Check for updates

Received: 8 November 2020 | Revised: 18 February 2021 | Accepted: 20 February 2021

DOI: 10.1111/1750-3841.15690

Journal of Food Science WILEY

HEALTH, NUTRITION, AND FOOD

Evaluation of the efficacy of probiotic VSL#3 and synbiotic VSL#3 and yacon-based product in reducing oxidative stress and intestinal permeability in mice induced to colorectal carcinogenesis

Bruna Cristina dos Santos Cruz[1] | **Luís Fernando de Sousa Moraes**[2] | **Letícia De Nadai Marcon**[1] | **Kelly Aparecida Dias**[1] | **Leonardo Borges Murad**[3] | **Mariáurea Matias Sarandy**[4] | **Lisiane Lopes da Conceição**[1] | **Reggiani Vilela Gonçalves**[5] | **Célia Lúcia de Luces Fortes Ferreira**[6] | **Maria do Carmo Gouveia Peluzio**[1]

[1] Nutritional Biochemistry Laboratory, Department of Nutrition and Health, Universidade Federal de Viçosa - UFV, Viçosa, Minas Gerais, Brazil

[2] Experimental and Dietetic Nutrition Laboratory, Department of Nutrition - Federal University of Pernambuco - UFPE, Recife, Pernambuco, Brazil

[3] Brazilian National Cancer Institute, Rio de Janeiro, Brazil

[4] Department of General Biology, Universidade Federal de Viçosa - UFV, Viçosa, Minas Gerais, Brazil

[5] Experimental Pathology Laboratory, Department of Animal Biology, Universidade Federal de Viçosa - UFV, Viçosa, Minas Gerais, Brazil

[6] Institute of Biotechnology Applied to Agriculture & Livestock (Bioagro), Universidade Federal de Viçosa - UFV, Viçosa, Minas Gerais, Brazil

Correspondence
Bruna Cristina dos Santos Cruz, Nutritional Biochemistry Laboratory, Department of Nutrition and Health, Universidade Federal de Viçosa - UFV, Viçosa, Minas Gerais 36570-900, Brazil.
Email: brunacruz09@yahoo.com.br

Previous address(es) [If research was conducted at a different affiliation than that listed above].

Abstract: The objective of the present study was to evaluate the effect of probiotic VSL#3 isolated or associated with a yacon-based product (synbiotic) on oxidative stress modulation and intestinal permeability in an experimental model of colorectal carcinogenesis. Forty-five C57BL/6J mice were divided into three groups: control (standard diet AIN-93 M); probiotic (standard diet AIN-93 M and multispecies probiotic VSL#3, 2.25×10^9 CFU), and synbiotic (standard diet AIN-93 M with yacon-based product, 6% fructooligosaccharides and inulin, and probiotic VSL#3, 2.25×10^9 CFU). The experimental diets were provided for 13 weeks. The probiotic and the yacon-based product showed antioxidant activity, with the percentage of DPPH radical scavenging equal to 69.7 ± 0.4% and 74.3 ± 0.1%, respectively. These findings contributed to reduce hepatic oxidative stress: the control group showed higher concentration of malondialdehyde (1.8-fold, $p = 0.007$ and 1.5-fold, $p = 0.035$) and carbonylated protein (2-fold, $p = 0.008$ and 5.6-fold, $p = 0.000$) compared to the probiotic and synbiotic groups, respectively. Catalase enzyme activity increased 1.43-fold ($p = 0.014$) in synbiotic group. The crypt depth increased 1.2-fold and 1.4-fold with the use of probiotic and synbiotic, respectively, compared to the control diet ($p = 0.000$). These findings corroborate the reduction in intestinal permeability in the probiotic and synbiotic groups, as measured by the percentage of urinary lactulose excretion (CON: 0.93 ± 0.62% × PRO: 0.44 ± 0.05%, $p = 0.048$; and CON: 0.93 ± 0.62% × SYN: 0.41 ± 0.12%, $p = 0.043$). In conclusion, the probiotic and synbiotic showed antioxidant activity, which contributed to the reduction of oxidative stress markers. In addition, they protected the mucosa from damage caused by chemical carcinogen and reduced intestinal permeability.

Practical Application: The relationship between intestinal health and the occurrence of various organic disorders has been demonstrated in many

 © 2021 Institute of Food Technologists®

studies. The use of probiotics and prebiotics is currently one of the main targets for modulation of intestinal health. We demonstrated that the use of a commercial mix of probiotic bacteria (VSL#3) isolated or associated with a yacon-based prebiotic, rich in fructooligosaccharides and inulin, is able to reduce the oxidative stress and intestinal permeability in a colorectal carcinogenesis model. These compounds have great potential to be used as a food supplement, or as ingredients in the development of food products.

KEYWORDS
beneficial bacteria, colorectal neoplasm, intestinal barrier, oxidative damage, yacon

1 | INTRODUCTION

Colorectal cancer (CRC) is a public health problem due to its high incidence and mortality rates worldwide. It is the third type of cancer most diagnosed and the second with the highest mortality. Only in 2020, there are projected to be 147,950 cases newly diagnosed of CRC in the United States (104,610 cases of colon cancer and 43,340 cases of rectal cancer) (Bray et al., 2018; Siegel et al., 2020).

Colorectal carcinogenesis is a complex process, driven by the progressive accumulation of genetic and epigenetic modifications, with activation of oncogenes and inactivation of tumor suppressor genes, that lead to dysregulation of apoptosis, differentiation, and cell proliferation (Greenman et al., 2007). Among the morphological changes that occur in the mucosa of the colon, it is initially observed the formation of aberrant crypt foci (ACF), which are putative preneoplastic lesions (Bird & Good, 2000; Khare et al., 2009).

Changes in the intestinal microenvironment, such as increased oxidative stress and the imbalance of the intestinal microbiota, precede the development of preneoplastic lesions and contribute to the progression of the CRC. Together, these disorders result in DNA damage, genetic mutations, endotoxemia, chronic inflammation, and increased intestinal permeability (Jahani-Sherafat et al., 2018).

Currently, specific bacterial genera and nondigestible compounds capable of modulating oxidative stress and microbiota have been deeply studied (Gagnière et al., 2016; Lucas et al., 2017). An inverse association between the consumption of probiotics, prebiotics, and the incidence of CRC has been demonstrated (Kich et al., 2016; Oh et al., 2020).

Probiotics are classically defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," and usually include the genera Bifidobacterium and Lactobacillus (Hill et al., 2014), whereas prebiotics comprise the indigestible food ingredients fermented by gut microorganisms that serve as selective substrate for their growth; include nondigestible carbohydrates, polyphenols, and polyunsaturated fatty acids (Gibson et al., 2017; Green et al., 2020). Synbiotics, in turn, are defined as the association between probiotic and prebiotic, that work synergistically in the colonization and survivability of beneficial microorganisms in colon; it is suggested that their use is more effective in preventing colorectal carcinogenesis than the use of probiotic or prebiotic separately (Rafter et al., 2007; Roberfroid, 2007).

VSL#3 is a commercial probiotic composed of eight bacterial species. This probiotic has a protective effect on intestinal barrier function, which is one of the important factors for treating multiple chronic diseases (Cheng et al., 2020). Studies have demonstrated the beneficial effects of VSL#3 on mechanical barrier function and control of inflammatory bowel diseases (Corridoni et al., 2012; Dai et al., 2012; Gionchetti et al., 2007); however, the evidence in the CRC is still insufficient.

Yacon (*Smallanthus sonchifolius*) is a tuberous root, source of fructooligosaccharides (FOS), which are inulin-type prebiotic fructans linked by β bonds with low degrees of polymerization, and inulin (Verediano et al., 2020). The yacon-based product (PBY) is a concentrate produced with yacon *in natura* and which has higher concentrations of prebiotics FOS and inulin when compared to fresh root (Rodrigues et al., 2011). Recently, the ability of PBY to reduce oxidative stress, reduce damage to intestinal crypts, and increase the production of short-chain fatty acids (SCFAs), particularly butyrate, in a colorectal carcinogenesis model was demonstrated (De Nadai Marcon et al., 2020; De Nadai Marcon et al., 2019).

Although studies show the benefits of using probiotics and prebiotics in the function of the intestinal barrier and control of oxidative stress, these effects are not clear in CRC. Here, we hypothesize that the prophylactic administration of the multispecies probiotic VSL#3 alone or as part of the novel synbiotic formulation VSL#3+PBY (considering its additional beneficial effects) could be able to

regulate intestinal barrier function and oxidative stress in the early stages of colorectal carcinogenesis. Thus, the present study aimed to investigate the effects of the probiotic VSL#3 and synbiotic VSL#3 + PBY on oxidative stress modulation and intestinal permeability in mice induced to colorectal carcinogenesis. Together, these data will contribute to elucidate the different pathways involved in the possible mechanisms of protection of probiotics and synbiotics.

2 | MATERIALS AND METHODS

2.1 | Probiotic

A commercial probiotic VSL#3® (Sigma Tau Pharmaceuticals, Inc.; acquired in 2016, valid 04/2018, lot number 604094) was acquired lyophilized, in sachets containing 450 billion viable bacteria, kept refrigerated throughout the experimental period, and reconstituted in water daily before administration. VSL#3 contains eight bacterial species: *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Streptococcus thermophilus*. The probiotic VSL#3 was administered at a dosage of 2.25×10^9 CFU/animal/day based on daily intake of about 10^9 CFU for an adult of 70 kg (Uronis et al., 2011). It is suggested that this dose is sufficient to increase intestinal health, in addition to ensuring a minimum count of 10^6 g^{-1} of bacteria in feces (Sarao et al., 2017; Zhang et al., 2016). Similar doses were observed in studies that used the probiotic VSL#3 to prevent or treatment of inflammatory bowel disease and colitis-associated cancer (Bassaganya et al., 2012; Chang et al., 2012; Mohania et al., 2013).

2.2 | Synbiotic

The synbiotic was composed of the probiotic VSL#3 and the yacon-based product (PBY). PBY is a concentrated yacon-based product, rich in prebiotics FOS and inulin. PBY was produced as described by Rodrigues et al. (2011). Briefly, the yacon root is sanitized, peeled, fractionated into smaller pieces, and completely crushed. After, it is taken to an open steam boiler to be concentrated until it reaches, approximately, 60°Bx (Patent Request: PI 1106621-0). The centesimal composition of PBY was determined according to the AOAC methodology (AOAC, 1997); FOS and inulin contents were determined by high-performance liquid chromatography (HPLC).

PBY was added to the standard rodent diet AIN-93 M (Reeves et al., 1993) to provide 6% FOS and inulin (Paula et al., 2012). This dose was defined based on previous studies in human and animal models, where several beneficial effects were observed, such as modulation of the immune system, reduction of constipation, increased integrity of crypts, and production of SCFA, without causing diarrhea (De Nadai Marcon et al., 2020; De Nadai Marcon et al., 2019; Sant'Anna et al., 2018).

TABLE 1 Composition of experimental diets

Ingredients (g 100 g^{-1})	AIN-93 M	AIN-93 M with PBY(6% FOS + inulin)
Cornstarch	33.20	28.55
Casein	16.50	16.40
Dextrinized starch	15.50	15.50
Sucrose	10.00	5.20
Soybean oil	4.00	4.00
Microfine cellulose	6.40	0.00
PBY*	0.00	25.40
Mineral mix	3.50	3.50
Vitamin mix	1.00	1.00
L-Cystine	0.18	0.18
Choline bitartrate	0.25	0.25
Energy density (kcal/g)	3.37	3.19

PBY: Yacon-based product. Centesimal composition and digestible content of carbohydrate, FOS, and inulin on PBY (100 g of product): fructose: 9.4 g; glucose: 6.45 g; sucrose: 3.05 g; FOS: 17.65 g; inulin: 5.95; total carbohydrate: 42.49 g; fibers: 1.64 g; humidity: 37.20 g; ashes: 1.55 g; lipids: 0.04 g; protein: 2.51 g.

Considering that 100 g of PBY contains 23.6 g of FOS and inulin, 25.4 g of PBY was added to every 100 g of standard diet (Table 1). For comparison purposes, the conversion of the PBY dose to humans was calculated using the body surface area normalization method (Reagan-Shaw et al., 2008). In our study, the average daily diet consumption was 4 g per mouse, which is equivalent to 1016 mg of PBY daily for an adult mouse of approximately 28 g (or 36.2 g PBY/kg body mass/day). Taking into account the average weight of an adult human is 70 kg, the equivalent average daily consumption per day for humans is 205.9 g, amount viable for consumption. In addition, because it is a concentrated product, PBY has advantages over yacon *in natura*: longer shelf life, FOS, and inulin stable for longer, regardless of seasonality and higher concentration of nutrients.

Carbohydrate, protein, and fiber contents were adjusted so that the experimental diets had a similar composition. The diets were prepared as pellets and stored at −20 °C.

2.3 | *In vitro* antioxidant activity of VSL#3 and PBY

In vitro antioxidant capacity of VSL#3 and PBY were evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

scavenger method (Brand-Williams et al., 1995). DPPH radical is captured by antioxidants in the sample, causing a decrease in absorbance that can be directly monitored in a spectrophotometer. Briefly, 1.5 ml of DPPH (methanolic solution) was added to 100 µl of probiotic stock solution. The samples were homogenized in a vortex mixer for 1 min and left to stand for 30 min in the dark. For the evaluation of PBY, 5 g of the product was homogenized in 30 ml of distilled water and then filtered with a filter paper. The analysis was performed as described above. DPPH radical scavenging activity (% AAI) was calculated by the equation: $\%AAI = [100–(A_{sample} - A_{blank})/(A_{control}) \times 100]$, where A = absorbance at 517 nm. The samples were analyzed in triplicate.

2.4 | Total phenolic content of PBY

Total phenolic content of PBY was determined using the Folin–Ciocalteu method (Mau et al., 2002). The absorbance was measured at 760 nm using a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Results were expressed as milligram of gallic acid equivalent (GAE) per gram of dry weight of PBY (mg GAE/g dw PBY). The samples were analyzed in triplicate.

2.5 | Biological assay

2.5.1 | Animals and experimental design

Forty-five male C57BL6/J mice, healthy, 8 weeks old, and body weight of approximately 22 g, were obtained from the Central Bioterium at the Biological Sciences and Health Center at Federal University of Viçosa, Minas Gerais, Brazil. The animals were collectively allocated in polypropylene cages, containing five mice each. Animals were kept under controlled conditions, at a temperature of 22 ± 2°C and humidity of 60 to 70% with a 12 hr light/dark cycle.

After a week of acclimatization, with free access to commercial diet (Purina®) and water, animals were divided according to body weight into three different groups ($n = 15$/group), to receive the following interventions, for 13 weeks, as previously described by Cruz et al. (2020a):

1. Control group (CON): standard diet AIN-93 M and 0.1 ml of water, via orogastric gavage;
2. Probiotic group (PRO): standard diet AIN-93 M and 0.1 ml of probiotic VSL#3 (2.25×10^9 CFU/animal), via orogastric gavage;
3. Synbiotic group (SYN): modified AIN-93 M diet, with PBY (6% FOS and inulin) and 0.1 ml of probiotic VSL#3 (2.25×10^9 CFU/animal), via orogastric gavage.

The interventions described above were started in the first experimental week. Standard and modified diets were offered *ad libitum*, and gavages administered in the morning, for 5 days a week (Arthur et al., 2013). From the third experimental week, the protocol for the induction of preneoplastic lesions (ACF) of CRC was initiated. All animals received an intraperitoneal injection of the colon carcinogen 1,2-dimethylhydrazine (DMH) (Sigma-Aldrich, Saint Louis, MO, USA), 20 mg/kg body weight, once a week, for 8 consecutive weeks (Gomides et al., 2014; Newell & Heddle, 2004).

After the end of the experimental period (13 weeks), the animals were anesthetized with 3% isoflurane (Isoflorine®, Cristalia, Itapira, Brazil) and sacrificed by cervical dislocation. Organs and tissues were collected, washed with cold 0.1 M phosphate-buffered saline (PBS, pH 7.2), and weighed. Cecum, liver, and serum were stored at −80 °C. Colon was fixed with Carson's formalin for histological analysis.

All experimental procedures were performed following the Directive 2010/63/EU, in compliance with the ethical principles for animal experimentation. The study protocol was approved by the Ethics Committee of the Federal University of Viçosa (CEUA/UFV, protocol n° 08/2017. Approval: May 9, 2017).

2.5.2 | Body weight and food intake

The animals were weighed weekly on a digital semianalytical scale. Food intake was measured daily and was calculated by the difference from the amount of diet offered (g) and the remaining amount (g). The coefficient of food efficiency (CFE) was calculated by the equation: CFE = weight gain (g)/total diet consumption (g).

2.5.3 | Colonic morphometry and histopathological score

Fragments of the colon were fixed with Carson's formalin for 24 hr (Carson et al., 1973). Slides of seven animals/group, containing 10 nonconsecutive sections (5 µm thick cuts) were stained with hematoxylin-eosin, and about 20 photos of each slide were obtained (Leica Microsystems®, Inc.) to assess intestinal morphometry and histopathological score. Crypt depth, thickness of the

submucosa, muscularis, and external muscularis layers were measured using the Image Pro Plus 4.5 software.

Histopathological score was calculated by the sum of the following criteria: crypt damage (score 0: none; 1: basal 1/3; 2: basal 2/3; 3: only surface epithelium intact; 4: complete loss of crypt and epithelium), severity of inflammation (score 0: none; 1: slight; 2: moderate; 3: severe), and injury depth (score 0: none; 1: mucosal; 2: mucosal and submucosal; 3: transmural) (Dieleam et al., 1994). The assessment was carried out by two examiners, independently.

2.5.4 | Biochemical analysis

After collection, the blood was centrifuged at 870 × *g* at 4 °C for 10 min. Serum markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), urea, and creatinine were quantified by colorimetric assay as recommended by the manufacturer of the kits (Bioclin®, Inc.). The measurements were performed in the BS-200 analyzer (Mindray®, Inc.).

2.5.5 | Intestinal permeability

The analysis of intestinal permeability was based on the urinary excretion of lactulose and mannitol (Jin et al., 2008). Briefly, in the last experimental week, the animals were fasted for 12 hr and received orogastric gavage (0.2 ml) with a solution containing 11.8 mg of lactulose and 8.9 mg of mannitol. Subsequently, the animals were fasted for another 6 hr and urine samples were collected for 24 hr and frozen at −80 °C. For analysis, the urine was thawed at room temperature, warmed in a 56 °C water bath for 10 min, centrifuged at 9720 × *g* for 7 min, and filtered in a 0.22 μm membrane filter. The urinary concentration of lactulose and mannitol was determined by HPLC (Shimadzu®, Quito, Japan) using a wave length of 220 nm, 300 mm × 7.8 mm diameter column, flow rate of 1 ml/min, pressure 54 Kgf, and acidified water (H_2SO_4, 0.005 M) as the mobile phase. The results were expressed as percentage of sugar excretion.

2.5.6 | Analysis of oxidation products and activity of antioxidant enzymes in hepatic and cecal tissues

Liver and cecum samples were weighed (150 mg) and homogenized in 1.5 ml of cold PBS (pH 7.4) using an Ultra-Turrax homogenizer (T10 basic UltraTurrax, IKA®, Brazil). The homogenate was centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was used for enzyme analysis, catalase (CAT) (Dieterich et al., 2000), superoxide dismutase (SOD) (Aebi, 1984; Buege & Aust, 1978), and glutathione-S-transferase (GST) (Habig & Jakoby, 1981); and for oxidation biomarkers assessment, malondyaldeide (MDA) (Wallin et al., 1993) and carbonyls protein (CP) (Levine et al., 1990). The results were normalized by total protein concentration of supernatant (Lowry et al., 1951).

2.6 | Statistical analysis

Statistical processing and analysis were performed using the Statistical Package for the Social Sciences 20.0 (SPSS Software IBM, Chicago, IL, USA), and graphs were constructed using GraphPad Prism 7 (GraphPad Software LLC, La Jolla, CA, USA). The normality of variables was determined by the Shapiro–Wilk test. The mean values of the three groups were compared by one-way analysis of variance (ANOVA) followed by Bonferroni multiple-comparison post hoc test, for parametric data. For nonparametric data, the Kruskal–Wallis test was applied, complemented by Dunn's multiple comparison test. $p < 0.05$ was considered to be statistically significant and the data are expressed as the mean ± standard deviation (SD).

3 | RESULTS AND DISCUSSION

3.1 | *In vitro* antioxidant activity and total phenolics

Oxidative stress results from excess free radicals (RF), such as reactive oxygen species (ROS), and can cause damage to lipids, proteins, and DNA. Although most organisms are able to deal with RF, the imbalance between production and elimination contributes to the development of diseases, including cancer. Thus, there is a growing search for natural antioxidant compounds for health promotion and disease prevention (Schieber & Chandel, 2014).

The antioxidant activity *in vitro* and *in vivo* of probiotics and prebiotics has been demonstrated for decades. In the present study, it was observed that probiotic VSL#3 has a high capacity to capture DPPH radicals (%AAI = 69.7 ± 0.4%). Kim et al. (2020) evaluated the *in vitro* antioxidant capacity of several isolated probiotics, and obtained a percentage of DPPH radical capture ranging between 22.2 ± 2.4% and 38.2 ± 1.6%. These results suggest that the use of multispecies probiotic, such as VSL#3, has potentiated antioxidant effects.

The antioxidant mechanisms of action of probiotics have not been fully clarified. However, it is described that probiotics can act on the redox status of the host through their ability to: chelate metal ions; stimulate the antioxidant system of the host, in addition to having its own antioxidant enzyme system; produce metabolites with antioxidant activity, such as glutathione and butyrate; mediate antioxidant signaling pathways, such as Nrf2-Keap1-ARE, NFκB, MAPK, and PKC; regulate enzymes that produce ROS; and regulate the intestinal microbiota, controlling excessive proliferation of harmful bacteria that cause endotoxin and significant oxidative stress (Wang et al., 2017).

For PBY, used in synbiotic formulation, the percentage of capture of the DPPH radical was 74.3 ± 0.1%. PBY contains flavonoids and phenolics compounds, which exhibit antioxidant and anti-inflammatory properties (Khajehei et al., 2018). These compounds protect biomolecules, such as DNA, against the damage caused by FR, reducing the risk of tumor development (Shahidi & Yeo, 2018).

The concentration of total phenolic compounds in PBY was 627.6 mg/L (mg GAE/100 g total solids). It is described that yacon roots have about 200 mg of phenolic compounds per 100 g of fresh matter (Gusso et al., 2015). Because it is a concentrated product, PBY has considerably higher concentrations of total phenolics. The demonstration of the *in vitro* antioxidant activity of the probiotic VSL#3 and PBY corroborates the beneficial results obtained in the evaluation of oxidative stress *in vivo*.

3.2 | *In vivo* study

3.2.1 | Body weight, food intake, and anatomical characteristics

The animals were weighed weekly to verify the influence of probiotic and synbiotic on body weight gain or loss. Similarly, food intake was evaluated daily. Body weight did not differ significantly between groups at the end of 13 experimental weeks (CON = 24.9 ± 1.3 g; PRO = 25.4 ± 1.5 g; SYN = 25.2 ± 1.4 g; $p = 0.647$).

Similar result was demonstrated by Leu et al. (2010), where animals that were induced to colorectal carcinogenesis and received probiotic or synbiotic did not show significant differences in body weight. This result indicates that the consumption of the probiotic VSL#3 or synbiotic VSL#3+PBY does not interfere with body weight. The similarity of weight can be justified by food intake, which also did not differ between groups in the last experimental week (CON = 3.8 ± 0.6 g; PRO = 4.1 ± 0.6 g; SYN = 3.9 ± 0.4 g; $p = 0.781$), as well as the CFE (CON = 0.007 ± 0.005; PRO = 0.012 ± 0.008; SYN = 0.010 ± 0.005; $p = 0.604$).

However, it was observed a significant increase in cecum weight in the group that received the synbiotic when compared to the CON (1.8-fold; $p = 0.000$) and PRO (1.5-fold; $p = 0.000$) groups, as well as colon weight (CON = 1.5-fold, $p = 0.000$; PRO = 1.3-fold, $p = 0.000$) (Figure 1a–c). These data corroborate previously published studies (Chang et al., 2012; Pattananandecha et al., 2016), including one that also used PBY (De Nadai Marcon et al., 2019). According to the study's authors, the fermentation of FOS and inulin present in PBY leads to the production of metabolites that stimulate the proliferation of healthy cells, such as butyrate, which is the main source of energy for colonocytes (De Nadai Marcon et al., 2019). In fact, we previously demonstrated the significant increase in SCFA and butyrate production in the group that received the synbiotic VSL#3 + PBY (Cruz et al., 2020a).

The trophic effect of butyrate reflects the increase in the weight of the cecum and colon and contributes to the maintenance and integrity of the mucosa. These findings are confirmed by changes in colon morphometry and reduced intestinal permeability.

3.2.2 | Colonic morphometry and histopathological score

Colon morphometry was influenced by probiotic and synbiotic use. The crypt depth increased 1.2-fold ($p = 0.000$) and 1.4-fold ($p = 0.000$) with the use of probiotic and synbiotic, respectively, compared to the CON group. The use of the synbiotic has a greater influence on the crypt depth, since there was also a significant difference when compared to the PRO group (1.1-fold; $p = 0.000$) (Figure 2a). Interestingly, the submucosal layer decreased 1.2-fold ($p = 0.025$) in the PRO group and 1.3-fold ($p = 0.000$) in the SYN group (Figure 2b). In the muscularis layer, a significant increase ($p = 0.000$) was observed only in the PRO group (1.5-fold and 1.3-fold compared to the CON and SYN groups, respectively); the CON and SYN groups did not differ (Figure 2c). Regarding the external muscularis layer, there was an increase in the PRO (1.5-fold; $p = 0.001$) and SYN (1.3-fold; $p = 0.000$) groups compared to the CON group (Figure 2d).

The increase in the colon layers in the SYN group justifies the difference in weight observed in this tissue, compared to the other groups (Figure 1b). Among the benefits of yacon, there is an increase in the crypts depth, with reduced intestinal permeability (De Moura et al., 2012). According to Leu et al. (2010), the increase in crypts depth is conditioned to the presence of fermentable substrates in

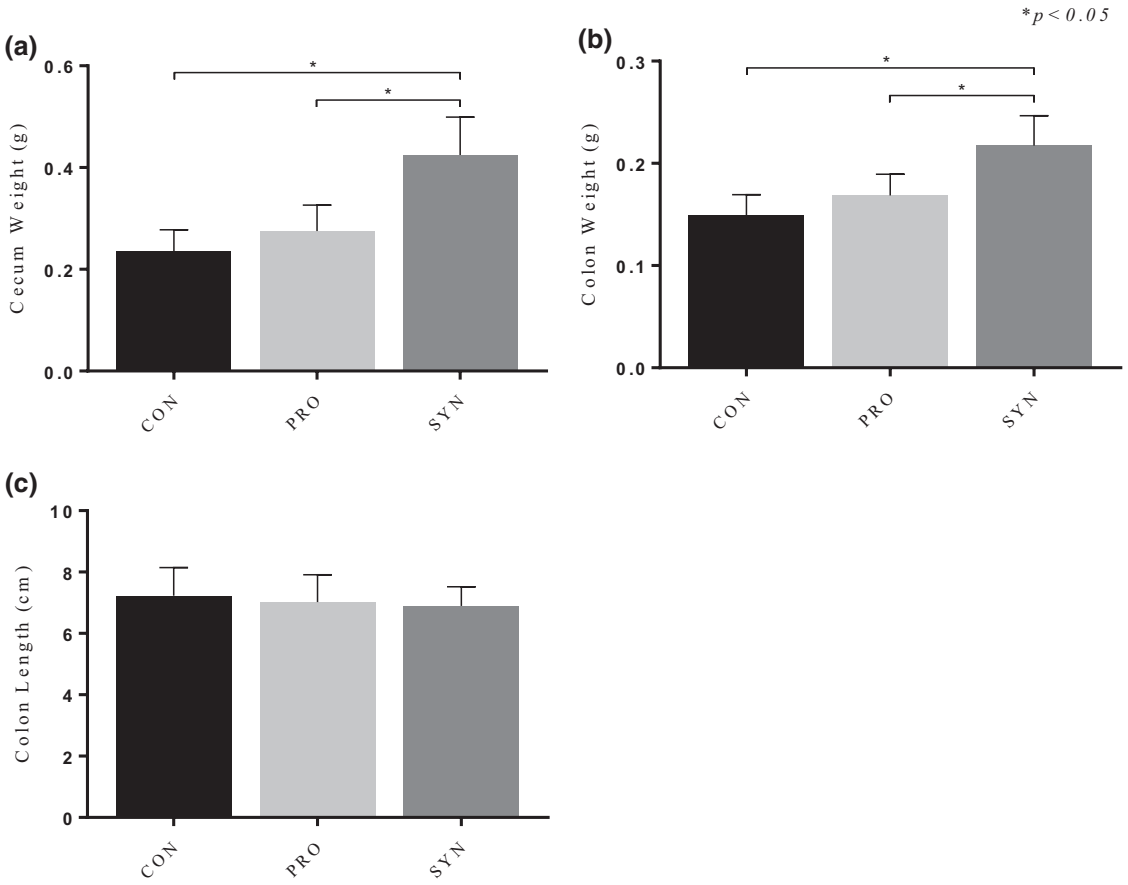

FIGURE 1 Effect of probiotic and synbiotic on (a) cecum weight, (b) colon weight, and (c) colon length in a colorectal carcinogenesis model. The data were expressed as mean ± SD ($n = 15$/group). Statistical difference between groups was analyzed by Anova test or Kruskal–Wallis test. (*) $p < 0.05$. CON, AIN-93 M diet; PRO, AIN-93 M diet and probiotic VSL#3®; SYN, AIN-93 M diet with PBY and probiotic VSL#3

the diet, such as FOS and inulin, since animals that receive restricted diets in prebiotics have smaller crypts.

The total histopathological score, calculated from the sum of the individual scores of the (1) crypt damage, (2) severity of inflammation, and (3) injury depth, was approximately 1.3-fold ($p = 0.000$) higher in the CON group compared to the others (Figure 3a). Similarly, when the parameters were evaluated alone, the CON group had a crypt damage score 2.4-fold higher than the PRO ($p = 0.003$) and SYN ($p = 0.000$) groups (Figure 3b).

Crypt damage, assessed by histopathological score, is a morphological alteration commonly observed during colorectal carcinogenesis. Mucosal damage occurs due to the genotoxicity of DMH and its active metabolites released into the colon (Genaro et al., 2019; Li et al., 2019). However, the use of probiotic and synbiotic can reduce the exposure of colon epithelial cells to genotoxic agents, as demonstrated in the present study.

Local toxicity and DNA damage induced by DMH are accompanied by the inflammatory process, with increased production of proinflammatory cytokines. Tissue inflammation favors tumor development as it provides substrates for cell proliferation (Lenoir et al., 2016). However, it is important to highlight that the inflammatory process is fundamental for the elimination of tumor cells in the early stages of the disease, as in the development of ACF (O'Donnell et al., 2019).

In the present study, we did not observe differences in the number of areas with inflammatory infiltrate between the groups; however, we previously demonstrated that the use of the synbiotic VSL#3 + PBY was able to reduce TNF concentrations (1.4-fold, $p = 0.028$) and increase interleukins 2 (1.5-fold, $p = 0.027$) and 4 (1.5-fold, $p = 0.035$), while the probiotic VSL#3 increased interleukin 4 levels (1.6-fold, $p = 0.044$) (Cruz et al., 2020a). Furthermore, the CON group presented deeper infiltrate areas, reaching the submucosal and muscularis layers (Figure 3c).

3.2.3 | Biochemical markers

Serum biomarkers were evaluated in order to verify alterations in liver and kidney functions. There were no

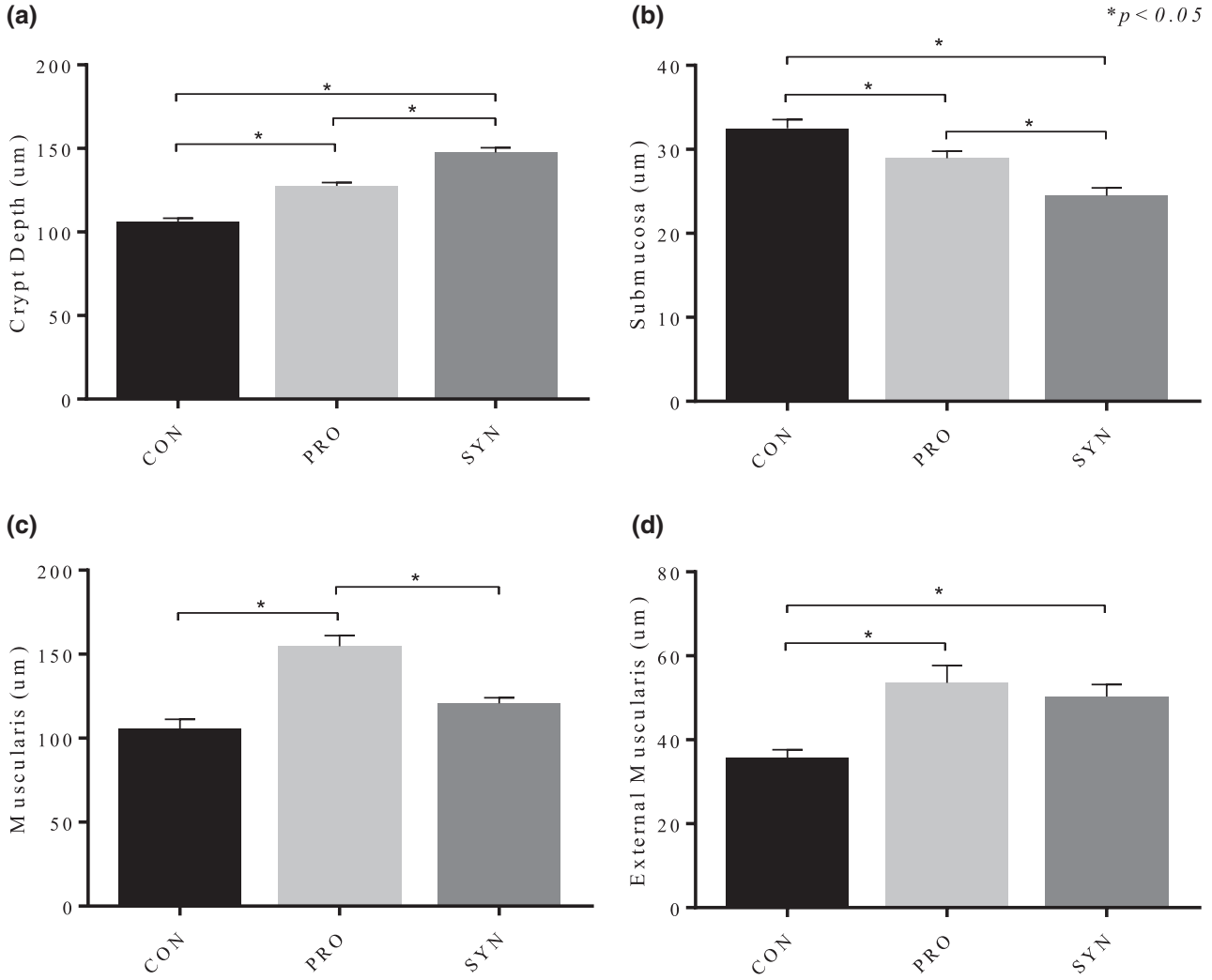

FIGURE 2 Effect of probiotic and synbiotic on intestinal morphometry in a colorectal carcinogenesis model. (a) Crypt depth, (b) submucosa layer, (c) muscularis layer, and (d) external muscularis layer. The data were expressed as mean ± SD ($n = 7$/group). Statistical difference between groups was analyzed by Anova test or Kruskal–Wallis test. (*) $p < 0.05$. CON, AIN-93 M diet; PRO, AIN-93 M diet and probiotic VSL#3®; SYN, AIN-93 M diet with PBY and probiotic VSL#3

differences between groups in serum levels of AST, ALT, alkaline phosphatase, GGT, urea, creatinine, and albumin (Figure 4). The results were within the limits recommended for the animal model used.

Similar results were observed by Sivieri et al. (2008), in a study with animals induced to preneoplastic lesions and who received probiotic for 42 weeks. De Nadai Marcon et al. (2019) also did not observe differences between serum markers of liver and renal function in mice induced to colorectal carcinogenesis and who received PBY for 8 weeks. Traditionally, the measurement of specific enzyme levels has served as a good indicator of tissue damage. The fact that there are no differences between the biomarkers evaluated may be the result of adaptations that have occurred due to stress conditions.

3.2.4 | Intestinal permeability

The intestinal permeability was measured by the urinary excretion of lactulose and mannitol, two nonmetabolizable sugars. Lactulose is absorbed via the paracellular route, while mannitol is absorbed via the transcellular route, through small aqueous pores, present in the intestinal epithelial cell membrane. Increased absorption of lactulose is indicative of increased permeability and evidences the occurrence of intestinal barrier dysfunction (Arrieta et al., 2006; Stewart et al., 2017).

Intestinal barrier dysfunction can increase the passage of antigens from the lumen to the intestinal mucosa and initiate an inflammatory process, increasing the risk of CRC (Mankertz & Schulzke, 2007; Molska & Regula, 2019).

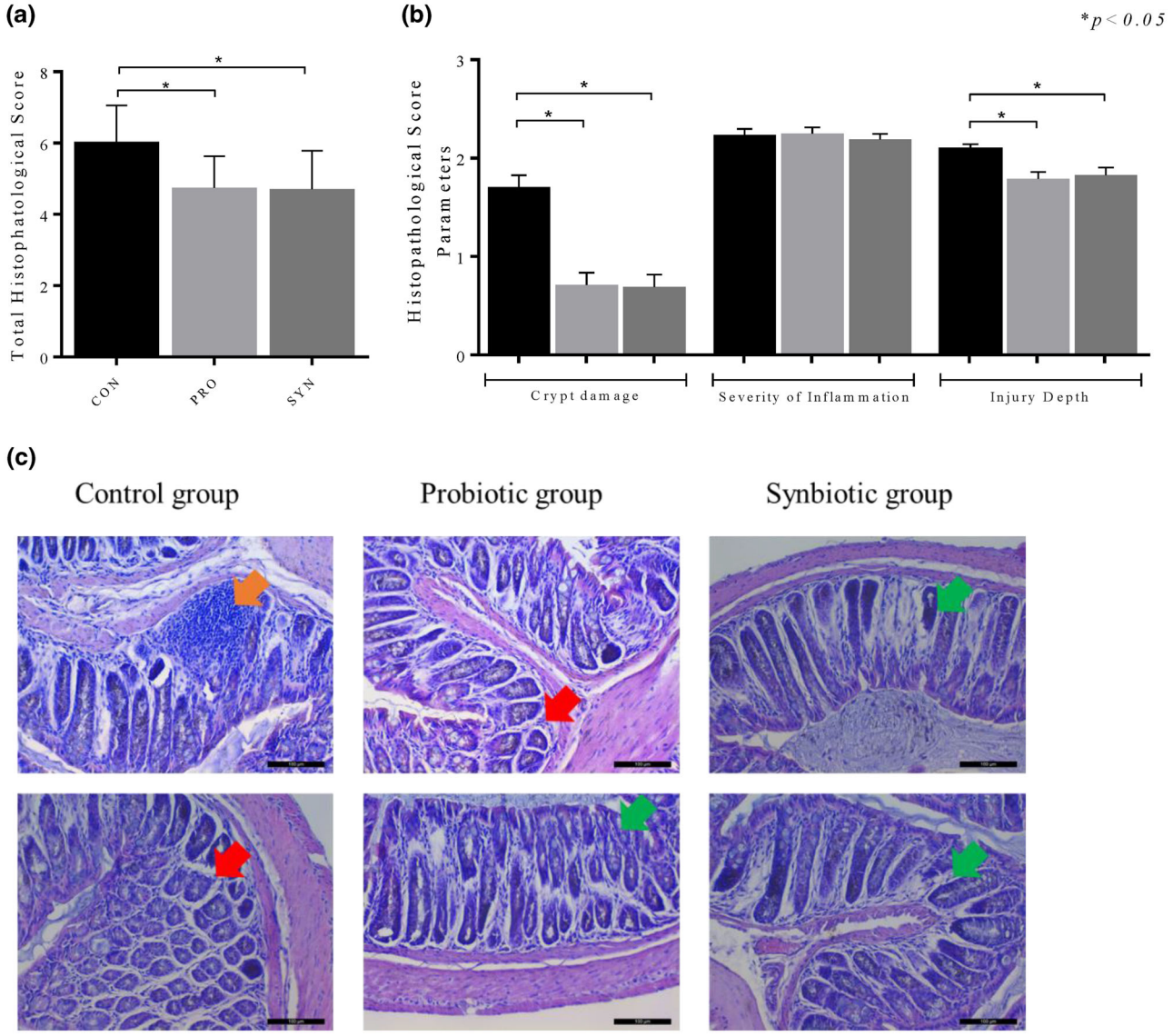

FIGURE 3 Effect of probiotic and synbiotic on histophatological score in a colorectal carcinogenesis model. (a) Total histophatological score, (b) histopathological score parameters, and (c) illustrative photomicrography of the colon of mice induced to carcinogenesis colorectal stained with hematoxylin-eosin (HE). Orange arrow: extensive area of inflammatory infiltrate (submucosa); red arrow: preserved colonic crypts and damaged crypt transition area; green arrow: preserved crypts and epithelium (scale bars, 100 μm). The data were expressed as mean ± SD ($n = 7$/group). Statistical difference between groups was analyzed by Anova test or Kruskal–Wallis test. (*) $p < 0.05$. CON, AIN-93 M diet; PRO, AIN-93 M diet and probiotic VSL#3®; SYN, AIN-93 M diet with PBY and probiotic VSL#3

Thus, intestinal barrier is an important target in the prevention and treatment of intestinal diseases (Lee, 2015).

The percentage of urinary lactulose excretion was higher in the CON group compared to the PRO (0.93 ± 0.62% × 0.44 ± 0.05%, $p = 0.048$) and SYN (0.93 ± 0.62% × 0.41 ± 0.12%, $p = 0.043$) groups. The PRO and SYN groups have not presented differences from each other. Monosaccharide mannitol was not identified in any of the urine samples.

Probiotics and synbiotics have been proposed as promising interventions to reduce intestinal barrier dysfunction. Studies have shown a reduction in intestinal permeability after using probiotic or synbiotic (Gotteland et al., 2001; Russo et al., 2012). One of the mechanisms by which the consumption of probiotics and synbiotics could regulate the function of the intestinal barrier is by stimulating the expression of tight junction proteins and increasing mucus production by the goblet cells (Karczewski et al., 2010).

SCFAs, produced from the fermentation of prebiotics, play a particularly important role in protecting the barrier of the intestinal mucosa. SCFAs activate 5′-adenosine monophosphate-activated protein kinase, which is a key agent in regulating energy metabolism in colonocytes,

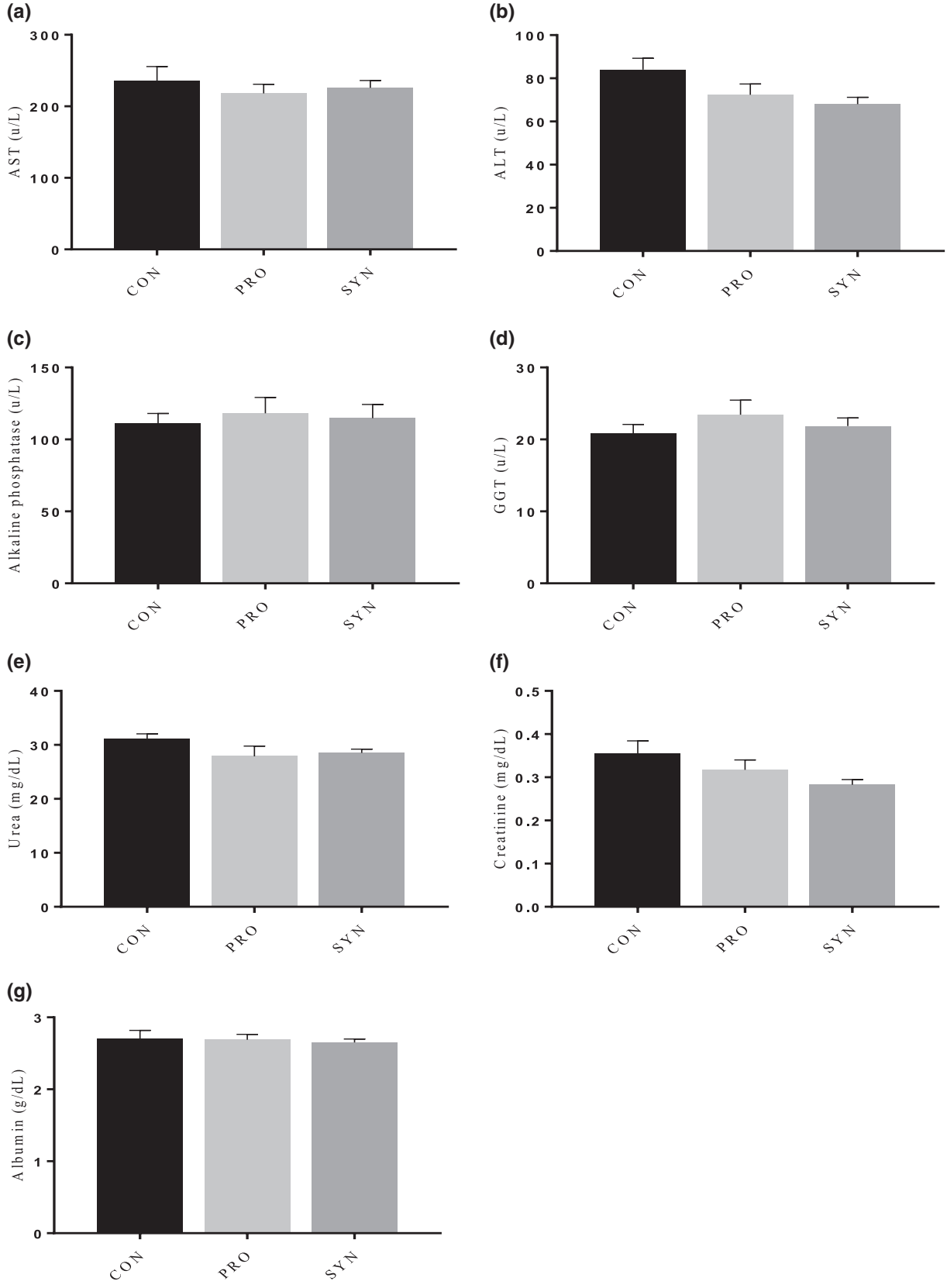

FIGURE 4 Effect of probiotic and synbiotic on biochemical markers in a colorectal carcinogenesis model. (a) Aspartate aminotransferase, (b) alanine aminotransferase, (c) alkaline phosphatase, (d) gamma-glutamyl transferase, (e) area, (f) creatinine, and (g) albumin. The data were expressed as mean ± SD ($n = 15$/group). Statistical difference between groups was analyzed by Anova test or Kruskal–Wallis test

TABLE 2 Effect of probiotic and synbiotic on oxidation products and antioxidant enzymes activity in C57BL/6 mice

	CON	PRO	SYN	*p*
MDA (nmol/mg protein)				
Liver	0.232 ± 0.06[a]	0.134 ± 0.06[b]	0.154 ± 0.03[b]	0.011[#]
Cecum	0.973 ± 0.23	0.817 ± 0.12	1.022 ± 0.27	0.280[*]
CP (nmol/ml)				
Liver	19.310 ± 7.02[a]	9.693 ± 6.56[b]	3.417 ± 1.49[b]	0.000[*]
Cecum	2.634 ± 1.19	2.262 ± 1.23	2.293 ± 0.74	0.584[#]
SOD (U/mg protein)				
Liver	19.903 ± 4.51	19.502 ± 1.60	21.519 ± 3.30	0.485[*]
Cecum	38.440 ± 2.58	42.881 ± 4.73	37.787 ± 7.09	0.773[*]
CAT (U/mg protein)				
Liver	4.059 ± 1.34[a]	4.405 ± 0.66[a,b]	5.827 ± 1.06[b]	0.014[*]
Cecum	0.359 ± 0.18	0.298 ± 0.07	0.370 ± 0.06	0.474[*]
GST (nmol/min/mg protein)				
Liver	71.667 ± 5.30	73.097 ± 3.46	71.148 ± 5.01	0.726[*]
Cecum	12.771 ± 1.23	13.526 ± 1.83	13.651 ± 2.90	0.701[*]

CAT, catalase; CP, carbonylated protein; GST, glutathione-S-transferase; MDA, malondyaldeide; SOD, superoxide dismutase. The data were expressed as mean ± SD (n = 8/group). Statistical difference between groups was analyzed by ANOVA[(*)] test or Kruskal–Wallis test[(#)], with $p < 0.05$. Different letters in the same line indicate statistical difference. CON, AIN-93 M diet; PRO, AIN-93 M diet and probiotic VSL#3; SYN, AIN-93 M diet with PBY and probiotic VSL#3.

leading to the strengthening of intestinal epithelial junctions and, consequently, to a strong and healthy barrier. Inhibition of histone deacetylase by butyrate increases mucus synthesis and mucosal thickness, as well as stimulates mucosal repair (Eslami et al., 2019; Molska & Regula, 2019). As previously demonstrated (Cruz et al., 2020a), the use of the synbiotic VSL#3 + PBY significantly increased SCFA concentrations, which may explain the reduction in intestinal permeability.

3.2.5 | Products of oxidation and antioxidant activity in hepatic and cecal tissues

Oxidative stress was evaluated in hepatic and cecal tissue, through the quantification of lipid and protein oxidation products, MDA and CP, respectively, and the evaluation of the activity of endogenous antioxidant enzymes (SOD, CAT, and GST). The intestine and liver are constantly exposed to RF, whether they are produced endogenously (by intestinal bacteria) or by exogenous sources, such as the DMH used to induce preneoplastic lesions (Brenner et al., 2015; Cruz et al., 2020b). Thus, considering the close relationship between oxidative stress and tumor development, an antioxidant system capable of maintaining the redox balance is essential.

In hepatic tissue, the CON group presented MDA concentration 1.8-fold (p = 0.007) and 1.5-fold (p = 0.035) higher than in the PRO and SYN groups, respectively; for CP, the results are even more expressive: 2-fold (p = 0.008) and 5.6-fold (p = 0.000) higher (Table 2). The reduction in MDA and CP concentration corroborates the results obtained in the evaluation of the *in vitro* antioxidant activity of VSL#3 and PBY.

CAT enzyme activity increased 1.43-fold (p = 0.014) in the SYN group. For the other enzymes, there were no differences between the groups. This enzyme is produced in large amounts in hepatic tissue, and composes one of the main defense systems against oxidative stress because it quickly converts H_2O_2 into H_2O and O_2 (Cheng et al., 2020); therefore, the increase in its activity indicates an increase in the endogenous antioxidant defense promoted by the synbiotic. In addition, some probiotic species, such as Lactobacillus present in the synbiotic formulation, are capable of producing CAT, which contributes to the increase of antioxidant response (Cruz et al., 2020b; Eslami et al., 2019).

In cecum, no significant differences were observed in oxidation products or enzyme activity for all groups. Some reasons support the differences observed between liver and cecum. The activation of DMH in its active metabolite occurs predominantly in the liver, with the formation of a large amount of FR. Additionally, dysbiosis and increased intestinal permeability will result in bacterial products in the portal blood, and the cells of the liver will be first to be exposed, thus, there is a great demand for the production of antioxidants in this organ specifically (Jackson et al., 2003).

The control of oxidative stress and the maintenance of the intestinal barrier are interconnected targets for the prevention of CRC. The use of probiotic and synbiotic

contributes to a reduction in oxidative stress and inflammation, which in turn leads to increased expression of the tight junction protein, such as claudin−3 and occludin, thereby restoring barrier function (Cruz et al., 2020b; Molska & Regula, 2019).

4 | CONCLUSION

The probiotic VSL#3 and the synbiotic VSL#3 + PBY showed antioxidant activity *in vitro* and *in vivo*, demonstrated by the significant reduction of markers of hepatic oxidative stress and increased activity of the enzyme catalase ($p < 0.005$). Through histopathological analysis, less damage was observed in the intestinal epithelium of animals that received probiotic or synbiotic, as well as the significant reduction ($p < 0.005$) in intestinal permeability, measured by the lower urinary excretion of lactulose. The crypt depth increased 1.2-fold and 1.4-fold with the use of probiotic and synbiotic, respectively, compared to the control. However, the synbiotic showed greater influence, since there was also a significant difference when compared to the group that received only the probiotic (1.1-fold; $p = 0.000$); this difference is justified by the trophic effect promoted by prebiotics. Reducing oxidative stress and permeability of the intestinal barrier are important ways to protect against CRC. Our findings suggest the promising use of probiotic and sybioticsas food ingredients for preventing CRC.

FUNDING

Our work was supported by the National Council of Technological and Scientific Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), and Foundation for the Support to the Researches in Minas Gerais (Fapemig).

AUTHOR CONTRIBUTIONS

B. Cruz was involved in conception, methodology, formal analysis, investigation, data curation, and writing (preparation, review, and editing). L. de Sousa Moraes and L. De Nadai Marcon were involved in methodology, formal analysis, and investigation. K. Dias was involved in formal analysis and investigation. L. Murad was involved in conception and writing (review and editing). M. Sarandy was involved in formal analysis and investigation. L. Conceição and R. Gonçalves were involved in methodology and resources. C. Ferreira was involved in conception, methodology, resources, and funding. M. Peluzio was involved in conception, methodology, resources, writing (preparation, review, and editing), supervision, project administration, and funding.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Bruna Cristina dos Santos Cruz https://orcid.org/0000-0003-1248-7066
Luís Fernando de Sousa Moraes https://orcid.org/0000-0003-4170-0233
Letícia De Nadai Marcon https://orcid.org/0000-0001-7007-7626
Kelly Aparecida Dias https://orcid.org/0000-0003-1199-0348
Leonardo Borges Murad https://orcid.org/0000-0001-8210-3000
Mariáurea Matias Sarandy https://orcid.org/0000-0003-1313-7877
Lisiane Lopes da Conceição https://orcid.org/0000-0001-8352-5539
Reggiani Vilela Gonçalves https://orcid.org/0000-0002-5831-3590
Célia Lúcia de Luces Fortes Ferreira https://orcid.org/0000-0002-0040-6938
Maria do Carmo Gouveia Peluzio https://orcid.org/0000-0003-4665-7043

REFERENCES

Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*, *105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

AOAC International. (1997). *Official methods of analysis* (16th edition). Gaitherburg: AOAC International.

Arrieta, M. C., Bistritz, L., & Meddings, J. B. (2006). Alterations in intestinal permeability. *Gut*, *55*(10), 1512–1520. https://doi.org/10.1136/gut.2005.085373

Arthur, J. C., Gharaibeh, R. Z., Uronis, J. M., Perez-Chanona, E., Sha, W., Tomkovich, S., Mühlbauer, M., Fodor, A. A., & Jobin, C. (2013). VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. *Scientific Report*, *8*(3), 2868. https://doi.org/10.1038/srep02868

Bassaganya-Riera, J., Viladomiu, M., Pedragosa, M., De Simone, C., & Hontecillas, R. (2012). Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. *PLoS One*, *7*(4), e34676. https://doi.org/10.1371/journal.pone.0034676

Bird, R. P., & Good, C. K. (2000). The significance of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Toxicology Letters*, *112–113*, 395–402. https://doi.org/10.1016/s0378-4274(99)00261-1

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/10.3322/caac.21492

Brenner, D. A., Paik, Y. H., & Schnabl, B. (2015). Role of gut microbiota in liver disease. *Journal of Clinical Gastroenterology*, *49*(Suppl 1), S25–S27. https://doi.org/10.1097/MCG.0000000000000391

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, https://doi.org/10.1016/S0076-6879(78)52032-6

Carson, F. L., Martin, J. H., & Lynn, J. A. (1973). Formalin fixation for electron microscopy: A re-evaluation. *American Journal of Clinical Pathology*, *59*(3), 365–373. https://doi.org/10.1093/ajcp/59.3.365

Chang, J. H., Shim, Y. Y., Cha, S. K., Reaney, M., & Chee, K. M. (2012). Effect of *Lactobacillus acidophilus* KFRI342 on the development of chemically induced precancerous growths in the rat colon. *Journal of Medical Microbiology*, *61*(Pt 3), 361–368. https://doi.org/10.1099/jmm.0.035154-0

Cheng, F. S., Pan, D., Chang, B., Jiang, M., & Sang, L. X. (2020). Probiotic mixture VSL#3: An overview of basic and clinical studies in chronic diseases. *World Journal of Clinical Cases*, *8*(8), 1361–1384. https://doi.org/10.12998/wjcc.v8.i8.1361

Corridoni, D., Pastorelli, L., Mattioli, B., Locovei, S., Ishikawa, D., Arseneau, K. O., Chieppa, M., Cominelli, F., & Pizarro, T. T. (2012). Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. *PLoS One*, *7*(7), e42067. https://doi.org/10.1371/journal.pone.0042067

Cruz, B., Sarandy, M. M., Messias, A. C., Gonçalves, R. V., Ferreira, C., & Peluzio, M. (2020b). Preclinical and clinical relevance of probiotics and synbiotics in colorectal carcinogenesis: A systematic review. *Nutrition Reviews*, *78*(8), 667–687. https://doi.org/10.1093/nutrit/nuz087

Cruz, B. C. S., Duarte, V. d. S., Giacomini, A., Corich, V., de Paula, S. O., da Silva Fialho, L., Guimarães, V. M., de Luces Fortes Ferreira, C. L., & Peluzio, M. d. C. G. (2020a). Synbiotic VSL#3 and yacon-based product modulate the intestinal microbiota and prevent the development of pre-neoplastic lesions in a colorectal carcinogenesis model. *Applied Microbiology and Biotechnology*, *104*(20), 8837–8857. https://doi.org/10.1007/s00253-020-10863-x

Dai, C., Zhao, D. H., & Jiang, M. (2012). VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. *International Journal of Molecular Medicine*, *29*(2), 202–208. https://doi.org/10.3892/ijmm.2011.839

de Moura, N. A., Caetano, B. F., Sivieri, K., Urbano, L. H., Cabello, C., Rodrigues, M. A., & Barbisan, L. F. (2012). Protective effects of yacon (*Smallanthus sonchifolius*) intake on experimental colon carcinogenesis. *Food and Chemical Toxicology*, *50*(8), 2902–2910. https://doi.org/10.1016/j.fct.2012.05.006

De Nadai Marcon, L., Moraes, L. F. S., Cruz, B. C. S., Teixeira, M. D. O., Bruno, T. C. V., Ribeiro, I. E., Messias, A. C., Ferreira, C. L. L. F., Oliveira, L. L., & Peluzio, M. C. G. (2019). Yacon (*Smallanthus sonchifolius*)-based product increases fecal short-chain fatty acids and enhances regulatory T cells by downregulating RORγt in the colon of BALB/c mice. *Journal of Functional Foods*, *55*, 333–342. https://doi.org/10.1016/j.jff.2019.02.039

De Nadai Marcon, L., Moraes, L. F. S., Cruz, B. C. S., Teixeira, M. D. O., Gomides, A. F. F., Ferreira, C. L. L. F., Oliveira, L. L., Oliveira, L. L., & Peluzio, M. C. G. (2020). Yacon (*Smallanthus sonchifolius*)-based product increases fecal short-chain fatty acids concentration and up-regulates t-Bet expression in the colon of BALB/c mice during colorectal carcinogenesis. *Food Science and Nutrition*, *6*(3), https://doi.org/10.24966/FSN-1076/100069

Dieleman, L. A., Ridwan, B. U., Tennyson, G. S., Beagley, K. W., Bucy, R. P., & Elson, C. O. (1994). Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology*, *107*(6), 1643–1652. https://doi.org/10.1016/0016-5085(94)90803-6

Dieterich, S., Bieligk, U., Beulich, K., Hasenfuss, G., & Prestle, J. (2000). Gene expression of antioxidative enzymes in the human heart: Increased expression of catalase in the end-stage failing heart. *Circulation*, *101*, 33–39. https://doi.org/10.1161/01.cir.101.1.33

Eslami, M., Yousefi, B., Kokhaei, P., Hemati, M., Nejad, Z. R., Arabkari, V., & Namdar, A. (2019). Importance of probiotics in the prevention and treatment of colorectal cancer. *Journal of Cellular Physiology*, *234*(10), 17127–17143. https://doi.org/10.1002/jcp.28473

Gagnière, J., Raisch, J., Veziant, J., Barnich, N., Bonnet, R., Buc, E., Bringer, M. A., Pezet, D., & Bonnet, M. (2016). Gut microbiota imbalance and colorectal cancer. *World Journal of Gastroenterology*, *22*(2), 501–518. https://doi.org/10.3748/wjg.v22.i2.501

Genaro, S. C., Reis, L. d. S. L. S., Reis, S. K., Rabelo Socca, E. A., & Fávaro, W. J. (2019). Probiotic supplementation attenuates the aggressiveness of chemically induced colorectal tumor in rats. *Life Sciences*, *237*, 116895. https://doi.org/10.1016/j.lfs.2019.116895

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Gionchetti, P., Rizzello, F., Morselli, C., Poggioli, G., Tambasco, R., Calabrese, C., Brigidi, P., Vitali, B., Straforini, G., & Campieri, M. (2007). High-dose probiotics for the treatment of active pouchitis. *Diseases of the Colon and Rectum*, *50*(12), 2075–2084. https://doi.org/10.1007/s10350-007-9068-4

Gomides, A. F., de Paula, S. O., Gonçalves, R. V., de Oliveira, L. L., Ferreira, C. L., Comastri, D. S., & Peluzio, M. (2014). Prebiotics prevent the appearance of aberrant crypt foci (ACF) in the colon of BALB/c mice for increasing the gene expression of p16 protein. *Nutricion Hospitalaria*, *30*(4), 883–890. https://doi.org/10.3305/nh.2014.30.4.7672

Gotteland, M., Cruchet, S., & Verbeke, S. (2001). Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indometacin in humans. *Alimentary Pharmacology & Therapeutics*, *15*(1), 11–17. https://doi.org/10.1046/j.1365-2036.2001.00898.x

Green, M., Arora, K., & Prakash, S. (2020). Microbial medicine: Prebiotic and probiotic functional foods to target obesity and metabolic syndrome. *International Journal of Molecular Sciences*, *21*(8), 2890. https://doi.org/10.3390/ijms21082890

Greenman, C., Stephens, P., Smith, R., Dalgliesh, G. L., Hunter, C., Bignell, G., Davies, H., Teague, J., Butler, A., Stevens, C., Edkins, S., O'Meara, S., Vastrik, I., Schmidt, E. E., Avis, T., Barthorpe, S., Bhamra, G., Buck, G., Choudhury, B., Clements, J., et al. (2007). Patterns of somatic mutation in human cancer genomes. *Nature*, *446*(7132), 153–158. https://doi.org/10.1038/nature05610

Gusso, A. P., Mattanna, P., & Richards, N. (2015). Yacon: Health benefits and technological applications. *Ciência Rural*, *45*(5), 912–919. https://dx.doi.org/10.1590/0103-8478cr20140963

Habig, W. H., & Jakoby, W. B. (1981). Glutathione-S-transferase (rat and human). *Methods in Enzymology*, *77*, 218–239.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, *11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66

Jackson, P. E., O'Connor, P. J., Cooper, D. P., Margison, G. P., & Povey, A. C. (2003). Associations between tissue-specific DNA alkylation, DNA repair and cell proliferation in the colon and colon tumour yield in mice treated with 1,2-dimethylhydrazine. *Carcinogenesis*, *24*(3), 527–533. https://doi.org/10.1093/carcin/24.3.527

Jahani-Sherafat, S., Alebouyeh, M., Moghim, S., Ahmadi Amoli, H., & Ghasemian-Safaei, H. (2018). Role of gut microbiota in the pathogenesis of colorectal cancer: A review article. *Gastroenterology and Hepatology from Bed to Bench*, *11*(2), 101–109.

Jin, W., Wang, H., Ji, Y., Hu, Q., Yan, W., Chen, G., & Yin, H. (2008). Increased intestinal inflammatory response and gut barrier dysfunction in Nrf2-deficient mice after traumatic brain injury. *Cytokine*, *44*(1), 135–140. https://doi.org/10.1016/j.cyto.2008.07.005

Karczewski, J., Troost, F. J., Konings, I., Dekker, J., Kleerebezem, M., Brummer, R. J., & Wells, J. M. (2010). Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum in vivo* and protective effects on the epithelial barrier. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *298*(6), G851–G859. https://doi.org/10.1152/ajpgi.00327.2009

Khajehei, F., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2018). Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a novel source of health promoting compounds: Antioxidant activity, phytochemicals and sugar content in flesh, peel, and whole tubers of seven cultivars. *Molecules (Basel, Switzerland)*, *23*(2), 278. https://doi.org/10.3390/molecules23020278

Khare, S., Chaudhary, K., Bissonnette, M., & Carroll, R. (2009). Aberrant crypt foci in colon cancer epidemiology. *Methods in Molecular Biology (Clifton, N.J.)*, *472*, 373–386. https://doi.org/10.1007/978-1-60327-492-0_17

Kich, D. M., Vincenzi, A., Majolo, F., Volken de Souza, C. F., & Goettert, M. I. (2016). Probiotic: Effectiveness nutrition in cancer treatment and prevention. *Nutricion Hospitalaria*, *33*(6), 1430–1437. https://doi.org/10.20960/nh.806

Kim, H., Kim, J. S., Kim, Y., Jeong, Y., Kim, J. E., Paek, N. S., & Kang, C. H. (2020). Antioxidant and probiotic properties of Lactobacilli and Bifidobacteria of human origins. *Biotechnology and Bioprocess Engineering*, *25*, 421–430. https://doi.org/10.1007/s12257-020-0147-x

Lee, S. H. (2015). Intestinal permeability regulation by tight junction: Implication on inflammatory bowel diseases. *Intestinal Research*, *13*(1), 11–18. https://doi.org/10.5217/ir.2015.13.1.11

Lenoir, M., Del Carmen, S., Cortes-Perez, N. G., Lozano-Ojalvo, D., Muñoz-Provencio, D., Chain, F., Langella, P., de Moreno de LeBlanc, A., LeBlanc, J. G., & Bermúdez-Humarán, L. G. (2016). *Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *Journal of Gastroenterology*, *51*(9), 862–873. https://doi.org/10.1007/s00535-015-1158-9

Leu, R. K. L., Hu, Y., Brown, I. L., Woodman, R. J., & Young, G. P. (2010). Synbiotic intervention of *Bifidobacterium lactis* and resistant starch protects against colorectal cancer development in rats. *Carcinogenesis*, *31*, 246–251

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B.-H., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology*, *186*, 464–478.

Li, S. C., Lin, H. P., Chang, J. S., & Shih, C. K. (2019). *Lactobacillus acidophilus*-fermented germinated brown rice suppresses preneoplastic lesions of the colon in rats. *Nutrients*, *11*(11), 2718. https://doi.org/10.3390/nu11112718

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Lucas, C., Barnich, N., & Nguyen, H. (2017). Microbiota, inflammation and colorectal cancer. *International Journal of Molecular Sciences*, *18*(6), 1310. https://doi.org/10.3390/ijms18061310

Mankertz, J., & Schulzke, J. D. (2007). Altered permeability in inflammatory bowel disease: Pathophysiology and clinical implications. *Current Opinion in Gastroenterology*, *23*(4), 379–383. https://doi.org/10.1097/MOG.0b013e32816aa392

Mau, J. L., Lin, H. C., & Chen, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agricultural and Food Chemistry*, *50*(21), 6072–6077. https://doi.org/10.1021/jf0201273

Mohania, D., Kansal, V., Sagwal, R., & Shah, D. (2013). Anticarcinogenic effect of probiotic dahi and piroxicam on DMH-induced colorectal carcinogenesis in Wistar rats. *Cancer Therapy and Pharmacology*, http://ivyunion.org/index.php/ajctp

Molska, M., & Reguła, J. (2019). Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. *Nutrients*, *11*(10), 2453. https://doi.org/10.3390/nu11102453

Newell, L. E., & Heddle, J. A. (2004). The potent colon carcinogen, 1,2-dimethylhydrazine induces mutations primarily in the colon. *Mutation Research*, *564*(1), 1–7. https://doi.org/10.1016/j.mrgentox.2004.06.005

O'Donnell, J. S., Teng, M., & Smyth, M. J. (2019). Cancer immunoediting and resistance to T cell-based immunotherapy. *Nature Reviews. Clinical Oncology*, *16*(3), 151–167. https://doi.org/10.1038/s41571-018-0142-8

Oh, N. S., Joung, J. Y., Lee, J. Y., Kim, Y. J., Kim, Y., & Kim, S. H. (2020). A synbiotic combination of *Lactobacillus gasseri* 505 and *Cudrania tricuspidata* leaf extract prevents hepatic toxicity induced by colorectal cancer in mice. *Journal of Dairy Science*, *103*(4), 2947–2955. https://doi.org/10.3168/jds.2019-17411

Pattananandecha, T., Sirilun, S., Duangjitcharoen, Y., Sivamaruthi, B. S., Suwannalert, P., Peerajan, S., & Chaiyasut, C. (2016). Hydrolysed inulin alleviates the azoxymethane-induced preneoplastic aberrant crypt foci by altering selected intestinal microbiota in Sprague–Dawley rats. *Pharmaceutical Biology*, *54*(9), 1596–1605. https://doi.org/10.3109/13880209.2015.1110597

Paula, H. A. A., Martins, J. F. L., Sartori, S. S. R., Castro, A. S. B., Abranches, M. V., Rafael, V. C., & Ferreira, C. L. L. F. (2012) The yacon product PBY: Which is the best dose to evaluate the functionality of this new source of prebiotic fructans? Functional Foods Forum Probiotics, Turku.

Rafter, J., Bennett, M., Caderni, G., Clune, Y., Hughes, R., Karlsson, P. C., Klinder, A., O'Riordan, M., O'Sullivan, G. C., Pool-Zobel, B., Rechkemmer, G., Roller, M., Rowland, I., Salvadori, M., Thijs, H., Van Loo, J., Watzl, B., & Collins, J. K. (2007). Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer

patients. *American Journal of Clinical Nutrition*, *85*(2), 488–496. https://doi.org/10.1093/ajcn/85.2.488

Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. *FASEB Journal*, *22*(3), 659–661. https://doi.org/10.1096/fj.07-9574LSF

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. https://doi.org/10.1093/jn/123.11.1939

Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, *137*(3 Suppl 2), 830S–837S. https://doi.org/10.1093/jn/137.3.830S

Rodrigues, V. C. (2011). Formulaçao, índice glicêmico e aplicação alimentar de um produto à base de yacon (*Smallanthus sonchifolius*). Tesis, 89 p.

Russo, F., Linsalata, M., Clemente, C., Chiloiro, M., Orlando, A., Marconi, E., Chimienti, G., & Riezzo, G. (2012). Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutrition Research (New York, N.Y.)*, *32*(12), 940–946. https://doi.org/10.1016/j.nutres.2012.09.010

Sant'Anna, M. S. L., Rodrigues, V. C., Araújo, T. F., Oliveira, T. T., Pelúzio, M. C. G., & Ferreira, C. L. L. (2018). Yacon product (PBY) modulates intestinal constipation and protects the integrity of crypts in Wistar rats. *Food Nutrition Science*, *9*, 1391–1407

Sarao, L. K., & Arora, M. (2017). Probiotics, prebiotics, and microencapsulation: A review. *Critical Reviews in Food Science and Nutrition*, *57*(2), 344–371. https://doi.org/10.1080/10408398.2014.887055

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology: CB*, *24*(10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Shahidi, F., & Yeo, J. (2018). Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. *International Journal of Molecular Sciences*, *19*(6), 1573. https://doi.org/10.3390/ijms19061573

Siegel, R. L., Miller, K. D., Goding Sauer, A., Fedewa, S. A., Butterly, L. F., Anderson, J. C., Cercek, A., Smith, R. A., & Jemal, A. (2020). Colorectal cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, *70*(3), 145–164. https://doi.org/10.3322/caac.21601

Sivieri, K., Spinardi-Barbisan, A. L. T., Barbisan, L. F., Bedani, R., Pauly, N. D., Carlos, I. Z., Benzatti, F., Vendramini, R. C., & Rossi, E. A. (2008) Probiotic *Enterococcus faecium* CRL 183 inhibit chemically induced colon cancer in male wistar rats. *European Food Research and Technology*, *228*, 231–237

Stewart, A. S., Pratt-Phillips, S., & Gonzalez, L. M. (2017). Alterations in intestinal permeability: The role of the "leaky gut" in health and disease. *Journal of Equine Veterinary Science*, *52*, 10–22. https://doi.org/10.1016/j.jevs.2017.02.009

Uronis, J. M., Arthur, J. C., Keku, T., Fodor, A., Carroll, I. M., Cruz, M. L., Appleyard, C. B., & Jobin, C. (2011). Gut microbial diversity is reduced by the probiotic VSL#3 and correlates with decreased TNBS-induced colitis. *Inflammatory Bowel Diseases*, *17*(1), 289–297. https://doi.org/10.1002/ibd.21366

Verediano, T. A., Viana, M. L., das Graças, V., Tostes, M., de Oliveira, D. S., de Carvalho Nunes, L., & Costa, N. M. (2020). Yacón (*Smallanthus sonchifolius*) prevented inflammation, oxidative stress, and intestinal alterations in an animal model of colorectal carcinogenesis. *Journal of the Science of Food and Agriculture*, *100*(15), 5442–5449. https://doi.org/10.1002/jsfa.10595

Wallin, B., Rosengren, B., Shetzer, H. G., & Cameja, G. (1993). Lipid oxidation and measurement of thiobarbituric acid reacting substances (TBARS) formation in a single microtitre plate: Its use for evaluation of antioxidants. *Analytical Biochemistry*, *208*, 10–15. https://doi.org/10.1006/abio.1993.1002

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant properties of probiotic bacteria. *Nutrients*, *9*(5), 521. https://doi.org/10.3390/nu9050521

Zhang, Y., Li, L., Guo, C., Mu, D., Feng, B., Zuo, X., & Li, Y. (2016). Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: A meta-analysis. *BMC Gastroenterology*, *16*(1), 62. https://doi.org/10.1186/s12876-016-0470-z

How to cite this article: Bruna Cristina dos Santos Cruz, Luís Fernando de Sousa Moraes, Letícia De Nadai Marcon, Kelly Aparecida Dias, Leonardo Borges Murad, Mariáurea Matias Sarandy, Lisiane Lopes da Conceição, Reggiani Vilela Gonçalves, Célia Lúcia de Luces Fortes Ferreira, Maria do Carmo Gouveia Peluzio. Evaluation of the efficacy of probiotic VSL#3 and synbiotic VSL#3 and yacon-based product in reducing oxidative stress and intestinal permeability in mice induced to colorectal carcinogenesis. *J Food Sci*. 2021;86:1448–1462. https://doi.org/10.1111/1750-3841.15690