Food and Agricultural Immunology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713422299

Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model

M.E. Bibas Bonet[a]; O. Meson[a]; A. de Moreno de LeBlanc[b]; C. A. Dogi[b]; S. Chaves[b]; A. Kortsarz[c]; A. Grau[c]; G. Perdigón[ab]
[a] Facultad de Bioquimíca, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina [b] Centro de Referencia para Lactobacilos (CERELA-CONICET), San Miguel de Tucumán, Argentina [c] Laboratorio de Investigaciones Ecológicas de las Yungas (LIEY), Facultad de Ciencia Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina

Online publication date: 07 May 2010

To cite this Article Bibas Bonet, M.E. , Meson, O. , de Moreno de LeBlanc, A. , Dogi, C. A. , Chaves, S. , Kortsarz, A. , Grau, A. and Perdigón, G.(2010) 'Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model', Food and Agricultural Immunology, 21: 2, 175 — 189
To link to this Article: DOI: 10.1080/09540100903563589
URL: http://dx.doi.org/10.1080/09540100903563589

Food and Agricultural Immunology
Vol. 21, No. 2, June 2010, 175–189

Taylor & Francis
Taylor & Francis Group

Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model

M.E. Bibas Bonet[a], O. Meson[a], A. de Moreno de LeBlanc[b], C.A. Dogi[b], S. Chaves[b], A. Kortsarz[c], A. Grau[c] and G. Perdigón[a,b]*

[a]*Facultad de Bioquimíca, Química y Farmacia, Universidad Nacional de Tucumán, 4000 San Miguel de Tucumán, Argentina;* [b]*Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, T4000ILC San Miguel de Tucumán, Argentina;* [c]*Laboratorio de Investigaciones Ecológicas de las Yungas (LIEY), Facultad de Ciencia Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, C.C. 34, 4107-Yerba Buena, Tucumán, Argentina*

(*Received 21 May 2009; final version received 16 December 2009*)

Prebiotics are non-digestible but fermentable oligosaccharides that can influence the composition and the activity of some intestinal bacteria to promote the health of the host. *Smallanthus sonchifolius* (yacon) contains beta-1,2-oligofructans as the main saccharides and its roots are consumed in South American countries. The aim of the study was to evaluate the prebiotic property of yacon root flour. Its influence on the intestinal microbiota and gut immune system were evaluated using a mice model. The results show the prebiotic effects of yacon root flour, stimulating the growth of bifidobacteria and lactobacilli and the intestinal immune system with increases in IgA and different cytokines. Cells from the innate response were mainly involved in the effect of yacon root flour. T cells were also activated and able to induce IL-10 and IFNγ production. The long term administration of yacon root flour maintained the intestinal homeostasis without inflammatory effect regulated mainly through IL-10 and IL-4 regulatory cytokines.

Keywords: intestinal microbiota; intestinal immunity; prebiotic; yacon

Introduction

In the last decades, there has been an increasing interest in the relation between colonic function and health (Burkitt & Trowell, 1975). It has been suggested that large amounts of unrefined plant foods, especially all starchy foods rich in dietary fibre, may offer protection against several intestinal diseases.

At first, dietary fibres were defined as indigestible plant cell wall materials that are predominantly carbohydrate in nature but include lignin. Later, the definition included all non-absorbable carbohydrates of plant origin (Trowell et al., 1976).

Dietary carbohydrates that escape digestion in the small intestine undergo bacterial fermentation in the colon. This process affects the microbial ecology of the gastrointestinal tract and influences gut metabolism and function. Prebiotics are non-digestible but fermentable oligosaccharides that are specifically designed to

*Corresponding author. Email: perdigon@cerela.org.ar

ISSN 0954-0105 print/ISSN 1465-3443 online

DOI: 10.1080/09540100903563589
http://www.informaworld.com

change the composition and on the activity of one or a limited number of bacteria of the intestinal microbiota with the prospect to promote the health of the host (Gibson & Roberfroid, 1995). Prebiotics specifically stimulate the growth of endogenous microbial population groups such as bifidobacteria and lactobacilli which are perceived as being beneficial to human health (Van Loo, 2004).

Dietary fibre and non-digestible oligosaccharides (NDO) are the main growth substrates of gut micro-organisms. Their fermentation results in the acidification of the colonic contents and the formation of short-chain fatty acid (sCFA) which act in different tissues and may play a role in the regulation of cellular processes.

Most research in the field of prebiotics has been done on inulin, fructooligosaccharides (FOS) and other NDOs including xylooligosaccharides, galactooligosaccharides and isomaltooligosaccharides. To serve as a bacterial substrate in the colon, a prebiotic must not be hydrolysed or absorbed in the upper part of the gastrointestinal tract (Schumann, 2002).

Smallanthus sonchifolius (yacon), originating from South America, has become popular in Japan and in New Zealand for its tubers which contain beta-1,2-oligofructans as the main saccharides. The plant is also successfully cultivated in Central Europe, in the Czech Republic in particular (Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). Yacon, as with many roots originating from the Andean region of South America, is used by the local inhabitants as sources of food energy. Traditionally, yacon roots and infusions from dried leaves were consumed by people suffering from diabetes or from various digestive disorders in South American countries such as Brazil. The percentage of FOS in yacon is 70–80% of its dry weight. Thus, yacon could be a potential prebiotic and exerts an effect on the intestinal ecosystem.

The aim of the present study was to evaluate the prebiotic property of yacon, and its influence after long-term daily consumption on the intestinal microbiota and gut immune system in mice.

Materials and methods

Purification of yacon root flour and analysis of the carbohydrate content

Yacon (*S. sonchifolius*) roots were obtained from plants (LIEY 97-1 clon) seeded during the 2002–2003 cycle in Sauce Huascho, Famaillá (Tucumán, Argentina) in a parcel of approximately 0.7 hectares. This territory is located at 451 m above sea level (26°59′ south and 65°27′ west). The roots were reaped in July after 276 days of sowing. The procedure to obtain the flour was the following: roots were peeled, cut in small pieces and introduced in a natural gas furnace with ventilation at 60°C during 24–48 h. The dry pieces were taken for milling in a hammer mill and the flour obtained after this procedure was used to analyse the carbohydrate content by HPLC, using a column Rezex RSO oligosaccharide Ag^{++}.

Animals and feeding procedure

Six-week-old BALB/c mice weighing 25–28 g were obtained from the random-bred colony maintained at the Institute of Microbiology (Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán) and divided into two

groups (test and control group). They were maintained in a room with a 12-h light/dark cycle at $18 \pm 2^{\circ}C$.

Mice from the test group (yacon group) were fed a solid conventional diet supplemented with 340 mg/kg day^{-1} of a flour obtained from the yacon roots during 75 consecutive days and water ad libitum. Control group received conventional diet and water in the same way as test group.

Haematological studies

In order to determine the effect of oral administration of yacon on the haematological response, mice from both control and test group were sacrificed at 15, 30, 45, 60 and 75 days and the blood was obtained by cardiac puncture. The number of erythrocytes and leukocytes was examined using the haemocytometric method. Smears stained with Giemsa solution were examined for relative values.

Histological studies and determinations of corporal, spleen and liver weights

Mice were sacrificed by cervical dislocation at the beginning of the experiment (day 0 or basal data), previous to the prebiotic administration and each 15 days until 75 days. Control mice, without special feeding were sacrificed at the same time points. Five mice per assay and period of time were used. The weights of the mice and their spleen and liver were determined. The small and large intestines were removed and washed with a saline solution (0.85% NaCl). Tissues were prepared for histological evaluation using the method described by Sainte-Marie (1962). Serial paraffin sections of 4 μm were made. Sections from the test and control groups were stained with haematoxylin–eosin for light microscopy examination. Histological scoring was based on a semi-quantitative scoring system proposed by Ameho et al. (1997) in which the following features were graded as follows: histological findings identical to normal mice (Score 0). Mild mucosal and/or submucosal inflammatory infiltrate (admixture of neutrophils) and oedema. Punctate mucosal erosions often associated with capillary proliferation. Muscularis mucosae intact (Score 1). Grade 1 changes involving 50% of the specimen (Score 2). Prominent inflammatory infiltrate and oedema (neutrophils usually predominating) frequently with deeper areas of ulceration extending through the muscularis mucosae into the submucosa. Rare inflammatory cells invading the muscularis propriae but without muscle necrosis (Score 3). Grade 3 changes involving 50% of the specimen (Score 4). Extensive ulceration with coagulative necrosis bordered inferiorly by numerous neutrophils and lesser number of mononuclear cells. Necrosis extends deeply into the muscularis propria (Score 5). Grade 5 changes involving 50% of the specimen (Score 6).

In order to study the mast cells associated to the gut lymphoid tissue, the histological sample was stained with toluidine blue in acetate–acetic acid buffer at pH 4.4.

Immunofluorescence assay for IgA-secreting cells, CD4+ and CD8+ T lymphocytes

The number of IgA+ cells, CD4+ and CD8+ T lymphocytes were determined by direct immunofluorescence assays using the tissue samples as explained above.

In order to study IgA+ cells, slides were incubated with α-chain monospecific antibody conjugated with fluorescein isothiocyanate (FITC, Sigma, St. Louis, USA). For CD4+ and CD8+ lymphocytes determinations, monoclonal antibodies conjugated with FITC were used (Cedarlane, Ottawa, Canada). The results are expressed as number of fluorescent cells counted in 10 fields of vision (1000 × of magnification).

Secretory IgA (s-IgA) in intestinal fluid

Intestinal fluid was collected from the small intestines of mice in 1 ml of 0.85% NaCl, centrifuged at 5000 *g* for 15 min at 4°C, using a refrigerated centrifuge (Presvac, Buenos Aires, Argentina). The supernatant was recovered and stored at −20°C until IgA determination.

ELISA test was used to measure the concentration of total s-IgA following a technique described previously (de Moreno de LeBlanc et al., 2008). The results are expressed as a concentration of s-IgA (μg/ml) in the intestinal fluid.

Cytokine-producing cell determination in histological sections

Tissue sections were used for immunofluorescence assays. Cytokine positive cells were detected by indirect immunofluorescence following the technique described by de Moreno de LeBlanc and Perdigón (2004). Rabbit anti-mouse IFNγ, IL-10, IL-12 and IL-4 (Peprotech, Inc., Rocky Hill, NJ, USA) polyclonal antibodies were used as primary antibodies. The sections were then treated with a dilution of goat anti-rabbit antibody conjugated with FITC (Jackson Immuno Research Labs. Inc., West Grove, USA). The results are expressed as number of fluorescent cells counted in 10 fields of vision (1000 × of magnification).

Microbiology

At the same period of time assayed for the immunological studies, mice were sacrificed for the microbiological determinations, the yacon root flour administration was stopped at 60th day, and 30 days after (90 days) the microbiota was again evaluated. The large intestines were aseptically removed, weighed and placed into sterile tubes containing 5 ml of peptone water (0.1%). The samples were immediately homogenised under sterile conditions using a homogeniser (MSE, UK). Serial dilutions of the homogenised samples were obtained and aliquots (0.1 ml) of the appropriate dilution were spread onto the surface of following agarised media: reinforced clostridial (RCA, Britania, Buenos Aires, Argentina) for total anaerobic bacteria; RCA containing 0.2% LiCl, colistin 4 mg L^{-1}, 1% aniline blue and after sterilisation adjusted to pH 5 with acetic acid (RCA-pH5) for isolation of bifidobacteria; Mann-Rogosa-Sharp Agar (MRS Britania, Buenos Aires, Argentina) for total lactobacilli and Mac Conkey (Britania, Buenos Aires, Argentina) for Enterobacteriaceae. This last culture media was aerobically incubated at 37°C for 24 h, the others plates were anaerobically incubated at 37°C for 72–96 h.

Statistical analyses

The results for the haematological and histological determinations were expressed as the mean of n experiments $\pm$ standard error of the mean (SEM). The Student t-test was used to calculate the statistical significance of the results comparing control and test group in each time point. For the microbiology and immunological assays, statistical analysis were performed using MINITAB 14 software (Minitab, Inc., State College, PA) by ANOVA GLM followed by a Tukey's post-hoc test, and $P < 0.05$ was considered significant. All values ($n = 15$) were the means of three independent trials (no significant differences were observed between individual replicates) $\pm$ standard deviation.

Results

Analysis of sugar content from yacon root flour

It was observed that the procedure to obtain the flour from the yacon roots modified the content of carbohydrates compared to the natural content of the roots. Fructose and glucose contents increased (near 100%). The disaccharide sucrose increased and there were also modifications in the degree of polymerisation (DP) of the FOS. The DPs predominant in the flour were 3 and 4. In the flour there were also FOS with a DP larger than 8 that were not present in the roots (Figure 1).

Studies of the effects induced by the long-term administration of yacon root flour

Effects on body, spleen and liver weight on the peripheral blood cells and histological studies

There were no significant differences for body, spleen and liver weight between test and control animals (Table 1). Macroscopically, neither spleenomegaly nor

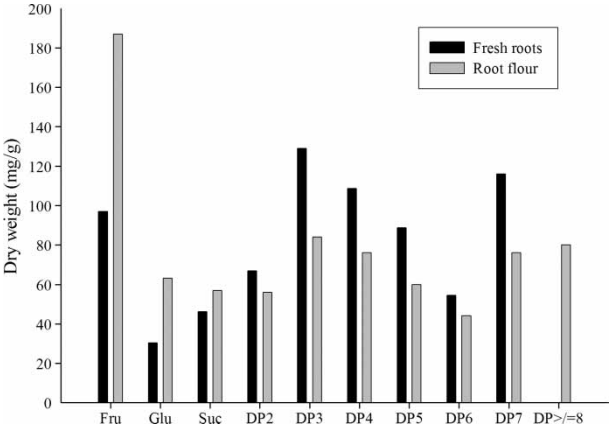

Figure 1. Sugar content of yacon root flour. The flour obtained from the roots was analysed for the carbohydrate content by HPLC. The results show the sugar content (expressed as dry weight) of yacon roots flour (grey bars) in comparison with the sugars in the fresh roots (black bars). Fru, fructose; Glu, glucose; Suc, sucrose; DP, degree of polymerisation.

Table 1. Effect of yacon administration on spleen, liver and body weight.

	Body weight (g)		Spleen weight (g)		Liver weight (g)	
	Control	Yacon	Control	Yacon	Control	Yacon
15	25 ± 3	24 ± 3	0.09 ± 0.01	0.08 ± 0.01	1.05 ± 0.03	1.00 ± 0.02
30	27 ± 3	25 ± 3	0.09 ± 0.01	0.09 ± 0.01	1.00 ± 0.02	1.00 ± 0.03
45	27 ± 2	26 ± 3	0.10 ± 0.01	0.09 ± 0.01	1.23 ± 0.05	1.20 ± 0.03
60	30 ± 3	29 ± 4	0.12 ± 0.01	0.11 ± 0.01	1.31 ± 0.02	1.33 ± 0.05
75	32 ± 4	31 ± 4	0.15 ± 0.01	0.14 ± 0.02	1.38 ± 0.01	1.39 ± 0.01

Note: mice from yacon group received orally the yacon root flour dissolved in water, once per day during 75 consecutive days. Each 15 days, five mice from yacon and control group were weighed and then sacrificed. The weight of their spleen and liver were also measured. Results are expressed as mean ± SD.

hepatomegaly was observed in animals that received yacon root flour. No influences in the peripheral blood cells were observed after yacon root flour administration. The values obtained for erythrocytes and leukocytes were similar for both test and control groups in all the time points studied ($4.9 \times 10^6 \pm 200$ erythrocytes/mm^3 and $5.2 \times 10^3 \pm 150$ leucocytes/mm^3). The percentage of different white blood cells was not modified by the yacon root flour administration compared to the control (24 ± 4% and 23 ± 4% for neutrophils, 70 ± 5% and 72 ± 3% for lymphocytes) both for control and test groups, respectively.

No side-effects such as inflammation were observed in response to long term administration of yacon root flour. The test group showed a slight cellular infiltration in the lamina propria of the small intestine without alteration in the histological structure of the villous in the slices stained with haematoxylin–eosin in relation with the control. The score was maintained near to 1 in all the animals analysed from this group (Figures 2A and B).

For mast cell staining, it was observed that yacon root flour administration increased the number of these cells in the lamina propria of the small intestine. This increase was observed in the mice from the test group in all the time points assayed (Figures 2C and D).

Effect of the oral administration of yacon root flour on the intestinal microbiota

Mice that received yacon root flour showed, in the first sample (15 days of administration), an increase in the bifidobacteria counts mean log CFU (7.7 ± 0.9) and in the lactobacilli counts mean log CFU (9.8 ± 0.3) compared to the control (3.1 ± 0.3 and 7.4 ± 0.2, for bifidobacteria and lactobacilli, respectively). After that, in the mice from test group, the count mean log for bifidobacteria diminished and reached a value less than two until the last sample (Figure 3B), similar to the control group (Figure 3A). Regarding to lactobacilli, they were maintained significantly increased in mice that received yacon root flour compared to the control (Figures 3A and B). When the prebiotic administration was stopped, this bacterial population did not show significant differences with the mice that had never received the special diet. The yacon root flour administration significantly diminished the enterobacteria counts mean log CFU at 60 days (4.2 ± 0.6) compared to the control (5.5 ± 0.6).

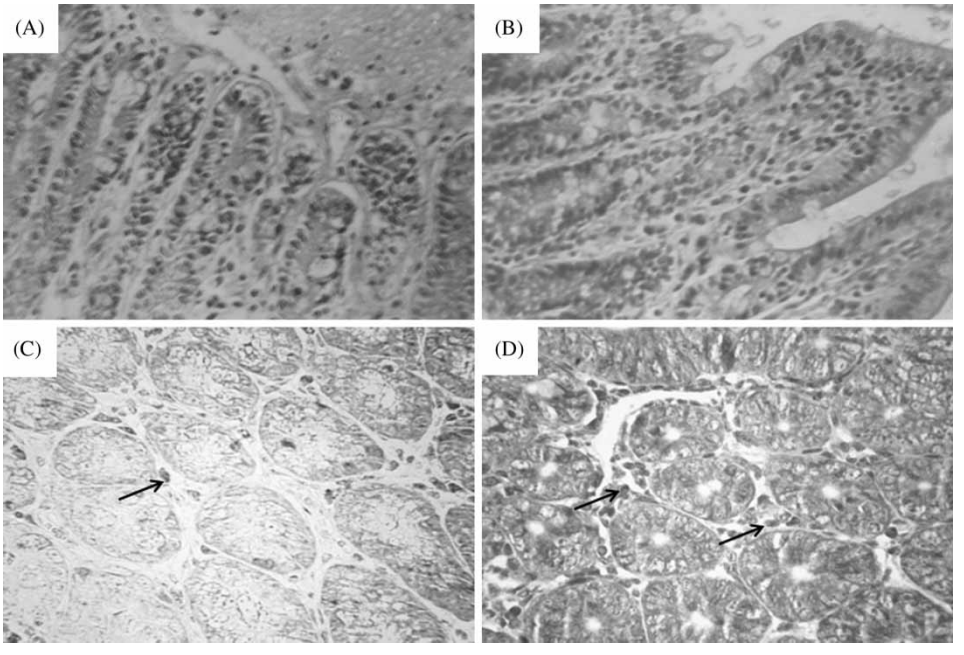

Figure 2. Histological studies in the intestinal tissues. Mast cells determination. The slides were stained with haematoxylin–eosin for the histological observations. No side-effects were observed in response to yacon root flour administration. The test group showed a slight cellular infiltration in the lamina propria of the small intestine (A) in relation with the control (B). For mast cells, the histological samples were stained with toluidine blue in acetate–acetic acid buffer at pH 4.4. The arrows show an example of mast cells (dark blue cells). Mice that received prebiotic flour (D) increased the number of mast cells in the lamina propria of the small intestine compared to the control group (C).

Thirty days after prebiotic administration, the counts for enterobacteria and lactobacilli were similar for both test and control groups (Figure 3).

Effects of yacon root flour on IgA+ cells and CD4+ and CD8+ T lymphocytes associated to the lamina propria of the small and large intestines

The number of IgA+ cells in the lamina propria of the small intestine increased significantly after 30 days of yacon root flour administration (98 ± 5) compared to the control (80 ± 5, basal value). For the test group, these cells remained significantly increased until the last sample (75 days, Figure 4A). For the large intestinal samples, the increases were significant in the mice that received yacon flour all the time points studied, compared to the control, basal value (Figure 4A).

There were no significant differences in the CD4+ T lymphocyte numbers in the mice administered with yacon root flour in all the time points assayed, compared to the control value (30 ± 5). The number of CD8+ T cells decreased significantly only for 30 days of yacon administration (18 ± 2) compared to the control (basal value, 25 ± 3). After that, this population did not show values significantly different to the control. For the control group, the values for IgA, CD4 and CD8 positive cells did not change significantly during the time of the experiment compared to the basal data.

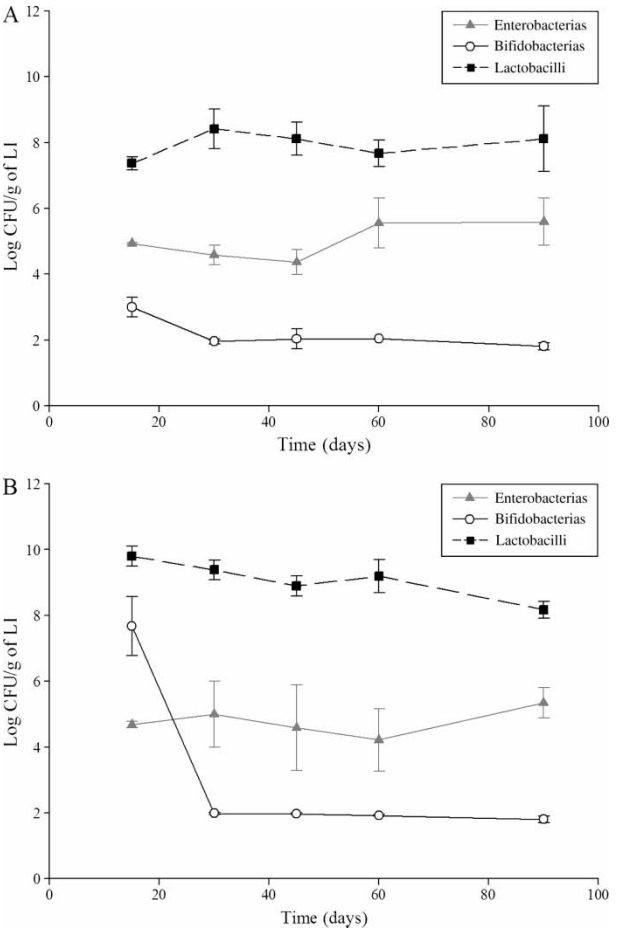

Figure 3. Intestinal microbiota of the large intestine. The large intestines were aseptically removed, homogenised under sterile conditions into sterile tubes containing peptone water. Serial dilutions of the homogenised samples were obtained and aliquots of the appropriate dilution were spread onto the surface of the following agarised media: Mac Conkey for enterobacteria; RCA-pH5 for bifidobacteria; MRS for total lactobacilli. Colony counts are expressed as $\log_{10}$ numbers of bacteria per gram of large intestine (LI). Each point represents the mean of $n = 15 \pm$SD. In order to simplify the analysis of the figure, the statistical analysis is shown only for the two media where significant differences were observed (MRS and RCA-pH5) comparing control group (A) and the group that received yacon root flour (B). [a,b,c]Means for each culture medium without a common letter differ significantly ($P < 0.05$).

Influence of yacon root flour administration on the levels of IgA secreted in the intestine fluids

Mice that received yacon root flour showed significant increased levels of s-IgA in the small intestine fluid at 15, 30 and 45 days (175.97 ± 8.3, 116.76 ± 7.9 and 106 ± 8.1, respectively), compared to the basal control data (81.05 ± 1.3). After 45 days, the prebiotic administration did not influence the s-IgA concentration. For the large intestine, in mice that received yacon root flour, the levels of sIgA in

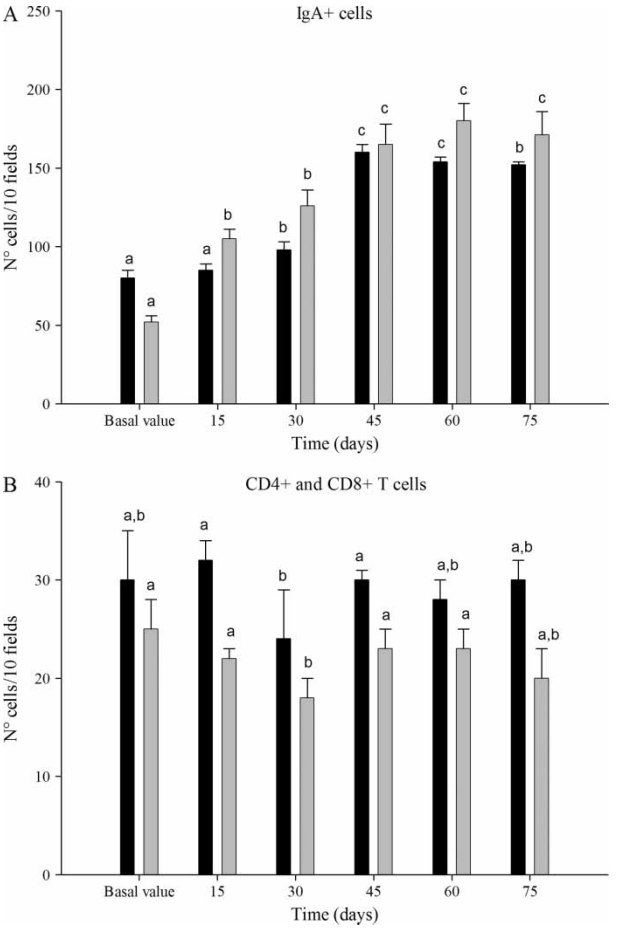

Figure 4. Effect of yacon root flour administration on IgA, CD4 and CD8 positive cells in the intestine. (A) IgA+ cells were counted in histological sections from small (black bars) and large (grey bars) intestine. (B) CD4+ (black bars) and CD8+ (grey bars) cells were counted in histological sections from small intestine. In order to simply the figure, the values for the control group were not included for all the time point assayed because this group did not show changes in the number of the cells analysed throughout the time and its values were not significant different to the basal values. Data correspond to the means ± SD of results of 15 animals from three separate experiments. Means for each IgA, CD4 or CD8 positive cells and part of the intestine without a common letter differ significantly ($P < 0.05$).

the fluid were maintained similar to the basal control data in all the time points studied (Figure 5).

Study of the cytokine positive cells on the intestinal tissues

The number of IL-12+ cells increased significantly in the small intestine in mice that received yacon root flour until the day 60 compared to the basal control. In the last sample (75 days) the number of cells for this cytokine diminished and it was similar to the basal values (Figure 6A). In the large intestine (Figure 6B), significant increase

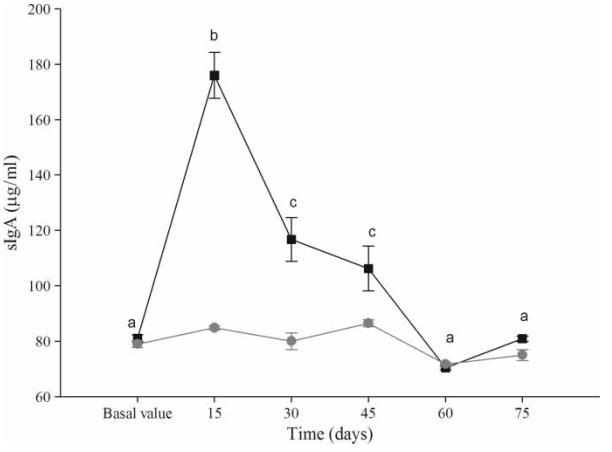

Figure 5. s-IgA in the intestinal fluid. ELISA was used to measure the concentration of total s-IgA in the small (black squares and line) or large (grey circles and line) intestine fluids obtained from mice in test and control groups and at different time points. Results are expressed as concentration (µg/ml). Each point represents the mean of $n = 15 \pm$SD mice from each group. In order to simplify the figure, the statistical analysis is shown only for the small intestine where significant differences ($P < 0.05$) were observed between mice that received yacon root flour and control group, which maintained the s-IgA concentration without significant differences to the basal value in all the time point assayed. [a,b,c]Different letters are used to show the significant differences.

for IL-12+ cells was only observed after 60 days of yacon root flour administration (34 ± 2), compared to the control (30 ± 1). Yacon root flour administration increased significantly the number of IFNγ+ cells at day 45 in the small intestine (38 ± 1) and at day 15 in the large intestine (31 ± 4), compared to the basal control (30 ± 2 and 18 ± 6, for small and large intestine, respectively, Figures 6A and B). For IL-10+ cells in the small intestine, the mice from the test group showed significant increases compared to the basal control, since 30 days of yacon root flour administration until the end of the experiment (Figure 6A). In the large intestine (Figure 6B), this increase for IL-10 was observed in the samples from 15th and 30th day (43 ± 2 and 40 ± 3, respectively) compared to the control (29 ± 2). The number of IL-4+ cells increased significantly in the small intestine of mice that received prebiotic in all the time points assayed (Figure 6A). In the large intestine, the numbers for these cells were maintained similar to the control in all the samples assayed (Figure 6B).

Discussion

FOS are polysaccharides composed of glucose and fructose units linked by a β1–2 bond. In the colon, FOS act as a substrate for the growth of the beneficial microbiota bifidobacteria and lactobacilli (Bornet & Brouns, 2002). The origins of the FOS and their DP are variable. Inulin is a heterofuctan (DP 10–60) obtained from chicory roots and it is one of the prebiotics more studied (Ramirez-Farias et al., 2008). Other FOS proposed as prebiotic have different degrees of polymerisation, lower than inulin and there are reports that related the DP with the microbiota stimulation. Some studies indicate that short-chain oligosaccharides (DP 2–5) are

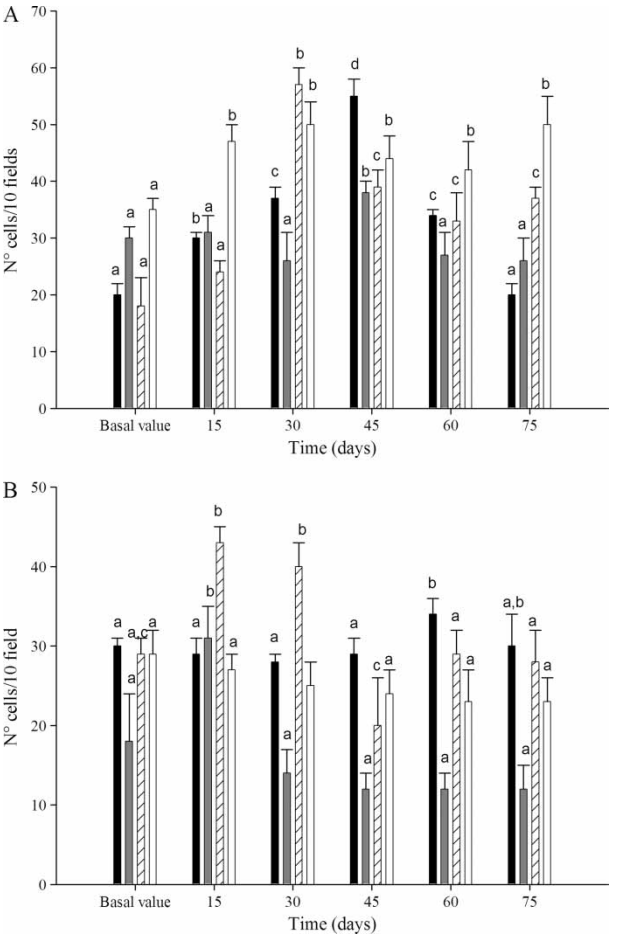

Figure 6. Effect of yacon root flour administration on cytokine-positive cells. Positive cells for each cytokine (IL-12, black bars; IFNγ, grey bars; IL-10, diagonal line bars; IL-4, white bars) were counted in histological sections from small (A) and large (B) intestine. Data correspond to the means ± SD of results from 15 animals. Means for each cytokine and part of the intestine without a common letter differ significantly ($P < 0.05$). The values for control group were not included for all time points because this group did not change significantly the number of cytokine positive cells throughout the time, remained similar to the basal data.

more efficiently metabolised in most of bifidobacteria (Rossi et al., 2005). In the present work, yacon roots were used as a source of FOS. The processing of the yacon roots to obtain the flour-induced modifications in the saccharides composition with changes in the DP, compared to the FOS of the natural yacon roots. Fructose and glucose are the predominant monosaccharides and more than 50% of total FOS has short-chain oligosaccharides (DP 2–5).

The potential use of yacon roots to stimulate the growth of health-promoting bacteria and exert beneficial effects on the gut immune system was analysed in the present work. It has been suggested that the increase in numbers of bifidobacteria could confer a beneficial effect on the stability of the intestinal microbiota

(Mitsuoka, 1990). In our in vivo study, mice that received the flour supplement obtained from the yacon root showed its prebiotic effect with increases for bifidobacteria at day 15 of yacon root flour administration. After that, no increase for this bacterial population was observed, compared to the mice that did not receive the prebiotic. The study for lactobacilli population showed that mice administered with the prebiotic increased this bacteria population in the large intestine. At difference of bifidobacteria; for lactobacilli the increases were maintained throughout the trial when mice received this prebiotic. This observation agrees with results obtained by other researchers where in vitro experiments confirmed that some lactobacilli utilise oligosaccharides with low DP (3 or 4) (Snart et al., 2006). These authors proposed that DP3 oligosaccharides were the substrates that produced the "lactobacillogenic" effect in the caecum of the rat assayed. In the present work, at the same time when lactobacilli increased with yacon root flour administration, enterobacteria diminished compared to the control group. This observation was similar to other studies performed by our group in which, when a probiotic product was administered to mice, they increased bifidobacteria or lactobacilli and decreased enterobacteria in the large intestine (de Moreno de LeBlanc et al., 2008). Another important observation was that when the prebiotic administration was stopped, the intestinal microbiota revert back to the same characteristics of the control (Figure 3). It is important when a dietary supplement is administered, that the changes obtained in the intestinal microbiota can be reversible.

Considering that the consumption of the yacon root flour increased bifidobacteria and lactobacilli populations, some immunological parameters were measured to analyse the influence of the development of these bacteria on the regulation of the immune system.

It is known that the response of the adaptive immune system to bacterial colonisation of the gut is the production of IgA by the gut-associated lymphoid tissues (Cebra, 1999). The role of the IgA+ cells in the intestine is undeniable (Lamm, 1976). Secretory IgA antibodies are major effector molecules in the mucosal system and their role as the first defence line against infections has been well demonstrated (Mazanec, Nedrud, Kaetzel, & Lamm, 1993). According to Lamm (1998), IgA can act in the lumen, the intraepithelial cells and in the lamina propria; we also analysed the number of IgA+ cells present in the lamina propria. The study of the IgA secreting cells showed that mice administered with the prebiotic root flour increased these cells in the large and small intestine (Figure 4). These cells can release the antibody in the lumen and the s-IgA levels increase in the intestinal fluid where they were also analysed. In regard to s-IgA, it was observed that in the small intestine fluid, IgA concentration increased up to 45 days of yacon root flour administration (Figure 5). After that, there were no increases for s-IgA, even when the number of IgA+ cells in the small intestinal tissue was maintained higher than the control basal data (Figures 4A and 5). In the large intestine fluid, no increases for s-IgA were detected in mice given the prebiotic. These findings showed clonal expansion of B lymphocytes in the lamina propria of both parts of the intestine; however, only at the beginning of the prebiotic administration, when the most impact is exerted by the growth of the beneficial bacteria with increases of lactobacilli and also bifidobacteria, increased levels of s-IgA in the small intestine were observed. These bacteria could influence B cell population to release the antibody reinforcing the barrier effect. Afterwards, the continuous administration of the prebiotic

maintained increased the IgA secreting cells but the s-IgA were not released. We think that IgA+ cells that were increased in almost all assayed periods, are an important source for these antibodies, which could be released against the different inflammatory challenges, for example, against an enteropathogens. We consider that the increase in the IgA+ B cells act as a surveillance mechanism in the gut due to their important function for this immunoglobulin at the mucosal level.

Other immune cell populations studied were the T lymphocytes. The number of CD4+ cells did not increase in the lamina propria of the small intestine but the CD4+/CD8+ cells ratio was higher in mice that received yacon root flour which showed decreased number of CD8+ cells, 15 and 30 days after probiotic administration. This finding was similar to others reported for the probiotic bacteria *Lactobacillus casei* CRL 431 where LAB antigens could interact with the immune cells and only activate T cells to stimulate other immune cells through the release of cytokine, but did not induce T cell proliferation (de Moreno de LeBlanc, Galdeano, Chaves, & Perdigón, 2005; Galdeano & Perdigón, 2006).

To study the importance of CD4+ cells or other immune cells participating in the innate immune response (i.e. macrophages, dendritic cells or mast cells) on the mucosal immunity stimulated by the prebiotic flour, the cytokine secreting cells were quantified. Two proinflammatory cytokines were studied: IL-12 and IFNγ. IL-12 drives the classical Th1 response producer of IFNγ (Stober, Schirmbeck, & Reimann, 2001). Mice given the yacon root flour increased IL-12+ cells in the lamina proria of the small intestine, and for the large intestine only on day 60. IFNγ+ cells only increased after 45 days in the small intestine and after 15 days in the large intestine. These cytokines are produced by activated cells and they are able to activate other cells during the inflammatory response. The lack of inflammation observed in the histological studies from mice given the prebiotic (Figure 2) suggests that these cytokines could be related with other effects or that the pro-inflammatory effect of these cytokines was modulated. This last observation could be attributed to the regulatory effect of the IL-10 and IL-4 that were also analysed.

IL-10 inhibits the production of TNFα, IL-1, IL-2 and IL-12 by macrophages and inhibits other functions of macrophage and T cell activation. Furthermore, IL-10 is very important as a regulator in the intestine. Berg et al. (1996) demonstrated the IL-10 role in intestinal inflammation. IL-10 also participates in the normal tolerance to indigenous bacterial flora and its lack is related to inflammation (Sydora, Tavernini, Wessler, Jewell, & Fedorak, 2003). In our study, IL-10+ cells were increased in the small intestine from mice that received yacon root flour from day 30 and the number of these cells were maintained higher than the control until the end of the experiment. In the large intestine, the increases for this cytokine were observed until day 30, which agrees with the increase for the pro-inflammatory cytokine IFNγ. These results show the role of IL-10 regulating IFNγ and IL-12 in the small and large intestine, respectively (Figure 6).

Another regulatory cytokine studied was IL-4 (Figure 6). The stimulatory effects of IL-4 are down-regulated by IFNγ, a cytokine whose functions are antagonised by IL-4 and vice versa (Feghali & Wright, 1997). In our study, IL-4-producing cells increased in the small intestine from mice that were given the prebiotic during all the experiments. The lack of T cell increases led us to study another cell population involved in IL-4 production as the mast cells. This last population was increased in the small intestine of mice that were given prebiotic (Figure 6) and could be

responsible for the high number of IL-4+ cells observed. The biological role of mast cell population at the mucosal level would be a source to initiate the adaptive response or to help the antigen clearance (Warrel, Cox, Firth, & Benz, 2003) more than as an effector cell to mediate the allergic process; that is restricted to special antigen structure or allergen.

The results obtained show the prebiotic effects of the yacon root flour, stimulating the beneficial population of bifidobacteria and lactobacilli. This fact could be related with the intestinal immune stimulation observed in mice that received the prebiotic: increases in IgA and different cytokines. The long term administration of yacon root flour maintained the intestinal homeostasis without inflammatory effects regulated mainly through IL-10 and IL-4 regulatory cytokines.

Yacon root flour has an immunomodulatory effect and this effect may be indirect because the prebiotic stimulates the growth of bifidobacteria and lactobacilli, which in turn can interact with the immune system at many levels, including cytokine production (Gibson & Roberfroid, 1995; Vintiñi et al., 2000). However, we cannot discard the direct effect of the FOS of the yacon root flour on the immune system. There are studies that suggest prebiotics by themselves may directly stimulate the mucosal immune system. Other in vitro experiments or in vivo studies using germ-free mice could be useful in order to study this possibility.

Acknowledgements

This work was financially supported by PICTO 04/503, Agencia Nacional de Promoción Científica y Tecnológica (ANPyCT-FONCYT) and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina.

References

Ameho, C.K., Adjei, A.A., Harrison, E.K., Takeshita, K., Morioka, T., Arakaki, Y., Ito, E., Suzuki, I., Kulkarni, A.D., Kawajiri, A., & Yamamoto, S. (1997). Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. *Gut*, *41*, 487–493.

Berg, D.J., Davidson, N., Kühn, R., Müller, W., Menon, S., Holland, G., Thompson-Snipes, L., Leach, M.W., & Rennick, D. (1996). Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *Journal of Clinical Investigation*, *98*(4), 1010–1020.

Bornet, F.R., & Brouns, F. (2002). Immune-stimulating and gut health-promoting properties of short-chain fructo-oligosaccharides. *Nutrition Reviews*, *60*(10 Pt 1), 326–334.

Burkitt, D.P., & Trowell, H.C. (1975). *Refined carbohydrate foods and disease: Some implications of dietary fibre*. London: Academic Press.

Cebra, J.J. (1999). Influences of microbiota on intestinal immune system development. *American Journal of Clinical Nutrition*, *69*(5), 1046S–1051S.

de Moreno de LeBlanc, A., Dogi, C.A., Galdeano, C.M., Carmuega, E., Weill, R., & Perdigón, G. (2008). Effect of the administration of a fermented milk containing *Lactobacillus casei* DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunology*, *9*, 27.

de Moreno de LeBlanc, A., Galdeano, C.M., Chaves, S., & Perdigón, G. (2005). Oral administration of *Lactobacillus casei* CRL 431 increases immunity in bronchus and mammary glands. *European Journal of Inflammation*, *3*(1), 23–28.

de Moreno de LeBlanc, A., & Perdigón, G. (2004). Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. *Medical Science Monitor*, *10*(4), BR96–BR104.

Feghali, C.A., & Wright, T.M. (1997). Cytokines in acute and chronic inflammation. *Frontier in Biosciences*, *2*, d12–d26.

Galdeano, C.M., & Perdigón, G. (2006). The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. *Clinical and Vaccine Immunology*, *13*(2), 219–226.

Gibson, G.R., & Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*(6), 1401–1412.

Lamm, M.E. (1976). Cellular aspects of immunoglobulin A. *Advances in Immunology*, *22*, 223–290.

Lamm, M.E. (1998). Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. *American Journal of Physiology*, *274*(4 Pt 1), G614–G617.

Mazanec, M.B., Nedrud, J.G., Kaetzel, C.S., & Lamm, M.E. (1993). A three-tiered view of the role of IgA in mucosal defense. *Immunology Today*, *14*(9), 430–435.

Mitsuoka, T. (1990). Bifidobacteria and their role in human health. *Journal of Industrial Microbiology*, *6*, 263–268.

Pedreschi, R., Campos, D., Noratto, G., Chirinos, R., & Cisneros-Zevallos, L. (2003). Andean yacon root (*Smallanthus sonchifolius* Poepp. Endl) fructooligosaccharides as a potential novel source of prebiotics. *Journal of Agricultural Food Chemistry*, *51*(18), 5278–5284.

Ramirez-Farias, C., Slezak, K., Fuller, Z., Duncan, A., Holtrop, G., & Louis, P. (2008). Effect of inulin on the human gut microbiota: Stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *The British Journal of Nutrition*, *101*(4), 541–550.

Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S., & Matteuzzi, D. (2005). Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. *Applied Environmental Microbiology*, *71*(10), 6150–6158.

Sainte-Marie, G. (1962). A paraffin embedding technique for studies employing immunofluorescence. *Journal of Histochemistry and Cytochemistry*, *10*, 150–156.

Schumann, C. (2002). Medical, nutritional and technological properties of lactulose. An update. *European Journal of Nutrition*, *41*(Suppl. 1), I17–I25.

Snart, J., Bibiloni, R., Grayson, T., Lay, C., Zhang, H., Allison, G.E., Laverdiere, J.K., Temelli, F., Vasanthan, T., Bell, R., & Tannock, G.W. (2006). Supplementation of the diet with high-viscosity beta-glucan results in enrichment for lactobacilli in the rat cecum. *Applied and Environmental Microbiology*, *72*(3), 1925–1931.

Stober, D., Schirmbeck, R., & Reimann, J. (2001). IL-12/IL-18-dependent IFN-gamma release by murine dendritic cells. *Journal of Immunology*, *167*(2), 957–965.

Sydora, B.C., Tavernini, M.M., Wessler, A., Jewell, L.D., & Fedorak, R.N. (2003). Lack of interleukin-10 leads to intestinal inflammation, independent of the time at which luminal microbial colonization occurs. *Inflammatory Bowel Diseases*, *9*(2), 87–97.

Trowell, H., Southgate, D.A., Wolever, T.M., Leeds, A.R., Gassull, M.A., & Jenkins, D.J. (1976). Letter: Dietary fibre redefined. *Lancet*, *1*(7966), 967.

Van Loo, J.A. (2004). Prebiotics promote good health: The basis, the potential, and the emerging evidence. *Journal of Clinical Gastroenterology*, *38*(6 Suppl), S70–S75.

Vintiñi, E., Álvarez, S., Medina, M., Medici, M., de Budeguer, M.V., & Perdigón, G. (2000). Gut mucosal immunostimulation by lactic acid bacteria. *Biocell*, *24*, 223–232.

Warrel, D.A., Cox, T.M., Firth, J.D., & Benz, E.J., Jr. (2003). *Oxford textbook of medicine* (Vol. 4). Oxford: Oxford University Press.