Research Article

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Yacón (*Smallanthus sonchifolius*) prevented inflammation, oxidative stress, and intestinal alterations in an animal model of colorectal carcinogenesis

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Abstract

BACKGROUND: Yacón (*Smallanthus sonchifolius*) roots store carbohydrate in the form of prebiotic fructooligosaccharides (FOS), which improve intestinal health. Yacon has the potential to prevent the intestinal barrier alterations associated with colorectal cancer (CRC). This study aimed to investigate the preventive effects of yacón flour (YF) on alterations promoted by CRC induced by 1,2-dimethylhydrazine in rats.

RESULTS: CRC increased tumor necrosis factor alpha levels (group CY = 10.2 ± 0.72; group C = 9.6 ± 1.0; group Y = 5.8 ± 0.54; group S = 5.95 ± 0.6 pg mL^{-1}) and short-chain fatty acid production, and decreased total antioxidant capacity (group CY = 4.7 ± 0.72; group C = 3.3 ± 0.3; group Y = 4.1 ± 0.47; group S = 6.7 ± 0.78 U mL^{-1}). Furthermore, YF treatment reduced intraluminal pH (group CY = 6.45 ± 0.47; group C = 7.65 ± 0.44; group Y = 6.75 ± 0.46; group S = 8.13 ± 0.2), lactulose/mannitol ratio, tumor necrosis factor-alpha (TNF-α)/interleukin (IL)-10 ratio, and increased secretory immunoglobulin A (group CY = 9.48 ± 1.46; group C = 10.95 ± 3.87; group Y = 15.95 ± 7.36; group S = 9.19 ± 1.52), but did not affect IL-10, IL-12, and TNF-α levels nor the IL-12/IL-10 ratio.

CONCLUSION: YF as a source of fructooligosaccharides may help to maintain the integrity of intestinal health, which is altered in induced CRC in rats.

Keywords: fructooligosaccharides; prebiotics; inflammation; intestinal barrier; oxidative stress

INTRODUCTION

According to the World Health Organization, approximately 9.6 million people worldwide died of cancer in 2018.[1] Cancer is an end-result of several successive cellular changes characterized by the formation and growth of abnormal cell populations that accumulate DNA mutations.[2] Colorectal cancer (CRC) is the fourth most common cause of death for men and the third for women worldwide, and by 2030 the global burden of CRC is expected to increase by 60%.[1–3]

CRC affects the colon and rectum. It is a multifactorial disease influenced by genetic and environmental factors as well as lifestyle factors, such as diet (low fiber and high red meat intake), physical inactivity, smoking, alcohol, and obesity.[4] CRC results from a sequence of mutations, activations, and deletions along a genetic pathway starting with the initial loss of the adenomatous polyposis coli (APC) tumor-suppressor gene leading to adenoma-carcinoma.[5,6] Moreover, intestinal health could be associated with the development of CRC since the transformation of epithelial cells with genetic mutations is associated with loss of epithelial barrier function. Cancer cells produce high demand of reactive oxygen species (ROS) due their constant proliferation, and as a result there is a less antioxidant capacity of the organism. Therefore, this oxidative damage formed contributes to change the epithelial barrier.[7,8] Hence, translocation of luminal bacteria into the lamina propria is facilitated. This translocation triggers inflammatory responses, activates myeloid cells, and further promotes epithelial-cell proliferation, which results in a gradual loss of functions and carcinogenesis.[5–7]

In this sense, bioactive compounds have been shown antioxidant effects, such as the phenolic compound hispidin that protects against DNA damage and hydroxyl radical formation,[9] anthocyanin from blackberry that suppresses ROS production, and improves the mitochondrial integrity.[10] Also, it was observed that the fermentation from intestinal bacteria increases the

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antioxidant capacity of polyphenols.[11] Therefore, the maintenance of intestinal health with bioactive compounds may be helpful in the prevention of CRC.[6] Yacón (*Smallanthus sonchifolius*) originates from the Andean region of South America and is consumed in its natural form, as a flour or syrup, and as an additive in various products. It is a rich source of fructooligosaccharides (FOS), which are inulin-type prebiotic fructans linked by β-(2-1) or β-(2-6) bonds with low degrees of polymerization that vary from three to ten units.[12] Previous studies reported the positive effects of consuming yacón on satiety,[13] antioxidant activity,[14] modulation of the immune system,[15] and hypolipidemic effect.[16]

Prebiotics are substrates that are selectively used by host probiotic microorganisms, which confer health benefits.[17] In the colon, prebiotics are fermented by beneficial bifidobacteria to produce short-chain fatty acids (SCFAs), namely, acetate, propionate, and butyrate.[18] Butyrate is a preferred energy source of colonocytes and has direct effects, such as, regulation of gene expression, epithelial barrier function, apoptosis, growth arrest, and cell differentiation.[19] Prebiotics also stimulate the production of anti-inflammatory cytokines and enhance intestinal barrier function by secreting antioxidative and anticarcinogenic compounds.[20] Therefore, yacón has a prebiotic effect by promoting the growth of probiotic bacteria, as evidenced by high levels of SCFA in cecal material, enhancing cell density, and forming crypts.[21]

A recent study by our group showed that yacón flour (YF) has potential beneficial effects on intestinal health when it is consumed after CRC develops.[22] Our hypothesis is that the intake of YF before the induction of colorectal carcinogenesis may be more effective in attenuating physiological changes caused by carcinogenesis. In this context, the aim of this study was to investigate the preventive effects of YF on the integrity of the intestinal barrier, inflammatory response, and total antioxidant capacity (TAC) resulting from colorectal carcinogenesis. Moreover, we investigated the beneficial effect of YF at 50 g kg^{-1} FOS in the diet, which is more tolerable and less prone to promote diarrhea than the 75 g kg^{-1} used in the previous study.

MATERIALS AND METHODS

Animals

Forty-four male adult Wistar rats (4 weeks old), with an average initial weight of 185.16 ± 19.99 g, were used. Animals were housed individually in stainless steel cages with a 12 h light/12 h dark cycle at room temperature (22 ± 2°C) with *ad libitum* water. The experiment was approved by the Ethics Committee of Animal Use of the Federal University of Espirito Santo (UFES), protocol number 017/2016 approved on August 5, 2016.

Experimental design

The 44 animals were divided into four experimental groups:

Group S: without CRC induction and without YF supplementation (n = 10);

Group Y: without CRC induction and with YF supplementation (n = 10);

Group C: with CRC induction and without YF supplementation (n = 12);

Group CY: with CRC induction and with YF supplementation (n = 12).

During the 16-week experimental period, groups S and C received the AIN-93M diet.[23] Groups Y and CY received the adapted AIN-93M diet that contained YF in a quantity sufficient to provide 5 g kg^{-1} FOS.[21] Figure 1 summarizes the experimental study.

CRC was induced in groups C and CY by subcutaneous injection of 25 mg kg^{-1} of 1,2-dimethylhydrazine (DMH) (Sigma®, St Louis, MO, USA) once a week, for five consecutive weeks (weeks 4–8). DMH was dissolved in sodium chloride (NaCl, 9 g kg^{-1}), ethylenediaminetetraacetic acid (EDTA, 150 g kg^{-1}) and the pH was adjusted to 6.5 immediately before use. The 8 week period immediately following the final injection was the interval for CRC development.[24]

In the last week of the experiment, the animals were housed in metabolic cages for 24 h and the urine was collected for intestinal permeability analysis. Individual food intake and body weight were recorded weekly and used for food efficiency ratio (FER) calculation at the end of the experiment: FER = (body weight gain/food consumption) × 100.

Yacón flour (YF) preparation

The yacón was obtained from a rural farmer at Santa Maria de Jetibá, ES, Brazil and was prepared as previously described.[15] The analyses of carbohydrates, proteins, fats, fiber, and ash were completed using the AOAC method.[25] FOS and inulin contents in the YF were determined (CERAT, São Paulo, Brazil) by high-performance liquid chromatography (HPLC) with a BIO-RAD brand HPX-87p column, Santa Maria de Jetibá, ES, Brazil (lead stationary phase) using purified water as the mobile phase. Samples were diluted in distilled water (1:100 *w/v*), centrifuged at 3000 × *g*, and then filtered through a Millipore polyvinylidene fluoride (PVDF) membrane with 0.22 µm pore size and 13 mm diameter. Samples were then injected into a liquid chromatograph, Varian brand Pro-STAR 410 model, with a refractive index detector and auto sampler (AUTO SAMPLER 410) with a flow of 0.6 mL min^{-1} and column temperature of 80 °C. This resulted in a sequence of peaks, which were compared with standard curves predefined in the equipment. YF analysis identified 280.95 g kg^{-1} FOS, 60.34 g kg^{-1} inulin, 40.52 g kg^{-1} protein, 3.3 g kg^{-1} lipids, 29.4 g kg^{-1} ash, 106.8 g kg^{-1} total fiber, 59.2 g kg^{-1} moisture, and 403.2 g kg^{-1} other carbohydrates.

Experimental diets

The experimental diets were prepared according to the recommendations of the American Institute of Nutrition, AIN-93M.[23] Groups S and C received the AIN-93M diet. The diet of groups Y and CY was supplemented with YF in amounts sufficient to provide 50 g kg^{-1} FOS. The YF used in this study contained 289.5 g kg^{-1} FOS, so 172.7 g of YF was added per 1 kg of AIN-93M diet. The amounts of casein, sucrose, starch, and dietary fiber in the diets of the experimental groups were adjusted to ensure that all groups had similar intakes of calories, carbohydrates, proteins, and fiber (Table 1).

Cytokine quantification by ELISA

Cytokine analyses were performed on the plasma of animals. Interleukin (IL)-10 and IL-12 cytokines were quantified using the commercial kit Milliplex® Map and tumor necrosis factor-alpha (TNF-α) was quantified using the EMD Millipore enzyme-linked immunosorbent assay (ELISA) kit (Bedford, MA, USA) in accordance with the manufacturer's recommendations. The results are expressed in pg mL^{-1}.

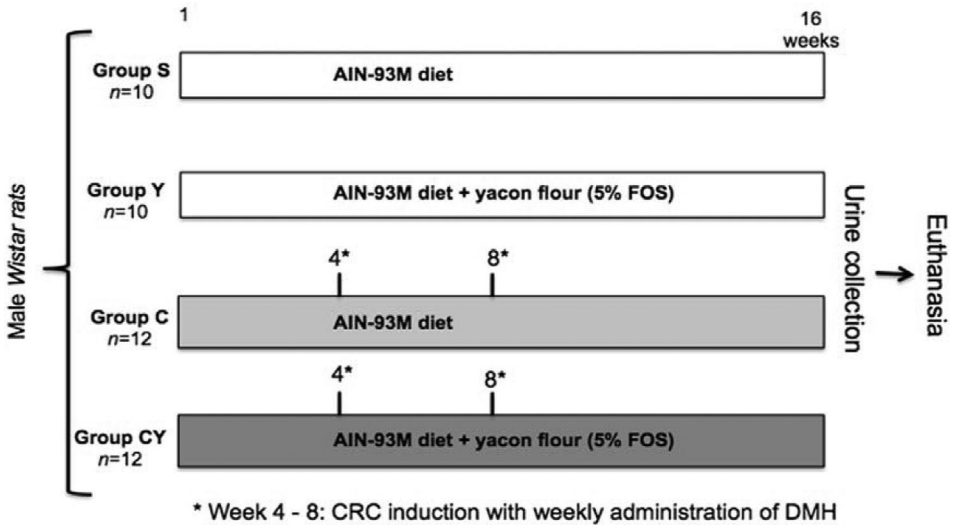

Figure 1 Diagram of the experimental study.

Total antioxidant capacity (TAC)

The TAC of the plasma was determined by the colorimetric assay 'Total Antioxidant Capacity Assay' (Cat. #E-BC-K136, Elabscience®, USA). The results are expressed as units of TAC (U) mL^{-1}.

Secretory immunoglobulin A (sIgA)

The cecal luminal content was diluted (1:10 *w/v*) with phosphate-buffered saline (pH 7.2), homogenized using a vortex (1 min), and centrifuged (3000 × *g*, 10 min). The suspension was collected and evaluated based on the Immunochron ELISA method using the Cloud-Clone® kit, in accordance with the manufacturer's recommendations for secretory immunoglobulin A (sIgA) determination. The results are expressed as ng mL^{-1}.

Table 1 Composition of AIN-93M diet and AIN-93M diet with yacon flour

Ingredients (g 100 g^{-1})	Groups: S and C (AIN-93M)	Groups: Y and CY (AIN-93M + yacon flour)
Casein	14.00	13.14
Dextrinized starch	15.50	15.50
Sucrose	10.00	7.24
Soy oil	4.00	4.00
Fiber (microfine cellulose)	5.00	0
Mineral mix	3.50	3.50
Vitamin mix	1.00	1.00
L-Cystine	0.18	0.18
Choline bitartrate	0.25	0.25
Maize starch	46.57	40.81
Yacon flour	0	17.27[a]
Caloric density (kcal g^{-1})	3.80	3.95

[a]Sufficient amount to provide 5% of fructooligosaccharides (FOS). S, group without colorectal cancer induction and without yacon flour ($n = 10$); C, group with colorectal cancer induction and without yacon flour ($n = 12$); Y, group without colorectal cancer induction and with yacon flour ($n = 10$); CY, group with colorectal cancer induction and with yacon flour ($n = 12$).

Intraluminal pH of the colon

The cecal luminal content was weighed, diluted in saline solution (1:10 *w/v*), and homogenized by vortexing for 15 s. The pH readings were then performed using a pH meter (Kasvi®).

Determination of short-chain fatty acids (SCFAs)

The SCFAs, acetate, propionate, and butyrate were evaluated in the colonic content of animals. Briefly, 100 mg of the colonic content was diluted in 2 mL of 0.1 mL perchloric acid with phenol solution (30 mg g^{-1}), mixed by vortexing for 5 min, centrifuged at 9000 × *g* for 10 min, and then filtered through a 0.45 μm membrane filter. Filtrates were placed in vials for analysis by HPLC.[26] Acetic acid, butyric acid, and propionic acid (Sigma-Aldrich, São Paulo, Brazil) were used as internal standards. The area under the curve was calculated and converted to milligrams per gram of colonic content. The R^2 values of the standard curve were 0.982 for propionate and 0.999 for acetate and butyrate.

Intestinal permeability

Intestinal permeability was determined in the last week of the experiment. For the analysis, animals were fasted for 12 h followed by oral gavage of 2 mL of a solution containing 200 mg of lactulose and 100 mg of mannitol. After administration, animals were placed in metabolic cages and fasted for another 5 h. Urine was collected for 24 h period. During this time, the volume was measured and recorded, and samples were stored at −80 °C. For the analyses of lactulose and mannitol, the urine was filtered through a 0.45 μm membrane filter and placed in vials for HPLC analysis. Lactulose and mannitol (Sigma-Aldrich) were used as internal standards. Lactulose and mannitol concentrations were obtained and converted to grams per liter to calculate the percentage of urinary excretion. The R^2 values of the lactulose and mannitol standard curves were 0.994 and 0.981, respectively. The lactulose/mannitol ratio was calculated by dividing the concentration of lactulose by the concentration of mannitol.[27]

Chromatographic conditions

The chromatographic analyses were based on the chromatographic conditions developed by De Sá *et al.*[28] All analyses were performed using a Shimadzu HPLC system (Kyoto, Japan). The

Table 2 Weight gain, food intake and food efficiency ratio of animals

Variable	Groups				*p*		
	S	Y	C	CY	YF	CRC	YF × CRC
Weight gain (g)	286.9 ± 15.9[a]	281.2 ± 12.98[a]	301.1 ± 10.8[a]	267.6 ± 10.85[a]	0.1346	0.9845	0.2861
Food intake (g)	164.9 ± 5.68[a]	163.2 ± 3.37[a]	164.1 ± 3.68[a]	154.7 ± 3.84[a]	0.1977	0.2785	0.3638
FER (%)	11.5 ± 0.38[a]	11.4 ± 0.44[a]	12.2 ± 0.45[a]	11.5 ± 0.41[a]	0.3548	0.3596	0.4684

Values expressed as mean ± standard error of mean (SEM). Same letters in the same line: groups are not significantly different ($P > 0.05$). *p*, 'two way' ANOVA of the effects of yacon flour (YF) and colorectal cancer (CRC) and the interaction of them (YF × CRC); S, group without CRC induction and without YF ($n = 10$); C, group with CRC induction and without YF ($n = 12$); Y, group without CRC induction and with YF ($n = 10$); CY, group with CRC induction and with YF ($n = 12$); FER, food efficiency ratio.

chromatographic system consisted of a degasser (Model DGU-20A), pump (Model LC-20AT), auto-sampler (Model SIL-20A), column oven (Model CTO-10AS), and UV-visible detector (model SPD-20AV) connected in series with a refractive index detector (Model RID-10A). The analytical column used was Aminex HPX-87H (300 cm × 8.7 mm) from BIO-RAD (Hercules, CA, USA). The mobile phase was sulfuric acid (H_2SO_4) 0.005 mol L^{-1} that was filtered through a 0.45 µm Millipore membrane and a vacuum pump (Tecnal®, Model TE-0582). The analyses were performed at 55 °C under isocratic conditions. The flow rate of the mobile phase was 0.6 mL min^{-1} for lactulose and mannitol and 1.0 mL min^{-1} for SCFA. The injection volume of the sample was 20 µL. Lactulose and mannitol were analyzed using a refractive index (RI) detector and SCFAs (acetic, propionic, and butyric acids) were analyzed by UV-visible detection at 210 nm.

Statistical analyses

The samples were analyzed using the Kolmogorov–Smirnov normality test. Samples without normal distribution were transformed using the $\log_{10}$ function. The groups were tested for the effects of YF and CRC, and/or their interactions, using two-way analysis of variance (ANOVA), followed by Newman–Keuls *post hoc* ($P < 0.05$). Data were expressed as mean ± standard error of mean (SEM) and considered significant at $P < 0.05$. Statistical analyses were performed using the GraphPad Prism® software (GraphPad Software Inc., Version 7, San Diego, CA, USA).

RESULTS

Food consumption, body weight gain, and food efficiency ratio

Food consumption, body weight gain, and food efficiency ratio were similar among the groups (Table 2).

Cytokines

CRC had a significant effect on TNF-α levels. In groups C and CY with induced CRC, TNF-α levels increased compared to TNF-α levels in groups S and Y, without induced CRC. In addition, group C had a higher TNF-α/IL-10 ratio than that in group Y (Table 3). The YF supplementation and CRC induction did not have a significant effect on IL-10 and IL-12 levels or the IL-12/IL-10 ratio.

Total antioxidant capacity (TAC)

A significant interaction between YF and CRC ($P < 0.05$) was observed. The animals in group S had the highest TAC (6.71 U mL^{-1}) of all experimental groups. The animals with induced CRC, with or without YF (groups C and CY), were similar (Table 3).

Secretory immunoglobulin A (sIgA)

Group Y presented the highest sIgA levels of all groups. Therefore, YF did increase sIgA levels, but an interaction between YF and CRC ($P < 0.05$) was observed. There was no difference between groups C and CY ($P < 0.05$), animals with CRC, thus, YF was not able to increase the sIgA levels in the context of CRC (Fig. 2).

Intraluminal pH of the colon

Groups Y and CY had similar intraluminal pH values that were lower than those of groups S and C ($P < 0.05$). Furthermore, group Y had a lower pH than group S ($P < 0.05$) (Fig. 2). Therefore, YF supplementation decreased intraluminal pH.

Short-chain fatty acids (SCFAs)

The groups without CRC (S and Y) had lower excretion of acetate, compared to groups with CRC (C and CY), demonstrating a significant effect of the CRC ($P < 0.05$). Group CY showed higher excretion of acetate than group C. Concerning propionate, group Y had a higher excretion than group S, showing a significant effect of YF ($P < 0.05$). Moreover, there was a significant effect of CRC, since the animals with CRC (groups C and CY) had higher excretion of propionate than the animals without CRC (group S). For butyrate, group C had higher excretion than group S, demonstrating the effect of CRC ($P < 0.05$). YF did not promote significant modifications of butyrate since groups S and Y did not differ between the groups with induced CRC (C and CY) (Fig. 3). A significant effect of CRC on total SCFA was also observed. Groups C and CY showed higher excretion of total SCFA when compared to the excretion levels in groups without CRC induction (groups S and Y).

Intestinal permeability

The urinary excretion of lactulose did not differ significantly among the experimental groups. There was, however, a significant interaction between YF and CRC ($P < 0.05$) with the urinary excretion of mannitol. Group S (without YF) and group Y (with YF) did not differ, but in the presence of CRC, group CY showed higher urinary excretion of mannitol than group C.

Group S had a higher lactulose/mannitol ratio than group Y, demonstrating the significant effect of the YF ($P < 0.05$). Moreover, there was an interaction between YF and CRC. So, there was no difference in groups with CRC supplemented or not supplemented with YF (C and CY) (Table 4).

Table 3 Concentration of IL-10, IL-12, TNF-α, IL-12/IL-10, TNF-α-/IL-10 and TAC

Variable	Groups				*p*		
	S	Y	C	CY	YF	CRC	YF × CRC
IL-10 (pg mL^{-1})	134.8 ± 37.53^{a}	95.66 ± 14.88^{a}	89.95 ± 11.6^{a}	118.8 ± 24.75^{a}	0.8345	0.6594	0.1743
IL-12 (pg mL^{-1})	9.2 ± 1.45^{a}	7.89 ± 1.58^{a}	8.97 ± 2.14^{a}	8.72 ± 1.97^{a}	0.7464	0.1957	0.9359
TNF-α (pg mL^{-1})	5.95 ± 0.6^{b}	5.8 ± 0.54^{b}	9.6 ± 1.0^{a}	10.2 ± 0.72^{a}	0.7446	<0.0001	0.5915
TNF-α/IL10 (pg mL^{-1})	0.069 ± 0.01^{ab}	0.043 ± 0.008^{b}	0.115 ± 0.026^{a}	0.059 ± 0.014^{ab}	0.0288	0.0932	0.4004
IL-12/IL10 (pg mL^{-1})	0.060 ± 0.016^{a}	0.054 ± 0.011^{a}	0.119 ± 0.041^{a}	0.099 ± 0.035^{a}	0.6726	0.1126	0.8224
TAC (U mL^{-1})	6.7 ± 0.78^{a}	4.1 ± 0.47^{b}	3.3 ± 0.3^{b}	4.7 ± 0.72^{b}	0.3136	0.0263	0.0022

Values expressed as mean ± standard error of mean (SEM). Different letters are groups significantly different ($P \leq 0.05$). *p*, 'two-way' ANOVA of the effects of yacon flour (YF) and colorectal cancer (CRC) and the interaction of them (YF × CRC). S, group without CRC induction and without YF ($n = 10$); C, group with CRC induction and without YF ($n = 12$); Y, group without CRC induction and with YF ($n = 10$); CY, group with CRC induction and with YF ($n = 12$); IL, interleukin; TNF-α, tumor necrosis factor-alpha; TAC, total antioxidant capacity.

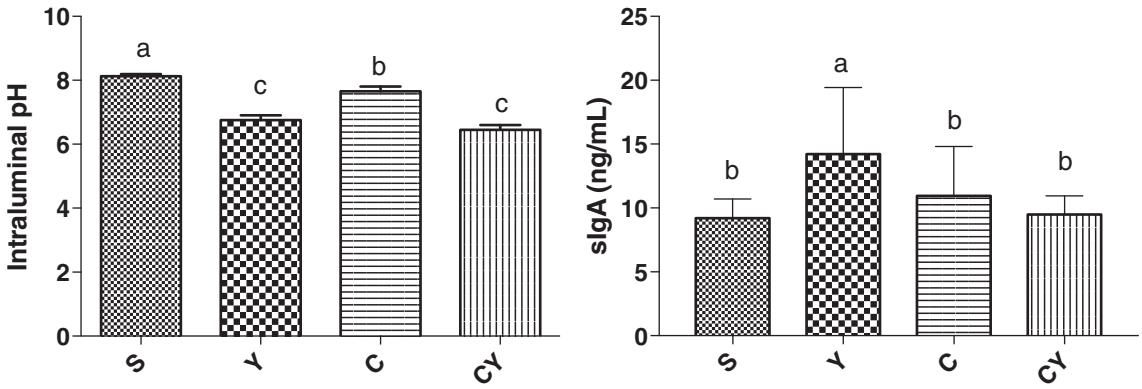

Figure 2 Levels of sIgA (ng mL^{-1}) and intraluminal pH of the colon.

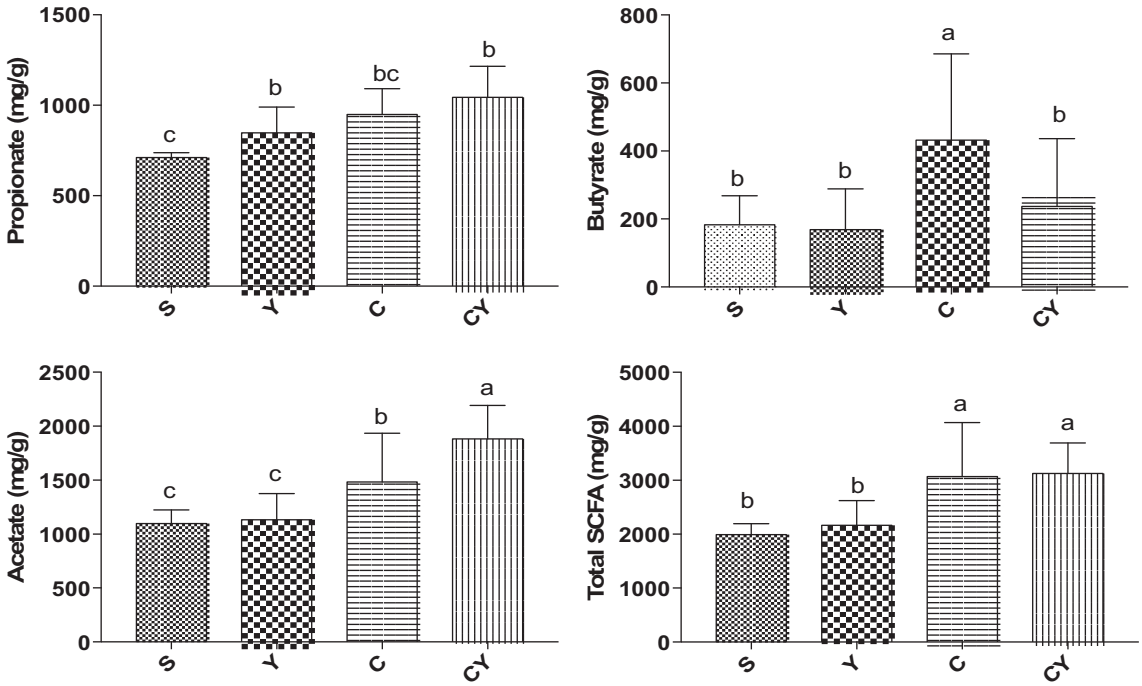

Figure 3 SCFA: excretion of acetate, butyrate and propionate (mg g^{-1}).

Table 4 Urinary excretion of lactulose, mannitol and the ratio between them

	Groups				*p*		
Variables	S	Y	C	CY	YF	CRC	YF × CRC
Lactulose (%)	2.5 ± 0.54[a]	0.9 ± 0.21[a]	1.9 ± 0.45[a]	2.06 ± 0.59[a]	0.3662	0.4081	0.0580
Mannitol (%)	12.1 ± 0.54[b]	12.0 ± 0.9[b]	13.3 ± 0.93[b]	18.2 ± 1.7[a]	0.0340	0.0022	0.0298
L/M ratio	0.2 ± 0.04[a]	0.07 ± 0.01[b]	0.01 ± 0.03[b]	0.09 ± 0.03[b]	0.0391	0.1640	0.0256

Values expressed as mean ± standard error of mean (SEM). Different letters in the same line: groups are significantly different ($P \leq 0.05$). *p*, 'two way' ANOVA of the effects of yacon flour (YF) and colorectal cancer (CRC) and the interaction of them (YF × CRC); S, group without CRC induction and without YF ($n = 10$); C, group with CRC induction and without YF ($n = 12$); Y, group without CRC induction and with YF ($n = 10$); CY, group with CRC induction and with YF ($n = 12$); L/M ratio, lactulose/mannitol ratio.

DISCUSSION

Yacón is a functional food that differentiates itself from most roots by storing its carbohydrate in the form of FOS, which, as a prebiotic, improves intestinal health.[29] The present study verified that YF improved intestinal health by reducing intraluminal pH of the colon, intestinal permeability, and TNF-α/IL-10 ratio and by increasing sIgA. However, colorectal carcinogenesis increased the levels of pro-inflammatory cytokines and SCFAs, which changed the TAC.

Carcinogenesis of the colon is associated with chronic inflammation of the epithelium with abnormal immune system activation and mucosal cell hyperproliferation.[5,25] In this study, the animals with induced carcinogenesis had greater TNF-α levels compared to the levels in healthy animals. TNF-α is a pro-inflammatory cytokine recognized as a critical tumor promoter related to tissue inflammation and process initiation, such as communication, differentiation, and cellular death. TNF-α accomplishes this by activating pathways like NF-kB, which act on anti-apoptotic signaling.[26,27] The increase in TNF-α levels observed in this study may be related to the presence of colorectal carcinogenesis. Indeed, animals with induction of other types of cancer, including stomach[33] and mammal cancer,[34] presented with an increase in TNF-α levels.[35]

Although yacón is considered a food with immunomodulatory capacity,[36] the levels of the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine IL-12 did not change after yacón supplementation. IL-10 is suspected to affect immunoregulation and inflammation by increasing $CD8^+$ T cell numbers and antigen presentation, and by inhibiting inflammatory mediators such as IL-12, which suppresses tumor growth.[37] However, the previous studies remain controversial with some authors reporting higher levels of IL-10,[38] while others did not observe any change in animals,[39] or even in humans,[15] after yacón consumption.

However, when analyzing the TNF-α/IL-10 ratio, which represents a balance between pro- and anti-inflammatory cytokines, there was a significant effect of yacón supplementation as evidenced by a lower ratio in group Y than in group C. IL-10 is produced by Th2 lymphocytes and inhibits macrophage-dependent cytokines, which are synthesized by Th1 cells that also produce TNF-α.[35] Thus, an auto-regulatory loop seemingly exists in which TNF-α stimulates IL-10 production, which, in turn, reduces TNF-α synthesis.[40] BALB/c mice supplemented with a yacón-based product (60 g kg^{-1} FOS) showed an increase in the percentage of regulatory T cells (T reg) in the colon,[41] and these cells also produce IL-10.[40]

YF is a source of phenolic acids, mainly caffeic and chlorogenic acids, which help to reduce oxidative stress, and studies have shown that it has high antioxidant capacity.[10–38] In the present study, the TAC of the plasma was reduced in groups with induced CRC (groups C and CY) and YF treatment was not able to decrease the oxidative damage caused by the carcinogenesis. Another study observed that intake of YF (80 g kg^{-1} FOS) improved the antioxidant capacity of healthy Wistar rats as evidenced by an increase in the content of phenolic components in the cecum.[43] Thus, because cellular damage by ROS is one of the main drivers of mutations,[44] cancer should be considered a disease with highly oxidative characteristics. This highly oxidative state of cancer may explain why YF did not change the TAC in animals with induced carcinogenesis.

Colorectal carcinogenesis is associated with intestinal dysbiosis resulting in impairment of mucosal barrier integrity.[45] Prebiotics have beneficial effects on the immunity of the intestinal mucosa.[20] In this experiment, YF increased sIgA in group Y animals. This corroborates other studies in animal models[39] and in preschool children.[15] Additionally, intake of yacón (75 g kg^{-1} FOS) after CRC induction increased the levels of sIgA,[22] which shows the action of the immune system against inflammation caused by the carcinogenesis. The increase in sIgA levels may be attributed to the FOS contents that are fermented in the cecum by bifidobacteria. It is known that sIgA acts as the first-line of defense to protect the epithelium from pathogens and toxins and help inhibit the colonization of pathogenic bacterium in the intestine and their penetration into the mucosa.[46]

FOS are selectively fermented by bifidobacteria and lactobacilli[12] and produce SCFAs as the major metabolic end-products, specifically acetate, propionate, and butyrate.[18] YF (75 g kg^{-1} FOS) intake after CRC induction increased butyrate and propionate production, which was associated with an increase in the depth of colonic crypts and the number of crypts.[22] Thus, an increase in acetate and butyrate contents in the cecum of rats was observed after 10 g kg^{-1} yacón intake, which was related to the reduced number of aberrant crypt foci and colon tumors.[47] SCFAs have anti-inflammatory effects. Butyrate, specifically, acts as the main source of energy to colonocytes, inhibits tumor cell growth and proliferation,[48] and inhibits the motility of colon cancer cells, thereby reducing metastasis.[49] In the present study, animals with induced colorectal carcinogenesis (with and without YF) had greater production of acetic, propionic, and butyric acids, and total SCFA.

It is known that tumor cells undergo metabolic changes during carcinogenesis to meet the energy requirements resulting from higher levels of proliferation. In cancerous colonocytes, there is a preference for glycolytic metabolism over oxidative phosphorylation, called the Warburg effect. Therefore, compared to normal

colonocytes that oxidize butyrate, butyrate is accumulated in cancer cells, generating higher concentrations of butyrate in cancerous epithelial cells.[45–48,50,51] A study with mice verified that butyrate increased the efficacy of stem cell generation and self-renewal, which were mediated by changing the reprogramming dynamics.[52] The authors explained that to relate the action of SCFAs on cancer, it is necessary to consider factors such as genetic background, cellular energetics, and environmental contexts.[53] Therefore, metabolic interaction at the luminal level may have occurred in the animals, resulting in SCFA increase after carcinogenesis induction.

In addition, the intake of prebiotics is beneficial to the physiological status of the intestines by decreasing the intraluminal pH, which is associated with better luminal moisture content.[54] In agreement with previous studies,[18–43] the present study showed that YF supplementation reduced the intraluminal pH, which may be due to FOS fermentation. The reduction in intraluminal pH inhibits the proliferation of pathogenic bacteria and the activity of microbial enzymes involved in the production of carcinogenic by-products.[55] CRC patients also show elevated intraluminal pH compared to healthy individuals.[55] In addition, luminal content acidification improves mineral absorption by increasing the solubilization of minerals.[56] Thus, improvement of the physiological status of the intestines by oral administration of polysaccharides is related to improvement of intestinal barrier function.[54]

The intestinal barrier is composed of epithelial cells linked by cell junctions known as tight, adhesion, and gap junctions, which are responsible for controlling the amount of luminal antigens that cross the epithelium. In this case, intestinal barrier dysfunction leads to an increase in intestinal permeability and bacterial translocation, which has the potential to release inflammatory mediators and immunological cells, and to exacerbate the development of inflammation.[57,58]

Moreover, in the present study, the interaction between YF and colorectal carcinogenesis decreased the lactulose/mannitol ratio in excreted urine, which represents a reduction in intestinal permeability. Yacón supplementation (75 g kg^{-1} FOS) also promoted beneficial effects that reduced intestinal permeability in animals with induced CRC,[22] which is relevant because higher intestinal permeability is related to inflammatory diseases.[59] FOS fermentation may act beneficially on the intestinal barrier integrity by increasing the production of mucin, which permits the passage of some substances while restricting others.[60] In addition to the effects attributed to FOS, chlorogenic acid, mainly the phenolic component found in yacón,[61] reduced the lactulose/mannitol ratio and increased the expression of tight junction proteins in colitic rats.[62] This serves as clear evidence that some component of yacón has distinct effects on the maintenance of intestinal integrity.

The SCFA, resulted from the fermentation of FOS by bifidobacteria, performs beneficial effects to colonic mucosa, promoting an improvement of the epithelial barrier integrity, with reduced intestinal permeability. Furthermore, immune system modulation is observed with greater production of antibacterial defensins, sIgA, and anti-inflammatory cytokines, mainly IL-10. In addition, prebiotics affect intestinal barrier integrity by increasing epithelial mucus production and maintaining the integrity of tight junctions that prevent bacterial translocation.[16,59,60]

CONCLUSION

The consumption of YF promotes beneficial effects on the intestinal health of animals with induced CRC in many ways including reduction of pH, intestinal permeability, TAC, and the TNF-α/IL-10 ratio. Therefore, the intake of YF at 50 g kg^{-1} FOS in the diet showed the potential to improve the intestinal barrier and mucosal immunity, particularly in healthy animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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