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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

THAÍSA AGRIZZI VEREDIANO

EFEITO PREVENTIVO DA YACON (*Smallanthus sonchifolius*) NAS ALTERAÇÕES INFLAMATÓRIAS, OXIDATIVAS E DA INTEGRIDADE INTESTINAL EM MODELO ANIMAL DE CARCINOGÊNESE COLORRETAL.

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Dissertação apresentada ao programa de Pós-Graduação em Ciência e Tecnologia de Alimentos do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, como parte das exigências para obtenção do Título de Mestre em Ciência e Tecnologia de Alimentos.

Orientadora: Profª. Dra. Neuza Maria Brunoro Costa
Coorientadoras: Profª. Dra. Mirelle Lomar Viana
Profª . Dra. Maria das Graças Vaz Tostes

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Aprovada em 26 de fevereiro de 2019.

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VEREDIANO, THAISA AGRIZZI. **Efeito preventivo da yacon (*smallanthus sonchifolius*) nas alterações inflamatórias, oxidativas e da integridade intestinal em modelo animal de carcinogênese colorretal.** 2019. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) – Universidade Federal do Espírito Santo, Alegre – ES. Orientadora: Profª. Dra. Neuza Maria Brunoro Costa. Coorientadoras: Profª. Dra Mirelle Lomar Viana, Profª. Dra. Maria das Graças Vaz Tostes.

Fatores dietéticos estão relacionados à carcinogênese colorretal. Yacon (*Smallanthus sonchifolius*) é um alimento funcional com atividade prebiótica, por ser fonte abundante de frutooligossacarídeos (FOS), que são fermentados pelas bactérias benéficas com efeitos positivos à saúde intestinal. O objetivo deste estudo foi investigar o efeito prebiótico da farinha de yacon (FY), como fonte de FOS, na integridade da barreira intestinal, na resposta inflamatória e no estresse oxidativo em modelo animal de câncer de cólon induzido (CRC). Ratos *Wistar* adultos foram divididos em 4 grupos: S (sem CRC e sem FY, *n*=10); C (com CRC e sem FY, *n*=12); Y (sem CRC e com FY, *n*=10); CY (com CRC e com FY, *n*=12). Animais dos grupos S e C receberam a dieta AIN-93 M e os animais do grupo Y e CY receberam a mesma dieta acrescida de FY em quantidades suficientes para fornecer 5% FOS, durante as 16 semanas do experimento. Entre as semanas 4 – 8, os animais do grupo C e CY receberam, por via subcutânea, 25 mg/ kg peso corporal de 1,2-dimetilhidrazina (DMH) uma vez por semana. Na última semana, foi realizada a coleta da urina de 24 h para análise da permeabilidade intestinal utilizando lactulose e manitol. Amostra sanguínea foi coletada para análise das interleucinas (IL) -10 e IL-12, fator de necrose tumoral alfa (TNF-α) e capacidade antioxidante total (CAT). O intestino grosso foi coletado para análise do pH intraluminal, ácidos graxos de cadeia curta (AGCC) e imunoglobulina A secretória (sIgA). Os resultados foram analisados utilizando-se Two-way ANOVA (análise de variância), seguida pelo teste Newman-Keuls ($p<0,05$), com auxílio do GraphPad Prism®, versão 7. Os animais induzidos ao câncer apresentaram aumento de TNF-α, AGCC (acetato, propionato e butirato) e redução da CAT. A farinha de yacon exerceu efeito significativo na redução do pH intraluminal e a relação lactulose/manitol, mas não apresentou efeito no nível de IL-10, IL-12 e TNF-α. Ainda, sIgA aumentou no grupo que recebeu a FY sem a indução do CC (Grupo Y). Observou-se aumento de manitol e CAT no grupo CY, evidenciando interação significativa entre FY x CRC. Assim, o consumo da farinha de yacon promoveu efeitos benéficos na saúde intestinal de animais induzidos ao câncer de cólon e também foi benéfica em parâmetros intestinais dos animais saudáveis.

Palavras-chave: frutooligossacarídeos, prebióticos, inflamação, imunidade de mucosa, barreira intestinal.

ABSTRACT

VEREDIANO, THAISA AGRIZZI. Preventive effect of **yacon (*Smallanthus Sonchifolius*) on inflammatory, oxidative and intestinal integrity alterations in animal model of colorectal carcinogenesis**. 2019. Dissertation (Master in Food Science and Technology) – Federal University of Espirito Santo, Alegre – ES. Advisor: Prof[a]. Dra. Neuza Maria Brunoro Costa. Co-advisors: Prof[a]. Dra. Mirelle Lomar Viana, Prof[a]. Dra. Maria das Graças Vaz Tostes.

Colorectal cancer (CRC) presents dietary factors related to its carcinogenic process. Yacon (*Smallanthus sonchifolius*) is a functional food with prebiotic activity as source of fructooligosaccharides (FOS), which are fermented by bifidobacteria with positive effects to the intestinal health. The present study investigated the prebiotic effect of yacon flour (YF), source of FOS, on intestinal barrier integrity, inflammatory response and oxidative stress in animal model of induced colorectal cancer. Wistar rats were distributed in 4 groups: S (without CRC and YF, n=10), C (with CRC and without YF, n=12), Y (without CRC and with YF, n=10), CY (with CRC and YF, n=12). Animals of groups S and C received AIN-93 M diet and animals of groups Y and CY received the same diet but added with YF in amounts to provide 5 % FOS, during the 16 weeks of experiment. From week 4 to 8, the animals of C and CY groups received, by subcutaneous via, 25 mg/ kg body weight of 1.2-dimethilydrazine (DMH) once a week. In the last week, 24 h-urine collection was performed for intestinal permeability analysis using lactulose and mannitol. Blood sample was collected to the analysis of interleukin (IL)-10 and IL-12, tumor necrosis factor- alpha (TNF-α) and total antioxidant capacity (TAC). Large intestine was collected for intraluminal pH, short chain fatty acids (SCFA) and secretory immunoglobulin A (sIgA) analysis. Normal distributed data were analyzed by Two-way ANOVA (analysis of variance) followed by Newmans-Keuls test ($p<0.05$), using GraphPad Prism®, version 7. The animals induced to cancer had higher TNF- α, SCFA (acetate, propionate and butyrate), and lower TAC. The yacon flour reduced the intraluminal pH and lactulose/mannitol ratio, but had no effect on IL-10, IL-12 and TNF- α levels. Also, the levels of sIgA were increased only in the group fed YF without CRC (group Y). It was observed an increase of mannitol and CAT in group CY, showing a significant interaction of YF x CRC. Therefore, yacon flour consumption promoted beneficial effects on the intestinal health of colorectal cancer animals. As a source of FOS, yacon showed to improve the intestinal barrier and mucosal immunity particularly in healthy animals.

Keywords: fructooligosaccharides, prebiotics, inflammation, mucosal immunity, intestinal barrier.

1. INTRODUÇÃO

Câncer refere-se a um conjunto de doenças caracterizadas pela formação e crescimento celular anormal que ameaça a vida do indivíduo por interferir nas funções vitais do organismo (LÓPEZ-LÁZARO, 2018). Entre os diferentes tipos, o câncer colorretal (CRC) destaca-se como o segundo tipo de câncer com maior incidência estimada para as mulheres e o terceiro para os homens. No Brasil, estimou-se 17.380 casos novos em homens e 18.980 em mulheres para cada ano do biênio 2018-2019 (INCA, 2018).

Alguns fatores de risco tem sido associados à maior incidência do CRC, como histórico familiar, doença inflamatória intestinal, consumo excessivo de álcool, alta ingestão de carnes vermelha e processadas, ausência ou baixa atividade física, obesidade e diabetes (SONG; CHAN, 2019). Além disso, a microbiota intestinal, que refere-se ao conjunto de microrganismos presentes no intestino, tem sido reconhecida como um fator essencial na tumorogênese de cólon (TILG et al., 2018).

A carcinogênese do cólon é um processo multifatorial iniciado pela combinação de mutações genéticas e a presença de microrganismos patogênicos na microbiota intestinal. Sugere-se que a microbiota em situação de disbiose, ou seja, o desequilíbrio entre microrganismos benéficos e patogênicos, ocasiona maior produção de metabólitos tóxicos bacterianos, como os lipopolissacarídeos (LPS), e consequente redução dos metabólitos das bactérias benéficas (bacteriocinas, peptideoglicanos), além de contribuir para a destruição dos tecidos de barreira intestinal. Por sua vez, a redução da integridade da barreira epitelial ocasiona a translocação de bactérias e metabólitos, que iniciam uma resposta inflamatória mediada por citocinas, principalmente interleucina (IL) 17 e IL-23 por uma ativação crônica da imunidade, sustentando o processo inflamatório crônico, o que contribui para a iniciação e proliferação dos tumores (GRIVENNIKOV et al., 2012; SUN; KATO, 2016; TILG et al., 2018).

Neste contexto, a utilização de nutrientes que modulem a microbiota intestinal constitui-se uma alternativa viável para redução do risco do desenvolvimento do câncer de cólon, sendo os prebióticos destaque nesta categoria (SEIDEL et al., 2017). Prebióticos são substratos seletivamente utilizados pelos microrganismos do hospedeiro conferindo benefícios à saúde (GIBSON et al., 2017). Os prebióticos podem ser encontrados naturalmente em diversos alimentos, como aspargo,

chicória, banana, tomate, cebola, alho, cereais, alcachofra, yacon ou incorporados em alimentos (MOHANTY et al., 2018).

A yacon (S*mallanthus sonchifolius*) é uma raiz tuberosa originária na região dos Andes considerada um alimento funcional, principalmente por ser rica em frutooligossacarídeos (FOS), os quais possuem propriedades prebióticas (CAETANO et al., 2016). O consumo de FOS está associado a diversas propriedades benéficas, como o aumento da biodisponibilidade de minerais (LOBO et al., 2014), regulação do apetite (SILVA et al., 2017), aumento da atividade antioxidante (SOUSA et al., 2015), modulação positiva do sistema imune (VAZ-TOSTES et al., 2014) e efeito hipolipidêmico (PEREIRA et al., 2016).

Os FOS são substratos para as bifidobactérias que realizam fermentação e promovem o crescimento das bactérias intestinais benéficas como *Lactobacillus e Bifidobacterium* (GIBSON et al., 2017). O principal produto metabólico da fermentação são os ácidos graxos de cadeia curta (AGCC), principalmente o acetato, propionato e butirato, que possuem efeito anti-inflamatório e auxiliam na redução do pH intraluminal (GIBSON et al., 2017;MCLOUGHLIN et al., 2017). Além disso, os AGCC auxiliam na melhora da saúde dos colonócitos com capacidade para reduzir o desenvolvimento de focos de criptas aberrantes, ou seja, lesões pré-neoplásicas, e tumores locais, reduzindo a carcinogênese de cólon (MOURA et al., 2012).

Neste contexto, estudos evidenciaram que a suplementação na dieta de animais com farinha de yacon foi capaz de aumentar a produção dos AGCC (CAMPOS et al., 2012; MOURA et al., 2012; GRANCIERI et al., 2017). Estudos *in vivo* realizados com modelo animal de CC induzido verificaram que a farinha de yacon foi eficaz em melhorar a integridade epitelial e reduzir os focos de criptas aberrantes após a indução dos tumores (GRANCIERI et al., 2017), assim como o simbiótico entre yacon e *Lactobacillus acidophilus* apresentou efeito quimiopreventivo contra a carcinogênese de cólon em animais (ALMEIDA et al., 2015).

Por outro lado, a melhoria do ambiente colônico promoveu o aumento dos níveis da imunoglobulina A secretória (sIgA) e de citocinas anti-inflamatórias (VAZ-TOSTES et al., 2014; MIYAGUCHI et al., 2015). No lúmen intestinal, as sIgA são as primeiras linhas de barreira que limita o acesso dos antígenos intestinais na circulação sanguínea e controla a microbiota intestinal, além de minimizar o contato

de componentes potencialmente carcinogênicos com os colonócitos (PABST, 2012). Dessa forma, o consumo de prebióticos contribui para a modulação positiva da resposta imune.

Assim, verifica-se que o avanço dos modelos experimentais e métodos de análises permitem obter um entendimento científico da relação entre a microbiota intestinal, integridade da barreira epitelial e resposta imune do hospedeiro, de modo que essas novas percepções auxiliam na descoberta de novos alvos terapêuticos no câncer de cólon (SUN; KATO, 2016). Neste sentido, o presente estudo teve por objetivo avaliar o efeito preventivo do consumo da farinha de yacon, fonte de frutooligossacarídeos, na carcinogênese de cólon induzido em ratos.

1.1 Referências bibliográficas

ALMEIDA, A. P. S.; AVI, C. M.; BARBISAN, L. F.; MOURA, N. A.; CAETANO, B. F. R.; ROMUALDO, G. R.; SILVIERI, K. Yacon (*Smallanthus sonchifolius*) and *Lactobacillus acidophilus* CRL 1014 reduce the early phases of colon carcinogenesis in male Wistar rats. **Food Research International**, v. 74, p. 48–54, 2015.

CAETANO, B. F. R.; MOURA, N. A.; ALMEIDA, A. P. S.; DIAS, M. C.; SIVIERI, K. BARBISAN, L. F. Yacon (*Smallanthus sonchifolius*) as a food supplement: Health-promoting benefits of fructooligosaccharides. **Nutrients**, v. 8, n. 436, p. 1–13, 2016.

CAMPOS, D.; BETALLELUZ-PALLARDEL, I.; CHIRINOS, R.; AGUILAR-GALVEZ, AN.; NORATTO, G.; PEDRESCHI, R. Prebiotic effects of yacon (*Smallanthus sonchifolius* Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity. **Food Chemistry**, v. 135, n. 3, p. 1592–1599, 2012.

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. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews: Gastroenterology and Hepatology**, v. 14, n. 8, p. 491–502, 2017.

GRANCIERI, M.; COSTA, N. M. B.; VAZ-TOSTES, M. G.; OLIVEIRA, D. S.; NUNES, L. C.; MARCON, L. N.; VERIDIANO, T. A.; VIANA, M. L. Yacon flour (*Smallanthus sonchifolius*) attenuates intestinal morbidity in rats with colon cancer. **Journal of Functional Foods**, v. 37, p. 666–675, 2017.

GRIVENNIKOV, S.I.; WANG, K.; MUCIDA, D.; STEWARD, C. A.; SCHNABL, B.; JAUCH, D.; TANIGUCHI, T.; YU, G. Y.; OSTERREICHER, C. H.; HUNG, K. E.; DATZ, C.; FENG, Y.; FEARON, E. R.; TRINCHIERI, G.; KARIN, M.Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. **Nature**, v. 491, n. 7423, p. 254–258, 2012.

INCA. Instituto Nacional do Câncer. **Estimativa 2018: Incidência de câncer no Brasil**. Instituto Nacional de Câncer José Alencar Gomes da Silva. Coordenação de Prevenção e Vigilância. - Rio de Janeiro, INCA, 2018. Disponível em: < http://www1.inca.gov.br/estimativa/2018/estimativa-2018.pdf>. Acesso em: 20 dez 2018.

LOBO, A. R.; GAIEVSKI, E. H. S.; DE CARLI, E.; ALVARES, E. P.; COLLI, C. Fructo-oligosaccharides and iron bioavailability in anaemic rats: The effects on iron species distribution, ferroportin-1 expression, crypt bifurcation and crypt cell proliferation in the caecum. **British Journal of Nutrition**, v. 112, n. 8, p. 1286–1295, 2014.

LÓPEZ-LÁZARO, M. Stem cell division theory of cancer. **Critical Reviews in Oncology / Hematology**, v. 123, p. 95–113, 2018.

MCLOUGHLIN, R. F.; BERTHON, B. S.; JENSEN, M. E.; BAINES, K. J.; WOOD, L. G. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. **The American Journal Of Clinical Nutrition**, v. 106, n. 1, p. 930–945, 2017.

MIYAGUCHI, Y.; TOMATSURI, T.; TOYODA, A.; INOUE, E.; OGAWA, Y. Effect of Yacon Tuber (*Smallanthus sonchifolius*)-derived Fructooligosaccharides on the Intestinal Flora and Immune System of OVA-sensitized BALB/c Mice. **Food Science and Technology Research**, v. 21, n. 2, p. 255–262, 2015.

MOHANTY, D.; MISRA, S.; MOHAPATRA, S.; SAHU, P. S. Prebiotics and synbiotics: Recent concepts in nutrition. **Food Bioscience**, v. 26, p. 152–160, 2018.

MOURA, N. A.; CAETANO, B. F. R.; SIVIERI, K.; URBANO, L. H.; CABELLO, C.; RODRIGUES, M. A. M.; BARBISAN, L. F. Protective effects of yacon (*Smallanthus sonchifolius*) intake on experimental colon carcinogenesis. **Food and Chemical Toxicology**, v. 50, n. 8, p. 2902–2910, 2012.

PABST, O. New concepts in the generation and functions of IgA. **Nature Reviews Immunology**, v. 12, n. 12, p. 821–832, 2012.

PEREIRA, J. DE A. R.; BARCELOS, M. F. P.; FERREIRA, E. B.; PEREIRA, R. C.; ANGELIS-PEREIRA, M. C. Changes in glucose levels and fecal excretion of lipids due to consumption of yacon flour. **Nutrition & Food Science**, v. 46, n. 6, p. 791–802, 2016.

SILVA, M., F., G.; DIONÍSIO, A. P.; CARIOCA, A. A. F.; ADRIANO, L. S.; PINTO, C. O.; ABREU, F. A. P.; WURLITZER, N. J.; ARAÚJO, I. M.; GARRUTI, D. D.; PONTES, D. F. Yacon syrup: Food applications and impact on satiety in healthy volunteers. **Food Research International**, v. 100, p. 460–467, 2017.

SEIDEL, D. V.; AZCÁRATE-PERIL, M. A.; CHAPKIN, R. S.; TURNER, N. D. Shaping functional gut microbiota using dietary bioactives to reduce colon cancer risk. **Seminars in Cancer Biology**, v. 46, p. 191–204, 2017.

SONG, M.; CHAN, A. T. Environmental factors, gut microbiota, and colorectal cancer prevention. **Clinical Gastroenterology and Hepatology**, v. 17, n. 2, p. 275–289, 2019.

SOUSA, S.; PINTO, J.; RODRIGUES, C.; GIAO, M.; PEREIRA, C.; TAVARIA, F.; MALCATA, F. X.; GOMES, A.; PACHECO, M. T. B.; PINTADO, M. Antioxidant properties of sterilized yacon (*Smallanthus sonchifolius*) tuber flour. **Food Chemistry**, v. 188, p. 504–509, 2015.

SUN, J.; KATO, I. Gut microbiota, inflammation and colorectal cancer. **Genes and Diseases**, v. 3, n. 2, p. 130–143, 2016.

TILG, H.; ADOLPH, T. E.; GERNER, R. R.; MOSCHEN, A. R. The Intestinal Microbiota in Colorectal Cancer. **Cancer Cell**, v. 33, n. 6, p. 954–964, 2018.

VAZ-TOSTES, M. G.; VIANA, M. L.; GRANCIERI, M.; LUZ, T. C. S.; DE PAULA, H.; PEDROSA, R. G.; COSTA, N. M. B. Yacon effects in immune response and nutritional status of iron and zinc in preschool children. **Nutrition**, v. 30, n. 6, p. 666–672, 2014.

2. OBJETIVOS

2.1 Objetivo geral

Investigar o efeito prebiótico da farinha de yacon, como fonte de frutooligossacarídeos, na integridade da barreira intestinal, na resposta inflamatória e na capacidade antioxidante total do plasma resultante do processo oncológico colorretal induzido.

2.2 Objetivos específico

- Avaliar o efeito da suplementação dietética com farinha de yacon no consumo alimentar e no peso corporal;
- Comparar as alterações na produção de citocinas pró e anti-inflamatórias e imunoglobulina A secretória (sIgA) após a suplementação com a farinha de yacon;
- Verificar se a suplementação com a farinha de yacon é capaz de modular a capacidade antioxidante total do plasma;
- Analisar a alteração do pH intraluminal do cólon após a administração da farinha de yacon;
- Avaliar a produção de ácidos graxos de cadeia curta após a administração da farinha de yacon;
- Investigar possíveis alterações da permeabilidade intestinal;

3. REVISÃO BIBLIOGRÁFICA

The prebiotic benefits of yacon (*Smallanthus sonchifolius)* in colorectal cancer

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ABSTRACT

Colorectal cancer is caused by genetic predisposition and lifestyle risk factors and is associated with altered homeostasis of the intestinal microbiota. Evidence suggests that chronic infection and inflammation contribute to carcinogenic mutagenesis and promote cancer initiation and progression. Food components with prebiotic properties, such as fructooligosaccharides (FOS) promote intestinal integrity and health benefits. Yacon (*Smallanthus sonchifolius*) is an abundant source of FOS, which are fermented by probiotic bacteria, improving the intestinal environment affected by colorectal cancer. In the current review, it is discussed the colorectal cancer and its inflammatory process of development. Also, some general aspects concerning yacon roots and its prebiotic property are described, and finely, the beneficial effects of yacon on colorectal cancer and the main mechanisms of action.

Keywords: Fructooligosaccharides; intestinal barrier; inflammation; immune system; neoplasms.

Introduction

Cancer is a generic term for a group of diseases characterized by the growth of abnormal cells beyond their usual boundaries. Colorectal cancer (CRC), that affects the colon and rectum, is one of the most common malignancies in the Western countries, being the fourth major cause of death in men and the third for women (WHO 2018). By 2030 the global burden of CRC is expected to increase in 60%, and its incidence has been rising in many low- and middle-income countries consequent to adoption of a Western lifestyle (Arnold et al. 2017).

Lifestyle factors and dietary habits have substantially changed in the last decades, and these might affect colorectal carcinogenesis at various steps through metabolic and inflammatory mechanisms (Song and Chan 2019). Some dietary habits influence the composition and function of the intestinal microbiota, which in turn induces modifications in the host gene expression, metabolic regulation, and local and systemic immune response, thereby influencing an inflammatory process leading to colorectal adenoma-carcinoma development (Tilg et al. 2018). Therefore, components that act directly on the microbiota may exert effects on the reduction of CRC risk.

Yacon (*Smallanthus sonchifolius*), a perennial herbaceous plant native to the Andean region, is known as a functional food due to be an abundant source of fructooligosaccharides (FOS). In its dry matter the major portion consists of FOS, which are fructans with linear short chains of fructose molecules. FOS are able to resist to salivary and intestinal enzymes in the upper gastrointestinal, acting as prebiotics on the colon (Caetano et al. 2016). Prebiotics are fermented by beneficial bacteria and restore the gut microbiota diversity and activity, by increasing the number of *Bifidobacteria* and *Lactobacillus* (Gibson et al. 2017).

The process of fermentation produces short chain fatty acids (SCFA), which promote a reduction of the intraluminal pH, showing beneficial effects. Also, prebiotics are suggested to increase the immunological system and to reduce the oxidative stress by modulating pro and anti-inflammatory cytokines. Therefore, diverse mechanisms of action are proposed whereby prebiotics seem to reduce the risk of CRC. Furthermore, changes on the microbiota composition improve the intestinal barrier, preventing bacterial translocation and further inflammation (Mcloughlin et al. 2017; Reis et al. 2017; Gibson et al. 2017).

Thus, the present review aims to discuss the role of yacon as a source of FOS on the intestinal health affected by colorectal cancer, summarizing data from experimental animals

and human's studies. To our knowledge, this is the first review to examine the effect of yacon, particularly, on intestinal parameters changed by colorectal cancer.

The studies included in this review were identified by an electronic literature search using Science Direct, Portal Periodicos Capes and PubMed database. The search strategy included combinations of the following description: yacon, *Smallanthus sonchifolius*, prebiotics, colorectal cancer, intestinal permeability, colorectal cancer microbiota, colorectal cancer inflammation, prebiotic colorectal cancer and immune system. The search included reviews, experimental, epidemiological, and clinical studies. Also, the search was filtered to limit only the past 10 years (2009-2019).

Colorectal cancer

Worldwide, colorectal cancer is the third most common cancer and the second most common causes of cancer-related deaths (WHO 2018). By the year of 2030, it is expected 2.2 million new cases and 1.1 million cancer death, showing a dramatic increase of 60 % (Arnold et al. 2017). In most part of the world the incidence of this type of cancer is higher in men than women and increases with age, being the median age of diagnosis about 70 years. Besides this, rates are higher in developed countries than in those less developed, since rates of CRC increase with industrialization and urbanization (Brenner, Kloor, and Pox 2014). The two main strategies for CRC screening are the fecal occult blood test and colonoscopy, and surgery is the main treatment according to the stage of classification (Kniery, Nishtala, and Steele 2016).

CRC in most cases (approximately 75%) is sporadic and occurs in people without genetic predisposition or family history, and it is inherited in few cases (Yamagishi et al. 2016). It is a multifactorial disease influenced by genetics, environmental and lifestyle risk factors (Song and Chan 2019; Labianca et al. 2010). Some risk factors have been established such as family story of colorectal cancer, inflammatory bowel disease, smoking, excessive alcohol consumption, high intake of red and processed meat, obesity and diabetes (Brenner, Kloor, and Pox 2014). Diet is considered the most important exogenous factor in the etiology of CRC, and meta-analysis suggests that the intake of high amounts of dietary fiber against low intake is related to reduction in the incidence of CRC, since fibers can modulate in a positive manner the composition and function of the gut microbiota (Figure 1) (Danneskiold-Samsøe et al. 2019; McRae 2018; Labianca et al. 2010).

Gut microbiota composition has been recognized to play an important role in colorectal carcinogenesis by acting as an inflammatory initiator, which contributes to tumor initiation and progression (Song and Chan 2019; Sun and Kato 2016). In this context, metagenomic studies indicate that *Fusobacterium nucleatum* is enriched in colorectal cancer, whereas *Bifidobacterium* and *Lactobacillus* spp. are depleted in cancer patients (Tilg et al. 2018; Borges-Canha et al. 2015). *In vivo* study found difference in intestinal bacterial microbiota between healthy and CRC rats, showing higher abundance of *Firmicutes*, *Proteobacteria* and *Actinobacteria*, and lesser butyrate-producers and probiotics in animals induced to tumor (Q. Zhu et al. 2014), in the same way similar results were found in study with CRC patients (Ohigashi et al. 2013).

CRC development is a multistep process called adenocarcinoma sequence that initiates when normal epithelium forms aberrant crypts and further advances into stages of early and late adenomatous polyps, invasive carcinoma, and metastasis (Irrazábal et al. 2014). There is a combination of mutation and microbes, being adenomatous polyposis coli (APC) loss of function an initiating event and the most frequent mutation. APC is a tumor suppressor and its mutation increases cell migration and leads to activation of β-catenin, which induces a proliferative state (Aghabozorgi et al. 2019; Brenner, Kloor, and Pox 2014; Grivennikov et al. 2012). Genetic mutations accrue in epithelial cells, leading to a loss of junction proteins, as tight junctions, and mucus, resulting in less mucosal integrity. This event facilitates bacterial translocation from the lumen to the lamina propria, where bacterial products activate inflammatory cytokines, such as IL-23 and IL-17 and result in a chronic inflammatory process and cancer progression (Grivennikov et al. 2012). Therefore, colorectal carcinogenesis may be associated to altered microbial metabolism with inflammation and oxidative stress that lead to DNA damage due to change of host defense mechanism, modifying apoptosis and cell cycle (Mandal 2018).

In this sense, food components, such as prebiotics, with potential to reduce the inflammatory process and improve the epithelial integrity act as chemo preventive agents in the early stages of colon carcinogenesis.

Prebiotics

The prebiotic was first defined in 1995 (Gibson and Roberfroid 1995), and since then some modifications on its definition have occurred. Recently, the International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the definition of a prebiotic as “a

substrate that is selectively utilized by host microorganisms conferring a health benefit", which must be confirmed in well-controlled studies in the target host (Gibson et al. 2017). Over the last 10 years, the global demand of prebiotics has increased to 500,000 t per annum (Mohanty et al. 2018). Prebiotic are widely found in foods as legumes, fruits and vegetables, being the main sources wheat, oatmeal, beans, chickpeas, onions, garlic, chicory, bananas, greens, asparagus, spinach, Jerusalem artichokes, berries and yacon (Mohanty et al. 2018; Schaafsma and Slavin 2015). Furthermore, prebiotics are stable under not extreme conditions of high temperature and low pH, so it can be incorporated into a variety of acidic products, such as yogurts, pasteurized fruit juices, as well as baked products, without been degraded (Charalampopoulos and Rastall 2012), or used as ingredient to improve structure and taste and to increase the intake of fiber (Schaafsma and Slavin 2015).

Inulin-derived fructans (fructooligosaccharides, FOS: inulin and oligofructose) and galactooligosaccharides (GOS) are the most extensively documented prebiotics to have health benefits in human. Other candidate prebiotics are under investigation, such as xylo-oligosaccharides (XOS), resistant starch and soybean oligosaccharides (SOS) (Rastall and Gibson 2015). GOS are present naturally in human milk, and its commercial form is synthesized from lactose using activity of β-galactosidase from microorganisms (Zhu et al. 2018). On the other hand, fructans are linear or branched fructose polymers linked by β (2-1) bonds on FOS and β (2-6) on inulin. The difference between them is the degree of polymerization (DP), that is, the number of individual units of monosaccharaides presented. So, the inulin molecule has the DP of 10 or more, and FOS of 3 to 10 (Schaafsma and Slavin 2015).

Prebiotics compounds are not hydrolyzed by the digest system due to the lack of hydrolytic enzymes to act on β-linkages, but the colon microbiota is able to degrade these bindings (Valcheva and Dieleman 2016a). FOS and GOS, the leading prebiotics available, have linkage bonds that can be degraded by β-fructanosidade and β-galactosidade enzymes, respectively, which are prevalent in bifidobacteria, so *Lactobacillus* and *Bifidobacterium* are stimulated primarily (Quigley 2019; Rastall and Gibson 2015). Therefore, prebiotics are fermented and the primary metabolic product of this are the short chain fatty acids (SCFA) mainly acetate, butyrate and propionate (Valcheva and Dieleman 2016a). Diverse studies verified an increase on SCFA production after prebiotics intake on animals (Grancieri et al. 2017; Campos et al. 2012), and humans (van der Beek et al. 2018; Paganini et al. 2017).

The process of fermentation reduces the pH of the colonic environment that limits the production of putrefactive compounds (Valcheva and Dieleman 2016a) and acts against the

promotion of potential opportunistic pathogens, such as *Clostridium* (Quigley 2019). Also, the more acidic luminal content due to fermentation of prebiotics improves the mineral solubility and increases intestinal absorption (Lobo et al. 2014). The consumption of 5g of GOS/100g of diet improved apparent absorption of Ca, Mg and Fe after 3 weeks of supplementation on rats (Maawia et al. 2016), although in human the results still are controversial (Weinborn et al. 2017; Vaz-Tostes et al. 2014).

Regarding diet, prebiotics and probiotics are the most commonly used substances in order to maintain a health microbiome or restore balance when bacterial homeostasis has been disturbed in diseases (Quigley 2019). Fructans lead to a modulation of the immune system (Peshev and Van den Ende 2014), with improvement of mucosal immunity through an increase in secretory immunoglobulin A (sIgA) levels and a preservation of an anti-inflammatory state (Choque Delgado et al. 2012). Also, the role of prebiotics in prevention of obesity has been studied, since prebiotics may decrease lipopolysaccharide (LPS) levels, leading to reduction of an inflammatory process (Choque Delgado and Tamashiro 2018). The ingestion of a single 24 g dose of inulin increased fat oxidation in overweight to obese men (van der Beek et al. 2018). Inulin fructans have a low caloric value, so replacing digestible carbohydrates with inulin may favor the maintenance and weight loss (Schaafsma and Slavin 2015). Also, it has been suggested that prebiotics act on the nervous system with beneficial effects on stress-related behaviors, supporting the concept of a brain gut microbiome axis (Osadchiy, Martin, and Mayer 2019; Burokas et al. 2017).

Prebiotic appropriate dose and safety must be enough to promote beneficial results without adverse effect such as excessive gas formation or non-selective utilization (Gibson et al. 2017). Often, inulin is well tolerated up to a level of 20 g/d (Schaafsma and Slavin 2015). On the other hand, 0.29 g FOS/kg body weight caused undesirable gastrointestinal side effects (abdominal distension and/or flatulence) in pre-menopausal women (Genta et al. 2009). The smallest daily dose of 0.14 g FOS/body weight had no intestinal discomfort in preschool children (Vaz-Tostes et al. 2014). Regarding the GOS, the high dose of 7.5g (~1g GOS/kg body weight) in infants did not promote side effects (Paganini et al. 2017). Among the prebiotics found naturally in foods, the class of FOS is abundant in a specific type of root, the yacon.

Yacon

Yacon (*Smallanthus sonchifolius*) root belongs to the Asteraceae family and is native to the Andean regions of South America (Caetano et al. 2016). Over the years, it cultivation has spread outside the region of origin to several countries, mainly Bolívia, Brazil, the Czech Republic, Italy, Japan, New Zeland, Peru and United States. One reason for this expansion is that yacon plant adapts to diverse climatic regions (develops in temperature ranging from 0 ^{0}C to 24 ^{0}C), altitudes (800 to 2,800 m above the sea) and different types of soils (Caetano et al. 2016).

Yacon is a perennial plant, which after planting generally takes 6 to 12 months to reach maturity with the aerial stems about 2.5 m in height. The roots are of different shapes and sizes that weigh between 200 to 500 g, thick of 10 cm and 15 - 20 cm long in the average. They come in different colors as brown, purple, cream and pink. Each plant produces bunches of 5-20 units, with average of 5 kg/plant (Cao et al. 2018; Choque Delgado et al. 2013).

Yacon roots have a sweet taste and a crunchy texture, so it has been described to resemble a taste of a fresh apple or watermelon. Often, it is consumed as a fruit, peeled and fresh, or in the form of beverage by extracting its juice (Choque Delgado et al. 2013). The cooked form is less used due to the browning reaction that happens once the tissue is damaged and exposed to the air. This reaction is related to the content of phenolic compounds and polyphenol oxidase activity, which can be prevented by some methods as dehydration, low temperature storage, use of antioxidants and removal of oxygen from the medium (Vasconcelos et al. 2015). Yacon in the form of flour has the advantage of concentrating the FOS contents and improving the shelf life, and can be used as a functional ingredient to formulate industrialized foods (Rodrigues et al. 2011).

Table 1 presents the chemical composition of yacon flour according to data compiled from different authors. Mostly, the composition of yacon roots consists of water and carbohydrates. However, different from most of the roots that store its carbohydrates as starch, yacon stores as fructooligosaccharides (FOS) (Ojansivu, Ferreira, and Salminen 2011). FOS are the main component in the root dry matter, and its concentration can vary from 25.7 % to 65 % widely. Yacon has small amount of vitamins and minerals and no starch (Choque Delgado et al. 2013). Besides the roots, yacon leaves and flowers can be considered a source of phenolic acids and flavonoids compounds, with appreciable antioxidant properties (de Andrade et al. 2014).

The dry matter of yacon roots is influenced by specific crop and location, and the

sugar level may vary depending on factors, such as location, farming, the growing season, harvest time and the post-harvest storage conditions (temperature and time), and the methodology used for quantification of these substances (Choque Delgado et al. 2013; Almeida, Abranches, and Ferreira 2013). It is noted that as the time after harvesting increases, fructans are hydrolized to mono- and disaccharides by action of the enzyme fructan hydrolase (Cao et al. 2018; Almeida, Abranches, and Ferreira 2013). FOS presented in yacon roots are non-reducing sugar, so they are not suceptible to the Maillard reaction (Almeida, Abranches, and Ferreira 2013). Also, yacon roots contains considerable amounts of phenolic compounds mainly caffeic and chlorogenic acid that help prevent degenerative processes caused by oxidative stress (Pereira et al. 2016).

As described in the previous topic, FOS are considered prebiotics for resisting to the action of digestive enzymes, reaching the colon intact. In the colon, they are fermented by bifidobacteria promoting beneficial effects to the health (Gibson et al. 2017). Yacon, as a source of FOS, showed prebiotic effects, promoting the growth of bifidobacteria and lactobacilli (Campos et al. 2012). Yacon is related to the control of excess body weight, showing to increase satiety in women (Silva et al. 2017), and to reduce body weight and body fat in adults with excess weight supplemented with 25 g of yacon flour for 6 weeks (Machado et al. 2018). Also, freeze-dried yacon roots (7.4 g FOS) had proved to decrease serum glucose in elderly individuals (Scheid et al. 2014), to reduce postprandial glucose peaks on health animals (Pereira et al. 2016), and to control hyperglycemia on diabetic mellitus animals (Oliveira, Braga, and Fernandes 2013). Over the last years, some authors reviewed in a detailed way these and others functional effects of yacon, considering others bioactive compounds as polyphenols (chlorogenic and caffeic acid) and antimicrobial substances (Khajehei et al. 2018; Jimenez, Rossi, and Sammán 2015; Choque Delgado et al. 2013).

Yacon x intestinal health x colorectal cancer (CRC)

Dietary habits exhibit a strong impact on the microbiota composition and provide risk for developing CRC, since intestinal bacteria have a fundamental role in the promotion and evolution of tumorigenesis (Tilg et al. 2018). Modulation of gut microbiota by prebiotics compounds may positively affect the cross-talk between immune system and microorganisms, which may be beneficial in preventing inflammation and CRC (Ambalam et al. 2016). In this sense, there is a connection between yacon (*Smallanthus sonchifolius*) roots, as a source of fructooligosaccharides (FOS), and CRC by attenuating intestinal parameters altered by

carcinogenesis (Figure 2).

In the colon, FOS are fermented by bifidobacteria, promoting beneficial changes on microbiota composition (Gibson et al. 2017). Tables 2 and 3 summarize the main studies carried out with yacon related to intestinal parameters presented in this section. Research performed with yacon flour (5% FOS) showed the growth of Bifidobacteria and Lactobacilli and higher levels of SCFA in guinea pig model (Campos et al. 2012). Also, yacon-based product providing 10g FOS/day improved the microbiota composition evidenced by higher counts of *Bifidobacterium* and lower *Clostridium*, and lower fecal pH in adult individuals with constipation (Sant'Anna et al. 2015). A more acid pH promotes health benefits by inhibiting the growth of opportunistic pathogens such as *Escherichia coli* and *Samonella spp.* Also, bifidobacteria produces antimicrobial substances as bacteriocins, which inhibit the growth or eliminate pathogenic bacteria from the intestinal lumen (Reis et al. 2017; Ambalam et al. 2016). In this sense, patients with CRC presented higher pH, alterations on the intestinal environment and decreased SCFA (Ohigashi et al. 2013), so the intake of prebiotic shows to be an alternative to improve these parameters altered by colon carcinogenesis.

In-vivo study with chemically-induced (1,2-dimethylhydrazine, DMH) animal model of colorectal cancer supplemented with yacon flour (YF) (7.5 % FOS) showed positive results on intestinal health such as reduction of luminal pH and preneoplastic lesions - ACF (aberrant crypt foci), and an increase of SCFA (Grancieri et al. 2017). ACF are considered biomarkers that anticipate the colonic carcinogenesis process (Moulahoum et al. 2018), and prebiotics are suggested to reduce its formation by modifying the colonic microbiota (Ji, Peng, and Wang 2018). Other authors also verified a reduction on preneoplastic lesions (ACF), cell proliferation and an increase of SCFA after YF (1%) ingestion on animals induced to CRC (de Moura et al. 2012). SCFA are byproducts primary produced from fermentation of prebiotics, presenting systemic anti-inflammatory effects (Mcloughlin et al. 2017). In specific, butyrate, that is the main energy source for colonocytes, may exert anticancer effects through the reduction of pro-inflammatory cytokine, inhibiting colon inflammation and oxidative stress, and by inducing cell apoptosis due to histone deacetylases inhibition (Chen and Vitetta 2018; Gonçalves and Martel 2016). Also, aqueous yacon extract (1% FOS) on rats chemically-induced to CRC promoted a reduction in leukocyte DNA damage and in colonic cell proliferation after the first DMH administration, and five months later was observed a reduction in ACF development and colon tumors (Almeida et al. 2015).

Yacon flour showed to reduce intestinal permeability (IP) in CRC animals (Grancieri et al. 2017). IP refers to the physical barrier function that the epithelium exerts on the intestinal

lumen, so that changes in intestinal mucosal integrity and barrier dysfunction result in higher permeability to toxins and pathogens, leading to bacterial translocation and inflammatory reactions (Witten, Samad, and Ribbeck 2018). FOS supplementation (6%) was able to reduce the intestinal permeability and inflammatory infiltrate and to promote less tissue damage in BALB/c mice induced to mucositis (Galdino et al. 2018). Some proposed mechanisms that FOS improve the intestinal barrier are the intracolonic pH acidification, changes in cellular junction proteins and production of mucins (Reis et al. 2017). Since colorectal neoplasms exhibit intestinal barrier deterioration, functional compounds able to improve the function of barrier can be beneficial to reduce the translocations of microbial products that trigger inflammation (Reis et al. 2017; Grivennikov et al. 2012).

Furthermore, FOS exhibit immunomodulatory effects on the intestinal health by influencing the growth of bifidobacteria or through a direct interaction with the immune system (Valcheva and Dieleman 2016b). The intestinal microbiota exerts a role in the maturation and tolerance of the immune system, acting by stimulating the production of anti-inflammatory and inhibiting the pro-inflammatory cytokines (Peshev and Van den Ende 2014). Choque-Delgado et al., (2012) verified reduction of proinflammatory cytokine IL-1β in peritoneal macrophages, and elevated fecal IgA in mouse supplemented with YF (3 or 5%), but no difference observed in interleukin (IL) 4, IL-10 and tumor necrosis factor-alpha (TNF-α) levels. On the other hand, YF increased IL-6 and secretory IgA without translocation of pathogens in animal model of intestinal infection with *Salmonella Typhimurium* (Velez et al. 2013). Yacon extract (5% FOS) decreased the levels of TNF-α in BALB/c mice sensitized with ovalbumin (Miyaguchi et al. 2015). TNF- α is an important pro-inflammatory cytokine associated to tissues inflammation by signaling process as communication, differentiation and cellular death and trigger of inflammatory cell infiltration. Usually, this cytokine is elevated in cancer patients, and is related to cancer cachexia (Annibaldi and Meier 2018; Patel and Patel 2017).

In vivo study in an animal model of colorectal cancer supplemented with yacon flour (7.5 % FOS) increased the levels of sIgA fecal (Grancieri et al. 2016). Positive effects were also observed on preschool children who received YF (0.14 g FOS/ kg body weight) during 18 weeks, showing an increase on serum levels of IL-4 and fecal sIgA (Vaz-Tostes et al. 2014). Thus, sIgA acts directly on intestinal homeostasis by serving as a first-line barrier to protect the epithelium from pathogens and toxins and binds antigens in the mucus layer preventing them to initiate inflammatory process (Pabst 2012). It was observed that perioperative CRC patients who received 30 g of prebiotics (mixed of FOS, xylooligosaccharide, polydextrose

and resistant starch) for 7 days improved serum immunological indicators as IgA, IgA, IgM and intestinal microbiota structure (Xie et al. 2018). Also, the use of preoperative symbiotic in CRC patients attenuated the inflammatory state, associated with reduction of morbidity (Polakowski et al. 2019).

Conclusions

Growing evidence suggest that lifestyle factors, mainly those related to dietary habits, are related to microbiota function and composition, which play an important role in the development and progression of CRC, acting as inflammatory initiator. Recent studies suggested that yacon consumption presents prebiotic effects on the intestinal health due to its abundant amount of FOS. In this sense, yacon has been related to protective effect on colorectal cancer by improving the immune system, the integrity of intestinal barrier, the microbiota composition and the inflammatory state. Considering the studies summarized in this review, animals studies showed some positive effects of yacon intake, mainly providing 5% or more of FOS, on intestinal parameters, such as reduction of pH, production of SCFA, increase of bifidobacteria and anti-inflammatory cytokines, reduction of intestinal permeability and pro-inflammatory cytokines. However, human studies are scarce and showed controversial results.

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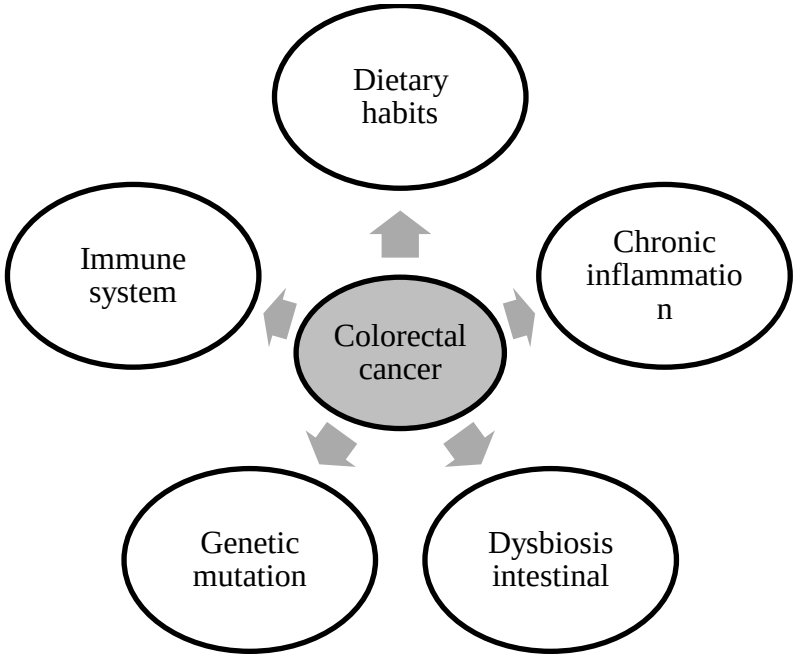

Figure 1. Risk factors related to colorectal cancer development.

Table 1. Composition of yacon flour.

FOS (%)	Carbohydrates (%)	Proteins (%)	Fats (%)	Reference
25.7	71.4	2.7	0.15	(Rodrigues et al. 2012)
34.7	85.5	2.8	0.9	(Machado et al. 2018)
35.18	85.33	3.23	0.99	(Rocha et al. 2018)
41.20	88.6	4.7	0.3	(Scheid et al. 2014)
52.20	77.98	4.52	0.33	(Grancieri et al. 2017)
65	82.72	2.7	0.17	(Fabersani et al. 2018)

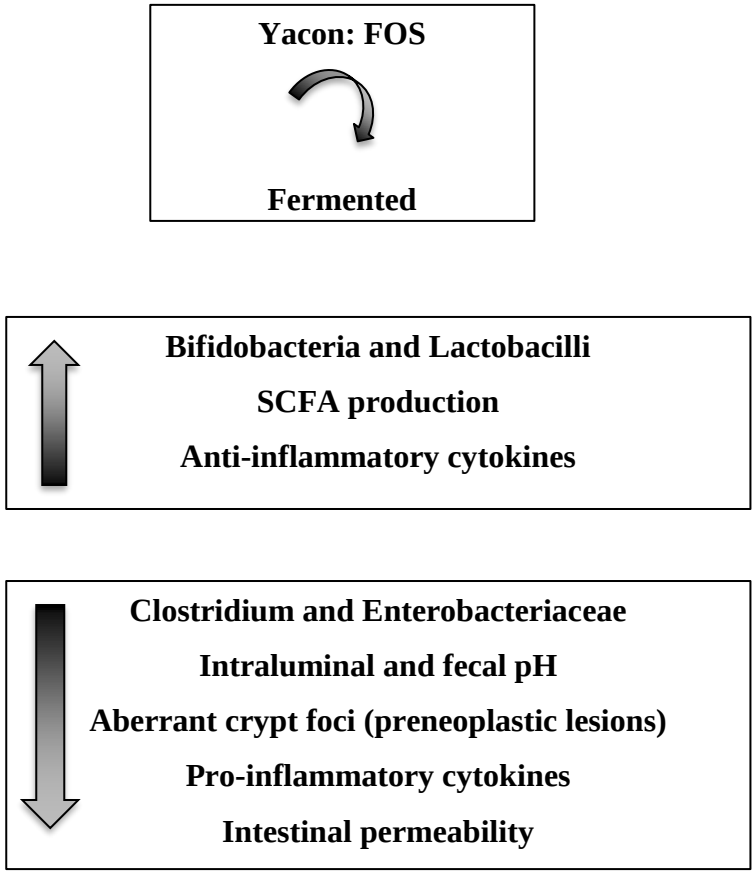

Figure 2. Proposed benefit effect that yacon improves intestinal parameters related to colorectal cancer.

FOS: fructooligosaccharide; SCFA: short chain fatty acids; sIgA: secretory immunoglobulin A.

Table 2. Animal studies with yacon related to colorectal cancer.

Animal model	**Source/ Dose/ Time**	**Main results**	**Reference**
Male *Wistar* rats induced to CRC by DMH	YF (7.5 % FOS) 8 weeks	Reduction of ACF, intestinal permeability, pH Higher SCFA	(Grancieri et al. 2017)
Male *Wistar* rats induced to CRC by DMH	YF (7.5 % FOS) 2 weeks	Increase of sIgA fecal	(Grancieri et al. 2016)
Female sensitized ovalbumin BALB/c mice	Yacon extract (5% FOS) 4 weeks	Increase of Lactobacilli and Bifidobacteria Decrease of TNF- α	(Miyaguchi et al. 2015)
Wistar rats induced to CRC by DMH	Aqueous yacon extract (1% FOS)	Reduction of DNA damage in leukocyte and in colonic cell proliferation	(Almeida et al. 2015)
BALB/c mice induced to intestinal infection model with *Salmonella Typhimurium*	YF (340 mg/ kg/ day) 45 days	Increase of sIgA and IL-6	(Velez et al. 2013)
Guinea pigs	YF (5% FOS) 8 weeks	Growth of bifidobacteria and lactobacilli Increase of SCFA, cell density and crypt formation	(Campos et al. 2012)
Male *Wistar* rats induced to CRC by DMH	YF (1%) and symbiotic (YF-1% + *Lactobacillus casei*) 13 weeks	Reduction on preneoplastic lesions, tumor multiplicity and cell proliferation Increase of SCFA	(Moura et al. 2012)

BALB/c mice	YF (3 or 5% FOS) 30 days	No difference on IgM, IgG, nitric oxid, IL-4, IL-10, TNF- α Reduction of IL-1β Increase of fecal sIgA	(Choque Delgado et al. 2012)

CRC: colorectal cancer; YF: yacon flour; FOS: fructooligosaccharide; ACF: aberrant crypt foci; DMH: 1.2-dimethylhydrazine; SCFA: short chain fatty acids; Ig: immunoglobulin; TNF-α: tumor necrosis factor-α; IL: interleukin.

Table 3. Human studies with yacon on intestinal health.

Individual	**Source/ Dose/ Time**	**Result**	**Reference**
Adults (20-75 yeas) with intestinal constipation *n*=48	Yacon based product (10 g FOS) 30 days	Decrease in the intensity of constipation Increase of bifidobacteria, Decrease of Clostridium, Enterobacteriaceae, and fecal pH No difference on SCFA	(Sant'Anna et al. 2015)
Preschool children *n*=117	YF (0.14 g FOS/body weight) 18 weeks	Increase of IL-4 and fecal sIgA	(Vaz-Tostes et al. 2014)

YF: yacon flour; FOS: fructooligosaccharide; SCFA: short chain fatty acids; Ig: immunoglobulin; TNF-α: tumor necrosis factor-α; IL: interleukin.

References

Aghabozorgi, Amirsaeed Sabeti, Amirhossein Bahreyni, Atena Soleimani, Afsane Bahrami, Majid Khazaei, Gordon A. Ferns, Amir Avan, and Seyed Mahdi Hassanian. 2019. "Role of Adenomatous Polyposis Coli (APC) Gene Mutations in the Pathogenesis of Colorectal Cancer; Current Status and Perspectives." *Biochimie* 157: 64–71.

Almeida, Ana Paula da Silva, Camila Martins Avi, Luís Fernando Barbisan, Nelci Antunes de Moura, Brunno Felipe Ramos Caetano, Guilherme Ribeiro Romualdo, and Kátia Sivieri. 2015. "Yacon (Smallanthus Sonchifolius) and Lactobacillus Acidophilus CRL 1014 Reduce the Early Phases of Colon Carcinogenesis in Male Wistar Rats." *Food Research International* 74. Elsevier B.V.: 48–54.

Almeida Paula, Hudsara Aparecida de, Monise Viana Abranches, and Célia Lúcia de Luces Fortes Ferreira. 2013. "Yacon (Smallanthus Sonchifolius): A Food with Multiple Functions." *Critical Reviews in Food Science and Nutrition* 55 (1): 32–40.

Ambalam, Padma, Maya Raman, Ravi Kiran Purama, and Mukesh Doble. 2016. "Probiotics, Prebiotics and Colorectal Cancer Prevention." *Best Practice and Research: Clinical Gastroenterology* 30 (1). Elsevier Ltd: 119–31.

Andrade, Eriel Forville de, Roberta de Souza Leone, Luciana N. Ellendersen, and Maria Lucia Masson. 2014. "Phenolic Profile and Antioxidant Activity of Extracts of Leaves and Flowers of Yacon (Smallanthus Sonchifolius)." *Industrial Crops and Products* 62. Elsevier B.V.: 499–506.

Annibaldi, Alessandro, and Pascal Meier. 2018. "Checkpoints in TNF-Induced Cell Death: Implications in Inflammation and Cancer." *Trends in Molecular Medicine* 24 (1). Elsevier Ltd: 49–65.

Arnold, Melina, Mónica S. Sierra, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. 2017. "Global Patterns and Trends in Colorectal Cancer Incidence and Mortality." *Gut* 66: 683–691. d

Beek, Christina M. van der, Emanuel E. Canfora, Anna M. Kip, Stefan H.M. Gorissen, Steven W.M. Olde Damink, Hans M. van Eijk, Jens J. Holst, Ellen E. Blaak, Cornelis H.C. Dejong, and Kaatje Lenaerts. 2018. "The Prebiotic Inulin Improves Substrate Metabolism and Promotes Short-Chain Fatty Acid Production in Overweight to Obese Men." *Metabolism Clinical and Experimental* 87. Elsevier Inc.: 25–35.

Borges-Canha, Marta, José Pedro Portela-Cidade, Mário Dinis-Ribeiro, Adelino F. Leite-Moreira, and Pedro Pimentel-Nunes. 2015. "Role of Colonic Microbiota in Colorectal Carcinogenesis: A Systematic Review." *Revista Espanola de Enfermedades Digestivas* 107 (11): 659–71.

Brenner, Hermann, Matthias Kloor, and Christian Peter Pox. 2014. "Colorectal Cancer." *Lancet* 383. Elsevier Ltd: 1490–1502.

Burokas, Aurelijus, Silvia Arboleya, Rachel D. Moloney, Veronica L. Peterson, Kiera Murphy, Gerard Clarke, Catherine Stanton, Timothy G. Dinan, and John F. Cryan. 2017. "Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice." *Biological Psychiatry* 82 (7). Elsevier Inc.: 472–87.

Caetano, Brunno F.R., Nelci A. de Moura, Ana P.S. Almeida, Marcos C. Dias, Kátia Sivieri, and Luís F. Barbisan. 2016. “Yacon (Smallanthus Sonchifolius) as a Food Supplement: Health-Promoting Benefits of Fructooligosaccharides.” *Nutrients* 8 (436): 1–13.

Campos, David, Indira Betalleluz-Pallardel, Rosana Chirinos, Ana Aguilar-Galvez, Giuliana Noratto, and Romina Pedreschi. 2012. “Prebiotic Effects of Yacon (Smallanthus Sonchifolius Poepp. & Endl), a Source of Fructooligosaccharides and Phenolic Compounds with Antioxidant Activity.” *Food Chemistry* 135 (3). Elsevier Ltd: 1592–99.

Cao, Yang, Zheng Ma, Hongxia Zhang, Yifan Jin, Yihe Zhang, and Frank Hayford. 2018. “Phytochemical Properties and Nutrigenomic Implications of Yacon as a Potential Source of Prebiotic: Current Evidence and Future Directions.” *Foods* 7 (59): 1–13.

Charalampopoulos, Dimitris, and Robert A. Rastall. 2012. “Prebiotics in Foods.” *Current Opinion in Biotechnology* 23 (2). Elsevier Ltd: 187–91.

Chen, Jiezhong, and Luis Vitetta. 2018. “Inflammation-Modulating Effect of Butyrate in the Prevention of Colon Cancer by Dietary Fiber.” *Clinical Colorectal Cancer* 17 (3). Elsevier Inc.: e541–44.

Choque Delgado, Grethel T., Rodolfo Thomé, Dirce L. Gabriel, Wirla M S C Tamashiro, and Glaucia M. Pastore. 2012. “Yacon (Smallanthus Sonchifolius)-Derived Fructooligosaccharides Improves the Immune Parameters in the Mouse.” *Nutrition Research* 32 (11). Elsevier Inc.: 884–92.

Choque Delgado, Grethel Teresa, and Wirla Maria da Silva Cunha Tamashiro. 2018. “Role of Prebiotics in Regulation of Microbiota and Prevention of Obesity.” *Food Research International* 113. Elsevier: 183–88.

Choque Delgado, Grethel Teresa, Wirla Maria da Silva Cunha Tamashiro, Mário Roberto Maróstica Junior, and Glaucia Maria Pastore. 2013. “Yacon (Smallanthus Sonchifolius): A Functional Food.” *Plant Foods for Human Nutrition* 68 (3): 222–28.

Danneskiold-Samsøe, Niels Banhos, Helena Dias de Freitas Queiroz Barros, Rosangela Santos, Juliano Lemos Bicas, Cinthia Baú Betim Cazarin, Lise Madsen, Karsten Kristiansen, Glaucia Maria Pastore, Susanne Brix, and Mário Roberto Maróstica Júnior. 2019. “Interplay between Food and Gut Microbiota in Health and Disease.” *Food Research International* 115. Elsevier: 23–31.

Fabersani, Emanuel, María Virginia Grande, María Victoria Coll Aráoz, Martin L. Zannier, Sara S. Sánchez, Alfredo Grau, Ruben Oliszewski, and Stella M. Honoré. 2018. “Metabolic Effects of Goat Milk Yogurt Supplemented with Yacon Flour in Rats on High-Fat Diet.” *Journal of Functional Foods* 49. Elsevier: 447–57.

Galdino, Flávia Mendes Peradeles, Maria Emília Rabelo Andrade, Patrícia Aparecida Vieira de Barros, Simone de Vasconcelos Generoso, Jacqueline Isaura Alvarez-Leite, Camila Megale de Almeida-Leite, Maria do Carmo Gouveia Peluzio, Simone Odília Antunes Fernandes, and Valbert Nascimento Cardoso. 2018. “Pretreatment and Treatment with Fructo-Oligosaccharides Attenuate Intestinal Mucositis Induced by 5-FU in Mice.” *Journal of Functional Foods* 49. Elsevier: 485–92.

Genta, Susana, Wilfredo Cabrera, Natalia Habib, Juan Pons, Iván Manrique Carillo, Alfredo Grau, and Sara Sánchez. 2009. “Yacon Syrup: Beneficial Effects on Obesity and Insulin

Resistance in Humans." *Clinical Nutrition* 28 (2): 182–87.

Gibson, Glenn R., Robert Hutkins, Mary Ellen Sanders, Susan L. Prescott, Raylene A. Reimer, Seppo J. Salminen, Karen Scott, et al. 2017. "The International Scientific Association for Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Prebiotics." *Nature Reviews: Gastroenterology and Hepatology* 14 (8). Nature Publishing Group: 491–502.

Gibson, Glenn R., and Marcel B. Roberfroid. 1995. "Dietary Modulation of the Human Colonie Microbiota: Introducing the Concept of Prebiotics." *Journal of Nutrition* 125: 1401–1412.

Gomes da Silva, Maria de Fátima, Ana Paula Dionísio, Antônio Augusto Ferreira Carioca, Lia Silveira Adriano, Claudia Oliveira Pinto, Fernando Antonio Pinto de Abreu, Nedio Jair Wurlitzer, Idila Maria Araújo, Deborah dos Santos Garruti, and Dorasilvia Ferreira Pontes. 2017. "Yacon Syrup: Food Applications and Impact on Satiety in Healthy Volunteers." *Food Research International* 100 (May). Elsevier: 460–67.

Gonçalves, Pedro, and Fátima Martel. 2016. "Regulation of Colonic Epithelial Butyrate Transport: Focus on Colorectal Cancer." *Porto Biomedical Journal* 1 (3): 83–91.

Grancieri, M, P. A. Machado, D. F. Oliveira, L.N. Marcon, M. G Vaz Tostes, N. M. B. Costa, M.D. C. Ignacchiti, L. C. Nunes, and M. L Viana. 2016. "Efeito Da Farinha de Yacon (Smallanthus Sonchifolius) Na Resposta Imunológica Intestinal No Câncer Colorretal." *Braspen Journal* 31 (4): 335–39.

Grancieri, Mariana, Neuza Maria Brunoro Costa, Maria das Graças Vaz Tostes, Daniela Silva de Oliveira, Louisiane de Carvalho Nunes, Letícia de Nadai Marcon, Thaísa Agrizzi Veridiano, and Mirelle Lomar Viana. 2017. "Yacon Flour (Smallanthus Sonchifolius) Attenuates Intestinal Morbidity in Rats with Colon Cancer." *Journal of Functional Foods* 37. Elsevier Ltd: 666–75.

Grivennikov, Sergei I., Kepeng Wang, Daniel Mucida, C. Andrew Stewart, Bernd Schnabl, Dominik Jauch, Koji Taniguchi, et al. 2012. "Adenoma-Linked Barrier Defects and Microbial Products Drive IL-23/IL-17-Mediated Tumour Growth." *Nature* 491 (7423): 254–58.

Irrazábal, Thergiory, Antoaneta Belcheva, Stephen E. Girardin, Alberto Martin, and Dana J. Philpott. 2014. "The Multifaceted Role of the Intestinal Microbiota in Colon Cancer." *Molecular Cell* 54 (2): 309–20. doi:10.1016/j.molcel.2014.03.039.

Ji, Xiaolong, Qiang Peng, and Min Wang. 2018. "Anti-Colon-Cancer Effects of Polysaccharides: A Mini-Review of the Mechanisms." *International Journal of Biological Macromolecules* 114. Elsevier B.V.: 1127–33.

Jimenez, María Eugenia, Analia Rossi, and Norma Sammán. 2015. "Health Properties of Oca (Oxalis Tuberosa) and Yacon (Smallanthus Sonchifolius)." *Food & Function* 6 (10). Royal Society of Chemistry: 3266–74.

Khajehei, Forough, Nikolaus Merkt, Wilhelm Claupein, and Simone Graeff-Hoenninger. 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* 23 (2): 1–19.

Kniery, Kevin, Madhuri Nishtala, and Scott R. Steele. 2016. “Staging, Prognosis, and Survivorship in Colon Cancer.” *Seminars in Colon and Rectal Surgery* 27 (4). Elsevier: 193–98.

Labianca, Roberto, Giordano D. Beretta, Basem Kildani, Laura Milesi, Federica Merlin, Stefania Mosconi, M. Adelaide Pessi, et al. 2010. “Colon Cancer.” *Critical Reviews in Oncology/Hematology* 74 (2). Elsevier Ireland Ltd: 106–33.

Lobo, Alexandre R., Eduardo H.S. Gaievski, Eduardo De Carli, Eliana P. Alvares, and Célia Colli. 2014. “Fructo-Oligosaccharides and Iron Bioavailability in Anaemic Rats: The Effects on Iron Species Distribution, Ferroportin-1 Expression, Crypt Bifurcation and Crypt Cell Proliferation in the Caecum.” *British Journal of Nutrition* 112 (8): 1286–95.

Maawia, Kaleemullah, Sanaullah Iqbal, Tahir Rasool Qamar, Pakeeza Rafiq, Azmat ullah, and Mansur ud Din Ahmad. 2016. “Production of Impure Prebiotic Galacto-Oligosaccharides and Their Effect on Calcium, Magnesium, Iron and Zinc Absorption in Sprague-Dawley Rats.” *PharmaNutrition* 4 (4). Elsevier B.V.: 154–60.

Machado, Adriane M., Nayara B.M. da Silva, José Benício P. Chaves, and Rita de Cássia G. Alfenas. 2018. “Consumption of Yacon Flour Improves Body Composition and Intestinal Function in Overweight Adults: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial.” *Clinical Nutrition ESPEN*.

Mandal, Paramita. 2018. “Molecular Mechanistic Pathway of Colorectal Carcinogenesis Associated with Intestinal Microbiota.” *Anaerobe* 49. Elsevier Ltd: 63–70.

Manzoni, Marla Simone Jovenasso, Elizeu Antonio Rossi, Nadiége Dourado Pauly-Silveira, Roseli Aparecida Pinto, Mariana Nougalli Roselino, Iracilda Zeppone Carlos, Marcela Bassi Quilles, Maria Beatriz de Abreu Glória, and Daniela Cardoso Umbelino Cavallini. 2017. “Consumption Effect of a Synbiotic Beverage Made from Soy and Yacon Extracts Containing Bifidobacterium Animalis Ssp. Lactis BB-12 on the Intestinal Polyamine Concentrations in Elderly Individuals.” *Food Research International* 99. Elsevier: 495–500. d

Maruvada, Padma, Vanessa Leone, Lee M. Kaplan, and Eugene B. Chang. 2017. “The Human Microbiome and Obesity: Moving beyond Associations.” *Cell Host and Microbe* 22 (5). Elsevier Inc.: 589–99.

Mcloughlin, Rebecca F, Bronwyn S Berthon, Megan E Jensen, Katherine J Baines, and Lisa G Wood. 2017. “Short-Chain Fatty Acids, Prebiotics, Synbiotics, and Systemic Inflammation: A Systematic Review and Meta-Analysis.” *The American Journal Of Clinical Nutrition* 106 (1): 930–45.

McRae, Marc P. 2018. “The Benefits of Dietary Fiber Intake on Reducing the Risk of Cancer: An Umbrella Review of Meta-Analyses.” *Journal of Chiropractic Medicine* 17 (2). Elsevier Inc.: 44–53.

Miyaguchi, Yuji, Takuya Tomatsuri, Atsushi Toyoda, Eiichi Inoue, and Yasuki Ogawa. 2015. “Effect of Yacon Tuber (Smallanthus Sonchifolius)-Derived Fructooligosaccharides on the Intestinal Flora and Immune System of OVA-Sensitized BALB/c Mice.” *Food Science and Technology Research* 21 (2): 255–62.

Mohanty, Debapriya, Snigdha Misra, Swati Mohapatra, and Priyadarshi Soumyaranjan Sahu.

2018. “Prebiotics and Synbiotics: Recent Concepts in Nutrition.” *Food Bioscience* 26: 152–60.

Moulahoum, Hichem, Belkacem Mohamed Amine Boumaza, Meriem Ferrat, Andras Laszlo Nagy, Diana Elena Olteanu, Abdelkader Bounaama, Simona Clichici, and Bahia Djerdjouri. 2018. “Aberrant Crypt Foci Are Regionally Affected by Zinc Treatment in a 1,2-Dimethylhydrazine Induced Colon Carcinogenesis Model.” *Journal of Trace Elements in Medicine and Biology* 47. Elsevier: 21–30.

Moura, Nelci A. de, Brunno F R Caetano, Kátia Sivieri, Luis H. Urbano, Claudio Cabello, Maria A M Rodrigues, and Luis F. Barbisan. 2012. “Protective Effects of Yacon (Smallanthus Sonchifolius) Intake on Experimental Colon Carcinogenesis.” *Food and Chemical Toxicology* 50 (8). Elsevier Ltd: 2902–10.

Ohigashi, Seiji, Kazuki Sudo, Daiki Kobayashi, Osamu Takahashi, Takuya Takahashi, Takashi Asahara, Koji Nomoto, and Hisashi Onodera. 2013. “Changes of the Intestinal Microbiota, Short Chain Fatty Acids, and Fecal pH in Patients with Colorectal Cancer.” *Digestive Diseases and Sciences* 58: 1717–26.

Ojansivu, Ilkka, Celia Lucia Ferreira, and Seppo Salminen. 2011. “Yacon, a New Source of Prebiotic Oligosaccharides with a History of Safe Use.” *Trends in Food Science and Technology* 22 (1). Elsevier Ltd: 40–46.

Oliveira, Gilberto Ornelas, Camila Pereira Braga, and Ana Angélica Henrique Fernandes. 2013. “Improvement of Biochemical Parameters in Type 1 Diabetic Rats after the Roots Aqueous Extract of Yacon [*Smallanthus Sonchifolius* (Poepp.& Endl.)] Treatment.” *Food and Chemical Toxicology* 59. Elsevier Ltd: 256–60.

Osadchiy, Vadim, Clair R. Martin, and Emeran A. Mayer. 2019. “The Gut-Brain Axis and the Microbiome: Mechanisms and Clinical Implications.” *Clinical Gastroenterology and Hepatology* 17 (2). Elsevier, Inc: 322–32.

Pabst, Oliver. 2012. “New Concepts in the Generation and Functions of IgA.” *Nature Reviews Immunology* 12 (12). Nature Publishing Group: 821–32.

Paganini, Daniela, Mary A. Uyoga, Colin I. Cercamondi, Diego Moretti, Edith Mwasi, Clarissa Schwab, Salome Bechtler, et al. 2017. “Consumption of Galacto-Oligosaccharides Increases Iron Absorption from a Micronutrient Powder Containing Ferrous Fumarate and Sodium Iron EDTA: A Stable-Isotope Study in Kenyan Infants.” *American Journal of Clinical Nutrition* 106 (4): 1020–31.

Patel, Hetal J., and Bhoomika M. Patel. 2017. “TNF-α and Cancer Cachexia: Molecular Insights and Clinical Implications.” *Life Sciences* 170. Elsevier Inc.: 56–63.

Pereira, Juciane Abreu Ribeiro, Meryene Carvalho Teixeira, Adelir Aparecida Saczk, Maria de Fátima Piccolo Barcelos, Marcelo Firmino de Oliveira, and Wilson César de Abreu. 2016. “Total Antioxidant Activity of Yacon Tubers Cultivated in Brazil.” *Ciência E Agrotecnologia* 40 (5): 596–605.

Pereira, Juciane de Abreu Ribeiro, Maria de Fátima Piccolo Barcelos, Eric Batista Ferreira, Rafaela Corrêa Pereira, and Michel Cardoso de Angelis-Pereira. 2016. “Changes in Glucose Levels and Fecal Excretion of Lipids due to Consumption of Yacon Flour.” *Nutrition & Food Science* 46 (6): 791–802.

Peshev, Darin, and Wim Van den Ende. 2014. “Fructans: Prebiotics and Immunomodulators.” *Journal of Functional Foods* 8 (1). Elsevier Ltd: 348–57.

Polakowski, Camila Brandão, Massakazu Kato, Vinicius Basso Preti, Maria Eliana Madalozzo Schieferdecker, and Antonio Carlos Ligocki Campos. 2019. “Impact of the Preoperative Use of Synbiotics in Colorectal Cancer Patients: A Prospective, Randomized, Double-Blind, Placebo-Controlled Study.” *Nutrition* 58: 40–46.

Quigley, Eamonn M.M. 2019. “Prebiotics and Probiotics in Digestive Health.” *Clinical Gastroenterology and Hepatology* 17 (2). Elsevier, Inc: 333–44.

Rastall, Robert A., and Glenn R. Gibson. 2015. “Recent Developments in Prebiotics to Selectively Impact Beneficial Microbes and Promote Intestinal Health.” *Current Opinion in Biotechnology* 32. Elsevier Ltd: 42–46.

Reis, Sandra A. dos, Lisiane L. da Conceição, Nathane P. Siqueira, Damiana D. Rosa, Letícia L. da Silva, and Maria do Carmo G. Peluzio. 2017. “Review of the Mechanisms of Probiotic Actions in the Prevention of Colorectal Cancer.” *Nutrition Research* 37: 1–19. doi:10.1016/j.nutres.2016.11.009.

Rocha, Daniela Mayumi Usuda Prado, Priscila Vaz de Melo Ribeiro, Ana Paula Silva Caldas, Bárbara Pereira da Silva, Alessandra da Silva, Alinne Paula de Almeida, Nayara Benedito Martins da Silva, Adriane Moreira Machado, and Rita de Cássia Gonçalves Alfenas. 2018. “Acute Consumption of Yacon Shake Did Not Affect Glycemic Response in Euglycemic, Normal Weight, Healthy Adults.” *Journal of Functional Foods* 44 (February). Elsevier: 58–64.

Rodrigues, Fabiana Carvalho, Adriano Simões Barbosa Castro, Hércia Stampini Duarte Martino, and Célia Lúcia de Luces Fortes Ferreira. 2011. “Farinha de Yacon (Smallanthus Sonchifolius): Produção E Caracterização Química.” *Revista Do Instituto Adolfo Lutz* 70 (3): 290–95. http://revista.ial.sp.gov.br/index.php?option=com_remository&Itemid=27&func=startdown&id=710.

Rodrigues, Fabiana Carvalho, Adriano Simões Barbosa Castro, Vívian Carolina Rodrigues, Sérgio Antônio Fernandes, Edimar Aparecida Filomeno Fontes, Tânia Toledo de Oliveira, Hércia Stampini Duarte Martino, and Célia Lúcia de Luces Fortes Ferreira. 2012. “Yacon Flour and *Bifidobacterium Longum* Modulate Bone Health in Rats.” *Journal of Medicinal Food* 15 (7): 664–70.

Schaafsma, Gertjan, and Joanne L. Slavin. 2015. “Significance of Inulin Fructans in the Human Diet.” *Comprehensive Reviews in Food Science and Food Safety* 14 (1): 37–47.

Scheid, M. M.A., P. S. Genaro, Y. M.F. Moreno, and G. M. Pastore. 2014. “Freeze-Dried Powdered Yacon: Effects of FOS on Serum Glucose, Lipids and Intestinal Transit in the Elderly.” *European Journal of Nutrition* 53 (7): 1457–64.

Song, Mingyang, and Andrew T. Chan. 2019. “Environmental Factors, Gut Microbiota, and Colorectal Cancer Prevention.” *Clinical Gastroenterology and Hepatology* 17 (2). Elsevier, Inc: 275–89.

Sant’Anna, Mônica de Souza Lima, Vivian Carolina Rodrigues, Tatiane Ferreira Araújo, Tânia Toledo de Oliveira, Maria do Carmo Gouveia Peluzio, and Célia Lúcia de Luces

Fortes Ferreira. 2015. “Yacon-Based Product in the Modulation of Intestinal Constipation.” *Journal of Medicinal Food* 18 (9): 980–86.

Sun, Jun, and Ikuko Kato. 2016. “Gut Microbiota, Inflammation and Colorectal Cancer.” *Genes and Diseases* 3 (2). Elsevier Ltd: 130–43. doi:10.1016/j.gendis.2016.03.004.

Tilg, Herbert, Timon E. Adolph, Romana R. Gerner, and Alexander R. Moschen. 2018. “The Intestinal Microbiota in Colorectal Cancer.” *Cancer Cell* 33 (6). Elsevier Inc.: 954–64.

Valcheva, Rosica, and Levinus A. Dieleman. 2016a. “Prebiotics: Definition and Protective Mechanisms.” *Best Practice and Research: Clinical Gastroenterology* 30: 27–37.

———. 2016b. “Prebiotics: Definition and Protective Mechanisms.” *Best Practice and Research: Clinical Gastroenterology* 30 (1): 27–37.

Vasconcelos, Christiane Mileib, Eduardo Basílio de Oliveira, Stephania Nunes Rossi, Luma Furtado Arantes, Rolf Puschmann, and José Benício Paes Chaves. 2015. “Evaluating Strategies to Control Enzymatic Browning of Minimally Processed Yacon (Smallanthus Sonchifolius).” *Food and Bioprocess Technology* 8 (9): 1982–94.

Vaz-Tostes, Maria das Graças, Mirelle Lomar Viana, Mariana Grancieri, Tereza Cecília dos Santos Luz, Heberth de Paula, Rogério Graça Pedrosa, and Neuza Maria Brunoro Costa. 2014. “Yacon Effects in Immune Response and Nutritional Status of Iron and Zinc in Preschool Children.” *Nutrition* 30 (6): 666–72.

Velez, Eva, Natalia Castillo, Oscar Mesón, Alfredo Grau, María E. Bibas Bonet, and Gabriela Perdigón. 2013. “Study of the Effect Exerted by Fructo-Oligosaccharides from Yacon (Smallanthus Sonchifolius) Root Flour in an Intestinal Infection Model with Salmonella Typhimurium.” *British Journal of Nutrition* 109 (11): 1971–79.

Weinborn, Valerie, Carolina Valenzuela, Manuel Olivares, Miguel Arredondo, Ricardo Weill, and Fernando Pizarro. 2017. “Prebiotics Increase Heme Iron Bioavailability and Do Not Affect Non-Heme Iron Bioavailability in Humans.” *Food & Function* 8 (5). Royal Society of Chemistry: 1994–99.

WHO. 2018. “10 Facts About Cancer.” *World Health Orgnization*. http://www.who.int/features/factfiles/cancer/en/index.html.

Witten, Jacob, Tahoura Samad, and Katharina Ribbeck. 2018. “Selective Permeability of Mucus Barriers.” *Current Opinion in Biotechnology* 52. Elsevier Ltd: 124–33.

Xie, Xiaoliang, Yaqin He, Hai Li, Dong Yu, Li Na, Ting Sun, Dong Zhang, et al. 2018. “Effects of Prebiotics on Immunological Indicators and Intestinal Microbiota Structure in Perioperative Colorectal Cancer Patients.” *Nutrition* 99 (2): F110.

Yamagishi, Hidetsugu, Hajime Kuroda, Yasuo Imai, and Hideyuki Hiraishi. 2016. “Molecular Pathogenesis of Sporadic Colorectal Cancers.” *Chinese Journal of Cancer* 35 (4). BioMed Central: 1–8.

Zhu, Fan, Colin Prosser, Ying Zhu, Don Otter, and Yacine Hemar. 2018. “Enzymatic Formation of Galactooligosaccharides in Goat Milk.” *Food Bioscience* 26. Elsevier Ltd: 38–41.

Zhu, Qingchao, Zhiming Jin, Wen Wu, Renyuan Gao, Bomin Guo, Zhiguang Gao, Yongzhi

Yang, and Huanlong Qin. 2014. “Analysis of the Intestinal Lumen Microbiota in an Animal Model of Colorectal Cancer.” *PLoS ONE* 9 (3): 1–10.

4. ARTIGO ORIGINAL

Preventive effect of yacon (*Smallanthus sonchifolius*) on intestinal health in animal model of colorectal cancer[1]

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ABSTRACT

Yacon (*Smallanthus sonchifolius*) is a source of prebiotic fructooligoccharides (FOS), which promote intestinal health. The present study investigated the preventive effect of yacon flour (YF) intake on the alteration promoted by carcinogenesis colorectal (CRC) induced by 1.2-dimethylhydrazine (DMH). Forty-four male adult Wistar rats were divided into four groups: S (without CRC and YF; n=10), Y (without CRC with YF; n=10), C (with CRC without YF; n=12), CY (with CRC and YF; n=12). Groups S and C received AIN-93 M diet and groups Y and CY received the same diet but added with YF (5% of FOS), for 16 weeks. At the end, CRC increased TNF-α levels, SCFA production and decreased TAC. Yacon reduced intraluminal pH and Lactulose/Mannitol ratio, increased secretory immunoglobulin A (sIgA), but did not change interleukin (IL)-10, IL-12 and TNF-α levels. Therefore, yacon flour may help to maintain the integrity of intestinal health degraded due to colorectal cancer induction.

Keywords: fructooligoccharides, prebiotics, inflammation, intestinal barrier, oxidative

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stress

Abbreviations: YF: yacon flour; CRC: colorectal cancer; TNF-α: tumor necrosis factor-alpha; TAC: total antioxidant capacity; sIgA: secretory immunoglobulin A; SCFA: short chain fatty acids; FOS: fuctooligosaccharides, L: Lactulose; M: Mannitol.

1. Introduction

According to the World Health Organization (WHO, 2018), 9.6 million people worldwide were estimated to die from cancer in 2018. Cancer is an end-result of several successive cellular changes characterized by the formation and growth of abnormal cell population with accumulation of DNA changes, called mutations (López-Lázaro, 2018). Among the different types, the colorectal cancer (CRC) is the fourth most common cause of death for men and the third for women worldwide (WHO, 2018).

Colorectal cancer reaches the colon and the rectum, and it is a multifactorial disease influenced by genetic, environment and lifestyle factors, such as diet (low fiber and high red meat intake), physical inactivity, smoking, alcohol, and obesity (Labianca, et al., 2010). CRC is considered a series of sequences caused by mutations, activations, and deletions along a genetic pathway from the initial loss of the adenomatous polyposis coli (APC) tumor-suppressor gene leading to adenoma-carcinoma (Grivennikov, et al., 2012; Ambalam, et al., 2016). Moreover, the composition of gut microbiota could be associated with the development of CRC, since the transformation of epithelial cells with genetic mutations is associated to loss of epithelial barrier function. Hence, luminal bacteria translocate into the lamina propria is facilitated, which activates myeloid cells and further promotes epithelial-cell proliferation, resulting in gradual loss of functions and tumorigenesis (Grivennikov, et al., 2012; Gallimore & Godkin, 2013).

In this context, food supplements able to modulate the gut microbiota may act as chemopreventive agent of CRC (Ambalam, et al., 2016). Yacon (*Smallanthus sonchifolious*) originates from the Andean region of South America, and is consumed in its natural form, flour, syrup, or added in products. It is a source particularly of fructooligosaccharides (FOS), that are an inulin-type of prebiotic fructans joined by β-(2-1) or β-(2-6) bonds with low degree of polymerization (DP), varying from 3 to 10 units (Ojansivu, Ferreira, & Salminen, 2011).

Prebiotics are a substrate that is utilized selectively by host microorganisms conferring health benefits (Gibson, et al., 2017). In the colon, prebiotics are fermentable by beneficial bifidobacteria and produce short chain fatty acids (SCFA), namely acetate, propionate and butyrate, as the main metabolic product of fermentation (Macfarlane & Macfarlane, 2011). Butyrate is a preferred energy source of colonocytes and demonstrates direct affect, such as regulatory effects on gene expression, epithelial barrier function, modulation of apoptosis, growth arrest and cell differentiation (Canani, Costanzo, & Leone, 2012). Also, prebiotics have immune function by stimulating the production of anti-inflammatory cytokines and enhancing intestinal barrier function by secreting antioxidative and anticarcinogenic compounds (Peshev & Van den Ende, 2014). Therefore, yacon has prebiotic effect by promoting the growth of probiotic bacteria, with high levels of SCFA in the cecal material and enhancement of cell density and crypt formation (Campos, et al., 2012).

Recent study by our research group found that yacon flour has potential to promote beneficial effects on the intestinal health by attenuating changes promoted after the CRC induction in an animal model (Grancieri, et al., 2017). Our hypothesis is that the intake of yacon flour before the colorectal cancer being induced can be more effective to attenuate physiological changes caused by the carcinogenesis. In this context, the aim of this study was to investigate the preventive effect of yacon flour in the integrity of the intestinal barrier, inflammatory response and total antioxidant capacity resulted from colorectal carcinogenesis.

2. Materials and methods

2.1 Animals

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

2.2 Experimental design

The 44 animals were divided into 4 experimental groups:

Group S: without colorectal cancer induction and without yacon flour supplementation, (n=10);

Group Y: without colorectal cancer induction and with yacon flour supplementation, (n=10);

Group C: with colorectal cancer induction and without yacon flour supplementation, (n=12);

Group CY: with colorectal cancer induction and with yacon flour supplementation, (n=12).

The period experimental was 16 weeks. During this period, groups S and C received the AIN-93 M diet, and groups Y and CY received the adapted AIN-93 M diet with yacon flour addition to in sufficient amounts to provide 5% of FOS (Campos, et al., 2012). Figure 1 summarizes the experimental study.

The CRC was induced in the C and CY groups by the weekly subcutaneous injection of 25 mg /kg body weight of 1.2-dimethylhydrazine (DMH- Sigma®), for 5 consecutive weeks (from the 4^{th} to 8^{th} weeks). DMH was dissolved in NaCl 0.9 %, 15 % EDTA with the pH adjusted to 6.5, and it was prepared before use immediately. The remaining 8 subsequent weeks were the interval for the CRC development (Rodrigues, et al., 2002).

In the last week of experiment, the animals were housed at metabolic cages for 24 h, and the urine was collected for the intestinal permeability analysis. Individual food intake and body weight were recorded weekly, so the mean of the total food intake, the mean of total weight gain and the food efficiency ratio (FER= (body weight gain/food consumptionx100) were calculated at the end of the experiment.

At the end of the study (16 weeks), animals were anesthetized by intraperitoneal administration of 0.2 mL /100 g body weight of anesthetic solution with 37.5 % ketamine, 25 % xylazine and 37.5 % of saline solution. After, the thoracic cavity was open and blood was collected with cardiac puncture by leading to hypovolemia and euthanasia of the animals consequently. The collected blood was centrifuged (3000 *g*, 10 min) to obtain the plasma, and stored at -80 ºC for cytokines analysis. Moreover, caecum was removed and the cecal content collected and stored

at -80 ºC for further analysis.

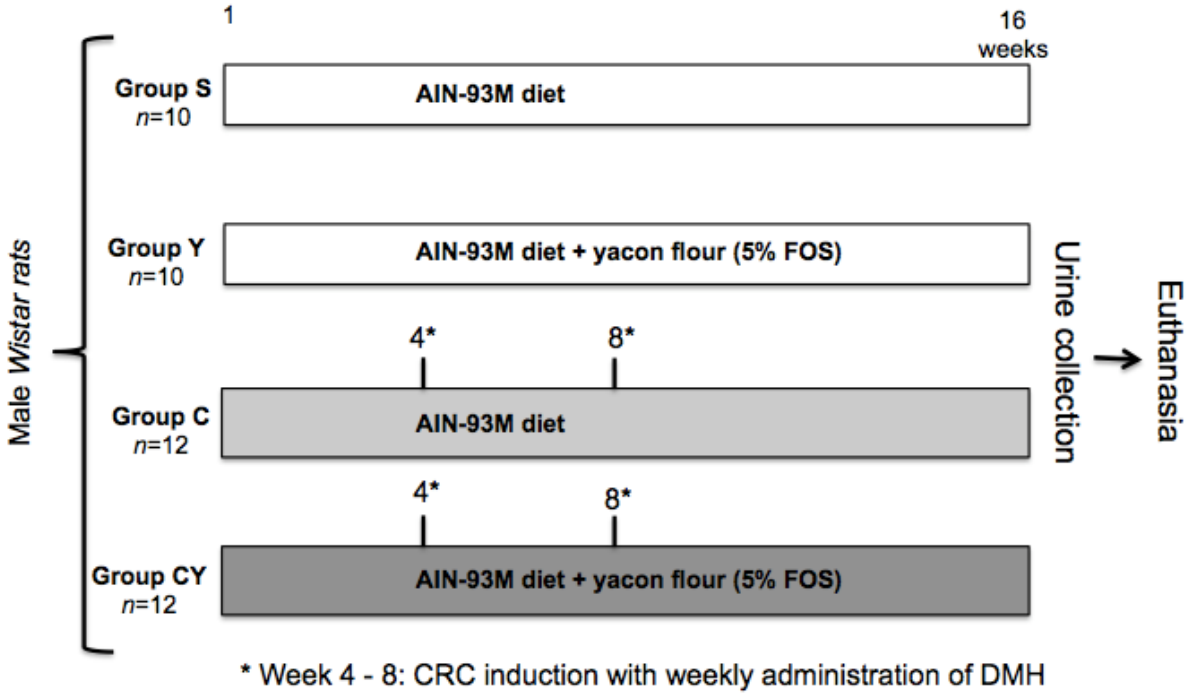

Fig. 1. Diagram of the experimental study.
S: group without colorectal cancer induction and without yacon flour (*n*=10); C: group with colorectal cancer induction and without yacon flour (*n*=12); Y: group without colorectal cancer induction and with yacon flour (*n*=10); CY: group with colorectal cancer induction and with yacon flour (*n*=12); YF: yacon flour; CRC: colorectal cancer. DMH: 1.2-dimethylhydrazine

2.3 Yacon flour preparation

The yacon was obtained from a rural producer from Santa Maria de Jetibá/ES. The yacon flour elaboration was realized as described by Vaz-Tostes, et. al., 2014. The analyses of carbohydrates, proteins, fats, fiber and ash were evaluated by the AOAC method (AOAC, 2012). FOS and inulin content in the yacon flour were determined by High Performance Liquid Chromatography (HPLC) with a BIO-RAD brand HPX-87p column (lead stationary phase) using purified water as the mobile phase. Samples were diluted (1g in 100 mL distilled water), centrifuged at 3000 *g* and then filtered through a Millipore brand polyvinylidene fluoride membrane (PVDF), with 0.22 µm pore size and 13 mm diameter. Then, samples were injected into the liquid chromatograph, Varian brand, Pro-STAR 410 model, with refractive index detector and auto sampler (AUTO SAMPLER 410) with a flow of 0.6 mL min^{-1} and

column temperature of 80 ^{0}C, projecting a sequence of peaks, which were compared with standard curves predefined in the equipment. Yacon flour analysis identified 28.95 % FOS, 6.34 % inulin, 4.52 % protein, 0.33 % lipids, 2.94 % ash, 10.68 % total fiber 5.92 % moisture, and 40.32 % other carbohydrates.

2.4 Experimental diets

The experimental diets were prepared following the recommendations of the American Institute of Nutrition, AIN-93 M, (Reeves, Nielsen, & Fahey, 1993). The groups S and C received the AIN-93 M diet. The diet of the groups Y and CY was supplemented with yacon flour (YF) in amounts sufficient to provide 5 % of FOS. The yacon flour contained 28.95 % of FOS, so it was added 17.27 g of YF/100 of diet. The content of casein, sucrose, starch and dietary fiber of the experimental groups was adjusted in order to all groups had the similar content of calories, carbohydrates, proteins and fiber (Table 1).

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

Ingredients (g/ 100 g)	Groups: S and C AIN-93 M	Groups: Y and CY AIN- 93 M + YF
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*
Caloric Density (kcal g^{-1})	3.80	3.95

* Sufficient amount to provide 5% of 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); YF: yacon flour; CRC: colorectal cancer.

2.5 Cytokines quantifications by ELISA

For cytokines analyses, blood samples were centrifuged at 3000 *g*, 10 min, 4 ^{0}C. IL-10 and IL-12 cytokines were quantified by using the commercial kit Milliplex® Map and TNF-α (tumor necrosis factor-alpha) by using the kit EMD Millipore ELISA, in accordance with the manufacturer's recommendations. The results were expressed in pg/mL.

2.6 Total antioxidant capacity

The total antioxidant capacity of the plasma was carried out by the colorimetric assay "Total Antioxidant Capacity Assay", Elabscience®. The results were expressed as unit of total antioxidant capacity (U)/ mL.

2.7 sIgA

The caecal luminal content, 100 mg, was diluted with 1 mL of phosphate-buffered saline (pH 7.2), homogenized by using a vortex, and centrifuged at 3000 *g* for 10 min. The suspension was collected and evaluated based on the Immunochron enzyme-linked immunosorbent assay (ELISA) method using the Cloud-Clone® kit, in accordance with the manufacturer's recommendations for sIgA determination. The results were expressed by ng/mL.

2.8 Intraluminal pH of the colon

The caecal luminal content was weighted, diluted in saline solution (1:10), and homogenized by vortexing. Then, the pH reading was performed using a pH meter (Kasvi®).

2.9 Determination of short chain fatty acids (SCFA)

Acetate, propionate and butyrate were evaluated in the colonic content of animals. To that, 100 mg of the colonic content were diluted in 2 mL of 0.1 mL

perchloric acid with 3% of phenol solution, and then mixed by vortexing for 5 min, centrifuged (9000 *g*, 10 min) and filtered through a 0.45 µm membrane filter and placed in vials for analysis by High Performance Liquid Chromatography (HPLC) (Kotani, et al., 2009). As internal standards, it was used acetic acid®, butyric acid® and propionic acid® (Sigma-Aldrich, São Paulo/SP- Brazil). The areas obtained by the curves were calculated and converted to mg/g of colonic content.

2.10 Intestinal permeability

The intestinal permeability was determined in the last week of the experiment. For the analysis, the animals were fasted for 12 h, and received 2 mL of a solution with 200 mg of lactulose and 100 mg of mannitol by gavage. After the administration, the animals were allocated in metabolic cages and fasted for another 5 h. The urine was collected during 24 h, and the volume was measured, recorded and stored at -80 ^{0}C. For the analyses of lactulose and mannitol, the urine was filtered in a 0.45 µm membrane filter and placed in vials for analysis by HPLC. As internal standards, it was used Lactulose® and Mannitol® (Sigma-Aldrich, São Paulo/SP- Brazil). Lactulose and mannitol concentrations were obtained and converted to g/L in order to calculate the percentage of urinary excretion. The Lactulose/Mannitol ratio was calculated by dividing the concentration of lactulose by the concentration of mannitol (Song, et al., 2011).

2.11 Chromatographic conditions

All analyses were performed on a Shimadzu HPLC system (Kyoto, Japan). The chromatographic system consists of: degasser (Model DGU-20A), pump (Model LC-20AT), auto-sampler (Model SIL-20A), column oven (Model CTO-10AS) and UV-Vis 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 x 8.7 mm) of BIO-RAD (California, USA). The mobile phase consisted of H_2S0_4 0.005 mol/L. This solution was filtered through a 0.45 µm Milipore membrane and filtered through a vacuum pump Tecnal® (Model TE-0582). The analyses were performed at 55 ^{0}C under isocratic conditions. Flow rate of mobile phase was 0.6 mL/min for lactulose and mannitol and 1.0 mL/min for short chain fatty acids. The injection

volume of sample was 20 µL. Lactulose and mannitol were analyzed by using refractive index detector (RI) and short chain fatty acids (acetic, propionic and butyric acids) were analyzes by UV-Vis detection at 210 nm.

2.12 Statistical analyses

The samples were tested by the Kolmogorov-Smirnov normality test. Samples with no normal distribution were transformed (using the $\log_{10}$ function). The groups were tested for the effects of yacon flour and colorectal cancer, and/or their interactions, by using "Two-way" ANOVA (variance analysis), followed by Newman-Keuls *post hoc* ($p<0.05$). Data were expressed as mean ± standard error (SE), and considered significant the value of $p<0.05$. Statistical analyzes were performed in the GraphPad Prism®, version 7 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1 Food consumption, body weight gain and food efficiency ratio

There was no significant difference in body weight gain, food intake and food efficiency ratio among the groups (Table 2).

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

	Groups				*p*		
Variable	**S**	**Y**	**C**	**CY**	**YF**	**CRC**	**YFxCRC**
Weight gain (g)	286.9±50.2[a]	281.2±41.0[a]	301.1±34.4[a]	267.6±34.3[a]	0.1346	0.9845	0.2861
Food intake (g)	164.9±17.9[a]	163.2±10.6[a]	163.1±11.6[a]	154.7±12.1[a]	0.1977	0.2785	0.3638
FER (%)	11.5±1.2[a]	11.4±1.39[a]	12.2±1.4[a]	11.5±1.31[a]	0.3548	0.3596	0.4684

Values expressed as mean ± standard deviation. Same letters in the same line: groups are not significantly different ($p> 0.05$). *p* = "two way" ANOVA of the effects of YF and CRC and the interaction of them. 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); YF: yacon flour; CRC: colorectal cancer; FER: food efficiency ratio.

3.2 Cytokines

After the experimental period, the supplementation with YF and the colorectal cancer induction did not have significant effect in IL-10 and IL-12 levels. However, the CRC presented significant effect in TNF-α levels. In groups C and CY, there was an increase of TNF-α levels when compared to the groups without colorectal cancer induced (groups S and Y) (Table 3).

3.3 Total antioxidant capacity

It was observed a significant interaction between YF and CRC ($p<0.05$). The animals in the S group had the highest value of TAC (6.71 U/mL) among all experimental groups. There was no difference between the animals induced to the CRC with or without YF (groups C and CY) (Table 3).

3.4 Secretory immunoglobulin A

The Y group presented the highest value among all groups, demonstrating the YF effect to increase de sIgA levels, but it was observed an interaction between YF x CRC ($p<0.05$). There was no difference between the C and CY groups ($p<0.05$), so in the animals with CRC the YF was not able of increasing the sIgA levels (Table 4).

3.5 Intraluminal pH of the colon

The YF supplementation led to a decrease in the intraluminal pH, since groups Y and CY, without difference between then, presented lower pH values when compared to groups S and C ($p<0.05$). Furthermore, it was observed a significant difference between group S and C ($p<0.05$), so the animals with colorectal cancer induced had a pH value lower than the healthy animals (Table 4).

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

Variable	Groups				P		
	S	Y	C	CY	YF	CRC	YFXCRC
IL-10 (pg/mL)	219.5±78.5[a]	203.9±66.8[a]	89.9±11.6[a]	253.5±82.7[a]	0.2101	0.3905	0.1977
IL-12 (pg/mL)	10.2±1.2[a]	8.6±1.6[a]	8.9±2.1[a]	9.5±2.0[a]	0.7956	0.9612	0.2399
TNF-α (pg/mL)	6.8±3[a]	5.8±1.7[a]	9.6±1.0[b]	10.2±0.7[b]	0.3100	<0.0001	0.8356
TAC (U/mL)	6.7±2.4[a]	4.1±1.5[b]	3.3±0.9[b]	4.7±2.2[b]	0.3136	0.0263	0.0022

Values expressed as mean ± standard deviation. Different letters are groups significantly different (p≤ 0.05) by "two-way" ANOVA of the effects of yacon flour and colorectal cancer and the interaction of them. 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); YF: yacon flour; CRC: colorectal cancer; IL: interleukin; TNF-α: tumor necrosis factor α; TAC: total antioxidant capacity.

Table 4. Levels of sIgA (ng/mL) and intraluminal pH of the colon.

Variable	Groups				*p*		
	S	Y	C	CY	YF	CRC	YFxCRC
pH	8.13±0.205[a]	6.754±0.466[b]	7.651±0.447[c]	6.457±0.474[b]	<0.0001	0.0059	0.4964
sIgA (ng/mL)	9.1±1.5[a]	15.9±7.3[b]	10.9±3.8[a]	9.4±1.4[a]	0.0708	0.1048	0.0064

Values expressed as mean ± standard deviation. Different letters in the same line: groups are significantly different (p≤ 0.05). *p* = "two way" ANOVA of the effects of FY and CRC and the interaction of them. 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); YF: yacon flour; CRC: colorectal cancer.

3.6 Short chain fatty acids (SCFA)

For the acetate, groups without CRC (S and Y) had lesser excretion compared to groups with CRC (C and CY), demonstrating a significant effect of the colorectal cancer ($p<0.05$). The CY group presented higher excretion than the group C. In relation to the propionate, the Y group had higher value when compared to the S group, showing significant effect of YF ($p<0.05$). Moreover, there was a significant effect of the CRC, since the animals with CRC (groups C and CY) had higher excretion than the animals without the colorectal cancer (group S). For the butyrate, the C group had higher excretion than the S group, demonstrating effect of the CRC

(p<0.05). The YF did not promote significant modifications, since the groups S and Y did not differ neither the groups induced to the CRC (C and CY) (Table 5). In relation to total SCFA, it was observed a significant effect of the CRC, so that the C and CY groups showed higher excretion when compared to the groups without the CRC induction (groups S and Y). The R_2 values of standard curve were 0.982 for propionate and 0.999 for acetate and butyrate.

3.7 Intestinal permeability

The urinary excretion of lactulose did not have significant difference among the experimental groups. There was a significant interaction between yacon flour and colorectal cancer (p<0.05) to the urinary excretion of mannitol. The S group (without YF) and the Y group (with YF) did not differ, but in the presence of colorectal cancer the CY group showed higher urinary excretion of mannitol than the C group.

For the ratio lactulose/mannitol (L/M), the S group had higher value than the Y group, demonstrating the significant effect of the yacon flour (p<0.05). Moreover, there was an interaction between FY x CRC, so there was no difference in groups with colorectal cancer supplemented or not with YF (C and CY), as presented in the Table 6. The R_2 values of lactulose and mannitol standard curve were respectively, 0.994 and 0.981.

Table 5. SCFA: excretion of acetate, butyrate and propionate (mg/g).

	Groups				p		
Variable	**S**	**Y**	**C**	**CY**	**YF**	**CRC**	**YFxCRC**
Acetate	1097±	1083±	1596±	2007±	0.1277	<0.0001	0.1045
(mg/g)	126.9^{a}	276.1^{a}	559.8^{b}	494.1^{c}			
Butyrate	182.9±	169.1±	525.1±	338±	0.2690	0.0071	0.3392
(mg/g)	85.76^{a}	120.2^{a}	380.2^{b}	371.5^{ab}			
Propionate	712.2±	847.5±	949±	1045±	0.0094	<0.0001	0.6407
(mg/g)	25.79^{a}	143.4^{b}	143.2^{bc}	170.8^{c}			
Total SCFA	1992±	2166±	3070±	3390±	0.3014	<0.0001	0.7586
(mg/g)	201.9^{a}	455.9^{a}	999.6^{b}	983.9^{b}			

Values expressed as mean ± standard deviation. Different letters in the same line: groups are significantly different (p≤ 0.05). *p* = "two way" ANOVA of the effects of FY and CRC and the interaction of them. 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); YF: yacon flour; CRC: colorectal cancer; SCFA: short chain fatty acids.

Table 6. Urinary excretion of lactulose, mannitol and the ratio between them.

Variables	Groups				*p*		
	S	Y	C	CY	YF	CRC	YFxCRC
Lactulose (%)	2.5±1.6[a]	0.9±0.6[a]	1.9±1.5[a]	2.5±2.8[a]	0.3662	0.4081	0.0580
Mannitol (%)	12.1±1.5[a]	12.0±2.8[a]	13.3±2.7 [a]	18.2±5.9[b]	0.0340	0.0022	0.0298
L/M Ratio	0.2±0.1[a]	0.07±0.05[b]	0.09±0.03[b]	0.09±0.1[b]	0.0391	0.1640	0.0256

Values expressed as mean ± standard deviation. Different letters in the same line: groups are significantly different (p≤ 0.05). *p* = "two way" ANOVA of the effects of FY and CRC and the interaction of them. 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); YF: yacon flour; CRC: colorectal cancer; L/M Ratio: ratio lactulose/mannitol.

4. Discussion

Yacon is a functional food that differentiates itself from most roots by storing its carbohydrate in the form of FOS, which act on intestinal health directly by acting as prebiotic (Choque Delgado, et al., 2013). Experimental studies with animals induced to colorectal cancer showed that yacon flour is a potential food to reduce deleterious effects of cancer on intestinal health (Grancieri, et al., 2017; Moura, et al., 2012). Therefore, the present study evaluated the chemopreventive effect of yacon flour on the inflammatory response, total antioxidant capacity and integrity of intestinal barrier in animal model of colorectal cancer.

The colon carcinogenesis is related to a process of chronic inflammation of the epithelium, with abnormal immune system activation and hyperproliferation (Sun & Kato, 2016; Grivennikov, et al., 2012). In this study, the animals induced to cancer presented an increase in the TNF-α levels compared to the healthy animals. TNF-α is a pro-inflammatory cytokine related to tissues inflammation and process signalization as communication, differentiation and cellular death, by being responsible to activate paths as NF-kB, which act on the anti-apoptotic signaling (Waters, Pober, & Bradley, 2013; Annibaldi & Meier, 2018). So, it was observed that the increase of TNF-α levels found in this study is related to the colorectal cancer presence. In the same way, animals induced to other types of cancer showed an increase in TNF-α levels, as

stomach (Wang, et al., 2014) and mammal cancer (Karnam, et al., 2017).

Although yacon is considered a food with immunomodulatory capacity (Paredes, et al., 2018), the levels of the anti-inflammatory IL-10 and the proinflammatory IL-12 cytokine did not change after yacon supplementation. It is proposed that the IL-10 presents effect in immunoregulation and inflammation, by increasing $CD8^+$ T cell numbers, antigen presentation, and inhibiting inflammatory mediators such as IL-12, which suppresses tumor growth (Teng, Darcy, & Smyth, 2011). However, the results found on the literature still are controversial, since some authors verified higher production of IL-10 (Bibas Bonet, et al., 2010), as other did not observe its modification in animals (Choque Delgado, et al., 2012) or in humans (Vaz-Tostes, et al., 2014) after yacon intake. The time of yacon supplementation, the animal model, the dose of FOS and the evaluation of systemic or local cytokine levels may interfere on these results.

The yacon flour is source of phenolic acids, mainly caffeic and chlorogenic acid, which help to reduce oxidative stress (Pereira, et al., 2016), so studies have been showed that it has high antioxidant capacity (Sousa, et al., 2015; Habib, et al., 2015). In this study, the total antioxidant capacity (TAC) of the plasma reduced in groups with colorectal cancer, but the YF was not able to decrease the oxidative damage from the carcinogenesis, evidencing the interaction between cancer with yacon flour. Another study (Jimenez, Rossi, & Sammán, 2015) observed that the intake of YF (8%) improved the antioxidant capacity, verified by an increase in the content of phenolic components in the cecum of healthy Wistar rats. In this sense, it should be considered that cancer is a disease with highly oxidative characteristics, since cellular damage by reactive oxygen species is one of the main drivers of mutations, so cancer cells are under constant oxidative stress (Gill, Piskounova, & Morrison, 2016), which may explain the yacon flour did not change the TAC in animals induced to cancer.

The colorectal carcinogenesis is associated with intestinal dysbiosis, imbalance between beneficial and pathogenic microorganisms, resulting in impairment of mucosa barrier integrity (Borges-Canha, et al., 2015). Thus, prebiotics present beneficial effects on the immunity of intestinal mucosa (Peshev & Van den Ende, 2014). In this experiment, the yacon flour increased the sIgA in animals of the Y group, corroborating other studies in animals models (Choque Delgado, et al., 2012) and in preschool children (Vaz-Tostes, et al., 2014). The increase in the sIgA

levels may be attributed to the FOS contents. sIgA acts as the first-line of defense that protects the epithelium from pathogens and toxins, helping to inhibit the colonization of pathogenic bacterium in the intestine and its penetration into the mucosa (Pabst, 2012). Although in the present study the microbiota composition was not evaluated, other authors verified an increase in sIgA levels correlated with an increase of bifidobacteria after yacon flour intake (Bibas Bonet, et al., 2010).

FOS are fermentable selectively by Bifidobacteria and Lactobacilli (Ojansivu, Ferreira, & Salminen, 2011), producing short chain fatty acids (SCFA) as metabolic product, mainly acetate, propionate and butyrate, that have potential to reduce the intraluminal pH (Macfarlane & Macfarlane, 2011). In the present work, yacon flour supplementation reduced the intraluminal pH, corroborating other studies (Grancieri, et al., 2017; Moura, et al., 2012). The reduction in the intraluminal pH inhibits the proliferation of pathogenic bacteria, and the activity of enzymes involved in the carcinogenic components production (Ohigashi, et al., 2013). Also, the luminal content acidification is beneficial to improve mineral absorption (Lobo, et al., 2011).

Grancieri et al., (2017) verified that the YF (7.5% FOS) intake after colorectal cancer induction increased butyrate and propionate production, being associated to an increase in the depth of colonic crypts and the number of crypts. Other authors also observed an increase in SCFA levels after yacon flour intake (Campos, et al., 2012; Moura, et al., 2012). SCFAs have anti-inflammatory effects (Mcloughlin, et al., 2017), and the butyrate acts as the main source of energy to the colonocytes, inhibits tumor cells growth and proliferation (Gonçalves & Martel, 2016), and inhibits the motility of colon cancer cells, reducing metastasis (Li, et al., 2017). However, in this study a distinct and unexpected behavior was observed, so that the animals induced to cancer (with and without yacon flour) had a higher production of acetic, propionic and butyric acids.

In this sense, it is known that tumor cells undergo metabolic changes during carcinogenesis to provide the energy requirements related to higher proliferation. So, it is observed an intensification of the glycolytic metabolism in detriment of the oxidative, a modification known as the Warburg effect. In this way, colorectal tumor cells prefer glucose to butyrate for energy supply (Gonçalves & Martel, 2016;Donohoe, et al., 2012;Burgess, 2012). Therefore, some metabolic interaction at luminal level may have been occurred in the animals, resulting in an increase of SCFA after cancer induction. However, no studies with similar results were found in

the literature in order to have a better understanding of the mechanisms involved in this metabolic modification.

Moreover, yacon flour intake and colorectal cancer induction were able to reduce the L/M ratio, since it was observed an interaction between them. The reduction of this ratio means a lower intestinal permeability, which refers to the physical barrier function that the epithelium exerts on the intestinal lumen to allow or not the passage of molecules (Witten, Samad, & Ribbeck, 2018). Another study verified beneficial effect of YF (7.5% FOS) to reduce the intestinal permeability in animals induced to CRC (Grancieri, et al., 2017), since a higher intestinal permeability is related to inflammatory diseases (Sina, Kemper, & Derer, 2018). Besides the effects attributed to FOS, the chlorogenic acid, mainly phenolic component found in the yacon (Pereira, et al., 2016), reduced the L/M ratio and increased the expression of tight junction proteins in colitic rats (Ruan, et al., 2016). So, for future studies, quantification of phenolic compounds is important for more conclusive relationships.

Prebiotics and probiotics act in a beneficial manner on the epithelial barrier reducing intestinal permeability, since SCFA produced as a result of fermentation by bifidobacteria prevent the growth of pathogenic microorganisms. Furthermore, immune system modulation is observed with higher production of antibacterial defensins, sIgA and anti-inflammatory cytokines, mainly IL-10. In addition, prebiotics have effects on the intestinal barrier integrity by increasing epithelial mucus production and maintain integrity of tight junction that prevent bacterial translocation (Stoidis, et al., 2011;Peshev & Van den Ende, 2014; Reis, et al., 2017).

5. Conclusion

The consumption of yacon flour promotes beneficial effects on the intestinal health of animals induced to colorectal cancer, such as reduction of pH, intestinal permeability and total antioxidant capacity. Also, as a source of FOS, yacon showed potential to improve intestinal barrier and mucosal immunity in healthy animals.

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References

Ambalam, P., Raman, M., Purama, R. K., & Doble, M. (2016). Probiotics, prebiotics and colorectal cancer prevention. *Best Practice and Research: Clinical Gastroenterology*, *30*(1), 119–131.

Annibaldi, A., & Meier, P. (2018). Checkpoints in TNF-Induced Cell Death: Implications in Inflammation and Cancer. *Trends in Molecular Medicine*, *24*(1), 49–65.

AOAC International. (2012). *Official Methods of Analysis* (19th ed.). United States: AOAC International.

Bibas Bonet, M. E., Meson, O., de Moreno de LeBlanc, A., Dogi, C. A., Chaves, S., Kortsarz, A., Grau, A., & Perdigón, G. (2010). Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model. *Food and Agricultural Immunology*, *21*(2), 175–189.

Borges-Canha, M., Portela-Cidade, J. P., Dinis-Ribeiro, M., Leite-Moreira, A. F., & Pimentel-Nunes, P. (2015). Role of colonic microbiota in colorectal carcinogenesis: A systematic review. *Revista Espanola de Enfermedades Digestivas*, *107*(11), 659–671.

Burgess, D. J. (2012). Warburg behind the butyrate paradox? *Nature Reviews Cancer*, *12*, 798.

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

Canani, R. B., Costanzo, M. Di, & Leone, L. (2012). The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. *Clinical Epigenetics*, *4*(4), 1–7.

Choque Delgado, G. T., da Silva Cunha Tamashiro, W. M., Maróstica Junior, M. R., & Pastore, G. M. (2013). Yacon (*Smallanthus sonchifolius*): A Functional Food. *Plant Foods for Human Nutrition*, *68*(3), 222–228.

Choque Delgado, G. T., Thomé, R., Gabriel, D. L., Tamashiro, W. M. S. C., & Pastore, G. M. (2012). Yacon (*Smallanthus sonchifolius*)-derived fructooligosaccharides improves the immune parameters in the mouse. *Nutrition Research*, *32*(11), 884–892.

Donohoe, D. R., Collins, L. B., Wali, A., Bigler, R., Sun, W., & Bultman, S. J. (2012). The Warburg Effect Dictates the Mechanism of Butyrate Mediated Histone Acetylation and Cell Proliferation. *Molecular Cell*, *48*(4), 612–626.

Gallimore, A. M., & Godkin, A. (2013). Epithelial Barriers, Microbiota, and Colorectal Cancer. *The New England Journal of Medicine*, *368*(3), 282–284.

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). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews: Gastroenterology and Hepatology*, *14*(8), 491–502.

Gill, J. G., Piskounova, El., & Morrison, S. J. (2016). Cancer , Oxidative Stress , and Metastasis. *Cold Spring Harbor Symposia on Quantitative Biology*, *LXXXI*, 163–175.

Gonçalves, P., & Martel, F. (2016). Regulation of colonic epithelial butyrate transport: Focus on colorectal cancer. *Porto Biomedical Journal*, *1*(3), 83–91.

Grancieri, M., Costa, N. M. B., Vaz Tostes, M. das G., de Oliveira, D. S., Nunes, L. de C., Marcon, L. de N., Veridiano, T. A. & Viana, M. L. (2017). Yacon flour (*Smallanthus sonchifolius*) attenuates intestinal morbidity in rats with colon cancer. *Journal of Functional Foods*, *37*, 666–675.

Grivennikov, S. I., Wang, K., Mucida, D., Stewart, C. A., Schnabl, B., Jauch, D., Taniguchi, K., Yu, G., Osterreicher, C. H., Hung, K. E., Datz, C., Feng, Y., Fearon, E. R., Oukka, M., Tessarollo, L., Coppola, V., Yarovinsky, Cheroutre, H., Eckmann, L., Trinchieri, G., & Karin, M. (2012). Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature*, *491*(7423), 254–258.

Habib, N. C., Serra-Barcellona, C., Honoré, S. M., Genta, S. B., & Sánchez, S. S. (2015). Yacon roots (*Smallanthus sonchifolius*) improve oxidative stress in diabetic rats. *Pharmaceutical Biology*, *53*(8), 1183–1193.

Jimenez, M. E., Rossi, A., & Sammán, N. (2015). Health properties of oca (*Oxalis tuberosa*) and yacon (*Smallanthus sonchifolius*). *Food & Function*, *6*(10), 3266–3274.

Karnam, K. C., Ellutla, M., Bodduluru, L. N., Kasala, E. R., Uppulapu, S. K., Kalyankumarraju, M., & Lahkar, M. (2017). Preventive effect of berberine against DMBA-induced breast cancer in female Sprague Dawley rats. *Biomedicine and Pharmacotherapy*, *92*, 207–214.

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

Labianca, R., Beretta, G. D., Kildani, B., Milesi, L., Merlin, F., Mosconi, S., Pessi, M. A., Prochilo, T., Quadri. A., Gatta, G., Braud, F., & Wils, J. (2010). Colon cancer. *Critical Reviews in Oncology/Hematology*, *74*(2), 106–133.

Li, Q., Ding, C., Meng, T., Lu, W., Liu, W., Hao, H., & Cao, L. (2017). Butyrate suppresses motility of colorectal cancer cells via deactivating Akt / ERK signaling in histone deacetylase dependent manner. *Journal of Pharmacological Science*, *135*(4), 148–155. h

Lobo, A. R., Cocato, M. L., Borelli, P., Gaievski, E. H. S., Crisma, A. R., Nakajima, K., Nakano, E. Y., & Colli, C. (2011). Iron bioavailability from ferric pyrophosphate in rats fed with fructan-containing yacon (*Smallanthus sonchifolius*) flour. *Food*

Chemistry, *126*(3), 885–891.

López-Lázaro, M. (2018). Stem cell division theory of cancer. *Critical Reviews in Oncology / Hematology*, *123*, 95–113.

Macfarlane, G. T., & Macfarlane, S. (2011). Fermentation in the Human Large Intestine. *Journal of Clinical Gastroenterology*, *45*(December), S120–S127.

Mcloughlin, R. F., Berthon, B. S., Jensen, M. E., Baines, K. J., & Wood, L. G. (2017). Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *The American Journal Of Clinical Nutrition*, *106*(1), 930–945.

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

Ohigashi, S., Sudo, K., Kobayashi, D., Takahashi, O., Takahashi, T., Asahara, T., Nomoto, K., & Onodera, H. (2013). Changes of the Intestinal Microbiota, Short Chain Fatty Acids, and Fecal pH in Patients with Colorectal Cancer. *Digestive Diseases and Sciences*, *58*, 1717–1726.

Ojansivu, I., Ferreira, C. L., & Salminen, S. (2011). Yacon, a new source of prebiotic oligosaccharides with a history of safe use. *Trends in Food Science and Technology*, *22*(1), 40–46.

Pabst, O. (2012). New concepts in the generation and functions of IgA. *Nature Reviews Immunology*, *12*(12), 821–832.

Paredes, L. L. R., Smiderle, F. R., Santana-Filho, A. P., Kimura, A., Iacomini, M., & Sassaki, G. L. (2018). Yacon fructans (*Smallanthus sonchifolius*) extraction, characterization and activation of macrophages to phagocyte yeast cells. *International Journal of Biological Macromolecules*, *108*, 1074–1081.

Pereira, J. A. R., Teixeira, M. C., Saczk, A. A., Barcelos, M. de F. P., Oliveira, M. F. de, & Abreu, W. C. de. (2016). Total antioxidant activity of yacon tubers cultivated in Brazil. *Ciência E Agrotecnologia*, *40*(5), 596–605.

Peshev, D., & Van den Ende, W. (2014). Fructans: Prebiotics and immunomodulators. *Journal of Functional Foods*, *8*(1), 348–357.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. (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. *The Journal of Nutrition*, *123*(11), 1939–1951.

Reis, S. A., da Conceição, L. L., Siqueira, N. P., Rosa, D. D., da Silva, L. L., & Peluzio, M. do C. G. (2017). Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutrition Research*, *37*, 1–19.

Rodrigues, M. A. M., Silva, L. A. G., Salvadori, D. M. F., De Camargo, J. L. V., & Montenegro, M. R. (2002). Aberrant crypt foci and colon cancer: Comparison between a short- and medium-term bioassay for colon carcinogenesis using

dimethylhydrazine in Wistar rats. *Brazilian Journal of Medical and Biological Research*, *35*(3), 351–355.

Ruan, Z., Mi, S., Zhou, L., Zhou, Y., Li, J., Liu, W., Deng, Z., & Yin, Y. (2016). Chlorogenic acid enhances intestinal barrier by decreasing MLCK expression and promoting dynamic distribution of tight junction proteins in colitic rats. *Journal of Functional Foods*, *26*, 698–708.

Sina, C., Kemper, C., & Derer, S. (2018). The intestinal complement system in in fl ammatory bowel disease : Shaping intestinal barrier function. *Seminars in Immunology*, *37*, 66–73.

Song, P., Zhang, R., Wang, X., He, P., Tan, L., & Ma, X. (2011). Dietary grape-seed procyanidins decreased postweaning diarrhea by modulating intestinal permeability and suppressing oxidative stress in rats. *Journal of Agricultural and Food Chemistry*, *59*(11), 6227–6232.

Sousa, S., Pinto, J., Pereira, C., Xavier Malcata, F., Bertoldo Pacheco, M. T., Gomes, A. M., & Pintado, M. (2015). In vitro evaluation of yacon (*Smallanthus sonchifolius*) tuber flour prebiotic potential. *Food and Bioproducts Processing*, *95*, 96–105.

Stoidis, C. N., Misiakos, E. P., Patapis, P., Fotiadis, C. I., & Spyropoulos, B. G. (2011). Potential benefits of pro- and prebiotics on intestinal mucosal immunity and intestinal barrier in short bowel syndrome. *Nutrition Research Reviews*, *24*(1), 21–30.

Sun, J., & Kato, I. (2016). Gut microbiota, inflammation and colorectal cancer. *Genes and Diseases*, *3*(2), 130–143.

Teng, M. W. L., Darcy, P. K., & Smyth, M. J. (2011). Previews Stable IL-10 : A New Therapeutic that Promotes Tumor Immunity. *Cancer Cell*, *20*(6), 691–693.

Vaz-Tostes, M. das G., Viana, M. L., Grancieri, M., Luz, T. C. dos S., Paula, H. de, Pedrosa, R. G., & Costa, N. M. B. (2014). Yacon effects in immune response and nutritional status of iron and zinc in preschool children. *Nutrition*, *30*(6), 666–672.

Wang, D., Sun, F., Lu, C., Chen, P., Wang, Z., Qiu, Y., & Pimentel-Nunes, P. (2014). Role of colonic microbiota in colorectal carcinogenesis: A systematic review. *Cell Host and Microbe*, *15*(3), 659–671.

Waters, J. P., Pober, J. S., & Bradley, J. R. (2013). Tumour necrosis factor and cancer. *Journal of Pathology*, *230*(3), 241–248.

WHO. (2018). 10 Facts About Cancer. Retrieved from http://www.who.int/features/factfiles/cancer/en/index.html

Witten, J., Samad, T., & Ribbeck, K. (2018). Selective permeability of mucus barriers. *Current Opinion in Biotechnology*, *52*, 124–133.

5. CONCLUSÃO

O presente estudo verificou o potencial prebiótico da farinha de yacon, como fonte de frutooligossacarídeos, em promover efeitos benéficos na saúde intestinal de animais induzidos ao câncer de cólon.

Observou-se que o câncer de cólon apresenta características inflamatórias que condicionam o organismo a alterações metabólicas, como aumento da citocina inflamatória TNF-α e dos ácidos graxos de cadeia curta e redução da capacidade antioxidante total. Os resultados do estudo sugerem que o consumo preventivo da farinha de yacon, contendo 5 % FOS, foi eficaz em reduzir o pH intraluminal e a razão lactulose/manitol. No entanto, não foi capaz de melhorar os parâmetros inflamatórios dos animais induzidos ao câncer. Ainda, verifica-se efeito prebiótico da farinha yacon em melhorar a imunidade de mucosa com aumento de sIgA nos animais saudáveis.

Nesse sentido, a farinha de yacon mostra potencial benéfico nesta área. Assim, estudos com distintas doses de FOS e tempos de intervenção são necessários para aprofundar as hipóteses estabelecidas. Ainda, considerando o vasto número de interações entre dieta, microbiota, resposta metabólica, sistema imune e barreira intestinal, estudos mais detalhados a nível metabólico são necessários a fim de se estabelecer mecanismos de ação dos prebióticos na carcinogênese de cólon. Por fim, sugere-se, pesquisas realizadas com humanos, que especificamente explore as alterações na composição da microbiota intestinal após a ingestão de prebióticos.

APÊNDICE

APÊNDICE 1. Metodologia detalhada

O estudo foi desenvolvido nos Laboratórios de Nutrição Experimental e Fisiologia Humana; Central Analítica; Operações Unitárias e Química dos Alimentos da Universidade Federal do Espírito Santo, *campus* Sul Capixaba.

A1.1 Ensaio biológico e delineamento experimental

Foram utilizados 44 ratos machos *Wistar* adultos, com aproximadamente 4 semanas (185,16g±19,99g), provenientes do Biotério Central da UFES.

Os animais foram mantidos em gaiolas individuais, com controle da iluminação (fotoperíodo de 12 horas claro e 12 horas escuro) e temperatura ambiente de 22 ± 2 ^{0}C e água *ad libitum*. A manutenção, assim como o uso dos animais no experimento, foi conduzida respeitando-se as normas estabelecidas pelo Colégio Brasileiro de Experimentação Animal (2006). O projeto foi aprovado pelo Comitê de Ética Animal (CEUA), protocolo n^{0} 17/2016 (Anexo 1).

Os animais foram divididos em quatro grupos:

I. Grupo S: sem indução de câncer de cólon e sem farinha de yacon; n=10

II. Grupo C: com indução de câncer de cólon e sem farinha de yacon; n=12

III. Grupo Y: sem indução de câncer de cólon e com farinha de yacon; n=10

IV.Grupo CY: com indução de câncer de cólon e com farinha de yacon; n=12.

Durante as 16 semanas do experimento, os animais dos grupos S e C receberam a dieta AIN-93 M e os animais dos grupos Y e CY receberam a dieta AIN-93 M adicionada com farinha de yacon em quantidade suficiente para fornecer 5% de FOS (CAMPOS et al., 2012).

O câncer de cólon foi induzido nos grupos C e CY entre a 4^{a} e 8^{a} semana, por meio de uma dose semanal de 25 mg/kg de peso corporal da droga 1,2-dimetilhidrazina (DMH-Sigma®). Nas 8 semanas subsequentes compreendendo o período para o desenvolvimento do CRC.

A DMH foi fornecida por injeção intraperitoneal preparada em capela de exaustão de gases imediatamente antes do uso, dissolvida em NaCl 0,9%, contendo

15% de EDTA como veículo e o pH ajustado para 6,5, na proporção de 1:1 (DMH:salina), conforme método utilizado por Rodrigues et al., (2002).

Na última semana, os animais foram mantidos em gaiolas metabólicas durante 24 horas, na qual foi coletada a urina de todo esse período para análise da permeabilidade intestinal (SONG et al., 2011).

Ao final do experimento de experimento (16 ª semana) os animais foram anestesiados pela administração intraperitoneal de 0,2 mL/100 g peso corporal de solução anestésica contendo 37,5% cetamina, 25% xilazina e 37,5% solução salina. Foi realizada a abertura da cavidade torácica e o sangue foi coletado por punção cardíaca em tubos heparinizados. Após, foram centrifugados (3000 *g*, 10 minutos, 4 ^{0}C) para obtenção do plasma e armazenados a -80 ºC até o momento das análises das citocinas pró e anti-inflamatórias e capacidade antioxidante.

O intestino grosso dos animais foi coletado no dia da eutanásia e o conteúdo luminal do ceco foi retirado para posterior análise de pH intraluminal, ácidos graxos de cadeia curta e imunoglobulina A, conforme metodologias descritas posteriormente.

A1.2 Elaboração da farinha de yacon

As raízes de yacon foram adquiridas diretamente de um produtor da cidade de Santa Maria de Jetibá – ES. Para a elaboração da farinha, as raízes tuberosas foram pré-higienizadas em água corrente e, logo após, sanitizadas em solução de hipoclorito de sódio a 200 ppm por 15 minutos. Depois, descascadas com auxílio de um descascador doméstico e fatiadas manualmente por meio da utilização de facas de aço inox em forma de laminas. Após esse procedimento, foram imersas em solução contendo 1% de ácido cítrico por 20 segundos, sendo drenadas e secas com auxílio de secadora de bandejas com circulação de ar (Polidryer,® Brasil), a 60 °C por 24 horas. Após, foram trituradas em liquidificador doméstico para obtenção da farinha. Ao final do processo, a farinha foi armazenada em embalagens laminadas de 2 a 5 kg e vedados à temperatura de 4 °C.

A análise da composição de frutooligossacarídeos da farinha foi realizada no CERAT – Centro de Raízes e Amidos Tropicais, Botucatu-SP, pelo método de Cromatografia Líquida de Alta Eficiência (HPLC). As análises de carboidratos, proteínas, lipídios, fibras e cinzas da farinha de yacon foram realizadas de acordo

com método *Official Methods of Analysis* – (AOAC INTERNATIONAL, 2012). Toda farinha utilizada no preparo das dietas pertencia ao mesmo lote de raízes da yacon.

A1.3 Dietas experimentais

As dietas experimentais foram elaboradas manualmente e os ingredientes foram adicionados em quantidades determinadas, seguindo as recomendações do *American Institute of Nutrition for Rodents* (REEVES; NIELSEN; FAHEY, 1993), de acordo com o protocolo AIN-93 M, para manutenção de animais adultos.

Os grupos S e C receberam a dieta AIN-93 M. A dieta dos grupos Y e CY foi suplementada com farinha de yacon (FY) em quantidades suficientes para fornecer 5 % de FOS. Visto que a farinha de yacon apresentava 28,95 % FOS, adicionou-se, aproximadamente, 17,27 g de FY/100g de dieta. O conteúdo de caseína, sacarose, amido e fibra da dieta dos grupos experimentais foi ajustado, a fim de que as dietas de todos os grupos tivessem as mesmas concentrações de energia, carboidratos, lipídeos, proteínas e fibras.

A1.4 Consumo alimentar e alteração ponderal

O consumo alimentar foi avaliado semanalmente por meio da diferença entre peso da ração ofertada e a sobra. O peso da ração foi aferido em balança semi-analítica (Radwag®). O peso dos animais foi aferido semanalmente, sempre no mesmo horário, utilizando balança semi-analítica (Radwag®).

O Coeficiente de Eficiência Alimentar (CEA) foi determinado por meio do ganho de peso do animal (g), pela diferença entre o peso inicial do final, dividido pelo consumo de dieta em cada grupo experimental.

A1.5 Dosagem das citocinas

Para dosagem das citocinas (TNF-α, IL-12 e IL-10), as amostras de sangue coletadas por punção cardíaca foram centrifugadas durante 10 min a 3000 *g* a 4 ºC e o sobrenadante congelado a -80 ºC até o uso.

A dosagem de citocinas (IL-10 e IL-12) foi realizada por kit comercial específico Milliplex® Map, com a plataforma de tecnologia Luminex® citocinas

Multiple Analyte Profiling. A leitura das amostras foi efetuada em leitor Luminex 200. Os resultados foram expressos em pg/mL.

A dosagem de TNF- α foi realizada pelo kit de Sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A leitura das amostras foi efetuada em leitor multiplaca Sat Fax (Awareness Technology®). Os resultados foram expressos em pg/mL.

A1.6 Capacidade antioxidante total do plasma

O plasma obtido pela centrifugação do sangue (3000 *g*, 10 minutos, 4 ^{0}C) foi congelado a -80 ^{0}C. A análise da capacidade antioxidante total do plasma foi realizada por meio de ensaio colorimétrico com o kit comercial específico “Total Antioxidant Capacity Assay Kit”, marca Elabscience®. A leitura das amostras foi efetuada em leitor de microplaca Thermo Scientific®, modelo Multiskan GO, em comprimento de onda de 520 nm. Os resultados foram expressos como unidade de capacidade antioxidante total (U)/ mL.

A1.7 Dosagem da imunoglobulina secretória A

Para análise da sIgA, foi coletado o ceco dos animais pelo corte nas junções gastroduodenal e ileocecal. O conteúdo do ceco foi retirado e congelado a -80 ^{0}C.
No momento da análise, o conteúdo do ceco foi diluído com solução tampão fosfato (PBS) pH 7,2, na proporção de 100 mg do conteúdo: 1 mL de PBS e então, centrifugadas (3000 *g*, 10 min, 20 ^{0}C). Em seguida, o sobrenadante foi coletado e utilizado para a dosagem da imunoglobulina secretória A, pelo método de ELISA, utilizando o kit comercial específico Cloud-Clone®, seguindo as recomendações do fabricante. A leitura das amostras foi efetuada em leitor de microplaca Thermo Scientific®, modelo Multiskan GO, em comprimento de onda de 420 nm. Os resultados foram expressos como ng/mL.

A1.8 Análise do pH Intraluminal do cólon

Foi realizada a remoção do intestino grosso dos animais para a remoção do conteúdo luminal do ceco, que foi pesado e diluído na proporção 1:10 em solução

salina (0,9%) e homogeneizado em vórtex. Em seguida, realizou-se a leitura do pH por meio de pHmetro de bancada (Kasvi®).

A1.9 Determinação dos ácidos graxos de cadeia curta (AGCC)

Foram avaliados os AGCC (acetato, propionato e butirato) presentes no conteúdo colônico dos animais. Para tal, 100 mg do conteúdo luminal foram diluídos em 2 mL de ácido perclórico 0,1 mM com 3% de fenol, agitados em vórtex por 5 minutos, centrifugados (9000 *g,* 10 minutos, 20 ^{0}C) e filtrados em filtro de membrana 0,45 µm, conforme descrito por Kotani et al., 2009, com modificações. Imediatamente após a extração foi realizada a análise por Cromatografia Líquida de Alta Eficiência (HPLC).

O sistema de cromatografia consistiu em desgaseificador (Modelo DGU-14A), bomba (Modelo LC-20AT), auto injetor automático (modelo SIL-20A), forno de coluna (Modelo CTO-10AS) e detector UV-Visível (modelo SPD-20AV). A coluna analítica utilizada foi a Aminex HPX-87H (300 cm x 8,7 mm) da marca BIO-RAD (Califórnia, EUA). As análises por HPLC foram realizadas a 55 °C, numa pressão de 120 kgf, sob condições isocráticas. A fase móvel empregada foi água em 0,005 mol/L de ácido sulfúrico; fluxo da fase móvel: 1,0 mL/min; com volume de injeção da amostra de 20 µL. O comprimento de onda de detecção foi 210 nm (SÁ et al., 2011).

Para a quantificação dos ácidos analisados, foram elaboradas curvas de calibração com concentrações crescentes de ácido acético (0,00156 a 0,1 Mol/L), butírico (0,00034 a 0,025 Mol/L), e propiônico (0,00156 a 0,1 Mol/L) (Sigma-Aldrich®, São Paulo), com a finalidade de se compor a equação da reta dos compostos analisados (y=ax + b).

Os ácidos foram analisados em HPLC e suas áreas sob a curva indicavam os valores de y em Mol/L e, uma vez aplicada a equação da reta, foi determinado os valores de x. Esse valor foi transformado e expresso em mg/g amostra.

A1.10 Determinação da permeabilidade intestinal

A determinação da permeabilidade intestinal foi realizada na última semana do experimento. Para a análise, todos os animais foram mantidos em jejum de 12 horas, sendo posteriormente administrado 2 mL de solução contendo 200 mg de

lactulose e 100 mg de manitol por gavagem. Após a administração, os animais foram alocados em gaiolas metabólicas e permaneceram em jejum por 5 horas e depois, retornaram a dieta e água *ad libitum.* Foi realizada a coleta de urina de 24 horas, com auxílio de filtro, funil e béquer, sendo o volume medido, registrado e armazenado em um frasco a -80 ºC até a análise laboratorial (SONG et al., 2011).

Para determinação das concentrações de lactulose e manitol, a urina coletada foi filtrada em filtros Millipore de 0,45 milímetros (São Paulo, Brasil) e cerca de 1,5 mL colocado em vials para Cromatografia Líquida de Alta Eficiência (HPLC). Para análise, foi utilizado o sistema de HPLC, da marca Shimadzu (Kyoto, Japão). O sistema de cromatografia consistiu em desgaseificador (Modelo DGU-20A), bomba (Modelo LC-20AT), injetor automático (modelo SIL-20A), forno de coluna (Modelo CTO-10AS) e detector de índice de refração (modelo RID-10A). A coluna analítica utilizada foi a Aminex HPX-87H (300 cm x 8,7 mm) da marca BIO-RAD (Califórnia, EUA). As análises por CLAE foram realizadas a 55 °C, numa pressão de 120 kgf, sob condições isocráticas. A fase móvel empregada foi água em 0,005 mol/L de ácido sulfúrico, fluxo da fase móvel: 0,6 mL/min com volume de injeção da amostra de 20 µL (SÁ et al., 2011).

Para a padronização do teste e o adequado conhecimento dos valores medidos na unidade g/L, concentrações crescentes de manitol (Sigma®) (0,01 a 0,5 mol/L) e de lactulose (Sigma®) (0,003 a 0,125 mol/L) foram avaliadas separadamente, com a finalidade de se compor a equação da reta dos compostos analisados (y=ax + b).

A urina foi analisada em HPLC e suas áreas sob a curva indicavam os valores de y em Mol/L e, uma vez aplicada a equação da reta, foi determinado os valores de x. Esse valor foi transformado para g/L para o cálculo das taxas de excreção da lactulose e do manitol pela equação:

[(x*volume de urina excretada em 12 h) / 0,1 g manitol ingerido]*100

[(x*volume de urina excretada em 12 h)/ 0,2g lactulose ingerida]*100

Para a relação entre os mesmos, dividiu-se a taxa de excreção da lactulose pela taxa de excreção do manitol.

A1.11 Análises estatísticas

As amostras foram testadas pelo teste de normalidade Kolmogorov-Smirnov. As amostras que não apresentarem distribuição normal foram transformadas (utilizando a função log). Os grupos foram testados para os efeitos da FY e do CC, e, ou as suas interações utilizando-se “Two-way” ANOVA (análise de variância) seguida por “post hoc” de Newman-Keuls (p<0,05). Os dados foram expressos como média ± desvio padrão (DP), sendo considerado significativo o valor de p<0,05. As análises estatísticas foram realizadas no programa estatístico GraphPad Prism®, versão 7 (GraphPad Software Inc., San Digo, CA, EUA).

Referências bibliográficas

AOAC INTERNATIONAL. **Official Methods of Analysis**. 19. ed. United States: AOAC International, 2012.

CAMPOS, D.; BETALLELUZ-PALLARDEL, I.; CHIRINOS, R.; AGUILAR-GALVEZ, AN.; NORATTO, G.; PEDRESCHI, R. Prebiotic effects of yacon (*Smallanthus sonchifolius* Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity. **Food Chemistry**, v. 135, n. 3, p. 1592–1599, 2012.

KOTANI, A.; MIYAGUCHI, Y., KOHAMA, M.; OHTSUKA, T.; SHIRATORI, T.; KUSU, F. Determination of short-chain fatty acids in rat and human feces by high-performance liquid chromatography with electrochemical detection. **Analytical Scienes**, v. 25, n. 8, p. 1007–1011, 2009.

REEVES, P. G.; NIELSEN, F. H.; FAHEY, G. C. 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. **The Journal of Nutrition**, v. 123, n. 11, p. 1939–1951, 1993.

RODRIGUES, M. A. M.; SILVA, L. A. G.; SALVADORI, D. M. F.; DE CAMARDO, J. L. V.; MONTENEGRO, M. R. Aberrant crypt foci and colon cancer: Comparison between a short- and medium-term bioassay for colon carcinogenesis using dimethylhydrazine in Wistar rats. **Brazilian Journal of Medical and Biological Research**, v. 35, n. 3, p. 351–355, 2002.

SÁ, L. R. V.; DE OLIVEIRA, M. A. L.; CAMMAROTA, M. C.; MATOS, A.; FERREIRA-LEITÃO, V. S. Simultaneous analysis of carbohydrates and volatile fatty acids by HPLC for monitoring fermentative biohydrogen production. **International Journal of Hydrogen Energy**, v. 36, n. 23, p. 15177–15186, 2011.

SONG, P.; ZHANG, R.; WANG, X.; HE, P.; TAN, L.; MA, X. Dietary grape-seed procyanidins decreased postweaning diarrhea by modulating intestinal permeability and suppressing oxidative stress in rats. **Journal of Agricultural and Food Chemistry**, v. 59, n. 11, p. 6227–6232, 2011.

ANEXO

ANEXO 1 - Certificado: Comissão de Ética no Uso de Animais – CEUA

UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS DA SAÚDE
COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

CERTIFICADO

Certificamos que o Projeto intitulado"Efeitos do Consumo Preventivo com frutooligossacarídeos derivados do Yacon na Barreira Intestinal, resposta inflamatória e estresse oxidativo em modelo animal de câncer de cólon**",Protocolo nº.17/2016, sob a responsabilidade de Mirele Lomar Viana que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata(exceto o homem), para fins de pesquisa científica(ou ensino)encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, do Decreto nº6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal(CONCEA),e foi aprovado "ad referendum" pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS(CEUA) DO(A) Centro de Ciências da Saúde-Maruípe-Vitória-ES em 05-08-2016.**

Vigência do Projeto	Início: Agosto/2016 Término:Agosto/2018
Espécie/Linhagem	Ratos Wistar
Nº de Animais	Experimento Piloto:0 Protocolo Experimental:40 Total:40
Peso/Idade	Peso: entre 300-450g Idade:Adulto
Sexo	Macho
Origem	Mamíferos

Vitória (ES), 05 de agosto de 2016.

Prof. Roger Lyrio dos Santos
Presidente da Comissão de Ética no Uso de Animais
CEUA/CCS/UFES