Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2025; 10(1): 124-133 Website: jnfs.ssu.ac.ir

The Effect of Synbiotic Supplementation on Self-Reported Aggression in Healthy Adult Men: A Randomized, Double-Blind, Placebo-Controlled Trial

Rahele Sadat Montazeri; MSc[1,2], Farzad Shidfar; PhD*[2], Fatemeh Sadat Hosseini-Baharanchi; PhD[3] & Golraste Kholasezadeh; MD[4,5]

[1] Student Research Committee, School of Public Health, Iran University of Medical Sciences, Tehran, Iran; [2] Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran; [3] Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran; [4] Department of Psychiatry, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [5] Research Center of Addiction and Behavioral Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran .

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 18 Oct 2023
Revised: 14 Apr 2024
Accepted: 21 Apr 2024

Corresponding author:
shidfar.f@iums.ac.ir
Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Teheran, Iran.

Postal code: 1449614535
Tel: +98 21 8 862 27 55

Keywords:
Aggression; Anger; Synbiotic; Adult.

ABSTRACT

Background: There is a positive relationship between aggressive behaviors and gut microbiota composition. The present study aimed to investigate the effect of synbiotic supplementation on self-reported aggression in healthy adult men. **Methods:** This double-blind randomized controlled clinical trial was conducted on 100 healthy adult men working at Iran University of Medical Sciences. Members of synbiotic group (SG) received 2 capsules daily containing 500 mg of synbiotic (Familact) and members of placebo group (PG) received 2 placebo capsules daily for 8 weeks. The intensity of aggression (verbal, physical aggression, hostility, and anger), depression, anxiety and stress, food intake, physical activity, and anthropometric indicators were measured at the beginning and end of the study. Statistical analysis of data was done using SPSS software version 26. P-value <0.05 was considered significant. **Results:** Thirty people completed the study. The synbiotic supplement caused a significant decrease in total aggression at the end of the intervention, compared to PG (*P*=0.04). Moreover, verbal aggression decreased in both groups compared to the beginning of the study, while it was significant in SG (*P*=0.005). There was no difference in the intensity of physical aggression, anger, and hostility after intervention. **Conclusion:** Synbiotics may be useful in reducing self-reported aggression in healthy adult men.

Introduction

Aggression is a behavior that aims to harm oneself. This behavior manifests in different ways; verbal and physical aggression represents behavioral aspect, hostility represents cognitive aspect, and anger represents its emotional aspect (Ramirez and Andreu, 2006). Anger is a destructive emotion that exposes us to potential dangers and has harmful effects that affect internal and external aspects of a person. The inability to manage anger, leads to personal discomfort, disturbance in public health and interpersonal relationships, incompatibility, and harmful consequences of aggressive behavior (Taylor and Novaco, 2005).

This paper should be cited as: Montazeri RS, Shidfar F, Hosseini-Baharanchi FS, Kholasezadeh G***. The Effect of Synbiotic Supplementation on Self-Reported Aggression in Healthy Adult Men: A Randomized, Double-Blind, Placebo-Controlled Trial.*** *Journal of Nutrition and Food Security (JNFS)*, 2025; 10 (1): 124-133.

[DOI: 10.18502/jnfs.v10i1.17764]

Various factors are involved in aggression etiology, including genetic, biological, psychological, and environmental factors (Weber *et al.*, 2019). Several studies have reported the structural and functional disorders related to aggression in some parts of the brain such as frontal cortex, amygdala, striatum, hippocampus, and hypothalamus, and imbalance in Hypothalamus-Pituitary-Adrenal axis (HPA) and cortisol levels (Choy and Raine, 2018, Nelson and Chiavegatto, 2001, Ruttle *et al.*, 2011). Serotonin is the main molecule in aggression. It has been shown that Serotonin-boosting drugs such as Serotonin precursors, reuptake inhibitors, and receptor agonists can reduce aggression (Nelson and Chiavegatto, 2001).

In recent years, many efforts have been made to control aggression, such as applying psychoanalytic methods, behavior modification, and drug therapy. However, these methods were not as successful as expected. A possible cause is related to nutritional factors affecting behavior that has not been considered in these treatment methods (Liu and Raine, 2011).

In the late 1990s, studies began to identify the role of diet and nutrition in aggression (Neugebauer *et al.*, 1999). For example, Meyer showed that prisoners with a lower omega-3 index are more aggressive (Meyer *et al.*, 2015). Interventional studies have reported the beneficial effects of multivitamin-minerals and essential fatty acids supplementation in reducing aggression in adults (Gajos and Beaver, 2016, Long and Benton, 2013, Sawada and Yokoi, 2010).

The role of gut microbiome in brain function and behavior has been considered. The relationship between behavior and microbiome is called brain-gut axis and includes neuronal, hormonal, and immunological pathways (Gato *et al.*, 2018). One of the proposed mechanisms of microbiome effect on brain and behavior is its ability to synthesize precursors of neurotransmitters such as serotonin, dopamine, and epinephrine. These molecules enter blood circulation through intestinal epithelium, pass through blood-brain barrier, and affect brain function and behavior (Aarts *et al.*, 2017). The effects of some bacterial species on behavior are mediated by autonomic nervous system and HPA axis (Dinan *et al.*, 2015). Gut microbiota can affect aggression by regulating levels of Corticotropin-Releasing Hormone (CRH) and cortisol hormone (Gato *et al.*, 2018). Another mechanism is the production of compounds with neuroactive properties called short-chain fatty acids by microbiota in intestine, such as acetate, propionate, and butyrate (Cenit *et al.*, 2017). Hanstock showed that rats with more propionate in their intestines have more aggressive and anxious behaviors (Hanstock *et al.*, 2004).

The intestinal microbiota is regulated by prebiotics and probiotics, which have beneficial effects on neuropsychiatric disorders such as anxiety, depression, Alzheimer's, Parkinson's, and autism (Alòs Alcalde, 2018). The combination of probiotic species and prebiotic fibers has synergistic effects, called synbiotics. The mutual effects between pro and prebiotics in the living organism improve the survival of probiotics. It has been shown that intake of synbiotic can improve the content of microbiota and maintain intestinal microbial balance (Waitzberg *et al.*, 2013). Messaoudi (Messaoudi *et al.*, 2011) and Mitrović (Mitrović, 2017), in two separate studies conducted on healthy adults, demonstrated the effects of probiotic supplementation in reducing anger. Given that synbiotics have beneficial effects on mental health and behavior and considering that to the best there is limited study carried out regarding the beneficial effect of synbiotic supplementation on different types of aggression in healthy adults, this study aimed to determine the effect of synbiotic on self-reported aggression in healthy adult men.

Materials and Methods

Participants

A hundred healthy adult men aged 18-75 years who were employee in Iran University of Medical Sciences participated in the study. This randomized, double-blind, placebo-controlled trial was conducted at Iran University of Medical Sciences, Tehran, Iran, from May 2021 to July

[DOI: 10.18502/jnfs.v10i1.17764]

2021. Patients were excluded if they refused to participate or had chronic heart disease, inflammatory disease, autoimmune diseases or thyroid diseases, peptic ulcer, allergies, mental disease, or used antibiotics and nutritional supplements within the past 2 months.

Study design

In the current study, participants were randomly allocated into two groups symbiotic group (SG, n=50) and placebo grope (PG, n=50). SG participants receive two synbiotic capsules daily (containing 500 milligrams of 7 probiotic species: Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophilus, 10^9 colony-forming units, along with 38 milligrams prebiotic Fructo oligosaccharide: Familact). PG members receive two capsules (contained 500 milligrams maltodextrin) daily with their lunch and dinner for 8 weeks). The physical properties of the placebo were indistinguishable in terms of shape, color, size, packaging, and smell, but contained no bacteria (All capsules were provided by Zist Takhmir Company, Tehran, Iran). A person who was blinded to the study gave the randomization sequence extracted from allocation software.

In the beginning, the participants were given a full explanation of the study goals and their general information were recorded including age, height, screen time (duration of watching TV, using computer and mobile phone), sleep duration (daytime), family history of aggression and anger, level of education, and occupation. Moreover, their weight, body mass index (BMI), physical activity, dietary intakes, aggression, depression, anxiety and stress status were measured by related questionnaires at the beginning and end of study. All participants were asked to not alter their physical activity level, or usual food intake, and not take any additional dietary supplements.

Dietary assessment

Participants were requested to record their dietary intakes for three days. Dietary records were based on estimated values in household measurements. To obtain the nutrient intakes of participants, Nutritionist IV software modified for Iranian food was used.

Physical activity assessment

The physical activity assessment was obtained to monitor participants' usual physical activity levels during the study. The validated short-form International Physical Activity Questionnaire (IPAQ) was used to measure the participants' physical activity. This questionnaire determines the intensity of activities in the past 7 days and reports results in categories (low activity levels, moderate activity levels or high activity levels). Scoring a high level of physical activity implies that the physical activity levels equate to about one hour of activity per day or more at least a moderate intensity activity level. Scoring a moderate level of physical activity implies that you are doing some activity more than half an hour of moderate intensity physical activity on most days. Scoring a low level of physical activity implies that you do not meet any of the criteria for moderate or high levels of physical activity (IPAQ Research Committee, 2005).

Anthropometric characteristics assessment

Anthropometric parameters were evaluated using standard protocols in participants with light clothes and without shoes. An electronic scale (Seca, Hamburg, Germany) was used to measure body weight to the nearest 0.1 kg. For height assessment, a nonelastic tape measure (Seca, Hamburg, Germany) was used. For calculating BMI, weight in kilograms was divided by height in squared meters.

Psychological status assessment

Depression, anxiety, and stress symptomatology were assessed in all study participants using the translated version of the Depression Anxiety Stress Scale-21 (DASS-21), which is validated and reliable in the Persian adult general population (Maleki *et al.*, 2005). The DASS-21 consists of 21 self-reported items with seven items in each of the three subscales for depression, anxiety, and stress. The respondent scores each item based on the frequency or severity of emotional experiences

[DOI: 10.18502/jnfs.v10i1.17764]

over the last week on a four-point scale ranging from 0 to 3. A score of 0 was considered for the item "no symptoms at all" and 3 for the item "very severe". The scores from the seven items in each subscale are summed to produce a single subscale score (Baker *et al.*, 2001).

Aggression assessment

The Persian version of the Buss-Perry Aggression Questionnaire (AQ; 1992) was used to assess the aggression before and after the intervention (Gallagher and Ashford, 2016). The AQ is a self-reported questionnaire with 29 items comprising four subscales of aggressive disorder including physical aggression (9 items), verbal aggression (5 items), anger (7 items), and hostility (8 items). Participants rated statements about their character using a five-point likert scale ranging from 1 (extremely uncharacteristic of me) to 5 (extremely characteristic of me). For example, "If I have to resort to violence to defend my rights, I will" (physical aggression); "I often get into fights" (verbal aggression); "I recover quickly after facing challenges" (anger); and "I sometimes feel that I have gotten a raw deal out of life" (hostility). Moreover, the scores were added to make an overall score (Buss and Perry, 1992). The validity of this questionnaire has been established among the Iranian population.

Ethical considerations

The study protocol was approved by the Medical Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.REC.1398.694). This study was registered on the Iranian website for registration of clinical trials (www.irct.ir; IRCT code: IRCT20091114002709N53).

Data analysis

The sample size was determined using Long study (Long, 2013). The probability of type 1 error was assumed to be 0.05 and the power of the study was 0.8. Using Procedure menu in PASS11 software, independent sample and difference sub-menu, 42 samples were required in each group. By applying about 20% dropout, 50 samples were required in each group and 100 samples in general.

IBM SPSS Statistics version 26 was applied to carry out all analyses. The normality of variables was examined by Kolmogorov-Smirnov test. Baseline variables were compared between treatment groups using Independent t-test and Mann–Whitney U-test. To compare changes from baseline to end of the intervention in each group, Paired t-test was used for the data with normal distribution, and Wilcoxon Signed-rank test was used for skewed data. Changes from baseline to the end of the intervention were compared using analysis of covariance (ANCOVA), with the baseline level as the covariate. Results were expressed as mean±SD. P-value<0.05 was considered as significant.

Results

After 8 weeks, 30 participants, including 17 in the SG and 13 in the PG, completed the study (**Figure 1**). The treatment compliance was 78.77% in SG and 71.1% in pPG. Baseline characteristics of participants in both groups are given in **Table 1**. At baseline, no significant differences were found among the two groups in terms of mean age, height, weight, BMI, sleep duration, screen time, family history of aggression, education, and occupation. Weight, BMI, and dietary intakes of study participants as confounding factors, had no significant difference between two groups before and after the intervention. Due to the non-cooperation of the participants, we could assess one-day food record data.

Comparing depression, anxiety, and stress status at baseline and during the study in each group, we observed no significant differences (**Table 2**).

According to **Table 3**, changes in the intensity of total aggression between SG and PG at the end of the study were significant (*P*=0.042) and after adjusting the data based on its initial values, remained significant (*P*=0.032).

Verbal aggression significantly decreased after the 8-week intervention in SG (*P*=0.005), while there was no significant difference in PG. These changes were not significant between the two groups. Moreover, when other confounding

[DOI: 10.18502/jnfs.v10i1.17764]

variables were controlled, there were no significant differences in the findings (**Table 3**; adjusted for baseline value). There were also no significant differences between the two groups in terms of physical aggression, hostility, and anger within or between the two groups, and controlling for confounders had no significant effect (**Table 3**).

Discussion

To the best of our knowledge, the present study is the first double-blind randomized clinical trial using placebo that investigated the effect of synbiotic supplementation on self-reported aggression in healthy adult men.

The results of this study showed that daily consumption of two capsules containing 500 mg of synbiotic supplement (Familact) for 8 weeks could significantly reduce total aggression compared to placebo in healthy adults. It also significantly reduced the severity of verbal aggression in the SG, but it was not significant compared to PG.

Animal studies have shown the beneficial effect of synbiotic on aggressive behaviors. For example, Hu, Rushen, and Cheng *et al*., in three separate studies, investigated the effect of a diet containing three specific species of Bacillus Subtilis on aggressive behaviors in white chickens and showed preventing effects of aggressive behaviors along with probiotic consumption for 2 weeks. Moreover, the plasma serotonin decreased in feeding chickens with probiotic (Cheng *et al.*, 2019, Hu *et al.*, 2018, Rushen, 1982). In 2022, Yeh *et al*. showed that 2 weeks of probiotic consumption in 45 dogs with behavioral problems such as aggression and anxiety had beneficial effects (Yeh *et al.*, 2022).

It has been shown that aggressive people have more plasma serotonin (Jiang *et al.*, 2022). Serotonin plays an essential role in regulating behavior in the Central Nervous System (CNS). The intestine contains the largest amount of serotonin in the body, which is mainly synthesized in enterochromaffin cells and distributed in blood circulation. These cells have neuron-like properties that can regulate the function of the CNS through the enteric nervous system and the vagus nerve (Liddle, 2019). Environmental serotonin can also affect serotonin receptors of vagus nerve in the digestive system and transmit information from intestinal nervous system to the CNS, i.e., the brain-gut axis (Yeh *et al.*, 2022). Microorganisms in the digestive tract interact with signaling system of the host cell through this axis (Cheng *et al.*, 2019).

Table 1. Baseline characteristics of study participants in two groups.

Variables	SG(n= 17)	PG(n= 13)	P-value
Age (year)	36.2±6.7	38.0±6.9	0.49
Height (cm)	179.2 ± 8.2	177.7 ± 8.4	0.62
Weight (kg)	87.2 ± 15.5	82.5 ±12.1	0.36
Body mass index (kg/m^2)	27.2 ± 4.6	26.0 ± 2.8	0.45
Screen time (h)			
<2	7 (41.2)	4 (30.8)	0.71
2≤	10 (58.8)	9 (69.2)	
Sleep duration (h)			
<8	10 (58.8)	9 (69.2)	0.71
8≤	7 (41.2)	4 (30.8)	
Family history of aggression			
Yes	5 (29.4)	2 (15.4)	0.43
No	12 (70.6)	11 (84.6)	
Education			
Non-academic	10 (58.8)	9 (69.2)	0.71
Academic	7 (41.2)	4 (30.8)	
Occupation			
Worker	3 (17.6)	4 (30.8)	0.67
Employee	14 (82.4)	9 (69.2)	

*Quantitative variables (mean±SD) and p-values are obtained from Independent sample t-test; Qualitative variables n(%) and p-values are obtained from Fisher's exact test; **SG**: Synbiotic group; **PG**: Placebo group.*

Modifying the microbiota in dysbiosis animals can reduce injurious behaviors (Yeh *et al.*, 2022). The study by Sylvia *et al*. showed a significant decrease in aggressive behavior after receiving antibiotics for 14 days in hamsters (Sylvia *et al.*, 2017). In 2021, Watanabe *et al*. showed that maintaining healthy gut microbiota of Germ-Free (GF) mice early in life is effective in reducing aggressive behavior throughout their lifetime (Watanabe *et al.*, 2021).

[DOI: 10.18502/jnfs.v10i1.17764]

The present study found no effect of synbiotic on anger and hostility. However, Steenbergen *et al*. examined the effect of probiotic consumption on cognitive reactivity in bad mood in 40 healthy people for 4 weeks by using Leidin Depression Susceptibility Scale and showed a significant reduction of aggressive thoughts in the group receiving probiotic (Steenbergen *et al.*, 2015). Messaoudi *et al*. also showed a significant decrease in the anger-hostility subscale on the HSCL-90 questionnaire after taking probiotic supplements in 66 healthy adults for 30 days. Moreover, urinary cortisol levels significantly reduced in the probiotic group, which indicates the role of probiotics in regulating HPA activity (Messaoudi *et al.*, 2011). In another study by Mitrović *et al*., the effects of the probiotic supplement were investigated in 110 adults aged 18 to 70 years for 5 weeks. The results showed that anxiety and mood (including anger) subscales on the POMS and SCL-90 questionnaire significantly improved in both probiotic and placebo groups. More improvement was also observed in people taking medicine or referring to a mental health specialist (Mitrović, 2017).

Table 2. Depression, stress, and anxiety of study participants.

Variable	SG(n= 17)	PG(n= 13)	P-value[a]
Depression			
Baseline	5.05±3.63[c]	4.85 ± 3.26	1
End	4.58 ± 4.92	4.26 ± 2.16	0.81
P-value [b]	0.6	0.44	
Stress			
Baseline	6.42 ± 3.49	5.61 ± 3.62	0.54
End	6.27 ± 5.11	5.14 ± 1.86	0.41
P-value	0.87	0.63	
Anxiety			
Baseline	3.23 ± 2.38	3.38 ± 2.18	0.85
End	4.00 ± 3.42	2.73 ± 1.85	0.24
P-value	0.33	0.21	

*[a]: Independent sample t-test; [b]: Paired sample t-test; [c]: Mean±SD; **SG**: Synbiotic group; **PG**: Placebo group.*

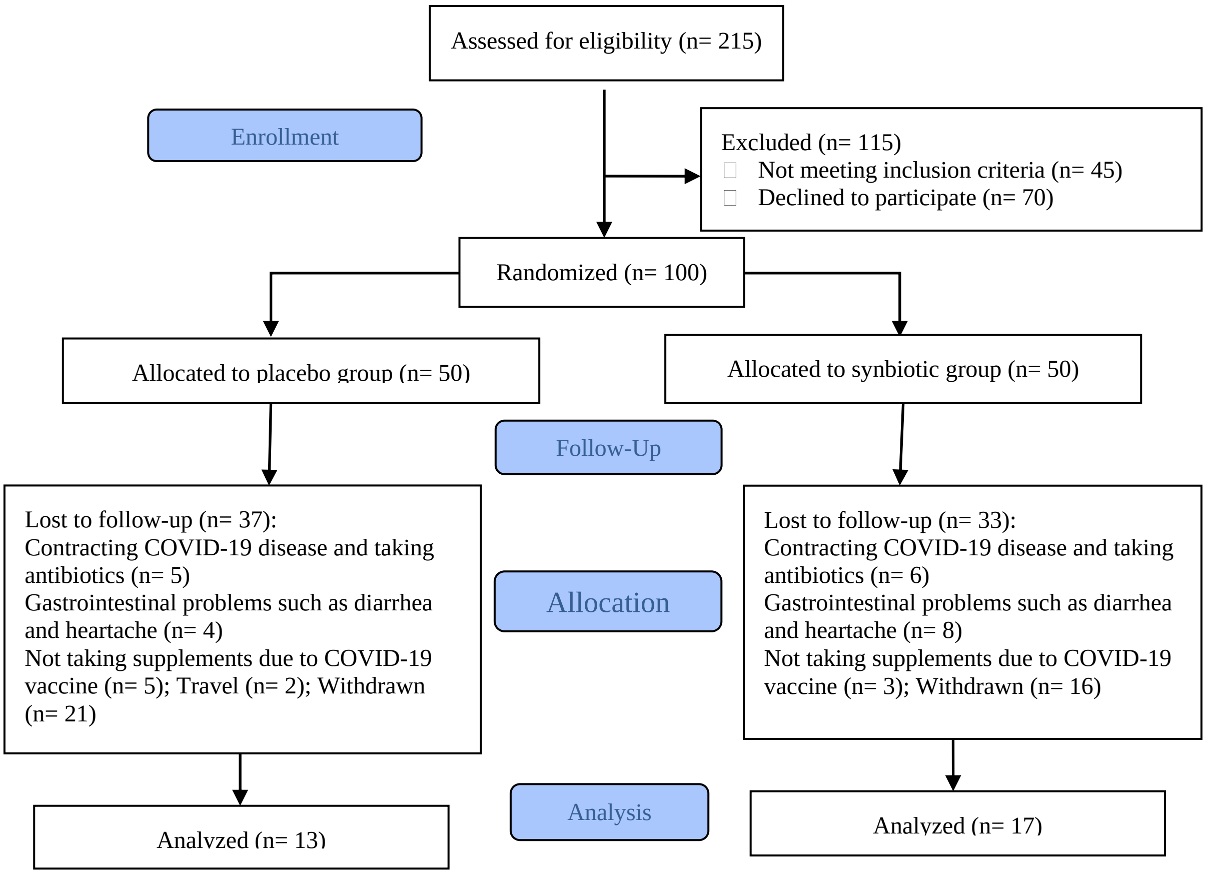

Figure 1. Flow diagram of the enrolled participants

[DOI: 10.18502/jnfs.v10i1.17764]

Table 3. Effect of synbiotic supplementation on different types of aggression score before and after intervention.

Variables	SG(n= 17)	PG(n= 13)	P-value[a]	P-value[d]
Total aggression				
Baseline	72.5±16.9[c]	68.8±17.7	0.56	
End	67.8±11.5	73.8±14.9	0.22	0.03
Change	4.7±13.3	-5.0±10.8	0.04	
P-value	0.16	0.12		
Physical aggression				
Baseline	21.1±5.8	18.9±4.7	0.27	
End	19.9±4.2	21.2±4.5	0.41	0.24
Change	1.2±6.0	-2.3±5.3	0.25	
P-value	0.41	0.14		
Verbal aggression				
Baseline	15.2±3.2	12.9±3.2	0.06	
End	12.9±.2.2	12.8±2.9	0.85	0.10
Change	2.2±2.8	0.1±4.4	0.12	
P-value	0.005	0.90		
Hostility				
Baseline	19.2±5.5	19.1±6.8	0.97	
End	3.9±8.7	19.7±5.5	0.57	0.45
Change	0.5±4.3	-0.5±5.2	0.54	
P-value	0.62	0.72		
Anger				
Baseline	18.5±5.0	16.9 ±6.2	0.44	
End	16.3 ±4.5	17.5±5.2	0.49	0.17
Change	2.2±4.7	-0.6±4.7	0.11	
P-value	0.07	0.65		

*[a]: Independent sample t-test; [b]: Paired sample t-test; [c]: Mean±SD; [d]: ANCOVA (adjusted for baseline value); **SG**: Synbiotic group; **PG**: Placebo group.*

In two separate studies with the same method, 33 healthy adults received synbiotic (all species of Lactobacillus and Bifidobacterium Longum + prebiotic Maltodextrin) for 6 weeks. The results showed that synbiotic intake changed the state of the microbiome towards a healthy mood and decreased the anger subscale of the POMS questionnaire. Also, the consumption of Maltodextrin in the control group produced Bifidobacterium bacteria (Calgaro *et al.*, 2021, Marotta *et al.*, 2019), which plays a role in GABA production (Cenit *et al.*, 2017). Anger-reducer herbal medicines can increase GABA levels in the brain (Xiaoju *et al.*, 2021).

The observed beneficial effects can be related to the role of probiotics in improving the microbial ecology and integrity of the digestive system, as well as more efficient signaling through biochemical pathways, including reducing inflammation, strengthening the immune system, regulating cortisol levels, and improving serotonin and GABA signaling (Wallace and Milev, 2017).

Alternatively, Benton *et al.* investigated the effect of consuming milk containing the probiotic Lactobacillus casei for 3 weeks on memory and mood in 132 healthy adults. Taking probiotic milk did not significantly change anger-hostile score and mood of participants, but mood improved in participants who had severe depression at the beginning of the study (Benton *et al.*, 2007). In another study, probiotic intervention for 14 weeks in 39 athletes did not have any effect on the anger-hostility subscale of the POMS questionnaire (Michalickova *et al.*, 2016). Murata *et al.* investigated the effect of Lactobacillus paracasei supplementation on cold symptoms and mood in healthy women and showed improvement in some positive mood states in the

[DOI: 10.18502/jnfs.v10i1.17764]

6th and 12th week after the intervention. However, there were no significant changes in adverse mood states such as anger-hostility, confusion-surprise, depression, fatigue, tension-anxiety (Murata *et al.*, 2018). In addition, taking a probiotic supplement with iron in 53 healthy female athletes for 12 weeks was unaffected by the anger-hostility subscale of the POMS questionnaire (Axling *et al.*, 2020). Different results of the mentioned studies can be influenced by the type and dose of the probiotic supplement, the duration of the intervention, and different sample sizes. Considering that anger does not always lead to aggression and acts differently in the present study, synbiotic supplementation reduced total aggression, but had no effect on anger intensity.

Based on these findings, the baseline mood of people was very important in the effectiveness of the intervention, since no effect was seen in people who were in good condition. Probably, nutritional intervention along with psychotherapy skills training has better improving effects on different types of aggression, and the use of a self-report questionnaire in addition to asking the individual's relatives, can provide more accurate results. It seems the socioeconomic status of participants can be effective in results. Moreover, fecal bacterial loads were not explored to evaluate the number of bacteria in the gut at the baseline and end of the intervention. Therefore, these results must be interpreted cautiously.

The present study has some limitations, including considerable dropout in both groups related to the COVID-19 epidemic, and a small number of participants answered the questionnaires a few weeks after the end of the intervention period.

Conclusion

Synbiotic supplementation among healthy adult men for 8 weeks had beneficial effects on aggressive behavior. However, it did not have any effects on physical aggression, anger, and hostility. Further studies with larger sample sizes are required to confirm these findings.

Acknowledgments

This study was approved by the School of Nutrition and Food Science, Iran University of Medical Sciences (code: IR.IUMS.REC.1398.694). We would like to thank the study participants for their cooperation, and Zist Takhmir Company for providing synbiotic supplementation (Familact) and placebo capsules.

Authors' contributions

R. Montazeri designed and conducted the research. R. Montazeri, G. Kholasezade and F. Shidfar provided essential materials. R. Montazeri and F. Hosseini analyzed data. R. Montazeri wrote, and F. Shidfar revised the manuscript. F. Shidfar had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest.

Funding

This study was financially by Iran University of Medical Sciences, Tehran, Iran.

References

Aarts E, et al. 2017. Gut microbiome in ADHD and its relation to neural reward anticipation. *PloS one.* **12 (9)**: e0183509.

Alòs Alcalde E 2018. Probiotics, Prebiotics and the Nervous System, https://hdl.handle.net/2445/124965.

Axling U, et al. 2020. The effect of Lactobacillus plantarum 299v on iron status and physical performance in female iron-deficient athletes: a randomized controlled trial. *Nutrients.* **12 (5)**: 1279.

Baker RA, Andrew MJ, Schrader G & Knight JL 2001. Preoperative depression and mortality in coronary artery bypass surgery: preliminary findings. *ANZ journal of surgery.* **71 (3)**: 139-142.

Benton D, Williams C & Brown A 2007. Impact of consuming a milk drink containing a probiotic on mood and cognition. *European journal of clinical nutrition.* **61 (3)**: 355-361.

Buss AH & Perry M 1992. The aggression questionnaire. *Journal of personality and social*

[DOI: 10.18502/jnfs.v10i1.17764]

psychology. **63 (3)**: 452.

Calgaro M, et al. 2021. Metabarcoding analysis of gut microbiota of healthy individuals reveals impact of probiotic and maltodextrin consumption. *Beneficial microbes*. **12 (2)**: 121-136.

Cenit MC, Sanz Y & Codoñer-Franch P 2017. Influence of gut microbiota on neuropsychiatric disorders. *World journal of gastroenterology*. **23 (30)**: 5486.

Cheng H-W, Jiang S & Hu J 2019. Gut-brain axis: Probiotic, Bacillus subtilis, prevents aggression via the modification of the central serotonergic system. In *Oral Health by Using Probiotic Products*. IntechOpen.

Choy O & Raine A 2018. Omega-3 supplementation as a dietary intervention to reduce aggressive and antisocial behavior. *Current psychiatry reports*. **20 (5)**: 1-5.

Dinan TG, Stilling RM, Stanton C & Cryan JF 2015. Collective unconscious: how gut microbes shape human behavior. *Journal of psychiatric research*. **63**: 1-9.

Gajos JM & Beaver KM 2016. The effect of omega-3 fatty acids on aggression: A meta-analysis. *Neuroscience & biobehavioral reviews*. **69**: 147-158.

Gallagher JM & Ashford JB 2016. Buss–Perry aggression questionnaire: testing alternative measurement models with assaultive misdemeanor offenders. *Criminal justice and behavior*. **43 (11)**: 1639-1652.

Gato WE, Posick C, Williams A & Mays C 2018. Examining the link between the human microbiome and antisocial behavior: Why criminologists should care about biochemistry, too. *Deviant behavior*. **39 (9)**: 1191-1201.

Hanstock TL, Clayton EH, Li KM & Mallet PE 2004. Anxiety and aggression associated with the fermentation of carbohydrates in the hindgut of rats. *Physiology & behavior*. **82 (2-3)**: 357-368.

Hu J, Chen H & Cheng H 2018. Effect of direct-fed microbials, Bacillus subtilis, on production performance, serotonin concentrations and behavioral parameters in a selected dominant strain of white leghorn hens. *International journal of poultry science*. **17 (3)**: 106-115.

IPAQ Research Committee 2005. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ)-short and long forms. *http://www. ipaq. ki. se/scoring. pdf*.

Jiang S, Hu J-Y & Cheng H-W 2022. The Impact of Probiotic Bacillus subtilis on Injurious Behavior in Laying Hens. *Animals*. **12 (7)**: 870.

Liddle RA 2019. Neuropods. *Cellular and molecular gastroenterology and hepatology*. **7 (4)**: 739-747.

Liu J & Raine A 2011. Malnutrition and externalizing behaviour. In *Lifetime nutritional influences on cognition, behaviour and psychiatric illness*, pp. 301-322. Elsevier.

Long SJ & Benton D 2013. A double-blind trial of the effect of docosahexaenoic acid and vitamin and mineral supplementation on aggression, impulsivity, and stress. *Human psychopharmacology: Clinical and experimental*. **28 (3)**: 238-247.

Maleki A, Asghari M & Salari R 2005. Credit terms of scale, depression, anxiety Vastrs DASS-21 in the Iranian population. *Journal of Iranian psychology*. **1 (4)**: 9-12.

Marotta A, et al. 2019. Effects of probiotics on cognitive reactivity, mood, and sleep quality. *Frontiers in psychiatry*. **10**: 164.

Messaoudi M, et al. 2011. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British journal of nutrition*. **105 (5)**: 755-764.

Meyer BJ, et al. 2015. Baseline omega-3 index correlates with aggressive and attention deficit disorder behaviours in adult prisoners. *PLoS One*. **10 (3)**: e0120220.

Michalickova D, et al. 2016. Lactobacillus helveticus Lafti L10 supplementation reduces respiratory infection duration in a cohort of elite athletes: a randomized, double-blind, placebo-controlled trial. *Applied physiology, nutrition, and metabolism*. **41 (7)**: 782-789.

Mitrović N 2017. Effects of a Probiotic

[DOI: 10.18502/jnfs.v10i1.17764]

Supplement on Anxiety and Mood Balance in Adults. Acadia University.

Murata M, et al. 2018. Effects of paraprobiotic Lactobacillus paracasei MCC1849 supplementation on symptoms of the common cold and mood states in healthy adults. *Beneficial microbes.* **9 (6)**: 855-864.

Nelson RJ & Chiavegatto S 2001. Molecular basis of aggression. *Trends in neurosciences.* **24 (12)**: 713-719.

Neugebauer R, Hoek HW & Susser E 1999. Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood. *JAMA.* **282 (5)**: 455-462.

Ramirez JM & Andreu JM 2006. Aggression, and some related psychological constructs (anger, hostility, and impulsivity) Some comments from a research project. *Neuroscience & biobehavioral reviews.* **30 (3)**: 276-291.

Rushen J 1982. The peck orders of chickens: how do they develop and why are they linear? *Animal behaviour.* **30 (4)**: 1129-1137.

Ruttle PL, et al. 2011. Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol. *Hormones and behavior.* **59 (1)**: 123-132.

Sawada T & Yokoi K 2010. Effect of zinc supplementation on mood states in young women: a pilot study. *European journal of clinical nutrition.* **64 (3)**: 331-333.

Steenbergen L, Sellaro R, van Hemert S, Bosch JA & Colzato LS 2015. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, behavior, and immunity.* **48**: 258-264.

Sylvia KE, Jewell CP, Rendon NM, John EAS & Demas GE 2017. Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters. *Brain, behavior, and immunity.* **60**: 51-62.

Taylor JL & Novaco RW 2005. Anger treatment for people with developmental disabilities: A theory, evidence and manual based approach. John Wiley & Sons.

Waitzberg DL, et al. 2013. Effect of synbiotic in constipated adult women–a randomized, double-blind, placebo-controlled study of clinical response. *Clinical nutrition.* **32 (1)**: 27-33.

Wallace CJ & Milev R 2017. The effects of probiotics on depressive symptoms in humans: a systematic review. *Annals of general psychiatry.* **16 (1)**: 1-10.

Watanabe N, et al. 2021. Effect of gut microbiota early in life on aggressive behavior in mice. *Neuroscience research.* **168**: 95-99.

Weber L, Kamp-Becker I, Christiansen H & Mingebach T 2019. Treatment of child externalizing behavior problems: A comprehensive review and meta–meta-analysis on effects of parent-based interventions on parental characteristics. *European child & adolescent psychiatry.* **28 (8)**: 1025-1036.

Xiaoju L, Wang H-j, Gao J, Wang X-y & Ning Y-x 2021. Baixiangdan Capsule and Shuyu Capsule Regulate Anger-out and Anger-in: GB1 Mediated GABA Can Regulate 5-ht Level in the Dorsal Raphe Nucleus. *Aging.* **15 (6)**: 2046.

Yeh Y-M, et al. 2022. Effects of Lactiplantibacillus plantarum PS128 on alleviating canine aggression and separation anxiety. *Applied animal behaviour science.* **247**: 105569.

[DOI: 10.18502/jnfs.v10i1.17764]