POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

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Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 2, pp. 269-272

EFFECT OF FRUCTANS WITH DIFFERENT DEGREES OF POLYMERIZATION ON BACTERIAL ENZYMES ACTIVITY, LIPID PROFILE AND ANTIOXIDANT STATUS IN RATS

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Key words: fructans, degree of polymerization, bacterial enzyme activity, antioxidant status, rat

The aim of the study was to compare the effect of two types of fructans characterised by a different degree of polymerization (long-chain inulin and short-chain fructooligosaccharides [FOS]), added as single dietary components on the activity of selected bacterial enzymes in the faeces and the caecal digesta, the antioxidant status and the serum lipid profile in laboratory rats. Twenty four male Wistar rats were randomly divided into three groups and for 4 weeks were fed a control casein and two experimental diets supplemented with fructooligosaccharides and inulin at a level of 7.5% of diet (C, FOS and IN groups, respectively). The inulin addition caused a significant decrease in faecal pH value throughout the study (pH measurements were taken after day 7, 14, 21 and 28 of feeding), whereas the FOS-diet significantly decreased pH of fresh faeces only after 3 weeks of the study ($p<0.05$ *vs.* the control). Both experimental diets beneficially diminished the activity of faecal β-glucosidase and β-glucuronidase as compared to the control animals. The decreased activity of bacterial β-glucosidase and increased activity of β-galactosidase in the caecal digesta was noted only in the case of dietary FOS. The lowest concentrations of TBARS in the liver, kidneys and serum were observed in the IN group ($p<0.05$ *vs*. the control), however the FOS diet also effectively reduced concentration of TBARS in the liver tissue and the serum. The dietary inulin significantly decreased the serum glucose level and increased the percentage of HDL-cholesterol in total cholesterol (*vs*. C and FOS groups). The results obtained demonstrate that the consumption of diet supplemented with inulin seems to be more health promoting and elicits more beneficial changes in microflora activity, lipid metabolism and antioxidant status in rats than that enriched with short-chain fructooligosaccharides.

INTRODUCTION

Low physical activity, obesity and ill-balanced diet are the main reasons of an increased risk of "civilizational diseases" incidence. This fact reflects the necessity of increasing the consumption of products rich in constituents which may play a beneficial role in the maintenance of human health and are recognised as functional food's ingredients. The concept of food for health is a leading trend that has been quickly becoming popular. A significant driving force in the "functional food" market place is consumer demand – the quest by consumers to optimize their health through food [Sangeetha *et al*., 2005].

"Fructans" is a general term used for naturally occurring plant oligo – and polysaccharides. Belonging to fructans inulin (IN) and fructo-oligosaccharides (FOS) are plant-derived carbohydrates with the benefits of soluble dietary fiber and different chain lengths (degree of polymerization). They are not digested or absorbed in the small intensine, but fermented by beneficial bacteria in the lower gut [Lopez-Molina *et al*., 2005]. FOS and IN can increase selective bacterial growth (*Bifidobacterium*) and short-chain fatty acids (SCFA) production in the caecal-colonic segments with local and systemic beneficial effects [Bouhnik *et al*., 1996; Cummings *et al*., 2001]. Positive systemic effects of the probiotic function of fructans could be reducing plasma glucose, triglyceride and total, LDL and VLDL cholesterol concentrations [Giacco *et al*., 2004]. The probiotic function of fructans is considered to be of significance to the process of colorectal carcinogenesis. Fructooligosaccharides are capable of diminishing the production of enzymes, such as β-glucosidase and β-glucuronidase which may exert toxic, carcinogenic or mutagenic effects in the colon [Grasten *et al*., 2002].

Antioxidant activity is mainly attributed to dietary polyphenols which have been an object of numerous researches [Hollman, 2001]. On the other hand, there is a lack of information dealing with an influence of various dietary fibres on the antioxidant status of the host. Laurrari *et al*. [1996] postulated the inclusion of antioxidant properties characteristics while assessing the physiological effect of different types of dietary fibre.

Long-chain inulin and short-chain FOS can provoke different effects not only in the gastrointestinal tract but differentiate the physiological effects in the whole organism depending on the length of the carbohydrate chain [Carabin & Flamm, 1999; Giacco *et al.*, 2004].

The study was aimed at determining the effect of dietary inulin and FOS on selected bacterial enzymes activities, lipid metabolism, and particularly on antioxidant status which is the new field of scientific endeavour connected with dietary fibre.

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MATERIALS AND METHODS

The experiment was conducted for 4 weeks on 24 male Wistar rats aged 4 weeks and weighing 75±2.5 g. The animals were randomly allocated to three experimental groups of 8 rats. The rats were fed a control casein diet (C) with a standard amount of mineral and vitamin mixture (according to AIN-93G Mineral Mix and AIN-93G Vitamin Mix). Experimental diets were supplemented with: inulin IN-diet, (Frutafit-Inulin Tex, Sensus, Roosendaal, The Netherlands) and fructooligosaccharides FOS-diet (Wako Pure Chemical Industries, Tokyo, Japan). The supplements were introduced instead of sucrose (Table 1). Amounts of supplements were prepared in a such way to assure the share of both types fructans at a dietary level of 7.5%. Frutafit-Inulin-Tex is a long-chain, sugar-free inulin product in which approximately 91% of the molecules is long-chain inulin (degree of polymerization >10). FOS-Wako is a short-chain FOS product in which approximately 95% of the molecules is short-chain oligofructose (DP 3-5). All animals were fed daily with a fresh diet *ad libitum* and had permanent access to tap water. Rats were housed in individual cages in a well-ventilated room (an air turnover of 15 per hour) with a constant temperature of 21-22°C and a 12-h dark/light cycle. The experimental protocol was approved by the Local Council for Animal Experiments in Olsztyn (Poland). Faecal pH was measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal) after 1st, 2nd, 3rd and 4th week of experimental feeding. At the termination of the study, the rats were anaesthetized with sodium pentobarbitone according to the recommendations for euthanasia of experimental animals. After laparotomy, blood samples were taken from the tail vein, then the caecum with contents and selected tissues (heart, lungs, liver and kidneys) were removed.

Bacterial enzyme activity was measured by the rate of *p*- or *o*-nitrophenol release from their nitrophenylglucosides according to the method described by Juśkiewicz *et al*. [2002] in fresh faeces (β-glucosidase, β-glucuronidase), which were taken after each week of experiment and in the cecal digesta on termination of the study (α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase). Enzymatic activity in the fresh feaces and cecal digesta was expressed as μmol product formed per an hour per gram of feaces or digesta.

In heparinized blood, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) was determined with the aid of enzymatic kits (Randox Laboratories Ltd., Crumlin, UK). Total antioxidant status (TAS) in the serum was estimated by using kit from Randox Laboratories Ltd. Extent of lipid peroxidation in selected tissues (kidneys, lungs, liver, heart, serum) was measured by quantifying malonodialdehyde formed in terms of thiobarbituric acid-reactive substances (TBARS) [Ohkawa *et al*., 1979].

Diagnostic sets from Alpha Diagnostics (Warsaw, Poland) were used for determining concentrations of cholesterol (total and high-density lipoprotein), triacylglycerol (TAG) and glucose.

Results were worked out statistically using one-way analysis of variance and significance of differences between groups was determined with Duncan's multiple range test at a significance level of $p<0.05$. Calculations were made with STATISTICA 6.0 (StatSoft Corp., Kraków, Poland).

TABLE 1. Composition of diets (%).

	C	FOS	IN
Casein	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2
Sucrose	7.5	-	-
Cellulose	0.6	0.6	0.6
Soybean oil	5	5	5
Lard	5	5	5
Cholesterol	0.5	0.5	0.5
Mineral mix[1]	3.5	3.5	3.5
Vitamin mix[2]	2	2	2
Inulin preparation[3]	-	-	8.3
FOS preparation[4]	-	7.9	-
Maize starch	60.9	60.5	60.1

[1]AIN-93G-MX according to Reeves [1997]; [2] AIN-93G-VM according to Reeves [1997]; [3] FOS-Wako Pure Chemical Industries, Tokyo, Japan (oligofructose - 95%); [4] Frutafit Tex, Sensus, Netherlands (long chain inulin - 91%); [3, 4] The fructan products to be compared were applied in diets at different doses so that the content of fructan fraction in a diet was alike, corresponding to 7.5% sucrose in the control group.

RESULTS AND DISCUSSION

The administration of an inulin preparation was accompanied by a significant decrease in the faecal pH values throughout the study measured at the end of each week, compared to the sucrose group (Table 2). In the case of the FOS administration, a significantly ($p<0.05$ *vs*. control) drop in the faecal pH was observed only after the third week of the

TABLE 2. pH value and bacterial enzymes activity in fresh feaces (U/g fresh feaces).

	C	FOS	IN	SEM
pH				
after I week	6.57[a]	6.39[a]	5.70[b]	0.098
after II week	6.42[a]	5.99[ab]	5.80[b]	0.103
after III week	6.98[a]	6.05[b]	5.84[b]	0.116
after IV week	6.89[a]	6.51[ab]	6.05[b]	0.102
β-Glucosidase[1]				
after I week	35.4[a]	3.24[b]	0.96[b]	3.53
after II week	28.2[a]	4.44[b]	4.68[b]	2.60
after III week	19.8[a]	2.52[c]	6.96[b]	1.72
after IV week	8.52[a]	0.48[c]	3.24[b]	0.86
β-Glucuronidase[1]				
after I week	128[a]	18.0[b]	9.48[b]	12.1
after II week	107[a]	25.6[b]	10.4[b]	9.79
after III week	72.6[a]	13.2[b]	18.4[b]	5.84
after IV week	32.4[a]	15.6[b]	9.24[b]	2.53

Means within each row with the same superscript (a, b, c) are not different at $p<0.05$; SEM - SD for all rats divided by the square root of rat number; n = 24; [1]μmol/h/g fresh faeces.

experiments. There has been an agreement amongst researchers that an improvement of the gastrointestinal ecosystem, especially its lower part, is considered as the main target of dietary supplementation with fructan mixtures [Juśkiewicz *et al*., 2005; Twomey *et al*., 2003]. The reduction in pH value was a result of microflora action utilizing fructans and producing fermentation end-products like lactic and short-chain fatty acids. Our results probably pointed out faster utilization of short-chain fructooligosaccharides by bacteria in the upper parts of the large intestine. Some authors have even observed that oligofructose, and to a lesser extent inulin, might be well utilized by bacteria present in the small intestine of monogastric animals [Willard *et al*., 1994].

Compared with the control group the supplementation of diets with both fructans resulted in a significant reduction of bacterial β-glucosidase and β-glucuronidase activities in fresh faeces, which was noted after each week of the study. It is interesting to indicate that when the FOS preparation was considered then the lowest activity of bacterial β-glucosidase was observed at the end of the study (4th week). Changes in its activity appeared to be differentiated throughout the experiment when the inulin preparation was used as the supplement, and the lowest activity of β-glucosidase was recorded after 7 days of dietary administration. It indicated that the fructans varying in the length of the carbohydrate chain differently affected the enzymatic activity of microflora, thus might have induced disparate adaptation period to a diet.

The inulin addition to the diet caused also a significant reduction in the activity of bacterial β-glucuronidase in the caecal contents in comparison to the control group (Table 3). Such an effect was not observed in the case of short-chain FOS. On the other hand, the latter preparation was associated with a significant decline in the activity of β-glucosidase as well as an increase in the β-galactosidase activity when compared to long-chain inulin. The enhanced β-galactosidase activity may be beneficial in lactose maldigestion which, in turn, may induce diarrhoea. Normal intestinal flora may influence carcinogenesis by producing enzymes that transform pre-carcinogens into active carcinogens. These enzymes include β-glucosidase, β-glucuronidase, azoreductase, and nitroreductase [Pool-Zobel *et al*., 2002]. Prebiotics might suppress the growth of undesired bacteria, thereby reducing the amount of unwished compounds in the intestine [Rowland *et al*., 1998]. The results obtained in the present study confirmed that fructan-type oligo- and polysaccharides can be very effective in a beneficial modification of the microflora activity. Inulin, when compared to FOS, seems to be more effective in diminishing the activity of caecal and faecal β-glucuronidase.

TABLE 3. Bacterial enzymes activity in the caecal digesta (μmol/h/g fresh digesta).

	C	FOS	IN	SEM
α-Glucosidase	9.72	13.4	12.1	0.94
β-Glucosidase	3.00^{ab}	1.32^{b}	4.80^{a}	0.59
α-Galactosidase	4.20	4.20	4.56	0.52
β-Galactosidase	26.9^{ab}	47.9^{a}	22.9^{b}	4.87
β-Glucuronidase	11.6^{a}	8.04^{ab}	6.00^{b}	0.86

Means within each row with the same superscript letter (a, b) are not different at $p<0.05$; SEM - SD for all rats divided by the square root of rat number; n = 24.

We did not find any statistically significant changes in the serum total antioxidant status (TAS), glutathione peroxidase (GPx) nor superoxide dismutase (SOD) activities among the groups examined (Table 4). The lowest concentration of TBARS in the liver, kidneys and blood serum was determined in rats fed with the inulin preparation ($p<0.05$ *vs*. the control). Also the FOS diet efficiently decreased the concentration of TBARS in the liver and blood serum ($p<0.05$ *vs*. the control). Compared to the FOS preparation, the dietary inulin exerted a stronger antioxidant effect in the body with reference to the lowest concentration of TBARS in selected tissues. Nicolle *et al*. [2004], on the basis of their research on lettuce consumption, have recently proposed that increasing HDL-cholesterol concentration and lower peroxidation level in rat's tissues, especially in heart measured by TBARS assay (associated with a substantial increase of the vitamin E/TAG ratio in plasma), may suggest a synergistic impact of both dietary antioxidants and fibre.

The IN diet caused a significant decrease in the concentrations of serum glucose ($p<0.05$ *vs*. the control and FOS groups) and an increase in the HDL-cholesterol concentration ($p<0.05$ *vs*. the C group). Moreover, it was characterised by the highest HDL/Total cholesterol profile ($P<0.05$ vs. the control and FOS groups). Such an effect of dietary inulin can be considered as beneficial for cardiovascular disease prevention [Nicolle *et al*., 2004].

The supplemented diets did not affect the concentrations of triacylglycerol (TAG) in the serum. However, Williams & Jackson [2002] reported on nine studies focusing on the re-

TABLE 4. Parameters of antioxidant status and lipid metabolism in rats.

	C	FOS	IN	SEM
Antioxidant status				
SOD (U/mL)	294.7	280.2	297.0	5.857
GPx (U/mL)	29.76	31.47	28.67	0.800
TAS (mmol/L)	1.128	1.128	1.155	0.014
TBARS				
Liver (μmol/100 g of tissue)	1.439^{a}	1.234^{b}	1.171^{b}	0.040
Heart (μmol/100 g of tissue)	1.224	1.183	1.180	0.022
Lungs (μmol/100 g of tissue)	1.474	1.381	1.476	0.049
Kidney (μmol/100 g of tissue)	2.385^{a}	2.026^{ab}	1.757^{b}	0.124
Serum (μmol/100 mL)	8.309^{a}	7.172^{b}	6.878^{b}	0.375
Serum				
Glucose (mg/dL)	276.7^{a}	272.5^{a}	238.5^{b}	5.925
TAG (mg/dL)	278.0	263.8	270.7	16.59
Cholesterol (mg/dL)	132.4	136.6	128.1	3.194
HDL (mg/dL)	41.5^{b}	45.9^{ab}	52.1^{a}	1.553
HDL/Total (%)	31.3^{b}	34.3^{b}	40.8^{a}	1.371

Means within each row with the same superscript (a, b) are not different at $p<0.05$; SEM - SD for all rats divided by the square root of rat number; n = 24; SOD - superoxide dismutase; GPx - glutathione peroxidase; TAS - total antioxidant status; TBARS - thiobarbituric acid-reactive substances; TAG – triacylglycerol.

sponse of blood lipids to inulin and FOS in human yielded differential results: among the aforementioned studies three have shown no effects on blood levels of cholesterol or triacylglycerol, four have shown modest reductions in total and LDL cholesterol, whilst another three have shown significant reductions in TAG. The studies on rats [Kok *et al*., 1996; Fiordaliso *et al*., 1995] showed a reduction of hepatic plasma triglycerides in normolipidaemic animals after the addition of inulin to diet. From currently available scientific evidence, inulin seems to be more effective in the control of lipid metabolism.

CONCLUSIONS

The results obtained in the conducted experiment indicated that long-chain inulin more beneficially influenced bacterial enzyme activity (lower activity of caecal and faecal β-glucuronidase), lipid metabolism (higher concentration and profile of HDL cholesterol fraction) and antioxidant status (lower TBARS concentration in the liver, kidneys and serum) in rats than short-chain FOS.

ACKNOWLEDGEMENTS

This study was financed by the State Committee for Scientific Research, Grant No. 2P06T 007 30.

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Received December 2007. Revision received and accepted February 2008.