INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY
Volume: 2: Issue-2: April-June -2011 IJABPT ISSN 0976-4550

HYPOCHOLESTEROLEMIC EFFECT OF THE ANOXYGENIC PHOTOTROPHIC BACTERIUM *RHOPSEUDOMONAS PALUSTRIS* MGU001 IN HEN LAYING EGGS.

Ramchander Merugu[1,3], M.P.Pratap Rudra[3], Veerababu Nagati[3], Thirupathaiah Atthapu[2]

Department of Biochemistry[1], Mahatma Gandhi University, Nalgonda.
Department of Pharmaceutical Chemistry[2], Mahatma Gandhi University, Nalgonda
Department of Biochemistry[3], Osmania University, Hyderabad.

ABSTRACT : The study was designed to investigate the effects of dietary *Rhodopseudomonas palustris* on the laying hen. The values were recorded after about a period of 60 days. Dietary supplementation of four day old cultures of *R.palustris* at 0.08% reduced cholesterol and triglycerides concentration in serum by 15.34% and 6.19% respectively. The hen egg-yolk recorded a reduction of about 17.18% in cholesterol concentration. Also, supplementation of *R.palustris* in diets increased high-density lipoprotein cholesterol level and decreased atherogenic index in serum. Reduction in the levels of cholesterol was also observed in liver, breast and thigh muscles. Diets fed with *R.palustris* may lead to the development of chicken and eggs containing less cholesterol. Significance of the above results with respect to the existing literature are discussed in this communication.
Key words: Hypocholesterol, R*hopseudomonas Palustris MGU*001 Eggs

INTRODUCTION

Eggs contain many essential nutrients, such as protein, calcium, phosphorus, retinol, *α*-tocopherol, folate, and other B vitamins. Egg yolk contains sterols, phospholipids and triglycerides. The yolk from a large egg may contain 213 mg of cholesterol (USDA, 1991). The risk of coronary heart disease can be reduced by consuming not more than 300 mg of cholesterol daily (Weggemans *et al*., 2001). Reduction of egg yolk cholesterol in chicken eggs is thus beneficial for human health. Animal biotechnologists have tried to reduce cholesterol by supplementation of chromium (Uyanick *et al*., 2002) cupric sulphate. (Pesti and Bakalli, 1998), garlic (Chowdury *et al*.,2002), turmeric powder (Emadi and Kermanshahi ,2006), tamarind (Chowdury *et al*., 2005) and probiotic microorganisms (Mohan *et al*.,1995 and Arun *et al*.,2006). *Rhodopseudomonas palustris* (Lee *et al.,*1990) and *Rhodobacter capsulatus* (Salma *et al*., 2006,Tsujii *et al*., 2007) were used for lowering cholesterol in rats and hens respectively. As anoxygenic phototrophic bacteria have high protein content, good amount of essential amino acids, vitamins, biological co-factors and less amount of nucleic acids (Sasikala and Ramana, 1995), a preliminary investigation on the use of *R.palustris* for lowering cholesterol in hens was conducted and the results are discussed in this communication.

MATERIAL AND METHODS

Phototrophic bacteria were isolated from the paddy fields by enrichment techniques by inoculating into the Biebl and Pfennig's medium and incubated anaerobically in the light. The cultures obtained by enrichment technique were streaked on to the solid medium repeatedly and colonies were picked up to inoculate into the liquid medium and maintained by subculturing. Bacteria thus isolated were identified by studying the cultural characteristics (colour, size and shape), utilization of carbon and nitrogen sources, vitamin requirements, absorption spectral analysis, bacteriochlorophyll and carotenoids with the help of Bergey's Manual of Systematic Bacteriology (1989). Tubes were inoculated with 1ml log phase cultures of anoxygenic phototrophic bacteria and incubated at 30±2º C under the light intensity of 2000 lux in fifteen ml screw cap tubes. Carbon source in the form of acetate was maintained at a concentration of 1.0% while ammonium chloride was used as a nitrogen source at 0.5%. Growth was determined by measuring optical density at 600 nm using UV-vis spectrophotometer. Absorption spectrum of whole cell was measured by sucrose method described by Truper and Pfenning (1981). Bacteriochlorophyll 'a' and carotenoid content of the culture were determined by the method suggested by Cohenbazire *et al.* (1957).

Cells of *R.palustris* were collected by centrifugation and were lyophilized. The dried powder was supplemented with the diets of hens at various concentrations of 0.02%,
0.04% and 0.06% and 0.08% of *R.palustris* powder for a period of 60 days. The basal diet of the hens (*Gallus gallus*) consisted (in percentage) of wheat (55.00), rice polish (19.00), soybean seed (10.50), fish meal (4.00), sesame meal (4.00), oyster shell (5.50), bone meal (1.50), common salt (0.25), Vitamin-mineral mixture (0.25). A total of 40 birds separated into five experimental groups with eight birds in each group (Control, 0.02%,0.04%,0.06% and 0.08% supplementation) were used in this study. They were exposed to a 16 hrs light and 8 hrs dark periods. Blood samples, egg, liver and muscle collection and processing were done according to the methods of Salma *et al*.,(2007). Cholesterol, triglycerides, HDL cholesterol concentrations were determined by enzymatically by commercially available reagent kits. The atherogenic index was calculated as the ratio of LDL-cholesterol to HDL-cholesterol.

RESULTS

Table 1 shows the morphological and cultural characteristics of the organism which has been isolated from paddy fields. Based on the characteristic absorption spectra and the production of lycopene and rhodopin, the organism was identified as *Rhodopseudomonas palustris.* Carotenoids are known to decrease the concentration of serum cholesterol, hence production of carotenoids was investigated. Carotenoid production by this organism at 4, 8, 12, and 16 days incubation showed that the maximum production of both red (88µg/100mg) and yellow (68µg/100mg) carotenoids took place on 4th day of incubation (table 2). Hence four days old cultures were supplemented at different concentrations. Decreased levels of reduction in the concentrations of cholesterol were observed at 0.02% , 0.04% and 0.06% supplementation when compared to 0.08%, hence the data is not presented. Dietary supplementation of *R.palustris* of about 0.08% reduced cholesterol and triglycerides concentration in serum by 15.34% and 6.19% respectively (table 3). The hen egg-yolk recorded a reduction about 17.18 % in cholesterol concentration. Cholesterol, triglycerides concentrations in serum and egg-yolk changed linearly in accordance with increasing levels of dietary *R.palustris*. Also, supplementation of *R. palustris* in diets increased high-density lipoprotein cholesterol level. Atherogenic index showed a decrease after 60 day feeding period. Increasing supplementation of *R.palustris* caused decreases in the levels of cholesterol (22.91%) and trigylcerides (30.95%) in the liver of hens. Compared to the decrease in liver cholesterol, reduction of breast (4.04%) and thigh (3.70%) muscle cholesterol was less (table 4).

Table 1: Cultural and morphological characteristics of *Rhodopseudomonas palustris* MGU001 isolated from rice paddy fields

Colour of the cell suspension	Reddish brown
Motility	+
Shape	rod
Size (in µm)	1.7
Arrangement	Singly
Amax	374, 409, 469, 472, 495, 525, 590, 806, 870
Predominant Carotenoid	Lycopene, Rhodopin
Predominant Bacteriochlorophyll	'a'

Table 2: Production of red and yellow carotenoids by *Rhodopseudomonas palustris*

Organism	Incubation period(in days)	pH	Biomass (in O.D)	Bacteriochlorophyll (mg/100mg)	Carotenoids (µg/100 mg)	
					Red	Yellow
	4	7.2	0.914	0.6113	88±3.6	68±2.6
Rhodopseudomonas palustris	8	7.8	1.306	0.6651	82±2.4	62±3.2
	12	8.2	1.086	0.5985	75±1.8	58±2.6
	16	8.3	0.872	0.5344	68±2.6	52±1.4

Table 3 : Effect of supplementation of *R.palustris* (0.08 %) on serum and eggs of hens after 30 and 60 days

Parameter	Control (Before supplementation)	After supplementation for 30 days	After supplementation for 60 days
Yolk Cholesterol (mg/g)	12.8 ±1.4	11.2±0.8	10.6±1.2
Serum Cholesterol (mg/dl) Total Cholesterol	176 ± 12.4	160 ± 7.8	149 ± 4.6
Triglycerides	710 ± 5.4	690 ± 6.4	666 ±3.8
HDL	16 ±1.6	18 ± 0.6	19 ±1.8
LDL	29 ± 0.6	26 ±1.2	22 ± 0.8
VLDL	132 ± 7.8	116 ± 8.8	108 ± 6.6
Atherogenic index	1.8 ± 0.2	1.44 ± 0.6	1.15 ± 0.2

Table 4: Effect of *R.palustris* on liver weight, cholesterol and triglyceride concentrations in liver and cholesterol concentrations in breast and thigh muscles of laying hens

Parameter	Control (Before supplementation)	After supplementation for 30 days	After supplementation for 60 days
Liver weight(g/100g)	2.6±0.14	2.4±0.28	1.9±0.22
Cholesterol (mg/g)	4.8±0.64	4.2±1.05	3.7±0.32
Triglycerides(mg/g)	16.8±1.4	14.2±0.88	11.6±1.08
Muscle cholesterol Breast(mg/100g)	54.4±1.4	53.6±2.1	52.2±2.4
Thigh (mg/100g)	59.4±1.6	58.6±1.1	57.2±2.2

DISCUSSION

The results obtained in the above study are in agreement with that of Salma *et al*.(2007), Tsujji *et al*. (2007) who also observed hypocholesterolemic effect of this bacterium in hens and rats. Chowdury *et al*.(2002, 2005) and Shim *et al*.(2004) have observed reduction in cholesterol using garlic, tamarind and *Codonopsis lanceolata* root respectively. The hypocholesterolemic effect of phototrophic bacteria has been attributed to the presence of carotenoids and the amino acids arginine, glycine and lysine which are known to have these effects. Lysine plays an important role in the production of carnitine which mediates the conversion of fatty acids into energy and helps to lower cholesterol. Yeum and Rusell (2002) reported that carotenoid rich diets are associated with reducing serum cholesterol. Sadia *et al*. (2010) investigations have revealed that the suppression of palmitic acid incorporation into total lipids, phospholipids, triacylglycerol and cholesterol in the liver could be responsible for hypocholesterolemic effect. Another possible mechanism could be the inhibition of hepatic β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and fatty acid synthetase enzymes by this organism. Such inhibition of enzyme activity was reported by Qureshi *et al*., (1983) when chickens were fed with garlic powder. Although, further work is required to know the exact mechanism for the observed hypocholesterolemic effect of this organism, it has become clear that the supplementation of phototrophic bacteria can lead to production of chicken and eggs containing less cholesterol.

REFERENCES

1. USDA. 1991. Nutrient content of foods. Dairy and Egg Products Handbook 8-1. United States Department of Agriculture, Washington, DC
2. Weggemans, R. M., P. L. Zock, and M. B. Katan. 2001. Dietary cholesterol from eggs increase the ratio of total cholesterol to high-density lipoprotein cholesterol in humans. A metaanalysis. Am. J. Clin. Nutr. 73:885–891.
3. Uyanik, F., Atasever, A., Ozdamar, S., Aydin, F., 2002. Effect of dietary chromium chloride supplementation on performance, some serum parameters and immune response in broilers. Biol. Trace Elem. Res. 90:99-115.
4. Pesti, G. M., and R. I. Bakalli, 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poultry Sci. 75:1086–1091.
5. Chowdhury, S.R., S.D. Chowdhury and T.K. Smith, 2002. Effects of dietary garlic on cholesterol metabolism in laying hens. Poult. Sci., 81: 1856-1862.
6. Emadi.M and Kermanshahi,H 2006.Effect of turmeric rhizome powder on performanace and carcass characteristics of broiler chickens. Int J Poult Sci 5:1069-1072
7. Chowdhury.S.R, D. K. Sarker, S. D. Chowdhury, T. K. Smith, P. K. Roy, and M. A. Wahid. 2005.Effects of dietary tamarind on cholesterol metabolism in laying hens. Poultry Science 84:56–60
8. Mohan, B., R. Kadirvel, M. Bhaskaran and M. Natarajan. 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. British Poultry Science 36 : 799 – 803.
9. Arun, K. P., Rama Rao, V. Savarm, V. L. N. Raju Mantena and R. Sharma Sita.2006. Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. Journal of Poultry Science 43: 235 – 240.
10. Lee, M. G., K. Michiharu, and Y. Kyoden. 1990. Hypocholesterolemic effect of phototropic bacterial cells in rats. J. Nutr. Sci. Vitaminol. 36:475–483
11. Salma U, Miha AG, Make T, Nishimura M, Tsujii H 2006. Effect of dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in broiler meat. Poult. Sci. 86: 1920-1926.
12. Tsujii, H., M. Nishioka, U. Salma, A. G. Miah, T. Maki, and M. G. Lee. 2007. Comparative study on hypocholesterolemic effect of *Rhodopseudomonas palustris* and *Rhodobacter capsulatus* on rats fed a high-cholesterol diet. Anim. Sci. J.78:535-540
13. Sasikala, C., Ramana, C.V., 1995. Biotechnological Potentials of Anoxygenic Phototrophic Bacteria. I. Production of Single-Cell Protein, Vitamins, Ubiquinones, Hormones and Enzymes and Use in Waste Treatment. *In*: Laskin, A.I., Bennett, J.W., Gadd, G.M. (Eds.), Advances in Applied Microbiology. Academic Press, New York,p.173-226
14. Bergey's Manual of Systematic bacteriology (1989). "Enrichment and isolation of purple non sulphur photosynthetic bacteria" . Eds:J.T.Stanley, M.P.Byrant, N.Pfennig and J.C.Holt.
15. Truper H.G. and Pfennig.N (1981). Characterisationa nd identification of anoxygenic phototrophic bacteria In: Starr MP. Stolp
16. Cohenbazire G., Sistrom W.R and Stanier R.Y. 1957.Kinetic studies of pigment synthesis by non sulphur purple bacteria J.cell.comp.physiol. 49: 25-68.
17. Shim, K. S., G. H. Park, C. J. Choi, and C. S. Na. 2004. Decreased triglyceride and cholesterol levels in serum, liver and breast muscle in broiler by the supplementation of dietary *Codonopsis lanceolata* root. Asian-australas. J. Anim. Sci. 17:511–513.
18. Yeum KJ and Russell RM.2002. Carotenoid bioavailability and bioconversion. Annual Review of Nutrition.22:483-504
19. Sadia Afrose, Md. Sharoare Hossain, Ummay Salma, Abdul Gaffar Miah, and Hirotada Tsujii 2010. Dietary Karaya Saponin and *Rhodobacter capsulatus* exert Hypocholesterolemic effects by suppression of hepatic cholesterol synthesis and promotion of bile acid synthesis in laying hens, Cholesterol: doi:10.1155/2010/272731
20. Qureshi, A. A., N. Abuirmeileh, Z. Z. Din, C. E. Elson, and W. C. Burger. 1983. Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. Lipids 18:343–348.
21. Salma, U., A. G. Miah, K. M. A. Tareq, T. Maki, and H. Tsujii. 2007. Effect of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. Poult. Sci. 86:714–719.