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CHOLESTEROL METABOLISM IN RATS UNDER EFFECTS OF CHITOSAN AND DEAE-CELLULOSE

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Comparative study on effects of chitosan and DEAE-cellulose on cholesterol metabolism in rats was presented here. Research results shown that chitosan significantly decreases the cholesterol level in the blood serum and in the liver. Here it has been discovered the tendency of decrease of relative expression of LDL-receptor mRNA. DEAE-cellulose had lower effect.

Results of study have shown that neither cholesterol supplementation nor aminated polysaccharides influenced activity of HMG-CoA reductase, which is the rate-limiting enzyme of cholesterol synthesis.

Key words: CHITOSAN, DEAE-CELLULOSE, CHOLESTEROL, LIPIDS, BLOOD SERUM, LIVER, CAECUM, LDL-RECEPTOR mRNA

Chitosan is a polymer of β-1,4-glucosamine prepared by alkaline N-deacetylation of chitin. Shells of marine crustaceans, which are waste products from food-processing, are the current source of chitin and chitosan. Chitosan has been widely used in industry as a cationic flocculating agent for waste-water treatment, paper additive, cosmetic ingredient and absorbable material [1]. Chitosan and its derivatives are useful excipients for the peroral administration of drugs [2, 3]. Cholesterol-lowering effects of chitosan have been reported in humans [4], rats [5] and poultry [6]. At present, chitosan is increasingly used as an over-the-counter cholesterol-lowering agent [7].

A potential cholesterol-lowering polysaccharide may be diethylaminoethyl-cellulose (DEAE-cellulose). The functional groups of DEAE-cellulose are protonated amino groups able to bind bile acids. As a result of its binding of bile acids in the intestine, the enterohepatic circulation of bile acids is broken and a consequential lowering of serum cholesterol occurs. Clas [8] reported that the DEAE-cellulose-chloride binding capacity *in vitro* was 219 mg of Na-glycocholate per g. Cholesterol-lowering capacity of DEAE-cellulose *in vivo* is not known. Thus, the purpose of this study was to compare chitosan and DEAE-cellulose in rats fed a cholesterol-containing diet.

Material and Methods

Chitosan and DEAE-cellulose were supplied by Sigma-Aldrich. Twenty-four female Wistar rats were housed individually in a temperature and humidity-controlled room. The rats were fed a rat diet ST-1 (Velaz, Czech Rep.) supplemented with cellulose, cholesterol and palm fat. Experimental diets were prepared by replacing cellulose with chitosan and DEAE-cellulose (Tab. 1). Diets were fed *ad libitum* for 4 weeks. Eventually, rats were sacrified, liver, spleen and caecum weighed, and samples of serum, liver, caecal contents and faeces analyzed.

Analyses were performed as described previously [9]. Data were statistically analyzed using one-way ANOVA and Tukey's test. The results are expressed as means ± SD.

Results and discussion

Cholesterol supplementation significantly increased cholesterol concentration in the liver but not in serum (fig. 1). There was no significant treatment effect on serum triacylglycerols and hepatic fat. Chitosan significantly decreased serum and hepatic cholesterol. No corresponding effect on weight of liver, spleen and caecum was apparent (fig. 2). Chitosan significantly decreased concentrations of dry matter and volatile fatty acids (VFA) in the caecum and feaces (fig. 3). DEAE-cellulose significantly reduced faecal VFA concentration. Activities of LDL-receptor mRNA were variable and tended to decrease in rats fed chitosan. Neither cholesterol supplementation nor aminated polysaccharides influenced activity of HMG-CoA reductase, which is the rate-limiting enzyme of cholesterol synthesis (tab. 2).

Table 1

Composition of control and experimental diets (g/kg)

Ingredient	Diet			
	1	2	3	4
Chitosan	-	-	60	-
DEAE-cellulose	-	-	-	60
Cellulose	60	60	-	-
Cholesterol	-	5	5	5
Palm fat	55	50	50	50
Diet ST-1*	885	885	885	885

Note: * — Diet ST-1 ingredients were soybean meal, meat and bone meal, fish meal, wheat, maize, oats, wheat bran, limestone, dicalcium phosphate, salt and supplements of vitamins, trace elements and amino acids

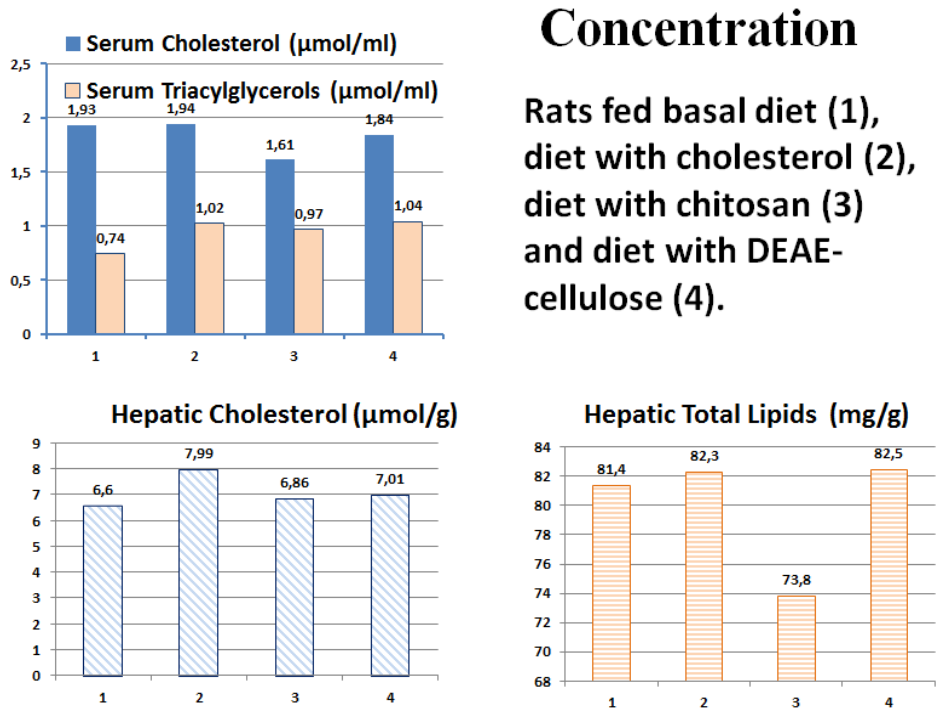

Fig. 1. Effect of chitosan and DEAE-cellulose on serum and hepatic concentrations of cholesterol and serum triacylglycerols, and fat in. rats fed basal diet (1), diet with cholesterol (2), diet with chitosan (3) and diet with DEAE-cellulose (4)

Moderate cholesterol-lowering effect of chitosan is consistent with previous reports [10, 11]. There are numerous papers on antimicrobial activity of chitosan [12]. In our experiment chitosan decreased metabolic activity of caecal and colonic bacteria as evidenced by significantly lower concentration of VFA in the caecal contents and feaces. Low dry matter concentration in the caecal contents and feaces indicates a significant water-holding capacity of chitosan.

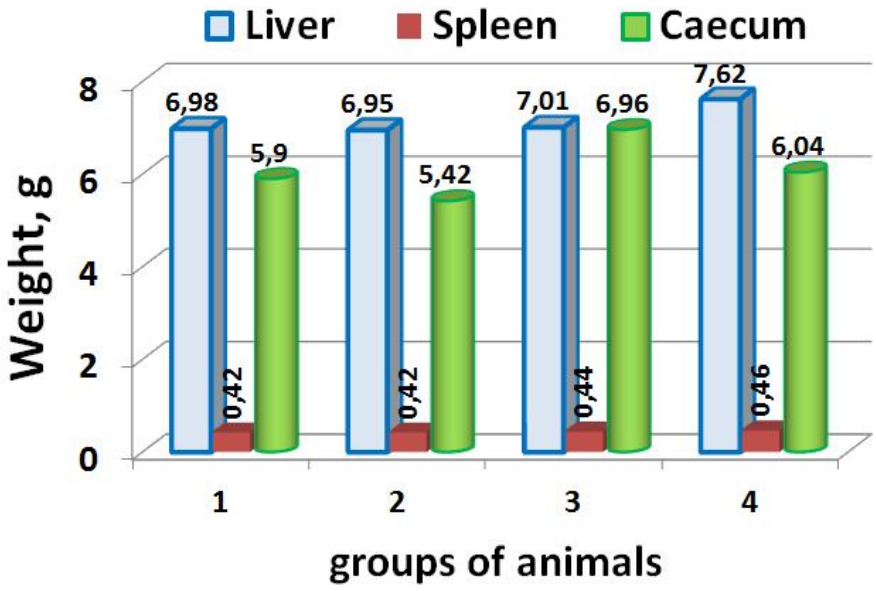

Fig. 2. Effect of chitosan and DEAE-cellulose on weight of liver, spleen and caecum of rats fed basal diet (1), diet with cholesterol (2), diet with chitosan (3) and diet with DEAE-cellulose (4)

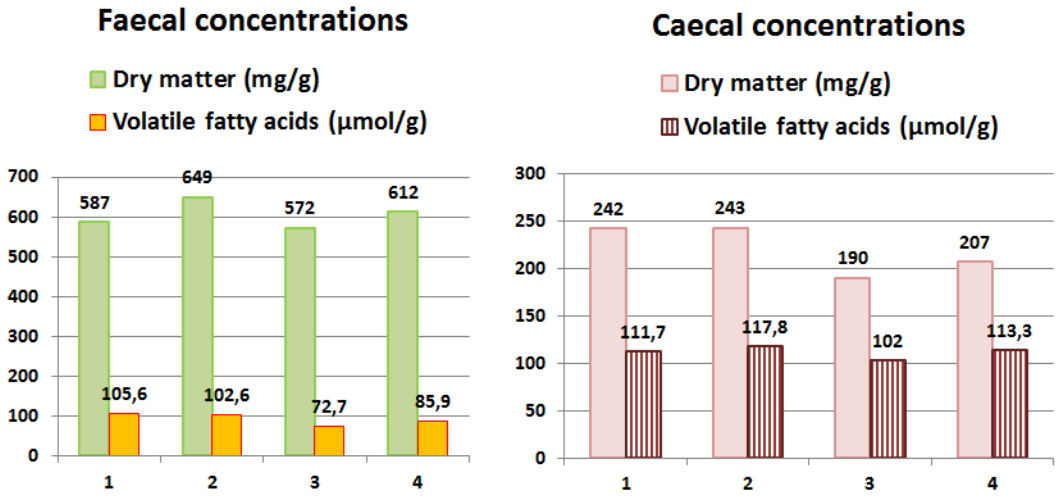

Fig. 3. Effect of chitosan and DEAE-cellulose on dry mater and volatile fatty acids concentration in caecal contents and feaces. Rats were fed basal diet (1), diet with cholesterol (2), diet with chitosan (3) and diet with DEAE-cellulose (4)

Rats were apparently healthy and experimental groups did not differ in the feed intake. The weight of liver and spleen in rats fed DEAE-cellulose was non-significantly increased, thus a minor metabolit burden in these rats can not be excluded.

DEAE-cellulose shares some similarities with chitosan. Both polymers are N-protonated polysaccharides, non-absorbable in the digestive tract, capable to bind bile acids *in vitro*.

However, as emphasized by Stedronsky [3, 9, 11], the correlation between measurements made *in vitro* and performance of bile acid sequestrants observed *in vivo* may be very poor.

Table 2

Relative expression of LDL-receptor mRNA and activity of HMG-CoA reductase in hepatic tissue of rats fed basal diet (1), diet with cholesterol (2), diet with chitosan (3) and diet with DEAE-cellulose (4)

	Diet			
	1	2	3	4
LDL-r mRNA*	1	0,90	0,67	0,87
	(0,84–1,19)	(0,74–1,10)	(0,52–0,86)	(0,67–1,12)
HMG-CoA / mevalonate**	6,44 ± 1,65	6,73 ± 1,93	6,35 ± 1,91	6,56 ± 2,53

Note: * — Measured by RT-PCR, ** —Ratio of 3-hydroxy-3-methylglutaryl-CoA to mevalonate was inversely related to HMG-CoA reductase activity [13]

Conclusion

In accordance with the literature, chitosan significantly decreased serum and hepatic cholesterol concentration in rats fed a diet supplemented with cholesterol. No lipid-lowering effects of DEAE-cellulose were observed.

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МЕТАБОЛІЗМ ХОЛЕСТЕРОЛУ В ЩУРІВ ЗА ДІЇ ХІТОЗАНУ І ДЕАЕ-ЦЕЛЮЛОЗИ

Р е з ю м е

Хітозан, який є полімером β-1,4-глюкозаміну, отримують через лужне N-дезацетилювання хітину, широко використовується в індустрії та є допоміжним матеріалом для перорального введення ліків. Використання його та ДЕАЕ-целюлози передбачається в якості знижувальних агентів холестеролу. Тому таку дію необхідно довести експериментально in vivo. У статті представлено порівняльне вивчення впливу хітозану і ДЕАЕ-целюлози на метаболізм холестеролу у щурів.

Експериментальні дані показали, що хітозан вірогідно понижує рівень холестеролу (ХС) у сироватці крові та у печінці. Тенденція до пониження рівня активації мРНК ЛПНЩ-рецептора була виявлена в дослідах, в той час як ДЕАЕ-целюлоза давала нижчий ефект. Результати досліджень показали, що ні добавка холестеролу, ні обох досліджуваних амінованих полісахаридів не впливають на активність HMG-CoA-редуктази, яка є ензимом, що лімітує швидкість біосинтезу холестеролу.

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МЕТАБОЛИЗМ ХОЛЕСТЕРОЛА В КРЫС ПОД ДЕЙСТВИЕМ ХИТОЗАНА И ДЭАЭ-ЦЕЛЛЮЛОЗЫ

А н н о т а ц и я

Сравнительное изучение влияния хитозана и ДЭАЭ-целлюлозы на метаболизм холестерола в крыс представлены в даной статье. Экспериментальные данные показали, что хитозан достоверно понижает уровень холестерола в сыворотке крови и в печени. Обнаружена тенденция к снижению уровня активации мРНК ЛПНП-рецептора, в то время как ДЭАЭ-целлюлоза дает ниже эффект.

Результаты исследований показали, что ни добавка холестерола, ни обоих исследуемых аминированных полисахаридов не влияют на активность HMG-CoA-редуктазы, которая является скорость-лимитирующим энзимом биосинтеза холестерола.

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