Fishery Technology
1998, Vol. 35(1) pp : 46 - 49

Hypocholesterolemic Effect of Chitin and its Hydrolysed Products in Albino Rats

P.T. Mathew and K.G. Ramachandran Nair
Central Institute of Fisheries Technology
Cochin - 682 029

Male albino rats (Wistar strain) were fed with diets supplemented with chitin, partially hydrolysed chitin and glucosamine hydrochloride (0.5% of the diet) for 13 weeks. During the course of the experimental feeding no significant difference in growth rate was observed for the above three groups compared to the animals of control group. There was significant decrease in lipid deposition, and cholesterol levels in muscle, serum, liver, kidney and heart of the experimental animals. It was more pronounced in the chitin- and PH chitin fed groups of animals. Difference in digestibility of chitin and derivatives were also not significant.

Key words: Chitin, partially hydrolysed chitin, glucosamine hydrochloride, hypocholesterolemic effect.

Chitin, a polymer of N-acetyl glucosamine, present in the head and shell waste of crustacea and chitosan, deacetylated chitin, have got numerous applications in diverse areas. The nutritional properties of these substances have been well documented. Growth promoting property of chitin in broiler chicken was reported by Nair *et al.* (1987) and effect of incorporation of chitosan as an ingredient in domestic animal feeds was studied by Hirano *et al.* (1990). Chitosan is a safe hypocholesterolemic agent, since its side effect is lower than that of cholestyramine, an anion exchange resin having bile acid lowering capacity (Gordon & Williford, 1984; Jennings *et al.*, 1988). The present study was undertaken to investigate the hypocholesterolemic effects of chitin, partially hydrolysed (PH) chitin and glucosamine in albino rats.

Materials and Methods

Chitin was prepared in the laboratory from the exoskeleton of prawn, *Penaeus indicus*. PH chitin was prepared by treating chitin with 3N HCl at room temperature for 3 h. It was washed free of acid, dried and powdered to a particle size 0.55 mm. Glucosamine hydrochloride was prepared according to the method of Kamasastri & Prabhu (1961). Four experimental diets were formulated; diet A- control, diet B- control + 0.5% chitin, diet C- control + 0.5% PH chitin and diet D- control + 0.5% glucosamine hydrochloride (Table 1). Protein content was kept at 10% level in all diets. 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. Animals were divided at random into four groups (6 each), adjusted to give mean average weight and were housed individually in cages. They were fed on weighed amounts of the test diets for 13 weeks and the weekly body weights were recorded.

After 13 weeks the animals were killed and the muscle, kidney and heart

Table 1. Composition of feeds (% by wt.)

	A	B	C	D
Casein	12.6	12.6	12.6	1.26
Refined oil	5	5	5	5
Shark liver oil	2	2	2	2
Vitamin mixture	1	1	1	1
Salt mixture	2	2	2	2
Glucose	25	24.5	24.5	24.5
Corn starch	52.4	52.4	52.4	52.4
Chitin	0	0.5	0	0
PH chitin	0	0	0.5	0
Glucosamine hydrochlorie	0	0	0	0.5

were dissected. A portion (approx. 2 g) of each was weighed and homogenised in chloroform-methanol (2:1) mixture to extract the lipids (Bligh & Dyer, 1959). About 3-4 ml of blood was collected from the rats by puncturing the heart. Portion of blood (2-3 ml) was put into small plastic centrifuge tubes kept at 4°C and centrifuged at 1000 g at 4°C for 20 min to give serum. Cholesterol content of serum was determined by the method of Hawk (1954). Portions (2 g) of liver and muscle of rats were homogenized in chloroform-methanol (2:1, v/v), and the mixture was centrifuged at 1000 g for 20 min at 4°C to give supernatant. The total cholesterol content of supernatant was determined by the method of Hawk (1954). Digestibility of chitin, partially hydrolyzed chitin and glucosamine was determined by the method of Blix (1934).

Results and Discussion

The animals were found healthy through out the experimental period. No adverse symptoms or mortality were observed either in the control or in the experimental groups. Feeding of rats with diets supplemented with chitin, PH chitin and glucosamine hydrochloride did not significantly affect the growth rate of rats during 13 weeks (Fig. 1).

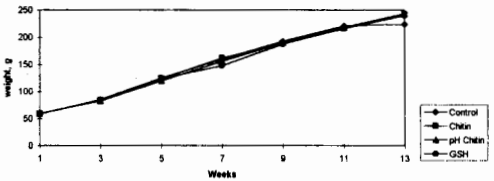

Fig. 1. Growth rate of rats

These results are similar to those obtained by Mathew *et al.* (1989) in which case also the effect of chitin on growth rate in rats was not significant. Chitin, PH chitin and glucosamine hydrochloride are found to be growth promoters in rabbits, chicks and piglets (Hirano, *et al*, 1990; Nair *et al.*, 1987). This shows that the growth promoting effect of chitin and its derivatives is not the same in all species. There was a significant lowering of lipid content of muscle and internal organs of the experimental animals compared to the control group. This was more pronounced in the case of animals fed on PH chitin (Table 2).

Cholesterol levels of serum and different organs of the four different

Table 2. Lipid content of different organs of rat (g/100 g)

	Liver	Kidney	Muscle	Heart
Control	18.53±2.1	17.69±1.61	16.07±0.57	15.35±0.82
Chitin	12.70±1.35	13.12±0.46	11.89±0.94	15.47±0.78
PH chitin	9.57±0.61	13.73±1.60	10.03±0.26	10.68±0.57
Glucosamine	10.11±0.40	14.43±0.72	11.88±1.67	13.64±0.78

Table 3. Total cholesterol in organs and serum of rat

	Liver (mg/100g)	Kidney (mg/100g)	Muscle (mg/100g)	Heart (mg/100g)	Serum (mg/100ml)
Control	460.0±42.26	465.2±26.28	112.7±11.43	223.3±5.05	86.2±0.94
Chitin	217.6±11.52	363.7±29.30	85.9±4.15	153.9±7.26	55.4±0.54
PH chitin	242.3±39.30	334.8±25.17	73.5±5.51	179.6±17.28	60.9±0.64
Glucosamine	376.8±52.71	347.5±5.76	104.6±5.76	170.3±12.24	83.3±1.468

groups of rats are presented in Table 3. Cholesterol levels were significantly low in all the three experimental groups. However glucosamine hydrochloride was found to be less effective than chitin or PH chitin. Digestibility was found to be maximum in the case of glucosamine hydrochloride. It was absorbed directly because it is a completely hydrolyzed product (Table 4). Therefore the low hypocholesterolemic effect of glucosamine hydrochloride is not due to its poor digestibility.

Table 4. Digestibility of chitin/partially hydrolyzed chitin and glucosamine hydrochloride (% absorption)

	0.5% Chitin	0.5% PH chitin	Glucosamine HCl
3rd week	92	95	98
6th week	93	96	98.2
12th week	93	96	99

Hypocholesterolemic effect of chitin and PH chitin has been attributed to the viscosity of chitin hydrolysates during digestion (Ikeda *et al.* 1993; Sugano *et al.*, 1992). Hypocholesterolemic effect of glucosamine incorporated diet is less than that of the chitin and PH chitin diets. Sugano *et al.* (1992) have reported that products of hydrolysis of chitin having a molecular weight of $7x10^3$ or more only have hypocholesterolemic effects. The ineffectiveness of glucosamine as a hypocholesterolemic agent suggests that a certain degree of polymerisation is required to induce cholesterol lowering activity (Sugano *et al.* 1992). This is in agreement with the results obtained in this study. It is not clear whether the mechanisms proposed for viscous fibre i.e., reduction of diffusion of micellar lipids to intestinal wall (Vahouny, 1982; Edwards, 1988 and Furda, 1990), is applicable to chitin and PH chitin.

The dietary effects of chitosan in adult men were reported by Maezaki *et al.*, (1993). A favourable effect was shown with a very low dose within a short period. The explanation given by Furda (1983) and Nauss *et al.* (1983) for the hypocholesterolemic effect of chitosan is based on the anion exchange capacity of chitosan. It is an anion exchanger which binds bile acids and fatty acids by ionic bond at pH lower than 6.0. Studies by Furda (1983) on rats indicated that the hypocholesterolemic action of chitosan is independent of its molecular weight within the tested viscosity range. According to Ikeda *et al.* (1993) chitosan hydrolysates with average molecular weights of 10,000 Dalton and above were more effective in faecal excretion of neutral steroids.

It therefore appears that products of hydrolysis of chitin above a particular molecular weight only are effective in controlling cholesterol metabolism. The results of this investigation show that

chitin and partially hydrolysed chitin are effective hypocholesterolemic agents. Glucosamine was not as effective as chitin or partially hydrolysed chitin. The effectiveness of chitin as a hypocholesterolemic agent appears to be due to its polymeric nature.

The authors are thankful to the Director, Central Institute of Fisheries Technology, Cochin-682029 for the facilities provided for the work.

References

Bligh, E.G. & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* 37, 911

Blix, G. (1934) *Acta Chem. Scand.* **2**, 467

Edwards, C.A. (1988) In *Dietary Fibre* (Kritchevsky D., Bonfield, C. & Anderson, J.W. Eds.) Plenum Press, New York, p.167

Furda, I.(1983) In: *Unconventional sources of dietary fibre* (Furda, I., Ed.) p.105 American Chemical Society, Washington, DC

Furda, I. (1990) In *New Developments in Dietary fibres* (Furda, I. & Brine, C.J., Eds.), p.67 Pleenum Press, New York

Gordon, D.T.& Williford, C.B.(1984) In *Chitin, Chitosan and related enzymes* (Zikakis, J.P., Ed.), p.97, Academic press, Orlando, FL.

Hawk, P.B. (1954) In *Practical Physiological Chemistry* (Hawk, P.B., Oser, B.L. & Summerson, W.H.,Eds.) 13th edition, p.584 Mcgraw-Hill Book Co., New York

Hirano, S., Itakura, C., Serino, H., Akiyama, J., Nanaka, S., Kanbara, N. & Kawakami, T. (1990) *J. Agric. Food Chem.* **38**, 1214

Ikeda, I., Sugano, M., Yoshida, K., Sasaki, E., Iwamoto, Y. & Hatano, K. (1993) *J. Agric. Food Chem.* **41**, 431

Jennings, C.D., Boleyn, K., Bridges, S.R., Wood, P.J. & Anderson, J.W. (1988) *Proc. Soc. Exp. Biol. Med.* **189**, 13

Kamasastri, P.V. & Prabhu, P.V. (1961) *J. Sci. Industr. Res.* **20**, 466

Maezaki, Y., Tsuji, K. & Nakagawa, Y. (1993) *Biosc. Biotech. Biochem.* **57**, 1439

Mathew, P.T., Nair, K.G.R., Madhavan, P. & Prabhu, P.V. (1989) *Fish. Technol.* **26**, 36

Nair, K.G.R., Mathew, P.T., Madhavan, P. & Prabhu, P.V. (1987) *Ind. J. Poult. Science*, **22**, 40

Nauss, J.L., Thompson, J.L.& Nagyvarey, J. (1983) *Lipids*, **18**, 714

Sugano, M.G., Yoshida, K., Hashimoto, M., Enomoto, K. & Hirano, S. (1992) In *Advances in chitin and chitosan,*(Brine, C.J., Sandford, P A. & Zikakis, J.P., Eds.) p.472, Elsvier Applied Sciences, London

Vahouny, G.V. (1982) *Fed. Proce.* **41**, 2801