Pak J Sci Ind Res 2002 ***45***(5) 334-336

Hypocholesterolaemic Effect of Leaf Protein Concentrate (LPC) on Blood Cholesterol of Rats and Poultry

Sakhawat Ali, Tahira Firdos, W H Shah* and F H Shah

Biotechnology, Environment and Food Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan

(Received 2 August 2000; accepted 26 June 2001)

Leaf protein concentrate (LPC) was extracted from barseem leaves and incorporated in the diet replacing casein in case of rats and fishmeal in poultry. A series of experiments were conducted on rats and poultry to determine the hypocholesterolaemic effect of LPC on the blood cholesterol of rats and poultry. Results indicated that incorporation of LPC decreased cholesterol level of blood in rats and poultry. Decrease in phospholipids has also been noticed with the increase in concentration of LPC. Decrease in cholesterol content in eggs laid by hens fed on diet containing LPC has also been observed.

Key words: Leaf protein concentrate, Hypocholesterolaemic, Rats, Poultry.

Introduction

Epidemiological studies have shown that elevated concentration of plasma cholesterol is a major factor in aetiology of coronary diseases (Malinow *et al* 1977). Purmova and Opletal (1996) and Hiroshi and Akira (1996) reported that mainly saponins from the plant of Salvia, Astragalus and Litchi lowered cholestlerol level in blood and could be effective in treatment of diseases of heart and circulatory system. Many workers (Malinow *et al* 1977; Oakenfull *et al* 1979; Hiroshi and Akira 1996) have reported that food containing saponins could be incorporated in formulating hypocholesterolaemic diets for human consumption. Sidhu and Oakenfull (1986) observed that saponins were stored as glycosides which occurred in a number of important food plants and were known to be hypocholesterolaemic. Some saponins formed an insoluble complex with cholesterol which prevented its absorption from the small intestine and caused an increase in faecal excretion of bile acids, resulting in the elimination of cholesterol. They concluded that control of plasma cholesterol and nutrient absorption through dietary saponins could provide substantial health and nutritional benefits in humans. Thus present study was carried to determine the hypocholesterolaemic effect of leaf protein concentrate (LPC) on rats and poultry.

Materials and Methods

LPC was prepared from barseem leaves (*Trifolium alexandrium*) according to the methods described by Shah *et al* (1988). Proximate composition of LPC was carried out according to AOAC (1984) methods (Table 1). Albino rats and poultry (laying hens) were used in experimental trials. They were housed in cages and were allowed to consume feed and water *ad libitum*. Blood samples were collected by direct heart puncture in the case of the rats and from jugular vein in the laying hens. The blood was centrifuged at 1500 rpm for 5 min to separate the serum and stored in deep freezer (-40 °C). Egg powder was prepared according to Muhammad *et al* (1988). Cholesterol was determined by method of Bowman and Wolf (1962) and phospholipids by Amic *et al* (1972).

Experiment No. I. Eight healthy adult male rats of 270-300 g body weight were randomly divided into two groups of same weight and size. They were housed in separate cages in the animal house of PCSIR Laboratories Complex, Lahore. Temperature was maintained at 20 ± 2 °C. Two diets were formulated and designated as A and B (Table 2). Diet A served as control containing no LPC and diet B was experimental containing LPC. The study was extended over a period of ten days. At the end blood samples were collected and analyzed for cholesterol contents.

Experiment No II. In the continuation of experiment no. 1 another experiment was conducted on rats. Sixteen male adult rats were randomly divided into four groups of similar weight and size. Four experimental diets were mixed and designated as A (control) containing zero LPC, diet B containing 2.5% LPC, diet C containing 5.0% LPC and diet D containing 7.5% LPC, replacing fish protein (Table 3). The study was extended over a period of 10 days. At the end blood samples were taken for the analysis of cholesterol and phospholipids.

Experiment No. III. Twenty-two laying hens were divided into two groups of eleven each. Two diets A and B were formulated where A served as control containing no LPC and B

*Author for correspondence

served as experimental containing LPC (Table 4). The study was extended over a period of eight months. At the end of the experiment blood samples and egg samples were analyzed for cholesterol and phospholipids.

Table 1
Proximate composition of leaf protein concentrate

Contents	Percentage
Dry matter	93.88
Protein (N x 6.25)	42.50
Ether extract	11.57
Ash	14.10
Crude fiber	3.30
Soluble carbohydrates	22.41

Table 2
Chemical composition of rat diet in experiment No. I

Ingredients	A (Control) (%)	B (Experimental) (%)
Casein	20.70	18.33
Leaf protein concentrate (LPC)	-	5.69
Corn starch	57.30	53.98
Glucose	5.00	5.00
Minerals	3.00	3.00
Vitamins	5.00	5.00
Corn oil	7.00	7.00
Crude fiber	2.00	2.00
Total	100.00	100.00

Table 3
Composition of experimental rations in experiment No. II

Ingredients	Rations			
	A	B	C	D
Maize	10.00	10.00	10.00	10.00
Wheat	21.00	21.00	21.00	21.00
Rice	30.00	30.00	30.00	30.00
Rice polishing	10.00	10.00	10.00	10.00
Corn gluten meal	10.00	10.00	10.00	10.00
Fish meal	10.00	7.50	5.00	2.50
Leaf protein concentrate	-	2.50	5.00	7.50
Molasses	3.00	3.00	3.00	3.00
Bone meal	0.50	0.50	0.50	0.50
Ground limestone	5.00	5.00	5.00	5.00
Vitamin mineral premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00

Results and Discussion

Chemical composition of leaf protein concentrate (LPC) is presented in Table 1. LPC contained 6.12 14.10, 42.50, 11.57 and 3.30 percent moisture, minerals, protein, ether extract and crude fiber respectively.

The average serum cholesterol content of rats (Experiment-I) given diet A (control) was 59.26 ± 0.89 mg 100 ml^{-1} and that of rats given diet B (containing LPC) was 55.84 ± 0.65 mg 100 ml^{-1} (Table 5). Results indicated that incorporation of LPC helped in lowering cholesterol. Decrease in cholesterol content might have been due to the presence of saponins in LPC. These findings are in accordance with Malinow *et al* (1977), Oakenfull *et al* (1979), Sidhu and Oakenfull (1986), Purmova and Opletal (1996) and Hiroshi and Akira (1996) who reported that saponins present in the plants lower cholesterol level.

In the continuation of Ist experiment another experiment (Experiment-II) was planned. Four groups were allotted four diets A, B, C, and D containing LPC at the rate of 0.0, 2.5, 5.0 and 7.5 percent.. The average serum cholesterol contents of rats given diets A, B, C and D were 76.17 ± 0.51, 71.83 ± 0.66, 67.10 ± 0.67 and 53.67 ± 0.79mg 100 ml^{-1} respectively (Table 6). A decrease in phospholipids is also noticeable with the increase in concentration of LPC. It is evident from the results that inclusion of LPC in rat diet resulted in decreasing cholesterol contents. Findings of this experiment confirmed the results of experiment -I

Table 4
Composition of experimental rations in experiment No. III

Ingredients	Rations	
	A (Control) (%)	B (Experimental) (%)
Maize	10.00	10.00
Wheat	21.00	21.00
Rice	30.00	30.00
Rice polishing	10.00	10.00
Corn gluten meal	10.00	10.00
Fish meal	10.00	5.00
Leaf protein concentrate	-	5.00
Molasses	3.00	3.00
Bone meal	0.50	0.50
Ground lime stone	5.00	5.00
Vitamin mineral premix	0.50	0.50
Total	100.00	100.00

Table 5
Average cholesterol concentration in rats in experiment No I

S.No.	Parameters	A (Control)	B (Experimental)
1.	No. of rats	4	4
2.	Days of experiment	10	
3.	Serum cholesterol (mg 100 ml^{-1})	76.17± 0.81	*55.84± 0.65
4.	Phospholipids	110.00± 0.47	*108.0± 0.28

Values are mean ± standard deviation of four determinations
* Significant at P< 0.05

Table 6
Average serum cholesterol of rats in experiment No. II

S.No.	Parameters	Rations			
		A	B	C	D
1.	No of rats	4	4	4	4
2.	Days of experiment	10	10	10	10
3.	Serum cholesterol (mg 100 ml^{-1})	76.17± 0.81	71.83± 0.66	67.10± 0.67	*53.67± 0.79
4.	Phospholipids	138.00± 0.10	136.00± 0.32	130.00± 0.43	125.00± 0.16

]Values are mean ± standard deviation of four determinations
*Significant at P< 0.05

Table 7
Cholesterol contents in poultry egg powder and in serum fed on LPC diets in experiment No. III

S. No	Description	Rations	
		A(Control)	(Experimental)
1.	No. of birds	11	11
2.	Days of experiment	224	224
3.	Cholesterol in egg powder (mg 100 ml^{-1})	121.11± 0.50	*108.88± 0.48
4.	Serum cholesterol (mg 100 ml^{-1})	190.00± 0.97	*170.00± 0.58

Values are mean ± standard deviation of four determinations
*Significant at P< 0.05

Average serum cholesterol contents in the blood of hens (Experiment-III) given diets containing LPC 0 and 5.0 % are presented in Table 7. Average serum cholesterol contents in the serum of hens given diet A (control) was 190 mg 100 ml^{-1} and in the case of hens given diet B (containing 5% LPC) was 170 mg 100 ml^{-1}, while average serum cholesterol contents of eggs of group A (control) was 121.11 mg 100 ml^{-1}, whereas it was 108.88 mg 100 ml^{-1} in the case of eggs laid by the hens given diet B (containing 5% LPC). It is evident from the results that incorporation of LPC in the diet significantly ($P< 0.05$) lowered cholesterol contents and phospholipids in the serum as well as in the eggs. These findings indicated that basal diet containing casein in the experiment-I, the cholesterol was 59.26 ± 0.89 mg 100 ml^{-1}. Supplementation of LPC was effective in lowering cholesterol and phospholipid contents. Similar results were observed when fish meal was replaced by LPC in experiment no. II. Hypocholesterolaemic effect was noticed with the increase in the LPC (Table 6). A decrease in cholesterol content in eggs laid by the hens given diet containing LPC was also observed (Table 7). These findings are in agreement with the results of Purmova and Opletal (1996) and Hiroshi and Akira (1996) who reported that plants containing saponins exerted a positive effect on the function of heart directly and inhibited the formation of lipid peroxides in the cardiac muscle, thus lowering cholesterol. It may be concluded from the result of present studies that hypocholesterolaemic effect in the blood and eggs was due to the parallel substitution of diets with leaf protein concentrate.

References

Amic J, Lairon D, Hauton J 1972 Technique de dosage automatique de phorthophosphate dune grande fiablilite *Clin Chem Acta* **40** 107-111.

AOAC 1984 Official Methods of Analysis of the Association of Analytical Chemists, Washington DC, USA.

Bowman R E, Wolf R C 1962 A rapid and specific ultramicro method for the determination of serum cholesterol. *Clin Chem* **20** 302-309.

Hiroshi U, Akira M 1996 Effect of soyabean saponins and soyabean protein on serum cholesterol fed chickens. *Anim Sci Telchnol* **67**(5) 415-422.

Malinow M R, McLaughlin P, Kohler G O, Livingston, A I 1977 Prevention of elevated cholesterolemia in monkey by alfa alfa saponins. *Steroids* **29** 105-110.

Muhamnmad N, Anwar M, Afzal M, Jamil M Ehteshamuddin A F M 1988. Egg powder by foam mat drying. *Pak J Sci Ind Res* **31**(4) 303.

Oakenfull D C, Fenwick E, Hood R L 1979 Effect of saponins on bile acid and plasma lipids in rats. *Brit J Nut* **42** 209-216.

Purmova J, Opletal L 1996 A new mechanism for the hypocholesterolaemic activity of saponins. Phytochemical aspects of circulatory system diseases. 5-saponins and possible use in prevention and treatment. *Ceska Stov Farm* **44**(5) 246-251.

Shah F H, Stahman M A, Sheikh A S 1988 Protein from grasses, *Annual Report PCSIR.Labs*, Lahore (Pakistan).

Sidhu G S, Oakenfull D C 1986 A mechanism for the hypocholesterolaemic activity of saponins. *Brit J Nut* **55** 643-649.