Effects of Chitosan—a Coagulating Agent for Food Processing Wastes—in the Diets of Rats on Growth and Liver and Blood Composition

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ABSTRACT

Effects of feeding free chitosan to rats at graded levels up to 15 percent of the diet for eight weeks was investigated. Animals receiving diets containing 5 percent or less of chitosan grew well at comparable rates. Progressive growth reductions occurred when chitosan was increased to 10 and 15 percent of the diet and enlargement of liver and kidneys was observed only in animals receiving the highest level of dietary chitosan. Liver moisture, protein, lipid, ash, and nucleic acids; blood hemoglobin and packed cell volume; and serum total protein, albumin, ceruloplasmin and transferrin were determined. Values for these components of liver and blood were altered significantly in the animals receiving the highest level of chitosan when compared to control animals. However, in animals receiving 5 percent or less of dietary chitosan none of these measures of tissue composition was different from controls, except for liver protein concentration of rats fed the 5 percent of chitosan diet. Animal feeds containing coagulated by-products are not expected to contain over 0.2% chitosan in the total diet. No adverse effects have been observed at this level in rat feeding studies. Therefore the tolerance level for dietary chitosan appears to be well above the levels expected to be in animal feeds containing by-products recovered from food processing wastes by coagulation with chitosan.

INTRODUCTION

Research on utilization of solid shellfish wastes has resulted in application of chitosan for coagulation of suspended solids in liquid waste effluents from a variety of processing operations (PENISTON and JOHNSON, 1970; BOUGH, 1975a,b; BOUGH, et al., 1975a,b). Chitosan is a polymer composed of glucosamine residues linked by β, 1-4 glucosidic bonds. It is a deacetylated derivative of chitin, which is the structural polymer of the exoskeleton of shellfish. Chitosan carries a net positive charge, and the polyelectrolytic character of this polymer is the basis for its use as a coagulating agent in physical-chemical waste treatment systems. The most recent applications of chitosan have

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been coagulation of activated sludge from a brewery and a vegetable cannery (BOUGH, et al., 1975a), poultry processing wastes (BOUGH, et al., 1975b), and egg breaking wastes (BOUGH, 1975b).

Sludge from waste treatment systems of food and beverage processing plants is a major disposal problem, and utilization of the dried sludge as a protein supplement in animal feeds may be a desirable solution in many instances. Utilization of chitosan as a coagulation agent in recovery of sludge or other waste by-products will result in its introduction into the food chain. Because of its electrolytic nature, the question of its effects, if any, on the consuming animal needs to be investigated.

ARAI, et al. (1968) found that chitosan caused no harmful effects in mice when fed at levels up to 18 g per kg body weight per day. In a preliminary study by BOUGH, et al. (1975a), significant growth retardation was not observed in rats, when chitosan was fed at levels less than 5 percent of the diet.

The present study was initiated to further investigate the effects of feeding chitosan to rats at graded levels to 15 percent of the diet. Data on growth, hematological and serum analyses, and liver composition, will be presented and discussed.

METHODS AND MATERIALS

Weanling Sprague-Dawley male rats (80 g average weight), obtained from Charles River Breeding Laboratories, were divided into groups of 10 and were fed the experimental diets for 58 days. The animals were housed individually and food and deionized water were supplied ad libitum. The control diet had the following percentage composition: soybean meal, 35.0; soybean oil, 20.0; dextrin, 15.0; sucrose, 11.43; mineral mix[a], 1.57; vitamin mix[b],

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[a]The mineral mixture provided per 100 g of diet, calcium phosphate, 878 mg; calcium carbonate, 556 mg; manganese sulfate monohydrate, 9.2 mg; sodium chloride, 124 mg; and potassium iodate, 28 mg.

[b]The vitamin mixture provided per 100 g of diet, vitamin A, 2000 units, vitamin D, 220 units; alpha tocopherol, 11 mg; ascorbic acid, 100 mg; inositol, 11 mg; choline chloride, 165 mg; menadione, 5 mg; p-amino benzoic acid, 11 mg; niacin, 10 mg; riboflavin, 2 mg; pyridoxine hydrochloride, 2 mg; thiamine hydrochloride, 2 mg; calcium pantothenate, 6.6 mg; biotin, 44 mcg; folic acid, 200 mcg; and vitamin B_{12}, 3 mcg.

1.0; dl-methionine, 1.0; and cellulose, 15.0. The test diets were prepared by adding chitosan[c] at 1.0, 2.5, 5.0, 10.0, and 15.0 percent of the diet at the expense of cellulose.

The animals were anesthetized with pentobarbital and exsanguinated by heart puncture. Blood samples were taken immediately for packed cell volume (PCV), using heparinized microtubes and for total hemoglobin (Hb) (EVELYN and MALLOY, 1938). The following determinations were made on the blood serum: total protein and albumin by the biuret method (NATELSON, 1971), ceruloplasmin (Cp) (SUNDERMAN and NOMOTO, 1970) and transferrin or total iron binding capacity (TIBC) (GOODWIN, et al., 1966 and STOOKEY, 1970).

Kidneys, spleen, and liver were removed and weights of these organs determined. The liver was sampled for determination of nucleic acids before being quick-frozen. Nucleic acids were extracted, as described by MUNRO and FLECK (1966). Ribonucleic acid (RNA) was determined by ultraviolet spectroscopy, and deoxyribonucleic acid (DNA) was determined by the method of CERIOTTI (1952). Liver protein content was measured by the method of LOWRY, et al. (1951), liver lipid as described by MILLER (1974), and liver ash by heating at 450°C for 20 hours in a muffle furnace.

The data were subjected to analysis of variance, and differences associated with dietary treatment were determined according to the multiple range tests of DUNCAN (1955).

RESULTS

The diets were consumed by all groups of animals, indicating that they had acceptable palatability. Chitosan intake was calculated by solving the regression equation for chitosan intake on body weight. Division of this solution by days on experimental diets results in daily chitosan intake (Table I). Those animals receiving diets containing 5 percent or less of chitosan grew well at comparable rates. Progressive significant growth reductions occurred when chitosan was increased to 10 and 15 percent of the diet. Efficiency of food utilization was also decreased by addition of chitosan to the diets at 10 and 15 percent levels.

Liver, kidneys, and spleen weights (Table II) were reduced only in the groups receiving 10 and 15 percent dietary chitosan.

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[c]The chitosan was obtained from Food, Chemical and Research Laboratories, Inc., Seattle, Washington and could be classified as free chitosan carrying an associated hydroxide ion. Before being added to the diets, it was ground to 20 mesh in a Wiley mill.

TABLE I

Weight gain, feed efficiency and chitosan intake of rats fed increasing levels of chitosan[1/]

% Chitosan in Diet	Chitosan Intake[2/]	Weight Gain g	Feed Efficiency[3/]
0	0.0	325[a]	.281[a]
1.0	0.55	334[a]	.282[a]
2.5	1.36	354[a]	.286[a]
5.0	2.87	337[a]	.268[a]
10.0	7.24	260[b]	.234[b]
15.0	16.50	163[c]	.149[c]

[1/] Values in a column followed by the same letter are not different at the 5% level of probability.
[2/] Chitosan intake is expressed as g of chitosan consumed per kg of body weight per day.
[3/] Feed efficiency is expressed as g of weight gain per g of food intake.

This decrease was in proportion to body weight for those animals receiving 10 percent dietary chitosan, but those receiving 15 percent of chitosan in the diet exhibited some organ enlargement, with the enlargement of the liver and kidneys being significant.

Calculations based on DNA content (SRIVASTAVA, et al., 1974) indicated a decrease in the number of hepatic cells in animals receiving the two highest levels of chitosan (Table III). The decrease in number of cells was in proportion to liver size as indicated by constant hepatic cell weight in all treatments. RNA concentration was significantly increased in animals receiving the highest level of chitosan. Protein concentration was higher in animals receiving 5 percent or more of chitosan. Lipid concentration was decreased in animals receiving the 10 and 15 percent levels of dietary chitosan, with the decrease being significant for those receiving 15 percent of chitosan in the diet. These animals also had a significantly higher concentration of water in the liver than was found in the other groups. Concentration of ash was constant in all treatment groups.

When the components measured were calculated on quantity per hepatic cell, there was generally good agreement between these values and values based on the whole tissue. However, differences were not significant because of more variation within treatments, since these values are based on a less precise measurement of number of cells rather than a more precise

TABLE II

Organ weights and percent of body weights of animals fed increasing levels of chitosan[1/]

% Chitosan in Diet	Liver		Kidneys		Spleen	
	Wt. g	% Body wt.	Wt. g	% Body wt.	Wt. g	% Body wt.
0	15.1^{ab}	3.73^{b}	2.72^{ab}	0.67^{b}	0.76^{a}	0.19
1.0	15.7^{a}	3.79^{b}	2.88^{a}	0.70^{b}	0.78^{a}	0.19
2.5	16.0^{a}	3.70^{b}	2.88^{a}	0.67^{b}	0.78^{a}	0.18
5.0	15.2^{ab}	3.63^{b}	2.79^{ab}	0.67^{b}	0.84^{a}	0.20
10.0	12.8^{b}	3.76^{b}	2.42^{b}	0.72^{b}	0.68^{ab}	0.20
15.0	10.0^{c}	4.31^{a}	1.95^{c}	0.82^{a}	0.54^{b}	0.21

[1/]Values in a column followed by the same letter are not different at the 5% level of probability.

measurement of organ weights.

Hematological status of the animals receiving 5 percent or less of chitosan was normal (Table IV), with both hemoglobin and packed cell volume decreasing significantly in the animals receiving 10 and 15 percent of dietary chitosan. Total serum protein was significantly lower than controls in the animals receiving the highest level of chitosan, but serum albumin and transferrin were not affected by dietary treatment. Ceruloplasmin was not affected consistently by dietary treatments, since it was elevated in the group fed 15 percent and decreased in the group fed 5 percent of chitosan compared to the control group. However, none of these differences were significant when compared to the control.

DISCUSSION

Chitosan is a glucosamine polymer similar in structure to cellulose. Since it probably would not serve as a source of energy, it was added to the rat diets at the expense of cellulose rather than one of the energy components. In order to raise the caloric density of the diets to meet the rat's needs, soybean oil was added at 20 percent of the total diet.

TABLE III

Several components of the livers from rats fed increasing levels of chitosan[1/]

% Chitosan in Diet	DNA mg/g	Cells Billions	Cell Weight ng	RNA		Protein		Lipid		Water		Ash	
				mg/g	pg/cell	mg/g	pg/cell	mg/g	pg/cell	mg/g	ng/cell	mg/g	pg/cell
0.0	1.30	6.35^{ab}	2.37	7.17^{b}	15.2	189^{bc}	444	44.8^{ab}	119	700^{b}	1.68	12.6	30.0
1.0	1.19	6.44^{ab}	2.56	7.01^{b}	18.0	189^{bc}	481	51.8^{a}	132	693^{b}	1.77	12.8	32.6
2.5	1.37	7.13^{a}	2.41	7.23^{ab}	17.4	185^{c}	444	50.4^{a}	122	696^{b}	1.68	12.8	30.7
5.0	1.27	6.24^{ab}	2.60	6.92^{b}	18.1	202^{a}	524	48.6^{a}	126	695^{b}	1.81	13.1	33.9
10.0	1.18	4.73^{bc}	2.75	7.47^{ab}	20.2	199^{ab}	541	42.3^{ab}	117	706^{b}	1.94	12.9	35.4
15.0	1.31	4.25^{c}	2.54	8.05^{a}	20.4	203^{a}	512	37.1^{b}	95	723^{a}	1.83	12.9	32.7

[1/]Values in a column followed by the same letter are not different at the 5% level of probability

TABLE IV

Several components of blood and serum from rats fed increasing levels of chitosan[1/]

% Chitosan	Hb %	PCV %	Protein %	Albumin %	TIBC mg/100 ml	CP mg/100 ml
0.0	15.8[a]	50.6[a]	6.33[a]	2.01	522	51.5[ab]
1.0	15.5[a]	49.6[a]	6.32[a]	2.15	613	47.4[b]
2.5	15.1[a]	49.3[a]	6.00[ab]	2.02	539	47.2[b]
5.0	15.0[a]	49.2[a]	6.17[a]	2.07	549	42.9[b]
10.0	14.0[b]	47.8[ab]	6.09[ab]	2.16	537	55.7[ab]
15.0	13.3[c]	46.2[b]	5.62[b]	2.19	613	62.6[a]

[1/]Values in a column followed by the same letter are not different at the 5% level of probability.

Those animals consuming diets containing up to 5 percent of chitosan thrived with a uniform growth rate, but growth of the animals on 10 and 15 percent of chitosan diets was not uniform. Some of these animals grew reasonably well, while others did not do well at all. Those that did not do well on these diets developed a dermatitis on the face and ears, paws, and around the anal area. This dermatitis was observed only in animals on the 15 percent of chitosan diet, with one or two exceptions. Diets containing 10 and 15 percent of chitosan were somewhat greasy, compared to the control diet, as chitosan did not absorb oil as well as cellulose. Those animals with dermatitis were greasy in appearance, and it may be possible that the oil of these diets solubilized some substance from chitosan, which caused the dermatitis.

Chitosan intake of the animals in this experiment was somewhat lower than that observed at comparable dietary levels in the preliminary study of BOUGH, et al. (1975a). This difference may be explained by the fact that maximum daily food intake was reached after about 5 weeks on the diets, at which time the previous study was terminated, yet growth continued throughout the present study. This would lower daily chitosan intake per kg body weight until the animals stopped growing.

Detrimental effects of dietary treatment began to appear at the 10 percent level of addition of chitosan to the diet or at a daily intake of 7.2 g per kg of body weight per day. This is less than half the level of 18 g reported by ARAI, et al. (1968)

as being detrimental for mice. As chitosan was increased further to 15 percent of the diet or 16.5 g daily intake per kg of body weight, severe detrimental effects were observed in growth and significant changes occurred in almost all of the biochemical evaluations made on the liver and blood.

Animals receiving the highest level of chitosan had reduced liver lipid concentration and in fact had very little or no depot fat. This and the very low feed efficiency indicated that these animals were using all the energy derived from the diet to maintain body functions. Elevated liver RNA and protein concentration in these animals indicated an increased rate of protein synthesis and retention. This would be expected to some extent since the liver is the major site of detoxication and metabolism of foreign substances. The other biochemical changes observed appear to be related to these phenomena. Probably hemoglobin and total serum protein synthesis was reduced partially as a result of liver protein synthesis and the liver moisture generally is related to lipid content in an inverse manner.

This study indicates that free chitosan may be incorporated into rat diets at levels of 5 percent or less with no adverse effects. This level is about 25 times that expected to be present in an animal feed containing activated sludge coagulated by chitosan (BOUGH, et al., 1975a). Coagulated sludge from that study contained 0.3-0.8 percent of chitosan on a dry weight basis, and if it was incorporated in an animal feed at a level of 20 percent, the chitosan concentration would be less than 0.2 percent of the final product. Also, chitosan present in coagulated sludge would not be expected to be in a free state, as that used in this experiment, but would be bound to the sludge in some manner. Therefore, this may give even more protection to the consuming animal, however this has not been shown experimentally. Further investigations are planned to study the cause of the dermatitis observed in these animals on the highest level of chitosan and the effects of feeding activated sludge and other by-products coagulated with chitosan.

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