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EFFECTS OF FRUCTOOLIGOSACCHARIDES AND LONG-CHAIN INULIN ON SERUM LIPIDS IN RATS

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Effects of fructooligosaccharides (FOS) and inulin on total-cholesterol, HDL-cholesterol, LDL+VLDL-cholesterol, triglycerides in serum of experimental rats were examined. The total number of forty eight animals were used in two experiments (Exp. 1 and Exp. 2). The rats were five--week-old and weighed 90–110 g. In Exp.1 and Exp. 2, twenty-four animals were randomly assigned to four experimental groups (6 rats each) and fed semi-purified diets (modified AIN-93G) providing four levels of either dietary fructooligosaccharides or inulin (0, 4, 8, and 12%), respectively. In the Exp. 1, no significant effects of dietary fructooligosaccharides on total serum cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were observed. In Exp. 2, dietary inulin induced significant increases in serum HDL-cholesterol concentrations and significantly decreased the level of triglycerides.

INTRODUCTION

Fructooligosaccharides and inulin are water soluble, short- and long chain, linear fructans, consisting of fructose monomers bound with β-2, 1-glycoside bonds [Incoll & Bonnet, 1993]. They occur in various edible plant parts (tubers, leaves or roots) as storage carbohydrates, accumulating mainly in the cells' vacuoles.

Fructans resist digestion in the human gastrointestinal tract because the human organism has no enzymes hydrolysing β-2, 1-glycoside bonds [Alles *et al.*, 1999; Andersson *et al.*, 1999; Kleessen *et al.*, 2001; Niness, 1999]. At the same time, fructans are fermented in the large intestine and induce the growth of endogenous bacteria colonizing this part of the human gastrointestinal tract. Consequently, their presence in a food product and expected health-related effects, give this product the status of functionality [Gibson, 1999; Roberfroid, 1999; 2001].

Despite the fact the fructans do not form viscous films in the gastrointestinal tract, and have no ability to bind bile acids in the gastrointestinal tract, it has been suggested that they may lower blood serum cholesterol and triglyceride levels [Prostak *et al.*, 2001a, b; Schneeman, 1999]. It was postulated that short-chain fatty acids (C_2, C_3 and C_4) formed during the fermentation of fructans in the large intestine may inhibit enzymes involved in triglyceride and cholesterol synthesis in the liver [Daubioul *et al.*, 2002]. Indeed, fructans may act hypolipeamically, as suggested by the results of research on animal models [Daubioul *et al.*, 2000; Delzenne, 2003; Delzenne & Kok, 1999, 2001]. In an experiment on rats [Delzenne & Kok, 1999], dietary fructooligosaccharides decreased serum and liver triglycerides.

Clinical studies with humans have not demonstrated similar results when compared with animal studies [Jackson *et al.*, 1999; Davidson & Maki, 1999; Williams, 1999]. Jackson *et al.* [1999], Davidson & Maki [1999] showed that fructans decrease the level of total cholesterol in human blood. However, they did not report any changes in serum triglyceride levels.

The objective of our study was to verify the hypotriglyceridemic and hypocholesterolemic effects of fructooligosaccharides and inulin in laboratory rats fed modified semi-purified AIN-93G diet providing increasing dietary levels (0, 4, 8 and 12%) of the fructans. Total cholesterol, HDL cholesterol, LDL+VLDL cholesterol, and triglycerides in blood serum of experimental animals were determined.

MATERIALS AND METHODS

Fructooligosaccharides (FOS) – "Raftilose" and inulin – "Raftiline" were commercial products obtained from Orafti (Belgium). As specified by the producer, the content of short-chain fructans in "Raftilose" was 93.2% (fructose units 8–10) and that of inulin in "Raftiline" was 90%. The semi-purified AIN-93G diets for rats (Table 1), with increasing concentrations of fructans (0, 4, 8 and 12%) were prepared according to Reeves *et al.* [1993]. In addition, the original soybean oil as a fat source was replaced by hypercholesterolemic lard (Table 1).

The experimental rats were obtained from the Department of Animal Nutrition, Institute of Animal Production in Kraków. They were albino males (Wistar strain), aged between 5–6 weeks with mean body weight of 90–120 g. The studies were carried out in compliance with ethical requirements and were approved by the Local Ethical Commission.

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TABLE 1. Composition of experimental diets with 4%, 8%, 12% addition of FOS or inulin (g/1000 g).

Ingredient (g)	Group I control	Group II	Group III	Group IV
Corn starch	533.97	533.97	533.97	533.97
Casein	200	200	200	200
Sucrose	100	100	100	100
Fibre	50	50	50	50
Lard	70	70	70	70
Vitamin mix	10	10	10	10
Mineral mix	35	35	35	35
Choline	1.017	1.017	1.017	1.017
Tert-butylo-hydroquinone	0.014	0.014	0.014	0.014
FOS or Inulin	0	40	80	120

Animals were kept individually in metal cages in a room maintained at constant temperature and humidity, under 12 h/12 h light/dark cycle. During the adaptation period (7 days), the animals were offered commercial GLM-1 diet and drinking water *ad libitum*. Two experiments were carried out. After the adaptation period, the animals in Exp. 1 were randomly divided into four groups (six rats in each) and fed four experimental diets (Table 1) providing graded levels of FOS (0, 4, 8 and 12%) for 21 days. In the parallel Exp. 2, the animals were fed four experimental diets (Table 1) providing graded levels of inulin (0, 4, 8 and 12%). The rats had free access to water and food and the diet intake was controlled daily. The rats were weighed at the beginning and at the end of experimental periods. Average diet intake was 12.3 g/rat/24 h in Exp. 1 and 11.9 g/rat/ 24 h in Exp. 2. On the last day of the experimental periods, the rats which had been deprived of food overnight (*i.e.* 14–16 h before sacrificed), were anesthetised by intraperitoneal injection of sodium thiopental (Biochemie, Vienna, Austria; 25 mg/100 g of body mass) and sacrificed by withdrawing blood from the heart. Blood samples were collected to separate tubes and centrifuged for 10 min at 4000 g to obtain blood serum.

The serum samples were analysed for total cholesterol (BioVendor kit No. 1085), HDL cholesterol (BioVendor kit No. 10855, method sensitivity 0.03-4.7 mmol/L), and triglycerides (Bio Vendor kit No.12805). LDL+VLDL cholesterol level was calculated from the difference between total cholesterol and HDL cholesterol.

One-way analysis of variance (ANOVA) was used to test the effect of fructans on total cholesterol, HDL-cholesterol, LDL+VLDL cholesterol, and triglycerides level in serum of rats. When differences were observed, the Duncan multiple range test ($p \leq 0.05$) was used to compare the difference between experimental groups. In addition, the relationships between increasing dietary fructan levels and serum lipids were described using linear regression analysis [Microsoft Excel, 1997].

RESULTS

A detailed data of the effect of fructans on body weight gain in rats has been published in a previous paper [Cieślik & Kopeć, 2003].

In the Exp. 1, no significant effects of dietary fructooligosaccharides (0, 4, 8 and 12%) on total serum cholesterol were observed (Table 2). The same was true for HDL-cholesterol, LDL+VLDL-cholesterol and serum triglycerides in rats.

TABLE 2. The effect of FOS on serum lipids concentration in rats*.

Group / % FOS	Total cholesterol	HDL cholesterol	LDL+VLDL cholesterol	Triglycerides
	(mmol/L)			
I – 0 %	2.16±0.13	1.52±0.10	0.64±0.09	0.81±0.05
II – 4%	2.17±0.13	1.55±0.04	0.62±0.10	0.61±0.10
III – 8%	2.00±0.09	1.48±0.08	0.54±0.06	0.78±0.09
IV – 12%	2.10±0.05	1.62±0.08	0.51±0.06	0.68±0.06
SE**	0.112	0.081	0.084	0.07

* there are no differences between experimental groups; ** SE – standard error

In the Exp. 2, dietary inulin (0, 4, 8 and 12%) did not alter the total serum cholesterol concentrations in rats (Table 3). On the other hand, HDL-cholesterol concentrations significantly ($p \leq 0.05$) increased (from 1.52 mmol/L to 1.76 mmol/L) with increasing dietary concentrations of inulin (from 0 to 12%). Moreover, the significant ($p \leq 0.05$) relationship between dietary inulin levels and HDL-cholesterol concentrations was described by the following linear regression equation: $y=0.01x+1.54$ (Figure 1), and the corresponding correlation coefficient ($r=0.461$) was statistically significant ($p \leq 0.05$). Serum LDL+VLDL-cholesterol concentrations were not significantly affected. However, we observed that they inconsistently tended to decrease

TABLE 3. The effect of inulin on serum lipids concentration in rats.

Group / % FOS	Total cholesterol	HDL cholesterol	LDL+VLDL cholesterol	Triglycerides
	(mmol/L)			
I – 0 %	2.16±0.14^{a}	1.52±0.10^{a}	0.64±0.09^{a}	0.81±0.48^{b}
II – 4%	2.13±0.13^{a}	1.66±0.09^{ab}	0.47±0.07^{a}	0.73±0.06^{ab}
III – 8%	2.17±0.11^{a}	1.73±0.02^{ab}	0.42±0.08^{a}	0.59±0.07^{a}
IV – 12%	2.26±0.15^{a}	1.76±0.05^{b}	0.51±0.10^{a}	0.65±0.06^{ab}
p	p=0.72	p≤0.05	p=0.065	p≤0.05
SE**	0.13	0.074	0.059	0.081

Data are expressed as means ± standard deviation (n=6), values in column with different letters (a, b, c) are significantly different at $p \leq 0.05$; ** SE – standard error

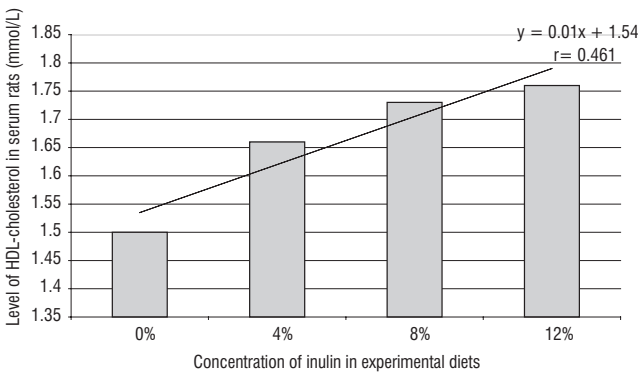

FIGURE 1. Effect of inulin on HDL cholesterol in serum of rats.

(p=0.065). Blood serum triglycerides significantly (p≤0.05) decreased in group III. The relationship between dietary inulin levels and triglyceride concentrations was significant (p≤0.05) and described by the following linear regression equation: y=-0.02x+0.787 (Figure 2). The corresponding correlation coefficient (r=0.437) was significant at p≤0.05.

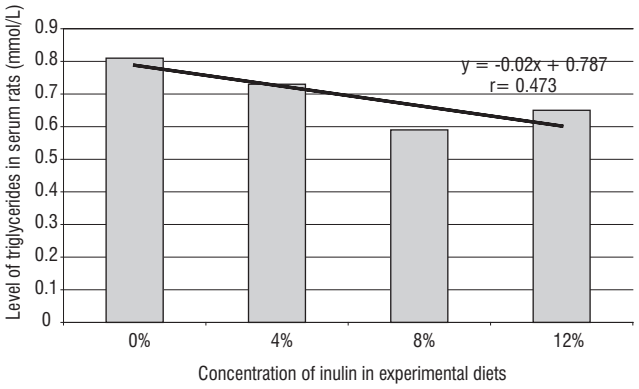

FIGURE 2. Effect of inulin on serum triglycerides in serum of rats.

DISCUSSION

The results of the Exp. 1 clearly indicate that an increasing dietary concentration of fructooligosaccharides had no effect on total cholesterol, HDL-cholesterol, LDL+VLDL--cholesterol and triglycerides in serum of rats (Table 2). It could be due to the experimental diets. Animals were fed diets with 63% carbohydrate (10% sucrose and 53% cornstarch) and 7 % fat (*i.e.* lard). It is likely that the ratio of sugar to starch was too low and lipid homeostasis was not destroyed. The level of cholesterol and triglycerides in serum of rats was correct, and short chain fatty acids were not able to reduce fatty acid synthesis in liver. On the other hand, literature data showed different kinds of diets used in similar experiments. Fiordaliso *et al.* [1995] reported that a normolipidemic diet with the addition of FOS decreased the level of total cholesterol and triglycerides in serum of normolipidemic rats. Agheli *et al.* [1998] used diets with 10% FOS, 57.5% sucrose, and 8.4% lard. Daubioul *et al.* [2000] showed that a diet containing 70% carbohydrates, 3.2% fat, and 10% FOS gave a hypolipidemic effect in plasma of obese Zucker rats. The authors did not specify the level of cholesterol and fat source in the diets.

In this study, the experimental diets were prepared on the basal AIN-93G diet. This diet for rats is recommended by the American Institute of Nutrition [Lien *et al.*, 2001; Reeves *et al.*, 1993]. It is very often used in experiments addressing the effect of different food ingredients on levels of cholesterol and triglycerides in rats [Ali *et al.*, 2004; Wiesenfeld *et al.*, 2003].

In contrast to our findings, dietary fructooligosaccharides exerted hypolipidemic effects both in animal and humans models (rats and hamsters). In rats, fructooligosaccharides were found to reduce triglyceridemia [Delzenne & Williams, 2002; Daubioul *et al.*, 2002]. Agheli *et al.* [1998] reported that diets with 10% FOS decreased triglycerides in the plasma of rats. Daubioul *et al.* [2000] showed that FOS (10% in diet) decreased LDL lipoprotein levels in plasma of obese Zucker rats.. In contrast, Fiordaliso *et al.* [1995] reported that FOS decreased the level of total cholesterol and triglycerides in serum of normolipidemic rats.

In eleven experiments on human subjects, consuming fructooligosaccharides, only three of them reported significant reductions in serum triglycerides whilst five trials reported modest reductions in total and LDL-cholesterol [Delzenne, 2003].

The results of Exp. 2 confirm, at least in part, the hypothesis that long-chain fructans (*i.e.* inulin) can exert a hypolipeamic effect and thus can be considered as potential functional food ingredients [Roberfroid, 2001]. At first, serum HDL-cholesterol concentrations in rats significantly increased (Table 3, Figure 1) with increasing dietary levels of inulin. Then, LDL+VLDL-cholesterol concentrations decreased inconsistently (Table 3). Finally, serum triglycerides decreased significantly (Table 3, Figure 2). The above hypolipeamic effects of inulin confirm previous findings reported by Trautwein *et al.* [1998], Williams [1999], and reviewed by Dalzenne & Williams [2002]. As indicated before, these hypolipeamic effects of inulin can be attributed to the action of fermentation products (*i.e.* short-chain fatty acids) of inulin in the large intestine by bifidobacteria. Short--chain fatty acids, mainly propionate, act as an inhibitor of hepatic lipid synthesis, including fatty acids and cholesterol [Beylot, 2005; Daubioul *et al.*, 2002; Delzenne & Kok, 2001].

Overall, the results obtained did not show any hypolipemic effects of fructooligosaccharides in laboratory rats fed a diet containing 63% carbohydrate (10% saccharose, 53% starch) and 7% fat (*i.e.* lard). At the same time, they confirmed the ability of inulin to exert both hypotriglyceridemic and hypocholesterolemic effects in these animals. Of interest are the effects of inulin on serum HDL-cholesterol and triglyceride concentrations. Increased concentrations of HDL-cholesterol are considered beneficial. It is known that HDL-cholesterol, involved in the reverse cholesterol transport, represents the only route of cholesterol elimination from the body. This function is associated with its antiatherogenic role in human tissues [Schaefer, 2002]. Likewise, the hypotriglyceridemic effect of inulin is positively health-related. Namely, elevated concentrations of serum triglycerides are considered to be an independent risk factor in the development of atherosclerosis in humans [Schaefer, 2002]. Finally, we conclude that inulin can be considered a functional food ingredient potentially exerting health-related (hypolipeamic) effects.

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WPŁYW FRUKTOOLIGOSACHARYDÓW ORAZ DŁUGOŁAŃCUCHOWEJ INULINY NA POZIOM LIPIDÓW W SUROWICY KRWI SZCZURÓW DOŚWIADCZALNYCH

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Celem pracy było określenie wpływu dodatku fruktooligosacharydów i inuliny na stężenie cholesterolu ogółem, cholesterolu frakcji HDL, cholesterolu frakcji LDL+VLD oraz triglicerydów w surowicy szczurów doświadczalnych. Badania przeprowadzono na 48 rosnących szczurach samcach szczepu Wistar. Zwierzęta podczas doświadczenia (21 dni) karmione były zmodyfikowaną dietą AIN-93G, w której olej sojowy zastąpiono smalcem, z dodatkiem FOS lub inuliny w ilości 0, 4, 8 lub 12%. W ostatnim dniu eksperymentu zwierzęta usypiano i bezpośrednio z serca pobierano krew. W otrzymanej surowicy krwi oznaczano enzymatycznie zawartość cholesterolu całkowitego, frakcji HDL oraz triglicerydów. Zawartość cholesterolu frakcji LDL+VLDL obliczono jako różnicę pomiędzy zawartością cholesterolu całkowitego i frakcji HLD. Nie stwierdzono wpływu FOS na stężenie cholesterolu ogółem, cholesterolu frakcji HDL oraz LDL+VLDL oraz triglicerydów w surowicy krwi szczurów (tab. 2). Natomiast w surowicy krwi gryzoni karmionych dietami z dodatkiem inuliny wykazano statystycznie istotny wzrost ilości cholesterolu-HDL oraz obniżenie poziomu triglicerydów (tab. 3, rys. 1, 2).