Effect of Vitamin C Administration on Serum and Egg-yolk Cholesterol Level of the Chicken

A. S. Al-Janabi, S. R. Al-Kattib^A and Z. D. Taha^B

Dept of Physiology, College of Veterinary Medicine, Baghdad University, Iraq. ^A Dept of Cytology, Biological Research Centre, Scientific Research Council, Baghdad, Jadiriyah, P.O. Box 2371/2.

^B Dept of Animal Resources, College of Agriculture, Baghdad University, Iraq.

Abstract

Ascorbic acid (vitamin C) was given to 144-days-old layer chickens mixed with their ration at three concentrations: 30, 60 and 90 p.p.m. for 6 months. Cholesterol levels in blood serum (CS) and egg yolk (CE) were measured every 6 weeks (four periods); there was a marked decrease in CS in most treated birds, especially those receiving the highest concentration of vitamin C. There was also a slight decrease in CE in most treated birds. Thyroidal weight showed a significant increase in most treated groups, especially those receiving the highest concentration of vitamin C.

Introduction

Egg yolk is considered to be one of the richest sources of cholesterol in the human diet (Bartov *et al.* 1971; Suky and Miller 1985). Hypercholesterolaemia has always been linked with cardiovascular diseases in man, e.g. atherosclerosis, hypertension and coronary heart disease (Alessandro *et al.* 1985).

Vitamin C has been shown to decrease cholesterol deposition in blood vessels in man (Gandzha et al. 1961; Fedorova 1960) and animals (Sokoloff et al. 1966; Bavina 1958).

In the present investigation the possibility of lowering the cholesterol content of both serum and egg yolk of chickens through vitamin C administration was examined.

Table 1. Percentage composition of the chick rationThe ration provided: crude protein 18.19%, crude fibre 2.62%, energy 2535 kcal of assimilated energy per kg, calcium 3.41% (Titus and Fritz 1971)				
Ingredient	Percentage	Ingredient	Percentage	

Ingredient Percentage		Ingredient	Percentage
Yellow maize	28.0	Barley flakes	8.0
Wheat	18.0	Protein concentrate ^A	8.0
Barley	14.5	Calcium carbonate	8 · 1
Soyabean	15.0	Sodium chloride	0.4

^A The protein concentrate contained 4% phosphorus.

Materials and Methods

Altogether 104 birds were used (Hybrid Layers, Hisex, Netherland). They were raised in the farms of the College of Agriculture, Baghdad University. They were kept in individual cages $(20.5 \times 47 \times 49.5 \text{ cm})$ in temperature-controlled $(20-30^{\circ}\text{C})$ closed houses $(5 \times 8 \times 3 \text{ m})$. The birds were exposed to 15 h of light starting from 5 a.m. Food and water were available *ad libitum*. The food given was the normal ration for layer chickens (State Animal Food Establishment, Baghdad); the composition of the ration is shown in Table 1. Vitamin C was added to the protein-concentrate of the ration at a rate of 875 mg kg⁻¹.

The experiment started in July, when the birds were 144 days old, and lasted for 6 months. All birds were vaccinated against Newcastle, Gumboro and fowl pox diseases. Birds were randomly divided into four equal groups (26 per group), one control group and three experimental groups. The latter received normal ration to which vitamin C (Analar L-ascorbic acid – provided by the State Organization of Drugs, Sammarra, Iraq) was added. The vitamin was thoroughly mixed with the ration before being given to the birds in three concentrations: 30, 60 and 90 p.p.m. (treated groups 1, 2 and 3 respectively). Every 6 weeks birds were weighed, and six from each group were bled through a knife incision in the jugular vein before being killed. Collected blood was allowed to clot at room temperature and prepared serum was kept at -20° C until required for estimation of cholesterol.

Eggs were collected over the same periods of 6 weeks as above and stored in a refrigerator (5°C) until required for estimation of cholesterol. Ten eggs from each group were randomly selected, and the egg yolk separated and processed according to the method of Dephir *et al.* (1960). Cholesterol content of both serum and egg yolk was determined photometrically (Franey and Elias 1968). All data were statistically analysed using analysis of variance (Snedecor and Cochran 1967).

Group			100 ml ⁻¹ serum) riod ^A	
	1	2	3	4
Control Treated group	249 ± 8	235 ± 5	231 ± 14	230 ± 9
No. 1 No. 2	$\begin{array}{c} 235 \pm 10 \\ 227 \pm 11 * \end{array}$	$\begin{array}{c} 228\pm14\\ 225\pm9 \end{array}$	$\begin{array}{c} 218\pm13\\ 221\pm12 \end{array}$	$\begin{array}{c} 217\pm12\\ 214\pm8 \end{array}$
No. 3	$221 \pm 9**$	220 ± 15	219 ± 8	$210 \pm 15*$

Table 2. Effect of dietary addition of vitamin C on serum cholesterol of laying hens Values are mean \pm s.e. *P < 0.05; **P < 0.01

^A Duration of each period was 42 days.

Results

Serum Cholesterol

In all experimental groups there was a general tendency for serum cholesterol to decrease in the different periods of the study. The magnitude of the decrease appears to be associated with the size of dose of the vitamin added (Table 2)—higher doses causing more pronounced decreases. The decrease was statistically significant (P < 0.05) in treated group No. 2 at the end of the first period and in treated group 3 at the end of the first (P < 0.01) and fourth periods (P < 0.05).

Table 3.	Effect of dietary addition of vitamin C on egg-yolk cholesterol of laying hens
	Values are mean \pm s.e.

Group			g gm ⁻¹ egg yolk) riod ^A	
	1	2	3	4
Control Treated group	$25\cdot 32\pm 0\cdot 43$	$25\cdot 31\pm 0\cdot 63$	$25 \cdot 44 \pm 0 \cdot 78$	$24 \cdot 52 \pm 0 \cdot 66$
No. 1	$24\cdot77\pm0\cdot55$	$24\cdot 59\pm 0\cdot 75$	$24 \cdot 46 \pm 0 \cdot 66$	$24 \cdot 30 \pm 0 \cdot 50$
No. 2	$24\cdot05\pm0\cdot39$	$24\cdot 13\pm 0\cdot 66$	$23\cdot 80\pm 0\cdot 70$	$23\cdot 62\pm 0\cdot 32$
No. 3	$23\cdot 96\pm 0\cdot 73$	$23\cdot 26\pm 0\cdot 60$	$23\cdot 89\pm 0\cdot 36$	$23 \cdot 82 \pm 0 \cdot 70$

^A Duration of each period was 42 days.

Egg-yolk Cholesterol

Addition of vitamin C caused a slight decrease in the cholesterol content of egg yolk in birds of all experimental groups. The magnitude of the decrease was again associated with the dose of the vitamin added (Table 3).

Table 4.	Effect of dietary addition of vitamin C on thyroid gland weight of laying hens
	Values are mean \pm s.e. * $P < 0.05$; ** $P < 0.01$

Group	Thyroidal weight (mg 100 g^{-1} birthweight) Period ^A				
	1	2	3	4	
Control Treated group	$7\cdot 90\pm 0\cdot 40$	$9\cdot10\pm0\cdot80$	$9\cdot 15\pm 0\cdot 50$	$9\cdot 80\pm 0\cdot 40$	
No. 1 No. 2	$8 \cdot 70 \pm 0 \cdot 50$ $9 \cdot 15 \pm 0 \cdot 85^*$	$8 \cdot 45 \pm 0 \cdot 55$ $9 \cdot 40 \pm 0 \cdot 65$	$7 \cdot 85 \pm 0 \cdot 45$ $9 \cdot 35 \pm 0 \cdot 70$	$8 \cdot 90 \pm 0 \cdot 70$ $9 \cdot 25 \pm 0 \cdot 50$	
No. 3	$11 \cdot 30 \pm 0 \cdot 50^{**}$	10.70 ± 0.55	10.40 ± 0.70	$8 \cdot 50 \pm 0 \cdot 50$	

^A Duration of each period was 42 days.

Thyroid Gland Weight

Addition of vitamin C caused an increase in thyroidal weight in comparison with the control birds, especially during the first two periods of the study (Table 4). The increase was statistically significant (P < 0.05) in treated group No. 2 during the first period compared with the control group or treated group No. 1 at the same period. The increase was more pronounced in treated group No. 3, where it was highly significant (P < 0.01) during the first period, compared with all other groups at the same period. Although the increase in thyroidal weight in treated group No. 3 was less marked in subsequent periods, it remained higher than all other groups during the second and third periods.

Group	Body weight (g) At the start Period ^A					
	of experiment	1	2	3	4	
Control Treated group	1472	1703 ± 33	1854 ± 20	1944 ± 26	2103 ± 35	
No. 1 No. 2 No. 3	1477 1484 1482	1742 ± 45 $1758 \pm 21*$ $1754 \pm 18*$	$1918 \pm 37^*$ $1920 \pm 34^*$ $1937 + 28^{**}$	$2054 \pm 37*$ $2049 \pm 16**$ 2114 + 25**	$2216 \pm 43^{*}$ $2243 \pm 39^{*}$ $2254 + 28^{**}$	

Table 5. Effect of dietary addition of vitamin C on body weight of laying hensValues are mean \pm s.e. *P < 0.05; **P < 0.01

^A Duration of each period was 42 days.

Body Weight

Addition of vitamin C caused an increase in body weight in all treated groups throughout all periods of the experiment. The increase was statistically significant (P < 0.05) in treated group No. 1 during periods 2, 3 and 4 and in treated group No. 2 during periods 1, 2 and 4; it was statistically significant (P < 0.01) in treated group No. 2 during the third period. The greatest body weight increase was obtained in group No. 3 (significant during all periods), with P < 0.05 during the first period and P < 0.01 during the last three periods (Table 5).

Discussion

This current study appears to be the first of its kind concerning the effect of vitamin C on cholesterol levels in both serum and egg yolk of poultry. Available literature contains ample evidence of the lowering effect of vitamin C on elevated blood cholesterol levels in man and several animals. In the human, for instance, Sedov (1956), Fedorova (1960), and Ginter *et al.* (1970), reported that vitamin C lowered blood cholesterol in patients suffering from hypercholesterolaemia. Vitamin C has been reported to prevent subsequent hypercholesterolaemia in animals fed a high-cholesterol diet (Sokoloff *et al.* 1966; Bavina 1958; Myasnikov 1958). But there are few reports on the effect of vitamin C administration on normal blood cholesterol levels. Anderson *et al.* (1958) failed to obtain a significant hypercholesterolaemia following vitamin C administration to humans with normal cholesterol levels.

It is not known why the lowering effect of vitamin C on serum cholesterol was more marked during the first period of our study. It may be due to the fact that egg production in this period (start of the egg-production cycle) is lower than in subsequent periods, with the result that we started with higher serum cholesterol levels, which gave vitamin C a better chance to exert its lowering effect than during the other periods where serum cholesterol was already relatively low. This seems to agree with results of other investigators, who reported that vitamin C had no effect on normal blood cholesterol levels (Sokoloff *et al.* 1966; Elwood *et al.* 1970).

The slight decrease in egg-yolk cholesterol in the vitamin-C-treated groups is probably due to the decrease in serum cholesterol, although its magnitude did not reflect the marked decrease in serum cholesterol. The reason for this is not clear and probably administration of yet higher concentrations of vitamin C is needed to achieve a pronounced effect on egg-yolk cholesterol content. Kechick and Sykes (1974) using much higher levels of vitamin C than ours (100 and 500 mg kg⁻¹ of ration) failed to obtain any marked change in several parameters studied at hot environmental temperature, including egg-shell quality.

It is not known how all these changes in cholesterol level are brought about. However, the data in Tables 4 and 5 suggest that the effects of vitamin C (especially at the highest dose) on thyroidal and body weights are associated with increased activity of the thyroid gland. Hyperthyroidism is associated with an increase in thyroidal weight (Ganong 1985). Searcy *et al.* (1961) showed that activation of the thyroid gland in man caused a decrease in serum cholesterol due to degradation of cholesterol to bile salts. Also, injection of thyroxine in rabbits caused a marked hypocholesterolaemia (Weiss *et al.* 1967; Kritchevsky *et al.* 1976). Ganong (1985) suggested that the effect of thyroid hormone on plasma cholesterol is mediated via increasing low-density-lipoprotein receptors. In the present study, increased thyroidal activity was accompanied by an increase in body weight; these results seem to agree with the finding of Thornton and Moreng (1959), who found a significant increase (P < 0.05) in body weight after the addition of vitamin C to layers kept at 27.8°C. In conclusion, the administration vitamin C to chickens appears to produce clear changes in blood cholesterol levels. Higher doses than those used are probably needed to induce pronounced changes in the egg-yolk cholesterol levels.

References

- Alessandro, M., Conti, S., Dimo, F., Paoli, S. G., Guili, B., Matano, M., and Seccareccia, F. (1985). Incidence of coronary heart disease in two generations of men exposed to different levels of risk factors. Acta. Cardiol. 40(3), 307-14.
- Anderson, J., Grande, F., and Keys, A. (1958). Dietary ascorbic acid and serum cholesterol. Fed. Proc. 17, 468.

Bartov, I., Bornstein S., and Budowski, P. (1971). Variability of cholesterol concentration in plasma and egg yolk of hens and evaluation of the effect of some dietary oils. *Poult. Sci.* 50, 1357-64.

Bavina, M. V. (1958). Effect of ascorbic acid on hypercholesterolemia. Circulation 17, 104.

•

- Daphir, N. J., Marion, W. W., and Ballonm, S. L. (1960). Influence of dietary fats and choline on serum and egg yolk cholesterol in the laying chickens. *Poult. Sci.* 39, 1459-66.
- Elwood, P. C., Hughes, R. E., and Hurley, R. J. (1970). Ascorbic acid and serum cholesterol. Lancet 2, 1197.
- Fedorova, E. P. (1960). The long range treatment of coronary atherosclerosis with ascorbic acid. Sov. Med. 25, 56.
- Franey, R. J., and Elias, A. (1968). Serum cholesterol measurement based on ethanol extraction and ferric chloride-sulfuric acid. Clin. Chim. Acta. 21, 255-63.
- Gandzha, I. M., Kovoleva, N. I., and Bronshtein, V. N. (1961). A comparative study of the effect of some medicinal agents in patients with atherosclerosis. *Vrach. Delo* 4, 6011.
- Ganong, W. F. (1985). Endocrinology and metabolism. In 'Review of Medical Physiology'. 12th Edn. (Lange Medical Publication: Los Altos, California.)
- Ginter, E., Kajaba, I., and Nizner, O. (1970). The effect of ascorbic acid on cholesterolaemia in healthy subjects with seasonal deficit of vitamin C. Nutr. Metab. 12, 76.
- Kechick, I. T., and Sykes, A. H. (1974). Effect of dietary ascorbic acid on the performance of laying hens under warm environmental conditions. Br. Poult. Sci. 15, 449-57.
- Kritchevsky, D., Tepper, S. A., and Story, J. A. (1976). Thyroid hormone and experimental atherosclerosis in rabbits. *Atherosclerosis* 23, 249.
- Myasnikov, A. L. (1958). Influence of some factors on development of experimental cholesterol atherosclerosis. Circulation 17, 99.
- Searcy, R. L., Larlucci, J. S., and Berquist, L. M. (1961). Hypolipemic effect of D. thyroxin therapy in coronary patients. Circulation 24, 1103.

Sedov, K. R. (1956). Prophylaxis and treatment of atherosclerosis by ascorbic acid. Ter. Arkh. 28, 58.

- Snedecor, G. W., and Cochran, W. G. (1967). 'Statistical Methods.' 6th Edn. (Iowa State University Press: Ames, IA.)
- Sokoloff, B., Hori, M., Saelhof, C., McConnell, B., and Tmai, T. (1966). Effect of ascorbic acid on certain blood fat metabolism factors in animals and man. J. Nutr. 91, 107-17.
- Suky, O. H., and Miller, L. T. (1985). Effect of dietary egg on variability of plasma cholesterol levels and lipoprotein cholesterol. Am. J. Clin. Nutr. 42(3), 421-31.
- Thornton, P. A., and Moreng, R. E. (1959). Further evidence on the value of ascorbic acid for maintenance of shell quality in warm environmental temperature. *Poult. Sci.* 38, 594-9.
- Titus, H. W., and Fritz, J. C. (1971). 'The Scientific Feeding of Chicken.' 5th Edn. (The Interstate Printers and Publishers Inc.: Illinois.)
- Weiss, F. J., Johnson, R. M., and Naber, E. C. (1967). Effect of some dietary factors and drugs and cholesterol concentration in the egg and plasma of the hen. J. Nutr. 91, 119-28.

Manuscript received 17 January 1987, revised 21 July 1987, accepted 10 August 1988

. f .