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Fructan extracts from wheat stem and barley grain stimulate large bowel fermentation in rats

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ABSTRACT

The benefits of inulin-type fructans for bowel health are well established, but less so for other fructan sources. In vitro data suggest that fructans extracted from cereals are readily fermented and produce favorable short-chain fatty acid profiles; however, whether this occurs in vivo is unknown. We hypothesized that in rats, fructans extracted from wheat stem and barley grain would have similar effects on fermentation as oligofructose (OF). Fifty-six male Sprague-Dawley rats were randomly assigned to 1 of 7 dietary treatments that contained either 2% or 5% fructan, provided by a barley grain fructan extract (BGFE), a wheat stem fructan extract, or OF or no added fructan (control). The duration of the feeding study was 14 days. Rats fed diets containing 5% fructan had higher cecal digesta weights; larger acetate, propionate, and total short-chain fatty acid pools; and lower pHs in comparison with the control group. In addition, only the 5% OF and 5% BGFE groups increased cecal butyrate pools, and 5% BGFE was the only group in which colonic digesta pH was lower than that of the control. Diets containing 2% fructan did not affect any of these fermentation end points. Whereas bifidobacteria numbers in cecal digesta of 2% and 5% OF were higher than that in the control group, they were not different from those in rats fed diets containing BGFE and wheat stem fructan extract. Barley grain and wheat stem fructans produced similar large bowel fermentation patterns to OF when fed to rats at 5% of the diet.

1. Introduction

There is considerable interest in the role inulin-type fructans can play in improving gut health and bowel function [1]. Fructans escape digestion in the small intestine because digestive enzymes are unable to hydrolyze the β-linked fructose units. In the colon, fructans are rapidly fermented by specific bacterial species, particularly bifidobacteria and lactobacillus that are preferentially stimulated to grow, thus causing significant changes in the composition of the gut microbiota by increasing the proportion of putative health-promoting bacteria or reducing the number of potentially harmful species [2,3]. Fructans increase large bowel digesta biomass and water content of the stools, thereby improving bowel habit [1].

Although fructans from chicory and Jerusalem artichoke are commonly studied for their effects on large bowel

Abbreviations: BGFE, barley grain fructan extract; DP, degree of polymerization; OF, oligofructose; qPCR, quantitative polymerase chain reaction; SCFA, short-chain fatty acid; WSFE, wheat stem fructan extract.

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fermentation, there are a variety of other fructan sources including cereals (eg, barley, wheat, and oat), forage grasses (eg, *Lolium* and *Festuca*), vegetables (eg, onion and lettuce), and tuberous ornamentals (eg, dahlia and tulip) [4]. Fructans in temperate cereals, such as wheat and barley, differ in molecular structure from inulin and oligofructose (OF) and contain a mixture of β-2,1-linkages and β-2,6-linkages between fructose residues, whereas inulin and OF are exclusively linear polyfructoses with β-2,1-linkages only. In addition, the degree of polymerization (DP) for cereal fructans is generally less than 20 [5], whereas that for chicory inulin ranges from 2 to 60, and for OF, it is much lower, ranging from just 2 to 8. Differences in DP between inulin and OF can affect fermentation rates and bifidobacteria numbers [6]. In addition, we have recently shown that the in vitro fermentation of OF and inulin by human fecal bacteria produced different molar ratios of short-chain fatty acid (SCFA) in comparison with alternative sources of fructans derived from barley grain and wheat stem [5].

The primary hypothesis of this study was that rats fed cereal fructans will have comparable effects on cecal fermentation, pH, and bacteria numbers as compared with those fed OF. The fructans were included at 2% and 5% of the diet to determine the minimum amount of each fructan necessary to elicit improvements in indices of bowel health.

2. Methods and materials

2.1. Rats and diets

Five-week-old male Sprague-Dawley rats (n = 56) of approximately 150 g were obtained from the Animal Resource Centre, Perth, Western Australia. Rats were housed in wire-bottomed cages in a room of controlled heating and lighting (23°C with a 12-hour light/dark cycle) and had free access to food and water. After arrival, the rats were adapted to a nonpurified commercial diet for 5 days. They were allocated randomly to 7 groups (n = 8 per group) and fed 1 of 7 diets (Table 1) for 2 weeks.

The diets, based on AIN-93G formulation, contained 2% or 5% fructan as OF (Beneo P95; Orafti, Oreye, France), a novel barley grain fructan extract (BGFE), or a wheat stem fructan extract (WSFE) (Table 1). The BGFE was isolated from mature mutant barley Himalaya 292 grains [8], and the WSFE was isolated from dried, ground wheat stem collected around anthesis. These fructan extracts were isolated and freeze-dried by procedures scaled up from those described previously [5], except that an additional charcoal clean-up step of the aqueous phase was included after removal of lipids, and extract components were not size separated. These extracts also contained lesser amounts of simple sugars, with the total carbohydrates in each fraction accounting for more than 90% of the mass (Table 1), and because lipid, protein, nitrogen, and insoluble residue was absent, the remaining fraction is likely to be tightly bound water. An additional diet was included, which did not contain any added fructan (control). The amount of fructan added to each diet was balanced using sucrose. All diets contained 5% α-cellulose as a fiber source. The diet and drinking water were freely available to the animals for the duration of the study.

The total soluble carbohydrate levels in the fructan extracts and diets were quantified by the anthrone method [9], adapted to allow measurement in a microtiter plate, using fructose as a standard. The composition profile was determined by high-performance anion-exchange chromatography using a CarboPac PA-100 column on a Dionex DX600 system (Sunnyvale, CA, USA), as described previously [10]. Analysis of the soluble carbohydrate extracts of the diets (Fig. 1) shows that the WSFE and BGFE diets comprised different compositions of sugars and fructo-oligosaccharides components than the OF diet and that the WSFE diet contained greater amounts of higher DP oligosaccharides than the BGFE diet.

Table 1 – Ingredient composition and energy density of the experimental diets fed to rats

	Control	OF		BGFE [a]		WSFE [b]	
		2%	5%	2%	5%	2%	5%
Ingredients (g/kg)							
OF	0	21.5	53.7	0	0	0	0
Barley fraction	0	0	0	31.9	79.6	0	0
Wheat stem fraction	0	0	0	0	0	31.7	79.2
Cornstarch	529.5	529.5	529.5	529.5	529.5	529.5	529.5
Casein	200	200	200	200	200	200	200
Sugar	100	78.5	46.3	68.1	20.4	68.3	20.8
Sunflower seed oil	70	70	70	70	70	70	70
α-Cellulose	50	50	50	50	50	50	50
L-Cystine	3	3	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin mix	10	10	10	10	10	10	10
Mineral mix	35	35	35	35	35	35	35
Energy density (kJ/g) [c]	15.8	15.2	14.3	15.2	14.3	15.2	14.3

[a] BGFE: 62.8 g fructan per 100 g (remainder, principally simple sugars [per 100 g]: 1.1 g glucose, 2.0 g fructose, 0.7 g maltose, 23.4 g sucrose).
[b] WSFE: 63.1 g fructan per 100 g (remainder, principally simple sugars [per 100 g]: 4.8 g glucose, 7.7 g fructose, 15.0 g sucrose).
[c] Energy content of the diets was calculated based on published values for each ingredient. Fructan was given an energy value of 6.3 kJ/g [7].