

Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women

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Abstract

AIM: To investigate the effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 and yogurt strains (BIO[®]) on adult women with constipation in Beijing.

METHODS: A total of 135 adult females with constipation were randomly allocated to consume for 2 wk either 100 g of the test fermented milk or 100 g of an acidified milk containing non-living bacteria (control). Stool frequency, defecation condition scores, stool consistency and food intake were recorded at baseline and after 1 and 2 wk in an intention-to-treat population of 126 subjects. In parallel, safety evaluation parameters were performed.

RESULTS: At baseline, no differences were found between groups. Following consumption of test product, stool frequency was significantly increased after 1 wk (3.5 ± 1.5 vs 2.4 ± 0.6 , $P < 0.01$) and 2 wk (4.1 ± 1.7 vs 2.4 ± 0.6 , $P < 0.01$), vs baseline. Similarly, after 1 and 2 wk, of test product consumption, defecation condition (1.1 ± 0.9 vs 1.9 ± 1.2 , $P < 0.01$ and 0.8 ± 1.0 vs 1.9 ± 1.2 , $P < 0.01$, respectively) and stool consistency (1.0 ± 0.8 vs 1.5 ± 1.1 , $P < 0.01$ and 0.6 ± 0.8 vs 1.5 ± 1.1 ,

$P < 0.01$, respectively) were significantly improved. Compared with the control group, stool frequency was also significantly increased (3.5 ± 1.5 vs 2.5 ± 0.9 , $P < 0.01$ and 4.1 ± 1.7 vs 2.6 ± 1.0 , $P < 0.01$, respectively), and defecation condition (1.1 ± 0.9 vs 1.6 ± 1.1 , $P < 0.01$ and 0.8 ± 1.0 vs 1.6 ± 1.1 , $P < 0.01$, respectively) and stool consistency (1.0 ± 0.8 vs 1.4 ± 1.0 , $P < 0.05$ and 0.6 ± 0.8 vs 1.3 ± 1.0 , $P < 0.01$, respectively) significantly decreased after 1 and 2 wk of product consumption. During the same period, food intake did not change between the two groups, and safety parameters of the subjects were within normal ranges.

CONCLUSION: This study suggests a beneficial effect of a fermented milk containing *B. lactis* DN-173010 on stool frequency, defecation condition and stool consistency in adult women with constipation constipated women after 1 and 2 wk of consumption.

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Key words: Probiotic; *Bifidobacterium lactis* DN-173010; Fermented milk; Constipation; Stool frequency; Stool consistency

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INTRODUCTION

In the Chinese National Product Standard of GB2746-1999, fermented milk is a product prepared with cow's milk or milk powder as raw material by degreasing, partly degreasing or non-degreasing and fermentation. Bacteria used to ferment milk, typically lactic acid bacteria (LAB), are bacteria that can produce lactic acid during the metabolism process. At present, common LAB used as fermentation agents include: *Streptococcus thermophilus*, *Lactobacillus delbrueckii sp bulgaricus*,

Lactobacillus acidophilus, and *Lactobacillus casei*. The health effects of yogurt validated by research include: regulation of intestinal and colon flora, prevention or treatment of diarrhea, regulation of immune function, decreasing symptoms of inflammatory bowel diseases, improvement of lactose intolerance, lowering blood cholesterol level, and prevention of certain types of cancers^[1,2]. These effects are a result of the yogurt characteristics due to the fermentation by yogurt symbiosis^[3].

Constipation is a common problem, and generally refers to less than 2-3 stools per week, accompanied by small, dry, and/or hard defecation and discharge difficulty. This trouble is commonly reported in many regions of the world including Asia^[4] North America^[5], and Europe^[6]. Functional constipation is caused by non-organic or drug factors. Constipation can result in some discomforts such as abdominal distension, abdominal pain, headache, dizziness and poor appetite^[7]. The symptoms of constipation can interfere with quality of life. An epidemiological study conducted in Beijing concluded that 6.1% of the adult population was suffering from the symptoms of functional constipation^[8]. The disorder is more common in women and elderly people^[9]. For example, in Beijing, the incidence of constipation in males and females is 1/4.59. High risk factors include anxiety, work fatigue, bad mood and working at a sedentary job^[10].

Probiotics are defined as live micro organisms that, when administered in adequate amounts confer a health benefit on the host^[11]. *Bifidobacterium lactis* DN-173010 survives complete transit through the digestive tract and is recovered live in stools in large quantities relative to the quantity initially ingested^[12-14]. Three separate clinical studies have demonstrated that daily consumption of fermented milk containing *B. lactis* DN-173010 in association with the yogurt starters *L. bulgaricus* and *S. thermophilus* for 15 d improves gastrointestinal transit. This effect is enhanced with increased daily intake (effect of three pots/d > two pots/d > 1 pot/day) in elderly subjects free of any gastrointestinal pathology^[15,16], and in a group of healthy women aged 18-45 years^[17]. An exploratory study was designed to examine the effect of this product, compared to heat-treated yogurt, on quality of life and symptoms in irritable bowel syndrome (IBS)^[18]. This large scale (267 IBS constipation predominant, Rome II criteria), double-blind, randomized, controlled, parallel group study over 6 wk demonstrated that daily consumption of this product alleviates bloating and improves digestive comfort as assessed by the Functional Digestive Disorders Quality of Life questionnaire. An increase in stool frequency was also observed in subjects with the lowest stool frequency (< 3 or 4 bowel movements/wk) without any change of stool consistency. A fifth study performed on fermented milk containing *B. lactis* DN-173010 alone also demonstrated a reduced transit time in healthy men and women^[19]. The results of these five studies support a strong link between improved stool frequency and the strain *B. lactis* DN-173010, and indicate that further research should be carried out to investigate the potential

use of this fermented milk product for improving stool parameters in subjects with constipation.

The aim of this study was to investigate the effect of a fermented milk (Bio[®]) combining *B. lactis* DN-173010 and yogurt strains on functional constipation parameters of adult women in Beijing.

MATERIALS AND METHODS

Study population

A total of 135 women, age 25-65 years old, were recruited in Beijing Hospital. They had a diagnosis of constipation according to the following criteria: less than three stools per week; increased stool hardness; non-organic constipation and habitual constipation.

The following were excluded from the study: Those unable to orally ingest or unable to administer according to instruction; those unable to express complaint clearly; those with constipation symptoms caused by surgical operation within 30 d; those with recent defecation difficulty due to severe organic diseases (colon carcinoma, severe enteritis, intestinal obstruction, inflammatory bowel disease); those with defecation difficulty accompanied by pain; those with acute gastrointestinal tract disease developing within 30 d; those who were pregnant or menstruating; with severe whole body diseases such as cardiovascular, liver, kidney and hematopoietic system; those taking short-term products which may have influenced the results.

Study protocol

One week before product consumption (baseline period), general conditions, safety evaluation parameters, dietary intake and defecation functional parameters (stool frequency, defecation condition scores, stool consistency) were recorded. Thereafter, eligible constipated women were randomly allocated to consume, daily for 2 wk, one pot of either the test product (67 cases) or the control product (68 cases). Subjects were stratified by age, daily eating habits, and constipation causes to the extent possible to ensure inter-group comparability, which could possibly have influenced the results. During the study period, subjects maintained their usual lifestyle and eating habits. The study was approved by the Ethical Review Committee of the Chinese Academy of Preventive Medicine, Institute of Nutrition and Food Hygiene. All subjects provided written informed consent before inclusion in the study.

Study products

The test product was a fermented milk (BIO[®], Danone (Shanghai) Consulting Co., Ltd, Shanghai, China), containing *B. lactis* DN-173010 [1.25×10^{10} colony forming unit (cfu) per pot], together with the two classical yogurt ferments, *S. thermophilus* and *L. bulgaricus* (1.2×10^9 cfu/pot).

The control product was an acidified milk without any ferments or probiotics. Both the test and control products were without flavor, and had similar appearance,

color, texture, taste and lactose concentration level. Each serving, corresponding to one pot, contained 100 g. Both products were specially prepared for the study and provided by Danone (Shanghai) Consultation Corp., Ltd.

Assessments and study criteria

The general conditions, such as the mental status, sleep, eating habit, and blood pressure, were evaluated 1 wk before product consumption.

The safety evaluation parameters blood, urine, and stool routine examinations [red blood cell (RBC) count, white blood cells (WBC) count, hemoglobin (Hb) in blood; RBC, WBC, protein in urine; stool properties, RBC and worm ova in feces], liver and kidney function examinations [glutamate pyruvate transaminase (GPT), glutamate oxalate transaminase (GOT), urea nitrogen, creatine, and blood sugar] were examined once 1 wk before and 1 and 2 wk after product consumption.

Chest X-ray, electrocardiography and abdominal B-ultrasound examinations were done 1 wk before product consumption.

Dietary intake was assessed after 1-2 d, 7-8 d, and 13-14 d of product consumption by food record method for 48 h, to monitor eating habits.

Defecation functional parameters: stool frequency, defecation condition scores and stools were recorded at baseline and 1 and 2 wk after product consumption.

According to extent of defecation difficulty, the defecation condition scores were categorized into four grades^[20]: Grade I (0 points): Normal defecation; grade II (1 point): Only bearing down and uncomfortable sensation. grade III (2 points): Obvious bearing down and uncomfortable sensation, or frequent defecation with difficult and little defecation, seldom abdominal pain or anal burning sensation; grade IV (3 points): Often abdominal pain or anal burning sensation to influence defecation. According to classification method of Bristol, stool consistency was classified into three grades^[20]: Grade I (0 points): Like sausage or snake, smooth and soft; like sausage, with fissure on the surface; grade II (1 point): Sausage-shaped, with lumps; noncohesive lumps, with coarse edges; grade III (2 points): Separating hard lumps, like fruit kernel (difficult discharge).

Statistical analysis

All analysis were conducted on the intention-to-treat (ITT) population, corresponding to subjects having consumed at least one pot of product.

Descriptive statistics were reported as mean \pm SD or frequency for all variables, unless otherwise stated. Statistical comparative analysis between two groups was performed by *t* test, χ^2 test and the sum of rank, by SPSS statistical software.

RESULTS

General information of the subjects

Female volunteers ($n = 135$) were recruited for the study;

Table 1 General basic information of subjects before product consumption

Parameters		Control group ($n = 63$)	Test group ($n = 63$)	<i>P</i> value
Eating pattern	Regular	59	56	0.344
	Irregular	4	7	
Appetite	Good	17	21	0.437
	Common	46	42	
	Poor	0	0	
Eating amount	Large	3	4	0.927
	Common	52	51	
	Small	8	8	
Age (yr)		46.4 \pm 6.7	46.4 \pm 9.8	0.992
Body weight (kg)		62.5 \pm 10.4	61.2 \pm 9.6	0.478
Stool frequency (n /wk)		2.4 \pm 0.6	2.4 \pm 0.6	0.746
Defecation condition score		1.9 \pm 1.2	1.9 \pm 1.2	0.914
Stool consistency		1.6 \pm 1.1	1.5 \pm 1.1	0.408

Table 2 Stool frequency (n /wk, mean \pm SD)

	<i>n</i>	Baseline	Week 1	Week 2
Control group	63	2.4 \pm 0.6	2.5 \pm 0.9	2.6 \pm 1.0 ^a
Test group	63	2.4 \pm 0.6	3.5 \pm 1.5 ^{b,c}	4.1 \pm 1.7 ^{b,c}

Note: Self comparison between before and after product consumption, ^b $P < 0.01$, ^a $P < 0.05$; compared with control group, ^c $P < 0.01$.

four cases in test group and five cases in control group were withdrawn from the study. The general conditions of the other 126 subjects in two groups are described in Table 1. A *t* test was used for age and weight, χ^2 test for eating pattern and amount, and non-parametric rank sum tests for the three defecation parameters, to compare difference between the two groups. There were no significant differences ($P > 0.05$).

Results of blood, urine and stool routine assays, and liver and kidney function examinations were all in normal range before and after product consumption, and there were no clinical changes between the two groups. The chest X-ray, electrocardiogram and abdominal B-ultrasound examinations indicated that the subjects were healthy.

Stool frequency of the subjects

The stool frequency at baseline and after 1 and 2 wk of product consumption is shown in Table 2. Non-parametric rank sum tests were used to compare the difference between groups.

At baseline, no differences were found between groups. Compared to baseline, stool frequency was significantly increased after 2 wk of control product consumption (2.6 \pm 1.0 *vs* 2.4 \pm 0.6, $P < 0.05$) but no differences were found after 1 wk. In the test product group, stool frequency was significantly improved after 1 (3.5 \pm 1.5 *vs* 2.4 \pm 0.6, $P < 0.01$) and 2 wk (4.1 \pm 1.7 *vs* 2.4 \pm 0.6, $P < 0.01$), respectively. Compared with control group, stool frequency was also significantly increased after 1 and 2 wk of product consumption (3.5 \pm 1.5 *vs*

Table 3 Defecation condition score (mean \pm SD)

	<i>n</i>	Baseline	Week 1	Week 2
Control group	63	1.9 \pm 1.2	1.6 \pm 1.1 ^a	1.6 \pm 1.1
Test group	63	1.9 \pm 1.2	1.1 \pm 0.9 ^{b,d}	0.8 \pm 1.0 ^{b,d}

Note: Self comparison before and after test, ^a*P* < 0.05, ^b*P* < 0.01; compared with control group, ^d*P* < 0.01.

Table 4 Stool consistency score (mean \pm SD)

	<i>n</i>	Baseline	Week 1	Week 2
Control group	63	1.6 \pm 1.1	1.4 \pm 1.0	1.3 \pm 1.0 ^a
Test group	63	1.5 \pm 1.1	1.0 \pm 0.8 ^{b,c}	0.6 \pm 0.8 ^{b,d}

Note: Self comparison before and after test, ^a*P* < 0.05, ^b*P* < 0.01; compared with control group, ^c*P* < 0.05, ^d*P* < 0.01.

Table 5 Food intake amount of the subjects (g, mean \pm SD)

	day 1-2		day 6-7		day 13-14	
	Control group	Test group	Control group	Test group	Control group	Test group
Staple food	341 \pm 110	324 \pm 117	350 \pm 109	334 \pm 115	351 \pm 117	353 \pm 123
Fruits and vegetables	287 \pm 227	341 \pm 235	296 \pm 212	322 \pm 223	305 \pm 244	318 \pm 201
Meat and egg	118 \pm 120	119 \pm 100	114 \pm 104	123 \pm 103	121 \pm 95	111 \pm 105
Total daily intake	745 \pm 343	784 \pm 322	761 \pm 294	779 \pm 316	776 \pm 344	783 \pm 315

2.5 \pm 0.9, *P* < 0.01 and 4.1 \pm 1.7 *vs* 2.6 \pm 1.0, *P* < 0.01, respectively) as shown in Table 2.

Defecation condition scores of the subjects

Defecation condition scores at baseline and after 1 and 2 wk of product consumption are shown in Table 3. Non-parametric rank sum tests were used to compare the difference between groups.

At baseline, no differences were found between groups. Compared to baseline, defecation condition score was significantly improved after 1 wk of control product consumption (1.6 \pm 1.1 *vs* 1.9 \pm 1.2, *P* < 0.05), but no differences were found after 2 wk. In the test product group, defecation condition score was significantly improved after 1 (1.1 \pm 0.9 *vs* 1.9 \pm 1.2, *P* < 0.01) and 2 wk (0.8 \pm 1.0 *vs* 1.9 \pm 1.2, *P* < 0.01), respectively. Compared with control group, defecation condition scores were also significantly improved (1.1 \pm 0.9 *vs* 1.6 \pm 1.1, *P* < 0.01 and 0.8 \pm 1.0 *vs* 1.6 \pm 1.1, *P* < 0.01), respectively, after 1 and 2 wk of product consumption.

Stool consistency scores of the subjects

Stool consistency at baseline and after 1 and 2 wk of product consumption is showed in Table 4. Non-parametric rank sum tests were used to compare the difference between groups.

At baseline, no differences were found between groups. Compared to baseline, stool consistency score was significantly decreased after 2 wk of control product consumption (1.3 \pm 1.0 *vs* 1.6 \pm 1.1, *P* < 0.05), but no differences were found after 1 wk. In the test product group, stool consistency score was significantly improved after 1 (1.0 \pm 0.8 *vs* 1.5 \pm 1.1, *P* < 0.01) and 2 wk (0.6 \pm 0.8 *vs* 1.5 \pm 1.1, *P* < 0.01), respectively. Compared with control group, stool consistency score was also significantly decreased after 1 and 2 wk of product consumption, (1.0 \pm 0.8 *vs* 1.4 \pm 1.0, *P* < 0.05 and 0.6 \pm 0.8 *vs* 1.3 \pm 1.0, *P* < 0.01), respectively.

Food intake of the subjects during product consumption

Food intakes of the subjects was surveyed by 48 h

dietary recall at initial stage (1st-2nd day), intermediate stage (6th-7th day) and end stage (13th-14th day) of product consumption. The mean daily intakes of staple food, fruits and vegetables, and meat and eggs of the three times were calculated and statistically analyzed by *t* test between the two groups (Table 5). Food intake throughout the study did not differ between groups.

DISCUSSION

Consumption of the fermented product tested in this study was well tolerated by all the participants, and no adverse effects were reported. An acidified milk was used as the control material in this study. Lactose in milk can cause intolerance characterized by rugitus, abdominal distension, abdominal pain, even diarrhea as a severe symptom. The incidence rate of lactose intolerance reaches 90% in Chinese adults. For example, Yang *et al*^[21] have shown that lactose intolerance occurred in 87% of the 7-8 and 11-13 years old Chinese children. In order to prevent diarrhea, due to milk intake in the control group, extrinsic lactase was added to control milk samples during the manufacturing process. This resulted in an equal lactose content between the test and control products.

Several reviews^[22-25] have described that some probiotics could improve lactose digestion and eliminate the symptoms of intolerance. A recent study has shown that a yogurt enriched with *B. lactis* DN-173010 and *B. longum* in capsules modifies the composition and metabolic activities of the colonic microbiota and alleviates symptoms in Chinese lactose-intolerant subjects^[26].

BIO[®] is a fermented milk product which contains a mixture of live bacterial cultures; *B. lactis* DN-173010 (1.25 \times 10¹⁰ cfu/pot) and yogurt starters *L. bulgaricus* and *S. thermophilus* (1.2 \times 10⁹ cfu/pot). It has been shown to increase slow transit and, therefore, was tested in women with constipation to determine whether it would modulate bowel habits. Food intake and blood parameters remained constant throughout the study.

The results of this study indicated that stool frequency was significantly increased by 40% and 58% after 1 and 2 wk of product consumption, respectively ($P < 0.01$). In addition, after 1 wk of consumption, defecation condition scores (31% and 50%, $P < 0.01$) and stool consistency (29%, $P < 0.05$ and 25%, $P < 0.01$) were also significantly improved from baseline values in women consuming test product.

Three separate clinical studies have already demonstrated that daily consumption of a fermented milk containing *B. lactis* DN-173010 improves gastrointestinal transit time in elderly subjects^[15,16], and in a group of healthy women with slow transit time^[17]. Stool frequency was also significantly improved after product consumption in IBS subjects compared to controls with respect to subjects with a stool frequency < 4 stools per week at baseline^[18]. A fifth study performed on fermented milk containing *B. lactis* DN-173010 alone also demonstrated a reduced transit time in healthy men and women with slow transit time^[19]. Our finding that probiotics may normalize bowel movements is in line with those in some previous studies^[27-30]. Some studies have shown that milk or yogurt fermented with different types of probiotics may increase the daily stool number in constipated subjects. In a double-blind, placebo-controlled study performed in 70 subjects with chronic constipation, a probiotic beverage containing *Lactobacillus casei* Shirota administered for a 4-wk period was significantly better than placebo in improving severity of constipation, stool frequency and consistency^[27]. Likewise, a preparation containing *Escherichia coli* Nissle 1917 (a probiotic strain) was compared to placebo in a double-blind clinical trial in 70 subjects with chronic constipation, showing that the *E. coli* preparation was significantly better than placebo in increasing stool frequency^[28]. Moreover, intake of a fermented milk product containing *L. casei* strain Shirota for 2 wk in a placebo-controlled double-blind cross-over design improved the state of bowel movements and stool quality in healthy subjects with a stronger tendency to constipation^[29]. Finally, in an open trial in elderly subjects, a commercial mixture of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* improved defecation frequency by 24%, but no reduction in laxative use was observed^[30]. To date, no clinical studies have been performed to measure defecation conditions/straining on constipated subjects. Few studies have been focused on the effects of probiotics on the intestinal function in healthy people, and the observed effects depended on the strain used. Three randomized, double-blind, placebo-controlled human clinical trials^[31-33] have been performed to investigate the effect of a fermented product containing probiotic strains. In these clinical studies, product consumption exerted a beneficial effect on the bowel functions, but with no significant effect compared to the placebo. Findings in previous studies are inconsistent possibly due to lesser statistical power, the use of different probiotic strains

and different subject population. Thus, probiotics may be effective in subjects with mild to moderate constipation and controlled and well-designed studies in this type of subjects are warranted^[34]. In any case, our study is the first showing the significant efficacy of fermented milk consumption on stool frequency and consistency, as well as defecation conditions in constipated subjects.

The patients' ability to achieve normal bowel habits without being in pain, and to control bowel movements, are important elements of physical well-being. This was shown by studies investigating the relationships between quality of life and gastrointestinal symptoms in persons with constipation^[35,36]. The surveys revealed an impaired quality of life in constipated individuals in comparison with healthy persons, depending on the severity of the constipation. In addition, Guyonnet *et al.*^[18] have shown that daily consumption of a fermented milk containing *B. lactis* DN-173010 improves quality of life and symptoms in IBS compared to heat-treated yogurt. These results support the hypothesis of a relationship between improved stool frequency, transit time, quality of life and a fermented milk containing *B. lactis* DN-173010, and indicate that further research should be carried out to investigate the potential use of this fermented milk product in improving quality of life in subjects with constipation.

In conclusion, the present large-scale study showed a beneficial effect of a fermented milk containing *B. lactis* DN-173010 on stool frequency and consistency, as well as on defecation conditions of women with constipation. Further studies are required to elucidate mechanisms of such effects to provide additional scientific evidence to support the use of such probiotic food to relieve constipation.

COMMENTS

Background

In recent years, probiotics have been studied for their efficacy on gastrointestinal disorders. *Bifidobacterium lactis* DN-173010, a probiotic strain, has already demonstrated health benefit on the gastrointestinal transit. Positive results have been obtained with consumption of a fermented milk containing *B. lactis* DN-173010 and yogurt strains on gut transit time in healthy people with normal to slow transit time. Equally, encouraging positive results have been obtained on stool frequency in Irritable Bowel Syndrome subjects with predominant constipation. These results indicate that research should be carried out to investigate the potential use of this fermented milk product in improving stool parameters in subjects with constipation.

Research frontiers

Constipation is reported in many regions in the world. An epidemiological study conducted in Beijing concluded that 6.1% of the adult population was suffering from the symptoms of functional constipation but no clinical study has been carried out in this population. This is why we decided to investigate the effect of a fermented milk (BIO⁺) combining *B. lactis* DN-173010 and yogurt strains on constipation functional parameters of adult women in Beijing.

Innovations and breakthroughs

This research demonstrates the first positive results on the ability of the *B. lactis* DN-173010 to improve stool frequency, stool consistency and defecation conditions in adult constipated women. It is believed to be the first study to investigate the effect of *B. lactis* DN-173010 led in another ethnic population with a different diet than European populations. Finally, this study shows the first evidence of the positive effect of *B. lactis* DN-173010 on constipated subjects.

Peer review

The contribution by Yue-Xin Yang *et al* studied the effect of a fermented milk containing *B. lactis* DN-173010 and yogurt strains (BIO[®]) in adult constipated women in Beijing China. The authors determined that the fermented milk containing *B. lactis* DN-173010 had a beneficial effect on stool frequency, stool consistency, and defecation of women with constipation. This is a well-written report.

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