DIGESTIBILITY AND HYPOCHOLESTEROLEMIC EFFECT OF CHITIN AND CHITOSAN IN ALBINO RATS

By

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Chitin and chitosan prepared from prawn head and shell waste were incorporated in the diets of albino rats (Wistar strain) in 0.5% and 1.0% levels and fed for a period of 13 weeks. At the end of the experimental feeding the cholesterol contents in the internal organs and serum were decreased in the chitin and chitosan fed animals compared to the control. The hypocholesterolemic effect was more significant in the serum than in the internal organs. This effect was more pronounced with chitosan than chitin.

INTRODUCTION

Chitin, a polymer of β (1-4) N-acetyl glucosamine is the second most abundant natural organic polymer available on earth. It is present in a vast majority of fungi as the principal fibrillar polymer in the cell wall. Arthropods are the best known and most important chitin producing animals. Besides the arthropods relatively large amounts of chitin is found in the setae of annelids, in the skeleton of colonies of bryozoa and in the shells and outer structures of many molluscan species. Although widely distributed, the most economic and available source of chitin is the head and shell of shrimps and crabs. Processes for the extraction of chitin from the shell and conversion to chitosan and other useful derivatives have been already developed (Madhavan & Nair, 1974, Somarin *et al.*, 1982, Muzzarelli and Tanfani, 1982). Various applications of chitin and its derivatives are well known now. It is non-toxic and it is reported that chitosan is as safe as sugar or salt (Asano *et al.*, 1978). Chitin in broiler diets containing whey has increased the digestibility and caused better feed conversion (Zikakis *et al.*, 1982). Chitin at the 0.5% level in broiler diet caused an additional 10-12% weight gain within 12 weeks (Nair *et al.*, 1987). The hypocholesterolemic effect of chitin and chitosan on albino rats is discussed in this paper.

MATERIALS AND METHODS

Chitin and chitosan were prepared from the head and shell of a single species of prawns, *Penaeus indicus*, by following the method of Madhavan & Nair (1974), dried and powdered in an ultracentrifugal mill to a particle size 0.5 mm to 1.0 mm and moisture, protein, fat and ash in the chitin and chitosan were estimated by the method of AOAC, 1978. Chitin was estimated according to the method described by Richards, 1978. Four experimental diets were formulated by incorporating chitin at 0.5% (Diet A), 1% (Diet B) and chitosan 0.5% (Diet C) in standard control diet (Diet D) keeping the concentration of protein at 10%. Mineral mixture and vitamin mixture were also added to the diets. Male weaning rats (Wistar strain) weighing 30-35 g were individually raised in cages on a basal diet. The animals were divided at random into four groups adjusted to give mean average

weight and were housed individually in cages. They were fed on weighed amounts of the test diets and water was supplied ad-libitum for 13 weeks and the average weekly body weights were determined.

After 13 weeks of feeding, the animals were slaughtered and muscle, kidney, heart and liver were collected. A portion (approximately 2 g) of each was weighed and homogenized in chloroform methanol (2:1 v/v) to extract the lipids and the volume was made up to 50 ml. Aliquots were pipetted and total cholesterol was determined by the method of Oser, 1976. About 3-4 ml blood was collected from the rats by puncturing the heart. It was centrifuged at 5000 rpm at 4°C for 20 minutes to separate the serum for determination of cholesterol. Haemoglobin in blood was determined by the method of Hunten and Brownford, 1956. Digestibility of chitin and chitosan was determined by the method of Elson and Morgan, 1933.

RESULTS AND DISCUSSION

The animals were healthy throughout the experimental feeding and no adverse symptoms or mortality was seen either in the control or in the experimental groups. Analysis of stools of experimental animals showed that nearly 90% of chitin and chitosan were digested (Table 4). The growth rates of animals are presented in Table 1. The results show that the experimental diets containing chitin and chitosan were also similar to the control diet as reported earlier by Mathew et al., 1989. The chitin diet did not show any growth promoting effect in contrast to that observed in broiler chicken (Nair *et al.*, 1987, Zikakis *et al.*, 1982). There was no mortality in either of the groups during the 13 weeks feeding. There was no significant difference in the weight of the internal organs of the animals

Table 1

Weight (g) of albino rats fed on experimental diets

Age in weeks	Diet A	Diet B	Diet C	Diet D
0	31.75+-4.86	31.75+-3.34	31.75+-3.50	31.75+-3.5
1	53+-5.24	52.75+-3.89	52.00+-3.25	49.5+-10.5
2	65+-3.08	59.25+-6.05	58.25+-4.50	63.5+-11.5
3	71.5+-3.04	63.5+-3.04	69.50+-4.82	72.75+-6.5
4	90.5+-6.02	82.75+-9.36	83.25+-5.65	89.5+-3.2
5	111+-7.58	104.75+-5.06	105.25+-5.15	104.75+-3.5
6	123.25+-11.00	115.25+-11.80	112.75+-6.50	125+-7.10
7	137.25+-13.5	132.5+-10.73	132.25+-7.52	136.75+-10.1
8	150.75+-14.25	150.75+-7.80	151.25+-6.50	152+-11.52
9	163+-13.15	162.25+-7.46	163.75+-7.2	168.25+-12.51
10	174+-11.72	166+-3.53	178.25+-5.1	181.5+-10.5
11	183+-8.83	184+-7.34	185.0+-7.51	186+-9.25
12	186.75+-14.16	194+-8.21	191.25+-7.8	195+-8.50
13	195+-13.05	204+-10.82	201+-8.50	204+-10.8

Mean + S.D. m = 5.

Table 2

Proximate composition of chitin and chitosan

	Chitin	Chitosan
Moisture	3.5	3.5
Fat	Nil	Nil
Protein	Nil	Nil
Ash	2	0.5
Chitin	94.5	Nil
Chitosan	Nil	96

resulting from diet. Table 3a shows the cholesterol levels of the animals fed on control and experimental feeds. The hypocholesterolemic effect of chitin and chitosan was remarkable in serum and not so pronounced in heart whereas in other internal organs such as liver and kidney, a nominal increase was noted in cholesterol content. Chitosan was found to reduce cholesterol in serum more significantly than chitin. The feed intake of animals in all the groups was almost similar. As seen from the Table, incorporation of 0.5% chitosan was found to be better than incorporation of 0.5 or 1% chitin in the diet for decreasing the serum cholesterol level in albino rats which is in agreement with the observations in broiler chicken by Hirano, 1980.

Table 3a

Total cholesterol

	Liver g/100 g	Muscle g/ 100 g	Kidney g/100 g	Heart g/100 g	Serum mg/ 100 ml
Control	944+-8.9	391.4+-5.16	345+-5.6	652+-14.3	67+-5.2
C + 0.5% chitin	960+-3.8	340+-7.9	370 = 4.2	550+-7.5	35+-2.1
C + 1% chitin	960+-10.47	373+-15.81	375+-8.7	530+-5.2	33+-4.2
C + 0.5% chitosan	950+-5.8	530+-7.5	650+-6.9	530+-5.2	33+-4.2

Mean + S.D. m = 5 C - Control

In the case of haemoglobin level in blood, there was slight increase in animals fed on chitin or chitosan diet compared to the controls as shown in Table 3b.

Mathew *et al.*, 1989, in their studies on feeding chitin at 0.5% level to rats found that the weight gain was slightly reduced compared to those fed on control diet, Gordan and Williford, 1984, reported that growth rate of rats was significantly reduced by feeding chitosan at levels between 2.5% to 20%. They also reported a significant reduction in haemoglobin content in animals fed diets with chitosan at levels of 2.5 to 5% compared to those fed on chitin diet. In the present experiments by feeding chitin and chitosan at 0.5% levels no significant change in haemoglobin content between the two groups fed on chitin and chitosan showing that low levels chitosan did not reduce haemoglobin levels and also that consumption of both chitin and chitosan at low levels increases the haemoglobin in blood.

Table 3b

Haemoglobin content of serum (g/100 ml)

Control	11.5+-0.31
C + .5% chitin	13.2+-0525
C + 0.5% chitosan	13.30+-0.47

Table 4

Digestibility of chitin/chitosan in rat faeces

	0.5% chitin %	1% chitin %	0.5% chitosan %
2 weeks	92	90	80
4 weeks	95	94	85.5
8 weeks	96	95	85
12 weeks	96	95.5	89

Certain proteins are supposed to play an important role in cholesterol depression in animals as reported by several workers; but the modus operandi of chitosan is not so far explained. The digestibility of chitosan was 80% after two weeks and was 89% by the 12th week, indicating an increase in digestibility of chitosan by adaptation to feeding. The digestion probably occurs by hydrolysis of chitosan by chitinase and chitosinase enzymes which are secreted by intestinal bacteria. In addition, plant ingredients present in commercial diets have chitinase activity. Hirano *et al.*, 1988, found from 910 to 1200 m u/100 g of dry diet and enzyme activity probably plays a minor role in the digestion of chitin and chitosan.

From the nutritional point of view, chitosan is very safe and readily available material for decreasing cholesterol in farmed animals by using it as a feed additive.

This observation opens up a new and very important medicinal application of chitosan.

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