Journal of Functional Foods 37 (2017) 666–675

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Yacon flour (*Smallanthus sonchifolius*) attenuates intestinal morbidity in rats with colon cancer

Mariana Grancieri [a,*], Neuza Maria Brunoro Costa [a], Maria das Graças Vaz Tostes [a], Daniela Silva de Oliveira [a], Louisiane de Carvalho Nunes [b], Letícia de Nadai Marcon [a], Thaísa Agrizzi Veridiano [a], Mirelle Lomar Viana [a]

[a] Department of Pharmacy and Nutrition, Center for Exact, Natural and Health Sciences, Federal University of Espirito Santo, Alto Universitário, S/N, Guararema, 29500-000 Alegre, ES, Brazil
[b] Department of Veterinary Medicine, Center of Agrarian Sciences and Engineering, Federal University of Espirito Santo, Alto Universitário, S/N, Guararema, 29500-000 Alegre, ES, Brazil

ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form 20 August 2017
Accepted 23 August 2017
Available online 31 August 2017

Keywords:
Fructooligosaccharides
Prebiotics
Intestinal permeability
SCFA
pH

ABSTRACT

Yacon flour (YF) (*Smallanthus sonchifolius*) is source of fructooligosaccharides (FOS) which are prebiotic with effects unknown in the colorectal cancer (CRC). This study investigated the intestinal effects of supplementation with 7.5% FOS from YF for 8 weeks on CRC induced by 1.2-dimethylhydrazine (25 mg/kg/-body weight) in male Wistar rats. The animals were divided in groups: S (without YF and without induced CRC, n = 10), Y (with YF and without induced CRC, n = 10), C (without YF and with induced CRC, n = 10) and CY (with YF and with induced CRC, n = 10). The animals that received YF had a percentage reduction of aberrant crypts focus in more than 40%, lower intestinal permeability, luminal content more acidic and with higher concentrations of SCFA. In addition, there was an increase in the depth and number of colonic crypts. In conclusion, we observed that YF promoted beneficial effects on the intestinal health of animals with induced CRC.

1. Introduction

The incidence of non-communicable chronic diseases and their aggravations, such as cancer is gradually increasing worldwide. Colon cancer includes tumors that affect the colon and rectum. It is one of the most prevalent cancers worldwide, being the third most common type among men and the second among women, with 55% of the cases occurring in developed regions (Ferlay et al., 2012).

This type of carcinoma, as well as most cancer types, results from the interaction between endogenous factors, such as genetic predispositions to the development of chronic bowel diseases and older age, and environmental factors, such as the diet (INCA. Instituto Nacional do Câncer. Estimativa, 2016). Moreover, changes in the intestinal microbiota, like dysbiosis, facilitate the development of inflammatory processes that can promote the activation of carcinogenic components and the production of mutagenic compounds, such as free radicals (Clark, Robien, & Slavin, 2012).

Some bioactive compounds are related to the modulation of the intestinal microbiota, acting beneficially in many disorders that affect the gastrointestinal tract (Toloudi et al., 2015), including colorectal cancer. Among these compounds are the fructooligosaccharides (FOS) which are considered prebiotics. Prebiotics are food ingredients that are non-digestible by the human organism that result in beneficial effects by selectively stimulating the growth and/or activating the metabolism of probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium* spp (Rodríguez-Cabezas et al., 2010; Saad, 2006).

FOS stimulate the intestinal colonization of bifidobacteria because it is a substrate for these microorganisms, improving the intestinal function and preventing systemic infections derived from bacterial translocation that can cause sepsis and even death (Westerbeek, van den Berg, Lafeber, Fetter, & van Elburg, 2011).

Abbreviations: YF, yacon flour; CRC, colorectal câncer; FOS, fructooligosaccharides; SCFA, short-chain fatty acids; ACF, aberrant crypts focus.

* Corresponding author at: Department of Pharmacy and Nutrition, Center for Exact, Natural and Health Sciences, Federal University of Espirito Santo, Alto Universitário, S/N, Guararema, P.O. Box: 16, 29500-000 Alegre, ES, Brazil.

E-mail addresses: marianagrancieri@gmail.com (M. Grancieri), neuzambc@gmail.com (N.M.B. Costa), mgvaztostes@gmail.com (M.G. Vaz Tostes), dani_ufv@yahoo.com.br (D.S. de Oliveira), louisianecn@gmail.com (L.C. Nunes), leticiadenadai@gmail.com (L.N. Marcon), thaisa.agrizzi@gmail.com (T.A. Veridiano), mirellelomar@gmail.com (M.L. Viana).

http://dx.doi.org/10.1016/j.jff.2017.08.039
1756-4646/

Consumption of FOS changes the intestinal histology by increasing the depth and the number of total and bifurcated crypts (Lobo, Colli, Alvares, & Filisetti, 2007; Lobo, Gaievski, De Carli, Alvares, & Colli, 2014). In addition, it can reduce the number and multiplicity of aberrant crypt foci (ACF), the number of invasive adenocarcinomas and cell proliferation rate in tumors (Moura et al., 2012).

FOS are fermented by bifidobacteria producing short chain fatty acids (SCFA), such as acetate, propionate and butyrate. Butyrate is a source of energy to enterocytes, and it is able to reduce chemoresistance and to block the action of histone deacetylases in tumor cells leading to apoptosis. In addition, butyrate modulates the expression of glutathione S- transferase, which detoxifies carcinogenic compounds and compounds associated with oxidative stress (Moura et al., 2012; Rajendran et al., 2011).

Sources of FOS and inulin are restricted and new sources of these prebiotics are being investigated due its beneficial effects on colon cancer. Yacon *(Smallanthus sonchifolius)* is Andean root rich in FOS, which can represent 40% to 70% of the dry matter (Santana; Cardoso, 2008).

In this context, the consumption of prebiotics can be a potentially beneficial alternative to reduce the intestinal damage in CRC. Although promising, the yacon beneficial effects on intestinal health has been little investigated. Therefore, our goal is to evaluate the effects of yacon flour consumption in intestinal parameters in rats with induced colon cancer.

2. Material and methods

2.1. Animals

Forty-six male Wistar (207 ± 5 g body weight) rats obtained from Central Biotery of Federal University of Espirito Santo were used. Throughout the experiment, the animals were kept in a room at 22±2 °C, under a 12-h light-dark cycle and received water *ad libitum*. The study was approved by the Ethics Committee of Animals Use of the Federal University of Espirito Santo, protocol number 004/2014.

2.2. Experimental design

Of the 46 animals, 20 were kept healthy and 26 animals were treated with a weekly subcutaneous injection of 25 mg/kg body weight of 1.2-dimethylhydrazine (DMH- Sigma®), during the first five weeks to induce CRC. In the subsequent 8 weeks, an interval was given for the development of CRC. DMH was prepared immediately before use, by being dissolved in NaCl 0.9%, 15% EDTA and the pH was adjusted to 6.5.

At the end of the tumor induction period, in the 13th week of experiment, two animals treated with CRC induction were euthanized and their intestines were collected and analyzed (Bird, 1987), for confirmation of ACF development. The remaining 24 animals with CRC induction were divided into groups C and CY, and the 20 animals without CRC induction were divided into groups S and Y, as described below:

I. Group S: animals without colorectal cancer induction and without yacon flour; n = 10
II. Group Y: animals without colorectal cancer induction and with yacon flour; n = 10
III. Group C: animals with colorectal cancer induction and without yacon flour; n = 12
IV. Group CY: animals with colorectal cancer induction and with yacon flour; n = 12

All animals received a basal diet (In Vivo®) during the initial 13 weeks. In the 8 following weeks, the animals of groups S and C received AIN-93M diet, and Y and CY groups received the adapted AIN-93M diet, with yacon flour addition to in sufficient amounts to provide 7.5% of FOS (Lobo et al., 2011).

Individual body weight and food consumption were recorded weekly, being calculated in the end of the experimental period the mean of the total food intake (total food consumption group/*n*), and the mean of total weight gain (total weight gain group/*n*) and food efficiency coefficient (FEC = (total weight gain group/total food consumption group)/100). In the last week of yacon flour intervention, urine was collected for intestinal permeability analysis.

In the 22nd week of the study, the large intestine of two other animals from each cancer-inducing group (C and CY) were evaluated to confirm the presence of neoplastic lesions in these groups. Then, finally, the other animals were anaesthetized by intraperitoneal administration of 0.2 mL/100 g body weight of anesthetic solution with 37.5% ketamine, 25% xylazine and 37.5% of saline solution. Afterwards, blood was collected by cardiac puncture leading to hypovolemia and consequent euthanasia of the animals. In addition, the large intestine was extracted, as well as its luminal contents in order to perform, respectively, histologic and short chain fatty acids and pH analyses (Fig. 1).

2.3. Yacon flour preparation and analyses

The yacon was purchased from a rural producer from Santa Maria de Jetibá- ES/ Brazil. The yacon flour preparation was carried out as detailed by Vaz-Tostes et al. (2014), and the analyses of carbohydrates, proteins, fats, fiber, ash and humidity were evaluated using the AOAC (1997). The analyses were performed in triplicate. All flour used to prepare the diets belonged to the same lot. FOS and inulin content in the yacon flour were determined by High Performance Liquid Chromatography (HPLC) with a BIO-RAD brand HPX-87p column (lead stationary phase) using purified water as the mobile phase. Samples were diluted (1 g in 100 ml distilled water), centrifuged at 12.000 rpm and then filtered through a Millipore brand polyvinylidene fluoride membrane (PVDF), with 0.22 μm pore size and 13 mm diameter. Then, samples were injected into the liquid chromatograph, Varian brand, Pro-STAR 410 model, with refractive index detector and autosampler (AUTO SAMPLER 410) with a flow of 0.6 ml min^{-1} and column temperature of 80 °C, projecting a sequence of peaks which were compared with standard curves predefined in the equipment. The yacon flour composition is presented in Table 1.

2.4. Experimental diet

The experimental diet was based on the AIN93-M diet, as recommended by the American Institute of Nutrition (Reeves, Nielsen, & Fahey, 1993). The diet of groups Y and CY was supplemented with 7.5% of FOS from yacon flour. Since the yacon flour contained 52.2% of FOS, 14.37 g of YF/100 g of diet was added. From the yacon composition, the diets, with and without yacon flour, were adjusted to present similar amounts of proteins, fibers, simple carbohydrates and calories (Table 2). Diets were stored in a refrigerator for a maximum period of fifteen days before consumption, in order to avoid the degradation of the FOS present in the diets.

2.5. Histological analysis

The large intestine was washed with distilled water, and a longitudinal incision was performed in the opposite mesenteric band with the inner mucosa of the intestine facing up, and fixed in 10% formalin for 24 h.

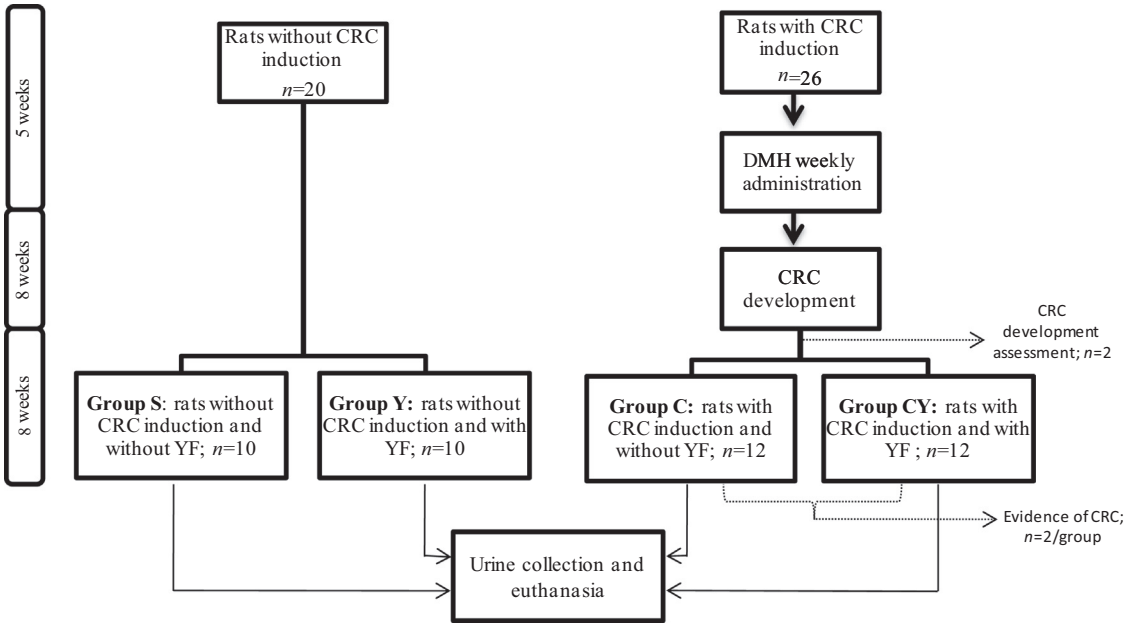

Fig. 1. Diagram of the study. CRC = colorectal cancer; DMH = 1.2-dimethylhydrazine; YF = yacon flour.

Table 1
Composition of yacon flour.

Components	Quantity (%)
Fructose	8.16
Glucose	3.76
Saccharose	7.25
FOS	52.20
Inulin	6.61
Fibres	10.68
Proteins	4.52
Lipids	0.33
Fibres	10.68
Moisture	5.92
Ash	2.94

Table 2
Composition of AIN-93M diet with and without supplementation with yacon flour.

Ingredients (g/100 g)	S and C groups AIN-93M	Y and CY groups AIN-93M + YF
Casein	14.0	13.14
Dextrinized starch	15.5	15.5
Saccharose	10.0	7.24
Soy oil	4.0	4.0
Fiber (microfine cellulose)	5.0	0
Mix Minerals	3.5	3.5
Mix Vitamínico	1.0	1.0
L-cystine	0.18	0.18
Bitartrate Choline	0.25	0.25
Maize starch	46.57	40.81
Yacon flour	0	14.37
Nutritional composition		
Yacon flour (kcal g^{-1})	0	1.65
Caloric Density (kcal g^{-1})	3.72	3.54

S = group without colon cancer induction and without yacon flour; C = group with colon cancer induction and without yacon flour; Y = group without colon cancer induction and yacon flour; CY = group with colon cancer induction and yacon flour. YF = yacon flour.

2.5.1. Aberrant crypt foci

After being fixed in formalin, the intestine was measured and equally divided in three fragments: proximal, medial and distal in relation to the caecum. Then, it was stained with a 1% solution of methylene blue for 1 min and washed with phosphate-buffered saline (PBS). The surface of the intestinal mucosa was observed with an optical microscope with a 4X objective lens to identify the ACF (Bird, 1987).

Two independent and trained observers carried out a blind count of the ACF on the mucosal surface of the large intestine (Henriques et al., 2009) with adjustments. They were quantified separately: foci with less than three aberrant crypts (ACF $\leq$ 3) and foci with more than three aberrant crypts (ACF > 3), the latter being indicative of more advanced neoplasic lesions. Moreover, the percentage reduction of total ACF, ACF > 3 and ACF $\leq$ 3 was evaluated. For this, the ACF percentage presented by the CY group was calculated and the median ACF in the control group (C) was considered 100%. This result was subtracted from 100% resulting in the ACF percentage reduction of the test groups compared to the control group.

The ACF images were digitized using a trinocular optical microscope Prolab® B-350, with a 4X magnification, coupled with a Dyno-eye® digital camera with Dean Capture 2.0 capture software.

2.5.2. Qualitative histology of colon

After counting the ACF, the mucosal tissue was immersed in ammonia-ethanol for 12 h. Then, it was washed in water, placed in cassettes and processed in histotechnician (OMA®) for 10 h. After this time, the intestines were included in paraffin blocks and then 5 μm thick histological sections were performed and stained with hematoxylin-eosin.

Afterwards, snapshots of the histological section slides were taken, with a dimension of 1280 × 1024 pixels, using the 4X objective. Each capture corresponded to a microscopic field. Five snapshots of each colonic segment (proximal, medial and distal) were taken, with a total of 15 microscopic fields on each slide. In addition, they were evaluated depth, apical and basal diameter of 3 crypts per field and values were expressed in μm. Also, counting of total number of crypts per microscopic field. These images were made with a trinocular optical microscope Prolab® B-350 coupled with a digital camera Dyno-eye® with capture software Dino Capture 2.0.

2.6. Intestinal permeability

The animals were kept in fasting for 12 h and then 2 mL of solution containing 200 mg of lactulose and 100 mg of mannitol was

administered by gavage. After the administration, the animals were kept in metabolic cages and fasted for 5 h. Urine collection was carried out for 24 h, its volume was measured, recorded and stored at −80 °C (Song et al., 2011). For lactulose and mannitol analyses, the animals urine was filtered through a 0.45 μm membrane filter and allocated in vials for analysis by HPLC method. We used Lactulose®, Mannitol® (Sigma-Aldrich, São Paulo/SP, Brasil) as internal standards. The areas obtained by the curves were calculated and converted to g/L in order to calculate the percentage of urinary excretion of lactulose and mannitol and the proportion of lactulose/mannitol (Vilela et al., 2008).

2.7. Determination of caecal short chain fatty acids (SCFA)

SCFA, acetate, propionate and butyrate in the colonic content of animals were evaluated. For the extraction of these fatty acids, 100 mg of luminal contents were diluted in 2 ml of 10% perchloric acid solution, which was mixed by vortexing for 5 min, centrifuged (9000*g*, 10 min) and filtered through a 0.45 μm membrane filter, as described by Kotani et al. (2009) with modifications. Immediately after the extraction, HPLC analysis was conducted, using Acetic acid®, Butyric acid® and Propionic acid® (Sigma-Aldrich, São Paulo/SP, Brasil) as internal standards. The areas obtained by the curves were calculated and converted to mg/g of feces.

2.8. Chromatographic conditions

The analyses of lactulose, mannitol and SCFA were performed on a Shimadzu HPLC system (Kyoto, Japan). The chromatographic system consists of: degasses (Model DGU-14A), pump (Model LC-10AT), auto- sampler (Model SIL-20A), column oven (Model CTO-10AS) and UV–Vis detector (Model SPD-10AV) connected in series with a refractive index detector (Model RID-10A). The analytical column used was Aminex HPX-87 H (300 cm × 8.7 mm) from BIO-RAD (California, USA). The HPLC analyses were performed at 55 °C, pressure of 1920 psi, under isocratic conditions. The mobile phase consisted of water in H_2SO_4 0.005 mM with injection volume of 20 μL (Sá, De Oliveira, Cammarota, Matos, & Ferreira-Leitão, 2011).

2.9. Intraluminal pH of the colon

After removing the large intestine of the animals, the caecal luminal contents were removed, weighed, diluted in a 1:10 proportion in saline solution, and homogenized by vortexing. Afterwards, pH reading was performed using a pH meter (Kasvi®).

2.10. Statistical analysis

The statistical analysis was performed using the statistical software Statistica®, version 10. The samples were tested by Kolmogorov-Smirnov normality test and the groups with a normal distribution were tested for the effects of yacon flour and colorectal cancer and/or their interactions using "Two-way" ANOVA (variance analysis) followed by Newman-Keuls post hoc ($p < 0.05$). Samples that did not show normal distribution were transformed (using the $\log_{10}$ function). Data were expressed as mean ± standard error (SE), and considered significant when $p < 0.05$.

3. Results

3.1. Food consumption, body weight gain and food efficiency coefficient

Colorectal cancer had influence on the animals food consumption ($p = 0.0011$). The C group showed statistically higher food consumption compared to the S and Y groups, but similar to the CY group (Table 3). There was no difference among the other experimental groups.

Table 3
Mean total food intake, weight gain and food efficiency coefficient of animals.

Variables	S	Y	C	CY	*p*		
					YF	CRC	YF × CRC
Weight gain (g)	78.06 ± 11.3[a]	64.25 ± 11.30[a]	91.29 ± 10.3[a]	88.96 ± 10.8[a]	0.4647	0.0904	0.6023
Feed intake (g)	1403.30 ± 39.1[b]	1357.25 ± 39.1[b]	1550.79 ± 35.7[a]	1475.96 ± 37.3[ab]	0.1183	0.0011	0.7059
FEC	5.55 ± 0.8[a]	4.55 ± 0.8[a]	5.93 ± 0.7[a]	6.13 ± 0.7[a]	0.7037	0.2506	0.5112

Values expressed as mean ± SE. Different letters in the same line: groups are significantly different ($p \leq 0.05$). *p* = "two way" ANOVA of the effects of FY and CRC and the interaction of them. S = group without colon cancer induction and without yacon flour (n = 10); C = group with colon cancer induction and without yacon flour (n = 10); Y = group without colon cancer induction and with yacon flour (n = 10); CY = group with colon cancer induction and with yacon flour (n = 10). YF = yacon flour; CRC = colorectal cancer. FEC = food efficiency coefficient.

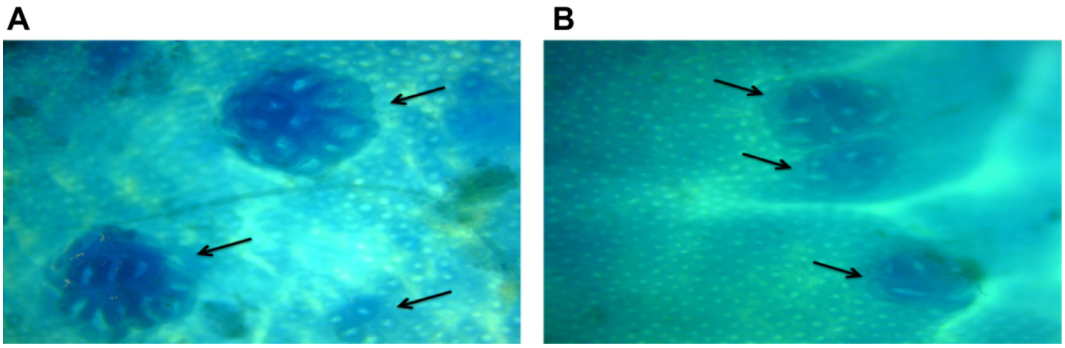

Fig. 2. Aberrant crypt focus presence of colon Wistar rats. A = mucosa area with two foci containing countless aberrant crypt and one a focus with >3 aberrant crypt of animal C group (group with colon cancer induction and without yacon flour). B = mucosa area with three foci containing >3 aberrant crypts of animal CY group (group with colon cancer induction and yacon flour). Objective: 4X. Colorant: Methylene blue.

Considering the body-weight gain and the FEC, yacon flour and CRC did not influence these variables, with no significant difference between the experimental groups ($p > 0.05$) (Table 3).

3.2. Histological analysis

3.2.1. Aberrant crypt foci

The CRC had an effect on the formation of ACFs ($p < 0.0001$). The cancer induction groups (C and CY) had a higher number of ACF, considering total ACF, ACF ≤ 3 and ACF > 3, when compared to those without cancer induction (S and Y). The same response pattern was observed for total ACF by segment of the colon ($p < 0.0001$) (Figs. 2 and 3).

In addition to the categorical analysis of ACF, the percentage reduction of ACF was calculated in the CY group compared to the C group. There was a reduction of 41.22%, 47.22% and 47.22% of total ACFs, ≤ 3 and >3 of the CY group when compared to the C group, respectively.

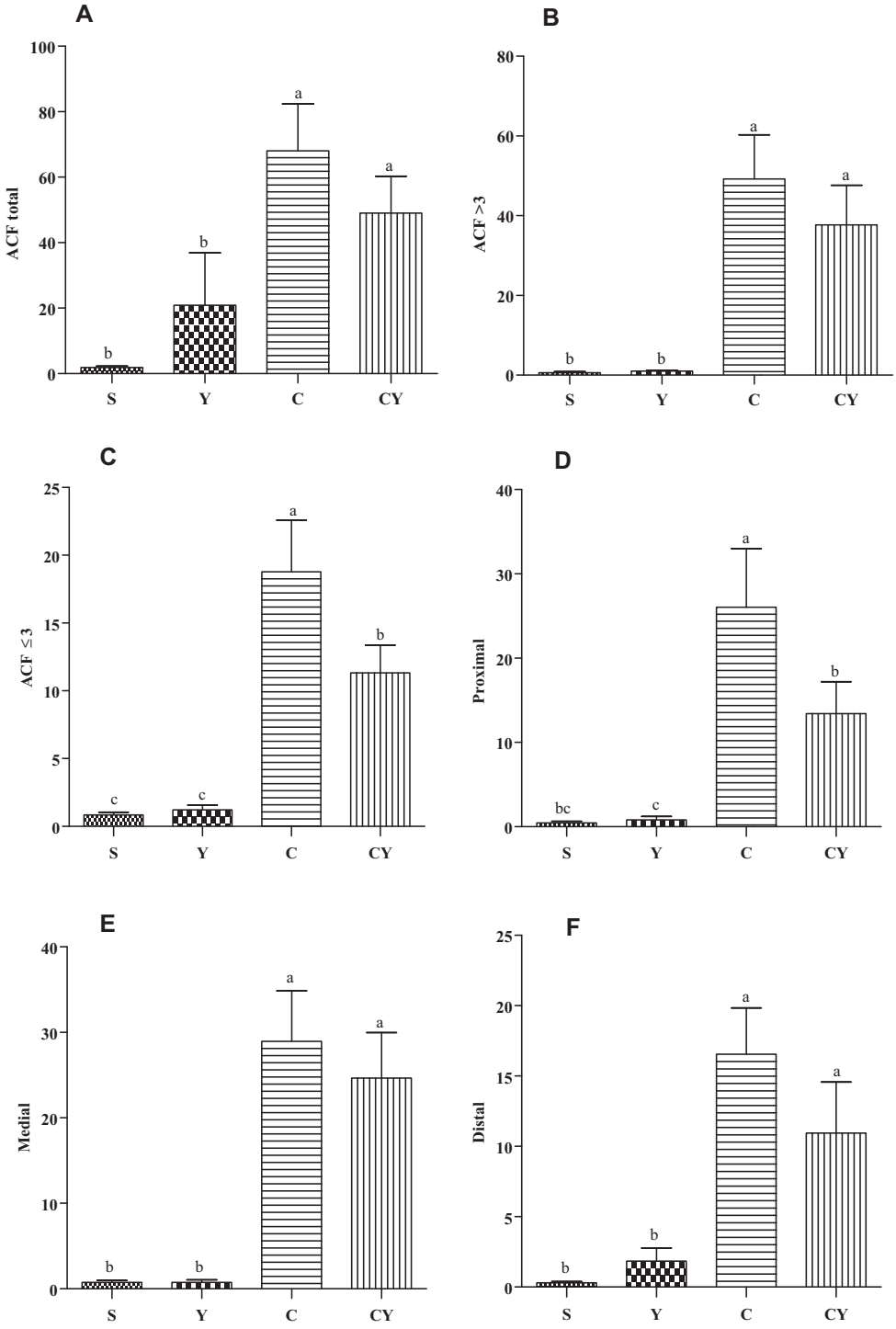

Fig. 3. Average values of aberrant crypt focus (ACF): total (A), more than three (B) and less than three (C) crypts per focus and total ACF in proximal (D), medial (E) and distal (F) the large intestine segment. Values expressed as mean ± SE. Different letters are groups significantly different ($p \leq 0.05$) by "two way" ANOVA of the effects of yacon flour and colorectal cancer and the interaction of them. S = group without colon cancer induction and without yacon flour (n = 10); C = group with colon cancer induction and without yacon flour (n = 10); Y = group without colon cancer induction and with yacon flour (n = 10); CY = group with colon cancer induction and with yacon flour (n = 10).

3.2.2. Qualitative histology of the colon

Yacon flour consumption and the presence of CRC did not affect the apical (AD) and basal (BD) diameters of colonic crypts. However, yacon flour was able to increase the number of crypts (NC) per microscopic field ($p = 0.0478$) and the depth of the colonic crypts (DC) ($p = 0.0020$). The Y and CY groups had a higher crypt depth when compared to the C group ($p = 0.0177$; $p = 0.0114$, respectively). The S group did not differ from any other experimental group (Fig. 4).

3.3. Intestinal permeability

The yacon flour exerted a significant effect by reducing levels of urinary mannitol excretion ($p < 0.0001$). The groups that consumed yacon flour (Y and CY) did not differ between them and presented the lowest mannitol urinary excretion values (Fig. 5). Considering the interaction, the factors, yacon flour and colorectal cancer, showed a significant interaction ($p = 0.0268$), therefore in the presence of yacon flour intake, there was a reduction of mannitol urinary excretion, even in the presence of neoplasia.

For the urinary excretion percentage of lactulose, the yacon flour had a significant effect in reducing the excretion of urinary lactulose ($p < 0.0001$). Groups without yacon flour consumption (C and S) had higher values compared to groups with consumption (Y and CY). For the ratio lactulose/mannitol (L/M), there was a positive effect of the yacon flour on intestinal permeability in CRC ($p = 0.0051$) (Fig. 5). The R^2 values of lactulose and mannitol standard curve were respectively, 0.998 and 0.997.

3.4. Short chain fatty acids

The yacon flour increased the luminal levels of propionate ($p < 0.0001$) and butyrate ($p = 0.0006$), but had no effect on acetate. While the acetate had its levels reduced in the presence of CRC ($p < 0.0001$) (Fig. 6). The R^2 values of the standard curve of propionate, butyrate and acetate were 0.999 for all SCFA.

3.5. Intraluminal pH

The groups that consumed yacon flour (Y and CY) showed a lower pH (more acidic) compared to groups that did not consume it (C and S), demonstrating the significant effect of this flour in reducing pH ($p < 0.0001$) (Fig. 6).

4. Discussion

The yacon is cultivated worldwide, and the Andean region is the largest producer and consumer of this tuberous root (Santana,

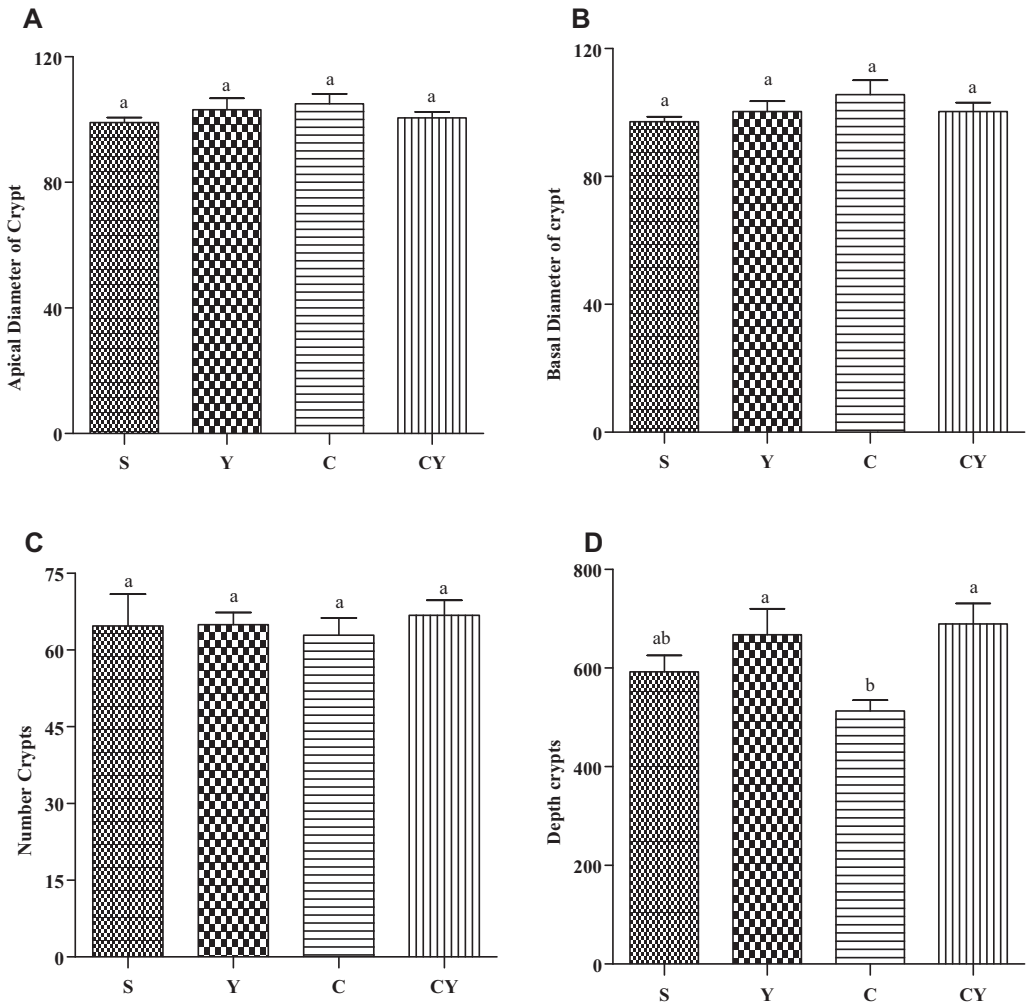

Fig. 4. Morphological characteristics of crypts (μm). Values expressed as mean ± SE. Different letters are groups significantly different ($p \leq 0.05$) by "two way" ANOVA of the effects of yacon flour and colorectal cancer and the interaction of them. S = group without colon cancer induction and without yacon flour (n = 10); C = group with colon cancer induction and without yacon flour (n = 10); Y = group without colon cancer induction and with yacon flour (n = 10); CY = group with colon cancer induction and with yacon flour (n = 10).

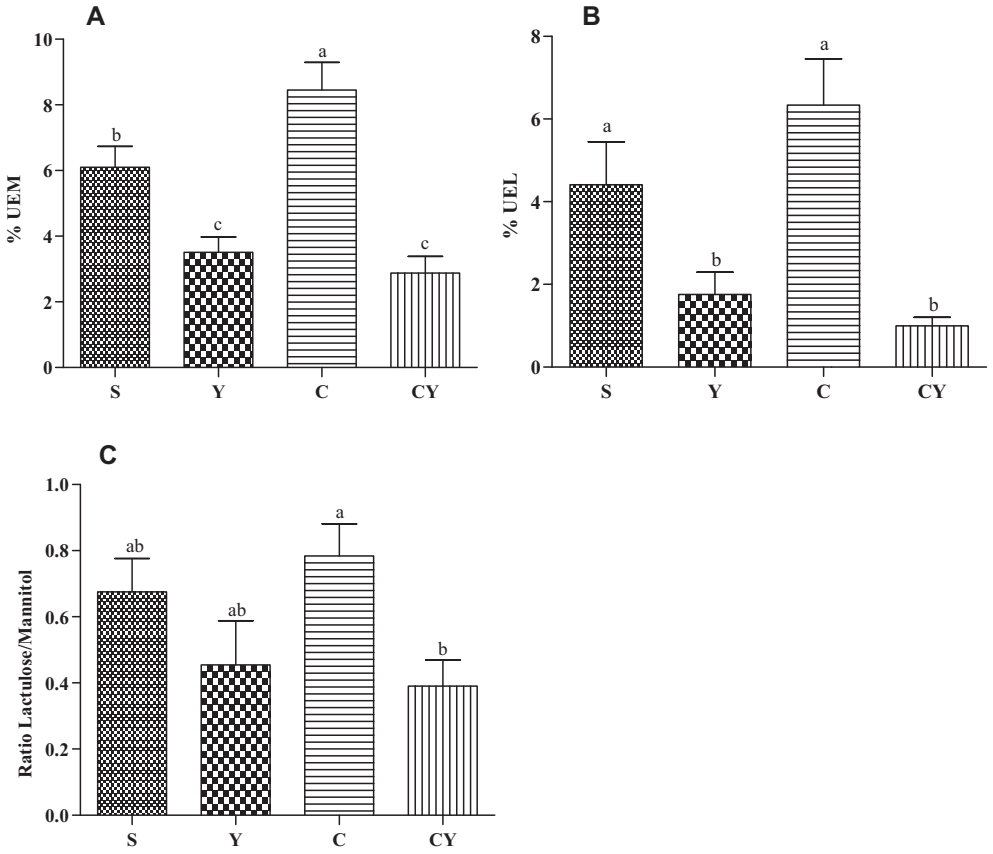

Fig. 5. Percentage of urinary excretion of lactulose and mannitol and the ratio between them. Values expressed as mean ± SE. Different letters are groups significantly different ($p \leq 0.05$) by "two way" ANOVA of the effects of yacon flour and colorectal cancer and the interaction of them. S = group without colon cancer induction and without yacon flour (n = 10); C = group with colon cancer induction and without yacon flour (n = 10); Y = group without colon cancer induction and with yacon flour (n = 10); CY = group with colon cancer induction and with yacon flour (n = 10). %UEM = urinary excretion of mannitol; %EUL = urinary excretion of lactulose.

Cardoso, 2008). Its consumption is associated with many health benefits, among them, its ability to modulate the intestinal microbiota, immune function (Vaz-Tostes et al., 2014) and effects on colorectal cancer (Moura et al., 2012).

In this study, colorectal cancer increased food intake of the animals and maintained weight gain and food efficiency coefficient FEC. These are plausible results, since malnutrition in colon and rectum cancer is rare, a fact that can be explained by the smaller change in food intake, lack of absorptive nutritional disorders, minimal metabolic changes and absence of obstructive factors or hormonal action for cachexia in this neoplasia (Fortes, Recôva, Melo, & Novaes, 2007; Moura et al., 2012).

Yacon flour intake reduced the intraluminal pH, corroborating other studies evaluating yacon flour effects in rats (Lobo et al., 2007; Rodrigues et al., 2012). The FOS fermentation by intestinal bacteria is able to produce SCFA, mainly acetate, propionate and butyrate in the colonic lumen, what reduces the intraluminal pH. The more acidic luminal content promotes benefits to the host due to the harming effects in the growth of opportunistic pathogens, for inducing membrane ruptures in gram-negative bacteria, such as *Escherichia coli* and *Salmonella spp,* inhibiting their growth (Alakomi et al., 2000). Moreover, a more acid pH improves the minerals solubility and increases intestinal absorption (Rodríguez-Cabezas et al., 2010).

The SCFA in feces is involved in many beneficial processes to the body, such as decreased absorption of cholesterol, stimuli to the growth of beneficial bacteria and increased mucus secretion (Rodríguez-Cabezas et al., 2010). Some studies suggest that SCFA can suppress inflammation and cancer by increasing local immune response, decreasing colon pH and promoting amine and ammonia excretion (Raman et al., 2013; Rolim, 2015). In this study, consumption of yacon flour increased production of propionic and butyric acids, suggesting beneficial effects of the flour. Other studies showed increased butyrate and acetate in rats with colorectal cancer after consuming yacon extract, but propionate levels did not change (Moura et al., 2012).

Among the benefits associated with higher levels of lumen SCFA is the ability to promote hypertrophy of intestinal mucosal cells, leading to increased surface area in the intestine. As evidenced in this study, with a dose of 7.5% of FOS, derived from yacon flour, there was an increase in the depth of colonic crypts and the number of crypts. It is believed that the increase in crypts occurs due to the fermentation of FOS, promoting increased absorptive surface area (Weaver, Martin, Story, Hutchinson, & Sanders, 2010).

Other studies showed, similar to this, the highest fermentation in the caecum, increase in diameter of the intestinal wall (Lobo et al., 2011), increased fission and cell proliferation in the crypts of the caecum (Lobo et al., 2014) in rats consuming 7.5% of FOS derived from yacon flour. At a dose of 6800 mg FOS/kg cecal, hypertrophy was observed in healthy rats (Genta, Cabrera, Grau, & Sánchez, 2005) and with 5% FOS, an increase in cell density and crypt formation in pigs colon (Campos et al., 2012). All these effects, like in our study, were linked to beneficial effects of FOS in the intestinal architecture.

The ACF found in the groups treated with DMH, C and CY groups, demonstrates the effectiveness of the carcinogenesis model described by Bird (1987). Similar from what is described in the literature, the ACF are observed more frequently in middle and distal colon (Henriques et al., 2009), in the present study the ACF were also mainly observed in the medial segments.

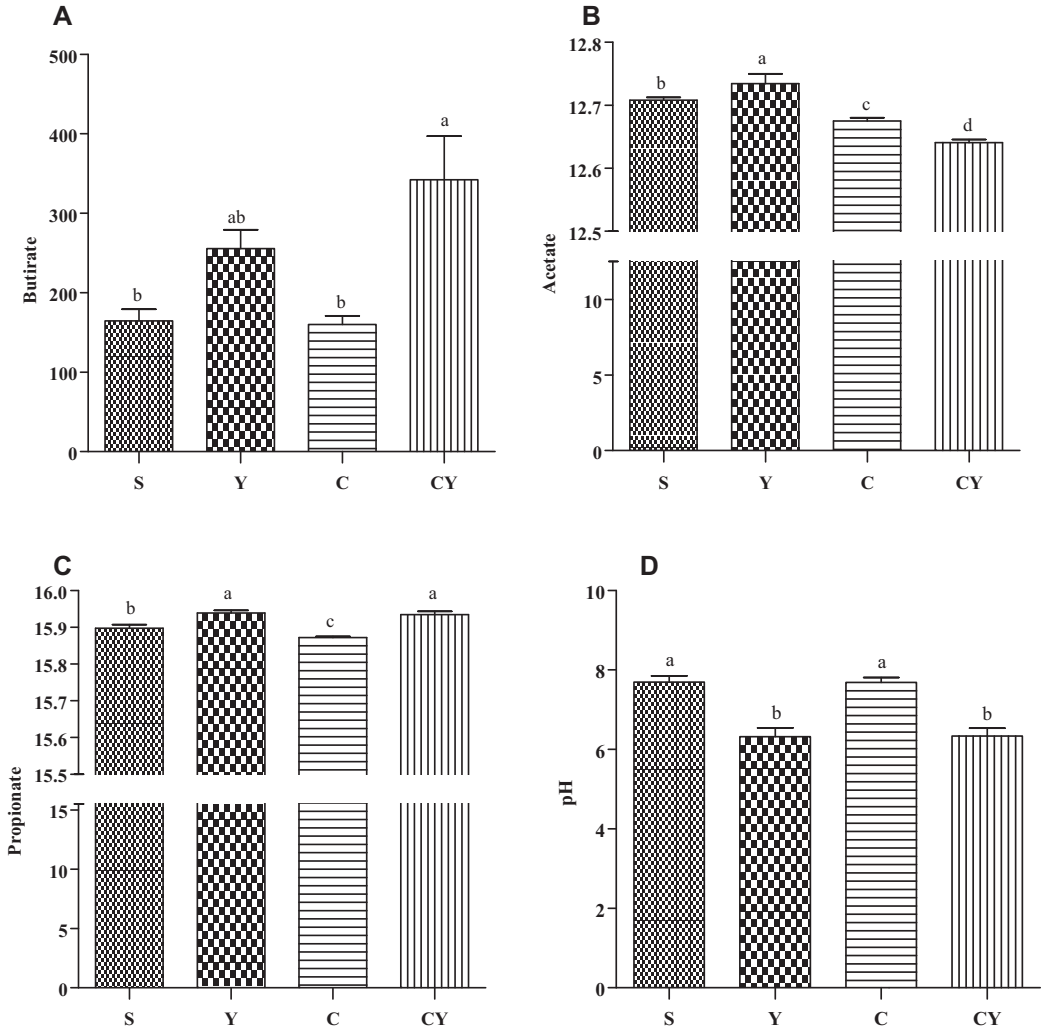

Fig. 6. SCFA excretion of acetate (mg/g), propionate (mg/g) and butyrate (mg/g) and intraluminal pH. Values expressed as mean ± SE. Different letters are groups significantly different ($p \leq 0.05$) by "two way" ANOVA of the effects of yacon flour and colorectal cancer and the interaction of them. S = group without colon cancer induction and without yacon flour (n = 10); C = group with colon cancer induction and without yacon flour (n = 10); Y = group without colon cancer induction and with yacon flour (n = 10); CY = group with colon cancer induction and with yacon flour (n = 10).

It is believed that prebiotics can reduce the process of carcinogenesis by inducing considerable change in the intestinal microbiota with increased number of bifidobacteria. Furthermore, prebiotics act to reduce the formation of ACF which are markers that anticipate the process of colonic carcinogenesis. Therefore, suggesting that they have the potential to suppress such cancer by modifying the colonic microbiota (Marques & Waitzberg, 2000).

Our study showed that the consumption of yacon flour decreased by more than 40% the values of all classes of ACF, highlighting the clinical performance of the consumption of this flour. In this study the fermentation of FOS resulted in increased production of butyrate which is associated with biological properties against colon carcinogenesis in rats (Pool-Zobel & Sauer, 2007). In carcinoma cells, butyrate has been shown to inhibit cell proliferation and angiogenesis, in addition to inducing apoptosis (Davis & Milner, 2009).

Probiotics, prebiotics or symbiotic can modulate the immunologic resistance of the host against inflammatory bowel diseases (Pool-Zobel, 2005; Pool-Zobel & Sauer, 2007), and they can provide protective effects against early biomarkers and development of tumors in the colon of rats treated with carcinogens (Moura et al., 2012).

In our study we found out that animals that did not induce CRC (S and Y groups) also had ACF in their intestines. This can have occurred once the animals at the end of the study were elderly, about 32 weeks old, and were male. It is known that the latter two are risk factors for the development of CRC (Barzi, Lenz, Labonte, & Lenz, 2013; INCA, 2016; Roshan, Tambo, & Pace, 2016).

Studies have shown that consumption of food sources of FOS and / or inulin increased the rate of apoptosis in the colon of rats (Hughes & Rowland, 2001), reduced the number and multiplicity of ACF, number of invasive adenocarcinomas and cell proliferation rate both in colonic crypts and in tumors, in addition to decreasing the multiplicity of invasive tumors when offered in addiction to an probiotic (Moura et al., 2012), indicating the beneficial effects of FOS fermentation on the intestinal mucosa and colon carcinogenesis.

In addition to these effects, FOS's ability to alter the intestinal microbiota is closely related to beneficial immunological and metabolic reactions, preventing imbalances that may result in increased intestinal permeability. This condition can cause bacterial translocation, inflammation and others complications. Moreover, the infiltrating lymphocytes and the intestinal mucosa start to produce proinflammatory cytokines, which have a key role in

angionenesis, inhibition of apoptosis, and stimulation of cell proliferation (Chalkias et al., 2011).

In the present study, the yacon flour was able to reduce the urinary excretion of lactulose and mannitol, even in the group with induced colon cancer (CY group), showing its capacity to decrease intestinal permeability. Therefore, improvement of the barrier function exercised by the mucosa resulted a reduction of antigen permeation and inflammatory reaction. Therefore, if a particular nutrient/food is able to improve intestinal permeability, it may provide clinical benefits in the treatment of intestinal diseases, such as colon cancer (Vilela et al., 2008).

The yacon flour may have this beneficial effect on intestinal permeability, since FOS fermentation by bifidobacteria stimulates the production of mucus and antimicrobial peptides, such as defensins and cathelicidins. Moreover, it promotes the production of SCFA and bacteriocins or microcins, and stimulates the proliferation of bifidobacteria which contributes to the intestinal barrier function by competing with pathogens for binding sites on epitelial cells and on the overlying mucosa layer. Also, FOS can increase the production and release of sIgA, and decrease changes in the tight junctions, so the junctions between enterocytes and colonocytes become narrow, making the passage of microorganisms and/or their toxins more difficult (Barton & Kagan, 2009).

Studies have shown that XOS prebiotic was ineffective on intestinal permeability in rats (Christensen, Licht, Leser, & Bahl, 2014) and that butyrate improved the intestinal barrier (Venkatraman, Ramakrishna, Pulimood, Patra, & Murthy, 2000). However, studies that evaluated the effects of yacon flour on intestinal permeability in rats did not exist, and our work is the first showing such benefits.

5. Conclusion

The consumption of yacon flour promoted beneficial effects on the intestinal health, attenuating changes promoted by colorectal cancer in an animal model, as it resulted in a reduction of intestinal permeability, increased luminal levels of SCFA, increased depth of colonic crypts, reduction of intraluminal pH, and reduction of ACF percentage. Many of these results were described for the first time, which may lead future works to elucidate the mechanisms of yacon flour action in these processes, such as the knowing of the intestinal microbiota composition.

Acknowledgements

The study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (grant numbers 486353/2013-3), and scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology, 66*(5), 2001–2005.

AOAC (1997). *Official methods of analysis of the AOAC* (12th ed). Washington.

Barton, G. M., & Kagan, J. C. (2009). A cell biological view of Toll-like receptor function: Regulation through compartmentalization. *Nature Reviews Immunology, 9*(8), 535–542.

Barzi, A., Lenz, A. M., Labonte, M. J., & Lenz, H. J. (2013). Molecular pathways: Estrogen pathway in colorectal cancer. *Clinical Cancer Research, 19*(21), 5842–5848.

Bird, R. P. (1987). Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. *Cancer Letters, 37*(2), 147–151.

Campos, D., Betalleluz-Pallardel, I., Chirinos, R., Aguilar-Galvez, A., Noratto, G., & Pedreschi, R. (2012). Prebiotic effects of yacon (*Smallanthus sonchifolius* Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity. *Food Chemistry, 135*(3), 1592–1599.

Cardoso, I. S. M. H. (2008). Raiz tuberosa de yacon (*Smallanthus sonchifolius*): potencialidade de cultivo, aspectos tecnológicos e nutricionais. *Ciência Rural, 38*(3).

Chalkias, A., Nikotian, G., Koutsovasilis, A., Bramis, J., Manouras, A., Mystrioti, D., et al. (2011). Patients with colorectal cancer are characterized by increased concentration of fecal hb-hp complex, myeloperoxidase, and secretory IgA. *American Journal of Clinical Oncology, 34*(6), 561–566.

Christensen, E. G., Licht, T. R., Leser, T. D., & Bahl, M. I. (2014). Dietary xylo-oligosaccharide stimulates intestinal bifidobacteria and lactobacilli but has limited effect on intestinal integrity in rats. *BMC Research Notes, 7*(1), 1.

Clark, M. J., Robien, K., & Slavin, J. L. (2012). Effect of prebiotics on biomarkers of colorectal cancer in humans: A systematic review. *Nutrition Reviews, 70*(8), 436–443.

Davis, C. D., & Milner, J. A. (2009). Gastrointestinal microflora, food components and colon cancer prevention. *The Journal of Nutritional Biochemistry, 20*(10), 743–752.

Ferlay J. et al. (2013). GLOBOCAN 2012 v1.0, *Cancer incidence and mortality worldwide: IARC cancer base no. 11*. Lyon, France: International Agency for Research on Cancer. Available from: <http://globocan.iarc.fr> (accessed on 10 dez 2015, Internet).

Fortes, R. C., Recôva, V. L., Melo, A. L., & Novaes, M. R. C. G. (2007). Hábitos dietéticos de pacientes com câncer colorretal em fase pós-operatória. *Revista brasileira de cancerologia, 53*(3), 277–289.

Genta, S. B., Cabrera, W. M., Grau, A., & Sánchez, S. S. (2005). Subchronic 4-month oral toxicity study of dried *Smallanthus sonchifolius* (yacon) roots as a diet supplement in rats. *Food and Chemical Toxicology, 43*(11), 1657–1665.

Henriques, V. T., Dias, C. M. G. C., Franceschini, S. D. C. C., Sabarense, C. M., Costa, N. M. B., Leite, J. I. A., et al. (2009). Ácidos graxos ômega-3 reduzem o desenvolvimento de lesões pré-neoplásicas. *Revista de Nutrição, 22*(2), 237–244.

Hughes, R., & Rowland, I. R. (2001). Stimulation of apoptosis by two prebiotic chicory fructans in the rat colon. *Carcinogenesis, 22*(1), 43–47.

INCA. Instituto Nacional do Câncer. Estimativa (2016). *Incidência de Câncer no Brasil*. Rio de Janeiro: Coordenação de Prevenção e Vigilância. <http://www.oncoguia.org.br/pub/material_apoio/material_apoio_299f_151205_2713.pdf> (accessed 10.12.15).

Kotani, A., Miyaguchi, Y., Kohama, M., Ohtsuka, T., Shiratori, T., & Kusu, F. (2009). Determination of short-chain fatty acids in rat and human feces by high-performance liquid chromatography with electrochemical detection. *Analytical Sciences, 25*(8), 1007–1011.

Lobo, A. R., Cocato, M. L., Borelli, P., Gaievski, E. H., Crisma, A. R., Nakajima, K., et al. (2011). Iron bioavailability from ferric pyrophosphate in rats fed with fructan-containing yacon (*Smallanthus sonchifolius*) flour. *Food Chemistry, 126*(3), 885–891.

Lobo, A. R., Colli, C., Alvares, E. P., & Filisetti, T. M. (2007). Effects of fructans-containing yacon (*Smallanthus sonchifolius* Poepp & Endl.) flour on caecum mucosal morphometry, calcium and magnesium balance, and bone calcium retention in growing rats. *British Journal of Nutrition, 97*(04), 776–785.

Lobo, A. R., Gaievski, E. H., De Carli, E., Alvares, E. P., & Colli, C. (2014). Fructo-oligosaccharides and iron bioavailability in anaemic rats: The effects on iron species distribution, ferroportin-1 expression, crypt bifurcation and crypt cell proliferation in the caecum. *British Journal of Nutrition, 112*(08), 1286–1295.

Marques, C. G., & Waitzberg, D. L. (2000). Fibras alimentares e cancer. In D. L. Waitzberg (Ed.), *Nutrição oral, enteral e parenteral na prática clínica* (3rd ed.. São Paulo: Atheneu.

Moura, N. A., Caetano, B. F., Sivieri, K., Urbano, L. H., Cabello, C., Rodrigues, M. A., et al. (2012). Protective effects of yacon (*Smallanthus sonchifolius*) intake on experimental colon carcinogenesis. *Food and Chemical Toxicology, 50*(8), 2902–2910.

Pool-Zobel, B. L. (2005). Inulin-type fructans and reduction in colon cancer risk: Review of experimental and human data. *British Journal of Nutrition, 93*(S1), S73–S90.

Pool-Zobel, B. L., & Sauer, J. (2007). Overview of experimental data on reduction of colorectal cancer risk by inulin-type fructans. *The Journal of Nutrition, 137*(11), 2580S–2584S.

Rajendran, P., Delage, B., Dashwood, W. M., Yu, T. W., Wuth, B., Williams, D. E., et al. (2011). Histone deacetylase turnover and recovery in sulforaphane-treated colon cancer cells: Competing actions of 14-3-3 and Pin1 in HDAC3/SMRT corepressor complex dissociation/reassembly. *Molecular Cancer, 10*(1), 1.

Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., et al. (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes, 4*(3), 181–192.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. Jr, (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition, 123*(11), 1939–1951.

Rodrigues, F. C., Castro, A. S. B., Rodrigues, V. C., Fernandes, S. A., Fontes, E. A. F., de Oliveira, T. T., et al. (2012). Yacon flour and Bifidobacterium longum modulate bone health in rats. *Journal of Medicinal Food, 15*(7), 664–670.

Rodríguez-Cabezas, M. E., Camuesco, D., Arribas, B., Garrido-Mesa, N., Comalada, M., Bailón, E., et al. (2010). The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats. *Clinical Nutrition, 29*(6), 832–839.

Rolim, P. M. (2015). Development of prebiotic food products and health benefits. *Food Science and Technology (Campinas), 35*(1), 3–10.

Roshan, M. H., Tambo, A., & Pace, N. P. (2016). The role of testosterone in colorectal carcinoma: pathomechanisms and open questions. *EPMA Journal, 7*(1), 22.

Sá, L. R. V., De Oliveira, M. A. L., Cammarota, M. C., Matos, A., & Ferreira-Leitão, V. S. (2011). Simultaneous analysis of carbohydrates and volatile fatty acids by HPLC

for monitoring fermentative biohydrogen production. *International Journal of Hydrogen Energy, 36*(23), 15177–15186.
Saad, S. M. I. (2006). Probiotics and prebiotics: The state of the art. *Revista Brasileira de Ciências Farmacêuticas, 42*(1), 1–16.
Song, P., Zhang, R., Wang, X., He, P., Tan, L., & Ma, X. (2011). Dietary grape-seed procyanidins decreased postweaning diarrhea by modulating intestinal permeability and suppressing oxidative stress in rats. *Journal of Agricultural and Food Chemistry, 59*(11), 6227–6232.
Toloudi, M., Apostolou, P., Chatziioannou, M., Eleni, K., Ioanna, V., Georgia, M., et al. (2015). A possible clinical benefit of the identification and characterization of colon cancer stem cells. *Asian Pacific Journal of Tropical Disease, 5*(1), 22–27.
Vaz-Tostes, M. G., Viana, M. L., Grancieri, M., dos Santos Luz, T. C., de Paula, H., Pedrosa, R. G., et al. (2014). Yacon effects in immune response and nutritional status of iron and zinc in preschool children. *Nutrition, 30*(6), 666–672.
Venkatraman, A., Ramakrishna, B. S., Pulimood, A. B., Patra, S., & Murthy, S. (2000). Increased permeability in dextran sulphate colitis in rats: Time course of development and effect of butyrate. *Scandinavian Journal of Gastroenterology, 35*(10), 1053–1059.
Vilela, G. E., De Lourdes, A. F. M., Oswaldo, G. T. H., Guerra, P. A., Aguirre, C. C. A., Martins, P. F., et al. (2008). Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission. *Scandinavian Journal of Gastroenterology, 43*(7), 842–848.
Weaver, C. M., Martin, B. R., Story, J. A., Hutchinson, I., & Sanders, L. (2010). Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. *Journal of Agricultural and Food Chemistry, 58*(16), 8952–8957.
Westerbeek, E. A., van den Berg, A., Lafeber, H. N., Fetter, W. P., & van Elburg, R. M. (2011). The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo-and acidic oligosaccharides on intestinal permeability in preterm infants. *British Journal of Nutrition, 105*(02), 268–327.