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DEPARTAMENTO DE CIÊNCIA DE ALIMENTOS

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“AVALIAÇÃO DOS EFEITOS DO CONSUMO REGULAR DE YACON
(*Smallanthus sonchifolius*) SOBRE O SISTEMA IMUNE MURINO”

Tese de doutorado apresentada à Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas para obtenção do título de Doutor em Ciências de Alimentos.

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Este exemplar corresponde à versão final da tese defendida por Grethel Teresa Choque Delgado, aprovada pela comissão julgadora em 16/03/2012 e orientada pela Profa. Dra. Glaucia Maria Pastore.

Glaucia Maria Pastore

Campinas

2012

**FICHA CATALOGRÁFICA ELABORADA POR LUCIANA P. MILLA – CRB8/8129-
BIBLIOTECA DA FACULDADE DE ENGENHARIA DE ALIMENTOS - UNICAMP**

Choque Delgado, Grethel Teresa,
C456a Avaliação dos efeitos do consumo regular de
yacon (*Smallanthus sonchifolius*) sobre o sistema
imune murino / Grethel Teresa Choque Delgado.--
Campinas, SP:[s.n.], 2012.

Orientador: Glaucia Maria Pastore
Tese (doutorado) - Universidade Estadual de
Campinas. Faculdade de Engenharia de Alimentos

1. Prebióticos. 2. Fructooligosacarídeos. 3.
Yacon. 4. Sistema imune. 5. Camundongo. I. Pastore,
Glaucia Maria. II. Universidade Estadual de Campinas.
Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Titulo em inglês: The effects of regular intake of yacon root (*Smallanthus sonchifolius*) on the murine immune system.

Palavras-chave em inglês (Keywords):

Prebiotics

Fructooligosaccharides

Yacon root

Immune system

Mice.

Área de concentração: Ciência de Alimentos

Titulação: Doutor em Ciência de Alimentos

Banca examinadora:

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Data de defesa: 16/03/2012

Programa de Pós Graduação: Ciência de Alimentos.

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AGRADECIMENTOS

Primeiramente agradeço a Deus, meu Pai, pelo seu amor e correção. Pela oportunidade de fazer este doutorado e por ter cuidado de mim durante esses anos.

Eterna gratidão ao meu pai Julio, que do céu continua a me abençoar e inspirar para enfrentar os desafios da vida.

À minha mãe, Glenda, pelo seu amor e pelas suas orações, as quais me fortaleceram e animaram durante a minha passagem pelo Brasil.

Aos meus irmãos Gleny e Cesar pelo apoio e motivação. Muito agradecida pelas suas palavras de conforto e de ânimo em todos os momentos desta corrida.

Aos meus tios e primos pelo seu carinho que mesmo de longe me incentivaram a continuar lutando pelos meus objetivos.

À minha orientadora Professora Glaucia M. Pastore por acreditar em mim e ter confiado em minha capacidade para desenvolver esta pesquisa.

À professora Wirla M.S.C. Tamashiro por abrir as portas, acolher-me e instruir-me nas pesquisas. Muito agradecida pela paciência nas horas de discussão dos resultados e correção dos manuscritos. A minha imensa gratidão pelo carinho e consideração para comigo.

Ao professor Mário Maróstica, pela ajuda nas horas escuras por ter me dado a luz para continuar o caminho.

Aos membros da banca examinadora, pelas correções e as oportunas sugestões que contribuíram para o aprimoramento desta tese.

À Dirce pela enorme ajuda na parte experimental da pesquisa, muito agradecida pela sua amizade e carinho. A você, minha grande admiração pela sua dedicação e responsabilidade em seu trabalho.

Ao meu querido amigo, Rodolfo, muito agradecida pelo apoio nos experimentos e por ter me ensinado as metodologias e a parte estatística, além da paciência na elaboração dos manuscritos.

Ao pessoal do IB do Departamento de Microbiologia e Imunologia: José Raimundo, Marcos Meneguetti e Luis Antonio Peroni.

Aos amigos do laboratório de Bioaromas: Dora e Nadir, muito agradecida pela amizade.

Ao pessoal do Departamento de Alimentos e Nutrição: Zueli, Susana Corrêa, Nice, Paula Poeta e Gláucia Carielo.

Ao pessoal do Laboratório de frutas e hortaliças do DTA: Ana, Bernadete e Adalton.

Às amigas e amigos: Mery Rendón, Cecilia Castro, Raquel Maciel, Fabiola Aliaga, Elizama Aguiar, Gustavo Obando, Salatir Rodrigues, Fábio Barros, Juliano Bicas, Caroline Bittencourt, Roberto Marcilio, Fabiana Rodrigues, Kazue Matsumoto, Selma Lopes, Igor Bresolin, Fernando Tello, Renan Chisté, Polyana Gurak, etc. Muito agradecida àqueles que estiveram ao meu lado durante a execução deste trabalho e minha gratidão por todos os momentos de alegria que passamos juntos.

Ao meu amigo Oscar Díaz pela amizade, pelo seu carinho e compreensão nas horas difíceis, pela escuta e pelos ânimos recebidos.

À minha amiga Lucia Márquez Cayro pelo incentivo e apoio, pelos conselhos, conversas e risadas, pelas palavras animadoras nos momentos mais complicados.

Aos meus irmãos da comunidade 9 da paróquia Nova Jerusalém em Jundiá, pela acolhida e pelo carinho para comigo. Agradeço por terem sido a minha família durante esses anos. Deus os abençoe e retorne em acréscimo.

À UNICAMP por todos os bens e serviços brindados, em especial ao Restaurante Universitário e ao CECOM.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de doutorado para o desenvolvimento desta pesquisa.

Aos funcionários da secretaria de Pós-graduação da Faculdade de Engenharia de Alimentos da UNICAMP, Cosme Perota e Marcos Sampaio, pela eficiência e empenho em seus serviços.

À Corn products Brasil, pela doação de frutooligossacarídeos (Nutraflora) para o desenvolvimento da pesquisa.

Ao Laboratório de Nutrição e Metabolismo e ao Laboratório de Ensaio Biológicos da FEA, pelo apoio das análises de composição centesimal e doação de materiais para a elaboração das dietas.

Ao Laboratório de Inflamação e Imunologia celular pela assistência nas análises de perfil imunológica.

E a todos que de alguma forma contribuíram para o desenvolvimento deste trabalho, minha imensa gratidão.

"Tudo posso naquele que me fortalece!" (Fl 4,13).

"Quanto mais amamos, mais parecidos com Deus nos tornamos!" V. Gozzi (I João 4,7-8).

"Um pouco de ciência nos afasta de Deus. Muito, nos aproxima" Louis Pasteur.

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LISTA DE ABREVIATURAS E SIGLAS

AOM	Azoxymethane
APCs	Antigen-Presenting Cells
BCG	Bacillus Calmette-Guérin
CEMIB	Centro multidisciplinar para investigação biológica na área de ciência em animais de laboratório
CEUA	Comissão de ética no uso de animais
CFU	Colony-forming units
ConA	Concanavalin A
CTLs	Cytotoxic lymphocytes
DCA	Deoxycholic acid
DNFB	Dinitrofluorobenzene
DSS	Dextran Sodium Sulfate
FAE	Follicle-associated epithelium
FOS	Fructooligosaccharides
GALT	Gut-associated lymphoid tissue
GF2	Kestose
GF3	Nystose
GF4	Fructofuranosyl nystose
GOS	Galactooligosaccharides
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HRPO	Horseradish peroxidase
IBDQ	Inflammatory Bowel Disease Questionnaire
IEL	Intraepithelial lymphocytes
IFN- γ	Interferon- γ
IgA	Imunoglobulina A
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IL-1 β	Interleucina 1 β
IL-4	Interleucina 4

IL-10	Interleucina 10
IL-17	Interleucina 17
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
NDOs	Non-digestible oligosaccharides
NDV	Newcastle Disease Virus
MICs	Minimal inhibitory concentrations
MOS	Mannanoligosaccharides
MRSA	Methicillin-resistant Staphylococcus aureus
PAMPs	Pathogen-Associated Molecular Patterns
PBS	Phosphate buffered saline
PP	Peyer's patches
SCFAs	Short-chain fatty acids
SPF	Specific pathogen free
STZ	Streptozotocin
SYN	Synbiotic
TNF- α	Tumor necrosis factor- α
UC	Ulcerative colitis
XOS	Xylooligosaccharides

RESUMO

O yacon (*Smallanthus sonchifolius*), raiz andina, tem se destacado pelo seu potencial prebiótico devido aos elevados teores de frutooligosacarídeos (FOS) e inulina em sua composição. O objetivo deste trabalho foi verificar se a alimentação com yacon alteraria o sistema imune de camundongas BALB/c e se sua ingestão regular seria capaz de modular a resposta intestinal induzida pela instilação retal de ácido trinitrobenzeno sulfônico (TNBS). Para avaliar os efeitos do consumo regular de yacon sobre o sistema imune murino, camundongas BALB/c com oito semanas de idade foram divididas em quatro grupos experimentais alimentados por trinta dias com dieta: controle AIN-93M; 5% de FOS comercial; 3% e 5% FOS de yacon. O peso corporal e consumo da ração foram acompanhados semanalmente. Os níveis de anticorpos IgA, IgM, IgG séricos e IgA fecal, a produção de óxido nítrico (NO) por macrófagos, a frequência de linfócitos T e B sanguíneos e a proliferação de células esplênicas *in vitro* foram avaliados. Para avaliar o efeito do consumo regular de yacon sobre a colite experimental, metade dos animais que ingeriram as dietas teste foi instilada por via retal durante sete dias consecutivos com solução contendo TNBS, enquanto que o restante recebeu veículo (etanol). Foram analisados o peso corpóreo, histopatologia dos intestinos, bem como a proliferação de células esplênicas e dosagem de citocinas. Os animais alimentados com yacon apresentaram respostas semelhantes aos camundongos que ingeriram as demais dietas em relação ao ganho de peso, consumo de ração, níveis de anticorpos séricos, frequência de linfócitos T e B sanguíneos e produção de NO por macrófagos. Entretanto, observou-se um aumento significativo tanto na secreção de IgA fecal quanto na proliferação de células esplênicas de animais alimentados com yacon. Os camundongos alimentados com yacon

sofreram menor perda de peso após instilação com TNBS do que os animais alimentados com AIN93M e FOS. Os animais que consumiram yacon apresentaram intestinos íntegros e funcionais, oposto ao observado nos animais dos demais grupos. Não foram encontradas diferenças significativas na proliferação de células esplênicas e produção de citocinas entre os diferentes grupos, salvo a maior produção de IL-17 e menor secreção de IL-10 nas culturas de células de animais alimentados com FOS. Nossos dados indicam que o consumo regular de yacon não acarreta efeitos adversos à fisiologia e sistema imune murino, levando à melhora no quadro de colite induzida por instilação com TNBS.

Palavras-chave: Prebióticos, Frutooligossacarídeos (FOS), Yacon, TNBS-colite, Sistema imune.

ABSTRACT

Yacon (*Smallanthus sonchifolius*), Andean root, has been recognized for its prebiotic potential due to its high levels of fructooligosaccharides (FOS) and inulin. The objective of this study was to verify if the regular consumption of yacon alters the immune system of BALB/c mice and if it may modulate intestinal inflammation induced by the rectal instillation of trinitrobenzene sulfonic acid (TNBS). To evaluate the effects of regular yacon consumption on the murine immune system, eight-week-old BALB/c mice were divided into four experimental groups fed for thirty days with diet: control AIN-93M, 5% commercial FOS, or 3% or 5% yacon FOS. Body weight and food consumption were monitored weekly. The serum levels of IgA, IgM and IgG and fecal IgA, the nitric oxide (NO) production by macrophages, the frequency of T and B lymphocytes in the blood and the proliferation of spleen cells (*in vitro*) were evaluated. To evaluate the effects of regular yacon consumption on experimental colitis, half of the animals was instilled rectally with a solution containing TNBS for seven consecutive days while the rest received vehicle (ethanol). We analyzed the body weight, histopathology of the intestines, spleen cell proliferation and cytokine production. The animals fed yacon showed responses similar to mice that consumed the other diets in relation to weight gain, food intake, serum antibody levels, frequencies of T and B lymphocytes in the blood and NO production by macrophages. There was a significant increase in both fecal IgA secretion and the proliferation of spleen cells from animals fed yacon. The mice fed yacon suffered less TNBS-induced weight loss than animals fed the FOS and AIN93M diets. The animals that consumed yacon also showed intact and functional intestines, as opposed to the other groups of animals. There were no significant differences in spleen cell proliferation or

cytokine production among groups, except for the increased production of IL-17 and lower IL-10 secretion in the cultures of cells from animals fed FOS. Our data indicate that the regular consumption of yacon does not cause adverse effects on the physiology or the murine immune system and that yacon consumption ameliorates colitis induced by TNBS instillation.

Keywords: Prebiotics, Fructooligosaccharides (FOS), Yacon root, TNBS-colitis, Immune system.

INTRODUÇÃO GERAL

A preocupação com uma alimentação mais adequada e a procura pelo bem-estar tem direcionado as pesquisas para a busca de alimentos que, além de fornecer nutrientes, possam trazer benefícios à saúde humana e à qualidade de vida. A alimentação é um fator importante no desenvolvimento e prevenção de diversas doenças. O aumento na incidência de doenças crônicas e degenerativas é mais um estímulo à investigação por alimentos com potencial terapêutico e preventivo. Nesse sentido, os alimentos funcionais oferecem uma grande oportunidade de se obter uma alimentação saudável (ORGANIZACIÓN PANAMERICANA DE LA SALUD (OPS), 2007; WATZL et al., 2005).

Os alimentos funcionais são aqueles que, além das funções nutricionais básicas, provocam efeitos benéficos, tanto metabólicos e/ou fisiológicos, à saúde (ANVISA, 2005). Entre os alimentos funcionais se incluem aqueles com propriedades prebióticas. Para um alimento ser considerado prebiótico, ele deve se ajustar aos seguintes critérios: 1) resistência à acidez gástrica; 2) ser fermentado pela microbiota intestinal; 3) estimular seletivamente o crescimento e/ou a atividade de bactérias intestinais associadas com saúde e bem-estar (GIBSON et al., 2004).

Os oligossacarídeos não digeríveis (NDOs, do inglês *non-digestible oligosaccharides*), constituem um grupo de prebióticos encontrados em plantas e vegetais como compostos de reserva energética. O consumo de prebióticos provoca a modulação de funções fisiológicas-chaves como a absorção de cálcio, o metabolismo de lipídios, assim como a modificação da microbiota intestinal para um padrão mais adequado (ROBERFROID, 2005).

O crescimento de bactérias benéficas de tipo *Bifidobacterium* e *Lactobacillus* decorrente do consumo de prebióticos (efeito bifidogênico) inibe o desenvolvimento de espécies bacterianas tanto putrefativas quanto patogênicas na região intestinal (POOL-ZOBEL et al., 2002). Os metabólitos de probióticos e a produção de ácidos graxos de cadeia curta por bifidobactérias estimulam a absorção de cálcio, o metabolismo de lipídios e a modulação das respostas imunes associadas à mucosa intestinal (ROBERFROID, 2005).

O Yacon (*Smallanthus sonchifolius*), raiz andina, é uma fonte potencial de prebióticos de tipo FOS e inulina (GRAEFE et al., 2004). Alguns estudos têm avaliado os efeitos benéficos do consumo de yacon como fonte de FOS em humanos e animais de experimentação. Recentemente, foi demonstrado que ratos diabéticos alimentados com farinha de yacon apresentaram redução significativa nos níveis séricos de colesterol LDL, contrastando com os animais alimentados com ração comum. Os resultados obtidos denotam o potencial hipolipidêmico do consumo de yacon em situações de alteração metabólica, como no diabetes (HABIB et al., 2011). Genta e colaboradores (2009) também demonstraram que o consumo de xarope de yacon por mulheres em fase pré-menopausa reduziu o índice de massa corporal e os níveis de LDL comparados com as pacientes que ingeriram placebo. Por conta de seus atributos prebióticos, o consumo de yacon pode ser capaz de modular as respostas imunes inflamatórias, especialmente as que ocorrem em nível intestinal.

A doença intestinal inflamatória, mais conhecida por sua sigla de origem inglesa IBD (*Inflammatory bowel disease*), é uma condição inflamatória observada em humanos geneticamente predispostos. Embora a causa não seja totalmente esclarecida, a IBD está associada às alterações na função da barreira intestinal, que incluem a redução da expressão

de proteínas de junção e aumento de apoptose das células epiteliais intestinais. Tais alterações são causadas por antígenos do lúmen derivados de bactérias não patogênicas que entram em contato com células do sistema imunológico da camada mucosa subjacente (HELLER et al., 2005; SCHULZKE et al., 2006).

A IBD pode ser considerada uma doença auto-imune cuja manifestação é vista como uma resposta imune anormal da mucosa aos antígenos da microbiota que são normalmente tolerados, dada sua proximidade e persistência (STROBER et al., 2002). Em uma situação de normalidade, as células T são ativadas e expandidas de modo polarizado (Th1, Th2, Th17) para destruir e proteger contra a invasão de patógenos entéricos no intestino. Contudo, quando este processo não é devidamente regulado, as células efectoras entéricas podem promover uma inflamação intestinal crônica (KOBOZIEV et al., 2010). Nesse sentido, a origem dos sintomas da colite resulta em uma resposta imune desequilibrada, com produção exacerbada de citocinas inflamatórias (BOUMA, STROBER, 2003).

A Doença de Crohn e a Colite Ulcerativa são as duas principais formas clinicamente definidas de IBD. Elas apresentam condições inflamatórias progressivas ou crônicas remitentes que afetam o trato gastrointestinal e a mucosa do cólon e estão associadas ao aumento de risco de câncer de cólon (KASER et al., 2010).

É conhecido que diversas substâncias naturais favorecem os processos envolvidos na imunidade. Contudo, a literatura carece de informações acerca dos possíveis benefícios decorrente do consumo regular de yacon sobre o sistema imune. Nesse sentido, o objetivo do presente estudo foi verificar se a ingestão de yacon altera o sistema imune de camundongos BALB/c, e se seu consumo regular é capaz de modular a inflamação intestinal induzida pela instilação retal de ácido trinitrobenzeno sulfônico (TNBS).

A presente tese está dividida em cinco capítulos na forma de artigos científicos. Os capítulos um, dois e três correspondem às revisões bibliográficas acerca dos efeitos benéficos do consumo de prebióticos, frutanos e yacon. O capítulo quatro apresenta os resultados a respeito dos efeitos do consumo regular de yacon sobre o sistema imune de camundongas BALB/c. No capítulo cinco estão expostos os resultados do efeito do consumo regular de yacon sobre o curso da colite induzida em camundongas BALB/c pela administração de TNBS. Ao final, são apresentadas as conclusões gerais e as sugestões de pesquisas futuras.

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CAPÍTULO 1

THE PUTATIVE EFFECTS OF PREBIOTICS AS IMMUNOMODULATORY AGENTS

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This manuscript was published to Food Research International 44 (2011) 3167–3173.

Abstract

The gradual increase of degenerative diseases observed in the last decades has been raising morbidity, incapacitation and mortality. The occurrence of these kinds of diseases is related to the aging of humanity as well as the unhealthy choices of individuals, particularly those dwelling in large urban centers, which are closely linked with poor nutrition, obesity, and tobacco and alcohol consumption. The introduction of functional compounds in the diet seems to be an attractive alternative to ameliorate the quality of life of all age groups. The prebiotics stand out because of their beneficial effects, favoring the growth of colonic microbiota, helping the gastrointestinal metabolism, and regulating the serum cholesterol and mineral absorption. Experimental data indicates that prebiotics could reduce the severity or incidence of degenerative diseases, such as neoplasias, diabetics, coronary diseases, and infectious diseases. They also seem to promote a positive modulation of the immune system. Their effects on the immune system could even be associated to increase of resistance to infection and microbicide capability, as well as to decrease in allergic reactions. This article's goal is to analyze the immunomodulatory potential of prebiotics observed in experimental and trial studies.

Keywords: Prebiotics, Bifidogenic effect, Immunomodulation, Immune disorders.

1. Introduction

In recent years, poor eating habits have had a pronounced impact on our health and quality of life. The excessive consumption of fats, especially saturated fats, surplus sugar and salt and low consumption of resistant starch and dietary fibers have led to a raised incidence of chronic degenerative diseases, such as cardiovascular problems, cancer, diabetes, and obesity ([OPS] Organización Panamericana de la Salud (OPS), 2007).

However, all around the world there is a growing interest on functional foods, that is, foods containing compounds benefiting physiological activities and strengthening the immune system, acting in the prevention and reduction of degenerative diseases (Watzl, Gierbach, & Roller, 2005).

The modulation of the immune system resulted from the ingestion of functional foods is a striking strategy for optimizing immunity and, consequently, improving health. The food components which have been most evaluated in immunonutrition research are the fatty acids omega-3, amino acids (arginine, taurine, glutamine, and cysteine), micronutrients (selenium, zinc), prebiotics, and probiotics (López-Varela et al., 2002; Singh, Gopalan, & Sibal, 2002; Choque-Delgado, Tamashiro, & Pastore, 2010).

Probiotics are live microorganisms, which confer beneficial effects to the host when administered in adequate amounts (FAO/WHO, 2001). The most studied genera are *Bifidobacterium* and *Lactobacillus*. The bacteria producing lactic acid, such as *Lactococcus* and *Streptococcus* could also be included in this group. Other promising probiotics include the genera *Bacillus*, *Bacteroides*, *Enterococcus*, *Escherichia*, *Faecalibacterium*, *Propionibacterium*, and the *Saccharomyces* yeast (Preidis & Versalovic, 2009).

Prebiotics are the functional compounds of foods, which play an important role on the prevention and treatment of gastrointestinal diseases. Prebiotics consumption could result in some advantages to the host, due to their selective metabolism in the intestinal tract (Gibson, Probert, Van Loo et al., 2004). The metabolites produced by healthy microbiota interact with the immune system in the prevention or reduction of intestinal inflammatory diseases (Damaskos & Kolios, 2008).

Products containing a mixture of probiotics and prebiotics are called synbiotic. They have the potential of promoting the survival and increasing the function of the probiotics and beneficial microorganisms residing in the microbiota. It has been demonstrated that the consumption of synbiotics could be responsible for alterations on the composition of microbiota in a more effective way when compared to the ingestion of probiotics alone (Gibson & Roberfroid, 1995; Preidis & Versalovic, 2009).

The food industry, mindful of these results, is already accruing value of their products by supplementing them with prebiotics. To understand what the actual beneficial properties of these compounds are and how they can improve human health, in this work we will evaluate the literature concerning the immunomodulatory effects of the intake of prebiotics, both in experimental models and in humans.

2. Prebiotics

In order for a food component to be considered a prebiotic, it must meet the following criteria: (1) resists gastric acidity; (2) is fermented by the intestinal microbiota; 3)

stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson et al., 2004).

Most of the studies about prebiotics have been focused on fructans, such as inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS), which are manufactured at relatively low cost, since they can be extracted from plants or produced by enzymatic synthesis. They are also valuable functional ingredients for the food industry with the potential to improve the sensory properties of food (Macfarlane, Macfarlane, & Cummings, 2006; Macfarlane, Steed, & Macfarlane, 2007). Fructans are found as reserve carbohydrates in plant species. The occurrence of these sugars in vegetables is scant as compared to that of starch (De Carvalho & Figueiredo-Ribeiro, 2001).

Fructans are found in almost every species in the *Asteraceae* family, many of which show economic importance, such as cichorium (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) (Carabin & Flamm, 1999; Figueiredo-Ribeiro, 1993).

Fructans are composed of one or as many as 70 units of fructose linked or not linked to a terminal sucrose molecule. Fructans can show a linear or ramified structure, with molecules united by fructosil–fructose bonds of type $\beta(2\rightarrow6)$, found in fructans of levan type, or $\beta(2\rightarrow1)$ bonds, which are found in inulin type fructans (Fig. 1a) (Carabin & Flamm, 1999; Roberfroid, Gibson, & Delzenne, 1993). Through enzymatic reactions, inulins give rise to FOS and oligofructoses. FOSs are non-digestible oligosaccharides (NDOs) and are considered non-caloric agents, since they are resistant to the hydrolytic action of digestive enzymes.

Upon reaching the colon, the FOSs are degraded by intestinal bacteria such as bifidobacteria, which produces short-chain fatty acids (SCFA) such as acetate, propionate,

and butyrate, as well as lactic acid, carbon dioxide, and hydrogen (Luo et al., 1996; Roberfroid et al., 1993). They play a very important role in health as a bifidogenic agent, regulating gastrointestinal tract, improving lipid metabolism, and increasing mineral absorption. They also reduce glucose levels of diabetics, modulate the immune system, decrease incidence of colon cancer and synthesis of triglycerides, among others (Kaur & Gupta, 2002; Kruger, Brown, Collet, Layton, & Chollum, 2003; Passos & Park, 2003, Thakur & Kumar, 2008).

GOS is present in human milk, which contains various oligosaccharides originating from lactose (Macfarlane et al., 2007). GOSs are mainly produced using lactose from milk serum after protein removal. In this way, GOS constitutes a byproduct of the dairy industry (Macfarlane et al., 2007). The GOS is mainly obtained by the action of β -galactosidase on lactose, resulting in the production of a lactose molecule with one or more galactosil residues linked by β 1-3, β 1-4 and β 1-6 (Fig. 1c). The β -galactosidase acts as a hydrolytic enzyme and also as a condensation enzyme, in this case, for a transglycosylation reaction (Gibson, 2008; Rabiou, Jay, Gibson, & Rastall, 2001; Sako, Matsumoto, & Tanaka, 1999; Santos, Simiqueli, & Pastore, 2009). Others substances classified as prebiotics are lactulose, Xylooligosaccharides (XOS) and mannanoligosaccharides (MOS).

Lactulose (4-0- β -D-galactopyranosyl-D-fructofuranose) is a disaccharide composed of galactose and fructose, found in milk and milk products which undergo thermal treatments, used in the baby food and pharmaceutical industries (Fig. 1b) (Saron, Sgarbieri, & Lerayer, 2005; Schumann, 2002). It can be used as a laxative, for preventing and treating chronic constipation, portal systemic encephalopathy, and other hepatic or intestinal disorders (Holsinger, 1999).

Xylooligosaccharides (XOS) are sugar oligomers formed by units of xylose found naturally in fruit, vegetables, milk, and honey (Fig. 1d). Its industrial production is carried out through lingo cellulosic materials. XOS can be used for various purposes, among which applications in the foods and pharmaceutical industries. Its ingestion favors the selective growth of *Bifidobacterium spp* which regulates intestinal functions. Moreover, XOS are moderately sweet, stable over a wide range of pH and temperatures and have organoleptic characteristics suitable for incorporation into foods (Alonso, Domínguez, Garrote, Parajó, & Vázquez, 2003). Trials with human subjects show that XOS can be considered as prebiotic and benefits the intestinal microbiota (Menezes & Durrant, 2008).

Mannan oligosaccharides are obtained from the cell walls of yeast (*Saccharomyces cerevisiae*). They not only promote the balance of the microbiota but also have immunomodulatory properties (Ferket, 2004).

The health promoting effects of prebiotics include its contribution to the nutrition of the host, inhibition of the growth of potential pathogens and promotion of beneficial microbiota. The latter causes fermentation of non-digestible fibers, energy saving, synthesis of B and K vitamins, metabolism of plant compounds and pharmaceuticals, production of SCFA and polyamines, improvement in gastrointestinal motility and function, cholesterol reduction, and stimulation of the local immune system (Hidaka, Eida, Takizawa, Tokunaga, & Tashiro, 1986; Penders et al., 2006; Shadid et al., 2007).

3. The immunomodulatory effect

Prebiotics act like growth factor to particular commensal bacteria, which inhibit the adherence and invasion of pathogens in the colonic epithelia by competing for the same

glycoconjugates present on the surface of epithelial cells, altering the colonic pH, favoring the barrier function, improving the mucus production, producing short-chain fatty acids and inducing cytokine production (Fig. 2) (Korzenik & Podolsky, 2006; Walker, 2000). The gut-associated lymphoid tissue (GALT) is the biggest tissue in the immune system comprising 60% of all lymphocytes in the body. It contains Peyer's patches (PP), lamina propria (LP) and intraepithelial lymphocytes (IEL) forming a unique immune network (Iijima, Takahashi, & Kiyono, 2001; Mowat & Viney, 1997). PP contains follicle-associated epithelium (FAE) that covers M-cells responsible for transporting the antigen onto the lymphatic tissue where dendritic, T and B-cells are found. Activated lymphocytes in the PP are drained to and expand in the mesenteric lymph nodes and transit through the blood stream into mucosal sites (Mowat, 2003). LP is the region between the epithelium and the muscle and contains mast cells, dendritic cells, macrophages and B- and T-cells (MacDonald, 2003).

The establishment of normal microbiota is fundamental for the development of the immune system. In germ-free animals, for example, there is a dramatic reduction in the number and function of lymphocytes in both the GALT and spleen and peripheral lymph nodes. In general, the host tolerates the presence of microorganisms that form the normal microbiota, developing an immune response in which elements such as regulatory T cells (Treg), Th1 and Th17 are present in a balanced manner resulting in a state of “physiological inflammation” (Sansonetti, 2011). Lymphocytes that were initially activated in GALT express addressins that direct them to other mucosal sites, such as salivary gland, mammary gland, cervix vagina uterus, and bronchus lymphoid associated tissue, composing the common mucosal immune system (Kaufman & Barouch, 2009). Thus, modulation of the

microbiota at the gastrointestinal tract by prebiotics and probiotics has a broad influence on the immune response of the host.

Several studies have highlighted the importance of intraluminal bacteria in maintaining the integrity of intestinal mucosa by modulating the inflammatory process in this microenvironment. Recent studies have associated certain compositions of intestinal bacteria with severe acute pancreatitis, including depletion of beneficial intestinal bacteria such as lactobacillus and colon proliferation of pathogens (reviewed by Hegazi, 2010). As discussed by these authors, a recent trial study showed that supplementation of isocaloric and isonitrogenous jejunal feeding with the high dose of prebiotics mix (insoluble and soluble fibers) improved clinical outcome and dampened systemic inflammatory disease in patients with severe acute pancreatitis (Karakan, Ergun, Dogan, Cindoruk, & Unal, 2007), although there was no data on the effects of prebiotics supplementation on the composition of the intestinal microbiota.

The administration of prebiotics has been associated with immunomodulatory effects encompassing innate, adaptive immunity as a result of the interaction with the microbiota (Vulevic, Drakoularakou, Yaqoob, Tzortzis, & Gibson, 2008). Most information about the effects of prebiotics on the immune system comes from experimental trials with inulin and FOS. The general observations are that inulin consumption increases the phagocytic capacity of macrophages and the production of secretory immunoglobulin A (IgA-s), which plays an important role in the defense of the gastrointestinal tract (reviewed in Van Loo, 2004). However, the enhancement of the specific response of delayed type hypersensitivity in an *influenza* vaccination model used as a marker for Th1 type immunity has been shown, in rats supplemented with a mixture of GOS/FOS (ratio 9:1; optimum at 5% w/w of total

diet) as well as the increase of the proportion of fecal bifidobacteria and lactobacilli (maximal effect at 10% w/w of total diet), whereas FOS/inulin (2% w/w of total diet) enhanced the specific response of delayed type hypersensitivity but did not affect the proportion of fecal bifidobacteria and lactobacilli (Vos et al., 2006).

Mice which consumed a diet rich in oligofructose and inulin showed greater activity in the *Natural Killer* (NK) splenic cells and increase in phagocytic activity of peritoneal macrophages. Furthermore, a decrease in total IgA in the feces was observed, when considering the total volume of feces produced (Kelly-Quagliana et al., 2003, Kudoh et al., 1999). Severe colitis was also experimentally controlled in a genetically susceptible host fed with a mixture of lactobacilli, bifidobacteria, and inulin (Schultz et al., 2004).

The addition of mannanoligosaccharides to the diet of turkey poult reduced the pro-inflammatory immune response induced by LPS (Ferket, 2002), as well as increased the humoral immune response in the vaccination of poultry against Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV) (Oliveira, Figueiredo-Lima, Faria Filho, Marques, & Moraes, 2009). However, the FOS and MOS intake (5 g/kg in addition to basal diet) resulted in a significant reduction in the proportion of B cells and in mitogen responsiveness of lymphocytes in cecal tonsil of chickens in comparison with those observed in birds treated with the antibiotic zinc bacitracin (Janardhana et al., 2009).

Although well designed clinical trials in humans are scarce, the specific effects of prebiotics have been investigated, particularly in inflammatory disorders in infants and elders. Some of the data obtained in these researches are summarized in Table 1. For example, Guigoz, Rochat, Perruisseau-Carrier, Rochat, and Schiffrin (2002), while studying the effects of supplementing with FOS (8 g/day) in elderly subjects, observed a

decrease in the expression and secretion of IL-6 by peripheral blood mononuclear cells. Another independent study showed that supplementation of diet of elders with GOS (5.5 g/day) resulted in significant reduction in the production of proinflammatory cytokine IL-6, IL-1 β , and TNF- α , as well as in increase of the activity of NK cells and production of IL-10, an anti-inflammatory cytokine (Vulevic et al., 2008).

In children and teenagers infected by HIV, ingestion of a supplement containing FOS (3,75 g/day) for three months resulted in an increase in lymphocyte proliferation specific to the BCG (Bacillus Calmette-Guérin) vaccine that was used to evaluate cellular immune response in these patients (Moreno, 2007). A diet enriched with a FOS:inulin mix (5%) was able to trigger and stimulate the gut mucosal immune system and supports the concept of using food supplements containing a FOS:inulin mix to enhance the oral vaccine efficacy. This study reflected the capacity of FOS mix to promote a Th1-like adjuvant effect which might have supported the strong specific humoral responses observed in FOS mix-fed mice. Any signs of immune suppression or other potential side effects in the study upon feeding with FOS mix were not discerned. The results showed that there was an improvement in the Salmonella vaccine protection rate upon feeding with the FOS mix (from 40 to 73%) (Benyacoub et al., 2008). In infants who lack the transfer of protective maternal SIgA from breast milk receiving standard formula containing a mixture of 0.6 g GOS+FOS (9:1)/100 mL until the eighth month, a tendency for increase in endogenous secretory IgA in the feces was observed (Bakker-Zierikzee et al., 2006).

Oligosaccharides were also able to alter the development of the postnatal immune system in a prospective clinical double-blind randomized trial controlled by placebo, conducted with 259 infants at risk for atopy. In the infants who ingested a mixture with 0.8

GOS+FOS (9:1)/100 mL, during the first six months of life, the cumulative incidence of atopic dermatitis was significantly reduced (Moro et al., 2006).

Some investigations have pointed out that the use of prebiotics, or other microbiota modulators, could contribute to the prevention of allergic diseases in human population. The normal intestinal microbiota associated with the gut intestinal mucosa represents the first line of defense against various potential pathogens. The recognition and presentation of the antigen in the GALT are fundamental for the construction of an immune response, whether to develop protection, tolerance, or disease (Canani, Passariello, Buccigrossi, Terrin, & Guarino, 2008; Chierici, Fanaro, Saccomandi, & Vigi, 2003; Gibson, 1999). The intestinal tract of the newborn is a sterile environment, which becomes colonized by microorganisms through breastfeeding. The absence or poor colonization of the intestinal mucosa is usually associated with the development of allergic conditions in childhood. The oligosaccharides and bifidogenic nucleotides in human milk interact with the sterile gastrointestinal tract of neonates, promoting the predominance of bifidobacterias (Ly, Litonjua, Gold, & Celedón, 2011; Macfarlane et al., 2007). The adequate colonization of intestines is necessary for the development of the immune system, especially in the synthesis and secretion of polymeric IgA, as well as balanced production of T helper cell response, and modulation of hypersensitivity reactions (Fanaro & Vigi, 2008). In this regard, it has been shown that allergic infants display an abnormal “adult-type” bifidobacterium flora, with high levels of *Bifidobacterium adolescentis* strain instead of the typical infant flora dominated by *Bifidobacterium bifidum* (He, Ouwehand, Isolauri et al., 2001).

It was recently demonstrated that the long-term consumption of orally administered lactulose (1 or 3%) had an immunomodulatory effect on the composition of T-cell subsets in gut-associated lymphoid tissue, secondary lymphoid tissues, and peripheral blood of probiotic fed calves, that could impact on calf health (Fleige, Preisinger, Meyer & Pfaffl, 2009).

Use of *Lactobacillus helveticus* M92 in combination with inulin, lactulose, or raffinose (10 g/l) resulted in immunomodulation, since the application of these synbiotic was able to stimulate the mucosa and the total humoral immune response in mice. The combination with inulin showed the best synbiotic effect over both the intestinal microbiota and the immune system. The concentration of both secretory IgA and total seric IgA in immunized mice was bigger than in the controls (Frece et al., 2009).

On the other hand, oral treatment with lactulose (10 g/day) during 4months had no beneficial effects in patients with intestinal bowel diseases such as ulcerative colitis (UC) and Crohn's disease, in particular with regard to clinical activity, endoscopic score or immuno histochemical parameters. According to the German version of the Inflammatory Bowel Disease Questionnaire (IBDQ), however, significant improvement of quality of life was observed in UC patients receiving lactulose compared to the control group (Hafer et al., 2007).

In another prospective, randomized, double-blind, placebo-controlled trial, healthy term infants with a parental history of atopy were fed either a prebiotic-supplemented (8 g/L short-chain GOS/large chain FOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 months of life. The scGOS/lcFOSmixture led to significant reduction in the cumulative incidence of allergic manifestations (atopic

dermatitis, recurrent wheezing episodes, and allergic urticaria) and in the number of infectious episodes (overall, upper respiratory tract infection, fever episodes, and infections requiring antibiotic therapy) during the 18 months after the termination of oligosaccharide supplementation (Arslanoglu et al., 2008).

Probiotics and prebiotics may have both adjuvant properties on immune defense, via up-regulating Th1 responses, and anti-inflammatory actions which prevent contact hypersensitivity responses, via downregulating Th1 responses. In this regard, an experimental study showed the dietary supplementation with FOS (50 g/kg diet) and *B. pseudolongum* was partially responsible for the reduction in the 2,4-dinitrofluorobenzene (DNFB) induced contact hypersensitivity response by the modulation of antigen-induced cytokine production. This effect was associated with an increased production of IL-10 and decreased production of interferon- γ , which might be mediated, at least in part, by increased proliferation of *B. pseudolongum* in the gastrointestinal tract. Although modulation of antigen-induced production of inflammatory and regulatory cytokines might be involved in the inhibitory effect of gut microbiota on contact hypersensitivity response, further studies remain to be performed, especially regarding IL-17 production (Sasajima et al., 2010).

A dietary intervention with a mix of prebiotics (scGOS/lcFOS/pAOS; 9:1:1) in mice orally sensitized with whey act as a protective effect on allergic symptoms. The prebiotic diet caused only a minor reduction in whey-specific Ig. However, partial ex vivo CD25⁺ Treg cell depletion abrogated the protective effects of the adoptive transfer with donor cells of whey-sensitized mice fed the prebiotic diet. The results suggest, at least in part, the involvement of whey-specific Treg cells in the transfer of tolerance (Schouten et al., 2010).

The effect of daily intake of a synbiotic on immune functions of colon cancer patients was investigated in a randomized double-blind, placebo-controlled trial with of colon cancer and polypectomised patients. No negative effects were observed in cancer and polyp patients with daily consumption of the synbiotic (SYN) encapsulated bacteria (1×10^{10} colony forming units of *Lactobacillus rhamnosus* and 1×10^{10} colony forming units of *Bifidobacterium lactis*) and a 10 g sachet of inulin enriched with oligofructose, for 12 weeks. The synbiotic supplement had only minor effects on the immune systems of the subjects in both groups. It is possible that SYN supplementation in humans preferentially affects the gut-associated lymphoid tissues rather than the systemic immune system (Roller, Clune, Collins, Rechkemmer, & Watzl, 2007).

Another set of data suggest beneficial effects of the prebiotic dietary inulin in colon cancer prevention. This effect seems to be related to the ability of short-chain fatty acids and secondary bile acid deoxycholic acid (DCA), derived from the culture of fecal inocula and mixture of inulin enriched with oligofructose, to inhibit growth and induce apoptosis in colon cancer cells. However, further studies are necessary to determine whether this mechanism could be involved with protection against colon cancer (Munjal, Glei, Pool-Zobel, & Scharlau, 2009).

It has been demonstrated that prebiotics such as inulin (5%) may increase Zn absorption, a mineral known to play a central role in the immune system since Zn-deficient states are characterized by suppressed immune function. Inulin also helped to conserve femur Zn concentrations during diet restriction, when dietary Zn was available despite reduced energy intake, but not during Zn deficiency when the availability of Zn was severely limited (Ryz, Meddings, & Taylor, 2009).

4. Conclusions

Several investigations have suggested that prebiotics can improve health and prevent chronic diseases such as obesity, atherosclerosis, hypertension, osteoporosis, diabetes, and cancer. Many bacteria residing in the gastrointestinal tract can be stimulated by the consumption of prebiotics, but most investigations show that the growth of bifidobacteria and, to a lesser extent, lactobacilli, is particularly favored. Although data from human studies is still scarce, the results from recent animal studies clearly suggest that prebiotics benefit the gastrointestinal tract by stimulating the immune system. The metabolites from bifidobacteria and lactic acid bacteria seem to cause positive regulation of the immune system, and thus reducing the incidence and severity of inflammatory diseases and allergies through the modulation of the functions of resident cells, particularly in the down regulation of the production of pro-inflammatory cytokines in those areas.

Nutritional intervention with prebiotics is an attractive option for modulating both the gastrointestinal tract and the immune system, particularly for some divisions of the population such as infants, elders, and the immunosuppressed. However, well designed clinical studies in humans are still necessary in order to investigate the optimal dosage, duration of treatment, and the specific effects of each prebiotic in different dietary matrices, in different populations showing different composition of the intestinal microbiota and immune response.

Acknowledgements

The authors thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico—“National Counsel of Technological and Scientific Development”) for the financial support.

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Legends:

Figure 1.- The structural formulas of prebiotics.

Figure 2.- Interactions of prebiotics, probiotics and immune system in the intestinal mucosa. Prebiotics promote the growth of healthy microorganisms in intestinal environment, which display immunomodulatory functions, and inhibit the adherence and invasion of pathogens in the colonic epithelia.

Table 1.- Trial of prebiotics in inflammatory disorders in human subjects.

Figures:

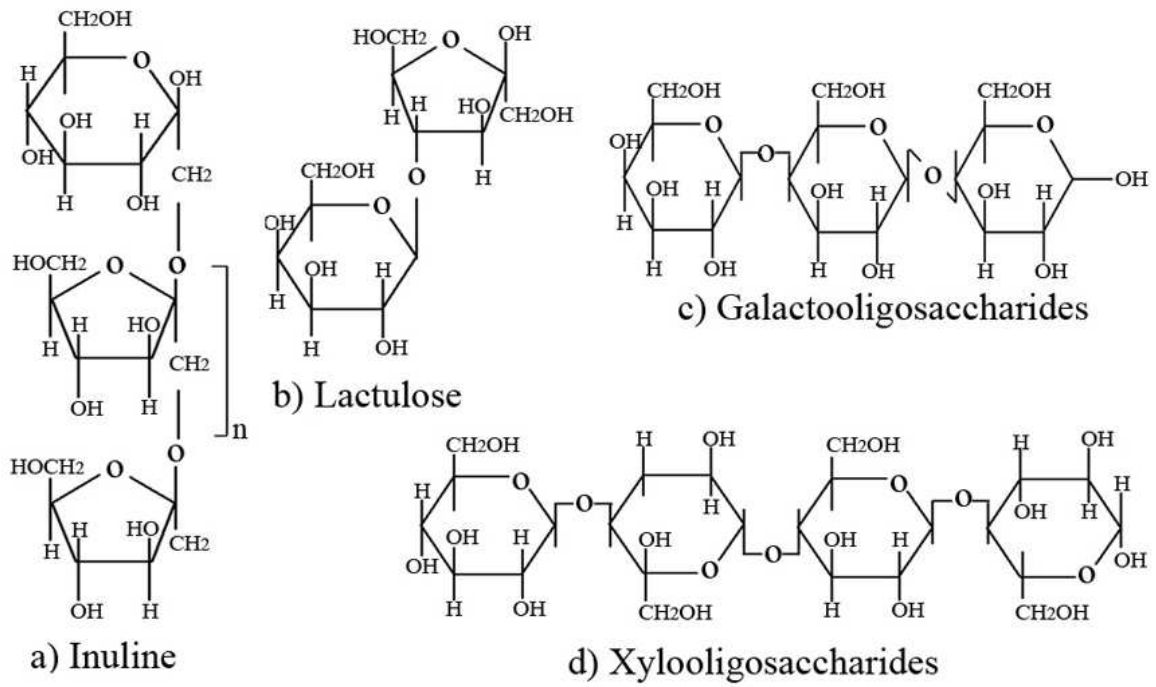


Figure 1

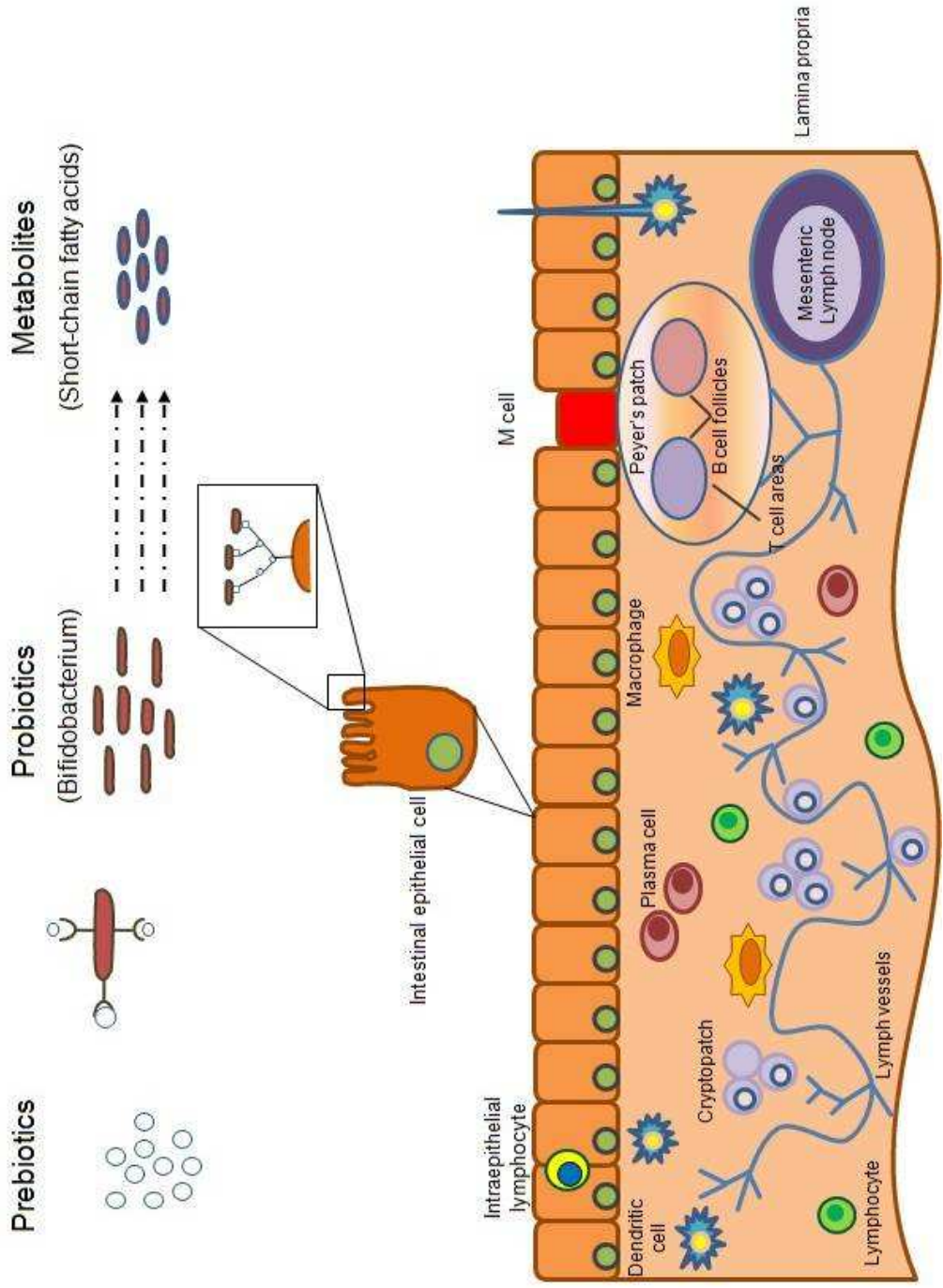


Figure 2

Table 1.

Prebiotic	Disease, author and year	Local, subject randomized and dose	Design and measures	Main findings	Inverse association to ID
FOS/inulin	IBD (Furrie et al, 2005)	Dundee, N= 18 (UC), Age: 24-67 y Dose: 6 g of FOS/inulin mix and <i>Bifidobacterium longum</i> (2x10 ¹¹ freeze dried) twice daily for four weeks.	DB, PC Clinical status scores, rectal biopsies, transcription levels of epithelium, CRP.	Sigmoidoscopy scores were reduced (p = 0.06), mRNA levels for human beta defensins 2, 3, and 4 were significantly reduced in the test group after treatment (p = 0.016, 0.038, and 0.008, respectively), TNF- α and IL-1 α , were also significantly reduced after treatment (p = 0.018 and 0.023, respectively). Biopsies had reduced inflammation and regeneration of epithelial tissue.	Yes
FOS/GOS	AD (van der Aa, et al, 2010)	The Netherlands, N=90 infants (AD), Age: 7 mo, Dose: 0.8 g/100 mL (9:1 scGOS:lcFOS) and 1.3x10 ⁹ CFU /100mL of <i>Bifidobacterium breve</i> M-16V by 12 weeks.	DB,PC SCORAD index, Microbiota composition	No difference in SCORAD score improvement between the SYN and PC. SYN did have a significantly higher percentage of bifidobacteria (54.7% vs. 30.1%, P<0.001) and significantly lower percentages of <i>Clostridium lituseburense</i> / <i>Clostridium histolyticum</i> (0.5 vs. 1.8, P = 0.02) and <i>Eubacterium rectale/ Clostridium coccooides</i> (7.5 vs. 38.1, P<0.001) after intervention than the PC. SCORAD score improvement was significantly greater in the SYN than in the PC (-18.1 vs. -13.5 points, P = 0.04).	No
Lactulose	IBD (Hafer, et al, 2007)	Germany; N=52, (UC/CD); Age: 30-38 y Dose: 10g lactulose daily,	Total= 31; 2 groups UC=14(L:7/C:7); CD= 17(L:8/C:9)	No beneficial effects in IBD patients in CAI, ES or IHP.	No

	Period: 4 mo.	IBDQ, Endoscopy, defecation frequency, orsomucoid, alpha1-antitrypsin, colonic biopsies.	Significant improvement of IBDQ in UC patients (L) compared to the C group (p = 0.04).	Yes
Oligofructose	Peru; N1=282, N2=349 Infants Age: 6–12 mo. Dose: oligofructose 0.55 g/15 g cereal and zinc (1 mg/15 g cereal), Period: 6 mo.	2 RBT 1) mean (\pm SD) days of diarrhea, 2) titers of antibody to <i>Haemophilus influenzae</i> type B	1) days of diarrhea were 10.3 ± 9.6 in the non supplemented cereal group and 9.8 ± 11.0 in the prebiotic-supplemented cereal group (P = 0.66). 2) days of diarrhea were 10.3 ± 8.9 in the group consuming cereal fortified only with zinc and 9.5 ± 8.9 in the group consuming cereal containing both zinc and prebiotics (P = 0.35). No effect on antibody response to Hib vaccination or on respiratory tract symptoms, antibiotic use, or use of health care resources.	No
HAMS	Australia, N=20 Age: 21-75 y Dose: 25 g HAMS/d and 5g of Bifidobacterium lactis, Period: 4 wk	DB, PC Rectal biopsy, feces, and serum samples, epithelial proliferation and crypt cellularity	Significantly different fecal stream bacterial community than did either the prebiotic (P = 0.032) or the probiotic (P = 0.001) No significant difference any other fecal, serum, or epithelial variables.	Yes
Psyllium (<i>Plantago ovata</i>)	Japan, N=10 (CD) Age: 27 ± 7 y Dose: 75 billion CFU daily of <i>Bifidobacterium/Lactobacillus</i> . and 9.9 g daily of psyllium. Period: 13.0 ± 4.5 mo	CDAI, IOIBD scores, blood sample	CDAI/IOIBD scores were significantly reduced after therapy (255–136, P = 0.009; 3.5–2.1, P = 0.03, respectively). High-dose PRO/PRE cotherapy can be safely and effectively used for the treatment of active CD.	Yes

FOS= fructooligosaccharides; GOS= galactooligosaccharides; HAMS= high-amylose maize starch; IBD= intestinal bowel disease; AD= atopic dermatitis; UC= ulcerative colitis; CD= Crohn's disease; ID= inflammatory diseases; N= sample; CFU= colony forming units; DB = double-blind; PC= placebo-controlled; IBDQ= quality of life index; C= control; L= lactulose; CAI= clinical activity indices; ES= endoscopic score; IHP= immunohistochemical parameters; RBT= randomized blinded trials; SCORAD= scoring atopic dermatitis; SYN= symbiotic; PRO= probiotic; PRE= prebiotic; CDAI= Crohn's disease activity index; IOIBD= international organization for the study of inflammatory bowel disease; CRP= C reactive protein; TNF α = Tumor necrosis factor; IL-1 α = interleukin 1 α ; Hib= Haemophilus influenzae type B.

CAPÍTULO 2

IMMUNOMODULATORY EFFECTS OF FRUCTANS

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This manuscript was published to Food Research International 43 (2010) 1231–1236.

Abstract

According to the Pan-American Health Organization (PAHO), there is a growing increase of degenerative diseases in countries of the Americas, bringing on disability and death of the population. As eating properly is the most effective way to reduce the risk of diseases, food scientists are seeking alternatives for more nutritional foods, mainly through the investigation of functional compounds in natural foods. Among the functional compounds, fructans stand out for their beneficial effects on the animal body, favoring the growth of bifidobacteria in the colon that improve gastrointestinal metabolism, as well as regulating the levels of serum cholesterol and the absorption of minerals. Besides, fructans seem to be involved in the positive modulation of the immune system, mainly in an increased resistance to infections and microbicidal activity as well as by the reduction of allergic reactions and cancer in experimental models. This paper aims to analyze literature data concerning the benefits of consuming the fructans contained in natural food, especially their immunomodulatory actions in both animals and humans.

Keywords: Functional foods, Prebiotics, Fructans, Immune system.

1. Introduction

In most countries throughout the Americas, chronic diseases have presented a greater increase in comparison to transmissible diseases, which brings on concern to both public and private health care entities. According to the Pan-American Health Organization (PAHO) (Organización Panamericana de la Salud (OPS), 2007), the death rate caused by chronic diseases will increase 17% in the next 10 years, especially the ones derived from diabetes, the incidence of which will increase around 80%. A significant increase in the number of overweight men and women in the Americas is expected up to 2015, mainly owing to bad eating habits. A great many of such obese people are going to catch diabetes.

In the last few years, the extensive substitution of natural foods by their industrialized peers has seriously affected health as well as the quality of life of human populations. Bad eating habits and the excessive consumption of fat, mainly saturated fats, excessive use of sugar and salt, and the low intake of starch and dietetic fibers have led to a high incidence of chronic degenerative diseases, such as cardiovascular diseases, cancer, diabetes, and obesity (Organización Panamericana de la Salud (OPS), 2007).

In the face of such issues, public health care policies should prioritize action towards changing people's habits, particularly as far as food quality, physical activity maintenance, and tobacco-use reduction are concerned. It is estimated that changes in eating habits alone should reduce the incidence of cancer at about 30–40% (Organización Panamericana de la Salud (OPS), 1997).

In that regard, an increasing worldwide interest has been developed towards functional food, i.e., food with compounds that provide physiological or metabolic activity

benefits and strengthen the immune system, acting in the prevention and decrease of degenerative disorders (Watzl, Gırrbach, & Roller, 2005).

Functional or nutraceutical foods can be found either in their natural or processed forms. The natural functional foods are the ones that preserve their innate functional nutrients and compounds. The processed foods, on their hand, are the ones to which functional ingredients have been aggregated in order to enhance their value (Roberfroid, 2005).

Fructans set an example of functional ingredients: also known as non-conventional sugars due to their prebiotic properties, fructans make up a good opportunity to add value to the product either in terms of functionality or in profitability for the food industry.

The regular intake of prebiotics such as fructooligosaccharides (FOS) and inulin improve a few physiological features, enhancing resistance against intestinal as well as extra-intestinal pathogens and promoting good immune response development, including the decrease of allergies (Fujitani et al., 2007; Vos et al., 2007).

The immune system is able to discriminate “self” molecules from “non-self” ones and to perceive the absence of the “self” element (Doan, Melvold, Viselli, & Waltenbaugh, 2008). The main activities of this system are the elimination of pathogens and the immunesurveillance against tumors. Such activities are developed by different, though integrated elements, which are known as innate and adaptive immune systems. The innate immunity comprises an ancestor set of defense mechanisms found in multicellular organisms whereas the adaptive immunity is evolutionarily more recent, having appeared in the vertebrate group (Abbas & Lichtman, 2005; Doan et al., 2008). Several scientific reports have shown an increase in immune system efficiency through the consumption of

functional foods, mainly fructans. On an experimental basis, it has been demonstrated that the regular intake of such compounds provides resistance against intracellular pathogens and lead to a decrease in digestive tract allergies. These effects seem to be correlated to the increase in bifidobacteria in the intestinal microbiota through fructan intake (Kelly-Quagliana, Nelson, & Buddington, 2003; Watzl et al., 2005).

Due to the large demand for quality eating habits, the inclusion of prebiotics of the fructan type in the production of different foods for human use will represent a great opportunity to innovate and add value to the functional food industry. This paper is aimed at examining the knowledge acquired about the features of fructans as well as at the effects from the regular intake of such prebiotics upon the immune system activities in both humans and animals.

2. Fructan composition

Fructans are reserve carbohydrates comprising 01–70 units of fructose, linked or not to a terminal sucrose molecule. Fructans can present a linear or branched structure with molecules bound together through $\beta(2\rightarrow6)$ fructosyl-fructose linkages found in fructans of the levan type or $\beta(2\rightarrow1)$ bonds found in fructans of the inulin type (Carabin & Flamm, 1999; Roberfroid, Gibson, & Delzenne, 1993). Fructans of the inulin type are divided in two compound groups: inulin and its hydrolysis products (oligofructose) plus the FOS (fructooligosaccharides), which are sucrosesynthesized. These carbohydrates have generally been differentiated by their polymerization level, which, in inulin, may vary from 2 to 70 monosaccharidic units with an average value around 10. The FOSs are used to describe fructans with a polymerization level lower than 10 (Carabin & Flamm, 1999). The

FOS present 1 or 4 fructose molecules connected in the β -(2 \rightarrow 1) sucrose position in Fig. 1. Their main types are: kestose (GF2), nystose (GF3) and 1- fructofuranosyl nystose (GF4) (Passos & Park, 2003; Sangeetha, Ramesh, & Prapulla, 2005).

The FOSs are non-digestible oligosaccharides (NDOs) considered as non-caloric agents as they are resistant against the hydrolytic action of saliva and small intestine enzymes. As they reach the colon, the FOS are degraded by intestinal bacteria as bifidobacteria, which produce short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, besides lactic acid, hydrogen and carbon dioxide (Luo & et al., 1996; Roberfroid et al., 1993).

Fructans are reserve carbohydrates found in about 36 thousand vegetable species. In the dicotyledon class, the fructans are found in almost every species of the Asteraceae family, several of which presenting economic relevance, such as the wild chicory (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) (Carabin & Flamm, 1999; De Carvalho & Figueiredo-Ribeiro, 2001). The fructooligosaccharides can also be found as natural compounds in asparagus, beetroot, garlic, chicory, onion, wheat, honey, banana, barley, tomato, and rye (Grajek, Olejnik, & Sip, 2005).

3. The immune system

The immune system of higher vertebrates comprises both innate and adaptive defense mechanisms capable of either preventing or limiting infections, in addition to eliminating tumor cells. The innate immunity comprehends an ancestor set of defense mechanisms that is found in most multicellular organisms, whereas the adaptive immunity is evolutionarily

more recent, having appeared in the vertebrate group. The cells involved in the innate immunity, such as macrophages, neutrophils, and dendritic cells present receptors for the recognition of molecular patterns in microorganisms, the so-called Pathogen-Associated Molecular Patterns (PAMPs), through which they interact with the microbes and phagocytose them. Next, the cells destroy these microbes through the action of proteolytic enzymes and oxygen free radicals generated in the inner part of the phagolysosomes (Janeway & Medzhitov, 2002; Reis & Sousa, 2004). The cells known as Natural Killers (NK) also play an important role in innate immunity by eliminating cells that have been altered by virus infection or malignant transformation. Several other factors contribute to the efficiency in the destruction of exogenous cells within innate immunity, with an impressive role played by the Complement System and the inflammatory process, which are responsible for the activation and recruitment of phagocytes towards the site where injury or infection is to be found (Abbas & Lichtman, 2005).

In addition to the huge efficiency of phagocytes to eliminate pathogens and exogenous substances from the organism, the internalization and antigenic processing followed by the exposition of peptidic fragments of antigens processed in its surface lead to the activation of adaptive immunity mechanisms. The phagocytes are called Antigen-Presenting Cells (APCs) as they accomplish this type of activity (Reis & Sousa, 2004).

The adaptive immunity is based upon lymphocyte activity, cells specialized in the recognition of molecular details of exogenous antigens through highly specific antigen receptors clonally distributed in its surface. The lymphocytes encompass two main cell types: lymphocytes B and T, which are developed in the bone marrow and thymus, respectively. Each T lymphocyte owns a single antigen receptor called TCR, composed of

two different polypeptidic chains (alpha and beta chains or gamma and delta chains) immersed in the cell surface through a carboxy-terminal hydrophobic region. In addition, the CD3 molecule is still to be found in association with the TCR, forming the antigen recognition complex. This molecule is indistinctly expressed in all T lymphocytes and acts in the transduction of cell activation signals triggered as the TCR and the specific antigen meet. The T lymphocytes only recognize the antigens that were processed and expressed as antigenic peptides associated with the molecules from the major histocompatibility complex (MHC) expressed in the surface of the APCs. The T lymphocytes are subdivided into two populations with different functional activities, i.e., TCD4+ and TCD8+ lymphocytes. The TCD4+ lymphocytes present a helper function (Th) in immune responses and interact with the APCs through the class II MHC molecules. The Th lymphocytes may be of the Th1 or Th2 types, each one producing, after activation, a given pattern of cytokines with diverse immunity functions. CD4+ T cells can leave the thymus already expressing the CD25 molecule; in this circumstance act as natural regulators of the immune response, and are called T reg cells. In the presence of IL-23, CD4+ T cells can differentiate into cells secreting IL-17, and then called Th17, which contributes to the pathogenesis of diseases such as asthma, rheumatoid arthritis, lupus and others (Winn, 2005). The TCD8+ lymphocytes interact with the APCs via antigenic peptides linked to the class I MHC molecules, turning into cytotoxic lymphocytes (CTLs), which are able to eliminate either infected or malignantly transformed target cells (Abbas & Lichtman, 2005).

The B lymphocytes, on their hand, present an antigen receptor called BCR in their surface. This receptor is composed of IgM and IgD-class immunoglobulins, which are able to establish a direct connection with a given portion of the antigen's native molecule, which

is called epitope or antigenic determinant. A few B lymphocytes can be directly activated through the antigen and be differentiated in antibody-releasing plasmocytes. In this case, the plasmocytes will always secrete IgM-class antibodies under the same specificity presented by the receptor found in the surface of its originating B cell. The antigens that activate B lymphocytes directly are called thymus-independent antigens.

However, most B lymphocytes are connected to the antigen to which they are specific and it is internalized by them. The endocytosed antigen is processed in small peptidic fragments that will be exposed in the surface of the B lymphocytes in association with the class II MHC molecules, becoming an APC for Th lymphocytes. The subsequent interaction between Th and B lymphocytes will result in the clonal expansion of both cells and in the differentiation of B lymphocytes into plasmocytes. Such plasmocytes may secrete immunoglobulins of the same specificity as the original molecule, though in diverse classes (IgG, IgA or IgE), depending on the cytokines released by the Th lymphocytes involved in their maturation (Janeway & Medzhitov, 2002).

4. Bifidogenic effect of fructans

According to Hidaka, Eida, Takizawa, Tokunaga, and Tashiro (1986), the benefits obtained from the consumption of Fructans for the human organism include the production of short-chain fatty acids (SCFAs) the main fuels of colonocytes, that lead to a decrease in the intestinal pH, a decrease in the production of putrefactive substances in the intestine, and an increase in the bifidobacteria population.

The microbiota is a complex ecosystem that shelters around 10^{14} microbes representing between 400 and 500 different species. From the microbiological point of view, the digestive tube can be divided into three main regions: stomach, small intestine, and colon. As far as the microbial population is concerned, the stomach presents a small number of bacteria owing to its low pH; a prevalence of facultative anaerobes is noticed, such as lactobacilli, streptococci, and yeast, which are found in the proportion of 100 colony-forming units (CFU) per mL. The small intestine presents a heavier bacterial load composed of facultative anaerobes as lactobacilli, streptococci, and enterobacteria, in addition to anaerobes such as *Bifidobacterium spp.*, *Bacteroides spp.*, and *Clostridia* at 10^4 and 10^8 CFU/mL. Meanwhile, the colon is the region that contains the largest quantity and diversity of microorganisms, i.e., around 10^{10} – 10^{12} CFU/mL. The main genera found in the colon are *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Bacillus*, *Peptostreptococcus*, *Fusobacterium*, and *Ruminococcus*.

The intestinal microbiota plays an important role in human health. The colonic microbiota is the predominant target of intestinal ecology in dietetic interventions (Fanaro, Chierici, Guerrini, & Vigi, 2003; Rastall, 2004; Shadid et al., 2007). The health-promoting effects include: (a) contribution to host's nutrition; (b) inhibiting the growth of potential pathogens; (c) non-digestible fiber fermentation; (d) energy economy; (e) synthesis of B and K complex vitamins; (f) metabolism of both plant and drug compounds; (g) production of short-chain fatty acids (SCFAs) and polyamines; (h) improvements in motility and gastrointestinal function; (i) cholesterol reduction, and (j) stimulation of the local immune system (Penders et al., 2006; Sanders & Gibson, 2006; Shadid et al., 2007).

A healthy microbiota is the one that is predominantly saccharolytic, with a significant prevalence of *Bifidobacterium* and *Lactobacillus*, since these genera do not contain known pathogens, produce a wide range of antimicrobial agents, and ferment mainly carbohydrates, unlike other groups, such as *Bacteroides* and *Clostridia*, which ferment proteins and amino acids as well (Macfarlane & Macfarlane, 2006; Rastall, 2004).

The butyric acid, which is the main product resulting from oligosaccharide hydrolysis, is the main energetic substrate for the colon mucosa, and seems to play a protective role against colon diseases, such as ulcerative colitis and cancer. Both propionic and acetic acids produced by these microorganisms seem to beneficially interfere with the metabolism of lipids and carbohydrates. The increase in SCFAs can also lead to pH decrease, which, on its hand, may stimulate intestinal absorption of minerals in the colon as well as increase local blood flow. Furthermore, oligosaccharide digestion products are able to suppress the *in vitro* activity of the nuclear transcription factor kappa B (NFκB), a key element for inflammatory responses (Rodríguez-Cabezas et al., 2002).

5. The immunomodulatory effect of fructans

Fructans (inulin and FOS) reach the large intestinal region where they are, to a greater or lesser extent, hydrolyzed and metabolized by the local microbiota, selectively stimulating the growth of certain bacterial species that are considered as being beneficial for the host. Therefore, the fermentation of such sugars achieve clinical meaning due to their important metabolic effects on large intestine physiology, showing the ability to affect the metabolism of carbohydrates, fats and minerals, and to act in immunologic function modulation (Delzenne, Daubiol, Neyrinck, Lasa, & Taper, 2002; Lobo & Filisetti, 2003;

Schley & Field, 2002; Scholz-Ahrens, Schaafsma, Vanden Heuvel, & Schrezenmeir, 2001; Scholz-Ahrens & Schrezenmeir, 2002; Wolever, 1995). The following information is about the benefits from the intake of prebiotic compounds of the fructan type for the physiology of both digestive and immune systems.

5.1. Gastrointestinal diseases

Evidence shows that the intake of prebiotics of the non-digestible oligosaccharide type (NDO) leads to the increment of fecal volume (1.5–2 g per NDO gram ingested), and to the normalization in the frequency under which feces are eliminated, thus regularizing intestinal functions of constipated individuals (Gibson, Beatty, Wang, & Cummings, 1995 Apud Van Loo et al., 1999). Research performed with laboratory animals showed that the daily oral administration of inulin produced an anti-inflammatory effect over the distal colitis pattern induced by Dextran Sodium Sulfate (DSS) in mice. As a result from the fermentation of inulin by the colonic microbiota, an increase in the production of short-chain fatty acids was observed in the colonic lumen as well as a mild acidification of its contents. A reduction in the local inflammatory activity and in damages caused by the inflammatory agent to the distal colon mucosa was simultaneously observed (Videla et al., 2001). The current research found out that inulin consumption increased the number of bifidobacteria and lactobacilli in the colon. In addition, it reduced the number of clostridium and bacteroids as well as other non-specific anaerobic microorganisms and aerobic microorganisms. The authors concluded that the increase in the number of lactobacilli after the intake of inulin significantly contributed to the anti-inflammatory effect ascribed to inulin (Videla et al., 2001).

5.2. Mineral absorption

The stimulation of fructan-induced absorption of minerals such as Ca, Mg, and Fe through inulin and transgalactosylated oligosaccharides has been repeatedly confirmed by studies performed with mice (Scholz-Ahrens & Schrezenmeir, 2002). It has also been noticed that, in humans, the consumption of fructans of the inulin type leads to an increase in Ca absorption, resulting in bone mineral density augmentation (revised by Van Loo et al. (1999)). Several studies actually prove that the daily intake of combined fructans in both short and long chain inulin types significantly increases calcium absorption, besides bone mineralization during the puberty growth period. The effects of these dietetic factors on calcium absorption seem to be modulated by genetic factors, including genetic polymorphism of the specific vitamin D receptor (Abrams et al., 2005). Moreover, studies have shown that the consumption of fructans of the inulin type also exerts impact on osteoporosis progression owing to the increase in calcium bioavailability with a meaningful increase in bone density and mineral mass (Roberfroid, 2000).

5.3. Neoplasias

The regular intake of pre- and probiotics also leads to a reduction in azoxymethane (AOM)-induced colon carcinogenesis in animal patterns. Although the active mechanisms of prebiotics are not very clear for carcinogenesis control, literature suggests that they may act through a combination of factors involving the increase in SCFA production, the reduction in proliferative cancer cell activity, and the reduction in the expression of certain enzymes involved in colon cancer pathogenesis (revised by Femia et al. (2002) and Van Loo et al. (1999)). In that regard, it has been noticed that chicory fructans – either of the

oligofructose or of the inulin types – cause apoptotic effects in the tumor cells of colon cancer induced in mice, thus proving their tumoricidal properties (Hughes & Rowland, 2001).

5.4. Cholesterol and triglyceride reduction

Evidences have indicated that moderate level ingestion of inulin or oligofructose affect the fat metabolism of experimental animals and humans (Delzenne et al., 2002; Van Loo et al., 1999). Hence, it has been demonstrated that oligofructose consumption along with fat-rich diets leads to the decrease in mice serum triglycerides owing to the extra-hepatic increase in the catabolism of triglyceride rich lipoproteins (Kok, Taper, & Delzenne, 1998). Likewise, studies performed in animal patterns with hyperlipidaemia present meaningful reductions in triacylglycerol and regular decrease in the levels of total cholesterol and LDL owing to oligofructose consumption (Williams & Jackson, 2002). The reduction in the risk of atherosclerotic cardiovascular disease is directly correlated to dyslipidaemia, especially in hypertriglyceridaemia and in insulin resistance, both associated with hypercaloric diets, mainly the carbohydrate-rich ones. Also in these cases, the regular consumption of fructans has brought on benefits related with either the reduction or prevention of cardiovascular disease (Roberfroid, 2000).

5.5. Immune system modulator

Changes in the composition and metabolic features of gastrointestinal tract bacteria resulting from a fructan-rich diet bring about positive effects upon the immune system

(Buddington, Donahoo, & Buddington, 2002). Research made with rodents demonstrated that diets with plenty of inulin and oligofructose increase the quantity as well as the diversity of lactic acid bacteria in the gastrointestinal tract, leading to a general improvement in animal health (Buddington et al., 2002). The lactic acid bacteria (LAB) stimulate various innate and acquired immune functions possibly through cytokine expression modulation (Buddington et al., 2002). Mice undergoing an oligofructose and inulin-rich diet presented greater activity of the splenic Natural Killer cells, increase in the phagocytic activity of peritoneal macrophages, in addition to a decrease in feces total IgA when the total fecal volume produced is taken into account (Kelly-Quagliana et al., 2003; Kudoh et al., 1999). It has been experimentally demonstrated that a FOS/inulin-based diet results in the rise of the IL-10 and IFN- γ production for cells in the Peyer's patches, which suggests that prebiotic-based supplements simultaneously activate different subpopulations of T lymphocytes and/or dendritic cells of the gastrointestinal tract (Watzl et al., 2005). Further studies demonstrated that mice undergoing a fructan-based diet (FOS/inulin) presented a reduction in colon cancer (Femia et al., 2002) as well as a reversion in the inhibition of NK cell activities provoked by the administration of the AOM carcinogen (Roller, Rechkemmer, & Watzl, 2004). Furthermore, mice fed with a diet containing fructans presented an increased resistance against infections caused by *Salmonella* and *Listeria* and against tumor development, which is consistent with the increase in NK cell functions and macrophages found in those animals (Buddington et al., 2002).

6. Conclusions

The general health condition of humans depends both on hereditary factors and environmental interferences in the organism, including life style and access to appropriate eating habits. The so-called functional foods have gradually become more popular due to the benefits that seem to be related to the consumption of such foods. Although functional foods do not constitute a panacea capable of healing diseases and other conditions that affect human beings, experimental evidences have been showing that their compounds have prebiotic properties, i.e., the ones that act in favor of the gastrointestinal tract functions. Among the most important compounds found in functional foods are the oligosaccharides of the fructan type acting as bifidogenic agents and immune system stimulators associated with the intestinal mucosa. Literature presents evidences pointing to the fact that the formation of a microbiota rich in bifidobacteria and lactic acid bacteria may regularize fat metabolism through triglyceride synthesis and mineral absorption, and such effects are positively correlated with the reduction of colon cancer incidence. The metabolites produced by bifidobacteria and lactic acid bacteria also play an important role in immune system regularization. There has been experimental evidence of their participation in the control of inflammatory diseases and allergic intestinal reactions through the modulation of resident cell functions, particularly in the downregulation of the pro-inflammatory cytokine production in these places. Although experimental results clearly suggest that fructans play a prominent role for the improvement in the functions of the digestive tract and immune system associated with the mucosae, the studies about the effect of fructans in daily diets for human beings are scarce and additional research is required to establish them more clearly.

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Legends:

Figure 1.- Structural formulas of fructans

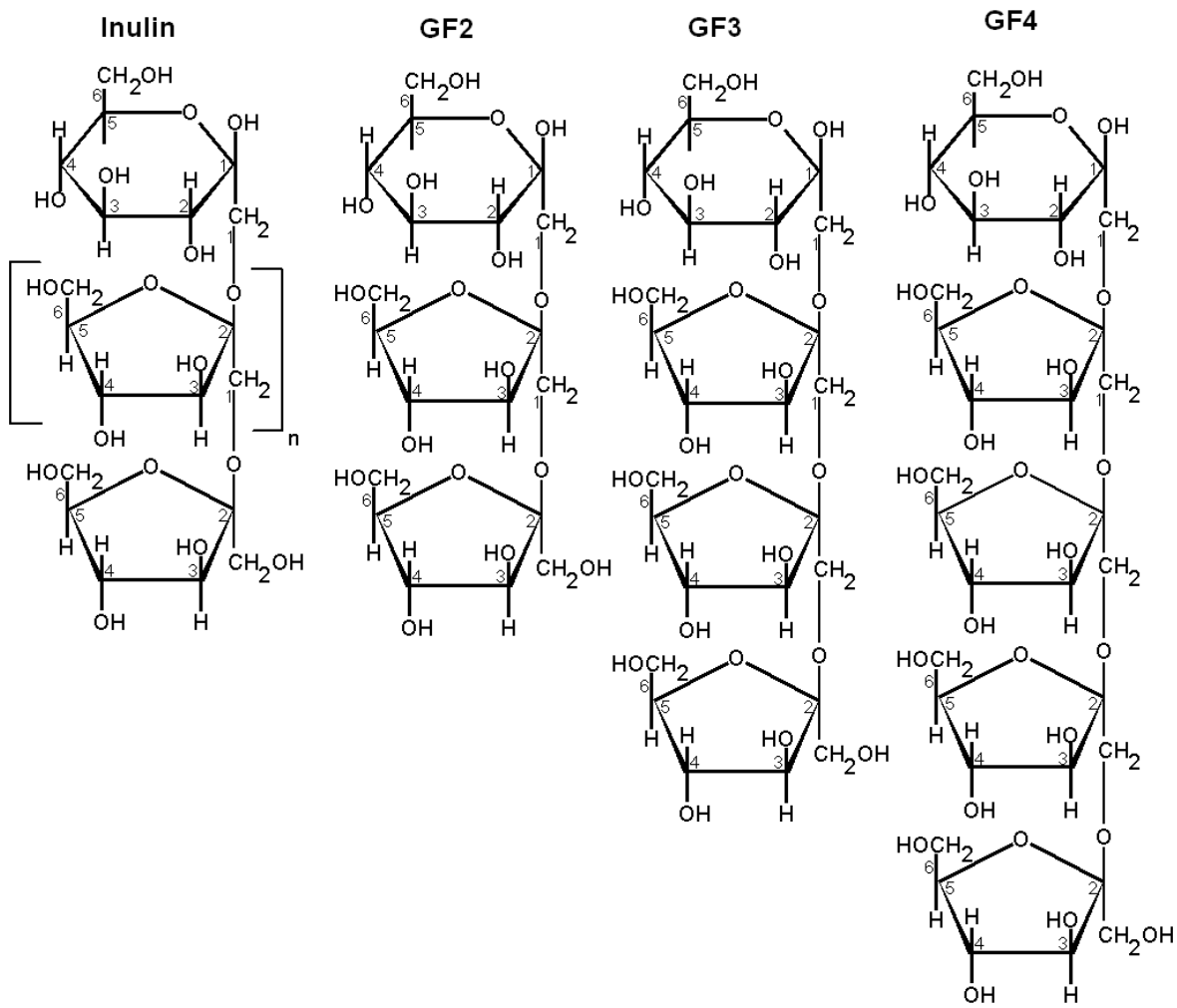


Figure 1

CAPÍTULO 3

YACON ROOT (*Smallanthus sonchifolius*): FUNCTIONAL PROPERTIES

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Abstract

Among the natural functional foods appears yacon (*Smallanthus sonchifolia*), an Andean tuberous root, which contains nutraceutical compounds of fructooligosaccharides (FOS) type, inulin and phenolic compounds. Consumption of FOS and inulin favors the growth of bifidobacteria in the colon, helping the gastrointestinal metabolism and regulating the serum cholesterol and mineral absorption. Besides, prebiotics promotes a positive modulation of the immune system, mainly translated into increment of resistance to infections, of microbicidal capacity and decrease of allergic reactions. Some studies show the potential of yacon as alternative food source for the necessary changes in the diet of our population. This article intends to describe the functional potential of yacon, aiming at encouraging its cultivation and industrial processing for the benefit of population health.

Keywords: Prebiotics, Fructans, Fructooligosaccharides, Phenolic Compounds.

1. Introduction

In recent years, extensive replacement of natural food by industrialized food has caused great impact on the health and quality of life of human population. Poor diet and bad eating habits have produced a high incidence of degenerative chronic diseases like cardiovascular problems, obesity, cancers and diabetes (ORGANIZACIÓN PANAMERICANA DE LA SALUD (OPS), 2007).

Before this problem, public policies in health must prioritize actions that lead to changes in habits of their populations, particularly in relation to the quality of food, maintenance of physical activity and reduction of tobacco use. It is estimated that only changes in eating habits would reduce cancer incidence by about 30-40% (ORGANIZACIÓN PANAMERICANA DE LA SALUD (OPS), 1997).

There is a worldwide increasing interest in nutraceutical foods, i.e., foods with properties that benefit physiological and metabolic activities, acting in the prevention and reduction of degenerative diseases (WATZL et al., 2005).

Consumption of prebiotics such as FOS and inulin enhances some physiological functions, promotes the immune response and increases the resistance to pathogens (VOS et al., 2007).

Yacon is an Andean plant that is attracting global attention for its prebiotics advantages and benefits, due to its high content of non-digestible oligosaccharides (NDOs) such as fructooligosaccharides and inulin, as well as phenolic compounds (GRAEFE et al., 2004; VALENTOVÁ et al., 2006) (Fig. 1).

The tuberous roots of yacon contain large amounts of inulin and fructooligosaccharides - even more than chicory and artichokes - and are used as natural sweeteners and syrups for digestive problems, particularly to balancing the intestinal microbiota. Traditional population attributes antidiabetic properties to yacon dry leaves, used in the preparation of teas used in low calorie diets.

In addition to prebiotics, yacon has a reasonable amount of phenolic compounds such as flavonoids, phenolic acids and tryptophan, which shows various defense functions of antioxidant, anti-inflammatory, antimicrobial and anticancer type. The phenolic compounds in yacon preserve biomolecules, such as DNA and lipids and proteins of cell membranes, against damage caused by free radicals (SIMONOVSKA et al., 2003).

Several studies show an increase in the efficiency of the immune system by the intake of nutraceuticals, especially fructans. Experimentally, it has been shown that regular consumption of these compounds confers resistance to infection by intracellular pathogens and leads to a reduction in allergies in the digestive tract. It seems to be correlated with the rise of bifidobacteria in the intestinal microbiota by the consumption of fructans (WATZL et al., 2005).

The absence of toxicity associated with beneficial metabolic activity are important aspects to consider when choosing the basic raw material for processing products containing sugar to be used in treating obesity and other aspects of diabetes (VALENTOVÁ et al, 2006). Since it has such properties, yacon has great potential to become a profitable niche market in systems of small-scale farming in Brazil, especially in São Paulo state, emphasizing the practice of organic cultivation (GRAEFE et al., 2004). In producer countries, there is a wide variety of products made of yacon such as flour,

dehydrated products, slices type "chips", tea (dried leaves), juices, purees, sweeteners in the form of syrup with high content of FOS (NATIONAL RESEARCH COUNCIL (NRC), 1989).

Due to the high demand for quality food, the inclusion of yacon as a source of prebiotic of fructan type in the production of different foods for human use represents a great opportunity for innovation and for adding value in the functional food industry. In this article, we intend to examine the current knowledge on the functional characteristics and the effects of regular consumption of yacon on human and animal physiology.

2. Yacon root

Yacon is plant from the Andes in South America, which is widespread from Venezuela to northwestern Argentina (ZARDINI, 1991). Yacon is cultivated between 900-3500m above sea level in Peru, between 600-2500m in Bolivia and Ecuador, and between 600-800m in Argentina (NATIONAL RESEARCH COUNCIL (NRC), 1989).

Yacon has been part of the diet of Andean people since pre-Columbian times. In the last decade of the 20th century, the cultivation of yacon has spread to several countries outside the Andean region, such as western New Zealand, Europe, USA and Japan, due to the medicinal properties of its roots and leaves (NATIONAL RESEARCH COUNCIL (NRC), 1989) (GRAU & REA, 1997). The Czech Republic has been a pioneer in the cultivation of yacon in Europe (VALENTOVÁ et al., 2005). In Brazil, yacon is becoming an important crop, especially in São Paulo.

According to Zardini (1991), the name yacon derives from the Quechua plant "yaku", which means water. But it is also known in other regions by other names such as (1) Arboloco, (2) Aricoma, (3) Jícama/chícama, (4) Yíquima, (5) Jiquimilla, (6) Llacon. In English, it is known as “yacon strawberry”, and in French is “poire de terre”.

Yacon was initially classified as *Polymnia* (*Compositae, Heliantheae*), but further studies ranked the species that grow in Central and South America in the genus *Smallanthus* (NATIONAL RESEARCH COUNCIL (NRC), 1989). The yacon employed in the diet of the indigenous tribes that inhabit the high mountains of the Andean region belongs to the species *Smallanthus sonchifolius*, of the family Asteraceae (GRAU & REA, 1997).

Yacon is sold in markets and fairs in the Andean region with apples, chirimoyas, avocados and pineapples and not along with tubers or roots (NATIONAL RESEARCH COUNCIL (NRC), 1989). In Peru, Bolivia, Ecuador or Argentina, it is usually eaten at religious festivals, such as Corpus Christi and All Souls' Day. Yacon is usually consumed peeled and fresh, like a fruit, and in fruit salad with bananas, oranges and papayas. It can also be baked in the oven or be consumed in the form of refreshing beverage by extracting its juice.

In the early 1990s, a Brazilian farmer born in Japan introduced this specie in the State of São Paulo. In the region of Capão Bonito/SP, the tubers of 60-80g are planted in beds of 1.0m wide by 0.4m high, spaced 1.4m between rows and 0.9m between plants (VILHENA, 1997).

The leaves of yacon have several soluble phenolic compounds in water that enable an epiphytic bacterial flora with very particular metabolic properties, inhibiting the attack of pathogens (VALENTOVÁ et al., 2005).

The roots of yacon can weigh between 200-500 grams each, and can reach up to 2000g. Each plant produces bunches of 5-20 units, giving an average of 5 kg/plant. The freshly harvested roots are insipid, but after about 3-5 days of exposure to the sun, they become juicy and sweet. The taste of sweet yacon has been described as similar to a fresh apple or watermelon (NATIONAL RESEARCH COUNCIL (NRC), 1989) (Fig. 2).

As to its chemical composition, the fresh roots of Yacon have 69-83% of moisture, 0.4 to 2.2% of protein and 20% of sugars (NATIONAL RESEARCH COUNCIL (NRC), 1989). The tuberous roots contain as a percentage: 3.59 of ash, lipids from 0.3 to 1.5, 3.88 of crude fiber, nitrogen free extractable of 85.19, K₂O 1.76, 0.18 to CaO, 0.13 MgO and 0.31 P₂O₅. It is about 35% of fructose and 58% of glucose + sucrose (HOLA & MICHL, 1996).

The oligosaccharides from yacon have repeated units of β -1.2-D-fructuranosil with terminal sucrose, being, therefore, oligosaccharides of inulin type. The major FOSs in yacon are kestose, and 1-nystose fructosilnystose, similar to those found in the tubers of Jerusalem artichoke (*Helianthus tuberosus*) (PASSOS & PARK, 2003).

Levels of sugars in yacon roots may vary depending on factors such as localization, farming, the growing season and harvest time and temperature in the post-harvest. In Brazil, it has been found 34-55% of FOS, 7-9% of glucose, 13-14% of fructose and 10-13% of sucrose (LOBO et al., 2007; CHOQUE et al., 2012). In Peru, researchers have found 40-

70% of FOS, 5-15% sucrose, 5-15% fructose and less than 5% glucose (dry basis) (PEDRESCHI et al., 2003; MANRIQUE & PARRAGA, 2005).

Yacon contains low-polymerization β -oligosaccharide, inulin, small amounts of vitamins and minerals and contains no starch. The most abundant minerals in yacon are calcium and potassium. In addition, the yacon juice is rich in free essential amino acids (KAPULER & GURUSIDDIAH, 1994). The tuberous yacon root is rich in bioactive substances such as phenolic compounds, ester derivatives, methyl esters, and glycosides. These compounds occur naturally in both leaves and roots.

Polyphenols of *Smallanthus sonchifolius* produce acrid or astringent flavor of the leaves and barks, as well as they affect an odor typical perception. Polyphenols are also substrates for the enzymatic browning of damaged tissues, becoming green or black substance by a condensation reacting of polyphenol compounds with amino acids and enzymatic polymerization of polyphenols. By contrast, the phenolic and flavonoid compounds can modulate lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis through antioxidant activity on super-oxide O_2 ion (ZHISHEN et al., 1999).

Yacon juice contains 850ppm of polyphenolic compounds such as chlorogenic acid, considered the main antioxidant of yacon along with tryptophan (TAKENAKA et al, 2003). The phenolic acids seem to be responsible for the biological activity of yacon, including effects anti-hyperglycemic, cito-protective and waste disposal. Total phenol levels in young leaves of yacon were lower than in leaves collected at the time of tuber harvest (VALENTOVA et al., 2006).

The yacon is rarely used in cooking because of its short lifetime and their rapid browning of juices and injured tissues. Yacon root quickly darkens in storage even at low

temperatures. After cutting and during processing, the dimming trend may be related to its phenolic content, especially the levels of caffeic and chlorogenic acids and endogenous activity of polyphenol oxidase. Although, the presence of polyphenol oxidase from yacon has been demonstrated, there are not any published studies on these enzyme properties (NEVES & DA SILVA, 2007).

3. The functional effects of yacon root

Yacon administered as dietary supplement is well tolerated and produces no negative response, toxicity or adverse nutritional effect. Besides that, its low glucose content and high contents of fructooligosaccharides allow the study of the possible effects in patients with metabolic diseases, such as diabetes and metabolic syndrome (GENTA et al., 2005). In this regard, yacon proved to have an effect in reducing temporal levels of glucose in normal and diabetic rats with hyperglycemia (AYBAR et al., 2001; VALENTOVÁ et al., 2005).

Because of its inulin and FOS content, it is possible that yacon presents the most important prebiotic characteristics, i.e., resistance to digestion in the upper digestive tract, being hydrolyzed and fermented only at the end of the path by colon bacteria. Thus, the intake of yacon could benefit the development of colonic microbiota by its potential natural bifidogenic effect. Recently, it was shown the tendency of certain probiotics, such as lactobacillus and bifidobacteria to ferment FOS in yacon roots; that is why this root has been considered a new option in prebiotics (PEDRESCHI et al., 2003). Bifidobacteria inhibit the growth of putrefactive bacteria in the colon, promote the absorption of Ca_2^+ and

PO_4^{3-} ions, and synthesis of B vitamins, besides stimulating the immune system (ROBERFROID, 2005).

Supplementation of diets with yacon flour in experimental diabetes has shown a link between FOS consumption and the insulin production, at least in part, via the stimulation of insulin release and/or insulin-like activity by FOS (HABIB et al., 2011). It seems that the regular intake of yacon flour exerts its lipid-lowering activities through the activation of the lipoprotein lipase enzyme and a slight increase in fasting plasma insulin levels (HABIB et al., 2011). For these characteristics, the saccharides in yacon, particularly β -(2 \rightarrow 1) fructooligosaccharides could indeed modulate the metabolic syndrome that occurs in type-2 diabetes and dyslipidemia, risk factors for atherosclerosis.

It has been shown that polyphenolic compounds may alter glucose metabolism and antihyperglycemic activity acting like antidiabetic agents. This effect on glucose metabolism may be mediated by the insulin-like effect or by improvement in antioxidant status (CHEMLER et al., 2007). In this regard, phenolic extracts of yacon leaves were able to reduce glucose production in rat hepatocytes by increasing glucokinase mRNA expression (VALENTOVÁ et al., 2004; VALENTOVÁ et al., 2007).

Among the phenolic compounds present in yacon roots, chlorogenic acid, tryptophan and derivatives of caffeine acid predominate. As natural antioxidants, these compounds are of great importance to human health, particularly in protecting cell membranes against damage from oxygen radicals and its consequences in cardiovascular disease and cancer (NEVES & DA SILVA, 2007). Antioxidant activity and cito-protective effect against tert-butylhydroperoxide in a model of oxidative damage in rats' hepatocytes were found in alcoholic extracts of yacon leaves (SIMONOVSKA et al., 2003). Trials in humans have

also reported the antioxidant activity of phenolic compounds present in yacon leaves, acting to prevent chronic diseases, such as atherosclerosis, a disease that involves the participation of free radicals in its development (VALENTOVÁ et al., 2005).

A search using diabetic rats showed that the hydro-ethanolic extract of yacon leaves significantly reduced serum glucose levels. Furthermore, treatment with the extract restored the activity of plasma enzymes and reduced diabetic animals' body weight gain. According to the authors, the inhibitory action of hyperglycemia induced by streptozotocin (STZ) can be attributed to the fact that yacon induces to an increase in plasma insulin concentration (BARONI et al., 2008).

Studies with yacon syrup, fructooligosaccharide source revealed that their consumption over a long period produced beneficial effects on obese and slightly dyslipidemic in pre-menopausal women's health with insulin resistance. The daily consumption of yacon syrup produced a significant reduction in body weight, waist circumference and body mass index. It was also observed reduction in fasting serum insulin and an index of homeostasis. Furthermore, ingestion of yacon syrup increased the frequency of defecation and satiety (GENTA et al., 2009).

A recent study evaluated the hypoglycemic activity of phenolic compounds in organic extracts from yacon leaves in Wistar rats. The results led to conclude that enhydrin, the main sesquiterpene lactone from yacon leaves and caffeic acids, chlorogenic acids and three dicaffeoilquinic (CAF, 3-CQ; 3,4-DCQ, 3,5 and 4,5-DCQ -DCQ) are related to the hypoglycemic effect, since they reduced levels of blood glucose in diabetic animals (GENTA et al., 2010).

Two new sesquiterpene lactones and eleven known melampolides were isolated from yacon leaves and showed nitric oxide inhibition (NO) by LPS in murine macrophages (HONG et al., 2008).

Low pH and production of SCFA due to the consumption of prebiotics result in the hypertrophy of the mucosal cells, enlargement of the intestinal surface and enhance solubility of mineral ions (ROBERFROID & DELZENNE, 1998). Yacon consumption for relatively short period also resulted in increased intestinal absorption of minerals and bone mass, favoring the biomechanical properties of bone in rats (LOBO et al., 2007). The enlargement of the caecal wall observed after the consumption of yacon seems to contribute to the increased mineral absorption in those animals. In fact, several studies show that daily consumption of inulin-type fructan combination of short and long chains increases significantly calcium absorption and bone mineralization during pubertal growth. Effects of these dietary factors on calcium absorption appear to be modulated by genetic factors, including genetic polymorphism of specific D-vitamin receptor (ABRAMS et al., 2005). It has been observed that consumption of inulin-type fructans also provides a reduction in osteoporosis progression by increasing the bioavailability of calcium with a significant increase in density and mass mineral bone (ROBERFROID, 2005).

Studies by Pinto and colleagues (2001) showed that yacon produces antimicrobial compounds and pesticides, which could explain the fact that in its cultivation it is hardly necessary to use pesticides. These authors reported the inhibition of aflatoxin production when aqueous and ethanol yacon extracts are added to *Aspergillus flavus* cultures (PINTO et al., 2001). Recently, Joung and colleagues (2010) showed that n-hexane fraction of yacon leaf extract have antimicrobial activity against six different strains of methicillin-

resistant *Staphylococcus aureus* (MRSA) in the presence of 4000 lux, with a minimal inhibitory concentrations (MICs) assay (15.6 g/ml). No activity was detected when the tests were performed in the absence of light.

4. Yacon root processing

There is great potential for commercial products that are generated from yacon. In this sense, the National Research Council ranked it as promising raw material based on its high content of fructans (ZARDINI, 1991). Due to its high concentration of fructose, yacon root is a fruitful source for development of natural sweeteners and syrups for people with digestive problems. Yacon leaves are consumed in teas for diabetic patients or those who suffer from digestive and kidney diseases.

Some studies have shown that incorporating inulin and fructans in various foods, especially baked goods, makes them acceptable to consumers because of their sensory characteristics. Yacon syrup has already been incorporated into delicious product formulations, considered "light" easy to chew and with porous texture. Likewise, products using inulin, low calorie, are known for their sensory characteristics such as neutral and mild flavor that increases palatability, stability and acceptability (MOSCATTO et al., 2006).

There are already some industrial products derived from yacon on market, such as: flour, candy, sliced-chip, teas, nutritional drinks and purees. Hisa and colleagues (1996) patented a method for preparing nutritional drinks from skim milk and yacon juice fermented by the action of lactic acid bacteria (*Lactobacillus plantarum*). According to the

authors, the method helps to counteract the astringent characteristic of yacon, and prevent from FOS degradation. In the 90s, drinks were developed in Japan from milled yacon subjected to the action of cellulase at 45°C for 2 hours. The juice was used as a sweetener in formulations ready to drink (KANEGAFUCHI et al, 1994). "Chancaca" is a concentrated sweet with high content of sugars that Andean natives prepared by boiling the root juice until crystallization and resulting in solid blocks of dark brown color (NATIONAL RESEARCH COUNCIL (NRC), 1989). Fructose from yacon has a sweetener power of 30-70% greater than sucrose, besides being a non-caloric sweetener. Finally, the presence of inulin in the yacon tubers is of great interest to inulinase's industrialization produced by microorganisms (CAZETTA et al., 2005).

5. Conclusions

According to OPS, the health status in humans depends on both hereditary and environmental factors, including lifestyle and access to adequate food. In this respect, the functional foods are gaining more supporters because the benefits are associated with their consumption. Yacon, a natural resource from Andes, has great potential as functional food for its high content of fructooligosaccharides and inulin that promote a good intestinal development in the colonic microbiota. In addition to the roots, yacon's leaves are also exploited because their phenolic compounds, to which has been attributed antioxidant properties that protect cell membranes against damage caused by oxygen radicals and its consequences in cardiovascular disease and cancer. Its regular use may benefit patients with digestive and kidney diseases, as well as those with diabetes and metabolic syndrome. The high levels of FOS and phenolic compounds represent a potentially profitable niche for

the yacon in the farming systems at small scale, characterized by ecological practices and local processing, thereby contributing to sustainable development of the region. An alternative production may be sweet syrups and natural sweetener for people with digestive problems and obesity, reaching a wider group of beneficiaries.

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Acknowledgement

We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - "National Counsel of Technological and Scientific Development").

Legends:

Figure 1.- Functional effects of yacon plant.

Figure 2.- Yacon plants. A) Vegetative and reproductive organs; B) Yacon roots; C) Slices of yacon root (Sources: A) <http://www.motherearthnews.com/multimedia/image-gallery.aspx?id=74438&seq=1>; B) <http://yacon.wordpress.com/about/>; C) G.T. Choque-Delgado).

Figures:

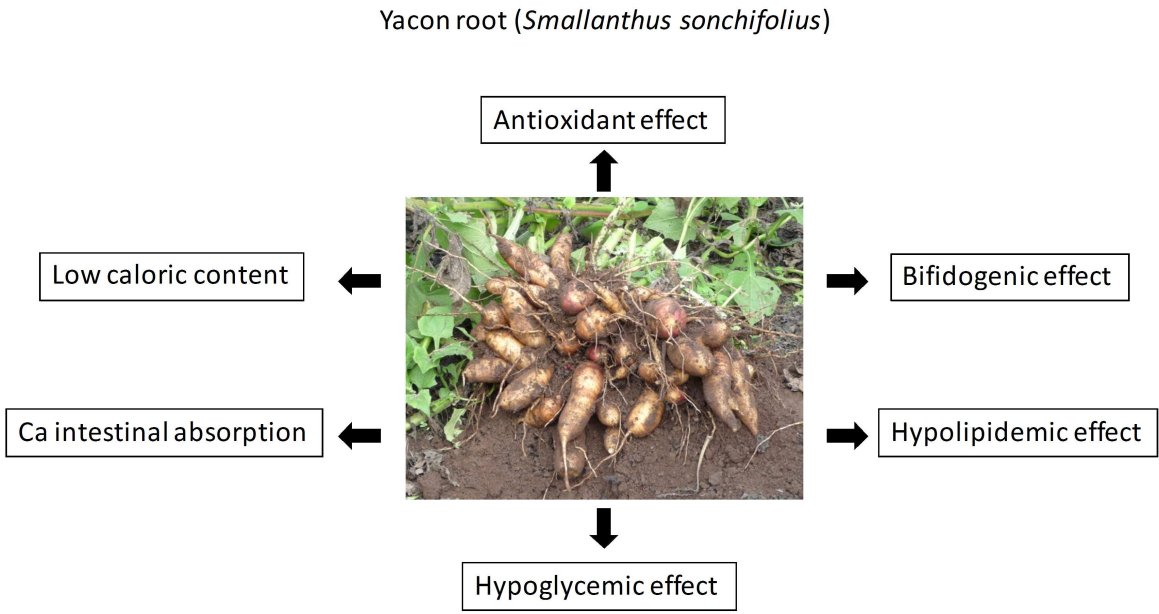


Figure 1



Figure 2

CAPÍTULO 4

THE EFFECTS OF REGULAR INTAKE OF YACON (*Smallanthus sonchifolius*)- DERIVED FRUCTOOLIGOSACCHARIDES ON THE MURINE IMMUNE SYSTEM

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This manuscript was submitted to Nutrition Research.

Abstract

Objective: Due to its high fructooligosaccharides (FOS) and inulin contents, the yacon root (*Smallanthus sonchifolius*) has excelled in traditional Andean medicine, where it is used as a substitute for cane sugar in diabetes and for obesity prevention. This study evaluates the physiological and immunological effects of incorporating yacon flour (*Smallanthus sonchifolius*) in the normal diet of mice.

Methods: BALB/c mice were fed an AIN-93M diet supplemented with 5% commercial FOS or either 3% or 5% yacon FOS. Thirty days later, weight gain and food intake, serum antibody levels of IgA, IgM, IgG, fecal levels of IgA, nitric oxide (NO) production in peritoneal macrophages, T and B lymphocytes frequency in peripheral blood, T lymphocyte proliferative capacity, and cytokine production were evaluated.

Results: Mice fed diets supplemented with commercial or yacon FOS showed no significant differences in weight gain and nitric oxide and cytokine production by peritoneal macrophages in comparison with the control group, except the IL-1 β production that was more reduced. Similarly, no differences were observed in serum immunoglobulin production or T and B-lymphocyte frequency in the peripheral blood and spleen. Significant increases were observed in the splenic T lymphocyte proliferation and IFN- γ production, as well as in fecal IgA levels in mice fed a diet containing 3 or 5% of yacon FOS in comparison with control group.

Conclusion: These results indicate that intake of yacon FOS do not alter the peripheral immune system equilibrium and promotes the production of fecal IgA.

Keywords: Yacon root; fructooligosaccharides; immunomodulation.

1. Introduction

The absence of a balanced diet containing carbohydrates, proteins, and lipids deprives the immune system of components necessary to create and sustain an effective immune response [1]. Dietary imbalance is associated with the development of chronic diseases, such as cardiovascular disease, type 2 diabetes, and cancer, greatly affecting the quality of life. "Immunonutrition" is a field of research that studies the relationship between food intake and a functional immune system [1,2,3]. Currently, research in this area is concentrated in on evaluating potential immunomodulators resulting from consuming functional diets.

Several studies have shown increased immune system efficiency following the consumption of functional foods such as fructans, which are non-digestible oligosaccharides [4,5]. The fructooligosaccharides (FOS) and inulin found in plant foods belong to fructans. The Andean yacon plant contains high levels of these compounds in the roots, while the leaves have high amounts of flavonoids, phenolic acids, and tryptophan. These components are able to stimulate immune defense by exercising antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [6,7,8].

The ingestion of FOS and inulin modulate key physiological functions such as calcium absorption, lipid metabolism, and modification of intestinal microbiota [9]. The growth of bifidogenic bacteria following FOS and inulin consumption, which inhibit the establishment of pathogenic and/or putrefactive bacteria, is directly related to colon cancer prevention in experimental models [10]. Similarly, it has been reported that these compounds promote increased resistance to infections and reduce allergies [11,12].

The immunomodulatory potential of the functional substances contained in the yacon root is not yet fully understood. This study aims to evaluate the physiological and immunological effects resulting from incorporating yacon flour in the diet of young mice.

2. Materials and Methods

2.1. Animals

Female mice from the BALB/c strain, aged 8 weeks, were obtained from the Multidisciplinary Center for Biological Research (CEMIB) at UNICAMP and were maintained throughout the experimental phase in specific pathogen free (SPF) conditions. The mice were housed in metabolic cages with a light/dark cycle of 12 hours at a temperature $22 \pm 2^{\circ}\text{C}$. The mice were given water and food *ad libitum*. Ethics Committee in the use of animals at UNICAMP (CEUA) approved this research protocol under license 1659-2.

2.2. Yacon

Yacon roots, cultivated in São Paulo, Brazil, were acquired at the CEASA market in Campinas, Brazil. The slices of yacon were frozen at -40°C and lyophilized at 40°C and $500 \mu\text{Hg}$ during 12 h (LP 1010; Liobras, São Carlos, Brazil) and then they were milled. Quantitative analyses were performed for proximate characterization of the lyophilized yacon, including determination of the protein, fat, carbohydrate, ash, fiber and water contents. The FOS content was determined by high-performance liquid chromatography (HPLC) using a Dionex Ion Chromatograph Model ICS-3000 (USA). Fructooligosaccharides were identified by the refraction index and categorized by

comparison with the retention standard of 1-kestose patterns (GF2), nystose (GF3) and fructofuranosyl nystose (GF4). Proteins were measured using the micro-Kjeldahl method [13]. The Bligh and Dyer method [14] was used to determine the lipid content. The crude fiber determination was made using the Scharrer & Akurschner method [15]. The moisture and ash contents were determined gravimetrically [16].

2.3. Diets and experimental groups

The basic maintenance diet was prepared according to the Reeves and collaborators [17] guidelines. For preparation of the diets containing FOS, the sucrose in the basic diet was replaced by lyophilized yacon flour containing 3% or 5% of FOS or 5% commercial FOS. Table 1 illustrates the final formulation of the diets.

Mice were divided into four experimental groups receiving the specified diets for 30 consecutive days. The control group (G1): AIN93M standard diet. Group 2 (G2): standard diet supplemented with 5% commercial FOS (Corn Products Brasil-Industrial Ingredients Ltda., São Paulo, Brazil). Group 3 (G3): standard diet plus yacon flour containing 3% FOS. Group 4 (G4): standard diet plus yacon flour containing 5% FOS. Feed consumption and the mice's weights were monitored weekly.

2.4. Serum and feces samples

Thirty days after receiving the specified diets, mice were bled; sera were individually separated and maintained at -20°C until use. Feces were individually collected and resuspended in PBS, 0.2 M, pH 7.4, at a 1:3 (w/v) ratio, vortex stirred and centrifuged at 200g for 10 min. The feces extracts and sera were immediately used in ELISA assays to detection of antibodies.

2.5. Macrophage cultures

Peritoneal macrophages were isolated from mice previously stimulated intraperitoneally with 3% thioglycollate (DIFCO, Franklin Lakes, NJ, USA) and cultured as indicated elsewhere [18]. The suspensions were adjusted to a concentration of 1×10^6 cells/mL in complete medium [RPMI 140 (Sigma) containing 10% FBS (Nutricel) and antibiotics (Sigma)]. Aliquots of 1 mL were plated in each well of 24 well plates (Corning) and incubated for 2 hours at 37°C with 5% CO₂. After removal of non-adherent cells, monolayers were incubated with LPS (1.0µg /mL) and IFN-γ (150 IU/ml) for 48 hours. Cells cultured in complete medium alone were used as controls. The culture supernatants were used to evaluate NO and cytokine production.

2.6. T lymphocyte proliferation

Proliferation assays were performed as indicated elsewhere [19]. Spleens were individually collected to prepare suspensions of erythrocyte free splenic cells. The cells were re-suspended in complete RPMI 1640 in 96-well plates (Corning) at a density of 2.5×10^5 cells per well and incubated for 48 hours at 37°C and 5% of CO₂ in the presence of 2.5 µg/mL Concanavalin A (Con-A, Sigma, USA). The supernatants were collected and stored at -80°C for cytokine dosages. Cell proliferation was assessed by the MTT method (4.5 -Dimethyl-2 thiazolyl-2,5-diphenyl-2H-tetrazolium bromide) read at 540 nm following formazan crystal dissolution. All samples were analyzed in sextuplicate. The absorbency results obtained from each treatment were expressed as ± SEM (Standard Error of the Mean) averages.

2.7. Flow cytometry analysis

To block nonspecific reactions, the cell suspensions (10^6 cells) were initially incubated with culture supernatant from clone 2.4G2 (anti-CD16/32), prepared in our laboratory. Then, cells were stained with either specific monoclonal antibodies or with the control isotypes, according to the manufacturer's recommendations (eBioscience; San Diego, CA. USA). Finally, the cells were re-suspended in 500 μ L of PBS containing 1% formaldehyde. The following antibodies were used: anti-CD3 (Clone 2C11, labeled with Percp-Cy5.5 or PE), anti-CD4 (clone GK1.5, rat IgG2b, labeled with FITC), anti-CD8 (in conjunction with PE clone 53-6.7, and rat IgG2), and anti-CD19 (clone 1D3, rat IgG2a kappa labeled with PE-Cy5.5). Rat IgG2 and IgG2b, labeled with PE, FITC or PE-Cy5.5 were used as isotopic controls. The preparations were analyzed using a FACS-Aria flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) located at the UNICAMP's Hematology Center. The event analyses were performed using FACSDIVA software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

2.8. Cytokine measurement

Cytokines IFN- γ , IL-4, IL-1 β , IL-10 (eBioscience; San Diego, CA. USA) and TNF- α (OptEIA, BD Biosciences; San Diego, CA. USA) were measured by ELISA in the culture supernatants using commercial kits, following the manufacturers' guidelines.

2.9. Detection of serum and fecal antibodies

Serum concentrations of IgM, IgG and IgA and fecal concentrations of IgA were measured by a capture ELISA developed in our laboratory, using commercially available antibodies (Sigma). 96-well microtitration plates (Nunc, Roskilde, Denmark) were coated

with a solution of polyclonal anti-murine immunoglobulin antibodies produced in goat, diluted in carbonate/sodium bicarbonate buffer, 0.1 M, pH 9.6. The plates were incubated overnight at 4°C and washed with PBS at 0.2 M, pH 7.4, containing 0.05% Tween 20. The free sites were blocked and the plates washed as above. The supernatant was used for fecal IgA detection. The serum and feces samples were added to the wells at various dilutions, and the plates were incubated for 1 h at 37°C. After washing, the specific anti-IgG, anti-IgM or anti-IgA antibodies were tagged with HRPO and added at predetermined dilutions. The reaction was developed by adding the chromogen substrate (0.03% H₂O₂ and 0.04% orthophenylenediamine in citrate-phosphate buffer, 0.05 M, pH 5.5) followed by incubation in the dark for 15 min. The reaction was stopped by adding 4 N H₂SO₄ to each well. The absorbance was read in a microplate reader (Multiskan MS, Labsystems, Helsinki, Finland) at a wavelength of 492 nm. The average concentrations of each immunoglobulin tested were calculated with a standard curve prepared with purified IgM, IgG and IgA (Sigma).

2.10. Nitric oxide measurement

Nitrite was measured using the Green specifications [20]. Briefly, aliquots of 50 µl Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naftylethylenediamine dihydrochloride in distilled water) were added to identical volumes of supernatants from cultures of macrophages, distributed previously in 96-well plates. After a 15 min incubation followed by plate agitation, the readings were performed in a spectrophotometric ELISA reader at 540 nm using sodium nitrite solutions (5 at 320 µM) as standards. The results were expressed in µM nitrite/ 1×10^6 cells/mL.

2.11. Statistical analysis

Statistical analysis on the results was performed using the ANOVA analysis of variance followed by Bonferroni's test using GraphPadPrism software. The minimum probability acceptable for differences between the averages was considered to be $p \leq 0.05$.

3. Results

3.1. Chemical composition of yacon flour and experimental diets

The results of the proximate analyses of the lyophilized yacon flour revealed a high carbohydrate proportion (86.13 %), proteins ($2.45 \pm 0.09\%$), lipids ($0.87 \pm 0.10\%$), ash ($2.53 \pm 0.14\%$), moisture ($8.02 \pm 0.08\%$), and crude fiber ($3.46 \pm 0.12\%$). The chromatography analyses by HPLC identified the presence of sugars such as glucose ($7.3 \pm 0.59\%$), fructose ($14.1 \pm 0.41\%$), and sucrose ($10.5 \pm 0.45\%$). The fructooligosaccharides GF2-GF4 accounted for $34.31 \pm 1.63\%$ of the sugars present in the mixture. Based on these findings, diets were prepared in which the sucrose content normally present in AIN93M was replaced by either 5% commercial FOS or 3% or 5% yacon FOS. The proximate analysis of these diets revealed no significant differences in their chemical compositions. However, the diets that included 3% or 5% yacon FOS had 8 kcal less sugar than the control diet (Table 2).

3.2. Food intake, body weight

The dietary intake of FOS-supplemented diets and the BALB/c mice's weights were evaluated by weekly weighing (Figure 1). There was no significant difference between the

weekly consumption of rations supplemented with FOS and the standard diet (Fig. 1 C). Similarly, mice fed a diet supplemented with FOS showed no significant variation in weight compared to control mice (Figure 1 A, B).

3.3. Levels of serum IgM, IgG, and IgA and fecal IgA

The levels of antibodies in serum and stool were analyzed in samples collected from mice fed either a diet containing FOS or a standard diet (Figure 2). There were no significant differences in serum IgG and IgA levels (Figure 2A, B), but there was a slight but statistically significant decrease in serum IgM in mice fed a diet containing 3% yacon FOS (Fig 2C). Fecal sample analysis showed a significant increase in the amount of IgA in samples collected from mice fed diets containing yacon FOS (Fig 2D).

3.4. Effects of yacon on the frequency and activation of lymphocytes

To verify the influence of yacon consumption on the peripheral distribution of T and B-lymphocytes, blood cells and spleens of mice fed with either the standard diet or the diets containing FOS were collected thirty days after the start of the experiment for analysis by flow cytometry. The results illustrated in Figure 3 show no significant differences in the proportions of these cell populations in either the blood (Figure 3A) or the spleen (Figure 3B) between the groups.

ConA-induced proliferation in splenic T lymphocytes was significantly higher in mice fed a diet containing yacon FOS than that observed in cells from mice fed a standard diet (Figure 3C). Following these findings, we observed a slight but significant increase in IFN- γ secretion in the cultures of spleen cells from mice fed 3% yacon FOS. There were no significant differences in IL-4 secretion observed (Figure 3 D, E).

3.5. Effects of yacon on peritoneal macrophage iNOS activity and cytokine production

To evaluate the possible effects of yacon FOS ingestion on peritoneal macrophage activity, iNOS and the cytokines IL-1 β , TNF- α , and IL-10 were measured in supernatants from thioglycollate-acquired macrophage cultures stimulated *in vitro* with LPS+IFN- γ . No significant changes in NO production were observed between macrophages obtained from the different dietary groups (Figure 4A). Similarly, no significant difference in IL-10 secretion was identified. However, IL-1 β secretion was found significantly reduced in the cells cultures derived from mice fed with rations containing either commercial or yacon FOS.

4. Discussions

According to Roberfroid and associates [21], prebiotic effects may be defined as “the selective stimulation of growth and/or activity (ies) of one or a limited number of microbial genus (era)/species in the gut microbiota that confer(s) health benefits to the host”. Thus, the incorporation of prebiotics in the diet leads to health benefits by changes in the gut's microbiota composition, especially an increase of the fecal concentrations of the bifidobacteria. The presence of healthy intestinal microbiota promotes a state of immune tolerance, which prevents the elimination of commensals and antigenic organisms from the diet, food allergies, and bowel disorders such as irritable bowel syndrome. Moreover, the consumption of prebiotics improves stool quality as measured by pH, SCFA, frequency and consistency, reduces the risk of infections and gastroenteritis, and increases Ca absorption, bone calcium accretion, and bone mineral density [9,22]. As observed in this study, yacon root flour contains reduced quantities of glucose and sucrose and high levels of FOS, which

is found in higher proportion in the yacon than in either chicory or Jerusalem artichokes (22.9/100 g and 13.5/100 g, respectively) [23,24]. Recent studies have shown positive effects resulting from yacon consumption. In adult women (31-49 years of age) with mild obesity and dyslipidemia, consumption of 0.14 or 0.29 g FOS/kg body weight over 120 days, resulted in decreased body weight, body mass index, and serum insulin, in addition to increased frequency of defecation and feelings of satiety [25]. The consumption of yacon flour containing 5% or 7% FOS for 27 days increased calcium absorption in Wistar rats, as well as an increase in the depth and number of intestinal crypts [26]. Habid and associates [27] showed hypolipidemic effects in diabetic Wistar rats that consumed yacon flour containing 340 or 6,800 mg FOS/kg body weight/d for 90 days. These effects on lipid metabolism were correlated with an increase in insulin-positive pancreatic cells within the exocrine parenchyma, although only a slight increase in plasma insulin levels was observed.

The partial or complete replacement of sucrose by the yacon flour in the diet resulted in similar levels of food intake and body weight as those observed in mice fed a standard diet. Similar observations have been reported in other experiments using diets supplemented with FOS [4,28]. The consumption of FOS (0.20 g /day/mouse) over 24 days by older female C57Bl/6J mice (33 to 35 weeks of age) from the second generation of mice fed with a diet poor in n-3-polyunsaturated fatty acids resulted in weight gain and better utilization of nutrients compared to the group fed a control diet [29].

In our study, we observed that the intake of a diet supplemented with commercial or yacon FOS resulted in no changes in serum levels of IgG and IgA. Literature data show that normal levels of IgM in sera of Balb/c mice are between 1.1 and 1.3 mg/mL [30]. In our

study, we found IgM levels ranging from 2.39 (\pm 0.149) and 1.73(\pm 0.097) mg/mL. Thus, although statistically significant, the difference in serum IgM levels observed between 3% yacon FOS and control groups seems to have no biological significance. However, the amount of fecal IgA was significantly higher in these groups compared to mice fed a standard diet. Hosono and associates showed that BALB/c mice, when fed 2.5% FOS, had increased fecal IgA levels as well as increased secretion of IgA by lymphocytes from the Peyer's patches of mice fed 2.5 % or 7% FOS, which were then cultured in bacterial extract [31]. Likewise, it was observed that the consumption of 5% FOS raised IgA levels in intestinal tissues extracts [32]. Other prebiotics, such as cicloinulooligosaccharides and isomaltooligosaccharides, have also been shown to increase fecal IgA levels in mice [33,34]. Conversely, inulin consumption did not significantly alter the levels of fecal IgA in mice [12].

The IgA can function as a high affinity system to neutralize toxins and pathogenic microorganisms or as a low affinity process to contain the dense microbiota content of the intestinal lumen [35]. Diets enriched in FOS and inulin can provoke and stimulate the intestine's mucosal immune system, and may improve the efficacy of vaccines administered orally [36]. In this study, we found increased fecal IgA in mice fed FOS in the absence of specific antigenic stimuli, which may be involved in the fixation of commensal microorganisms in the intestinal lumen.

Although we did not observe any diet-related changes in the frequency of T and B cells in the blood or spleen, our results showed that splenic lymphocytes from mice fed diets containing yacon FOS were more responsive to the ConA, in terms of proliferative response. Lymphocytes from mice fed AIN93-3% yacon also presented a more elevated

production of IL-4 and IFN- γ than splenic lymphocytes from mice fed the standard diet, although differences were statistically significant to IFN- γ . Thus, it seems to us there was no biological significance in these differences.

Despite of macrophages from mice fed FOS produced low levels of IL-1 β in response to *in vitro* stimulation with LPS, the levels of TNF- α were slightly more elevated in those groups. In consequence, no alterations in NO and IL-10 levels could be observed in macrophages supernatants, thus indicating a balanced production of pro- and anti-inflammatory mediators in macrophages by the consumption of yacon.

Several studies convey the importance of healthy microbiota in maintaining the intestinal tract's physiological and immunological functions, including inducing tolerogenesis toward exogenous antigens, such as those present in the diet [37]. The immune response against pathogens is characterized by the recognition of molecular patterns combined with strong innate responses, followed by an adaptive response to eliminate the offending agent, which often results in damage to the host's tissues. The response toward components of the symbiotic microbiota, however, is characterized by a complex integrated system of microbial recognition and inhibition of immune effector activation [38]. This process involves both the maintenance of a significant number of macrophages and dendritic cells in a state of immaturity and an appropriate balance between regulatory T (Treg) lymphocytes and "inflammatory" T lymphocyte subsets, such as Th1 and Th17 [39]. Similarly, the levels of fecal antibodies play an important role in digestive tract homeostasis. IgA is only induced at high levels in the intestines of animals with intestinal microorganisms. In germ-free mice, the numbers of IgA-producing cells are decreased almost two times [38]. Our results showed that regular consumption of yacon

FOS by mice presents no adverse effects and not alter the peripheral immune system balance, as well as encourage the production of intestinal IgA. Despite of yacon is being used in folk medicine for long time, well-designed clinical studies testing the effects of regular yacon consumption in humans are still necessary.

5. Conclusions

Our results show that regular consumption of yacon by BALB/c mice showed no adverse side effects to the whole-organism physiology, particularly to the immune system balance. This paper is the first to describe that consumption of yacon flour rich in FOS promotes increased levels of intestinal IgA.

Acknowledgements

We would like to thank to Marcos Cesar Meneguetti for animal care. Also we thanks to the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - "National Counsel of Technological and Scientific Development").

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Legends:

Table 1.- Formulation of experimental diets. Four different diets were prepared as follow:

A) AIN93M, control diet, (B) 5% FOS, (C) 3% yacon FOS, (D) 5% yacon FOS. In diets B, C, and D, sucrose was replaced by FOS or yacon flour. For diet D, the starch was also replaced.

Table 2.- Chemical composition of the experimental diets. The four diets were gravimetrically analyzed for moisture, ash, and total solids contents. Protein was measured by the micro-Kjeldahl method [13], Total lipids were measured by the Bligh & Dyer method [14]. The calories were calculated using the conversion factor for proteins 4.0, carbohydrates 4.0, and lipids 9.0.

Figure 1.- The effects of regular yacon consumption on weight of BALB/c mice: A) The average weekly consumption during the trial period was evaluated. B) The mouse weight was monitored weekly, during the diet intake. C) The percentage of weight gain variations, relative to the start of feeding. Data were analyzed using the ANOVA analysis of variance followed by the Bonferroni's test in comparison with the group that consumed AIN93 in the same period. Values of $p \leq 0.05$ (*) were considered significant (n=18).

Figure 2.- The effects of regular yacon consumption on the antibody levels in serum and feces. A-C) Serum IgG, IgM, IgA and fecal IgA, respectively. The data collected were then analyzed by the ANOVA analysis of variance followed by Bonferroni's test, with values $p \leq 0.05$ considered significant. (*) represents $p \leq 0.05$ compared with the AIN93M group (n=6). The data are representative of two independent experiments.

Figure 3.- The effect of regular yacon consumption on peripheral immune system. A) T and B-lymphocyte frequency in peripheral blood. B) T and B-lymphocyte frequency in spleen. C) Con-A induced proliferation of spleen cells. D-E) Levels of IL-4 and IFN- γ in culture supernatants. Statistical analysis was conducted using the ANOVA analysis of variance followed by Bonferroni's test, with values of $p \leq 0.05$ considered significant (n=6). (*) represents $p \leq 0.05$ compared with the AIN93M group, and (#) represents $p \leq 0.05$ when compared with the FOS group.

Figure 4.- The effects of the regular yacon consumption on NO and cytokine production by *in vitro* stimulated peritoneal macrophages. A) NO production, as measured by MTT method. B-D) Levels of IL-1 β , TNF- α and IL-10, respectively. Statistical analysis was conducted using the ANOVA analysis of variance followed by Bonferroni's test. Values of $p \leq 0.05$ were considered significant (n=6). (*) represents $p \leq 0.05$ compared with the AIN93M group, and (#) represents $p \leq 0.05$ compared with the FOS group.

Tables:

Ingredients (g/Kg)	AIN 93M	FOS 5%	YACON 3%	YACON 5%
Cornstarch	465,69	465,69	465,69	425,69
Casein (>= 85%)	140,0	140,0	140,0	140,0
Dextrinized cornstarch	155,0	155,0	155,0	155,0
Sucrose	100,0	52,5	7,0	0,0
FOS	0,0	47,5	0,0	0,0
Yacon root	0,0	0,0	93,0	140,0
Soybean oil (no additives)	40,0	40,0	40,0	40,0
Fiber	50,0	50,0	50,0	50,0
Mineral mix	35,0	35,0	35,0	35,0
Vitamin mix	10,0	10,0	10,0	10,0
L-cystine	1,8	1,8	1,8	1,8
Choline bitartrato	2,5	2,5	2,5	2,5
Tert-butylhidroquinona	0,008	0,008	0,008	0,008

Table 1

Components	AIN93M	FOS 5%	YACON 3%	YACON 5%
Moisture	8,02±0,28	8,13±0,53	9,85±0,48	9,60±0,00
Total Solids	91,98±0,28	91,87±0,53	90,15±0,48	90,40±0,00
Protein	11,48±0,31	11,47±0,03	13,08±0,77	12,11±0,12
Lipids	4,27±0,13	4,09±0,20	4,53±0,13	4,18±0,08
Ash	2,62±0,10	2,68±0,05	3,12±0,17	2,93±0,18
Carbohydrate	73,61	73,63	69,42	71,19
Energy Kcal /100g	378,78	377,19	370,78	370,77

Table 2

Figures

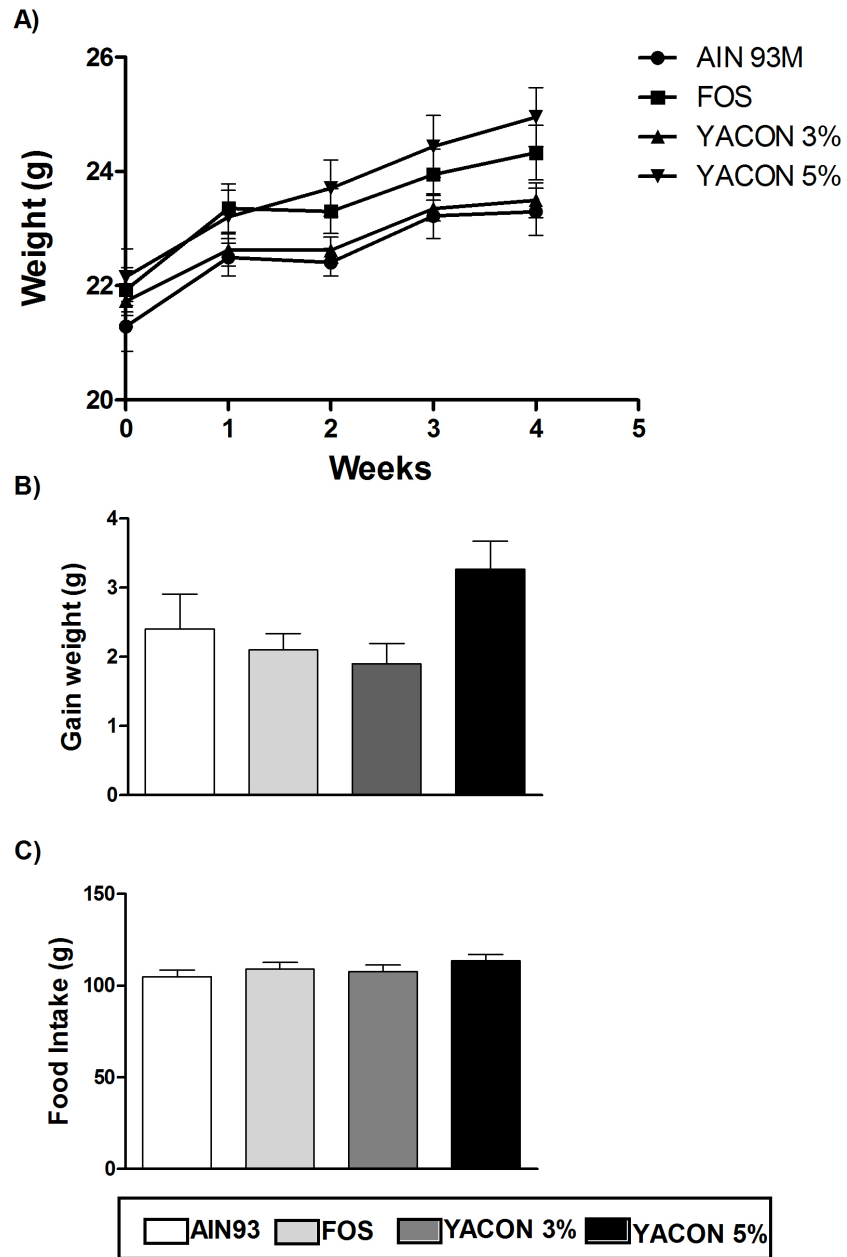


Figure 1.

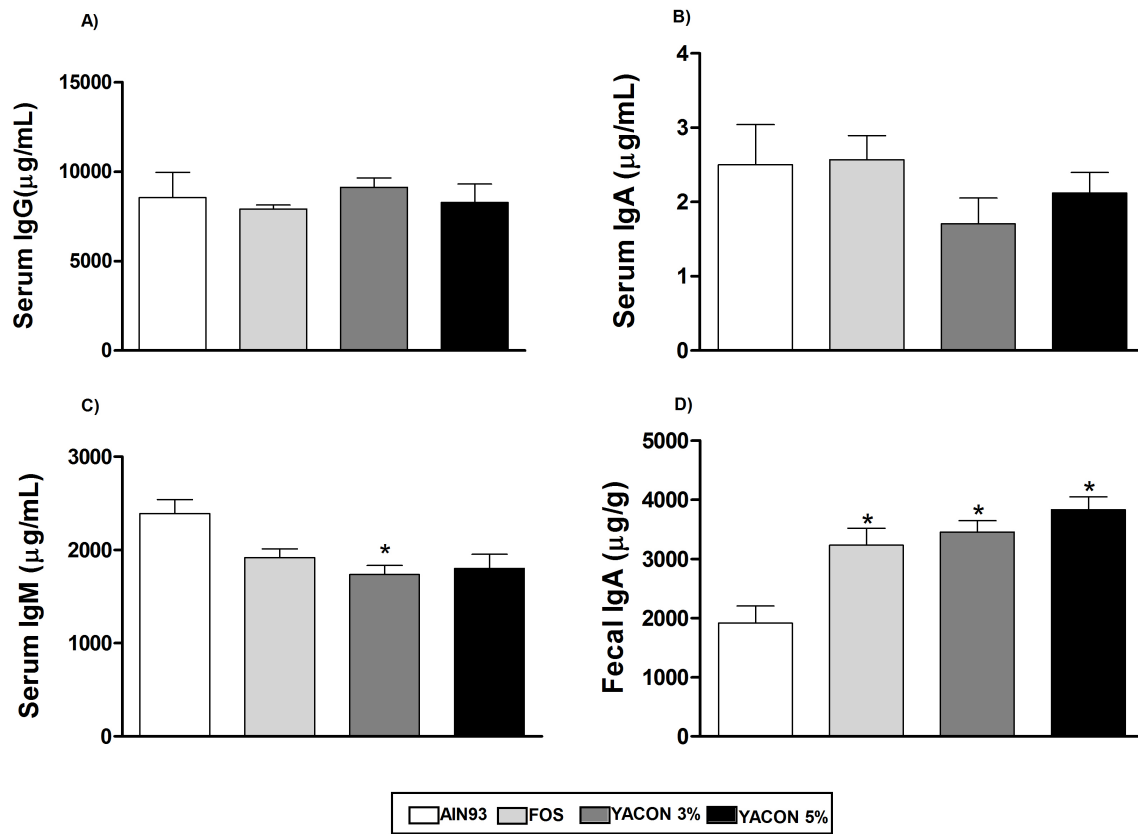


Figure 2

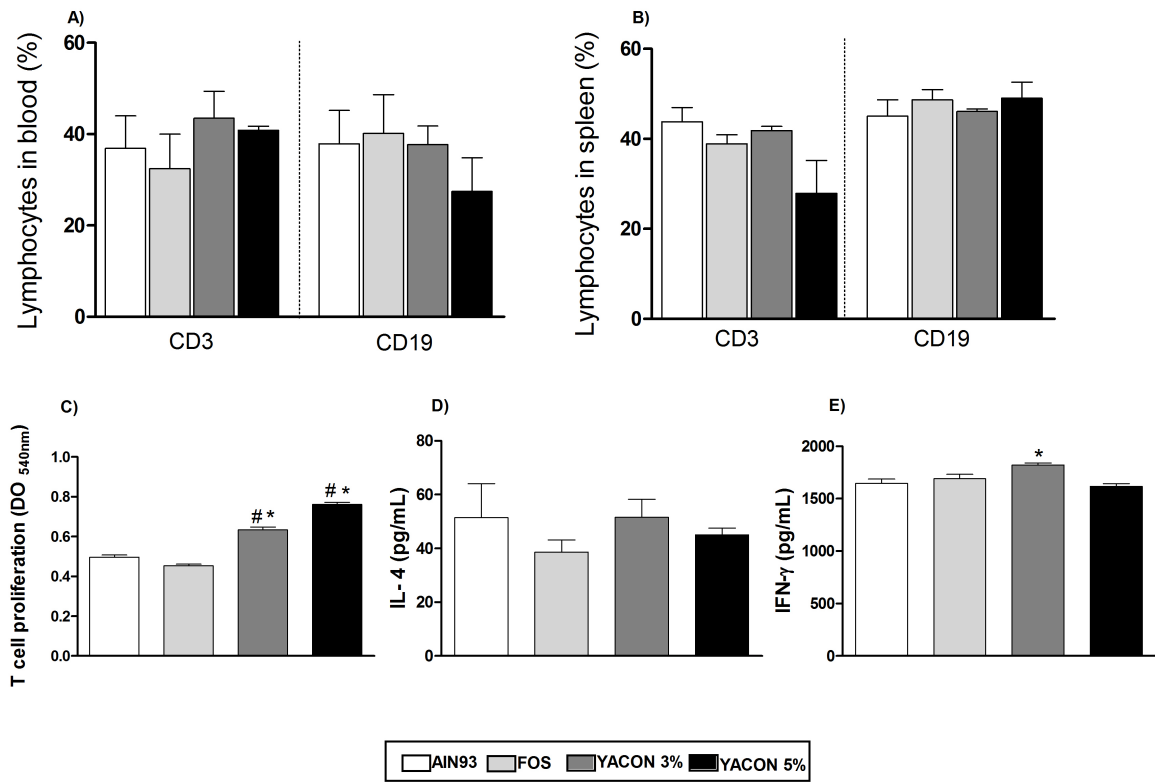


Figure 3

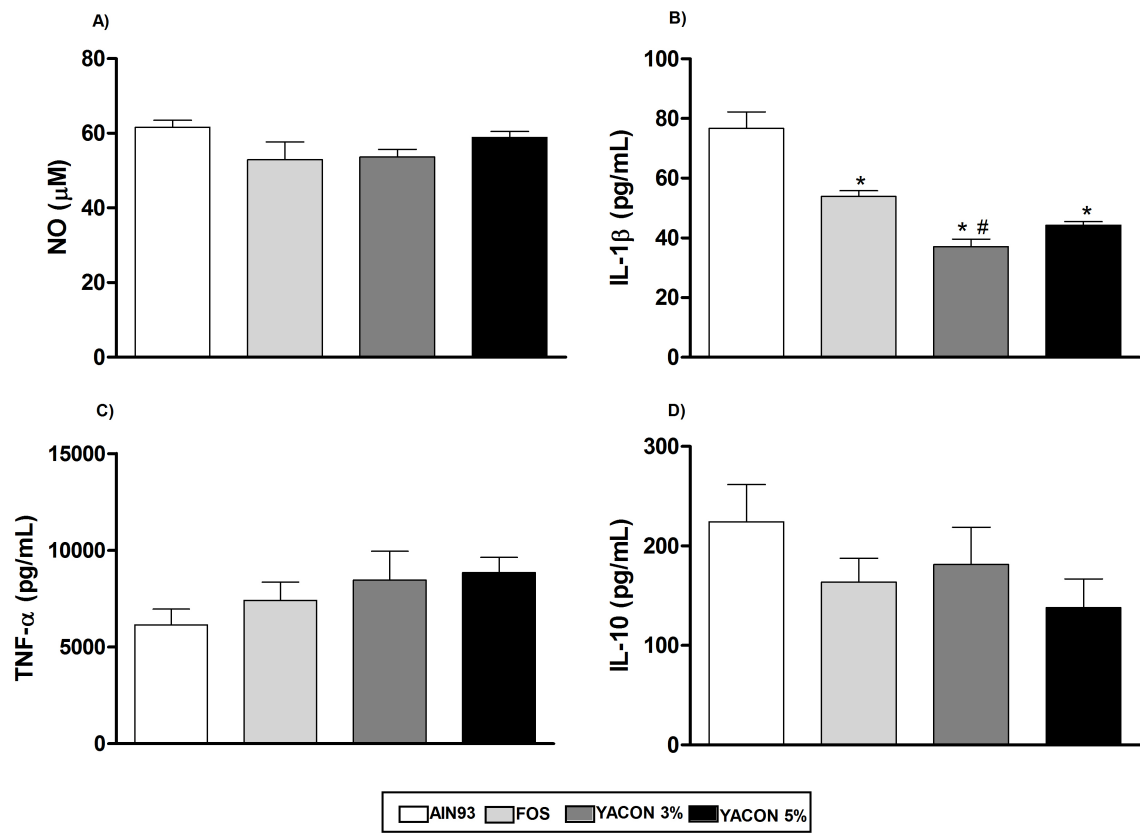


Figure 4

CAPÍTULO 5

THE EFFECTS OF THE REGULAR INTAKE OF YACON ROOT (*Smallanthus sonchifolius*) ON TNBS-INDUCED COLITIS IN MICE

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This manuscript was submitted to Plant Foods for Human Nutrition.

Abstract

Fructooligosaccharides (FOS) have been shown to improve intestinal function, increase resistance against pathogens and promote immunomodulation. The yacon root contains a high content of FOS and inulin. The aim of this study was to evaluate the effects resulting from the incorporation of yacon flour into the diet of mice with TNB-induced colitis. Six-week-old female BALB/c mice were fed (1) the control diet AIN-93M, (2) a 5% commercial FOS diet and (3) a 5% yacon FOS diet. Thirty days later, half of each group was challenged by the intrarectal administration of TNBS, while the others received vehicle (ethanol). The mice challenged with TNBS and fed a diet containing 5% yacon FOS suffered significantly less weight loss compared to animals fed the control diet or commercial FOS. Spleen T lymphocytes of mice with colitis did not respond to TNBS *in vitro*. The intake of the AIN93M, FOS and yacon diets did not induce significant changes in Con-A induced T cell proliferation. The production of the cytokines TNF- α and IFN- γ by these cultures was high in all experimental groups. IL-17 was increased in groups fed FOS, while IL-10 was lower. The macroscopic and histological analysis of sections of the large intestine from yacon FOS-fed mice with colitis showed a lower degree of inflammation and better preservation of the structure of the intestinal wall in comparison with the other groups. Altogether, our data indicate that the regular consumption of yacon FOS results in the moderate protection of the mouse mucosal immune system against inflammatory agents.

Keywords: yacon root (*Smallanthus sonchifolius*), (FOS), colitis, mucosa, IBD.

1. Introduction

The mucosal immune system is continuously exposed to massive amounts of foreign antigens derived from microbes and the diet. This environment is usually a non-inflammatory Th2 type, as evidenced by the predominance of IgA and IgG and the secretion of the cytokines IL-4, IL-10 and TGF- β by NKT cells and intestinal epithelial cells [1]. In contrast to the peripheral immune system, the microenvironment of mucosal surfaces inhibits the development of inflammatory immune responses in a scenario where the tolerance of foreign materials is the norm rather than the exception [2].

Breaking of tolerance to microbial antigens has been identified as one of the main factors leading to the development of intestinal disorders such as Crohn's disease and ulcerative colitis, which are collectively called Inflammatory bowel disease (IBD) [1,3]. The reduced expression of junction proteins and increased apoptosis of epithelial cells, which allows luminal antigens derived from non-pathogenic microorganisms to come into contact with cells of the mucosal immune system, have also been implicated in the pathogenesis of colitis [4-6]. The activation of dendritic cells (DCs) by flagellin derived from commensal bacteria appears to be one of the initial events in the development of IBD in humans and experimental models [7]. The activation of DCs via Toll like receptors results in the increased production of inflammatory cytokines such as TNF- α and a reduction in the regulatory cytokines normally present in this microenvironment, such as TGF- β and IL-10. Consequently, there is a marked reduction in the number of regulatory T cells from the CD4⁺ CD25⁺ Foxp3⁺ subpopulation, which favors the formation installation of an inflammatory environment [8,9].

The conventional therapeutic approaches for IBD involve the use of drugs that down-regulate the immune system, such as corticosteroids, aminosalicylates, azathioprine or infliximab (anti-TNF- α) [6,10].

Recently, new therapeutic approaches for IBD that include the consumption of probiotics [11], prebiotics [12-14] and symbiotics [15] have shown promising results. The aim of these therapies is the manipulation of the intestinal microbiota into a more appropriate profile with regard to the diversity of species and products derived from microbial metabolism [16].

Prebiotics like FOS and inulin have shown a more immediate potential therapeutic use [17]. It has been observed that the consumption of these compounds leads to an increase in bifidobacteria in the intestinal tract, with beneficial consequences for the mucosal immune system [18,19]. FOS/inulin is present in high amounts in many plants used as food, such as chicory, Jerusalem artichokes, asparagus, honey, onions, garlic, barley and bananas, among others [20-22]. The yacon, a root used in traditional Andean medicine for the prevention of diabetes and metabolic syndrome, also represents an abundant source of FOS/inulin [23,24].

The literature, however, lacks studies on the effects that the regular consumption of yacon has on the immune system in inflammatory conditions of the intestinal tract, such as IBD. This manuscript aims to evaluate the physiological and immunological effects of regular consumption of yacon on TNBS-induced colitis in young mice.

2. Material and Methods

2.1. Animals

Female BALB/c mice were obtained from the Multidisciplinary Center for Biological Research (CEMIB) at UNICAMP. The animals were housed in metabolic cages and were kept under specific pathogen free (SPF) conditions with a light / dark cycle of 12 hours, temperature of $22 \pm 2^{\circ}\text{C}$ and water and food *ad libitum*. The experimental protocols were approved by the Ethics Committee on the Use of Animals (CEUA), UNICAMP, under no. 1659-2.

2.2. Yacon

The roots of yacon were grown in the region of São Paulo, Brazil, and subjected to freeze-drying and grinding to obtain the flour that was maintained at -80°C until use. The chemical composition of yacon flour was similar to that reported previously [25].

2.3. Diets

The control AIN93 maintenance diet was prepared according to the specifications of Reeves and colleagues [26]. In the experimental diets, sucrose and starch were replaced by FOS or yacon flour, as described previously [25]. Six-week-old BALB/c mice were divided into the following groups (n = 12): (1) control diet AIN93M; (2) 5% commercial FOS diet and; (3) 5% yacon FOS diet. Mice were fed with these diets for 4 weeks before the induction of colitis.

2.4. Induction of colitis

Colitis was induced in 10-week-old BALB/c mice according to Neurath and colleagues [27] with modifications. Briefly, half of each group of mice was challenged by the rectal administration of 0.5mg TNBS/100µl 50% ethanol, and the other half received only the vehicle (100µl 50% ethanol) for seven consecutive days. The food intake and weight of the mice were monitored weekly for the four weeks before the induction of colitis and daily for seven days after the induction of colitis.

2.5. T lymphocyte proliferation

The spleens were collected individually for the preparation of erythrocyte-free cell suspensions. The cells were resuspended in RPMI medium with 10% fetal bovine serum, seeded into 96-well plates (Corning, MA, USA) at a density of 2.5×10^5 cells per well and incubated for 48 hours at 37°C and 5% CO₂ in the presence of 2.5µg/mL Concanavalin A (Con-A, Sigma, USA) or 50µg/mL TNBS-ovalbumin (OVA) previously prepared in our laboratory [28]. After incubation, the supernatants were collected and stored at -80°C for cytokine analysis. Cell proliferation was determined by the addition of MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) to the cultures. After the dissolution of formazan crystals, the plates were read in a spectrophotometer at 540nm. All tests were performed in sextuplicate. The proliferation results were expressed as the mean \pm SEM absorbance obtained for each treatment.

2.6. Cytokine measurement

IFN- γ , IL-4, IL-10, IL-17 (eBioscience; San Diego, CA. USA) and TNF- α (OptEIA, BD Biosciences; San Diego, CA. USA) were quantified by ELISA using commercial kits according to the manufacturer's instructions.

2.7. Histological analysis

The intestines were removed, sectioned in the region of the cecum and photographed. The intestinal segments were fixed in 1% buffered paraformaldehyde, embedded in paraffin, cut using a microtome (cutting thickness of 6 μ m) and stained with hematoxylin/eosin (H/E) for histopathological analysis.

2.8. Statistical analysis

The experimental data were analyzed using ANOVA followed by Bonferroni's test. Analyses were performed with GraphPadPrism software, with $p \leq 0.05$ considered statistically significant.

3. Results and Discussion

TNBS-induced colitis is an experimental model that mimics many inflammatory events observed in human diseases such as ulcerative colitis and Crohn's disease. It is characterized by weight loss, bloody diarrhea and disorganization of the intestinal architecture [29,30]. FOS oligosaccharides are well known for their beneficial effects on the body, particularly on the composition of intestinal microbiota and the modulation of the

mucosal immune system [18,31]. Recent evidence has shown that these compounds play an important role in preventing the clinical manifestations of experimental colitis [32,33]. In a previous study, we showed that substituting the sucrose and starch in the standard diet with an equivalent amount of yacon FOS showed no significant alterations in weight gain and the humoral immune responses associated with the intestinal mucosa [25]. Data obtained in this work confirm our initial findings, as BALB/c mice fed a diet containing commercial or yacon FOS had food intake and weight gain levels comparable to those of animals fed the standard AIN93M diet (Fig. 1). Colitic mice fed either the standard diet or commercial FOS showed an accentuated loss of weight, whereas those fed yacon FOS showed no alteration in comparison with animals in control group (Fig. 2B and C). Ribeiro [34] showed that the yacon meal contains high levels of phenolic acids, and is well known that phenolic compounds may act synergistically with FOS to produce its beneficial effects. Thus, the association between FOS and phenolic compound in yacon flour may be responsible for the protection against cachexia observed in those group of mice fed diet containing yacon.

Macroscopic examination of intestinal pieces shows that yacon-based diets seem to protect mice against TNBS-induced inflammation as characterized by the absence of bloody feces, integrity of intestinal walls and formation of stool. The intestines of mice fed yacon FOS showed no infiltrating leukocytes, as opposed to what we observed in the intestines of mice that consumed the standard diet or commercial FOS. The epithelial layer of the large intestine of mice fed a diet including yacon was also better preserved than the epithelial layers in the other groups with colitis (Fig. 2A). The benefits of prebiotic consumption have been attributed to the way in which these compounds are metabolized in the intestinal tract [35]. It has been shown that the ingestion of such food favors an increase in the proportion of symbiotic bacteria such as *Lactobacillus* and *Bifidobacterium*, which

produce short chain fatty acids specially butyrate, in the intestinal flora [36,37]. The metabolites produced by these intestinal bacteria play an important role in the intestinal mucosal immune system, favoring the development of an environment that inhibits inflammatory responses [38,39]. The destruction of the epithelial layer of the intestinal mucosa is an important element in the establishment of colitis in both humans and experimental models [1,2]. It is possible that the protection against the establishment of a more severe TNBS-induced colitis in yacon-fed mice may due to a prebiotic effect of yacon. In previous study, we observe an elevation of the levels of fecal IgA in absence of infection and inflammation that support this idea [25]. New researchs, however, are necessary to determine the effects that regular yacon consumption has on the intestinal microbiota and their products in both mice and humans.

To assess the systemic effects of FOS and yacon consumption on the adaptive immune response, spleen cells were stimulated with ConA or TNBS to determine cell proliferation and the production of pro- and anti-inflammatory cytokines. Figures 3 and 4 summarize the results obtained in these experiments. These figures show that stimulating splenic lymphocytes with TNBS did not cause spleen cells from any of the experimental groups to proliferate (Fig. 3A). Likewise, pro- and anti-inflammatory cytokine levels in these cultures did not differ significantly from unstimulated cultures of spleen cells from mice fed standard diet or a diet containing commercial FOS (Fig. 3B-F). However, TNF- α and IFN- γ were found augmented in the supernatants of TNBS-stimulated cultures of spleen cells from mice fed yacon FOS. It has been shown that TNBS-induced colitis in BALB/c mice is a Th2 model of the disease, and sometimes a mix Th2/Th1 model. The balance of Th2/Th1 response may be related to the contribution of normal microbiota and its mitogens (LPS, CpGs, etc) in activation or not of the IL-12 pathway in antigen

presenting cells [2]. However, consumption of yacon favors the imbalance between Th2/Th1 responses with the predominance of a Th1 response. On the other hand, IL-17 that now has been implicated as the main responsible for damages in bowel diseases [40] was not significantly altered by consumption of FOS.

The regular consumption of commercial FOS and yacon FOS did not affect the proliferative capacity of T lymphocytes in response to ConA, showing that cells from different experimental groups of mice were equally responsive to non-specific stimulation (Fig. 4A). However, stimulation with ConA resulted in the increased production of Th1-type cytokines (TNF- α and IFN- γ) in the cultures of spleen cells from all experimental groups, regardless of the diet consumed (Fig. 4B and C). Surprisingly, IL-17 production was significantly higher in the cultures of splenocytes from mice fed FOS (Fig. 4D). Moreover, the production of IL-10 was lower in the cultures of cells isolated from mice fed a diet containing commercial FOS or yacon FOS (Fig. 4F). Arribas and colleagues [32] also found that the consumption of FOS protects against the deleterious effects of TNBS-induced colitis. However, the effect observed by these authors was associated with reduced levels of colonic pro-inflammatory cytokines, myeloperoxidase and nitric oxide. The contradictory results we obtained may be attributed to the source of cells used in the study. Further studies are essential to clarify the interference of yacon consumption on the intestinal immune response, particularly in lymphocytes from intestinal sites such as mesenteric lymph nodes, Peyer's patches and lamina propria.

5. Conclusion

Taken together, our data show that the regular consumption of yacon FOS reduces the clinical signs of colitis in mice by mechanisms that remain to be elucidated but may be related to changes in the microbiota and the cell response profile in the intestinal microenvironment.

Acknowledgement

We would like to thank to Dirce Lima Gabriel for the support in the experiments and Marcos Cesar Meneguetti for animal care. Also we thanks to the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - "National Counsel of Technological and Scientific Development").

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Legends:

Figure 1.- The effects of regular yacon consumption on body weight and food intake. Six-week-old BALB/c mice (n=12) consumed diets containing 5% commercial FOS, 5% yacon FOS or a control diet (AIN-93M) for thirty consecutive days. The results were analyzed using ANOVA followed by Bonferroni's test. P values ≤ 0.05 (*) in comparison with the group that consumed AIN93M were considered significant.

Figure 2.- Effects of regular consumption of yacon on clinical signs of colitis. Six-week-old BALB/c mice (n=12) consumed diets containing 5% commercial FOS, 5% yacon FOS or a control diet (AIN-93M) for thirty consecutive days. For the induction of colitis, half of each group of mice was immunized and challenged with 0.5 mg TNBS/100 μ l 50% ethanol per rectum, while the controls received the vehicle (ethanol) for seven consecutive days. A) Macroscopic and histological evaluation of the large bowel (intestines were dissected, fixed in paraformaldehyde, embedded in paraffin and processed for histological analysis with H/E). Magnification: 400X . B-C) Body weights at the week of colitis induction and at the end of the experiment. Data were analyzed using ANOVA followed by Bonferroni's test. P values ≤ 0.05 (*) in comparison with the group that consumed AIN93M were considered significant.

Figure 3.- The proliferation and cytokine production of antigen-specific T cells. Six-week-old BALB/c mice (n=12) consumed diets containing 5% commercial FOS, 5% yacon FOS or a control diet (AIN-93M) for thirty consecutive days. For the induction of colitis, half of

each group of mice was immunized and challenged with 0.5 mg TNBS/100µl 50% ethanol per rectum, while the controls received the vehicle (ethanol) for seven consecutive days. (A) At the end of the experiment, spleen cells were collected and placed in culture in the presence of a specific stimulus (50µg/mL TNBS-OVA). (B-F) The culture supernatants were collected and assayed for TNF- α , IFN- γ , IL-17, IL-4 and IL-10. Statistical analysis was performed by ANOVA followed by Bonferroni's test. P values ≤ 0.05 were considered significant; (#) represents a p value ≤ 0.05 compared with the AIN93 group; (*) represents a p value ≤ 0.05 compared with the baseline group (no stimulus).

Figure 4.- The proliferation and cytokine production of T cells stimulated with ConA. Six-week-old BALB/c mice (n=12) consumed diets containing 5% commercial FOS, 5% yacon FOS or a control diet (AIN-93M) for thirty consecutive days. For the induction of colitis, half of each group of mice was immunized and challenged with 0.5 mg TNBS/100µl 50% ethanol per rectum, while the controls received the vehicle (ethanol) for seven consecutive days. (A) At the end of the experiment, spleen cells were collected and placed in culture in the presence of 2.5µg/mL ConA. (BF) The culture supernatants were collected and assayed for TNF- α , IFN- γ , IL-17, IL-4 and IL-10. Statistical analysis was performed by ANOVA followed by Bonferroni test. P values ≤ 0.05 were consider significant; (#) represents a p value ≤ 0.05 compared with the AIN93M group; (*) represents a p value ≤ 0.05 compared with the baseline group (no stimulus).

Figures

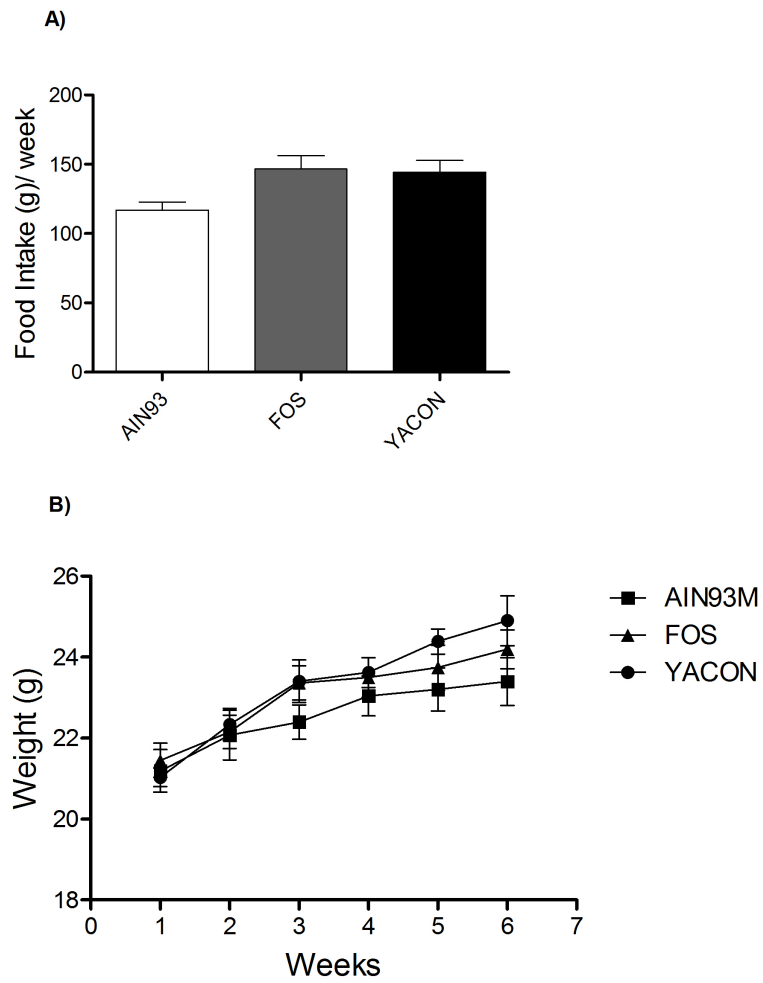
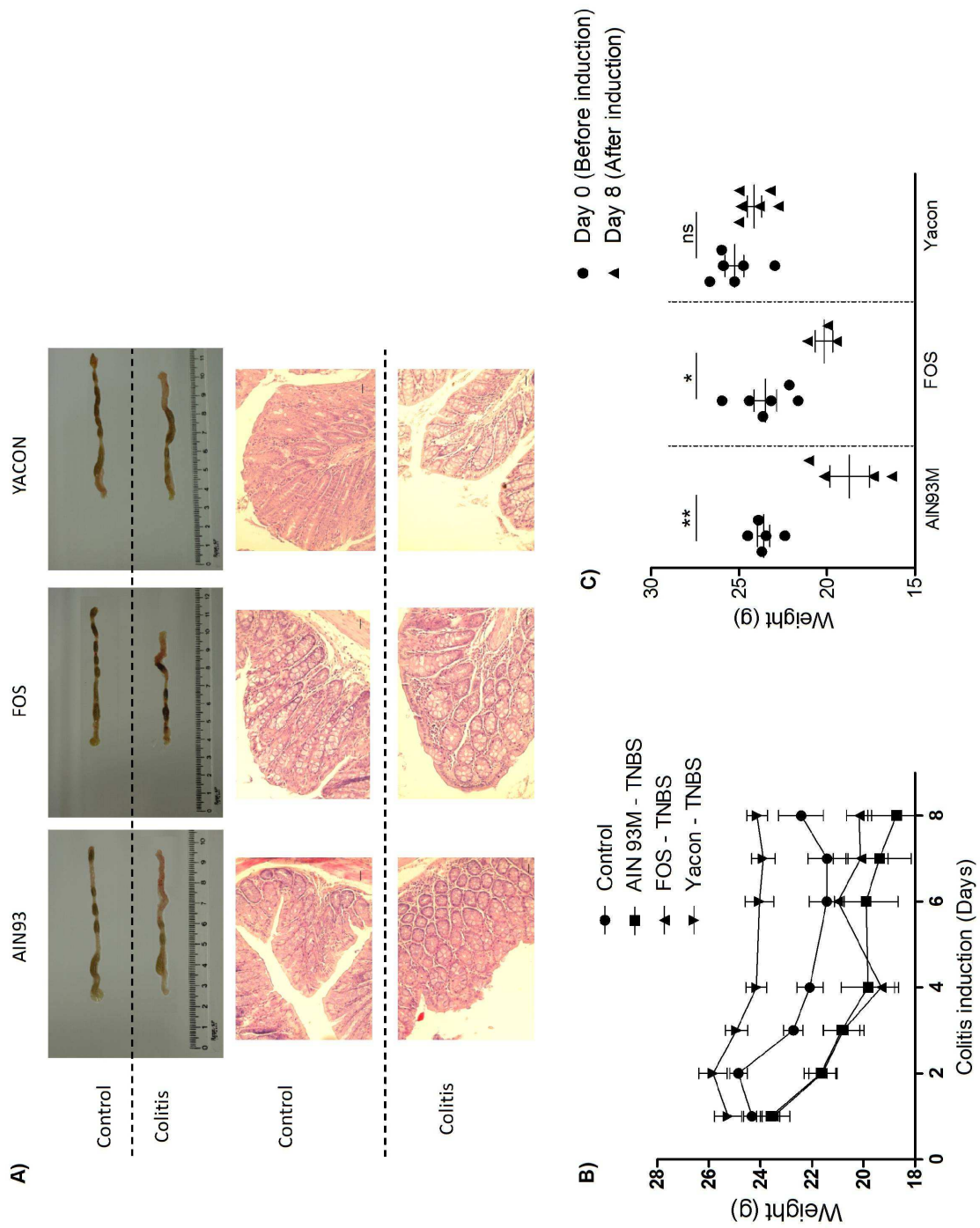


Figure 1

Figure 2



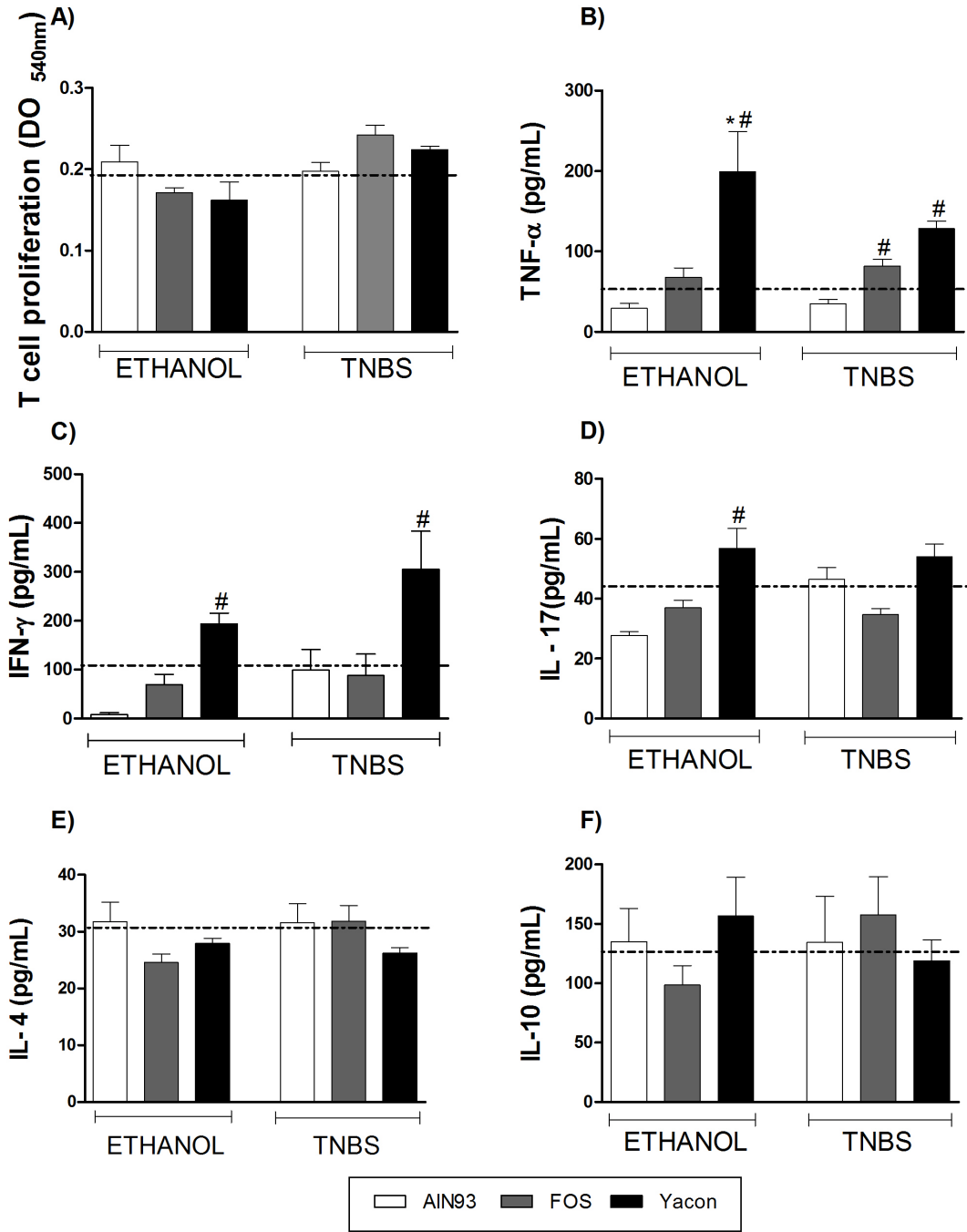


Figure 3

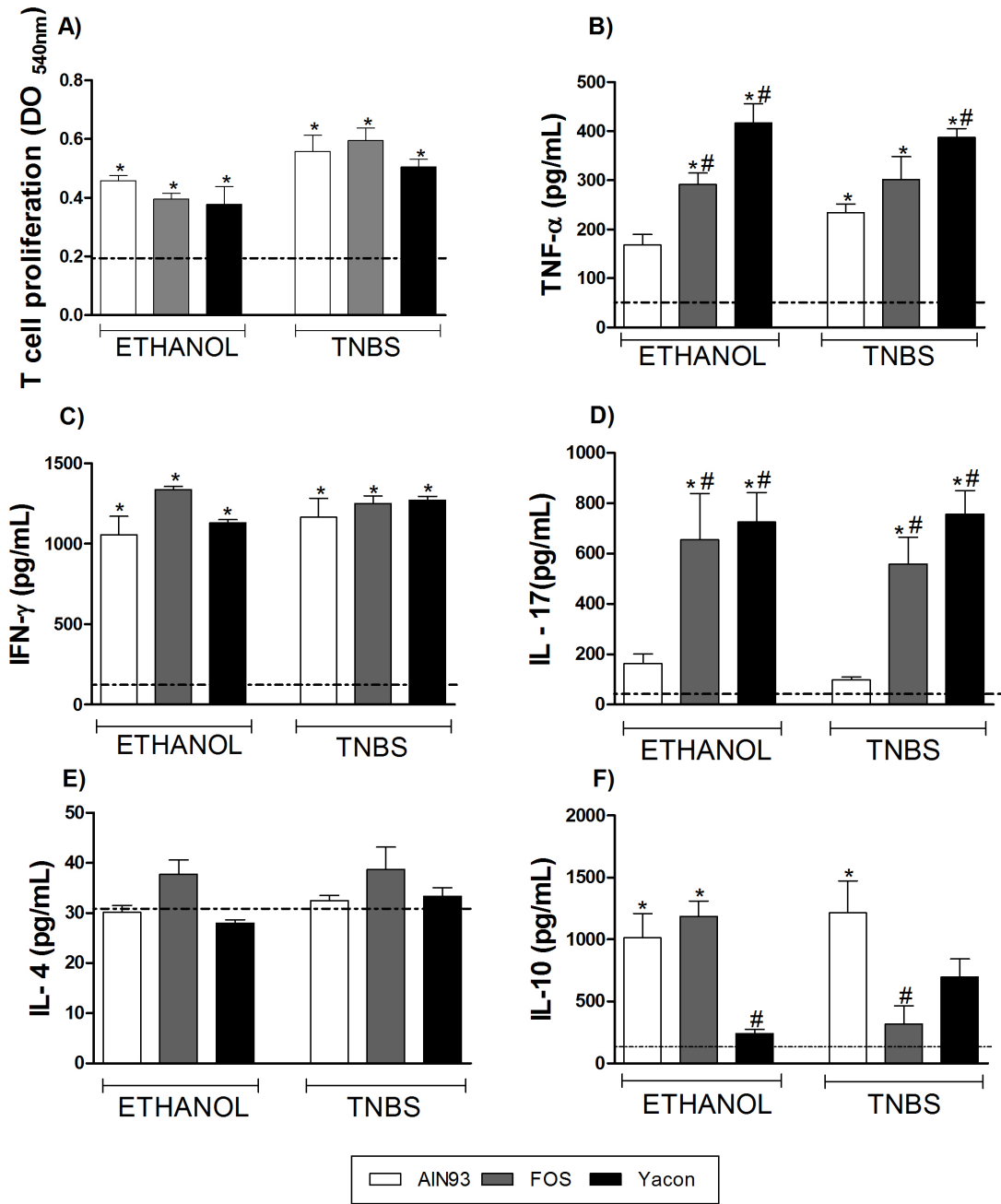


Figure 4

CONCLUSÕES GERAIS

Considerando que os frutanos de tipo inulina e frutooligosacarídeos agem como agentes bifidogênicos, nos propusemos neste trabalho a estudar o efeito do consumo regular de yacon sobre o sistema imune murino em condições fisiológicas e após a indução de doença inflamatória intestinal provocada pela administração de TNBS.

Os resultados obtidos no presente estudo indicaram que:

- O consumo regular de yacon não acarreta efeitos deletérios ao sistema imune murino. Os animais que consumiram dieta suplementada com FOS de yacon mantiveram os mesmos padrões de perfil celular, secreção de citocinas, níveis séricos de anticorpos e proliferação celular observados nos animais alimentados com dieta padrão;
- O yacon parece possuir propriedades prebióticas, favorecendo a expansão de microbiota mais adequada, uma vez que o consumo de dieta contendo yacon levou à elevação dos níveis de IgA intestinal na ausência de infecção;
- A alimentação com dieta contendo FOS de yacon previne pelo menos parcialmente o desenvolvimento de reações inflamatórias no trato intestinal. Camundongos alimentados com yacon tornaram-se menos susceptíveis ao desenvolvimento de sintomas clínicos da colite experimental induzida por TNBS, embora os parâmetros imunológicos não tenham sido completamente modulados.

Desta forma, consideramos que o yacon possui grande potencial como alimento funcional devido a sua elevada concentração de FOS e inulina e pelos efeitos ora observados sobre o sistema imune em murinos.

SUGESTÕES DE PESQUISAS FUTURAS

Os estudos de consumo de yacon em humanos são escassos e novas pesquisas são necessárias para avaliar seu potencial imunomodulador em uma situação de desafio, como nas doenças inflamatórias do trato intestinal. Ainda se faz necessário determinar as dosagens ótimas, durações de tratamento e os efeitos específicos de consumo de yacon em matrizes e populações diferentes, como infantes, idosos e imunosuprimidos. Além disso, é importante avaliar os efeitos do consumo regular de yacon sobre a composição da microbiota intestinal murina e humana.

ANEXO 1: Aprovação da Comissão de Ética no Uso de Animais



UNICAMP



CEEA/Unicamp

**Comissão de Ética na Experimentação Animal
CEEA/Unicamp**

CERTIFICADO

Certificamos que o Protocolo nº 1659-2, sobre "Avaliação do efeito modulatório do consumo regular de Yacon (Polymnia sonchifolia) sobre o sistema imune", sob a responsabilidade de Profa. Dra. Glaucia Maria Pastore / Grethel T. Choque Delgado, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal – CEEA/Unicamp em 06 de novembro de 2008.

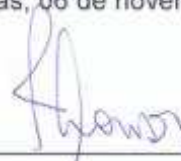
CERTIFICATE

We certify that the protocol nº 1659-2, entitled "Evaluation of the modulatory effect about regular consumption of Yacon (Polymnia sonchifolia) on the immune system", is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on November 6, 2008.

Campinas, 06 de novembro de 2008.



Profa. Dra. Ana Maria A. Guaraldo
Presidente



Fátima Alonso
Secretária Executiva

DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha dissertação /tese de Doutorado intitulada "AVALIAÇÃO DOS EFEITOS DO CONSUMO REGULAR DE YACON (*Smallanthus sonchifolius*) SOBRE O SISTEMA IMUNE MURINO":

não se enquadra no § 3º do Artigo 1º da Informação CCPG 01/08, referente a bioética e biossegurança.

Tem autorização da(s) seguinte(s) Comissão(ões):

CIBio – Comissão Interna de Biossegurança , projeto No. _____, Instituição: _____

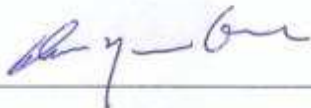
CEUA – Comissão de Ética no Uso de Animais, projeto No. 1659-2, Instituição: UNICAMP

CEP - Comissão de Ética em Pesquisa, protocolo No. _____, Instituição: _____

** Caso a Comissão seja externa ao IB/UNICAMP, anexar o comprovante de autorização dada ao trabalho. Se a autorização não tiver sido dada diretamente ao trabalho de tese ou dissertação, deverá ser anexado também um comprovante do vínculo do trabalho do aluno com o que constar no documento de autorização apresentado.*



Aluno: Grethel Teresa Choque-Delgado



Orientador: Gláucia Maria Pastore

Para uso da Comissão ou Comitê pertinente:

Deferido Indeferido

Carimbo e assinatura



Profa. Dra. ANA MARIA APARECIDA GUARALDO
Presidente da Comissão de Ética no Uso de Animais
CEUA/UNICAMP

Para uso da Comissão ou Comitê pertinente:

Deferido Indeferido

Carimbo e assinatura