H. Wei, L. Lu and Y. Han, Gut microbiota modulates the inflammatory response and cognitive impairment induced by sleep deprivation, *Mol. Psychiatry*, 2021, **26**(11), 6277–6292.

45 H. Jiang, Z. Ling, Y. Zhang, H. Mao, Z. Ma, Y. Yin, W. Wang, W. Tang, Z. Tan, J. Shi, L. Li and B. Ruan, Altered fecal microbiota composition in patients with major depressive disorder, *Brain, Behav., Immun.*, 2015, **48**, 186–194.

46 G. J. Grosicki, B. L. Riemann, A. A. Flatt, T. Valentino and M. S. Lustgarten, Self-reported sleep quality is associated with gut microbiome composition in young, healthy individuals: a pilot study, *Sleep Med*, 2020, **73**, 76–81.

47 Z. Liu, Z. Y. Wei, J. Chen, K. Chen, X. Mao, Q. Liu, Y. Sun, Z. Zhang, Y. Zhang, Z. Dan, J. Tang, L. Qin, J. H. Chen and X. Liu, Acute Sleep-Wake Cycle Shift Results in Community Alteration of Human Gut Microbiome, *mSphere*, 2020, **5**(1), e00914–19.

48 I. Haimov, F. Magzal, S. Tamir, M. Lalzar, K. Asraf, U. Milman, M. Agmon and T. Shochat, Variation in Gut Microbiota Composition is Associated with Sleep Quality and Cognitive Performance in Older Adults with Insomnia, *Nat. Sci. Sleep*, 2022, **14**, 1753–1767.

49 F. Magzal, T. Shochat, I. Haimov, S. Tamir, K. Asraf, M. Tuchner-Arieli, C. Even and M. Agmon, Increased physical activity improves gut microbiota composition and reduces short-chain fatty acid concentrations in older adults with insomnia, *Sci. Rep.*, 2022, **12**(1), 2265.

50 T. Teame, A. Wang, M. Xie, Z. Zhang, Y. Yang, Q. Ding, C. Gao, R. E. Olsen, C. Ran and Z. Zhou, Paraprobiotics and Postbiotics of Probiotic Lactobacilli, Their Positive Effects on the Host and Action Mechanisms: A Review, *Front. Nutr*, 2020, **7**, 570344.

51 H. Wang, C. Braun, E. F. Murphy and P. Enck, Bifidobacterium longum 1714 Strain Modulates Brain Activity of Healthy Volunteers During Social Stress, *Am. J. Gastroenterol.*, 2019, **114**(7), 1152–1162.

52 H. L. Drake, A. S. Gössner and S. L. Daniel, Old acetogens, new light, *Ann. N. Y. Acad. Sci.*, 2008, **1125**, 100–128.
53 M. S. Frolova, I. A. Suvorova, S. N. Iablokov, S. N. Petrov and D. A. Rodionov, Genomic reconstruction of short-chain fatty acid production by the human gut microbiota, *Front. Mol. Biosci.*, 2022, **9**, 949563.
54 M. L. Garron and B. Henrissat, The continuing expansion of CAZymes and their families, *Curr. Opin. Chem. Biol.*, 2019, **53**, 82–87.
55 S. Macfarlane and G. T. Macfarlane, Regulation of short-chain fatty acid production, *Proc. Nutr. Soc.*, 2003, **62**(1), 67–72.
56 N. Reichardt, S. H. Duncan, P. Young, A. Belenguer, C. McWilliam Leitch, K. P. Scott, H. J. Flint and P. Louis, Phylogenetic distribution of three pathways for propionate production within the human gut microbiota, *ISME J.*, 2014, **8**(6), 1323–1335.
57 O. Ojo, Q. Q. Feng, O. O. Ojo and X. H. Wang, The Role of Dietary Fibre in Modulating Gut Microbiota Dysbiosis in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomised Controlled Trials, *Nutrients*, 2020, **12**(11), 3239.
58 Y. Luo, B. Zeng, L. Zeng, X. Du, B. Li, R. Huo, L. Liu, H. Wang, M. Dong, J. Pan, P. Zheng, C. Zhou, H. Wei and P. Xie, Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus, *Transl. Psychiatry*, 2018, **8**(1), 187.
59 F. Magzal, C. Even, I. Haimov, M. Agmon, K. Asraf, T. Shochat and S. Tamir, Associations between fecal short-chain fatty acids and sleep continuity in older adults with insomnia symptoms, *Sci. Rep.*, 2021, **11**(1), 4052.
60 A. M. Heath, J. J. Haszard, B. C. Galland, B. Lawley, N. J. Rehrer, L. N. Drummond, I. M. Sims, R. W. Taylor, A. Otal, B. Taylor and G. W. Tannock, Association between the faecal short-chain fatty acid propionate and infant sleep, *Eur. J. Clin. Nutr.*, 2020, **74**(9), 1362–1365.
61 Z. Karaca, A. Grossman and F. Kelestimur, Investigation of the Hypothalamo-pituitary-adrenal (HPA) axis: a contemporary synthesis, *Rev. Endocr. Metab. Disord*, 2021, **22**(2), 179–204.
62 L. D. Asarnow, Depression and sleep: what has the treatment research revealed and could the HPA axis be a potential mechanism?, *Curr. Opin. Psychol*, 2020, **34**, 112–116.
63 L. Rudzki, L. Ostrowska, D. Pawlak, A. Malus, K. Pawlak, N. Waszkiewicz and A. Szulc, Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study, *Psychoneuroendocrinology*, 2019, **100**, 213–222.
64 A. R. Romijn, J. J. Rucklidge, R. G. Kuijer and C. Frampton, A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression, *Aust. N. Z. J. Psychiatry*, 2017, **51**(8), 810–821.
65 S. Kim, K. Jo, K. B. Hong, S. H. Han and H. J. Suh, GABA and l-theanine mixture decreases sleep latency and improves NREM sleep, *Pharm. Biol*, 2019, **57**(1), 65–73.
66 L. Yu, X. Han, S. Cen, H. Duan, S. Feng, Y. Xue, F. Tian, J. Zhao, H. Zhang, Q. Zhai and W. Chen, Beneficial effect of GABA-rich fermented milk on insomnia involving regulation of gut microbiota, *Microbiol. Res.*, 2020, **233**, 126409.
67 P. Strandwitz, K. H. Kim, D. Terekhova, J. K. Liu, A. Sharma, J. Levering, D. McDonald, D. Dietrich, T. R. Ramadhar, A. Lekbua, N. Mroue, C. Liston, E. J. Stewart, M. J. Dubin, K. Zengler, R. Knight, J. A. Gilbert, J. Clardy and K. Lewis, GABA-modulating bacteria of the human gut microbiota, *Nat. Microbiol*, 2019, **4**(3), 396–403.