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Original

Randomised, double-blind and placebo-controlled study of the effect of a synbiotic dairy product on orocecal transit time in healthy adult women

A. Malpeli[1], S. González[2], D. Vicentin[3], A. Apás[2] and H. F. González[1]

[1]IDIP-Instituto de Desarrollo e Investigaciones Pediatricas "Prof Dr. Fernando Viteri". Hospital de Niños de La Plata (MS/CICPBA). Argentina. [2]CERELA-Centro de Referencia para Lactobacilos (CONICET-Tucumán). Argentina. [3]Departamento de Investigación, Innovación y Desarrollo. SanCor CUL. Argentina.

Abstract

Objective: **To evaluate oro-cecal intestinal transit time (ITT) before and after administration of a dairy product containing Bifidobacterium BB12, *Lactobacillus casei* CRL 431 and fiber in healthy women.**

Methods: **A prospective, randomised, double-blind and cross-over study with a 4-phase design (run-in: time 0 [T0], two intervention periods: time 1 [T1] and time 3 [T3] and a wash-out: time 2, [T2]) was performed. Participants were asked about bowel movement and fiber consumption. ITT was assessed by the carmine red dye method.**

Results: **Mean age was 40.7 years (n = 102 healthy women; 83 completed the study). In women with initial ITT (IITT) ≥ 48 h consuming the synbiotic product, mean IITT and final ITT (FITT) was 86.9 ± 38.5 h and 51.2 ± 29.8 h (-40.9%), as compared to women consuming the control yoghurt (IITT, 80.8 ± 31.7 h; FITT, 69.5 ± 31.5 h; -13.8%) (p = 0.001). IITT in women with functional constipation consuming the control yoghurt was 57.0 ± 30.0 h; such figure increased 2.8 h after yoghurt consumption (FITT, 59.8 ± 3 0.2 h; +4.9%). Conversely, IITT in women who received the synbiotic yoghurt was 69.0 ± 49.6 h, with a -27.5% decrease 19 h later (FITT, 50.0 ± 27.5 h; p = 0.023). Enteric lactic flora stabilization was significantly higher in women who initially consumed the synbiotic product (p < 0.1).**

Conclusion: **ITT decreased significantly after consumption of the synbiotic product. Such beneficial effect was more evident in women with IITT ≥ 48 h and with functional constipation.**

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Key words: *Functional constipation. Synbiotic. Women. Intestinal transit time.*

Correspondence: Horacio F. González.
IDIP. Instituto de Desarrollo e Investigaciones Pediátricas
"Prof. Dr. Fernando Viteri".
Hospital de Niños de La Plata (MS/CIC-PBA).
Calle 63, n.º 1069.
1900 La Plata, Argentina.
E-mail: horaciofgonzalez@gmail.com

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ESTUDIO ALEATORIZADO, DOBLE-CIEGO Y CONTROLADO POR PLACEBO DEL EFECTO DE UN SIMBIÓTICO SOBRE EL TRÁNSITO INTESTINAL EN MUJERES ADULTAS SANAS

Resumen

Objetivo: **Evaluar el tiempo de tránsito intestinal (TTI) antes y después de consumir yogur con Bifidobacterium BB12, *Lactobacillus casei* CRL 431 y fibra (simbiótico).**

Métodos: **Estudio prospectivo, cruzado, aleatorizado y doble-ciego con 4 fases: preparación (tiempo 0); intervención (yogur o simbiótico; tiempo 1 y tiempo 3); sin intervención (tiempo 2). Evaluamos la frecuencia de defecación y el consumo de fibra. El TTI se estimo con rojo carmín.**

Resultados: **La edad promedio fue 40,7 años (n = 102 mujeres sanas; 83 completaron el estudio). En mujeres con TTI inicial (TTII) ≥ 48 h que consumieron el simbiótico, el TTII y el TTI final (TTIF) fue 86,9 ± 38,5 h y 51,2 ± 29,8 h (-40,9%), respectivamente, comparado con el de mujeres que consumieron el producto control (TTII, 80,8 ± 31,7 h; TTIF, 69,5 ± 31,5 h; -13,8%) (p = 0,001). En mujeres con constipación funcional que consumieron el producto control, el TTII fue 57,0 ± 30,0 h, valor que aumentó 2,8 h después de consumir el producto (TTIF, 59,8 ± 30,2 h; + 4,9%). El TTII en mujeres que consumieron el simbiótico fue 69,0 ± 49,6 h, registrándose una disminución de 27,5% 19 h después del consumo (TTIF, 50,0 ± 27,5 h; p = 0,023). La normalización de la flora láctica entérica fue significativamente mayor en mujeres que consumieron el simbiótico al inicio (p < 0,1).**

Conclusión: **El TTI disminuyó significativamente después de consumir el simbiótico. Dicho efecto beneficioso fue más evidente en mujeres con TTII ≥ 48 h y constipación funcional.**

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Palabras clave: *Constipación funcional. Simbiótico. Mujeres. Tiempo de tránsito intestinal.*

Introduction

Bowel disorders vary from person to person and are influenced by cultural factors.[1] The most common functional gastrointestinal disorders are irritable bowel syndrome, constipation and bloating, and they have a higher prevalence in women.[1] Constipation is classified into 3 main classes: normal-transit, slow-transit, and defecatory disorders, being normal-transit constipation the most prevalent class (59%).[2] Patients with this disorder only have three bowel movements or fewer in a week; stool frequency is normal, yet they believe they are constipated. In these patients, constipation is likely to be due to a perceived difficulty with evacuation or the presence of hard stools; they may also experience bloating and abdominal pain or discomfort.[3]

The presence of pain and slow-transit is favored by lifestyle habits, particularly low fiber intake, insufficient water intake and scarce practice of physical activity.[2] Therefore, constipation and bloating can be handled with diet and healthy habits, although dietary fiber supplements or laxatives can also be used.[4] In this context, the use of prebiotics and probiotics in every day diet has been shown to be effective.[5-9]

Probiotics are defined as food or drugs containing live microorganisms, such as *Lactobacillus, Bifidobacterium* and *Streptococcus*,[10] that exert a beneficial physiological effect on the host.[11] Prebiotics are short-chain carbohydrates resisting digestion by gastric acid and pancreatic enzymes; to be effective, they must reach the cecum, where they are fermented by bacteria and may positively influence intestine function.[12]

The term synbiotic is used to define products containing both prebiotics and probiotics, in which the prebiotic compound selectively favors the probiotic compound. They are functional foods that stimulate the growth of lactic microflora,[13] reason why dairy products are the vehicle of choice for these compounds.[14] The positive effect of some prebiotics and probiotics on different gastrointestinal dysfunctions, such as gastrointestinal infection, constipation, lactose intolerance, inflammatory bowel disease, and probably colon cancer, is supported by evidence in the literature.[15,16]

In this study, we evaluated oro-cecal intestinal transit time (ITT) in healthy women before and after administration of a dairy product containing *Bifidobacterium* BB12, *Lactobacillus casei* CRL 431 and fiber, as compared to placebo administration. We further determined the effect of both products on stool consistency and frequency, as well as symptomatic improvement (bloating and abdominal pain) at the end of the study.

Materials and methods

Design of the study

The study was prospective, randomised, double-blind and cross-over, and was carried out at IDIP – Instituto de Desarrollo e Investigaciones Pediátricas "Prof. Dr. Fernando E. Viteri" (La Plata's Children Hospital, Buenos Aires, Argentina). It had a 4-phase design, *i.e.*, 4 consecutive periods of 15 days each: preparation or run-in (time 0; T0), and two intervention periods (time 1 and 3; T1 and T3) separated by a wash-out (time 2; T2). Participants were randomly assigned to one of two groups according to the last two digits of their identity card number (odd or even). Study group 1 received standard yoghurt (T0), control yoghurt (T1), standard yoghurt (T2), and synbiotic yoghurt (T3). Study group 2 was given standard yoghurt (T0), synbiotic yoghurt (T1), standard yoghurt (T2), and control yoghurt (T3).

Subjects

A total of 102 healthy adult volunteer women aged 21-60 years and living in the city of La Plata, Province of Buenos Aires, Argentina, were recruited through street interviews. Inclusion criteria were women with slow-transit perception, abdominal pain and ITT > 24 h and willing to participate in the study. Exclusion criteria were use of medication that could affect intestinal transit, and/or diagnosis of any disease.

Women were asked to answer a questionnaire about dietary habits and bowel movement. Those with slow-transit perception and/or abdominal pain (bloating) or slow-transit (functional constipation) according to Rome III criteria[17] were invited to participate in the study and further asked to assess ITT by the carmine red dye method *(see Dye method)*. Only those volunteers with ITT > 24 h were included in the study (n = 83).

Before the study, a semi-quantitative survey about frequency of consumption was made to assess dietary fiber intake.

Yoghurt, cultured and/or fermented milk and laxative consumption was stopped 15 days before and during the study period. Participants were then instructed to go on their habitual diet.

All subjects gave their informed consent to participate in the study. The study protocol was approved by IDIP´s Institutional Research Protocol Review Board.

Dairy products

All products (synbiotic, control and standard yoghurt) were elaborated two weeks before each phase by San-Cor CUL Ltd (Sunchales, Argentina) and handed out in pots containing 125 g of yoghurt with the same appearance and taste.

The synbiotic yoghurt contained 0.625 g of prebiotics (inulin and oligofructose), the probiotic *Bifidobacterium lactis* BB12 (10^9-10^{10} colony forming units [CFU]), the probiotic *Lactobacillus casei* CRL 431 (1 x 10^9-6 x 10^9 CFU), *Lactobacillus bulgaricus* (10^9-10^{10} CFU), and *Streptococcus thermophilus* (10^9-10^{10} CFU).

The control and the standard yoghurt contained *Lactobacillus bulgaricus* (10^9-10^{10} CFU) and *Streptococcus thermophilus* (10^9-10^{10} CFU), and the same organoleptic characteristics as those of the synbiotic product. The only difference between them was in the color of the label.

Participants collected the corresponding product weekly throughout the study, and were instructed to consume 2 yoghurts per day during each phase.

Dye method

ITT estimations in each study period were done with carmine red dye, a safe and non-invasive method currently used.[18,19] Women were given1 g carmine red in capsules and had to register date and time of consumption on a record sheet. They also had to register date and time of elimination of the dye in stools, as determined by the change of color to intense orange or red. The procedure was repeated twice in each study phase, and the mean of both measurements was used to determine ITT in each participant. Women were asked not to eat food that could change the color of their stool, such as beetroot.

Stool analysis

Stool samples were collected at T0 (run in), T1 (synbiotic or control period) and T2 (wash-out). T3 was not considered because samples could be influenced by the products ingested during the previous periods.

Each stool sample was weighed and resuspended in 0.1% sterile peptone water. Thereafter, adequate dilutions for the corresponding cultures in differential media were made. Final counts were referred to CFU per gram of feces (CFU/g).

The culture media used were BHI agar for total aerobic bacteria (aerobic incubation), BHI for total anaerobic bacteria (anaerobic incubation), LBS agar (lactobacilli selective agar) for lactobacilli, KF agar for enterobacteria and streptococcus, Mc Conkey for enterobacteria, and modified HHD for bifidobacteria.

Statistical analysis

In all cases, repeated measures analysis of variance was used to determine the effect of the synbiotic product. χ^2 was used to compare proportions in intestinal symptom improvement.

Results

Mean age was 40.7 years; 83 out of the 102 healthy women completed the study and 19 dropped out (6 due to initial ITT (IITT) < 24 h, 6 due to causes unrelated to the study, 5 due to intercurrent disease without digestive function compromise [influenza, respiratory disease], 1 due to constipation and 1 due to bloating).

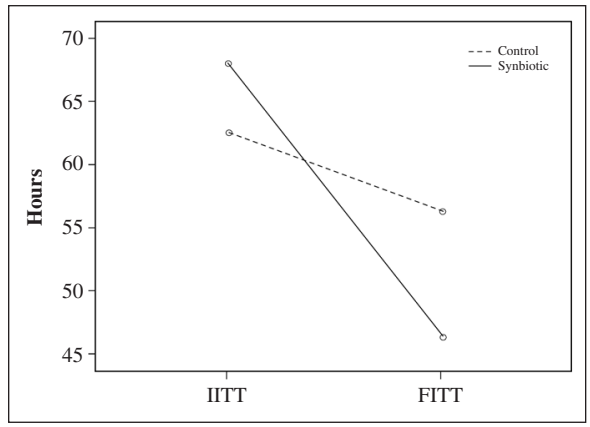

Fig. 1.—Changes in intestinal transit time (ITT) after synbiotic and control yoghurt consumption. (IITT: initial intestinal transit time; FITT: final intestinal transit time).

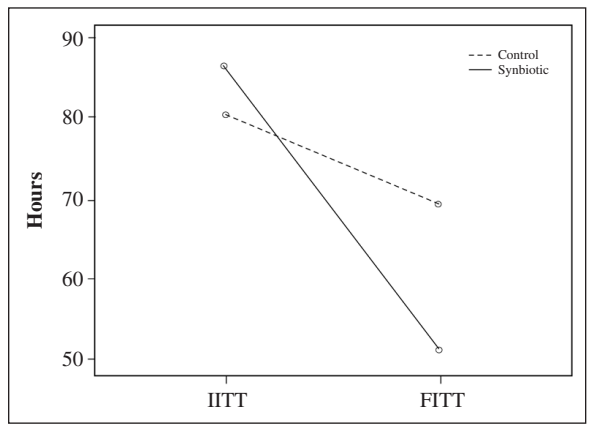

Fig. 2.—Changes in intestinal transit time (ITT) after synbiotic and control yoghurt consumption in women with initial ITT ≥ 48 h. (IITT: initial intestinal transit time; FITT: final intestinal transit time).

Mean IITT and FITT was 68.0 ± 39.8 and 46.4 ± 26.5, respectively, in women consuming the synbiotic product (ITT decrease of 16.6 ± 34.4 h; -27.6%). In women consuming the control yoghurt, mean IITT and FITT was 62.6 ± 34.3 h and 56.4 ± 30.7 h, respectively (ITT decrease of 3.68 ± 28.2 h; -6.36%; p = 0.005; fig. 1).

In women with IITT ≥ 48 h and consuming the synbiotic product, mean IITT and FIIT was 86.9 ± 38.5 h and 51.2 ± 29.8 h, respectively (-40.9%). In those receiving the control yoghurt, mean IITT and FITT was 80.8 ± 31.7 h and 69.5 ± 31.5 h (-13.8%; p = 0.001; fig. 2).

Results of the consequences of dairy product consumption in women with functional constipation are shown in figure 3. At the beginning of the study, 63 women with functional constipation were selected, 35 consumed control yoghurt and 28 the synbiotic product. IITT in the former was 57.0 ± 30.0 h; such figure increased 2.8 h after yoghurt consumption (FITT, 59.8 ±3 0.2 h; +4.9%). Conversely, IITT in women who ingested the synbiotic yoghurt was 69.0 ± 49.6 h, with a -27.5% decrease 19 h later (FITT, 50.0 ± 27.5 h; p = 0.023; fig. 3).

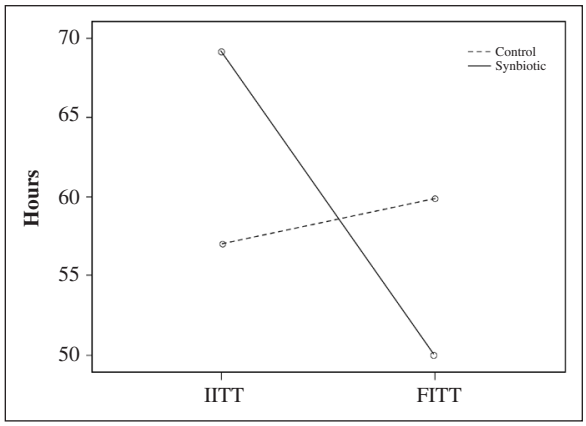

Fig. 3.—Changes in intestinal transit time (ITT) after synbiotic and control yoghurt consumption in women with functional constipation at the beginning of the study. (IITT: initial intestinal transit time; FITT: final intestinal transit time).

Changes in other bowel habits were concerned with voiding frequency, stool consistency and bloating. Stool consistency was not significantly different in either group (synbiotic vs. control). In the case of women who consumed the synbiotic product, there was a marked improvement in abdominal bloating as compared with those consuming the control yoghurt ($p = 0.04$). Even though not significant, there was a trend toward improvement in the frequency of voiding in women who consumed the synbiotic product ($p = 0.07$).

Results of the dietary survey indicated that consumption of fiber in the diet or with the dairy product with prebiotics was 10.7 and 1.25 g/day, respectively.

Changes in stool consistency were within the framework of intestinal flora dynamic balance in both study groups. Enteric lactic flora stabilization was significantly higher in Group 2 ($p < 0.1$) as compared to Group 1 (fig. 4). Whereas women in Group 2 had a significant decrease in gram-positive cocci and enterobacteria from T1 onwards ($p < 0.1$), no significant differences were detected in women from Group 1. In Group 1, there were no significant differences in bifid flora between T0 and T1/T2, whereas in Group 2 such differences were significant from T1 and remained until the end of the study ($p < 0.1$; fig. 5).

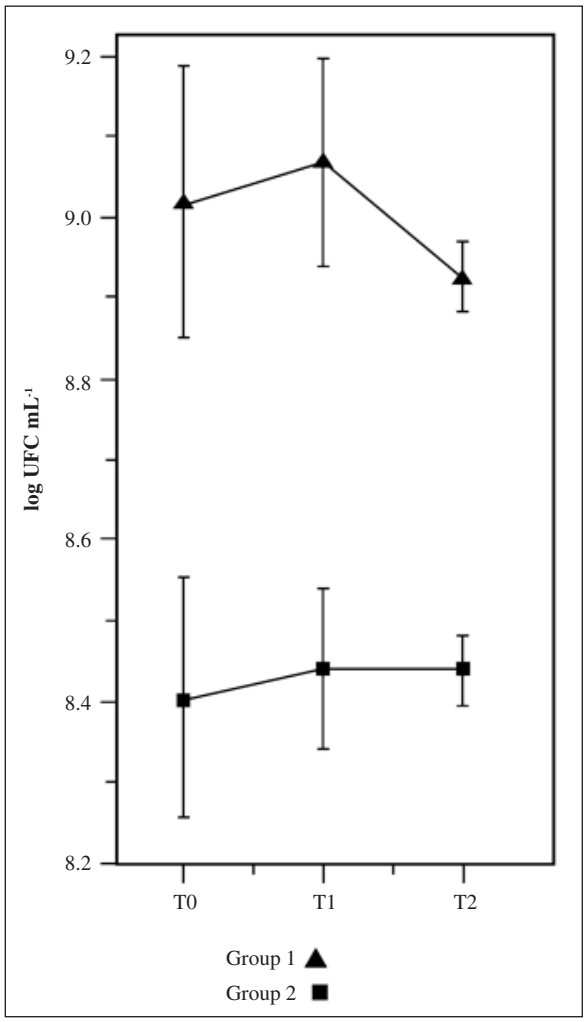

Fig. 4.—Lactobacilli count from feces cultured in LBS media.

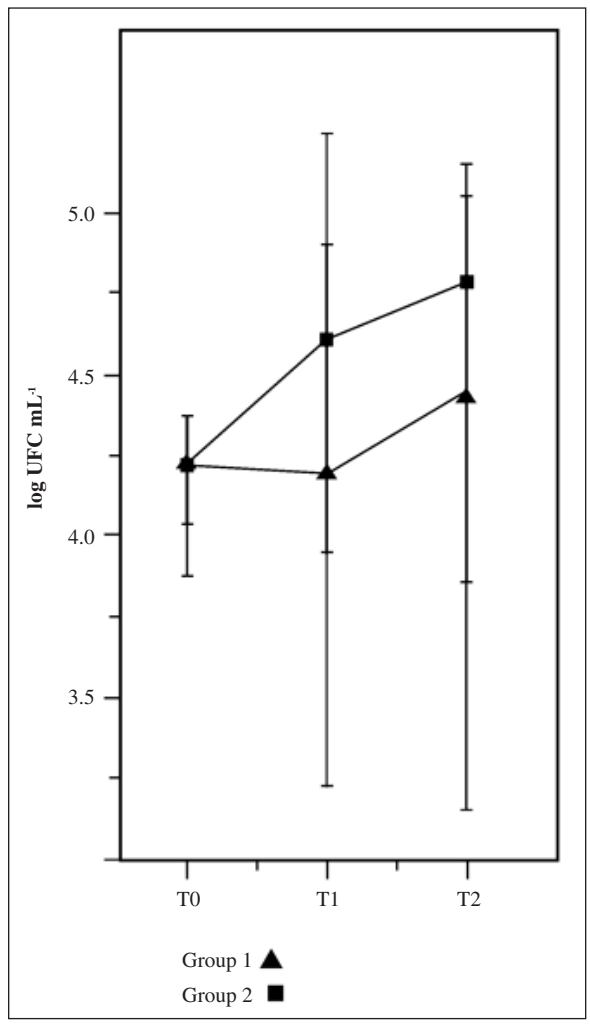

Fig. 5.—Bifidobacteria count from feces cultures in modified HHD media.

Discussion

Our results show the beneficial effect of synbiotic yoghurt consumption on ITT in healthy adult women, particularly in women with IITT > 48 h or functional constipation, who had the highest benefit.

Previous reports have demonstrated the beneficial effect of the probiotics used in our study on intestinal microflora. A significant increase of fecal bifidobacteria has been reported in intestinal microflora of elderly people after 2-week consumption of BB12-containing yoghurt.[20] Another study on elderly people showed that BB12 yoghurt consumption improved intestinal microflora, suggesting that probiotic qualities of this strain are optimal for adult people.[21]

On the other hand, our study represents the first investigation about consumption of *Lactobacillus casei* CRL 431 and its effect on people suffering constipation. This strain was tried in adult lactose-intolerant people;[22] although it was not the main aim of the study, in this report the protocol included measurement of oro-cecal transit time, which showed a statistically significant decrease.

A review on prebiotics (oligofructose, galactooligosaccharides and lactulose) showed that they are the most effective products for improving intestinal microflora, with increased levels of bifidobacteria and lactobacilli.[12]

In the study by Marteau and Boutron-Ruault, increased intestinal peristalsis due to prebiotic consumption was the result of bacterial growth stimulation and of the osmotic effect exerted when passing through the intestinal tract.[15]

The relationship prebiotic/laxative effect is still a matter of discussion because research protocols were not comparable and study samples diverse.[12]

A review of randomized controlled studies evaluating the efficacy and safety of probiotics for the treatment of functional constipation in adults determined that *Escherichia coli* Nissle 1917, *Lactobacillus casei* Shirota and *Bifidobacterium lactis* DN-173010 were the most effective strains. All studies agreed on higher stool frequency and decreased stool consistency; however, the clinical relevance of such findings is still under discussion.[23]

In other reports of constipated women who consumed the probiotic *Bifidobacterium lactis* DN-173010, a 3.7-h decrease (-6.7%) in colonic transit time and an 8-h decrease (-11.3%) in women with IITT of 40 h was observed.[8] Although we measured oro-cecal transit with carmine dye, results showed a marked decrease in transit time.

Another paper evaluating the effect of a synbiotic product *(Bifidobacterium lactis* DN-173010 + inulin) on evacuatory habits in women with functional constipation showed a significant increase in weekly stool frequency in women consuming the synbiotic (6.1 ± 2.7 stools/week) as compared to those in the control group (5.0 ± 2.6 stools/week), together with improved parameters of bowel movement (quality of feces, excessive straining, pain associated to evacuation).[24] Our findings are similar to those mentioned above, even when the observational methods used were different.

Synbiotic food is also used in constipated patients with irritable bowel syndrome. Dughera et al.[25] suggest promisory positive effects on clinical manifestations and intestinal function in treated patients; their data show improvements in abdominal pain and bloating as well as increased stool frequency. Similar results were reported by Colecchia et al.,[26] indicating that the synbiotic product also increased stool frequency and improved symptoms.

Other strains of probiotics have been studied to determine their effect on stool frequency and intestinal microflora; the intake of a dairy product containing *Bifidobacterium lactis* FK120 improved fecal microflora and promoted bowel movement in young healthy women[27] and healthy volunteers.[28]

The observed changes in microflora indicate that consumption of a synbiotic product at the beginning of the trial (Group 2) caused statistically significant changes in the microflora (increased bifidobacteria and decreased enterobacteria) from T1 onwards ($p < 0.1$), that remained until the end of the trial.

Some authors correlate symptoms, abdominal pain and bloating with decreased lactobacilli and bifidobacteria in intestinal microflora.[29] However, as suggested by Dughera et al.,[25] studies on the use of probiotics to relieve symptoms are controversial; while some authors say that the use of lactobacilli is effective for symptom treatment,[30] others state that probiotics are effective with bifidobacteria and not lactobacilli.[31] Further studies in comparable population samples are needed to evaluate such effects.

Conclusion

Our results suggest that the synbiotic product designed by our group caused a significant decrease in ITT, and that such beneficial effect was more evident in women with IITT ≥ 48 h and in those suffering functional constipation.

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