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Feeding with dairy *Propionibacterium acidipropionici* CRL 1198 reduces the incidence of Concanavalin-A induced alterations in mouse small intestinal epithelium

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ABSTRACT

Plant lectins are specific carbohydrate-binding proteins widespread in human and animal diets that cause antinutritional effects. Specific intestinal receptors and microbiota may interact with these dietary components leading to important changes on intestinal physiology. It has been proposed that probiotic microorganisms with suitable surface glycosidic moieties could bind to dietary lectins favoring their elimination from the intestinal lumen or inhibiting their interaction with epithelial cells. In this work, we assessed the effects of Concanavalin A (Con A) on some morphological and physiological parameters related to intestinal functionality such as small bowel architecture, main microflora components and disaccharidase activities of Balb/c mice after long term feeding with this lectin alone (8 mg/kg/day of Con A for 3 weeks) or with the simultaneous consumption of *Propionibacterium acidipropionici* CRL 1198 (5×10^8 CFU/mice/day). Long-term consumption of Con A reduced food efficiency suggesting the alteration of the digestion/absorption function of the intestine in the presence of lectin. This effect could be due to both histological alterations of the intestinal epithelium such as shortening and shedding of microvilli and physiological changes like the decrease of disaccharidase activities. Con A feeding increased enterobacteria and enterococci populations whereas lactobacilli, bifidobacteria and propionibacteria were not affected. Consumption of propionibacteria at the same time than Con A, reduced the incidence of Con A-induced alterations in Balb/c mice and may be an effective tool to avoid undesirable lectin–epithelia interactions in both animals and humans.

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1. Introduction

Foods and the metabolites generated during digestion and gastrointestinal transit exert a major role in the consumer's health. Many antinutritional and/or potentially toxic compounds are daily ingested by humans and animals. Lectins are nonimmune proteins or glycoproteins that bind specifically to carbohydrate moieties expressed on the cells surface commonly affecting cellular physiology (Sharon & Lis, 1995). They are present in a wide range of food items such as vegetables, fruits, cereals, beans and mushrooms (Nachbar & Oppenheim, 1980) so their ingestion could be significant. Since lectins are highly resistant to inactivation by cooking and by digestive processes, it is likely that the intestinal epithelium is exposed to many of these proteins that have retained at least some of their biological activity.

Plant lectins from the Leguminosae family, are considered as antinutritive or toxic substances since they lead to some harmful reactions after binding to the membrane glycosyl groups of the intestinal epithelial cells (Vasconcelos & Oliveira, 2004). In this sense, it has been observed that Concanavalin A, the lectin from Jack bean (*Canavalia ensiformis*), causes morphological and physiological changes in the intestinal epithelium that could reduce the absorptive function and nutrient utilization, and increases cellular proliferation and turnover (Fitzgerald et al., 2001; Lorenzsonn & Olsen, 1982). Miyake, Tanaka, and McNeil (2007) have reported that Con A impairs the normal protecting events of intestinal cells by inhibiting the rapidly repairing membrane disruptions by exocytotic reaction. In consequence the failure in the subpopulation of mucus-secreting cells reduces the mucosa lubrication and increases the mechanical stress suffered by the epithelial cells. Con A also exerts antinutritional effects and suppresses food consumption but stimulates intestinal epithelium growth in rats (Baintner, Kiss, Pfuller, Bardocz, & Pusztai, 2003; Fitzgerald et al., 2001; Lorenzsonn & Olsen, 1982). It was also found that Con A affects cell growth of colorectal cancer cell lines in a dose-dependent manner (Kiss et al., 1997). Because of the damage they produce on the epithelial cells and their hypertrophic effects, some lectins such as Con A, would act as tumor promoters (Evans et al., 2002; Kiss et al., 1997; Ryder, Smith, & Rhodes, 1992; Ryder, Smith, Rhodes, Parker, & Rhodes, 1994). On the contrary, Fitzgerald et al. (2001) have reported that Concanavalin A infused into rats' stomachs induces the proliferation of the intestinal epithelium without producing crypt branching. Therefore, short-term exposition

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to lectins that stimulate growth has been proposed as a useful therapy for the hypoplastic intestine. However, effects of long-term consumption of potentially toxic lectins should be evaluated.

Metabolism of ingested food begins with digestion and subsequent absorption of dietary nutrients. The process of digestion is mediated by different hydrolases secreted into the gastrointestinal tract or produced by the enterocytes in the intestinal brush border membrane. In the case of food carbohydrates, disaccharides and some oligosaccharides are hydrolyzed to monosaccharides by a few small intestinal brush border disaccharidases before their transport across the microvillus membrane. Lactase (lactase-phlorizin hydrolase, EC 3.2.1.23-62) is the enzyme essential for hydrolysis of lactose in milk. Sucrase (sucrase–isomaltase, EC 3.2.1.48-10) is an α-glucosidase that hydrolyzes sucrose, maltotriose and about 80% of dietary maltose, whereas maltase (maltase–glucoamylase, EC 3.2.1.20) digests the remaining maltose to glucose. Trehalase (α,α-trehalose glucohydrolase EC 3·2·1·28) hydrolyzes the disaccharide trehalose into its two glucose molecules.

Classical propionibacteria are microorganisms of interest for their role as starters in cheese technology as well as for their functions as probiotics (Cousin, Mater, Foligné, & Jan, 2010; Zárate, Babot, Argañaraz-Martinez, Lorenzo-Pisarello, & Pérez Chaia, 2011). *Propionibacterium acidipropionici* CRL 1198 is a dairy strain with probiotic potential as it modifies in a beneficial manner the composition and metabolism of the intestinal microflora (Perez Chaia, Nader de Macias, & Oliver, 1995; Perez Chaia, Zárate, & Oliver, 1999; Perez Chaia & Zárate, 2005; Lorenzo-Pisarello, Gultemirian, Nieto-Peñalver, & Perez Chaia, 2010). It also resists the manufacture process of a Swiss-type cheese (Perez Chaia & Zárate, 2005) so that this dairy product could be considered as a suitable vehicle for its delivery to the host. Development of cheeses as vehicle for probiotics, like propionibacteria, represents a valuable alternative to yogurt and fermented milks and a research topic of growing interest for industry (Ranadheera, Baines, & Adams, 2010).

In previous studies, we have determined that this strain has the ability to bind and remove some dietary lectins from media, preventing their cytotoxic effects on intestinal epithelial cells (Zárate & Perez-Chaia, 2009). Furthermore, it decreases *in vivo* the incidence of colonic lesions in a murine model fed with Concanavalin A (Zárate et al., unpublished results). In consequence, dairy propionibacteria would be intended to protect the intestinal mucosa from the toxic effects of dietary lectins by avoiding their interaction with epithelial cells. The aim of the present investigation was to assess the effects of long term feeding of mice with Con A on some morphological and physiological parameters related with the small bowel functionality as well as the potential of dairy propionibacteria to prevent any deleterious modification.

2. Materials and methods

2.1. Lectin

The lectin used in this study, Concanavalin A (Con A, from *C. ensiformis*) was provided by Sigma Chemical Co, St Louis, MO, USA.

2.2. Microorganism and culture conditions

The microorganism used in this study, *P. acidipropionici* CRL 1198 was obtained from Laboratorio de Ecofisiología Tecnológica-CERELA (CRL: Centro de Referencia para Lactobacilos, CERELA, Tucumán, Argentina). The strain was stored at −20 °C in 10% (w/v) reconstituted non-fat milk (NFM) containing 0.5% yeast extract. Before using, it was activated by three successive transfers every 24 h at 37 °C, in LAPTg broth (1% tryptone, 1% yeast extract, 1.5% meat peptone; 0.1% Tween 80, 1% glucose, pH:6.5) sterilized at 121 °C for 15 min. For mice feeding trials, an appropriated volume of a 24 h culture was harvested by centrifugation, washed in sterile saline solution (0.9% NaCl) and suspended in the same solution at a concentration of 10^8 CFU mL^{-1} in order to be added to the drinking bottles.

2.3. Animals and experimental procedures

The experimental protocol used in this study was approved by the Ethical Committee of CERELA (Centro de Referencia para Lactobacilos). For Assay 1, sixty male 5-weeks-old BALB/c mice, each weighing 24–26 g, from the inbreed colony of CERELA, were divided into four groups. They were housed in plastic cages with wire-tops in a room under controlled environmental conditions of 25 °C and a 12:12 h light–dark cycle and were allowed free access to a conventional solid balanced diet with the following composition (g/kg): water, 120; proteins, 230; carbohydrates, 538; lipids, 50; vitamins, 22; minerals, 40 (Cargill, Argentina). After 1 week of acclimatization groups were subjected to one of the following treatments: Group 1 was used as Control without treatment; mice of Group 2 were daily gavaged during 3 weeks, with 8 mg/kg of Concanavalin A dissolved in 0.9% NaCl containing 5 mM $CaCl_2$, 5 mM $MnCl_2$ and 5 mM $MgCl_2$, pH 7.2. Animals from Group 3 were allowed to drink *ad libitum* a suspension of *P. acidipropionici* CRL 1198 instead of water, provided in the drinking bottles during 3 weeks. That represented a daily dose of c.a. 5×10^8 bacteria mL^{-1}. Mice of Group 4 were subjected at the same time to the treatments of Groups 2 and 3. Three animals per group were sacrificed by cervical dislocation, on days 7, 14, and 21 during treatments and at days 1 and 7 after cessation of them in order to obtain organs and the small bowel walls.

For Assay 2, fifteen male 6-weeks-old BALB/c mice without any treatment were used.

2.4. Measurement of food intake, organ and body weights and intestinal permeability

The daily food intake of animals of Assay 1 was determined by difference between weights of food supplied every day and the remnant in the feeder 24 h later. Body weights were recorded twice per week since the beginning of treatments. Animals were fasted overnight before sacrifice and organs (stomach, small bowel, liver, spleen and cecum) were carefully removed post mortem and weighed.

On the morning of each day of sacrifice mice were inoculated by gavage with a marker molecule solution containing Na-fluorescein (NaF; Merck, Darmstadt, Germany) with low molecular mass (376 Da) and that diffuses passively through cell membranes. The marker molecule had been dissolved in 0.9% NaCl and was given to mice at 10 mg of NaF/kg of body weight. Blood samples for analyses of marker molecule absorbed were taken by cardiac puncture 1 h after administration. The amount of Na-fluorescein in plasma, in relation to standard dilutions of Na-fluorescein dissolved in PBS buffer, was measured by spectrofluorometry, using a filter set of 485 nm for excitation and 530 nm for emission (Varian-Cary Eclipse).

2.5. Tissue collection and preparation of intestinal mucosa homogenates

The euthanized animals were laid on their backs to the dissecting boards and the abdomen of each animal was opened up after an incision along the mid line. The small bowel was carefully brought out, and a 2-cm-long, whole-thickness segment from the middle of the intestine was taken for histological analyses. The remaining parts were flushed out with cold saline solution, slit opened longitudinally with sterile scissors, and used to prepare the mucosal homogenates. The intestinal mucosa was scrapped off gently with a sterile glass slide and homogenized in 4 parts of cold PBS as previously described (Zárate, Morata de Ambrosini, Pérez Chaia, & González, 2002). Homogenates were used for microbiological analysis or decanted to remove large debris and supernatants stored at −70 °C for further assays of enzyme activities.

2.6. Light and electron microscopy

The 2 cm-long fragments of intestines were divided in two parts and fixed with 10% paraformaldehyde for 24 h at room temperature and with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4 °C for further analysis by optical and electron microscopy respectively. After fixation, one set of samples was dehydrated and embedded in paraffin according to standard histological methods (Sainte-Marie, 1962). Serial sections of 4 μm in thickness were stained with hematoxylin–eosin and observed at 40× and 100× magnification under a light microscope for histomorphological analysis. The other set of samples was washed three times with 0.1 M cacodylate buffer at 10 min-intervals and postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer for 2 h. Cells were then washed three more times with the same buffer. Dehydration was carried out with ascending concentrations of acetone (35, 50, 75, 95, and 100%). The cells were finally dried in a critical point drier (HCP-2, Hitachi, Tokyo, Japan) for 20 min and coated with gold using a scanning electron microscopy coating unit (E5100). The samples were examined with a transmission electron microscope (Zeiss, EM 109, Germany).

2.7. Bacteriological analysis

Counts of major aerobe-facultative anaerobe groups of intestinal bacteria adhered to the mucosa were assessed by plating on selective media as previously described (Perez Chaia & Zárate, 2005). An aliquot of mucosal homogenates was transferred to sterile tubes, serially diluted with PBS and spread on Slantez–Bartley agar (Oxoid) aerobically incubated at 37 °C for 3 days and Violet Red-Bile-Glucose agar (Oxoid) aerobically incubated at 37 °C for 24 h for counts of *Enterococcus* and *Enterobacteriaceae*, respectively. Rogosa agar (Merck) containing glacial acetic acid (1.32 mL·L–1), Columbia modified agar medium and Sodium Lactate Agar, anaerobically incubated at 37 °C for 5 days (Huang, Kotula, & Adams, 2003) were used for counts of *Lactobacillus*; *Bifidobacterium*, and *Propionibacterium* respectively. Confirmation of bacterial genera from selective media was based on morphology, Gram stain, fermentation product formation and biochemical tests.

2.8. Effect of Con A on enzyme activities

Disaccharidase activities (lactase, sucrase, maltase and trehalase) of mucosal homogenates were determined according to the method of Dahlqvist (1984), by incubating the homogenates with the substrate (lactose, sucrose maltose or trehalose 56 mM in 0.2 M sodium phosphate buffer, pH 6.0) during 60 min at 37 °C. The glucose released by hydrolysis was measured with a glucose-oxidase assay kit according to the manufacturer's instructions (Wiener Lab; Argentina) and protein contents were estimated by the method of Bradford using bovine serum albumin as standard. Enzyme activities in homogenates were expressed as micromoles of glucose released per minute per mg of protein (μmol min^{-1} mg^{-1}).

Assay 2 was performed to in order to determine the immediate effect of Con A on disaccharidase activities and the possible interaction with the enzyme and/or the substrate. For this objective, five mice without any treatment were sacrificed and their intestinal mucosa were obtained as described above and pooled. Then, the disaccharidase activities were assessed in the following conditions; *C*: no Con A addition to the reaction mixture (control), *L*: Con A addition (400 μg/mL) to the reaction mixture, *LE*: Con A pre-incubated for 30 min with the enzyme (homogenate) before initiating the reaction; *LS*: Con A pre-incubated for 30 min with the substrate before initiating the reaction; *P*: propionibacteria pre-incubated for 30 min with saline solution before addition of supernatant to the reaction; *LP*: Con A pre incubated with propionibacteria for 30 min before addition of supernatant to the reaction.

2.9. Statistical analysis

The results informed are the average of the data obtained from three independent trials of each experiment. Significant differences between means were determined by Tukey's test after analysis of variance (ANOVA) with Minitab Statistic Program, release 14 for Windows. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Measurement of food intake, organ and body weights and intestinal permeability

Daily food intake was similar in mice from control and treatment groups ($P>0.05$), with a mean value of food consumption of 5.22 ± 0.29 g per mouse per day. Total body weights increased by 10–12 g during the study (28 days) and all groups showed a linear body weight gain (Fig. 1). However, the Con A-treated mice gained less weight during the 21 days of lectin administration ($P<0.05$). In consequence, the body weight gain and food efficiency at the end of the study were lower in the group treated only with lectin (Table 1) ($P<0.05$). Besides, no obvious differences in the weights of the different organs with the exception of stomachs were found (Table 1). Treatments with Con A significantly increased the stomach size and weight during and after feeding in the animals of Group 2 and to a lesser extent that of animals in Group 4 that received simultaneously lectin and propionibacteria (Table 1 and Fig. 2) ($P<0.05$). Consumption of propionibacteria at the same time than lectin prevented in a significant manner this effect ($P<0.05$). Other organs such as liver, small bowel and cecum enlarged during the first week of treatment with Con A solely ($P>0.05$), but the effect was naturally reversed by the second week of feeding (Fig. 2) whereas the spleens were not affected at all (Table 1).

Feeding with lectins and/or propionibacteria did not affect the intestinal permeability and absorption *in vivo* of small molecules since no differences in the plasma levels of NaF were observed between groups at 60 min after gavage during or after treatments (Table 1).

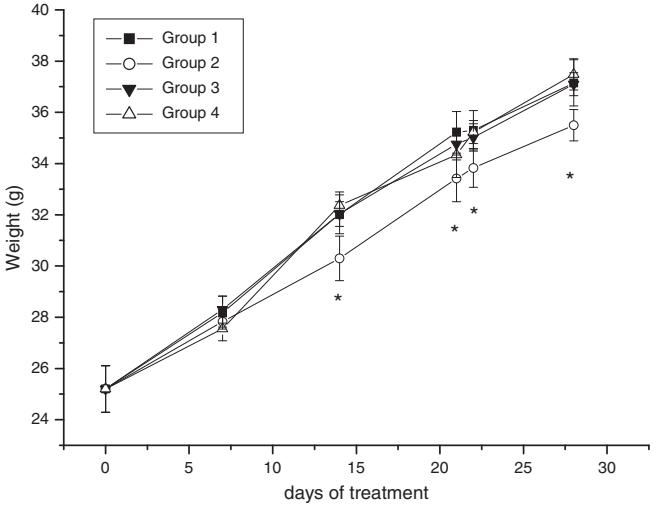

Fig. 1. Body weight gain of six-week-old Balb/c mice during 21 days of feeding with Concanavalin A, *Propionibacterium acidipropionici* CRL 1198 or lectin plus propionibacteria up to seven days after cessation of treatments .Group 1: Control; Group 2: Con A; Group 3: *Propionibacterium acidipropionici* CRL 1198; Group 4: Con A + *P. acidipropionici* CRL 1198. Values are means ± SD of three independent experiments. The asterisk indicates significant differences with the control group (G1) ($P<0.05$).

Table 1
Effect of feeding with Concanavalin A, *P. acidipropionici* CRL 1198 and lectin plus propionibacteria on body and organs weights, and food efficiency of Balb/c mice one week after treatments were finished (day 28).

Treatment groups	Food intake (g/mouse/day)	Body weight gain (g)	Food efficiency*	Stomach (g)	Small bowel (g)	Liver (g)	Spleen (g)	NaF in plasma (ng/mL)
G1: control	$5.27 \pm 0.45^{\dagger,a}$	11.95 ± 1.48^{a}	2.27	0.83 ± 0.04^{a}	2.38 ± 0.17^{a}	2.04 ± 0.28^{a}	0.11 ± 0.01^{a}	3.83 ± 0.29^{a}
G2: Con A	4.88 ± 1.03^{a}	10.13 ± 1.13^{b}	2.07	1.90 ± 0.19^{b}	2.41 ± 0.19^{a}	1.65 ± 0.23^{a}	0.12 ± 0.02^{a}	4.01 ± 0.46^{a}
G3: CRL 1198	5.35 ± 0.80^{a}	11.90 ± 0.87^{a}	2.28	0.73 ± 0.05^{a}	2.43 ± 0.16^{a}	1.87 ± 0.12^{a}	0.10 ± 0.00^{a}	3.81 ± 1.42^{a}
G4: Con A + CRL 1198	5.10 ± 0.80^{a}	12.28 ± 1.23^{a}	2.40	1.25 ± 0.21^{c}	2.41 ± 0.18^{a}	1.67 ± 0.12^{a}	0.13 ± 0.05^{a}	4.15 ± 0.63^{a}

† Values are means ± SD. Means in a column with different superscript are significantly different. ($P<0.05$).
* Ratio between body weight gain and food consumed per day.

3.2. Histomorphological studies of intestinal tissues

The morphological appearance of the mucosa of the small bowels 1 day post-treatments (day 22) under the light and electron microscope is illustrated in Figs. 3 and 4. Panels (a) (40×) and (b), (c) (100×) of Fig. 3 show the normal appearance of villus of control mice without any treatment whereas panels (d) to (f) show the histomorphology of villus of mice that consumed 8 mg/kg/day of Con A. Continuous feeding with Con A elevated epithelial cell proliferation in the mice intestine as was evidenced by the higher cellularity of the epithelium lining the villus and the disarrangement and stratification of nuclei. Mice that consumed 5×10^8 CFU/day of *P. acidipropionici* CRL 1198 alone or with the simultaneous administration of Con A showed no remarkable differences with respect to the control (data not shown). Long feeding with Con A also altered in a significant manner the ultrastructure of enterocytes by producing a shortening and shedding of microvillus as could be seen in Fig. 4(a) and (b). The histomorphological modifications induced by Con A were reverted 1 week after cessation of lectin treatment and were greatly prevented by consumption of propionibacteria at the same time than Con A (Fig. 4c and d).

3.3. Main bacteria adhered to the intestinal mucosa

Counts of relevant bacteria adhered to the mucosa of the small bowel of mice from the different groups during treatments are shown in Fig. 5. Con A feeding increased both enterobacteria and enterococci populations whereas lactobacilli, bifidobacteria and propionibacteria were not modified in a significant manner.

A number lower than 5×10^3 CFU g^{-1} of propionibacteria were detected in Groups 1 and 2, which were not fed with these microorganisms, whereas Groups 3 and 4 that received *P. acidipropionici*

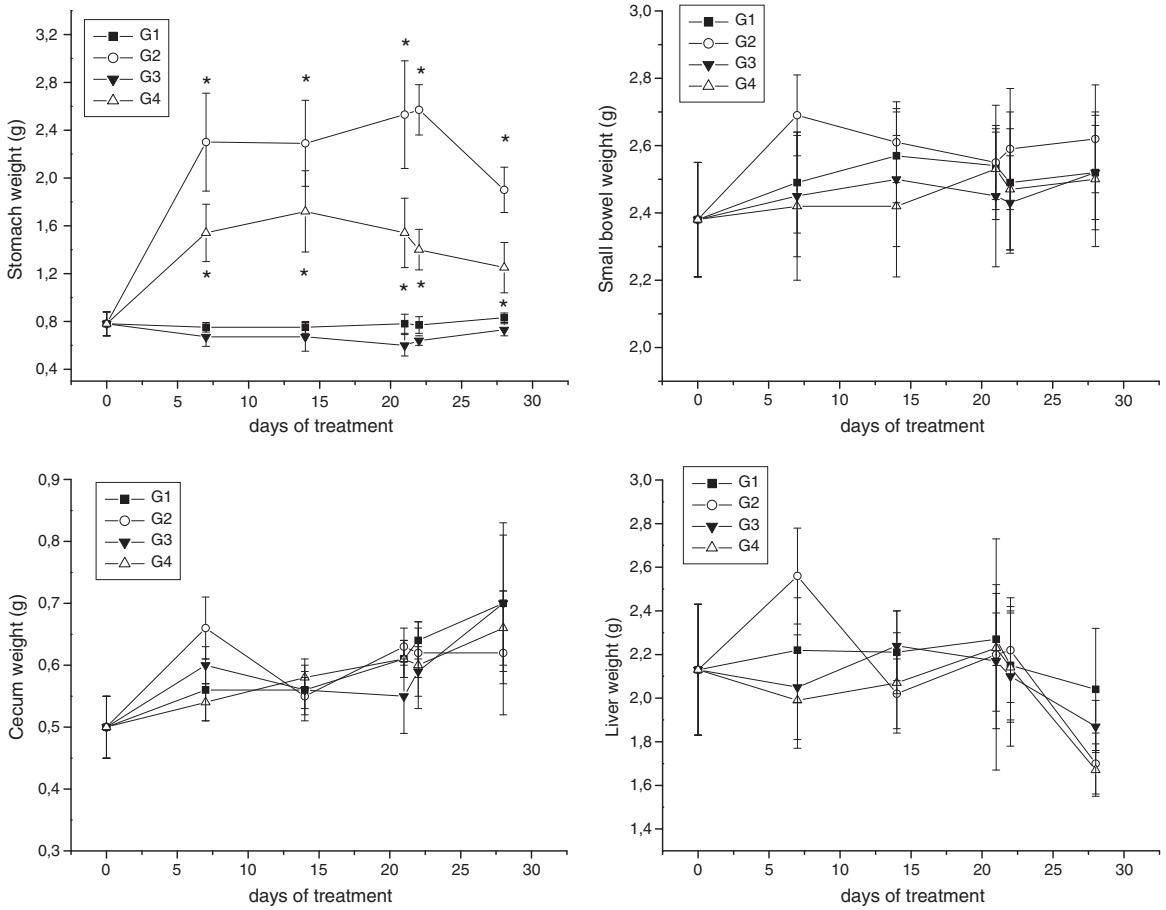

Fig. 2. Organs weights of six-week-old Balb/c mice during 21 days of feeding with Concanavalin A, *Propionibacterium acidipropionici* CRL 1198 or lectin plus propionibacteria up to seven days after cessation of treatments. Group 1: Control; Group 2: Con A; Group 3: *Propionibacterium acidipropionici* CRL 1198; Group 4: Con A + *P. acidipropionici* CRL 1198. Values are means ± SD of three independent experiments. The asterisk indicates significant differences with the control group (G1) ($P<0.05$).

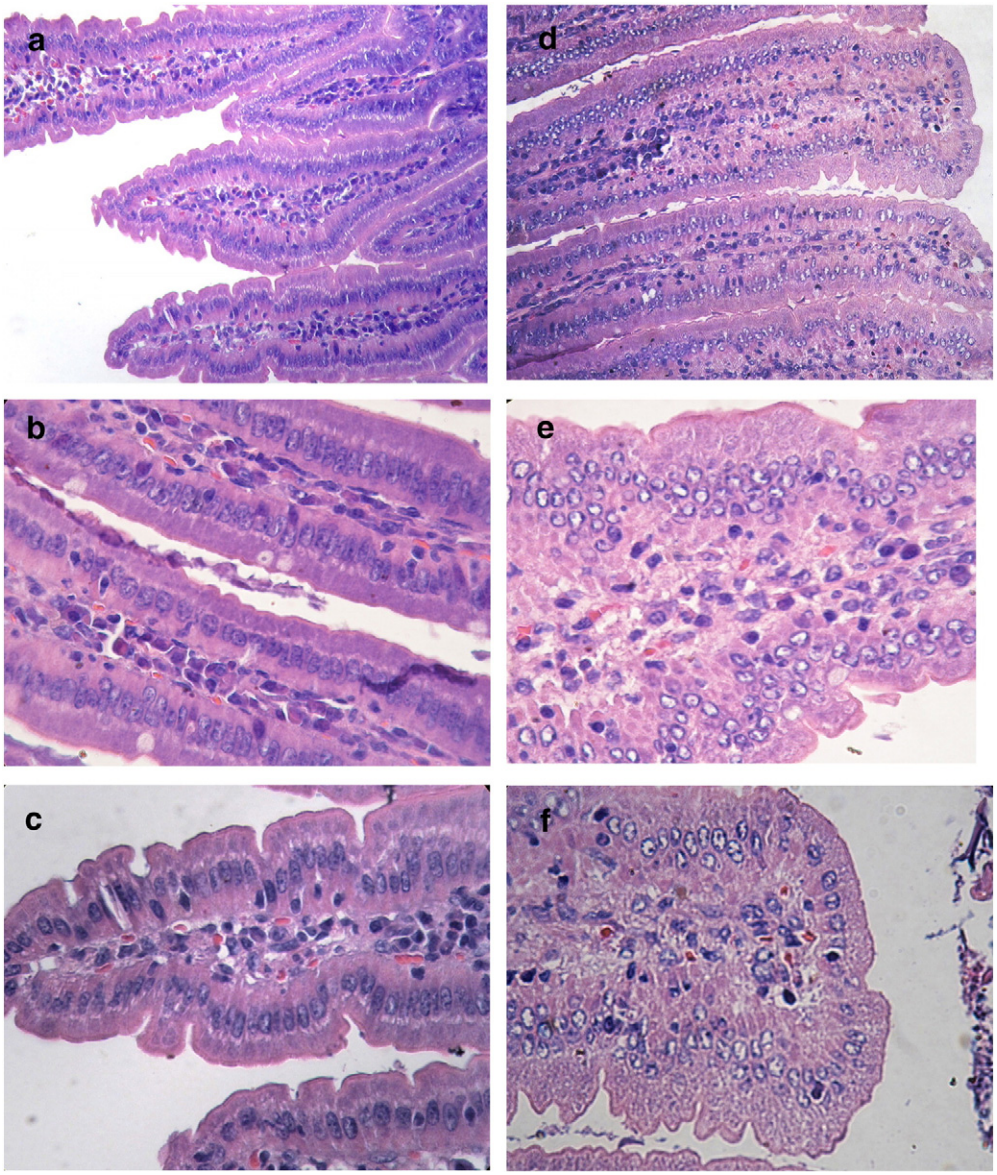

Fig. 3. Representative light microscopy photomicrographs of histological slides stained with Hematoxylin–Eosin of the small intestine of Balb/c mice without any treatment (Group 1) (panels a, b and c) and those fed during three weeks with Con A (Group 2) (panels c, d and e). Magnification: panels (a) and (d), 40×; panels (b), (c), (e), (f), 100×.

CRL 1198 during 3 weeks showed an increase in this counts up to 3×10^6 CFU g^{-1}. Simultaneous feeding of Group 4 with Con A did not inhibit the adhesion of propionibacteria. One week after the end of propionibacteria consumption, their number in Groups 3 and 4 fell to the range of 10^3 to 10^4 CFU g^{-1} found in Groups 1 and 2 showing that the strain is gradually washed out as was reported in previous studies (Perez Chaia et al., 1995, 1999), Inclusion of *P. acidipropionici* CRL 1198 in the diet prevented the microbial modifications induced by Con A (Group 4) *i.e.* the enterobacteria proliferation and to a lesser extent that of enterococci.

3.4. Intestinal disaccharidase activities

In mice without any treatment (Group 1), disaccharidase activities did not show significant changes with age during the 4 weeks of the study although there was a tendency for lactase activity to decrease (Fig. 6). The pattern displayed by the control group (Group 1) was not affected by consumption of propionibacteria (Group 3). However long feeding with Concanavalin A led to a significant decrease in the specific activities of lactase, sucrase, and trehalase whereas the activity of maltase was decreased to a lesser extent ($P<0.05$) (Fig. 6). In general, consumption of Con A supplemented with propionibacteria (Group 4) resulted in activities similar to those of Groups 1 and 3 (Fig. 6). One week after treatments were finished lactase and maltase activities of Con A treated mice were completely restored whereas sucrase and trehalase were still below control values ($P<0.05$).

In assay 2, the immediate effect of Con A on disaccharidase activities and the possible interaction with the enzyme, the substrate and the surface of propionibacteria were determined by pre incubating the lectin with these molecules or microorganisms prior to starting reactions. As seen in Fig. 7, Con A decreased lactase, sucrase and maltase activities in comparison with the control (*L vs C*) by interference with enzymes as was evidenced by the reduction observed by preincubation with homogenates before starting enzymes reactions (*LE vs C*). On the contrary trehalase activity seemed to be initially favored by Con A (assay 2) being this effect abolished after prolonged exposure of the mucosa to lectin (Assay 1; Fig. 6). The supernatant of propionibacteria had no effect on disaccharidase activities whereas supernatants of preincubation of propionibacteria with Concanavalin A exerted an effect less pronounced than lectin alone (*LP vs L*).

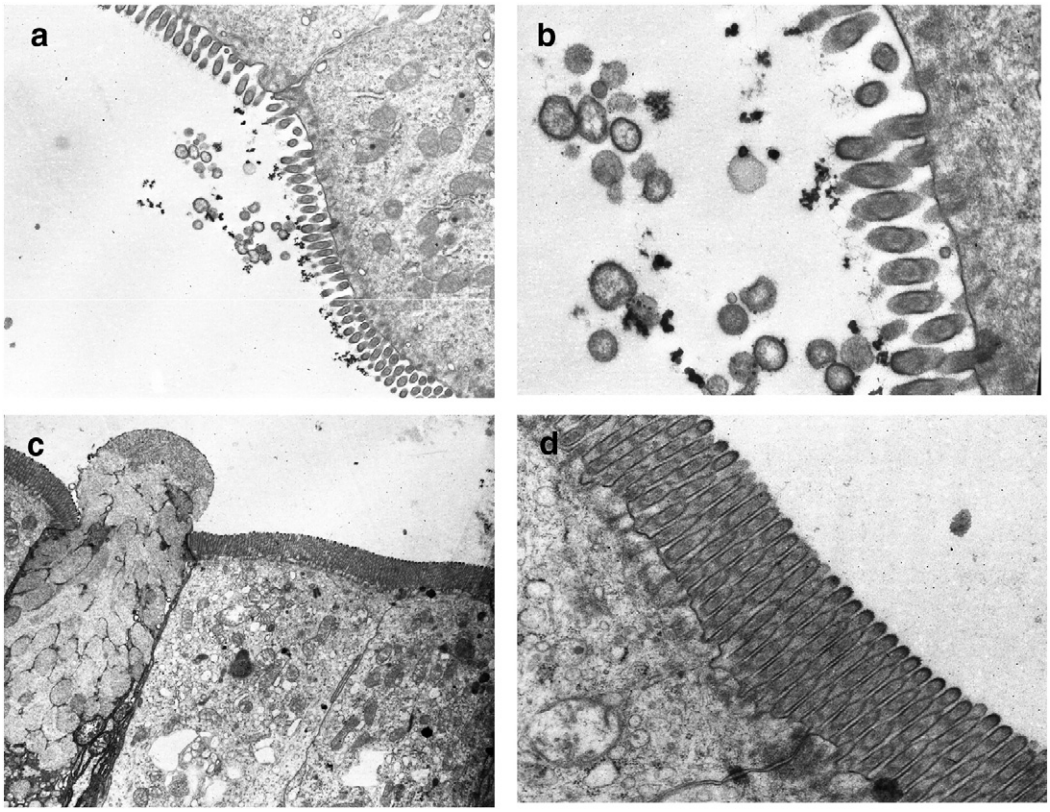

Fig. 4. Transmission electron microscopy photomicrographs of the microvillous surface of the small bowel of mice fed with Con A (Group 2) (panels a–b) and those that consumed lectin plus propionibacteria (Group 4) (panels c–d).

4. Discussion

Legumes (members of the Leguminosae family) are common components of the human and animal diets. In several developing countries of Latin America and Africa where animal protein is limited, beans are consumed in large quantities as a major source of dietary protein and some vitamins, minerals, and complex carbohydrates (Deshpande, 1992). However, in addition to these nutritional components, legumes also contain some antinutritional factors such as protease inhibitors, polyphenols, phytic acid and lectins, among others (De Mejía & Prisecaru, 2005).

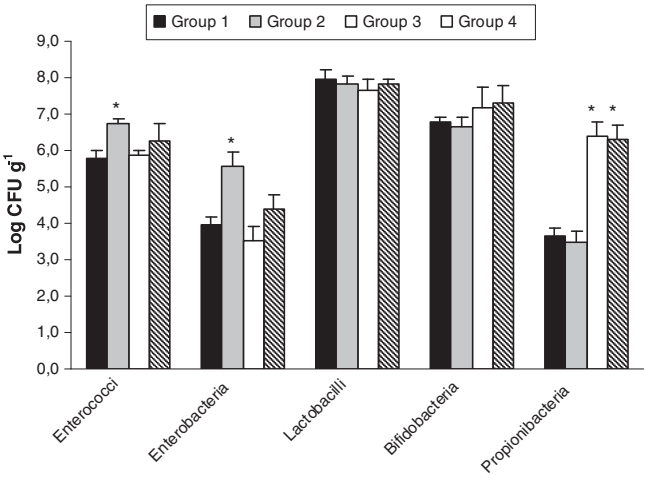

Fig. 5. Major aerobe-facultative anaerobe bacteria counted in the intestinal walls of Balb/c mice during and after treatments with Concanavalin A, *P. acidipropionici* CRL 1198 and lectin plus propionibacteria. Each bar represents the mean number of log colony-forming units (CFU) per gram of exfoliated mucosa. Group 1: Control; Group 2: Con A; Group 3: *Propionibacterium acidipropionici* CRL 1198; Group 4: Con A + *P. acidipropionici* CRL 1198. Values are means ± SD. The asterisk indicates significant differences with the control group (G1) ($P<0.05$).

Lectins are proteins which interact selectively and reversibly with specific residues of carbohydrates present in glycoconjugates (Sharon & Lis, 1995). Although their biological relevance as recognition molecules is unquestionable, their physiological role and impact on health are controversial since both beneficial and deleterious effects have been ascribed to different lectins (De Mejía & Prisecaru, 2005; Vasconcelos & Oliveira, 2004). It is well known that most plant lectins are highly resistant to degradation by gastric acid and enzymes of the small bowel, so after consumption, they reach the intestinal lumen in a bioactive state and bind to specific carbohydrates expressed on the glycocalyx of enterocytes affecting cellular physiology (Sharon & Lis, 1995). In this sense, significant amounts of ingested Con A were recovered unaltered from the cecal content of rats 4 h after its oral administration and from feces (90% recovery) 4 days later (Nakata & Kimura, 1985); whereas peanut lectin (PNA) has been extracted from human feces after peanut ingestion retaining hemagglutinating and pro-proliferative activity (Ryder, Jacyna, Levi, Rizzi, & Rhodes, 1998; Ryder et al., 1992). Lectins binding to the intestinal epithelium have also been showed by immunohistochemical staining, as a homogeneous lining along the gut mucosa up to 3 days after PHA inoculation in suckling rats (Linderoth et al., 2006).

According to the dose, the duration of exposure and other factors (such as the glycosylation status of the epithelium), the same lectin could exert toxic or beneficial effects. As an example, it is known that the consumption of raw or incompletely cooked red kidney beans induces acute gastroenteritis (Weinman, Allan, Trier, & Hagen, 1989) and it has been reported that the ingestion of *Phaseolus vulgaris* lectin (PHA) by rats, during 5 days, resulted in a significant less growth, protein utilization and glucose absorption (Donatucci, Liener, & Gross, 1987). This impairment in absorption does not appear

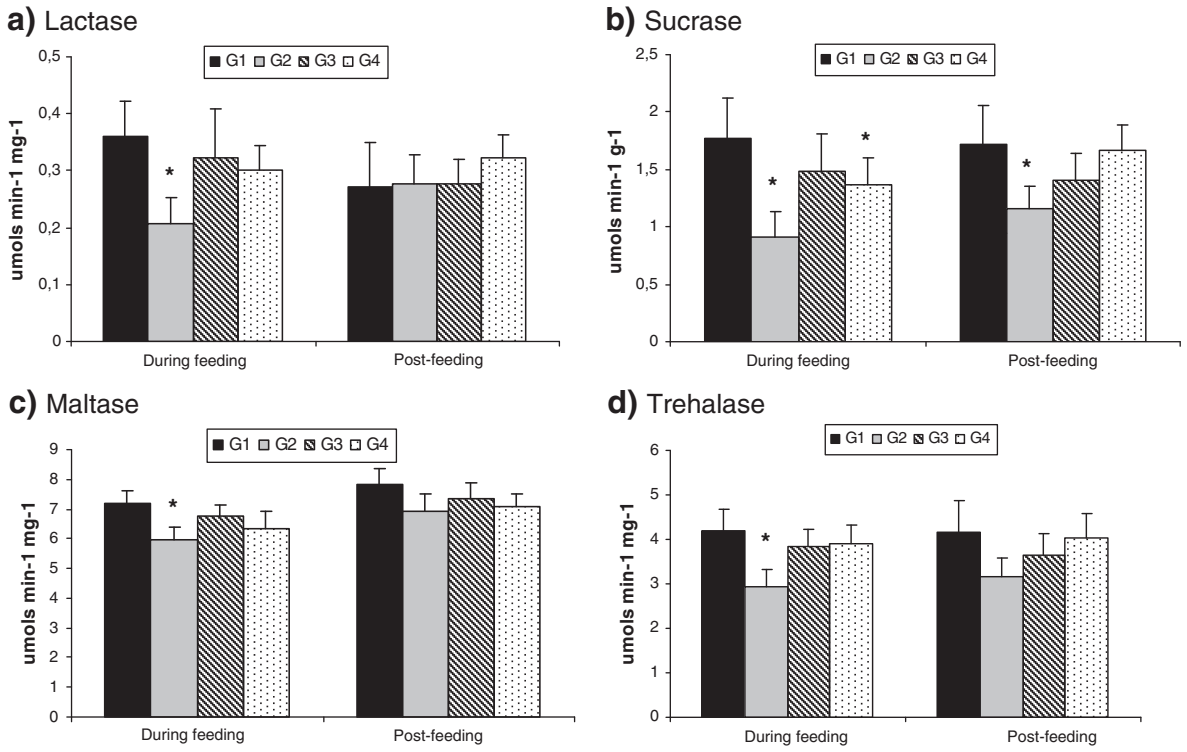

Fig. 6. Effect of Concanavalin A, *P. acidipropionici* CRL 1198 and lectin plus propionibacteria feeding on the disaccharidase activities of intestinal mucosa homogenates of Balb/c mice. G1: Control; G2: Con A, G3: *P. acidipropionici* CRL 1198, G4: Con A + CRL 1198. Values are means ± SD. The asterisk indicates significant differences with the control group (G1) ($P<0.05$).

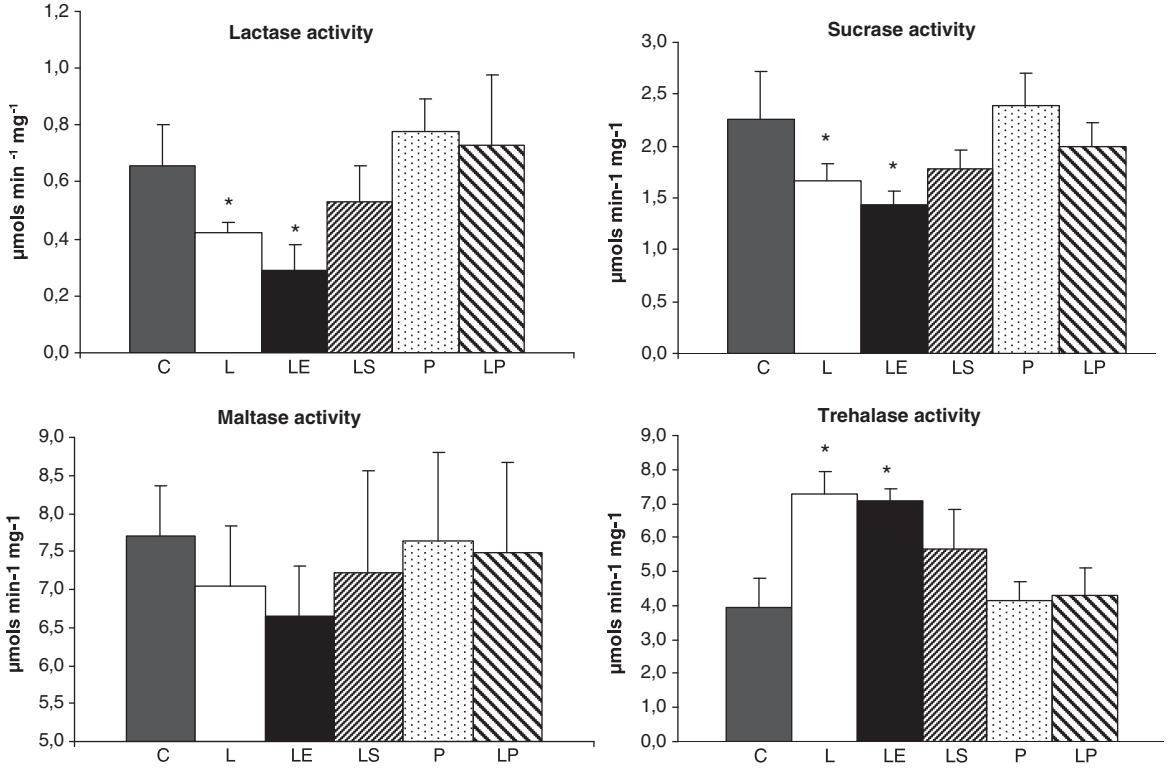

Fig. 7. Effect of immediate exposure of the intestinal mucosa homogenates of Balb/c mice to Concanavalin A and/or *P. acidipropionici* CRL 1198 supernatants on their disaccharidase activities. *C*: Saline solution (without Con A) added to the reaction mixture (Control); *L*: Con A added to the reaction mixture (400 µg/mL); *LE*: Con A pre-incubated for 30 min with the homogenate (enzyme) before starting the reaction; *LS*: Con A pre-incubated for 30 min with the substrate before starting the reaction; *P*: propionibacteria pre-incubated for 30 min with saline solution before addition of supernatant to the reaction. *LP*: Con A pre incubated with propionibacteria for 30 min before addition of supernatant to the reaction. The values are expressed as means ± standard deviation.

to be unique to glucose, as rats fed diets containing the lectin from kidney beans or Con A also exhibit malabsorption of such diverse nutrients as lipids, amino acids, vitamin B-12, calcium and sucrose (Ayyagari, Raghunath, & Rao, 1993; Banwell et al., 1983). The precise mechanism responsible for this impairment in the absorptive capacity of the intestines seems to be related with the profound changes in the morphology and ultrastructure of the intestine (lesions) and even the overgrowth of flora induced by the ingestion of lectins (Banwell et al., 1983; Banwell, Howard, Kabir, & Costerton, 1988). On the contrary, single or few oral inoculations of PHA to suckling rats and piglets induced structural and functional maturation of the gut evidenced as a decreased villi height, increased proliferation and crypt depth and a shift in the expression of enterocytes disaccharidases to an adult-type characterized by a decreased lactase and increased sucrase and maltase activities. In consequence, PHA has been proposed as an inductor of precocious maturation of the gut in order to better prepare the farm animals to digest and absorb the post weaning feed, as well as a treatment of mammals having an immature intestine and a therapy for individuals with a defective growth of the mucosa during total parenteral nutrition (Fitzgerald et al., 2001; Linderoth et al., 2005, 2006; Prykhod'ko, Fed'kiv, Linderoth, Pierzynowski, & Westrom, 2009; Radberg et al., 2001; Thomsson, Rantzer, Westrom, Pierzynowski, & Svendsen, 2007).

In the present study, long-term inoculation of adult Balb/c mice with Concanavalin A resulted in a less food efficiency since food consumption was not affected but animals gained less weights during this treatment, suggesting an alteration of the digestion/absorption function of the intestine in the presence of lectin. Some macroscopic alterations were also observed such as a huge enlargement of stomachs; and cecums with soft and gassy contents but without developing diarrhea. A higher cellularity but no lesions was observed at the optical microscopic level, however, alterations were induced at the ultrastructural level since a marked shortening and shedding of microvilli were evidenced in the lectin treated group. Similar results were reported previously by Lorenzsonn and Olsen (1982) who observed in the jejunum of normal rats, an increased shedding of brush border membranes, acceleration of cell loss and shortening of villi as acute effects after an intraluminal injection of Con A or WGA.

The structural modifications induced by Con A could be related to the microbial changes observed, such as the overgrowth of enterobacteria and enterococci populations. It is known that immature enterocytes of the small intestinal villi possess membrane receptors with a lot of polymannosylated residues whereas mature cells express receptors with complex type carbohydrate side chains and only few terminal mannose residues. Since Con A feeding seemed to-induce proliferation and turnover of the intestinal epithelium, it could be possible that some of the complex-type glycosyl side chains of cellular glycans of mature enterocytes were replaced by mannosylated residues that served as new adhesion sites for enterobacteria and/or the dietary lectin (Pusztai et al., 1993). Although Con A and members of enterobacteria possess the same sugar specificity and should compete for the same receptors, no inhibition of enterobacteria was exerted by Con A suggesting that probably too many receptors are available for binding. Microbiota proliferation could also be due to a stimulating effect of Con A on bacterial growth similar to the observed for *Bacillus cereus* (Lau & Chan, 1984). As was previously reported, propionibacteria prevented enterobacteria overgrowth in mice (Perez Chaia et al., 1995, 1999; Perez Chaia & Zárate, 2005) but the mechanism involved (like SCFA production) was not assessed in the present study. One week after treatments were finished, bacterial status of the small intestine of all groups reverted to control values.

Although the Jack bean is not a regular component of human diets, Con A is a good model to study the behavior of members of the mannose binding lectins family, which include, among others, lectins found in lentils and kidney beans. On the contrary, Jack bean represents an interesting alternative foodstuff for poultry. Although this legume may serve as a primary source of protein and energy for chicken, it has been reported that almost 10% of raw seeds in the diet (that is equivalent to approximately 0.3% of pure Con A), is enough to produce depressed feed intake and stunted growth of animals. Furthermore, continuous feeding of chicken with a diet containing Jack bean for 6-weeks, produced histological changes on the mucosa, with villi showing vascular degeneration and inflammatory reaction in the lamina propria followed by Con A endocytosis and transferal to the general circulation (Mendez, Vargas, & Michelangeli, 1998).

With respect to physiological effects, since lectins interact in the intestine with the mucosa membrane; it has been observed that the processes that take place at this level, such as hydrolysis of dietary components and nutrients transport may be affected leading to a low nutritional status. In suckling rats and piglets it has been reported that PHA affected brush border disaccharidases by decreasing lactase and increasing maltase and sucrase activities as an indication of enterocytes maturation (Linderoth et al., 2005; 2006; Radberg et al., 2001; Thomsson et al., 2007) whereas in adults rats and broiler chicken, lectins like Con A and other crude legumes decreased sucrase, maltase and pancreatic α amylase activities leading to nutrients malabsorption (Infante, García, Carmona, & Rivera, 2008; Nakata & Kimura, 1985; Rueda, Leon, Castañeda, Mendez, & Michelangeli, 2007).

In our study, disaccharidase activities were assayed during a period of 1 month of life of Balb/c mice. Only lactase activity seemed to be affected by aging and decreased during the 4 weeks of the study whereas the other disaccharidases remained stable. It is known that enzyme activities decline with age in a manner that likely involves a posttranscriptional process and this fact may have important implications for the digestion of carbohydrates by elderly (Ming-Fen, Robert, Robert, & Stephen, 1997). However, it is probable that 1 month is a short period within the adult lifetime of mice.

All the disaccharidases assessed were affected by Con A to some extent. Mice daily inoculated with Con A showed a great decrease in lactase, sucrase and trehalase activities whereas maltase seemed to be less affected. The immediate contact of Con A with the enzymes (assay 2), that could be resembled to an acute effect, inhibited in the same manner sucrase, lactase and maltase activities whereas trehalase was apparently activated indicating that long term effects could be different for some enzymes. The inhibition observed could be ascribed to interaction of Con A with the enzymes (*L* and *LE* in Fig. 7) whereas inhibition by interaction with the substrates was negligible in the conditions assayed (*LS*). Since the concentration of the disaccharides used in the reactions was five-fold higher than that of lectin, it should be discounted that any possible direct binding between the lectin and nutrients could make them unavailable for hydrolysis. In consequence, the observation that Con A pre or co-incubated with the enzymes decreased the hydrolysis of the corresponding disaccharides would suggest that binding of Con A to the brush border membrane hindered the accessibility of substrates to the active site of enzymes involved in disaccharide metabolism (Kim, Brophy, & Nicholson, 1976). Thus, it could be supposed that Con A at concentrations likely to be reached following the consumption of improperly processed lectin-containing foods, would have an immediate effect on uptake of some nutrients at intestinal level.

Different attempts have been made in order to prevent or counteract the deleterious effects of toxic or antinutritional dietary compound on the GIT, being of particular interest those that focus on a suitable complementary diet. In this sense, Ramadass, Dokladny, Moseley, Patel, and Lin (2010) have reported that the consumption of sucrose may reduce the toxic effects of legume lectins such as red kidney beans by protecting barrier function, bacterial overgrowth and bacterial translocation. In the same way, it has been proposed, that a high dietary intake of galactose-containing carbohydrates, such as galactose-containing vegetable fiber, would offer

protection against binding and proliferative effects of galactose-N-acetylgalactosamine-binding dietary lectins (such as PNA) on colonic neoplastic epithelium by removing free lectin from the colonic lumen (Evans et al., 2002; Ryder et al., 1998).

It is also known that plant lectins have the ability to interact with bacterial surfaces. In the intestinal ecosystem, where a complex microbiota inhabits free within the lumen or adhered to the mucosal surface, lectins can interact with the bacterial cells depending on the carbohydrates expressed on their cell walls. This lectin–bacteria interaction may interfere with the epithelial cell–lectin recognition process preventing some toxic effects. With this concept in mind, some bacteria with appropriated surface glycosidic moieties or adhesins could be consumed as a part of human or animal diets to interfere with the interaction between lectins and intestinal epithelial cells.

In a previous *in vitro* study (Zárate & Perez-Chaia, 2009), we observed that dairy propionibacteria were able to bind and remove different dietary lectins from the medium, decreasing their cytotoxicity on exfoliated colonocytes. In the present *in vivo* study we assessed the ability of one strain of propionibacteria daily consumed with Concanavalin A, to prevent some of the deleterious effects caused by this lectin on mice.

Long feeding with dairy *P. acidipropionici* CRL 1198 did not alter the structure of the small bowel nor the ultrastructure of enterocytes being the function of the brush border membranes not disturbed. Daily ingestion of these propionibacteria at the same time than Con A prevented the transient enlargement of organs and the disruption of brush border membranes being the disaccharidase activities conserved at all. Since the protective effects attained were observed during simultaneous feeding it could be suggested that the bacteria counteracted the effect of Con A on the mucosa by avoiding interaction of lectin with intestinal cells by at least one of two mechanisms: blockage of Concanavalin A receptors by the bacteria on the mucosa surface and/or binding of Concanavalin A on their surface contributing to reduce the amount of free lectin able to bind to the intestinal mucosa. It has been reported that propionibacteria have the ability to bind and remove toxic compounds like aflatoxins *in vivo* in the intestinal lumen of chicken (El-Nezami, Mykkanen, Kankaanpaa, Salminen, & Ahokas, 2000). However, probiotic properties are strain-dependent traits so that positive effects observed with one strain of propionibacteria like *P. acidipropionici* CRL 1198, could not be extrapolated to other microorganisms of the same species or genus.

5. Conclusion

In conclusion, results confirmed that Con A, the lectin present in raw jack bean seeds, may interfere with the digestive process in mammals, like mice, by disrupting brush border membranes and impairing carbohydrates digestion that leads, in turn, to the risk of colonic fermentation and osmotic diarrhea. This fact may help to explain the poorer growth observed on animals fed on diets containing this lectin. Consumption of suitable propionibacteria, like *P. acidipropionici* CRL 1198, at the same time than Con A administration, reduced the incidence of Con A-induced alterations in Balb/c mice and may be an effective tool to avoid lectins–epithelia interactions and its undesirable effects both in animals and humans. This positive effect could be adjudged to a barrier effect of *P. acidipropionici* CRL 1198 adhered to the mucosa (Zárate et al., 2002), or the binding of free lectin within the intestinal lumen (Zárate & Perez-Chaia, 2009). Further studies are actually ongoing to clarify the mechanism underlying this event and the effect of exogenous lectin on the mucous layer. Since it is the first report of dairy propionibacteria as *in vivo* protecting agent against dietary toxic lectins, investigations on their potential as probiotic detoxifying agents are strongly encouraged.

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