Probiotic infant cereal improves children’s gut microbiota: Insights using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®)

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ABSTRACT
Infants gut microbiota can be modulated by many factors, including mode of delivery, feeding regime, maternal diet/weight and probiotic and prebiotic consumption. The gut microbiota in dysbiosis has been associated with innumerable diseases. In this sense, early childhood intestinal microbiome modulation can be a strategy for disease prevention. This study had the purpose to evaluate the effect of an infant cereal with probiotic (Bifidobacterium animalis ssp. lactis BB-12®) on infants intestinal microbiota using SHIME®, which simulates human gastrointestinal conditions. The ascending colon was inoculated with fecal microbiota from three children (2–3 years old). NH₄⁺ short chain fatty acids (SCFAs) and microbiota composition were determined by selective ion electrode, GC/MS and 16S sequencing, respectively. After treatment, butyric acid production increased (p < 0.05) 52% and a decrease in NH₄⁺ production was observed (p < 0.01). The treatment stimulated an increase (p < 0.01) of Lactobacillaceae families, more precisely L. gasseri and L. kefiri. L. gasseri has been associated with the prevention of allergic rhinitis in children and L. kefiri in the prevention of obesity. Thus, infant cereal with BB-12® is able to stimulate the growth of L. gasseri and L. kefiri in a beneficial way, reducing NH₄⁺ and increasing the production of SCFAs, especially butyric acid, in SHIME®.

1. Introduction

The intestinal microbiota consists of a complex and diverse system of microorganisms that colonize the gastrointestinal tract (GT) (Frick & Autenrieth, 2012). The number of microorganisms that inhabit the GT has been estimated in the literature at over 10^14, which is ~10 times the number of human cells and over 100 times the amount of genomic content in the human genome (Thursby & Juge, 2017). The count of microorganisms present in the stomach is usually below 10^1, due to acidic pH, with a significant increase in the small intestine (10^4) to 10^7 CFU/mL due to favorable conditions such as slow intestinal transit time, nutrient availability and favorable pH (Payne, Zihler, Chassard, & Lacroix, 2012). The intestinal microbiota is mainly composed of strict anaerobic bacteria, which outperform the facultative anaerobic and aerobic bacteria (Walsh, Guineane, O’Toole, & Cotter, 2014). The most abundant species are the members of phyla Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (Gerritsen, Smidt, Rijkers, & De Vos, 2011).

Microbiota at the beginning of life has low microbial diversity and instability, however this same microbiota evolves to stability in the first 24 months of life (Rautava, Luoto, Salminen, & Isolauri, 2012). Bifidobacteria are generally dominant in the first months of life, especially in breastfed children, due to the bifidogenic effect of breast milk. Meta-genomic analyses show that in adults and children the main components of colon microbiota are Bacteroidetes, followed by various genera belonging to phylum Firmicutes, such as Eubacterium spp., Ruminococcus spp. and Clostridium spp. The composition of intestinal microbiota is influenced by several factors, including the form of delivery (cesarean section or normal delivery), feeding, host genetics, use of antibiotics, and immunological factors (Russell et al., 2011; Scott, Gratz, Sheridan, Flint, & Duncan, 2013). Diet has a significant impact on the intestinal environment, including intestinal transit time, pH and changes in the composition of the microbial community (Scott et al., 2013).
Intestinal microbiota plays a key role in the host’s health status, since it exerts important functions on the immunological, physiological and metabolic processes of the human body (Gerritsen et al., 2011), especially in the first 1000 days, the period from conception to the 2nd year of life, which is a critical window period with implications for long-term health, programming health and the future risk of illness of an individual (Butel, Waligora-Dupriet, & Wydau-Dematteis, 2018). Microbiota acts on the synthesis of B and K vitamins, on resistance against colonization of pathogenic microorganisms and on short chain fatty acid (SCFAs) synthesis (Davila et al., 2013; Gerritsen et al., 2011; Sekirov, Russell, Antunes, & Finlay, 2010). In addition, it is responsible for modulating the immune system, promoting maturation of immune cells and maintenance of motor functions of the gastrointestinal tract (Clemente, Ursell, Parfrey, & Knight, 2012; Round & Mazmanian, 2009; Zhuang et al., 2019).

Species and strains representing Lactobacillaceae families and Bifidobacteria have been used as probiotics, with the aim of colonizing the intestine of children and modulating the host’s immune response (Enomoto et al., 2014). According to Hill et al. (2014) the term “probiotics” says that microorganisms confer health benefits to individuals when administered in appropriate amounts, the true probiotic should preferably be of human origin, safe and free of vectors capable of transferring antibiotic resistance and pathogenicity or toxicity factors. In addition, the probiotic must have a high survival capacity under intestinal conditions (acid pH, enzymes, bile salts, etc.). It must also exhibit antagonism against pathogens, stimulate the immune system and, finally, have demonstrable beneficial effects on the host. The continuous activity, viability and growth efficiency of the probiotic must be demonstrated after the technological treatment (Plaza-Diaz, Fontana, & Gil, 2018; Plaza-Díaz, Ruiz-Ojeda, Gil-Campos, & Gil, 2019).

Several studies have demonstrated benefits of treatment with probiotics in atopic children and therefore modulation of the baby’s intestinal microbiota (Vandenplas, Huys, & Daube, 2015; Rather et al., 2016; D’Ellios et al., 2020; Andrade et al., 2020). In addition, evidence from different studies has shown that the occurrence of a disease is often preceded by early changes in microbiota (Zhuang et al., 2019).

Bifidobacteria are considered one of the key genera of the intestinal tract, representing approximately 3% of the microbiota in healthy adult humans (Solano-Agular et al., 2008). BB-12® has been widely used in infant formula, food supplements and fermented dairy products around the world. This strain is technologically suitable, expressing fermentation activity, high aerotolerance, good stability and high tolerance to acids and bile, also as lyophilized products in food supplements. Furthermore, BB-12® has no implications on taste, appearance or mouthfeel of foods and is able to survive in probiotic foods until consumed (Jungersen et al., 2014).

The health benefits of BB-12® includes, for example, bile salt hydrolase and strong adhesion properties, demonstrated through clinical research (Laparra & Sanz, 2009; Matsumoto, Otishi, & Benno, 2004; Vernazza, Gibson, & Rastall, 2006; Vinderola & Reinheimer, 2003). Besides the inhibition of pathogens, it is known that BB-12® promotes an improvement in epithelial barrier function and immune interactions, a better intestinal function and consequently a protective effect against diarrhea, thus reducing the side effects of antibiotic treatment (Collado, Grzeskowiak, & Salminen, 2007; López, Gueimonde, Margolles, & Suárez, 2010; Martins et al., 2009). In terms of immune function, clinical studies have shown that BB-12® increases body’s resistance to common respiratory tract infections, reducing the incidence of acute respiratory tract infections (Rizzadini et al., 2012).

Probiotic microorganisms are usually consumed as dairy products, such as fermented milk and yogurts (Hekmat, Soltani, & Reid, 2009). However, alternative food matrices, such as infant cereals, could be a good option as vehicles for probiotic consumption, particularly for infants and young children, as infant cereal represents one of the first foods introduced at the beginning of complementary feeding period in many countries (Klerks et al., 2019; Roess et al., 2018).

Infant cereals represent an excellent source of energy, providing substantial amounts of carbohydrates and protein (Agostoni et al., 2008) and also contribute as an important source of vitamins and minerals (Fardet, 2010), specially, iron (Finn et al., 2017; Grimes, Szmyk-Gay, Campbell, & Nicklas, 2015).

In vitro fermentation studies have been developed as tools to study human gut microbiota under highly controlled conditions, thus allowing dynamic sampling over time in reactors that mimic different regions of the colon. The colonic fermentation models have advantages when compared with clinical trials or animal models such as low cost, higher reproducibility, no need of ethical approval and, depending on the experiment, they can be conducted in a shorter time. Colonic models enable the cultivation of human gut microbiota derived from fecal samples under simulated physiological conditions (Pham & Mohajeri, 2018). Thus, these systems allow researchers to study the effects of prebiotics and probiotics on gut microbiota, the fermentation spectra of prebiotics, the survival capacity and function of probiotics throughout the gastrointestinal tract (Bianchi et al., 2018), and the synthesis of polyphenols that modulate the microbial community (Wu et al., 2018).

Our research group has been using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) for several years. Thus, we were able to advance in the studies correlating intestinal microbiota, probiotics, prebiotics and bioactive compounds, contributing to scientific knowl-edge on the following topics: the impact of Enterococcus faecium CRL 183 (Sivieri et al., 2014) and Lactobacillus acidophilus CRL 1014 on the intestinal microbiota (Sivieri et al., 2013), influence of a fermented vegetable drink with Lactcaseibacillus casei on the intestinal microbiota (Bianchi et al., 2014), as well as the prebiotic effect of fructo-oligosaccharide (Sivieri et al., 2014), “multifunctional” milk-based drink of fermented goat (Freire et al., 2017) and probiotic ice cream (Rodrigues et al., 2020) in the modulation of the intestinal microbiota.

Therefore, the evaluation of the influence of infant cereals on the modulation of the intestinal microbiota is still little explored. As so, the aim of this work was to evaluate the effect of infant cereal with Bifidobacterium animalis ssp. lactis BB-12® on children’s gut microbiota using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®), which simulates human gastrointestinal conditions, from the stomach portion to colon.

2. Experimental methods

2.1. Simulated digestion in the dynamic colonic model

The Simulator of Human Intestinal Microbial Ecosystem (SHIME®) is a computer controlled simulator and consists of 5 closed compartments representing the stomach, small intestine, ascending colon, transverse colon and descending colon (Molly, Woestyne, Smet, & Verstraete, 1994). In this experiment, SHIME® was adapted for the triplication of the ascending colon, where the transverse and descending colon have been substituted according to Rodrigues et al. (2020).

The volumetric capacity, pH, temperature (37 °C) and retention time (24 h) were controlled (Possemiers, Verthé, Uyttendaele, & Verstraete, 2004). Anaerobiosis of the system was achieved by the addition of nitrogen and the pH value corrected in each vessel using hydrochloric acid or sodium hydroxide accordingly, to be in the range from 5.6 to 5.9 (Molly et al., 1994; Possemiers et al., 2004).

The compartments were colonized with feces from three healthy children volunteers, inclusion criteria were ages 2–3 years, without food allergy or intolerance and exclusion criteria were dietary supplements and medication for gastrointestinal or metabolic disease, probiotics or prebiotics in the last 3 months, and antibiotics in the last 6 months. 24-hour total feces were collected and kept at 4 °C until its collection by the researcher. They were homogenized and a portion was stored in a sterile plastic tube (10 g) and held at −80 °C until analysis. According to Possemiers, Marzorati, Verstraete, and Van de Wiele (2010) the 40 g of
Abbeele et al., 2019), which is a carbohydrate-based medium [4.0 g/L starch (Maizena, S. comus)]. The samples were mixed in a stomacher and centrifuged at 3000 rpm for 15 min. The supernatant was collected and 10 mL added to each of the last 3 compartments together with 500 mL of sterile feed medium (van den Abbeele et al., 2019), which is a carbohydrate-based medium [4.0 g/L starch (Maizena, S. comus)].

2.3. Metabolic Activity: Ammonium (NH₄⁺) analysis and short chain fatty acids (SCFAs)

After the simulated digestion, the samples from the colon compartment (n = 3) were collected and stored at −20 °C. The ammonium ions (NH₄⁺) were quantified according to Bianchi et al. (2014) using a specific ion meter (Model 710A, Orion) coupled to a selective ammonia ion electrode (Model 95-12, Orion).

Short chain fatty acids were analyzed according to the protocol adopted by Duque, Monteiro, Adorno, Sakamoto, and Silvéri (2016), with modifications. The samples (n = 3, second week of the colon reactors) were centrifuged (14,000g, 5 min) followed by the dilution of 100 μL of the supernatant in 1900 μL of ultrapure water. Next, NaCl (1 g) and crotonic acid (100 μL) were added, as well as isobutanol (70 μL) and 2 M H₂SO₄ (200 μL). Analytical curves were constructed from stock solutions of the acids of interest (acetic, propionic and butyric). The SCFAs analysis was conducted using a HP-5975 model gas chromatograph (Agilent Technologies, Santa Clara-CA, USA) equipped with a split/splitless injector, a mass selective detector and a HP-7683B automated sampler. Separation of the SCFAs took place through a ZB-WAX column (60 m × 0.25 mm × 0.25 μm) (Phenomenex, Torrance-CA, USA). The GC was performed using the column set at 35 °C, 2 °C/min, 38 °C; 10 °C/min, 75 °C; 35 °C/min, 120 °C (1 min); 10 °C/min, 170 °C (2 min); 40 °C/min, 170 °C (2 min), and the temperature of the injector and the detector was maintained at 220 °C and 250 °C, respectively. Helium was used as the carrier gas, the flow rate was set at 1 mL/min (Hoving, Heijink, van Harmelen, van Dijk, & Giera, 2018).

2.4. Survival of Bifidobacterium animalis ssp. lactis –BB12® under simulated conditions of the stomach and duodenum in the SHIME®

During treatment period with the probiotic infant cereal formulation, samples were collected from the reactors corresponding to the stomach and duodenum in order to verify the survival of Bifidobacterium animalis ssp. lactis –BB12®. One mL of samples from each reactor were suspended in 9 mL of sterile peptone water. Serial dilutions were carried and plated in MRS agar supplemented with sodium propionate (3 g.L⁻¹) (Sigma-Aldrich®, USA) and lithium chloride (2 g.L⁻¹) (Merck®, Germany). The plates were incubated in anaerobiosis (Probar, Brazil) at 37 °C for 72 h (Vinderola & Reinnermann, 1999).

2.5. Microbiological analysis employing 16S rRNA gene sequencing

The microbiological analysis employing 16S rRNA gene sequencing (n = 2) was performed by Next-generation Sequencing (NeoProspecta Microbiome Technologies, Brazil) on what specific primers amplified the V3–V4 region of the 16S rRNA, 341F and 806R of 1 ng of DNA (Caporaso, Lauber, Walters, Berg-Lyons, Lozupone, Turnbaugh, Fierer, & Knight, 2011; Wang & Qian, 2009). The analysis of the sequences and the identification of the taxonomic units were based on the methodology of Giorgo et al. (2010) and Hong et al., (2006). The PCR were carried out in triplicate using a Platinum Taq (Invitrogen USA) under the following conditions: initial denaturation at 94 °C for 3 min; 20 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 30 s, extension at 65 °C for 90 s and final extension at 65 °C for 10 min. The PCR products were purified using AMPure beads (Beckman coulter) (Christoff, Fernanda, Sereita, Rodrigues, Lucio, & Moraes, 2017) and quantified by fluorometry using Qubit® 2.0 Fluorometer (Invitrogen, USA) (Fagen et al., 2012). The sequencing libraries were prepared according to NeoProspecta Microbiome Technologies, and the sequencing was carried out using the MiSeq platform (llumina).

Bioinformatics analyses were performed using the QIIME program (Quantitative Insights Into Microbial Ecology) according to procedures described by Caporaso et al. (2010). Taxonomic descriptions were generated based on the database NCBI taxonomy (http://www.ncbi.nlm.nih.gov) for the RDP database entries using the set of scripts listed in the TaxCollecter pipeline Giorgo et al. (2010). The strings were filtered and classified according to similarity. A minimum of 80% similarity was considered for domain and phylum identification, 90% for order and class identification, 90% for family, 95% for gender and 99% similarity for species identification (Savy et al., 2018).

2.6. Statistical analysis

Comparison of normally distributed data of the different control and treatment weeks on microbial metabolic markers and microbial community parameters were performed with a Student’s T-test for pairwise comparisons. Differences were significant if p < 0.05, using the statistical software Prism 7.0 (Software MacKiev© 1994–2016).

3. Results

3.1. Metabolic activity

Fermentation activity correlated with NH₄⁺ and SCFAs profiles (acetate, propionate and butyrate), as shown in Fig. 1. A reduction (p < 0.05) in the production of ammonia ions was observed in treatment studied with values below 10 ppm. Levels of acetic (14.76–12.42 mmol/L) and propionic acid (5.69–2.03 mmol/L) had a decrease during the treatment with probiotic infant cereal when compared to control. Therefore, butyrate levels significantly increased (p < 0.001) (0.89–1.70 mmol/L) in the ascending colon during treatment (see Fig. 2).
3.2. Survival of *Bifidobacterium animalis* subsp. *lactis* – BB12® under the simulated conditions of the stomach and duodenum in the SHIME®

A reduction in microbial counts of two logarithmic cycles was observed during the stomach simulation phase (Fig. 3), on what the pH was in the range of 2.5–2.9. After this decline, probably caused by the abrupt change in pH in the stomach phase, the strain remained stable ($p < 0.01$) in the duodenal phase.

3.3. Changes in the gut microbiota during the treatment with probiotic infant cereal

The identification of the microbiota in each experimental period was analyzed by next-generation sequencing. A total of 29,863 operational taxonomic units (OTU) were identified during control period and four main phyla were identified: Bacteroidetes (64.07%), Firmicutes (16.30%), Actinobacteria (12.77%) and Proteobacteria (6.86%) (Fig. 4). The treatment promoted an increase in OTU (42,505) and four main phyla were identified: Firmicutes (86.58%), Bacteroidetes (13.32%), Actinobacteria (0.001%) and Proteobacteria (0.09%). The treatment with probiotic infant cereal showed high richness (average Chao 1 of 14) but low diversity (average Shannon of 0.40) compared to controls (averages of 12 and 1.0 for Chao1 and Shannon, respectively).

High relative abundance of *Lactobacillaceae* (36.85%) to Firmicutes phylum and *Enterobacteriaceae* (28.61%) and Enterobacterales (14.31%) to Proteobacteria phylum were found during the control period. An increase in the relative abundance of *Lactobacillaceae* (36.85% to 85.63) and decrease of *Enterobacteriaceae* (28.61–0.047%) were observed during the treatment with probiotic infant cereal (Fig. 5). The main species of *Lactobacillaceae* stimulated during treatment were: *Lactobacillus gassert* (0.96–3.33%), *Lentilactobacillus kefiri* (*Lactobacillus kefiri*) (0.003–0.11%) and *Ligilactobacillus salivarius* (*Lactobacillus salivarius*) (0.23–0.74%) (Fig. 6).

Additionally, spearman correlation was used to associate the differentially abundant taxa with SCFAs at the family level. The relative abundance of *Lactobacillaceae* ($p = 0.001$) had a strong positive correlation with production of butyric acid (Fig. 7).

4. Discussion

In this study, the effect of the consumption of a infant cereal with probiotic (BB-12®) on infants microbiota was evaluated using a SHIME® model. To the best of our knowledge, this is the first study evaluating the benefits of the consumption of a probiotic in an infant cereal matrix.

A significant decrease in ammonia ions ($\text{NH}_4^+$) was observed throughout the treatment in relation to the control. $\text{NH}_4^+$ is one of the most important sources of nitrogen, it is obtained through protein breakdown and/or amino acid metabolism and is also produced by intestinal bacteria (Davila et al., 2013). Then, ammonia is transported through the portal circulation to periportal hepatocytes where 90% of...
the ammonia enters the urea cycle and is converted into urea. Yet, ammonia is not excreted, due to its low water solubility, the remaining 10% is transported to perennial hepatocytes where the ammonia is condensed with glutamate to glutamine through glutamine synthetase (GS) (Savy et al., 2018). According to Scott et al. (2013), the release of ammonia ions may be associated with increased metabolic activity of some species of *Bifidobacterium*, among others, that participate in the deamination processes. The reduction of ammonia in the colon is considered beneficial, because these ions in high quantity can alter the morphology, beyond the metabolism of the intestinal cells, increasing the synthesis of DNA and promoting the tumoral development (Davila et al., 2013). Hughes, Kurth, McGilligan, McGlynn, and Rowland (2008) demonstrated that ammonia can increase the cellular permeability in the colonocytes, causing several diseases in the host. The presence of ammonia can reduce the absorption and use of short chain fatty acids (SCFAs) by the colonocytes (He et al., 2019). Furthermore, the blood level of ammonia should remain very low because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system. The peak ammonia level and duration of hyperammonemia are the main risk factors for neurological deficits related to hyperammonemia and death. In children, hyperammonemia is mainly caused by severe liver failure and congenital metabolism errors (Mohiuddin & Khattar, 2020).

According to Cinquin, Le Blay, Fliss, and Lacroix (2006) the SCFAs proportion (acetate:propionate:butyrate) observed in infants feces (ratio close to 75:19:6) is different than those observed previously in adults feces (ratio close to 60:20:20) (Bridgman et al., 2017). Interestingly, the SCFAs proportion observed in the colon vessels in control period was

Fig. 5. Relative abundance of bacterial family (%) in the microbiota of children during all experiments on the SHIME® model. Control period; Treatment.
Butyrate has beneficial effects on human intestinal homeostasis (Canani et al., 2011; Macia et al., 2015), host immunological functions (Kim et al., 2016) and is considered a resource compound with promoting effects on various neurological and neuropsychiatric disorders, besides being the main source of energy for colonocytes and being involved in maintaining colonic health (Bourassa, Alim, Bultman, 2014). An excess of propionate and an inability for it to convert to methylmalonyl-CoA through propionyl-CoA causes propionic acidemia (Hosseini, Grootaert, Verstraete, & Van de Wiele, 2011). However, beneficial effects were attributed to propionic, such as; decreased serum cholesterol levels (Illman et al., 1988), upregulation of GLP-1 (Zhou et al., 2008), greater feeling of fullness, less hunger and reduced desire to eat (Roujolop, Boelrijk, & te Giffel, 2008).

In the stomach reactor, there was a decline in the viability of B. animalis - BB12®. However, this viability remained constant in the duodenum reactor. The presence of foods and food ingredients such as dietary fiber have a protective effect on the viability of microorganisms during their passage through the stomach and duodenum (Sendra, Sáez-Barberá, Fernández-López, & Pérez-Alvarez, 2016). The infant cereal product reached the colon with $10^5$ CFU log/g. According to Hill et al. (2014), the recommended use of the term probiotic in products is when living microorganisms provide a minimum viable and sufficient count of well-defined strains and evaluated with a reasonable expectation of providing benefits for the well-being of the host (ANVISA, Brazil, 2018). Survival capacity in aggressive GI conditions, overcoming the presence of a mixed solution with bile salts and other digestive factors, is crucial for host colonization by probiotics (Koskenniemi et al., 2011). Therefore, the infant cereal with probiotic proved to be a good matrix for carrying B. animalis - BB12®. In addition, bifidobacterial strains are frequently used as probiotic microorganisms and thus incorporated in several fermented products (Meira et al., 2015; Taverniti, Scabiosi, Arioli, Mora, & Guglielmetti, 2014; Verruck, Prudencio, Vieira, Amante, & de Mello Castanho Amboni, 2015). The administration of an infant cereal with probiotic caused a positive change in the pattern of relative abundance percentage of microbial family during the different experimental periods. Comparing with control period, a high prevalence of Lactobacillaceae was observed after administration of the infant cereal with probiotic. It is important to highlight that the treatment and ascending colon conditions can favor the growth of saccharolytic bacteria such as Lactobacillaceae (Liu et al., 2018). Lactobacillaceae family showed a positive strong correlation with butyric acid production. Increasingly, whole grain is becoming one of the choices as a carrier for probiotics. This is mainly because the formulation of probiotics with cereals products offers consumers benefits from both probiotics and cereal. Thus, the use of cereals in formulations increases the dietary value of the product, as well as being used as fermentable substrates for intestinal bacteria (Lamsal & Faubion, 2009; Pereira et al., 2019). Interestingly, the probiotic infant cereal treatment stimulated the increase of L. gasseri, which is involved in the prevention and treatment of children with asthma and allergic rhinitis (Chen et al., 2010). The...
study conducted by Zubiria et al. (2017), showed that the administration of L. gasseri SB2055 to mice fed a 10% fat diet resulted in reduced expression of pro-inflammatory genes such as CCL2 and CCR2 in fat tissue, preventing body weight gain and fat accumulation. An increase in L. kefiri and L. salivarius were also observed after the probiotic infant cereal treatment. Recently, L. kefiri was associated with obesity prevention and cholesterol control, and the mechanisms involved are the direct reduction of excessive lumen cholesterol to decrease the influx of fat and the positive regulation of genes coding for PPAR-α, CPT1 and FABP4 in fat tissue to increase the oxidation of fatty acids, moreover, L. kefiri is also associated with obesity and anti-inflammatory effects (Kim et al., 2017). Supplementation with L. salivarius was associated with improvement of the quality of life of children affected by atopic dermatitis (Niccoli et al., 2014). It is important to highlight that atopic dermatitis is a chronic inflammatory skin disease with multifactorial etiopathology, which is most prevalent in childhood and appears in the first 5 years of life, with about 60% of cases appearing between 0 and 6 months (Monti et al., 2011).

However, some limitations were also observed, such as the duration of the study (7 days), the in vitro study and the lack of previous studies demonstrating the biological effects between the infant cereal and microbiota. Despite the limitations, we could conclude that the dynamic view of microbiota and microbial metabolites demonstrated by treatment with infant cereals with Bifidobacterium animalis ssp. lactis – BB12® opened new perspectives on the interaction of probiotics with microbiota and metabolites. The results indicate that the infant cereal with probiotic has a positive impact on the microbiota with a decrease in the production of ammonia ions, increase in butyric acid, and stimulates the growth of L. kefiri, L. gasseri and L. salivarius with potential benefits to children and promoting a healthy microbiota. Finally, a clinical trial is essential to confirm the results found in the dynamic colonic reactor (SHIME®).

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'Natália Partis Perina, Thaís Moreno Tomé, Elaine Mosquera and Tamara Lazarini are employees of Nestlé Brazil, that financially supported the study and manufactures the infant cereal used in this study'.

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INTRODUCTION:


