EFFECT OF PROBIOTIC, PREBIOTIC AND SYNBIOtic ON COLON AND CECUM MICROBIOTA OF RATS

Celia L. L. Ferreira¹, Elisa Teshima² and Neuza M. B. Costa³

¹Department of Food Technology and ³Department of Nutrition and Health, Federal University of Viçosa, 36570.000 Viçosa - Minas Gerais., ² State University of Bahia, Feira de Santana, 44031-580 - Bahia, Brazil.

[Received November 1, 2007; Accepted December 11, 2007]

ABSTRACT: Prebiotics, probiotics and synbiotics have been considered alternatives for the maintenance of a healthy gut. A large number of data exists corroborating beneficial effects upon consumption of these functional food ingredients but experimental works evaluating the three approaches in the same study are rare. In this work we evaluated the implications on the increase of bifidobacteria and decrease of E. coli, as well as the modulation of some groups of the rat native microbiota upon administration of newly isolated human Bifidobacterium breve strains (UFVCC 1087-1100), a fructooligosaccharide (FOS) Meioligo®, and a synbiotic containing the bifidobacteria and FOS. A 28-day experiment included forty female adult Wistar rats, randomly assigned to one of the four groups (control, prebiotic, probiotic, synbiotic). The three treatments increased bifidobacteria counts in cecum and colon (P<0.01). However, the synbiotic treatment was superior in bifidobacteria counts (P<0.01). Escherichia coli numbers were lower (P<0.01) in both segments of prebiotic and synbiotic groups. The weight index of the animals in all groups were the same (P>0.05), indicating no splenomegaly or hepatomegaly. The data strongly indicated that consumption of Meioligo® and Bifidobacterium breve (UFVCC 1087-1100), modulates favourably the bifidobacteria levels (P<0.01) and reduce E. coli numbers in the gut of the rat.

KEYWORDS: Bifidobacteria, Intestinal microbiota, Modulation, Prebiotic, Probiotic, Synbiotic

INRODUCTION
The human large intestine comprises a complex microbiota where more than one hundred species both beneficial and potentially deleterious bacteria are maintained in a balance (Mitsuoka, 1990). These nutritionally and physiologically diverse ranges of bacteria promote normal intestinal function, offering the host protection against infections (Salminen et al., 1998). Disruption of the colonic microbiota, due to pathogens, (Gorbach et al., 1987; Gill, 2003), dietary antigens (Salminen et al., 1998), or other harmful substances (Ouwehand et al., 2003) can, however, lead to intestinal dysfunction. Whilst some indigenous bacteria can be pathogenic such as proteolytic clostridia and bacteroides, others may offer health-promoting attributes. Native genera such as Bifidobacterium and Lactobacilli exert powerful anti-pathogenic activities and are mainly responsible for “colonization resistance” in the gut (Gibson, 2004). Their significance as a natural means to resist possible pathogenic microorganisms was identified by Metchnikoff during research on cholera in the 19th century and in early 20th century while observing the Bulgarian peasants and their habit to consume large amounts of a soured milk. This soured beverage rich in lactic acid bacteria (LAB) provided the intestines with natural protection against putrefactive microorganisms, responsible for accumulation of toxic compounds and onset of diseases (Metchnikoff, 1908). He hypothesized, for the first time, that longevity was related to a healthy intestine and highlighted the importance of the lactobacilli group. One century later, the importance of a well-balanced gut microbial ecosystem is becoming more widely recognized for the host health. Currently, it is well established, that some medical conditions such irritable bowel syndrome (IBS) and recurrent Clostridium (C) difficile colites lack effective and safe approaches for treatment and prevention (Sanders and Gibson, 2006). In this context, the health benefits and efficacy of probiotics (live selected microorganisms originated from human intestines), prebiotics (non digestible food ingredients selective for beneficial indigenous microorganisms stimulating the growth and/or their activity) and synbiotics (products containing both, pre and probiotic) are in the present time, attracting much attention and broad range research including immune function, mineral bioavailability, IBS, diarrhoea of different origins, and gut performance (Gibson...
by the Ethical Committee of the UFV and the animals were assigned to one of four treatments: 1) control diet; 2) control diet + concentrated bifidobacteria (probiotic group - PRO); 3) control diet + 5% of FOS (Meioligo®, Meiji Seika Kaisha, Tokyo, Japan) (prebiotic group - PRE); or 4) control diet + concentrated bifidobacteria + 5% of FOS (Meioligo®, Japan) (synbiotic group - SYN). Animals were individually housed in suspended wire-mesh-bottom cages in an environmentally controlled room at 25°C, with a 12 hour light-dark cycle. Animals in each group were given distilled water ad libitum and AIN-93G diet for 28 days (Reeves et al. 1993). The animals from the PRO and SYN treatments received an oral dose of 0.1mL of the concentrated bifidobacteria (10^{10} UFC mL^{-1}) daily. The control animals, received 0.1mL oral doses of distilled water daily, to maintain the same stress level among the groups.

**Sampling procedure**

After 28 days of treatment, the animals were sacrificed in a CO₂ chamber. A ventral midline incision was made and the heart, liver, kidney, spleen, cecum and colon were excised. Immediately after removal, the organs were weighted. Cecal and proximal colonic contents were collected and 1g was diluted in peptonized reduced water (peptone 1g; NaCl 8g; tween 80 1g; cystein, 0.5g; glycerol 10g; water 1000mL). The reagents and ingredients were purchased from Sigma Aldrich (Steinheim, Germany), unless otherwise stated. The diluted samples were frozen immediately in liquid nitrogen and stored at -80°C. The remaining cecal contents were diluted in distilled water and pH was measured.

**Bacterial enumeration**

Cecal and colon diluted samples were thawed at room temperature, serially 10-fold diluted in peptonized reduced water, and 100 μL of each dilution plated on appropriate agars. *Bifidobacterium* ssp. were enumerated on RB agar (Hartemink et al., 1996); *Lactobacillus* ssp. on LAMVAB agar (Hartemink et al., 1997b); total anaerobes (TAN) on brain heart infusion (BHI – Difco Laboratories, Detroit, MI, USA) agar plus 5% (v/v) of sheep blood; total aerobes on BHI agar and *E. coli* on MacConkey sorbitol agar (Difco Laboratories). The plates for total anaerobes and bifidobacteria counts were incubated at 37°C in anaerobic jar (Gas Pak system; BBL, Sparks, USA), for 48 and 72 h, respectively. Total aerobes, lactobacilli and *E. coli* plates were incubated at 37°C for 24, 48 and 24 h, respectively. Results of colony counts were expressed as Log_{10} CFU.g^{-1} of wet weight.

**Organ weight indexes**

The heart, kidney, spleen and liver weight indexes were derived from the formula: organ weight (mg). g^{-1} of rat body weight, according to Zhou et al. (2000).

**Data Analysis**

Data were analyzed by ANOVA for a randomized block design. When significant (P<0.05) differences were detected,
individual means were compared by Tukey test.

RESULTS
All the animals were healthy and alive until the day on which they were sacrificed. The bacterial concentration in colon (CO) and cecum (CE) of animals is shown in Table 1. Total aerobes (TA) Log10 counts in both CO and CE follow the same trend. Animals from the control [CO (8.44 ± 0.23); CE (8.04 ± 0.64)] and PRO [CO (8.44 ± 0.23); CE (8.04 ± 0.64)] groups were higher (P<0.01) than PRE [CO (6.65± 0.35); CE (5.98 ± 0.40)] and SYN [CO (7.20 ± 0.68); CE (6.58 ± 0.65)] groups. In CO, total anaerobes (TAN) Log10 counts were not affected (P>0.05) by the diets whereas in the CE, TAN Log10 counts was lower (P<0.01) in PRE (9.05 ± 0.10) and higher (P<0.01) in SYN (10.45 ± 0.26) when compared to the control (9.72 ± 0.50) or PRO (9.58 ± 0.23) groups. Higher counts (P<0.01) of bifidobacteria in CO and CE were observed in the animals from the three treatments, comparing to the control. The highest (P<0.01) Log10 counts of bifidobacteria was found in the SYN group [CO (9.14± 0.06); CE (9.14± 0.06)] but were similar (P>0.05) in the animals from the PRO [CO (8.72± 0.33); CE (8.85± 0.33)] and PRE [CO (9.05± 0.10); CE (8.65± 0.10)] groups. The Log10 counts in the animals from the PRO [CO (6.38 ± 0.86) and CE (5.28 ± 0.76)] were numerically lower than the control [CO (7.31 ± 1.67); CE (6.86± 1.70)] but did not differ (P>0.05).

As shown in table 2, pH values of the cecal contents of rats in the control (7.24±0.32) were higher (P < 0.05) than the pH values of the PRO and SYN groups (6.85 ± 0.32 and 6.80 ± 0.30, respectively). In addition, the cecal weights (g.100g) of the animals from the groups PRE (2.89 ± 0.18) and SYN (2.88 ± 0.39) were higher (P<0.01) than animals from PRO (2.14 ±0.14) and control (2.10 ± 0.20). In the four groups, the weight index of the organs of the animals (table 3) did not differ (P>0.05).

### TABLE 1. Bacterial levels in caecum and colon content of rats fed some dietary adjuncts

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Control</th>
<th>Prebiotic</th>
<th>Probiotic</th>
<th>Synbiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO (Total anaerobes)</td>
<td>9.72 ± 0.50b</td>
<td>9.05± 0.10c</td>
<td>9.58±0.23b</td>
<td>10.45±0.26a</td>
</tr>
<tr>
<td>CE (Bifidobacteria)</td>
<td>7.06 ± 0.13d</td>
<td>8.72 ± 0.23d</td>
<td>8.49 ± 0.39b</td>
<td>9.14± 0.06a</td>
</tr>
<tr>
<td>CO (Total anaerobes)</td>
<td>8.04 ± 0.64c</td>
<td>5.98 ± 0.40b</td>
<td>8.27 ± 0.50c</td>
<td>6.58 ± 0.65b</td>
</tr>
<tr>
<td>CE (Lactobacilli)</td>
<td>6.28 ± 0.73d</td>
<td>4.39 ± 0.60b</td>
<td>6.48 ± 0.48c</td>
<td>5.19± 0.56b</td>
</tr>
<tr>
<td>E. coli</td>
<td>6.86 ± 1.70a</td>
<td>4.11±0.72b</td>
<td>5.28 ± 0.76ab</td>
<td>4.00 ± 1.39b</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 10 rats.
2 Wet weight content
3 Not significant (P>0.05).
4 Means in the same row not sharing superscript letters, differ (P<0.01).

### TABLE 2. Cecal weight and cecal pH of rats (n=10) tested with control, prebiotic, probiotic, and synbiotic diets for 28 days

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>Control</th>
<th>Prebiotic</th>
<th>Probiotic</th>
<th>Synbiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caecal weight, g.100g</td>
<td>2.10 ± 0.20b</td>
<td>2.89 ± 0.18b</td>
<td>2.14 ± 0.14b</td>
<td>2.88 ± 0.39b</td>
</tr>
<tr>
<td>Caecal pH</td>
<td>7.24 ± 0.32d</td>
<td>6.96 ± 0.27ab</td>
<td>6.85 ± 0.32ab</td>
<td>6.80 ± 0.30b</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 10 rats. * (P<0.05); ** (P<0,01)
2 Means in the same row not sharing superscript letters, differ.

### TABLE 3. Organ weight indexes of rats tested with control, probiotic, prebiotic and synbiotics diets

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>Control</th>
<th>Prebiotic</th>
<th>Probiotic</th>
<th>Synbiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>17.22 ± 1.82</td>
<td>17.68 ± 0.99</td>
<td>16.88 ± 1.09</td>
<td>17.73± 0.68</td>
</tr>
<tr>
<td>Liver</td>
<td>42.89 ± 3.62</td>
<td>43.41 ± 2.16</td>
<td>44.38 ± 2.95</td>
<td>45.69 ± 2.22</td>
</tr>
<tr>
<td>Heart</td>
<td>19.07 ± 1.29</td>
<td>18.94 ± 1.00</td>
<td>18.85 ± 1.31</td>
<td>18.86 ± 0.70</td>
</tr>
<tr>
<td>Kidney</td>
<td>21.34 ± 1.29</td>
<td>22.40 ± 0.78</td>
<td>21.37 ± 1.04</td>
<td>22.38 ± 1.40</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 10 rats.
2 Not significant (P>0.05).
DISCUSSION
This study provided evidence in a murine model that, consumption of a probiotic “pool” of Bifidobacterium breve (UFVCC 1087 to 1103) originated from the faeces of breast fed children, probiotic FOS (Meioligo®), and of a symbiotic containing both probiotic organisms and FOS, modulated the Log_{10} counts of TA, TAN, lactobacilli, bifidobacteria and E. coli in the cecum, whereas in the colon, TAN counts were similar in all treatments. The successful proliferation of bifidobacteria observed in the CE and CO of the PRE and SYN animals seems to be attributable to the availability of FOS which is not digested in the rat small intestine (Gibson and Roberfroid, 1995) and directly reaches the large intestine as carbon source for the endogenous and/ or exogenous species. The data also strongly indicated that the PRE and SYN consumption were more effective in antagonizing E. coli. The SYN diet increased also the numbers of TAN in the cecum, with a concomitant lowering in TA, indicating a shift towards anaerobic bacterial species in the large intestine. An interesting observation was that the TAN levels in this group was maintained, while the bifidobacteria concentration increased, suggesting a dominance of Bifidobacterium in detriment to another anaerobic genera. Likewise, studies indicated that under consumption of prebiotic, indigenous murine strains such as from Bifidobacterium animalis could increase up to 20% the microbiota of cecum (Dinoto et al., 2006). Similar modulation was found by Gibson et al. (1995) in a human evaluation where the subjects received 15 g of FOS daily. The authors reported an increase in the levels of Bifidobacterium in detriment to another anaerobic genera e.g. Bacteroides, Fusobacterium and Clostridium.

The higher Bifidobacterium spp. population could have increased the production of acetate and lactate, decreasing the intestinal pH and consequently inhibiting the growth of potentially pathogenic and putrefactive bacteria (Gibson and Roberfroid, 1995). In our study, populations of E. coli in CE and CO were reduced in the PRE, PRO and SYN groups. The data also indicated that the E. coli reduction could not have been related only to a decrease in the pH, since the pH in CE of both, PRO and SYN groups had the same value, but a more pronounced antagonism was seen in the SYN group. A factor probably related to the stimulation of the endogenous bifidobacteria should have been involved in the antagonism observed. Thus, the stimulated endogenous strains of bifidobacteria, more adapted to the gut environment could have inferred more pronounced inhibition when compared to the PRO animals that received only the exogenous bifidobacteria. Convincing results published by Ballongue et al. (1993), indicated that non human strains of bifidobacteria administered to volunteers could not alter the gut levels of Clostridium, Bacteroides and E. coli, while in the volunteers receiving strains of human origin, the reduction of these microbial species decreased significantly, suggesting specificity as an important condition for the inhibition to E. coli. In the present work, although less pronounced, the exogenous probiotic strains also exerted inhibition to E. coli. However, the mechanisms by which PRE, PRO and SYN inhibit pathogens, are not completely understood at the present time.

Although it has not been the scope of the present work to address the mechanisms by which E. coli has been inhibited, data have been accumulated corroborating the role of prebiotic ingredients and probiotic bacteria involved in the beneficial effects observed. In this context it is well established that probiotic bacteria had the ability to inhibit adhesion of E. coli and Salmonella enterica serotype Typhimurium to epithelial cells in vitro (Gill, 2003). Furthermore, many probiotic strains can produce one or more antimicrobial substances in vitro, including hydrogen peroxide, organic acids such as acetate and lactate, diacetyl, bacteriocins or bacteriocin-like molecules (Ouwehand et al., 2003). Competitive binding to receptors or stimulation of host factors such as mucin production have also been indicated as involved in the mechanism of inhibition exerted by PRE, PRO and SYN (Mack et al., 1999; Lee and Puong, 2002). The antimicrobial effects in the PRE are induced principally via their selective stimulation of indigenous beneficial strains, which secrete antimicrobial compounds, modulate immune function and inhibit pathogens. Similarly, prebiotic oligosaccharides may block common receptor sites for gut pathogens, through their presence in the lumen. Probably more than one of the described conditions was involved in the inhibition to E. coli observed in the present experimentation. The beneficial effects of these functional ingredients hardly can be explained by a unifying hypothesis that is based on a single variable or mechanism and remains valid to suggest a combination of factors as responsible for what is observed.

Bifidobacteria usually have the GRAS status, however, the strains evaluated in this study were newly isolated, and as such, have to be submitted to the safety parameters described in the guidelines of FAO/WHO (2002), where infectivity is listed as important component in safety studies on probiotic bacteria. Splenomegaly and hepatomegaly are indirect indicators of infection (Zhou et al., 2000a) and in the present study it was not found any macroscopic changes in the spleen or in the liver’s morphology of the animals treated with PRO, PRE and SYN. Zhou et al. (2000b) had also documented a similar result in spleen weight index of mice fed Bifidobacterium lactis. The safety of the newly isolated human strains of B. breve and of the FOS tested in this study was evidenced through the lack of splenomegal, hepatomegal as well as with the similarity of the weight index of the other organs evaluated.

CONCLUSION
The Bifidobacterium breve strains (UFVCC 1087 to 1100) and the prebiotic (Meioligo®), fed to rats in the form of PRO, PRE and SYN were able to beneficially modulate the gut microbiota translated mainly as an increase in the bifidobacteria counts and a decrease in E. coli. The stimulus exerted by the Meioligo® present in the PRE and SYN groups was more effective than that exerted by the exogenous bacteria (human origin), which emphasizes the importance of strain specificity when designing probiotic products. The strains and FOS evaluated in this study were safe for no splenomegal and hepatomegal was detected indicating that the testing FOS and bifidobacteria strains did not alter the health status of the host. It could be suggested that the consumption of PRE and SYN herein evaluated could beneficially affect the
gastrointestinal microbiota and the most effective strategy to increase bifidobacteria levels and antagonize E. coli in the low intestinal tract is through the consumption of the SYN, PRE and PRO, in that order. Studies of this nature constitute a first step in the guidance of the industry in the process of food development for a healthy gut. It is worth noting however that, the beneficial effect of these functional ingredients changes with the food matrix. Therefore, the strains and the FOS studied, should also be evaluated in the final product carrier.

ACKNOWLEDGEMENT
The Fundação de Amparo à pesquisa do Estado de Minas Gerais (FAPEMIG), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Brazil financially supported this project.

CONFLICT OF INTEREST STATEMENT
We, the authors, do not have financial interest in the present manuscript.

REFERENCES


