J. Nutr. Sci. Vitaminol., **38**, 609–613, 1992

Note

Effect of Dietary Chitin on Cholesterol Absorption and Metabolism in Rats

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(Received June 15, 1992)

Summary The effect of chitin at the level of 5% in the diet on cholesterol absorption and metabolism was studied in Wistar rats fed on diet containing beef tallow (7%) and cholesterol (1%). When compared with pair-fed controls, rats fed on diet containing chitin had: (1) similar weight gain and feed efficiency, (2) lower apparent protein digestibility, (3) equivalent liver steatosis, (4) reduced levels of liver triglycerides and cholesterol, (5) similar levels of serum and fecal cholesterol, (6) higher excretion of triglycerides in feces.

Key Words dietary chitin, liver cholesterol, serum cholesterol, fecal triglycerides, protein digestibility

Several reports on the effect of a variety of dietary fibers on blood cholesterol levels have appeared recently. Even though chitosans have been shown to be able to lower blood cholesterol levels (*1–6*), there are undesirable side effects due to their interference with nutrient absorption (*7*).

In the present paper the effect of dietary chitin on liver, blood and fecal lipids was studied.

Materials and methods. Chitin: Chitin was purchased from Sigma Chemical Co. (St. Louis, MO). Its degree of deacetylation was 16%.

Experimental design: Four-week-old male Wistar rats were used. They were fed on commercial diets for 7 days. The average initial weight was 75.7 ± 3.1 g. The animals were divided into four groups, each with 6 rats. Groups 1, 2, and 3 were

Table 1. Composition of the diets.

Ingredients	Control diet (g)	Cholesterol diet (g)	Cholesterol+chitin diet (g)
Casein	20	20	20
Beef tallow	—	7	7
Soy oil	10	1	1
Cholesterol	—	1	1
Starch[1]	53	23	18
Sucrose	10	40	40
Cellulose	1	1	1
Chitin	—	—	5
Salt mixture[2]	5	5	5
Vitamin mixture[2]	1	1	1
Protein content (%)	18.7	18.5	19.0
Energy (kcal/g)	4.22	4.13	3.95

[1] Corn starch (Maizena, Refinações de Milho Brasil Ltda.). [2] AOAC (*8*).

fed *ad libitum* on control, cholesterol, and cholesterol+chitin diets, respectively (Table 1). The fourth group, fed on cholesterol diet, was pair-fed with group 3 as follows. The food intake of each animal of group 3 was determined daily, taking into account the spilled food. The next day, every animal in group 4 received the amount of food consumed by its pair from group 3. The animals were maintained in individual all-wire cages. Feces and spilled food were collected daily on a sheet of paper placed underneath the cage. Feces were freed of food and fur, weighed, dried in a ventilated oven at 60°C and kept frozen. The animals were weighed weekly. The experiment lasted for 42 days. The diets were finely powdered, thoroughly mixed, and kept at 4°C. Feed efficiency (FE) and apparent protein digestibility (Dapp) were determined.

Analytical methods: After 42 days of experiment, the rats were killed under ether anesthesia. Blood was drawn by cardiac puncture and the serum was collected by centrifugation. The liver was weighed. Fragments of liver, heart, brain and small intestines were put in 4% formaldehyde solution and processed for routine histological examination. The rest of the liver was frozen. Total lipid extraction from liver and feces was performed with ethyl ether in a Goldfish apparatus. Determination of cholesterol levels in liver, serum and feces was done with a kit (Boehringer Mannheim Bioquímica S.A.) based on the method described by De Hoff *et al.* (*9*). Triglycerides were determined with a kit which is based on Soloni's method (*10*) manufactured by Labtest (Belo Horizonte). Phospholipid level was calculated by difference between total lipid and cholesterol plus triglyceride values (*11*).

Statistical analysis: Analysis of variance and Tukey test at a level of 5% of probability were applied (*12*).

Table 2. Body and liver weights and liver and serum lipid levels of rats fed on: control diet (group 1); diet containing cholesterol and beef tallow (group 2); diet containing cholesterol, beef tallow and chitin (group 3); and diet containing cholesterol and beef tallow, paired with the rats fed on diet containing chitin (group 4). Refer to Table 1 for composition of diets.

Parameter	Group[1]			
	1	2	3	4
Body weight	308±30[2,a]	335±12[a]	333±23[a]	305±21[a]
Liver weight (mg)/body weight (g)	3.6±0.2[b]	4.9±0.3[a]	5.1±3[a]	4.9±0.5[a]
Liver lipids (mg/g of tissue)				
Total ether extract	51.5±9.0[c]	139.0±13,0[a]	99.8±12.0[b]	131.3±27.2[a]
Triglycerides	21.0±2.8[c]	46.9±4.6[a]	35.0±1.5[b]	49.2±3.2[a]
Cholesterol	5.1±0.6[c]	12.5±2.9[a]	8.0±2.1[c]	11.6±3.3[ab]
Phospholipids	25.1±8.5[b]	79.4±8.9[a]	56.9±10.9[a]	70.5±23.0[a]
Serum lipids (mg/100 dl)				
Triglycerides	118±9[b]	181±21[a]	161±13[a]	180±17[a]
Cholesterol	83±11[a]	120±25[a]	113±15[a]	122±28[a]

[1] Each group consisted of 6 rats. Initial weight: 75.3±3.1 g. The experiment lasted 6 weeks. [2] Same superscript letters indicate no statistical difference at the level of 5% by Tukey test.

Results and discussion. Total food intake, total weight gain and FE were about the same in all groups of rats. Previous works (*1*, *7*) have shown that weight gain and ingested food by Wistar rats were not affected by the addition of chitin at a level of 2% in the diet. Dapp however was decreased from 93 to 82% upon addition of chitin to the diet. Fleming and Lee (*13*) reported decreased Dapp in rats caused by several types of fibers (cellulose, pectin, xylan, and blended fibers).

Table 2 shows the values of relative liver weight and of some lipid parameters in liver and serum. The significantly higher liver weights in all animals fed on diets containing beef tallow and cholesterol may be due to the intense and diffuse microvascular steatosis found in histopathological evaluation. Dietary chitin at the level of 5% did not protect the animals against hepatic steatosis under the conditions of this experiment. The values of relative liver weight reported here are similar to the ones reported elsewhere (*3*). The results depicted in Table 2 are also in agreement with those of Garg *et al.* (*14*), who showed that dietary cholesterol did not affect liver weight but elicited an increase in liver cholesterol. Similar results were reported by Kobayashi *et al.* (*2*), who studied the effect of dietary cholesterol and chitosan on liver lipids.

Table 2 also shows that livers from animals fed on beef tallow and cholesterol (groups 2 and 3) had higher levels of total lipids, triglycerides and cholesterol when compared with the controls (group 1). The data shown indicate that dietary chitin (group 3) protected the animals against accumulation of cholesterol and triglycerides in liver.

Table 3. Fecal weight and fecal lipid levels of rats fed on the different diets listed in Table 2.

Parameters	Group[1]			
	1	2	3	4
Fecal dry weight (g/day)	$0.90\pm0.13^{2,c}$	1.24 ± 0.06^{b}	2.26 ± 0.14^{a}	1.28 ± 0.09^{b}
Ether extract (mg/day)	53.8 ± 10.9^{b}	264 ± 28.1^{a}	295 ± 46.6^{a}	292 ± 37.1^{a}
Triglycerides (mg/day)	30.3 ± 5.5^{c}	91.9 ± 8.4^{b}	122 ± 14.6^{a}	94.4 ± 13.5^{b}
Cholesterol (mg/day)	3.7 ± 0.7^{b}	65.2 ± 5.9^{a}	76.8 ± 6.5^{a}	70.9 ± 11.8^{a}
Phospholipids (mg/day)	19.8 ± 7.2^{b}	106 ± 20.3^{a}	96.2 ± 32.4^{a}	127 ± 35.2^{a}

[1] Each group consisted of 6 rats. Initial weight: 75.7 ± 3.1 g. The experiment lasted for 6 weeks. [2] Same superscript letters indicate no statistical difference at the level of 5% by Tukey test.

Dietary beef tallow and cholesterol led to higher levels of serum triglycerides and cholesterol. Sugano *et al.* (*6*), working with four-week-old rats, reported that chitosan with different degrees of viscosity showed cholesterol-lowering properties. It was suggested that the amino sugar of chitosan appears to interact with bile acid and/or cholesterol in the intestinal lumen and to stimulate fecal excretion of steroids, thereby interfering with the absorption process (*6*).

The levels of total lipids, cholesterol and triglycerides in feces (Table 3) were always higher in animals fed on beef tallow and cholesterol (diets 2, 3, and 4) than in control group (diet 1). Chitin produced a higher excretion of triglycerides. Sugano *et al.* (*4*) reported that chitosan, but not chitin, raised fecal excretion of neutral steroids. Nauss *et al.*, based on *in vitro* studies, suggested that the binding of micellar lipids with chitosan interfere with the absorption of triglycerides (*15*).

The results of this work suggest that chitin may be effective in controlling lipid absorption from intestines. Since chitin is a neutral polysaccharide, its interference with the absorption of cations may be less pronounced than that observed with chitosan. Since the chitin used contained 16% of chitosan, there remains the possibility that the observed effects might be due to the latter. However, the low level of chitosan (0.8%) may be an argument to rule out this hypothesis. A higher proportion of chitin in the diet, the use of chitin with a higher degree of purity, a longer period of experimentation, and a higher number of animals may give more precise information on the parameters measured here.

This work was supported by Financiadora de Estudos e Projetos (FINEP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). The technical help of Márcio Eustáquio Silva is greatly acknowledged.

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