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Consumption effect of a synbiotic beverage made from soy and yacon extracts containing *Bifidobacterium animalis* ssp. *lactis* BB-12 on the intestinal polyamine concentrations in elderly individuals

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ABSTRACT

This study aimed to investigate the effect of a synbiotic beverage made from soy and yacon (*Smallanthus sonchifolius*) extracts containing *Bifidobacterium animalis* ssp. *lactis* BB-12 on healthy elderly individuals' intestinal polyamine concentrations. A randomized, double-blinded, placebo-controlled trial has been conducted with twenty-nine volunteers (over 65 years of age) who either had a daily intake of 150 mL of synbiotic (synbiotic group — S) or placebo (placebo group — P) beverages. Both had the same nutrient composition, except that a probiotic culture was added to the synbiotic beverage. Total experiment time was 8 weeks, which was divided into 3 consecutive phases: a prefeeding period (2 weeks), followed by a feeding period (4 weeks) and a postfeeding period (2 weeks). Stool samples were collected at 3 time periods. Fecal concentrations of polyamines, putrescine (PUT), cadaverine (CAD) and spermidine (SPD) that were obtained during the synbiotic and placebo consumption period were significantly higher ($p < 0.05$) than those found during the pre-consumption baseline level period. No significant differences in the number of bifidobacteria, clostridia, or enterobacteria were observed in any of the two groups at the three time periods. Similarly, no significant effect on the production of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and anti-inflammatory interleukin-10 (IL-10) was induced by the synbiotic or placebo beverages consumption. The results herein indicate that both the synbiotic and the placebo beverage consumption have increased polyamines levels, which are often reduced in elderly individuals, without influencing inflammatory responses. In addition, both placebo and synbiotic beverages seems to contribute by maintaining increased polyamines levels.

1. Introduction

The core microbiota of elderly individuals has been characterized by unusual phylum proportions and extreme variability (Claesson et al., 2011). Because of its crucial role in the host's physiology and healthy condition, age-related differences in gut microbiota composition may be related to diseases progression and a frail elderly population (Biagi et al., 2010). In this context, a growing understanding of the gut microbiota impact on human health has resulted in attempts to manipulate its composition by using probiotics and prebiotics, both from prophylactic and therapeutic perspectives (Biagi, Candela, Fairweather-Tait, Franceschi, & Brigidi, 2012).

More recently, a study has demonstrated that the intake of arginine with the probiotic strain LKM512 can prevent aging-induced quality of life deterioration via polyamines upregulation (Kibe et al., 2014). Furthermore, it has been demonstrated that once intestinal polyamines concentrations were increased by LKM512 yogurt ingestion, it inhibited rat's senescence and enhanced their longevity, as well as inhibited elderly individuals' systemic inflammation, and reduced mutagenicity in healthy adults' guts (Matsumoto & Benno, 2004; Matsumoto, Kurihara, Kibe, Ashida, & Benno, 2011; Matsumoto, Ohishi, & Benno, 2001).

As in plants, polyamines are also ubiquitous amongst mammalian cells, including human cells, which are involved in a wide range of vital cellular processes, playing essential roles in human development, metabolism and physiological functions, hence polyamines are essential to maintaining good health conditions at all life stages (Hunter & Burrit,

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2012; Kantaria & Gokani, 2011). Notwithstanding, there is a consensus in literature about a decrease in polyamine concentrations in the body due to aging, although this may be tissue-specific condition. The effect that it has on human health is still being investigated; however, enhanced polyamine intake appears to have a positive effect on human health, e.g. aging process, as opposed to immunosenescence and inflammaging (a chronic inflammatory status) (Binh, Soda, & Kawakami, 2011; Hunter & Burrit, 2012; Soda et al., 2009).

Polyamines, including putrescine, spermidine, and spermine, are amongst the most important bacterial metabolites in the intestine (Matsumoto, Sakamoto, & Benno, 2009). In fact, it has been acknowledged presently that there are three sources of polyamines in humans: intracellular de novo synthesis of polyamines, dietary polyamines and polyamines produced as metabolites by the gut microbiota (Hunter & Burrit, 2012).

Nowadays, it is well-established that *B. animalis* ssp. *lactis* BB-12 is a common commercial probiotic product that can be used in foods. Studies on BB-12 ingestion have shown that it offers many potential health benefits (Palaria, Johnson-Kanda, & O'Sullivan, 2011). However, to the best of our knowledge, no study has reported the effect of the strain BB-12 ingestion on fecal production of polyamines.

Therefore, the aim of the present double-blind, randomized, controlled feeding trial was to study the effects of ingesting a new low-calorie synbiotic beverage made from yacon (prebiotic source) and soy extracts, containing probiotic *Bifidobacterium animalis* ssp. *lactis* BB-12 (Manzoni, Cavallini, Pauly-Silveira, Roselino & Rossi, 2012) on elderly individuals' fecal polyamines concentration. It was found relevant to ascertain whether the synbiotic beverage consumption has also had an effect on stimulated cytokine production. In addition, intestinal bifidobacteria, clostridial, and enterobacterial numbers were determined.

2. Materials and methods

2.1. Synbiotic and placebo beverages production

The synbiotic and placebo beverages were processed at UNISOJA (Development and Production Unit for Soybean Derivates) in the Food Nutrition Department of the School of Pharmaceutical Sciences, UNESP, Araraquara (SP, Brazil). Procedures for preparing the beverages were described in detail by our previous report (Manzoni et al., 2012). They had the same nutrient composition, except that a probiotic culture was added to the synbiotic beverage (BB-12®-Probiotic-culture-Probio-Tec®, Christian Hansen, São Paulo, Brazil). Yacon and soy extracts were used as the raw material to produce the placebo and the synbiotic beverage. The chemical composition of the beverages is presented in Table 1. Microbiological analyses of the synbiotic product showed that the beverage exhibited *Bifidobacterium* ssp. counts of 10^{10} CFU per 100 mL of product (Manzoni et al., 2012). 150 mL of the synbiotic and placebo beverage were packaged in appropriate polypropylene plastic food containers. The products were stored under refrigeration (4 ± 1 °C) until consumption. The products were produced weekly and freshly delivered to each volunteer.

Table 1
Chemical composition of the synbiotic and placebo beverages.[a]

Component	(g/100 g)
Moisture	92.96 ± 0.01
Total solids	7.25 ± 0.01
Protein	2.91 ± 0.17
Fat	1.42 ± 0.01
Ash	0.30 ± 0.03
Total carbohydrate	2.41 ± 0.07
FOS content	0.82 ± 0.00
Energy value (kJ/100 g)	148.22

[a] Manzoni, Cavallini, Pauly-Silveira, Roselino, and Rossi (2012). Note. Synbiotic and placebo beverages had the same nutrient composition, except that a probiotic culture (*Bifidobacterium animalis* ssp. *lactis* BB-12) was added to the synbiotic beverage.

2.2. Participants

The study protocol and consent procedures were approved by the Ethical Committee of the School of Pharmaceutical Sciences at the São Paulo State University (Process number 37/2007). Before conducting this study, written informed consents forms were filled out by all volunteers. Participants were recruited from the Open University of the Elderly (São Carlos, São Paulo, Brazil). A total of forty healthy elderly volunteers have been selected after physical examination, but only twenty-nine of them completed the study (fifteen in the placebo group and fourteen in the synbiotic group). The average age of the volunteers in the placebo group was 71 years old, while that of the volunteers in the synbiotic group was 67. They have no antibiotic treatment for 3 months prior to the study period and no chronic or acute diseases. Volunteers were advised to maintain their normal life style, but were requested to refrain from consuming foods with probiotics. It is important to mention that there was no report on adverse events. The withdrawals in the synbiotic and placebo groups, six and five, respectively, were due to pneumonia with subsequent antibiotic treatment (one), and/or personal reasons.

2.3. Experimental design

This is a double-blind study in which the volunteers were randomly divided into two groups that either received the synbiotic (synbiotic group — S) or placebo beverages (placebo group — P). Total experiment time was 8 weeks and was divided into 3 consecutive phases: a prefeeding period (2 weeks), followed by a feeding period (4 weeks) and a postfeeding period (2 weeks). Three stool samples were collected: one during the initial period (week 1), another one at the end of the ingestion period (week 6) and one at the end of the wash out period (week 8), which were stored at − 80 °C until analyses.

2.4. Fecal polyamines concentration determination

For extracting the polyamines from the each stool sample, frozen feces were 7-fold diluted in 5% trichloroacetic acid (TCA). The samples had been shaken for 10 min by using a vortex mixer, which were centrifuged at 10,000 × *g* for 10 min at 4 °C after homogenization. The supernatant was collected and the sediment was extracted twice with 7.0 mL and 6.0 mL of 5% TCA, respectively. The combined supernatant was filtrated in a millipore membrane mesh (0.45 μm). The amines (putrescine, cadaverine, spermidine, and spermine) were separated by ion-pair reversed-phase HPLC and quantified fluorimetrically after post-column derivation with o-phthalaldehyde (Cirilo et al., 2003). The liquid chromatography was performed on a LC-10AD system connected to a RF-551 spectrofluorimetric detector at 340 and 445 nm of excitation and emission, respectively, and a CBM-10AD controller (Shimadzu, Kioto, Japan). A 300 × 3.9 mm i.d. and 10 μm reversed-phase μBondapaq C18 column, was used with a μBondapaq C18 guard-pack insert (Waters, Milford, MA, USA). The mobile phases were: A, solution of 0.2 *M* sodium acetate, and 15 mM 1-octanesulfonic acid sodium salt adjusted to pH 4.9 with glacial acetic acid; and B, acetonitrile. The flow rate was set at 0.8 mL/min and the gradient was: 13 min at 11% B, 19 min at 30% B; 24 min at 11% B, and 45 min at 11% B. The post-column derivatization reagent was delivered at 0.4 mL/min. It consisted of 1.5 mL Brij-35, 1.5 mL mercaptoethanol and 0.2 g o-phthalaldehyde dissolved in a 500 mL solution of 25 g boric acid and 22 g KOH (pH adjusted to 10.5 with 3% KOH). The column and the post-column reaction apparatus were at room temperature (22 ± 1 °C). The amines were identified by a comparison of their retention time in samples with standard solutions and also by adding the suspected amine to the sample. Amine levels were calculated by direct