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Cholesterol in eggs from different species of poultry determined by capillary GLC

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This study compared two methods of egg cholesterol extraction and determination by gas liquid chromatography (GLC), validated by comparison with standard reference material (SRM 1845). The two extraction procedures were of comparable accuracy with dried whole egg samples of 0·25–0·5 g, but the direct saponification method was rapid, reduced occupational hazard, and quantitative precision was within 2% coefficient of variation. The validated procedure was used to determine cholesterol in eggs of several domestic avian species. Eggs produced by commercial types of chicken (White Leghorn) had the lowest cholesterol (11·5–11·8 mg/g yolk) compared to 13·0, 14·6, 15·2, 15·6, 16·5, 16·8 and 18·1 mg/g for Rhode Island Red chicken, guinea fowl, New Hampshire chicken, duck, pea fowl, domestic turkey and wild turkey eggs, respectively. Yolk cholesterol concentration of commercial White Leghorn chicken eggs was not influenced by age. However, eggs from older birds contained about 23% more cholesterol on a per egg basis (220 versus 179 mg) because of the larger yolk.

INTRODUCTION

Cholesterol (5-cholesten-3β-ol) is an essential structural component of cell membranes and lipoproteins and serves as the precursor for steroid hormones and bile acids (Yeagle, 1988). There is an association between blood levels of cholesterol and the risk of coronary heart disease in humans (Stamler *et al.*, 1986) and premature development of atherosclerosis (Oliver, 1990). The main dietary modifications needed to reduce plasma LDL-cholesterol concentration involve decreased intake of dietary cholesterol and total fat (Expert Panel, 1988; USDHHS, 1988). One of the implications of these dietary guidelines for public health policy is the need for food labels showing the total fat, saturated fat and cholesterol content.

Eggs are an excellent source of nutrients; their fatty acid profile can be modified, which has great culinary advantages, but egg yolk is extremely high in cholesterol. The cholesterol content of eggs is a potential disadvantage for the use of eggs in some human diets. Reliable data for the cholesterol concentration in eggs of different species are essential for the assessment of their dietary value and to enable the public to make informed decisions.

The determination of dietary intake of cholesterol was based on an estimated cholesterol concentration of 274 mg/egg (USDA, 1976) until 1989. This value was based mainly on data obtained by colorimetric assays which are variable. These assays overestimated yolk cholesterol by 28% (Bair & Marion, 1978) to as high as 200% (Nichols *et al.*, 1963) when compared to values obtained by high performance liquid chromatography (HPLC) (Beyer & Jensen, 1989*a*,*b*). In contrast, yolk cholesterol values comparable to those obtained by HPLC or gas–liquid chromatography (GLC) (Beyer *et al.*, 1989) have also been reported (Rangachar *et al.*, 1970; Sainz *et al.*, 1983).

The yolk cholesterol concentration of chickens' eggs determined by HPLC, ranged from 10·97 to 11·7 mg/g (Beyer & Jensen, 1989*a*,*b*). Egg yolk cholesterol concentration determined by gas–liquid chromatography yielded a value of 12·3 mg/ml (Beyer *et al.*, 1989) which when adjusted for yolk density of 1·035 (Burley & Vadehra, 1989) resulted in a yolk cholesterol content of 11·9 mg/g. These new values are reflected in the revised cholesterol content of 213 mg/egg (USDA, 1989).

The procedures used in these two methods employ chloroform and methanol which are highly toxic and powerful irritants. Filtration in the extraction process for the HPLC method (Beyer & Jensen, 1989*a*) introduces appreciable amounts of vapor into the atmosphere (Christie, 1982). During the development of the two methods (Beyer & Jensen, 1989*a*; Beyer *et al.*, 1989)

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an appropriate cholesterol standard (cholesterol in egg yolk matrix) was not available to assess accuracy. The HPLC method used pure cholesterol from the National Bureau of Standards (NBS). Since the publication of these procedures, NBS has released Standard Reference Material 1845 (SRM 1845) intended for use in evaluating the reliability of analytical methods for the determination of cholesterol in eggs and similar foods. Comparison of sample preparation (direct saponification *versus* saponification of lipid extract), using SRM 1845 (19 mg cholesterol/g) showed that direct saponification is more accurate (19·1 *versus* 14·6 mg/g) (Van Elswyk *et al.*, 1991) and implied that the HPLC procedure, based on a lipid extract (Beyer & Jensen, 1989*a*), underestimated yolk cholesterol concentration.

The present study describes a GLC method and two methods of extraction that have been tested for egg cholesterol by comparison with SRM 1845. The two extraction methods were of comparable accuracy with yolk samples of 0·25–0·5 g, but the direct saponification method is selective, precise, rapid, and easy to perform. It did not require the relatively expensive solid-phase extraction cartridges nor did it employ chloroform and/or methanol for extraction, which reduced occupational hazards. The method was used to determine yolk cholesterol in eggs of different species and breeds of poultry.

MATERIALS AND METHODS

Apparatus

This consisted of a gas chromatograph (Hewlett-Packard Model 5890) equipped with flame ionization detector (FID) and integrator (Hewlett-Packard Model 3392A). Operating conditions were: split mode used with a 1 : 40 split ratio; helium carrier gas, flow rate 26·6 ml/min, head pressure 9·5 psi; 1 μl injection; injector 300°C; oven 280°C; detector 300°C. The gas chromatograph was fitted with a 15 m × 0·25 mm i.d. DB 5 (J & W Scientific, Folsom, CA) capillary column containing a film 0·1 μm thick.

Reagents

These were: cholesterol in whole egg powder, SRM 1845 (National Institute of Standards & Technology, Gaithersburg, MD) with certified cholesterol concentration of 19·0 ± 0·2 mg/g; cholesterol standard stock solution (5·0 mg/ml) diluted with chloroform to obtain working cholesterol solutions in the range 1·0–5·0 mg/ml; an internal standard solution of 5-α cholestane in chloroform (4 mg/ml); chloroform and methanol (2 : 1 (v/v)) for lipid extraction and 0·74% potassium chloride solution and anhydrous sodium sulfate for washing and drying the lipid extract; potassium hydroxide solution (1·6 g/ml) and ethanol (dehydrated 200 proof) used for saponification; hydrochloric acid (7·4 N) and petroleum ether (BP 35–60°C) for extraction of cholesterol after saponification; pyridine (AR grade with KOH pellets added when first opened) and derivatization reagent consisting of *N,O*-bis (trimethylsilyl) acetamide + trimethylcholorosilane + trimethylsilylimidazole, 3 : 2 : 3 (Sylon BTZ–Supeloco, Bellefonte, PA).

Sample source and preparation

All eggs were obtained from birds maintained under farm conditions and fed a complete diet. A sample of 12 eggs was taken at random from a day's collection of eggs at Clemson University Poultry Farm. White Leghorn, Rhode Island Red, and Barred Plymouth Rock eggs were from commercial strains maintained in flocks of about 100 birds each. New Hampshire (LJ Dreesen strain) and domestic turkey (Beltsville Small White) eggs were from closed flocks maintained for research. Quail and duck eggs were obtained from commercial farms and pheasant and pea fowl eggs from private breeders and flocks at the university farm. Eggs were weighed and boiled for 5 min. The shell and albumen were removed, the yolk rolled on a paper towel to remove moisture and weighed. The entire yolk was triturated and samples were stored at −20°C. Samples and the SRM 1845 were allowed to equilibrate to room temperature prior to analysis.

Lipid extraction

To compare direct saponification *versus* saponification of lipid extract, SRM 1845 was extracted with chloroform : methanol (20 ml/g). The internal standard was added prior to extraction. The lipid extract was washed with potassium chloride solution and dried with anhydrous sodium sulfate (Christie, 1982). The entire extract was dried and saponified.

Saponification

A modification of the method of Tsui (1989) was used. Internal standard (0·5 ml cholestane solution) was placed in culture tubes (25 × 150 mm) with Teflon-lined screw caps. The tubes were left open under the fume hood overnight to allow the chloroform to evaporate. The tubes containing the internal standard were weighed and a sample of about 0·25–0·5 g of SRM 1845 or ground egg yolk placed in each tube, with care being taken to deposit the sample at the bottom of the tube; the sample weight was obtained by difference. A half-inch stir bar was placed in each tube followed by the addition of 3 ml ethanol and 1 ml KOH solution. The contents were not mixed to prevent adherence of sample to the sides of the tube and out of contact with the ethanolic KOH. The tubes were capped tightly and placed in a boiling water bath set on a magnetic hotplate/stirrer. The contents in all the tubes were constantly stirred during the 1 h saponification. After an hour, the tubes were removed from the water bath and allowed to cool.

Extraction

After the tubes cooled, 2·5 ml hydrochloric acid, 10 ml water, and 10 ml petroleum ether were added. The contents in the tubes were vigorously mixed on a vortex mixer for 30 s and the tubes centrifuged at 2000 rpm for 10 min. An aliquot of the upper, clear petroleum ether phase was transferred into 102 × 75 disposable culture tubes. The extract was concentrated by overnight evaporation under the fume hood. The sides of the tube were washed down with about 2 ml petroleum ether and dried under nitrogen.

Derivatization

A modification of the method of Park and Addis (1985) was used. The concentrated petroleum ether extract was completely dried under nitrogen, 250 μl dry pyridine and 250 μl Sylon BTZ were added to each tube, and the mixture vigorously mixed on a vortex mixer for 30 s. The tubes were corked, covered with aluminium foil and incubated in an oven at 80°C for 5 min. A properly derivatized sample will be golden yellow to reddish-brown in color, clear, and show no phase separation. The reaction mixture was directly injected into the capillary column of the gas chromatograph.

Test of method

The method was tested by evaluating the method of sample preparation, the precision of the GLC analysis alone, the linearity of the procedure, and the precision of the entire procedure (Slover *et al.*, 1983). The effect of direct saponification *versus* saponification of lipid extract and variations in sample size (1, 0·5, and 0·25 g) were determined by a 2 × 3 factorial experiment using three replicates of SRM 1845 for each treatment combination. To assess stability of the method, derivatives were stored at 4°C in tightly stoppered, covered glass vials.

Statistical analysis

Data obtained were subjected to analysis of variance (Mead & Curnow, 1983). The criterion (*F*-statistic) used throughout for detecting statistically significant effects was based on a level of protection against type I error set at $P < 0.01$ or $P < 0.05$.

RESULTS

Method of sample preparation

The cholesterol contents of SRM 1845 prepared by direct saponification or saponification of lipid extract are shown in Table 1. A sample size × method of sample preparation interaction was not detected. The method of sample preparation did not influence accuracy of determination but a sample size of 1 g reduced accuracy regardless of sample preparation method.

Table 1. Comparison of extraction method and sample size for the determination of egg cholesterol[a]

Sample size (g)	Cholesterol contents (mg/g)		Mean[b]
	Direct saponification	Saponification of lipid extract	
1·0	18·4	18·6	18·5[A]
0·5	18·9	18·9	18·9[B]
0·25	19·2	19·2	19·2[C]
SEM	± 0·11		± 0·08
Mean	18·9	18·9	
SEM	± 0·06		

[a] Whole egg powder, SRM 1845 (National Institute of Standards & Technology, Gaithersburg, MD) with certified cholesterol concentration of 19·0 ± 0·2 mg/g was used.
[b] Means with different superscripts are significantly different ($P < 0.01$).

Test of method and stability of derivative

Typical GLC separations of cholestane and cholesterol are shown in Fig. 1. There were no signs of interference with either separation or quantitation and each run was completed in 5 min. Sequential chromatographs of the same sample of SRM 1845 gave a mean value of 18·9 ± 0·06 mg/g with a coefficient of variation of 0·3%. Repeatability of the method determined by replicate runs of SRM 1845 in 26 separate batch assays gave a mean value of 18·8 ± 0·27 mg/g and coefficient of variation of 1·5%. Five samples derivatized and assayed at 24-h intervals gave a mean value of 18·8 mg/g and coefficient of variation of 1·01%. The relationship between area ratio and cholesterol concentration in the range 1–5 mg/ml was linear with a correlation coefficient of 0·98% (Table 2).

Cholesterol content of egg yolk

The yolk cholesterol concentrations and egg cholesterol contents for 12 species of poultry are given in Table 3. Cholesterol/100 g edible egg was lowest in White

Table 2. Precision of egg cholesterol (SRM 1845) analysis by capillary GLC

	n	Mean (mg/g)	Variability[a] (%)
Precision of GLC analysis	5	18·9	0·32
Precision of method	26	18·8	1·44
Stability of derivative[b]	5	18·8	1·01
Linearity[c]		$r = 0.98$	

[a] Coefficient of variation.
[b] Samples were derivatized and stored at 4°C in foil covered glass vials and analyzed at 24-h intervals for 6 days.
[c] Relationship between area ratio (area of cholesterol: area of cholestane) and cholesterol concentration in the range 1–5 mg/ml.

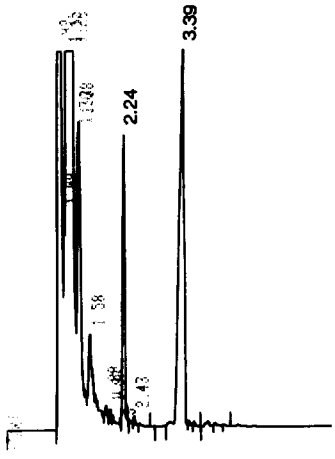

Fig. 1. Typical GLC chromatogram of internal standard 5-α cholestane (2·24 min) and cholesterol in whole egg powder, SRM 1845 (3·39 min).

Leghorn eggs and highest in wild turkey eggs. Duck and guinea fowl eggs contained more cholesterol per gram of yolk than commercial chicken eggs. However, on a whole-egg basis, guinea fowl egg was comparable to a 57 g commercial chicken egg. Significant differences were detected between different breeds of chicken. Egg cholesterol was higher in American breeds and, within this group, New Hampshire eggs from a closed research flock had more cholesterol than eggs of commercial Barred Plymouth Rock or Rhode Island Red chickens.

Yolk cholesterol content (mg/g yolk) of White Leghorn eggs did not vary with egg size (11·5 *versus* 11·8 mg/g yolk for 57·0 and 63·2 g eggs, respectively). The increase in egg size is associated with an increase in yolk size and this yielded higher cholesterol per egg for large eggs (179 *versus* 220 mg). A concomitant increase in egg white compensated for the larger yolk and a significant difference was not detected in cholesterol on an edible-egg basis (350 *versus* 386 mg/100 g edible egg).

DISCUSSION

To our knowledge, this is the first report of the cholesterol concentration in eggs of several species of domestic bird determined by a procedure validated for accuracy and precision by comparison with standard reference material (SRM 1845). The yolk cholesterol content (mg/g) of White Leghorn egg (similar to commercial eggs) was similar to values obtained with GLC analysis (Beyer *et al.*, 1989), HPLC assay (Beyer & Jensen 1989*a,b*), and values obtained by colorimetric procedures reported by Rangachar *et al.* (1970) and Sainz *et al.* (1983). The higher cholesterol content in the American breeds of chicken when compared to White Leghorn eggs is in agreement with previous results (Edwards *et al.*, 1960; Rangachar *et al.*, 1970; Sainz *et al.*, 1983), although the absolute amounts are different.

Table 3. Cholesterol content of eggs from different species and breeds of poultry[a]

Type of poultry	Egg weight (g)	Yolk weight (g)	Yolk cholesterol (mg/g)	Egg cholesterol	
				Whole egg (mg)	Edible egg (mg/100 g)
Chicken (*Gallus domesticus*)					
American breeds					
Barred Plymouth Rock	$60·0^{G}$	$18·3^{F}$	$13·3^{B}$	244^{G}	454^{C}
New Hampshire	$62·5^{GH}$	$20·5^{G}$	$15·2^{EF}$	311^{H}	556^{E}
Rhode Island Red	$65·4^{H}$	$18·4^{F}$	$13·0^{B}$	239^{FG}	409^{B}
Mediterranean breeds					
White Leghorn (32 weeks old)	$57·0^{F}$	$15·5^{E}$	$11·5^{A}$	179^{DE}	350^{A}
White Leghorn (60 weeks old)	$63·2^{GH}$	$18·6^{F}$	$11·8^{A}$	220^{F}	386^{AB}
Duck (*Anas platyrhynchos*)	$91·5^{K}$	$30·6^{I}$	$15·6^{F}$	476^{K}	593^{EF}
Guinea fowl (*Numida meleagris*)	$42·4^{D}$	$12·9^{D}$	$14·7^{DE}$	188^{E}	507^{D}
Pea fowl (*Pavo cristatus*)	$114·8^{L}$	$39·8^{J}$	$16·5^{G}$	657^{L}	632^{FG}
Pheasant					
Chinese Ringneck (*Phasianus cholchius torquatus*)	$25·2^{B}$	$8·2^{B}$	$15·7^{F}$	128^{B}	568^{E}
Lady Amherst (*Chrysolophus amherstiae*)	$29·9^{C}$	$11·0^{C}$	$14·0^{CD}$	154^{C}	577^{E}
Reeves (*Syrmaticus reevesi*)	$32·0^{C}$	$12·3^{D}$	$13·2^{B}$	163^{CD}	568^{E}
Silver (*Lophura nycthemera*)	$46·2^{E}$	$19·5^{FG}$	$13·5^{BC}$	261^{G}	633^{FG}
Quail					
Bobwhite (*Colinus virginianus*)	$9·6^{A}$	$3·5^{A}$	$13·2^{B}$	47^{A}	574^{E}
Japanese (*Corturnix coturnix*)	$10·8^{A}$	$3·1^{A}$	$14·7^{DE}$	45^{A}	495^{CD}
Turkey					
Domestic (*Meleagris gallopavo*)	$77·3^{J}$	$23·4^{H}$	$16·8^{G}$	393^{I}	576^{E}
Wild (*Meleagris gallopavo silvestris*)	$73·7^{I}$	$23·1^{H}$	$18·1^{H}$	419^{J}	643^{G}
Pooled SEM	± 0·85	± 0·32	± 0·24	± 6·2	± 12·0

[a] Means within a column with different superscripts are significantly different ($P < 0·05$). Each value represents the mean ($n = 12$) of a duplicate determination.

Species listed in increasing concentration of cholesterol/g yolk were chicken (White Leghorn), Bobwhite quail, pheasant, guinea fowl, Japanese quail, duck, pea fowl, domestic turkey, and wild turkey with an overall range of 11·5 to 18·1 mg/g. This is in contrast to the results of Bair and Marion (1978) which listed birds in the following order: guinea fowl, chicken, pheasant, Japanese quail, turkey, and duck. Our values for cholesterol in turkey eggs were higher than those reported by Bair and Marion (1978) and lower than those of Turk and Barnett (1971), and wild turkey eggs contained more cholesterol than eggs of the domestic turkey. Cholesterol contents of guinea fowl eggs were higher than the values reported by Bair and Marion (1978) and lower than those of Turk and Barnett (1971) and Oguntona & Hughes (1988) based on colorimetric procedures. Eggs used in this study were obtained from the same source as those used by Oguntona and Hughes (1988). Japanese quail eggs contained more cholesterol than Bobwhite quail and a level similar to those reported by Bair and Marion (1978), higher than the 11·8 mg/g reported by Tomita *et al.* (1975) and the 12·8 mg/g reported by Bitman and Wood (1980), and considerably lower than the concentration reported by Lepore and Marks (1965) and Turk and Barnett (1971).

These discrepancies in egg cholesterol values for the same species may be ascribed to the inaccuracy and lack of precision of the widely used, nonspecific Liebermann–Burchard acid-colorimetric procedures used in past studies (Beyer & Jensen, 1989*a*), unavailability of standard reference material prior to 1989, and possibly selection pressure.

Our results with respect to sample preparation and extraction of yolk cholesterol are at variance with the report of Van Elswyk *et al.* (1991). We failed to detect an effect of method of extraction on the accuracy of cholesterol determination in yolk, while Van Elswyk *et al.* (1991) reported a significantly lower value for the lipid extract when compared to direct saponification. The method of cholesterol extraction can influence cholesterol determination in fishery-based food products (Kovacs *et al.*, 1979). However, in the case of egg yolk our results are in agreement with those of Bitman and Wood (1980), who were unable to detect differences in egg cholesterol determined on a lipid extract or by direct saponification.

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