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Development of Novel Method for the Estimation of Total Cholesterol in Chicken Eggs

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ABSTRACT

Background: Cholesterol estimation in eggs was mostly done by High performance liquid chromatography (HPLC) and gas chromatography which are tedious, expensive and time consuming methods. Thus, there is a need to determine cholesterol content by an easy spectrophotometric method which is not expensive and tedious. The present study was aimed to determine the cholesterol content in chicken eggs spectrophotometrically so as to provide better information about the nutritional quality of an egg.

Methods: The experiment was conducted on 24 healthy layer birds of Dahlem Red (DR). 45 egg samples were randomly collected fresh in the morning. Total cholesterol was determined by enzymatic colorimetric method by using biochemical estimation kit (Agappe Diagnostics Ltd.). The pure yolk was separated and determined enzymatically for cholesterol estimation. The method is newly evaluated, rapid, simple and accurate.

Result: The methodology designed herein can provide specific, accurate and suitable method for estimation of total cholesterol in chicken eggs. The average cholesterol content of Dahlem red was 12.59±0.09 mg/ml in egg yolk or 195.66±2.80 mg/egg yolk.

Key words: Cholesterol, Coronary diseases, Dahlem red, Spectrophotometric.

INTRODUCTION

An avian egg is a highly integrated biological system. The nutritive value and functional properties of an egg makes it an important item in human diet. Eggs and egg products form an integral part of the food chain. It is the only animal protein source in developing countries that is available to the general population in affordable price. Egg is source of protein, liposoluble vitamins A, D, E, K, vitamin B-complex such as cyanocobalamin, thiamine, riboflavin, niacin and pyridoxine. Among minerals; it contains iron, calcium, potassium, sodium, phosphorous and zinc. Egg is also an important source of dietary cholesterol which increases serum cholesterol that ultimately causes the appearance of coronary diseases. Egg contains good and bad cholesterol. The consumption of cholesterol rich food by diabetic patients, cancer carriers and hypercholesterolemia patients can raise the risk of coronary diseases (Aquino *et al.* 2010). The association of cholesterol with coronary diseases has raised the concerns by the consumer and somewhat lowered it's consumption in a certain section of society (Bragagnolo *et al.* 2002).

Dahlem Red is a brown egg-laying layer chicken which was donated to India for introduction to tropical layer production. It gained more importance for rural poultry production because of the coloured plumage and brown colour of the egg and is now being utilized as a female line for producing egg type chicken for free-range conditions (Panda *et al.* 2012). Dahlem red is known for its high disease tolerance and immune competence (Kundu *et al.* 1999). The present study is focused on determining total cholesterol content in chicken eggs.

Simple and easy methods were not available for cholesterol estimation which is an important area of interest as higher cholesterol content is directly related with artherosclerosis and coronary diseases. In the earlier method, total lipids were extracted with chloroform: methanol 2:1 forming a biphasic layer according to modified Folch method (Folch *et al.* 1957, Washburn and Nix, 1974 and Washburn, 1989). Then, chloroform extract is taken to estimate cholesterol by colorimetry method (Aquino *et al.* 2010). Hexane is used for extraction of unsaponifiable matter. Hexanic extract with acetic acid saturated with ferrous sulphate and then concentrated sulphuric acid is used to estimate cholesterol spectrophotometrically. In this method cholesterol estimation is compromised as hexane interferes with cholesterol because it is a chemical method and thus cholesterol is underestimated. Methods such as HPLC and gas chromatography are highly sophisticated and other chemical methods are not much reliable. Estimation of cholesterol in eggs was done spectrophotometrically and

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showed the values within the range and the procedure was found easy and less time consuming.

MATERIALS AND METHODS

Experimental birds

Total 45 egg samples were randomly collected fresh in the morning. The study was carried out during the period of February 2019 to June 2020, in the Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, Palampur. Total twenty four healthy layer birds of Dahlem Red breed of 30-32 weeks of age were reared under deep litter system in separate pens with nest boxes and were offered *ad libitum* drinking water and standard layer feed presented in Table 1.

Sample preparation

The egg was broken on a flat surface and was allowed to stand for five minutes. Carefully albumen and yolk were separated and yolk was transferred and rolled on Whatmann filter paper No. 1 to remove all the sticking albumen residues and chalazae. Pure yolk was poured in the graduated beaker after rupturing the yolk membrane with rat tooth forceps. Volume of yolk was recorded. The sample solution was prepared by diluting 100 µl of yolk sample with 100 µl of distilled water and mixing well using a vortex mixer. From diluted yolk sample 10 µl of yolk sample was taken for estimation.

Principle of cholesterol estimation

Total cholesterol was determined by enzymatic colorimetric method (cholesterol oxidase peroxidase method) (Arntz *et al.* 1979; Fleg, 1973; Siedel *et al.* 1981 and Allain *et al.* 1974) using biochemical estimation kit (Agappe Diagnostics Ltd.). This method was based on the principle that free cholesterol and fatty acids are formed by the hydrolysis of cholesterol ester by cholesterol esterase. Free cholesterol is further oxidized by cholesterol esterase into 4-cholesten-3-one and hydrogen peroxide. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to form a red coloured quinoneimine dye complex. Intensity of colour formed is directly proportional to the amount of the cholesterol present in the sample.

Method of cholesterol estimation

Cholesterol estimation kit contained 2 types of reagents. The first reagent contained Pipes buffer (pH 6.9) - 50 mmol/L, phenol- 24 mmol/L and sodium Cholate- 0.5 mmol/L. The second reagent contained cholesterol esterase- >200 U/L, cholesterol oxidase- >250 U/L, peroxidase- >1000 U/L and 4 amino antipyrine- 0.5 mmol/L. A working solution was prepared by dissolving the content of first reagent with equal content of second reagent. The standard solution was prepared by mixing 1 ml of working reagent with 10 µl of cholesterol standard which was provided in the kit. The test solution was prepared with 1 ml of working reagent and 10 µl of egg yolk sample. The blank was prepared by taking 1 ml of working reagent. The blank, standard and test solutions were incubated at 37°C for 5 minutes. Total cholesterol was estimated by recording absorbance of standard, test against blank at a wavelength of 505 nm by using biochemical estimation kit (Agappe Diagnostics Ltd.) in a semi-automated clinical chemistry analyzer (Microlab 300) based on the above principle. Finally, mg/ml cholesterol in egg yolk was estimated by multiplying with the dilution factor. Cholesterol values in mg/egg yolk, cholesterol values in mg/ml in egg yolk and yolk volume are presented in Table 2.

Statistical analysis

The results obtained were analyzed for calculating mean±standard error.

RESULTS AND DISCUSSION

The egg yolk volume of Dahlem Red was 15.54±0.21 ml and the average cholesterol content of Dahlem red egg in our study was 195.66±2.80 mg/egg yolk or 12.59±0.09 mg/ml in egg yolk. Egg yolk cholesterol by gas liquid chromatography was 11.5-11.8 mg/g egg yolk in White Leghorn and 13 mg/g egg yolk in Rhode Island Red (Maurice *et al.* 1994) which was lower and almost within the present observed range, respectively. Egg yolk cholesterol by gas chromatography was 23.4±1.7 mg/g egg yolk in laying hen at 21 weeks of age and 20.4±1.6 mg/g egg yolk in laying hen at 56 weeks of age (Nielsen, 1998) which were higher than the present observed range. The egg yolk cholesterol levels in most of the poultry breeds were 180-190 mg in egg yolk (Naviglio *et al.* 2012) and present results are reliable.

Egg yolk cholesterol value by method of Ingr and Simeonová (1983) using the Bio–La-test and the photometric detection based on Liebermann Burchard reaction was 153.45±12.39 mg/g egg yolk to 263.90±14.83 mg/g egg yolk in hen (Vorlova *et al.* 2001) which was in agreement with the present study. Egg yolk cholesterol by HPLC was 12 mg/g egg yolk in Brazilian chicken egg (Bragagnolo *et al.* 2002) which was slightly lower than present observed range. Egg yolk cholesterol by gas chromatography and (Zak, 1977) procedures found was 12.07±0.06 mg/g in egg yolk (Rowghani *et al.* 2007) which was similar to the present estimated value. Egg yolk concentration by enzymatic method (Colestat Enzymatic kit, Wiener lab, Argentina) after lipid extraction was 0.65 g/100 g egg yolk in eggs of Araucana hen (Gultemirian *et al.* 2009) which was lower than the present observed range. Egg yolk cholesterol by gas

Table 1: Standard layer feed (Crumegg-1).

Ingredient	Standard layer feed (Crumegg-1)
Moisture (Maximum)	10.00%
Crude protein (Minimum)	18.00%
Crude fibre (Maximum)	6.00%
Crude fat (Minimum)	3.00%
Calcium (Minimum)	3.50%
Phosphorus (Minimum)	0.40%
Metabolic energy (Minimum)	2800 Kcal/kg

Table 2: Cholesterol values in mg/egg yolk, cholesterol values in mg/ml egg yolk and yolk volume (ml).

Cholesterol in mg/egg yolk	Cholesterol in mg/ml in egg yolk	Yolk volume (ml)
172.12	13.24	13.0
188.10	12.54	15.0
160.68	12.36	13.0
208.63	13.46	15.5
191.70	12.78	15.0
167.04	13.92	12.0
187.04	13.36	14.0
204.48	12.78	16.0
174.72	12.48	14.0
179.82	13.32	13.5
186.60	12.44	15.0
186.31	12.02	15.5
214.20	12.60	17.0
204.80	12.80	16.0
214.08	13.38	16.0
219.84	13.74	16.0
189.00	12.60	15.0
222.12	12.34	18.0
227.24	11.96	19.0
186.30	12.42	15.0
166.32	11.88	14.0
188.48	11.78	16.0
175.20	11.68	15.0
181.20	12.08	15.0
176.39	11.38	15.5
177.30	11.82	15.0
199.20	13.28	15.0
184.73	12.74	14.5
193.44	12.48	15.5
192.60	12.84	15.0
192.30	12.82	15.0
215.90	12.70	17.0
215.22	12.66	17.0
240.84	13.38	18.0
220.32	12.96	17.0
224.40	13.20	17.0
213.18	12.54	17.0
189.12	12.61	15.0
196.16	12.26	16.0
190.40	11.90	16.0
187.24	12.08	15.5
224.68	12.48	18.0
191.68	11.98	16.0
208.00	13.00	16.0
175.50	11.70	15.0

Mean±standard error of cholesterol in mg/egg yolk = 195.66±2.80 mg/egg yolk, of cholesterol in mg/ml in egg yolk = 12.59±0.09 mg/ml egg yolk and of yolk volume in ml = 15.54±0.21 ml.

chromatography was 341 mg/100 g egg yolk in conventional eggs and 489 mg/ 100 g egg yolk in organic eggs (Matt *et al.* 2009) which was lower than the present observed range. Egg yolk cholesterol determined by using the described method in Biochemical Analysis and Food Analysis was 20.27 mg/g egg yolk in laying hen egg (Canogullari *et al.* 2009) which was higher than the present observed range.

Egg yolk cholesterol by HPLC method and colorimeter method was 12.71±1.54 mg/g and 12.17±1.36 mg/g, respectively in chicken egg yolk (Aquino *et al.* 2010). Egg yolk cholesterol by gas chromatography using a polar capillary column was 120-193 mg/egg (Naviglio *et al.* 2012) which was within the present observed range. Egg yolk cholesterol estimated by (Allain *et al.*1974) method was 12.06±0.71 mg/g egg yolk in village hen egg and as 13.02±0.69 mg/g egg yolk in broiler egg (Jeyamala and Thangadurai, 2018) which was within the present observed range.

CONCLUSION

The results concluded that the value of total cholesterol obtained by this methodology was accurate and specific. Such information will be helpful to the consumers for the consumption of lower cholesterol eggs as well as helpful to farmers to compare and choose suitable breed for poultry farming under semi intensive system and for better marketability of eggs.

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