

## Variations in the Cholesterol Content of Egg Yolk

CHOLESTEROL has been implicated as a possible cause of atherosclerosis. Egg yolk, which is a prolific source of cholesterol, is therefore viewed with suspicion in many parts of the world. The validity of these assumptions has yet to be proved; the aim of the work described here was to characterize the "normal" cholesterol content of egg yolk and to attempt to reduce this content by feeding cholestyramine; the latter attempt failed.

Fifteen Thornber 606 light hybrid pullets were obtained from an accredited Thornber's agent at 4 months of age. They were immediately housed in individual cages in a battery unit. Lighting was artificial, and was programmed to help the birds to start laying after 5.5 months (that is, 8 h of light at the beginning, increased by 30 min/week up to 17 h). The room in which the battery unit was kept was maintained at 65° F. Five birds were fed on each of three diets: (A) Spillers intensive layers mash; (B) Spillers intensive layers mash + 1 per cent cholestyramine; (C) Spillers intensive layers mash + corn oil + 1 per cent cholestyramine.

The first five eggs laid by each pullet were collected and the cholesterol content of the yolks was determined. After this, every fifth egg was analysed until the thirtieth egg. Five more eggs from each pullet were analysed during the middle of the laying season (at about the hundredth egg), and again when egg production began to fall off at the end of the laying season. Eggs were allowed to stand at room temperature for one week before aliquots of yolk were taken. The aliquots were freeze-dried and extracted in chloroform:methanol (2:1 v/v). The cholesterol content of portions of the extract was determined using a colorimetric method<sup>1,2</sup> involving ferric chloride and sulphuric acid.

Blood samples were taken by cardiac puncture when the birds were first obtained (before feeding commenced) and again midway during the laying season. Samples were taken by syringe and immediately placed in heparinized tubes. Plasma was separated by centrifuging, extracted in chloroform:methanol, and portions of the extract were analysed for cholesterol as before.

Table 1. VARIATIONS IN CHOLESTEROL CONTENT OF EGG YOLK DURING THE FIRST 2 MONTHS OF THE LAYING SEASON (CHOLESTEROL CONTENTS EXPRESSED AS MEAN ± STANDARD DEVIATION, PERCENTAGE OF DRY MATTER)

Diet Egg No.	Spillers intensive layers mash	Spillers intensive layers mash + 1% cholestyramine	Spillers intensive layers mash + 10% corn oil + 1% cholestyramine
1	5.46 ± 0.10	4.78 ± 0.05	4.39 ± 0.53
2	3.44 ± 0.16	3.78 ± 0.54	3.79 ± 0.55
3	3.22 ± 0.18	3.53 ± 0.49	3.58 ± 0.70
4	2.80 ± 0.24	3.42 ± 0.31	3.37 ± 0.25
5	2.92 ± 0.02	3.07 ± 0.43	3.43 ± 0.35
10	2.77 ± 0.52	2.79 ± 0.29	2.76 ± 0.51
15	2.39 ± 0.01	2.57 ± 0.23	—†
20	2.20 ± 0.02	2.62 ± 0.18	—†
25	2.26 ± 0.09	—†	—†
30	—†	2.34 ± 0.20	2.22 ± 0.15
No. of observations	3*	5	5

\* Some eggs were lost from this group, and the final analysis was performed on only three eggs for each egg number.

† These values were not analysed statistically, for the Titan program used required equal numbers of observations for each set. Because some eggs were lost through breakage in transit, some values are therefore missing.

Significance of results: there is a progressive and significant decrease in yolk cholesterol content within each group. Full statistical data are available on request.

The cholesterol content of egg yolk is given in Tables 1 and 2. In Table 1, the birds are divided into groups according to their diet. There are no statistically significant differences between the groups, but there is a significant decrease in cholesterol content with increasing number of eggs laid within each group. In Table 2, only five birds are shown; these have not been divided into groups, for this table is meant to show the variation in

Table 2. COMPARISON OF THE YOLK CHOLESTEROL CONTENT OF MID-SEASON AND END OF SEASON EGGS FROM FIVE BIRDS. MEAN ± STANDARD DEVIATION, EXPRESSED AS PERCENTAGE OF DRY MATTER

Bird*	Mid-season eggs (five observations)	Significance in Student's <i>t</i> test	End of season eggs (five observations)
G: 597	2.40 ± 0.17	At 5% level	2.76 ± 0.19
G: 593	2.45 ± 0.09	At 5% level	3.08 ± 0.69
G: 595	2.44 ± 0.25	At 5% level	2.84 ± 0.43
G: 596	2.32 ± 0.18	At 5% level	3.12 ± 0.27
G: 598	2.45 ± 0.07	Not significant	2.51 ± 0.15

\* G: 597 received a control diet; the other birds received a diet with added cholestyramine. There is no statistically significant difference between the cholesterol content of yolks of mid-season eggs from G: 597 and those of other birds.

Table 3. EFFECT OF CHOLESTYRAMINE ON PLASMA CHOLESTEROL LEVELS IN LAYING FOWL

Description	Plasma cholesterol levels mg/100 ml. (mean ± standard deviation, five observations)
(A) Thornber 606 pullets on Spillers intensive layers mash. Mid-lay	205.0 ± 28.1
(B) Thornber 606 pullets on Spillers intensive layers mash + 1% cholestyramine. Mid-lay	40.8 ± 15.9
(C) Thornber 606 pullets on Spillers intensive layers mash + 10% corn oil + 1% cholestyramine. Mid-lay	52.0 ± 27.1
(D) Thornber 606 pullets, 4 months old	98.8 ± 14.9

Significance of results: A versus B, C or D, significant at 0.1 per cent in Student's *t* test.

cholesterol content of eggs laid by the same birds at different stages during the laying season.

The cholesterol content of plasma is shown in Table 3. Cholestyramine caused a highly significant decrease in the cholesterol content of plasma, but had no effect on the cholesterol content of yolk. This may indicate that the deposition of cholesterol in egg yolk as it forms is not a passive diffusion from plasma, but that either an active synthetic process is taking place in the ovaries or active transport must occur across the yolk membrane.

The first few eggs laid by a pullet are small and contain small yolks. These yolks have a higher percentage content of cholesterol than yolks from later eggs, but the actual quantity of cholesterol in the egg is approximately the same (about 250 mg in Thornber 606). There must therefore be a mechanism which ensures that this quantity of cholesterol is deposited in all eggs, irrespective of their size or the cholesterol content of the plasma of the laying bird, and this amount of cholesterol must approximate to the amount required by the developing embryo. There is a slight difference in the cholesterol content of eggs between various strains of birds: Rhode Island Reds, mid-lay, 3.84 ± 0.77 per cent (five observations); Thornber 505, mid-lay, 2.87 ± 0.68 per cent (twelve observations); Thornber 606, mid-lay, 2.78 ± 0.22 per cent (twelve observations), but the variation from egg to egg, even between birds of the same strain, is sufficient to prevent there being any difference statistically. It does seem that large heavy bodied birds which produce large chicks tend to deposit more cholesterol in egg yolk, but a point of importance here is that some large birds produce smaller eggs than light hybrids which have been specifically bred for laying. The percentage of cholesterol in the yolk would have to be higher in this case to ensure that the quantity of cholesterol necessary for development of the embryo was present.

I thank the British Egg Marketing Board for financial assistance, Houghton Poultry Research Station for permitting me to run this experiment there, the University Mathematical Laboratory for use of the Titan computer and Dr D. E. Bowyer for his programming.

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Received October 30, 1968; revised January 2, 1969.

<sup>1</sup> Crawford, N., *Clin. Chim. Acta*, **3**, 357 (1958).

<sup>2</sup> Jones, D., thesis, Univ. Cambridge (1968).