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Research Article

JOURNAL OF BIOTECHNOLOGY AND BIOMEDICINE

ISSN: 2642-9128

A Probiotic to Improve Sleep Quality during COVID-19 Pandemic: A Questionnaire Study

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Abstract

Sleep quality has been related to the gut microbiome and probiotics were shown to improve sleep quality, especially in people under physical or mental stress. COVID-19 results in such mental stress, leading to sleeping disorders, especially in people working in health care. A large dataset of quality of life data from people working in pharmacies or doctors´ offices was collected before and after intake of a free sample of a multi-species probiotic during a marketing survey. 9841 anonymous datasets were available for analysis. Group differences and univariate and multivariate logistic regression analysis was performed. COVID-19 related data were obtained from publicly available datasets and matched to quality of life data via the postal code of the participants. We aimed to relate self-reported quality of life, especially sleep quality data from a marketing survey to objective criteria of COVID-19, such as incidence and mortality figures at the time of the survey. The intake of only a two-week course of a multi-species probiotic markedly improved sleep quality, mental health, vitality, stress resistance and performance. Improvement in the different categories of quality of life was influenced by sex, age, initial intestinal complaints, the initial stress level, the initial sleep quality, initial vitality and mental health. Quality of life was reduced during COVID-19 compared to pre-pandemic values of the general population. We also found some associations of changes in quality of life in relation to COVID-19 incidence rates in employees of pharmacies and doctors´ offices, however to a lesser extent than expected. Although not derived from a clinical trial, this analysis of marketing survey data provides evidence that there is a link between the gut microbiome, sleep and quality of life suggesting that gut microbiome modulation could be a valuable therapeutic approach in sleeping disorders.

Keywords: COVID-19; Probiotic; Pandemic; Quality of Life; Sleep

Introduction

Sleep is an important factor for mental and physical recovery and is essential for a range of physiological functions such as neural cell growth, synaptogenesis and memory function [1]. Recently, quality of sleep was linked to alterations in the gut microbiome, allowing the hypothesis, that sleep and thereby quality of life can be improved by microbiome modulation [2]. Sleep deprivation leads to changes in gut microbiome composition through the brain-gut-microbiome axis [3]. The linkages between gut microbiome compositions, sleep physiology, immune system and cognition is a bi-directional interplay [4]. Probiotics can alter the gut microbiota composition and can mitigate positive effects on psychiatric symptoms via the microbiome gut-brain axis by metabolites such as short-chain fatty

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Citation: Angela Horvath, Jolana Wagner-Skacel, Verena Stiegelbauer, and Vanessa Stadlbauer. A Probiotic to Improve Sleep Quality during COVID-19 Pandemic: A Questionnaire Study. Journal of Biotechnology and Biomedicine 6 (2023): 80-91.

Received: March 01, 2023
Accepted: March 06, 2023
Published: March 24, 2023

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DOI:10.26502/jbb.2642-91280073

acids (SCFA) that can pass the blood-brain barrier [5] and thereby comprise a potential strategy to improve quality of life, especially in mentally challenging times. Evidence on the effect of probiotics to improve quality of sleep is available from several small-scale clinical studies in different populations. The benefit seems to be stronger in people under physical or mental stress [6-16]. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic and its social and health consequences has influenced the life of everybody and the whole world is experiencing a state of emergency [17]. Mental stress symptoms (including sleeping disorders, depression, somatization and anxiety) are logical consequences to such a threat [18]. Stress related symptoms such as anxiety, sleeping disorders or depressive symptoms increased in the COVID-19 pandemic [19], especially in female health care workers [20]. Quarantine measures, with its infection fears, frustration, boredom, inadequate supplies, inadequate information, or financial loss have negative psychological effects, including sleep disturbances [21]. A company performed a large marketing survey during the COVID-19 pandemic in over 10.000 employees of pharmacies and doctors´ offices. Health care workers are likely to be under additional work-related pressure during the pandemic [22]. From this survey, a large dataset of quality of life data from people before and after intake of a free sample of a multispecies probiotic is available. We aimed to relate self-reported quality of life, especially sleep quality data to objective criteria of COVID-19, such as incidence and mortality figures at the time of the survey. By analyzing this large-scale survey, we aim to understand whether a medically relevant multispecies probiotic can positively affect wellbeing during stressful live events.

Materials and Methods

Study Participants

Between April 2020 and June 2021 a marketing survey was conducted in Germany by Institut AllergoSan Deutschland (Grünwald, Germany). The survey and a free 2-week sample of the multispecies probiotic OM-NiBiOTiC SR-9 with vitamin B, a multispecies probiotic that was designed to target the gut brain axis, containing 7500000000 colony forming units of *Lactobacillus casei W56, Lactobacillus acidophilus W22, Lactobacillus paracasei W20, Bifidobacterium lactis W51, Lactobacillus salivarius W24, Lactococcus lactis W19, Bifidobacterium lactis W52, Lactobacillus plantarum W62* and *Bifidobacterium bifidum W23 as well as 0.21 mg vitamin B2, 0.21 mg vitamin B6 and 0.375µg vitamin B12,* was distributed to doctors' offices and pharmacies by sales representatives of the company. Participating persons were asked to fill in a questionnaire (either electronically via a QR code or on paper) and received a 2-week package of the multispecies probiotic. They were asked to take the probiotic for the following 2 weeks in the morning and after that complete the second part of the questionnaire. To improve compliance participants were sent an email reminder for the second part of the questionnaire after 2 weeks. Since this survey was planned as a marketing survey and not as a clinical study, no research ethics committee was involved beforehand and the survey was not registered as a clinical trial. The coincidence with the COVID-19 pandemic triggered the interest in additional analyses of this dataset and the anonymous dataset was handed over to AH and VaS for independent analysis and data interpretation. Data collection was anonymous; individual datasets were identified by consecutive numbers. The questionnaire (supplementary file 1) contained statements and questions from five categories: sleep quality, vitality, psychological health, stress resistance and performance. The allocation of the questions/statements to these categories is shown in Table 1. Each statement/question was answered on a 6-point Likert-like scale: Always, mostly, often, sometimes, rarely, never. For the merging of the individual questions into a category, the answer options were given points between 1 and 6, analogous to the evaluation strategy of the SF36 questionnaire. For questions with a positive connotation (e.g. "You fall asleep quickly" or "How often have you been full of energy?") the answer "always" was given 6 points and "never" was given 1 point), for questions with a negative connotation (e.g. "You sleep poorly, wake up often." or "How often have you been discouraged and sad?") the answer "never" was given 6 points and "always" 1 point. The points of all statements/questions within a category were added up. In this way, better quality of life was associated with a higher score. In order to achieve better comparability between the categories, all total scores were expressed as a percentage of the highest possible score. Two categories were taken from the well validated SF36 (vitality and mental health) [23] the other three categories were designed for this survey and subjected to face and content validity tests by 5 people unrelated to the study before fielding the questionnaire.

Statistical Analysis

Only datasets with complete quality of life data were considered for analysis, whereby data sets with implausible quality of life data points (e.g. <1 or >6) were excluded. Improbable meta-data (e.g., height below 110 or above 260 cm) were excluded and data entries with obviously wrong units (e.g., 1.67 for height instead of 167) were corrected, without excluding the whole dataset. For the statistical evaluation, the quality of life categories were handled as continuous variables and described with mean and standard deviation. Group differences were examined using Student t-test. Paired t-tests were performed to test the influence of the probiotic on these metrics. A p-value of <0.05 was considered to be statistically significant. Multivariate linear regression models were used to a) predict the improvement in quality of sleep based on demographic characteristics, initial quality of life, initial stress level and initial gastrointestinal

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DOI:10.26502/jbb.2642-91280073

Table 1: Quality of life categories and the related questions in the survey.

Category	Questions (number in the questionnaire)
Sleep quality	You wake up well rested in the morning. (3) You sleep badly, wake up often. (5)
	You fall asleep quickly. (15)
	You can sleep through the night. (16)
Vitality (SF36)	How often within the past weeks have you been ...
	... full of spirits? (17)
	… full of energy? (21)
	… exhausted? (23)
	… tired? (25)
Mental health (SF36)	How often within the past weeks have you been...
	… very nervous? (18)
	... so depressed that nothing could cheer you up? (19)
	… calm and relaxed? (20)
	... discouraged and sad? (22)
	… happy? (24)
Stress resistance	You are full of energy. (2) You are stressed. (4)
	You feel mentally tense. (6) You feel hopeless. (7)
	You feel mentally exhausted. (8th) You feel frustrated. (9)
	You have a good quality of life. (10) You have trouble relaxing. (11)
	You are easily irritated. (14)
Performance	You can handle new tasks well. (1) You avoid taking risks. (12)
	You are concentrated when working. (13)

problems and b) explore if changes in stress level and gastrointestinal problems during the intervention were associated with an improvement in sleep quality. Therefore, univariate linear regression was used to pre-screen potential predictors. Significant predictors ($p<0.05$) were included in either the predictive (a) or the explorative (b) multivariate model. Multicollinearity was estimated with the Variance Inflation Factor (VIF). VIF up to 4 was considered acceptable for inclusion in the multivariate model. In order to test the influence of local COVID-19 incidence rates on sleep quality and other quality of life parameter, the daily incidence for Germany, the 7-day incidence for each German province, the COVID-19 death count and the additional death counts per day as published by the Robert-Koch institute (Berlin, Germany) were matched with our data set according to the day of inclusion (i.e. completion of the first questionnaire) and the postal code/respective province. Spearman correlation analysis with Benjamini-Hochberg correction was applied to test the association between the initial quality of life and subjective stress levels, stomach and intestinal problems, and COVID-19 incidence and mortality, as well as the duration of the pandemic. To account for delayed response to COVID-19 incidence and non-linear associations, the weekly average of quality of life parameters were plotted against the corresponding incidence numbers in the respective province and a locally weighted regression model was fitted to visually assess similarities in the resulting curves.

Results

Cohort Characteristics

The invitation for participation in the survey was distributed during the COVID -19 pandemic, 14558 persons started to fill in the survey, 9881 finished the survey and after data cleaning 9841 datasets were available for analysis. Characteristics of the study cohort are shown in table 2. When comparing the results of vitality and mental health, with the normal values for the SF36 questionnaire obtained in the SF36 Health Survey study in Germany [24], scores of both categories were significantly reduced during the pandemic (vitality: 61.8±19.2 vs. 45.7±17.4; $p<0.001$; mental health: 72.8±17.3 vs. 53.0±14.2; $p<0.001$).

Sleep Quality

The average sleep quality of all participants before taking the probiotic was 52.3 ± 19.9% of the maximum score and increased significantly after taking the probiotic for 2 weeks to 62.8 ± 18.6% ($p <0.001$). The mean improvement was 10.5 ± 15.8 percentage points. Also, vitality, mental health,

Table 2: Characteristics of study participants.

	participants (n=9841)
Sex (female/male)	8952/889 (91%/9%)
Age (years)	42,7 ± 12,0
Weight (kg)	70.4 ± 15.6
Height (cm)	168.8 ± 7.4
Workplace n(%)	
pharmacy	8149 (82.4%)
doctor´s office	778 (7.9%)
other	914 (9.3%)
Current stress level (scale 1-10)	7.1 ± 2.1
stomach problems	
no (n/%)	4604 (46.8%)
light/medium (n/%)	4475 (45.5%)
strong/very strong (n/%)	762 (7.7%)
intestinal problems	
no (n/%)	3875 (39.4%)
light/medium (n/%)	4980 (50.6%)
strong/very strong (n/%)	986 (10.0%)
Adherence n(%)	
taken daily (n/%)	6246 (63.5%)
forgot to take it 1-2 times (n/%)	3224 (32.8%)
forgot to take it 3+ times (n/%)	369 (3.7%)
not given	2 (0.0%)

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performance and stress resistance improved significantly during the intervention, as shown in (Figure 1).

Before the intervention, men had significantly better sleep quality compared to women ($p < 0.001$). Both sex groups showed a significant increase in sleep quality while taking the probiotic (both $p < 0.001$), however, the difference between the sexes did not reach statistical significance after the intervention had ended, indicating a stronger response in women. Similar to sleep quality, men showed higher initial scores but women showed a more pronounced improvement during the intervention. Details are given in Table 3. Participants also reported decreased stress levels, stomach and intestinal problems after the intervention, as shown in Figure 2.

Sex was also identified as a significant influencing factor on sleep quality by linear regression analysis ($b = 2.19$; $p < 0.001$), however, the explained variance was relatively small ($R^2 = 0.0014$). Other influencing factors, especially other quality of life items, better explained the change in sleep quality. Details are given in Table 4.

The potential predictors for the improvement in quality of sleep – sex, age, BMI, initial stomach complaints, initial intestinal complaints, initial stress level, initial sleep quality, initial stress resistance, initial vitality, initial mental health and initial performance – were included in a multivariate linear regression model. The initial stress resistance was excluded because of moderate multicollinearity (VIF = 4.11). The resulting model showed that women benefit slightly more from taking the probiotic. The improvement in sleep quality decreases with age. People with stronger initial intestinal complaints experienced a higher increase in sleep quality than people with less pronounced intestinal complaints. The increase in sleep quality was the greater, the higher the initial stress level and the lower the initial sleep quality was perceived. Interestingly, and in contrast to the

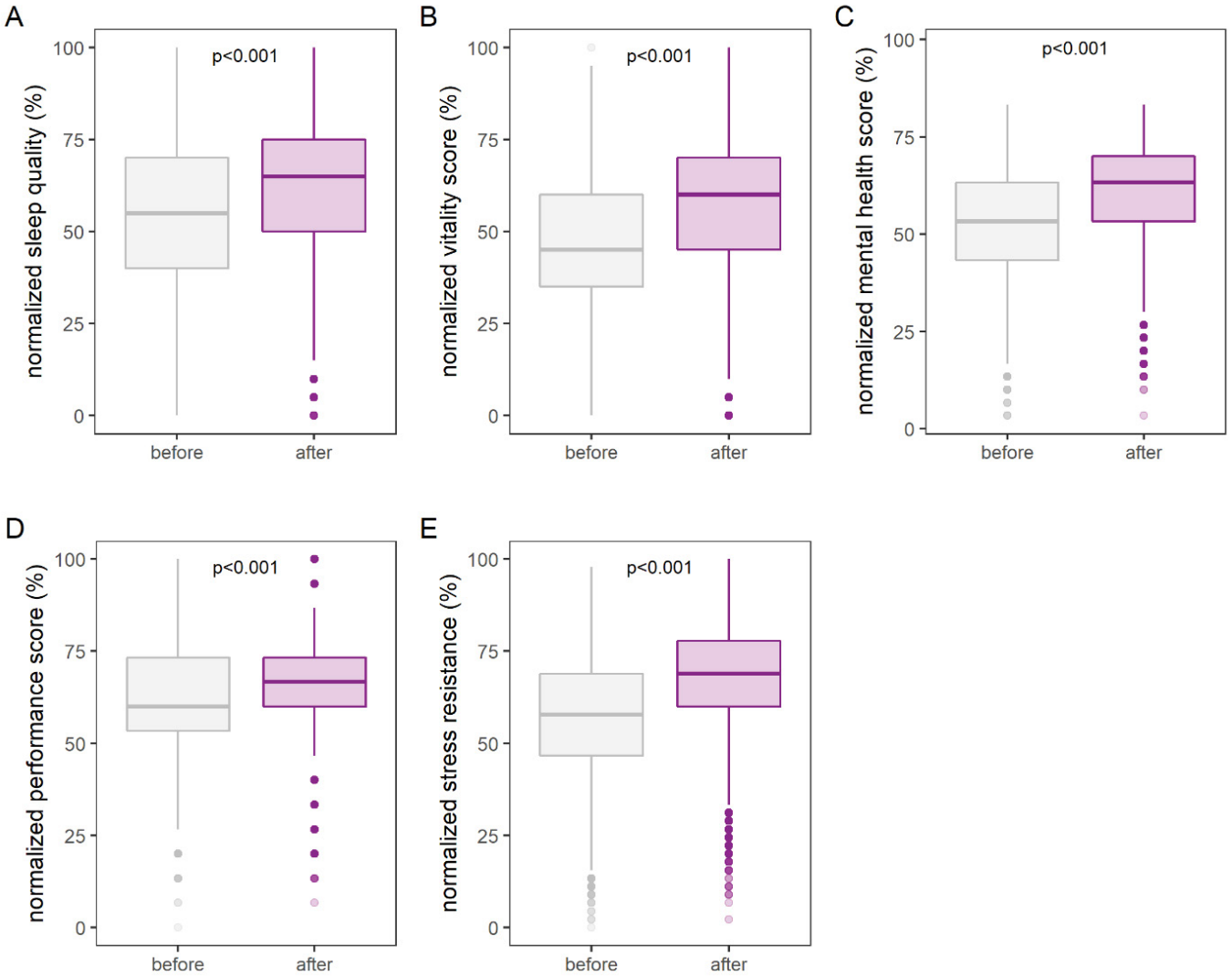

Figure 1: Quality of life improved after two weeks of intake of OMNi-BiOTiC® SR-9. Normalized scores before and after intervention for (A) sleep quality; (B) vitality; (C) mental health; (D) performance and (E) stress resistance.

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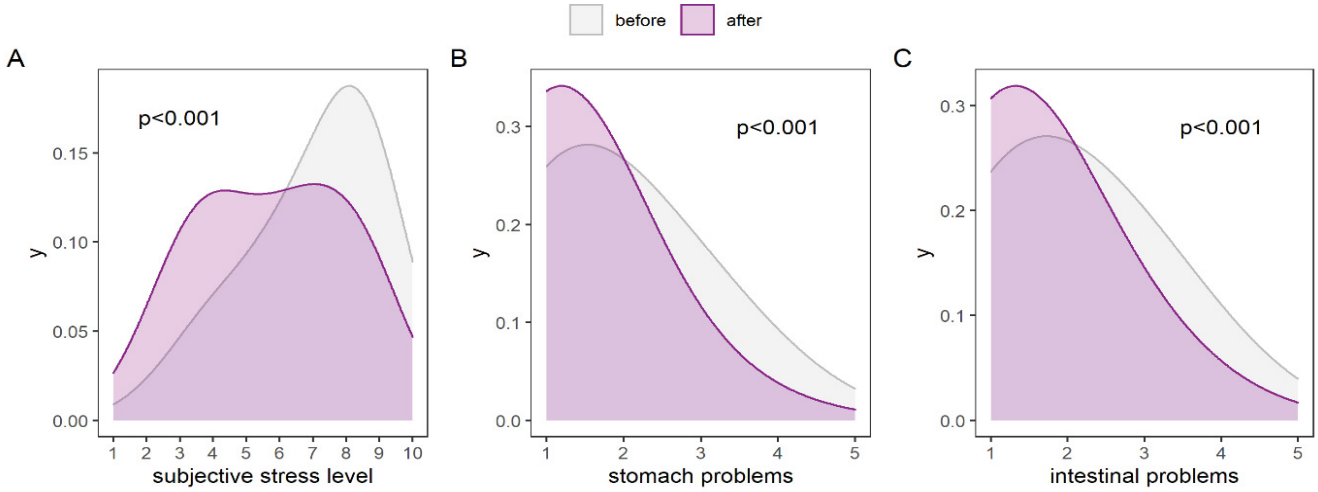

Figure 2: Subjective stress levels, stomach and intestinal problems before and after the intervention.

Table 3: Quality of life scores before and after intervention according to sex.

Before intervention				After intervention			Change		
Category	**Women**	**Men**	**p**	**Women**	**Men**	**p**	**Women**	**Men**	**p**
Sleep quality	52.1 (20.0)	54.9 (19.1)	<0.001	62.8 (18.6)	63.4 (18.4)	0.3	10.7 (15.9)	8.5 (15.7)	<0.001
Vitality	45.5 (17.4)	47.4 (17.2)	0.002	58.8 (17.0)	58.7 (16.9)	0.8	13.3 (15.7)	11.3 (15.7)	<0.001
Mental health	52.9 (14.2)	54.5 (13.9)	0.001	61.6 (12.3)	61.1 (12.8)	0.2	8.8 (11.3)	6.6 (11.6)	<0.001
Performance	62.1 (13.1)	64.1 (14.0)	<0.001	65.5 (12.5)	66.5 (13.5)	0.03	3.5 (11.9)	2.5 (12.6)	0.02
Stress resistance	56.9 (15.7)	58.4 (16.0)	0.008	67.8 (14.0)	67.2 (15.0)	0.3	10.8 (12.5)	8.8 (13.2)	<0.001

Table 4: Results of the univariate regression analysis to identify possible influencing factors on the improvement of sleep quality.

Factor	Regression coefficient	P-value	Explained variance (%)	Included in the multivariate model
sex (female)	2.16	<0.001	0.14	yes, predictive
age	-0.029	0.029	0.038	yes, predictive
Adherence: forgot to take the probiotic				
1-2 times	-1.78	<0.001	0.47	yes, explorative
3+ times	-4.47	<0.001		
weigth	0.034	<0.001	0.1	no (incl. in BMI)
heigth	-0.072	<0.001	0.1	no (incl. in BMI)
BMI	0.18	<0.001	0.28	yes, predictive
place of work				
doctor´s office	0.88	0.14	0.0021	no
other	0.071	0.9		
Initial stomach complaints#	1.24	<0.001	0.62	yes, predictive
change in stomach complaints	-1.98	<0.001	1.7	yes, explorative
initial intestinal complaints#	0.79	<0.001	0.26	yes, predictive
change in intestinal complaints	-1.74	<0.001	1.33	yes, explorative
initial stress level #	1.3	<0.001	2.982	yes, predictive
change in stress level	-1.92	<0.001	6.855	yes, explorative
initial quality of sleep#	-0.38	<0.001	22.87	yes, predictive
initial stress resistance#	-0.21	<0.001	4.49	yes, predictive*
initial vitality #	-0.19	<0.001	4.22	yes, predictive
initial mental health #	-0.19	<0.001	2.84	yes, predictive
initial performance#	-0.099	<0.001	0.67	yes, predictive

#before the intervention.
*Excluded from further analysis because of collinearity.

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Table 5: Multivariate linear regression model for predicting sleep quality improvement (predictors are sorted according to p-values, independent predictors are shown in bold).

Predictor	Regression coefficient	standard error of the regression coefficient	adjusted p- value	VIF
constant	26.02	1.59	<0.001	
initial sleep quality	-0.39	0.008	<0.001	1.29
initial stress level	0.48	0.08	<0.001	1.43
age	-0.062	0.012	<0.011	1.06
sex (female)	1.17	0.49	0.017	1.01
initial vitality	0.027	0.012	0.026	2.17
initial mental health	0.03	0.014	0.034	2.09
initial intestinal complaints	-0.31	0.17	0.06	1.48
BMI	0.053	0.029	0.07	1.03
initial performance	-0.0064	0.012	0.6	1.26
initial stomach complaints	0.017	0.17	0. 9	1.52

Table 6: Multivariate linear regression model for potential influencing factors of sleep quality improvement (covariates are sorted according to p-values, independent covariates are shown in bold).

Covariate	Regression coefficient	standard error of the regression coefficient	adjusted p- value	VIF
constant	4.03	0.22	<0.001	
Change in stress level	0.6	0.01	<0.001	1.04
Change in stomach problems	-0.65	0.15	<0.001	1.32
Adherence: forgot to take the probiotic				
1-2 times	-0.98	0.3	0.001	1.02
3+ times	-2.14	0.74	0.004	1.03
Change in intestinal problems	-0.26	0.15	0.08	1.32

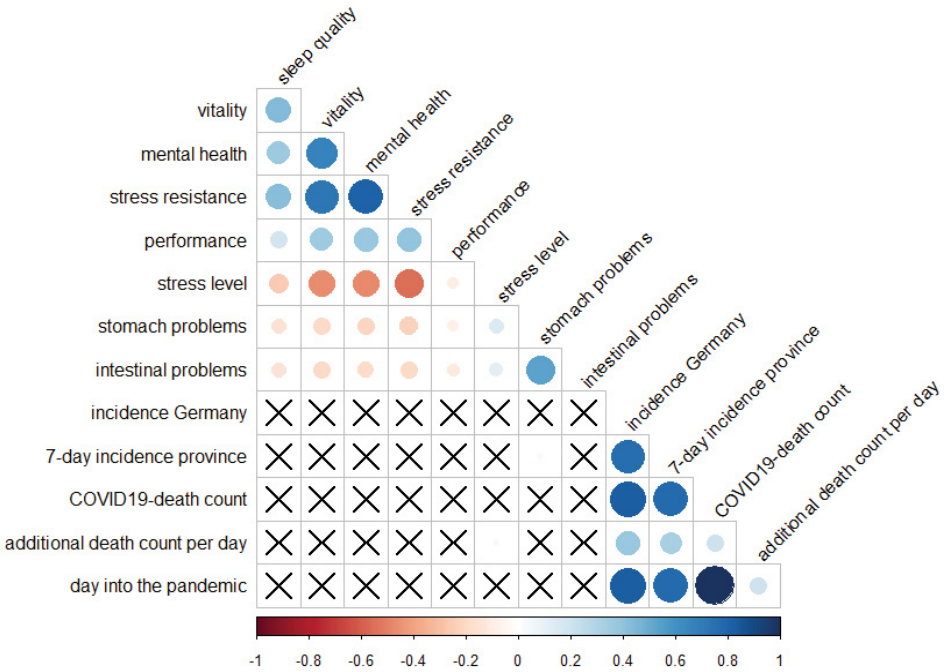

Figure 3: Correlation matrix of quality of life and COVID-19 parameters. Benjamini-Hochberg corrected Spearman correlations are shown. Strength and direction of the correlation are indicated by the size and color of the circles, respectively. Non-significant correlations are marked with X.

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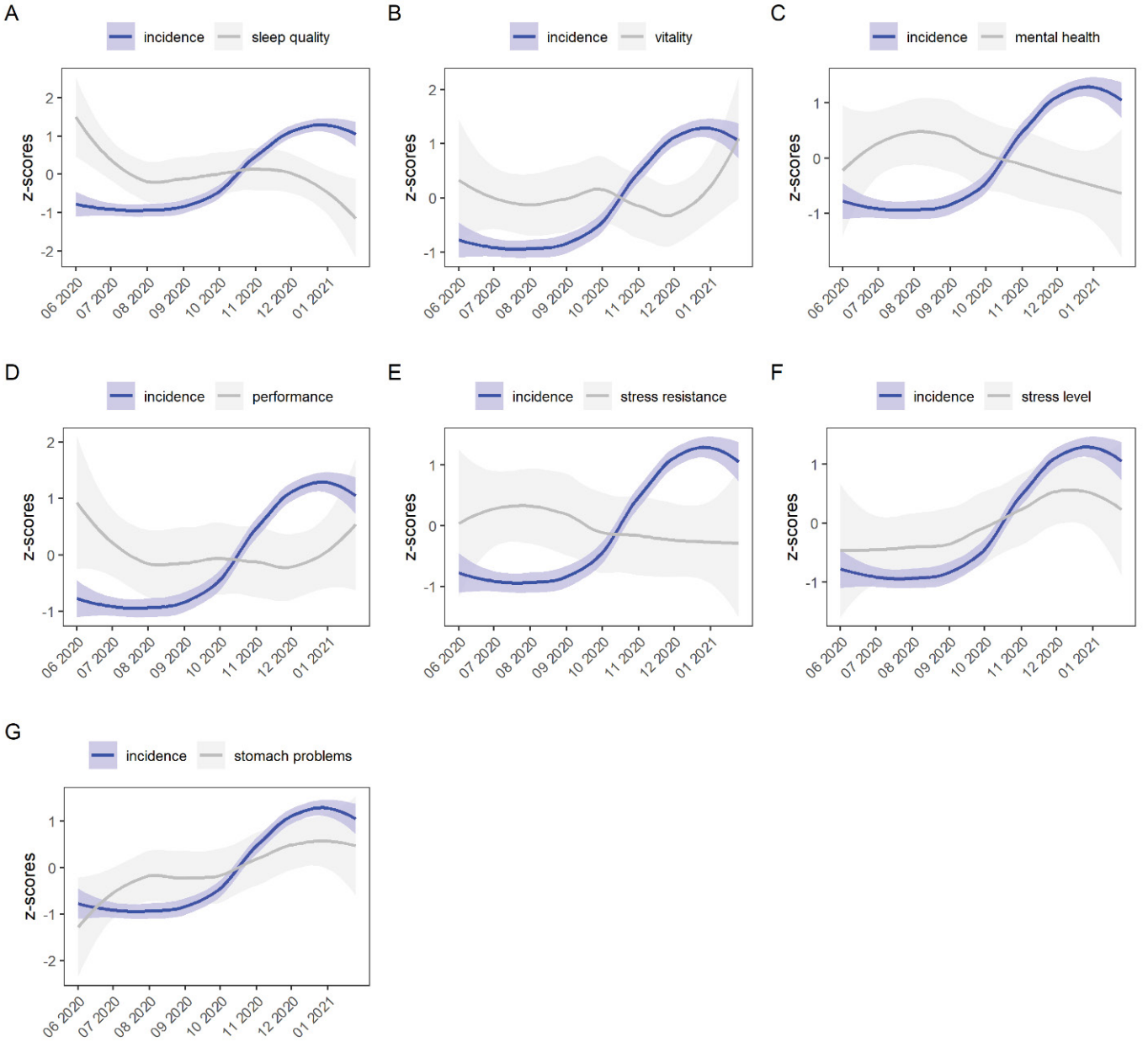

Figure 4: Quality of life development during the study period in relation to local incidence rates; (A) quality of sleep; (B) vitality; (C) mental health; (D) performance; (E) stress resistance; (F) stress levels; (G) stomach problems.

univariate regression models, there was a positive association between the increase in sleep quality and the initial vitality as well as the initial mental health in the adjusted model, i.e. the more vital a person was before the intervention, or the better the mental health, the better he or she responded in terms of sleep quality to the probiotic. According to this, sex, age, initial intestinal complaints, the initial stress level, the initial sleep quality, initial vitality and mental health are independent predictors for the response to the probiotic intervention with regard to sleep quality, while the BMI, initial stomach complaints and the initial performance did not play a significant role. This model was highly significant ($F = 299.1$; $p < 0.001$) and explained 23.5% of the observed variance. Details are given in (Table 5).

In order to explore which factors were associated with the increase in sleep quality and therefore potentially contributed to the improvement, a second multivariate linear regression model was tested that included changes in intestinal problems, changes in stomach problems, changes in stress levels and continuity of intake. The improvement in quality of sleep was the more pronounced the more stomach problems as well as stress levels improved, and the more regular the intake of the probiotic was. The change in intestinal problems was not independently associated with the improvement in

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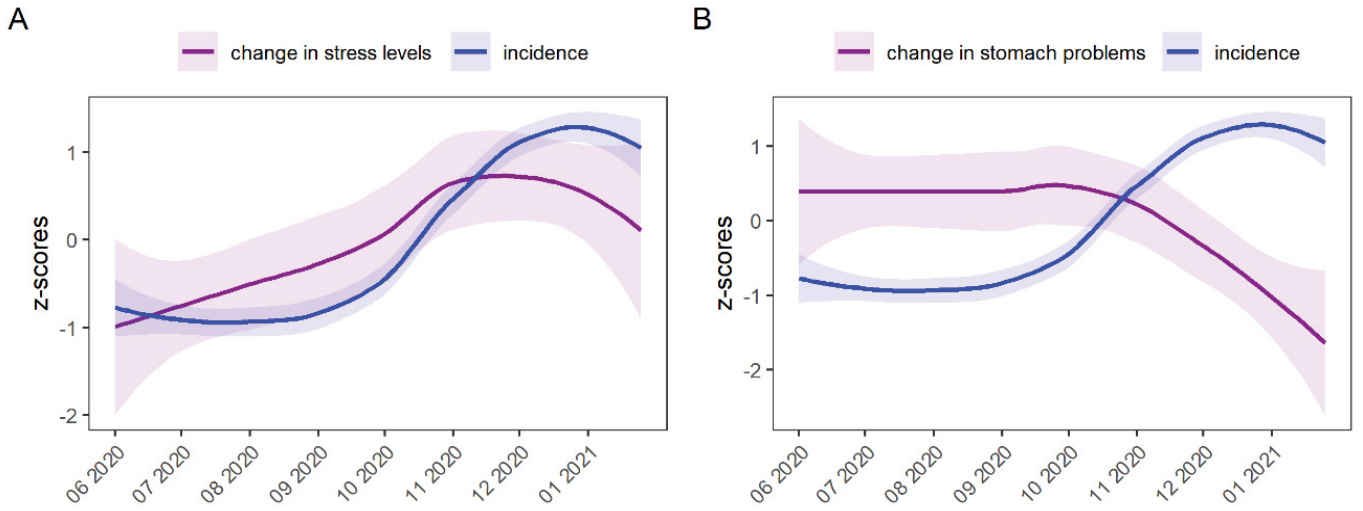

Figure 5: Changes during the intervention in subjective stress levels (A) and stomach problems (B) in relation 215 to local COVID-19 incidence rates.

quality of sleep. The model was highly significant ($F = 234.3$; $p < 0.001$) and explained 8.6% of the observed variance. Multicollinearity was negligible. Details are given in Table 6.

Association of Baseline Quality of Life with COVID-19 Incidence Rates

Based on the postal code provided by the participants and the daily COVID-19 statistics published by the Robert Koch Institute (Berlin, Germany) for Germany in total and for German provinces separately, the incidence and mortality related to COVID-19 at the day of study inclusion (1st questionnaire completed) was matched to each data set to assess potential associations of COVID-19 and stress levels. In total, postal code matched COVID-19 related incidence and mortality data were available for 6924 participants. Although quality of life parameters correlated well with each other, and COVID-19 statistics expectedly correlated well among themselves, there was no relevant correlation between quality of life and COVID-19 parameters, as shown in Figure 3. The change in quality of life before and after the intervention yielded similar results (data not shown).

The visual assessment of the time series showed that while sleep quality, performance and vitality did not show a comparable pattern as the incidence curve, the subjective stress level and the severity of stomach problems corresponded well with COVID-19 incidence. Mental health and stress resistance were highest, when the incidence was low and steadily decreased to varying degrees when local incidence rates increased, see Figure 4. Average scores of intestinal problems were invariable with time, and were therefore excluded from the analysis.

Furthermore, the improvement of stress levels and stomach problems also corresponded to the local incidence rate in the week of inclusion. While the most pronounced improvements in stress levels were observed when the incidence was increasing, improvements in stomach complaints steeply declined with rising incidence rates, as shown in Figure 5.

Discussion

We conducted an analysis of data obtained during a large marketing survey with nearly 10.000 datasets that was performed during the COVID-19 pandemic in 2020 in Germany and contained information on quality of life before and after the intake of a free sample of a multispecies probiotic. Our analysis showed reduced quality of life during the COVID-19 pandemic in comparison to pre-pandemic values of the general population. We also found some associations of changes in quality of life in relation to COVID-19 incidence rates in employees of pharmacies and doctors´ offices. The intake of a two-week course of a multistrain probiotic markedly improved sleep quality, mental health, vitality, stress resistance and performance. Improvement in the different categories of quality of life was influenced by sex, age, initial intestinal complaints, the initial stress level, the initial sleep quality, initial vitality and mental health. Although not derived from a clinical trial, this dataset therefore provides valuable evidence that there is a link between the gut microbiome, sleep and quality of life and that microbiome modulation is an interesting therapeutic strategy. The outbreak of the COVID-19 pandemic impacts on quality of life. A large study from Denmark showed a negative impact on mental health scores and sleep quality [25]. Sleep quality is of critical importance for mental and physical health. A review of 43 studies revealed lower sleep quality, lower psychological well-being and more anxiety and depression as negative consequences of the pandemic [26]. In people with underlying psychiatric diseases, the effect of the pandemic on sleep quality is even worse [27]. Poor sleep quality due to sleep fragmentation or short sleep duration

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DOI:10.26502/jbb.2642-91280073

does not only negatively affect cognitive, mood and motor functions, but can also have negative cardiovascular, inflammatory and metabolic consequences [28,29]. Factors associated with poor sleep quality during the pandemic may be host intrinsic, like female sex or sleep breathing disorders, as well as extrinsic, like home confinement [30]. In addition, in the past years, it has been shown that the gut microbiome and its metabolites exhibit diurnal rhythmicity, which predominantly respond to the feeding/fasting cycle and this oscillatory nature can be disturbed by persistent jet lag, an obesogenic diet, allowing to hypothesize that the gut microbiome composition impacts on sleep quality and that the gut microbiome may be an interesting therapeutic target to improve sleep quality and subsequent negative consequences of disturbed sleep [2]. The microbiome communicates with the brain via the microbiome-gut-brain axis, utilizing neural, immune and endocrine mechanisms [31]. Strategies to modulate the gut microbiome include probiotics, prebiotics, fecal microbiome transplantation or specific metabolites [31]. Probiotics are considered as a useful, safe and relatively low-cost microbiome modulatory strategy in several disorders [32]. So far, some studies in healthy adults or adults with gastrointestinal disorders were conducted to assess the effects of probiotics on sleep quality [6-16]. Since these studies included different target populations, used different probiotics for varying periods of time and have a small sample size (between 19 and 156 participants in these 11 studies), it has so far been difficult to draw firm conclusions. It seems that probiotics exhibit stronger effects in people who are mentally or physically stressed (e.g professional athletes or academically stressed students). Notably, most studies showed beneficial effects on at least some aspects of sleep [6-16]. Since, by coincidence, a large marketing survey for a probiotic product took place during the COVID-19 pandemic, these data, although not attempted to be used as a clinical study dataset, represent a valuable data source to generate hypotheses on how stress, sleep and the gut microbiome are interrelated and if microbiome modulation could be a therapeutic target to treat sleep disorders in working people. Here we present data from the so far largest observation on probiotic effects on self-reported quality of life, including sleep quality. The probiotic that was used in this market survey is composed of nine bacterial strains, namely *Lactobacillus casei W56*, *Lactobacillus acidophilus W22*, *Lactobacillus paracasei W20*, *Bifidobacterium lactis W51*, *Lactobacillus salivarius W24*, *Lactococcus lactis W19*, *Bifidobacterium lactis W52*, *Lactobacillus plantarum W62* and *Bifidobacterium bifidum W23*. The product influences brain activation patterns in resting state and in response to emotional memory and emotional decision-making tasks in healthy volunteers [33]. Furthermore, the product shows beneficial effects on symptoms and biomarkers of inflammation and gut permeability in patients with irritable bowel syndrome, psoriatic arthritis and depression [5,34,35]. Another multispecies probiotic, that overlaps in 4 strains with the product from this study, improved sad mood in healthy volunteers [36,37]. These data provide a scientific rational allowing us to conclude from our dataset that this multispecies probiotic can have positive effects on the quality of life of working people affected by stress. We could show that all aspects of quality of life that were studied in this survey – sleep quality, vitality, mental health, stress resistance and performance – improved significantly after the intervention as compared to the baseline assessment. The subjective improvement was largest for vitality, followed by sleep quality, stress resistance and mental health, whereas the effect on performance, albeit significant, was less impressive. The lack of a control group is of course a limitation of our dataset; however, we believe that it is unlikely that this result occurred by chance due to the large number of datasets available. In-depth analysis of factors influencing the perceived effect showed that age, gender, BMI, baseline levels of sleep quality, vitality, mental health, stress resistance and performance, baseline gastrointestinal complaints, baseline stress levels and changes in the different parameters influenced results in differential ways. For example, younger age was associated with a larger improvement of sleep quality, whereas older age was associated with stronger improvement of vitality and stress resistance. Sex affected all baseline scores, which were better in men. Women however, reported a stronger improvement in all categories, which even led to the loss of significant difference at the second evaluation for vitality, mental health and stress resistance. Interestingly, a higher BMI was associated with a stronger increase in vitality, mental health, performance and stress resistance. Since age, sex and BMI influence microbiome composition, our observation may be explained by the fact that probiotics have different effects depending on the host´s microbiome composition [38,39]. Since the dataset is from an uncontrolled dataset, there is also a chance of observing a "placebo response". Younger age and female sex were described as factors associated with a stronger "placebo response" [40], however, since in our analysis older age was associated with a stronger improvement of two categories, no relation with age was observed in two categories and only in one category younger age was associated with a stronger improvement, this seems unlikely. Among patient-based predictors, better symptom scores at baseline were associated in literature with a stronger placebo response [40] – since we observed the opposite more frequently (worse baseline symptom scores were associated with stronger improvement), this also opposes to the hypothesis that we observed a "placebo effect". Higher initial stress levels, using a self-rating scale – were associated with a stronger improvement in sleep quality and performance but less improvement in mental health. In a review of non-medical interventions to improve sleep quality in people without a diagnosed sleep disorder, the improvement

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DOI:10.26502/jbb.2642-91280073

of sleep quality was higher when the initial sleep quality was poorer [41]. We expected to see a strong association of COVID-19 related factors (incidence rates, mortality) on quality of life, since the target group were employees of pharmacies and doctors' offices, whom we expected to experience COVID-19 related stress at work, which we could not verify in our dataset. This may however be explainable because in the questionnaire there was no link to COVID-19 or because the individual perception of the stress caused by COVID-19 is not related to incidence or mortality rates but rather to individual factors. Interestingly the response to the probiotic showed some relation to COVID-19. Perceived stress levels improved more when COVID-19 figures were increasing, whereas stomach complaints improved less with rising COVID-19 figures – indicating differential mechanisms of improvement of min relation to external stressors. This may also show an unconscious attempt to control the crisis better with intake of probiotics leading to the perception of better self-efficacy. This study has several limitations. First, the study was not designed as a clinical trial, does not contain a control group, and was not conducted according to the principles of clinical trials but as a marketing survey. As already stated, a placebo effect cannot be excluded; likewise, completing a quality of life questionnaire can lead to participant's attention to this topic and unintended behavioral changes as confounders. Furthermore, other confounders, such as changes to diet, alcohol intake, physical activity, changes to work pattern, weather, have not been assessed in this study. The questionnaire used to assess different categories of quality of life was partly taken from the well validated SF36 questionnaire (2 dimensions) but other parts were designed by the marketing team of the company and therefore are not independently validated. Although this study was not designed as a clinical trial, we believe that it is of value due to the large number of obtained datasets.

Conclusion

In conclusion, we provide evidence from a marketing survey that even in the stressful period of the COVID-19 pandemic, people who are likely to have increased stress levels, show an improvement in quality of life after only two weeks of intake of a probiotic that was specifically designed to target the gut-brain axis. This result clearly warrants further controlled clinical trials to define the role of probiotics in improving quality of life.

Supplementary Materials

The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Supplementary file 1: questionnaire.

Author Contributions

Conceptualization, all authors; methodology, VeS; formal analysis, AH; resources, VeS; data curation, AH; writing—original draft preparation, AH, JWS, VaS.; writing—review and editing, all authors.; visualization, AH.; supervision, VaS.; funding acquisition, VaS. All authors have read and agreed to the published version of the manuscript.

Funding

The marketing survey was funded by Institut AllergoSan Deutschland (Grünwald, Germany). The analysis of the dataset was funded by the Center for Biomarker Research in Medicine (CBmed), a COMET K1 center funded by the Austrian Research Promotion Agency (Project 3.23).

Institutional Review Board Statement

Not applicable. This study is the analysis of data a marketing survey.

Informed Consent Statement

Consent to analyze the obtained data was obtained from all subjects involved in the study.

Data Availability Statement

Data are available upon reasonable request from the corresponding author.

Conflicts of Interest

VStiegelbauer is an employee of Institut AllergoSan Austria.

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