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Effect of Laying Hen's Age and Oviposition Time on Egg Cholesterol Content

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Abstract

The effect of the age and oviposition time on yolk cholesterol content and total yolk cholesterol was investigated in laying hens. Three hundred, twenty-two weeks old, ISA-Brown hens were raised in layer battery cages under the confined system for 16 weeks. Of which, oviposition time was recorded in the morning and afternoon egg collections. These egg samples were randomly collected nine times at a one-week interval based on hen's age. At each time, fifteen eggs laid in the morning and a similar number laid late in the afternoon were collected. Yolk samples were prepared followed by saponification and extraction of lipids. Yolk cholesterol content was determined using a spectrophotometer. Total yolk cholesterol was then calculated. The age of laying hens showed a significant effect; as the age progressed, yolk cholesterol content was decreased (14.44-14.32 mg/g yolk); whereas, total yolk cholesterol was increased (178.04-214.75 mg/egg). The oviposition time showed a significant effect as reflected by the lower yolk cholesterol content of morning eggs (12.53 mg/g yolk) compared to afternoon eggs (16.23 mg/g yolk). Furthermore, morning eggs had significantly lower total yolk cholesterol (176.63 mg/egg) than afternoon eggs (221.14 mg/egg). Hence, morning eggs are superior to meet the interest of table egg producers and consumer demand.



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Introduction

Eggs play a pivotal role in the human diet and nutrition worldwide [1]. Evidence has shown that egg yolk, in particular, provides excellent functional properties to a variety of food products, including mayonnaise, ice cream and bakery items [2]. However, chicken eggs contain a high amount of cholesterol, which located exclusively in the yolk [3]. Moreover, the cholesterol content of the chicken eggs continues to receive substantial attention [4]. This is justified in the sense that eggs are regarded as important contributors to serum cholesterol [5]. The biosynthesis of cholesterol in chicken occurs chiefly in the liver, where it is regulated by various dietary factors [6]. Nevertheless, it was long proved that as the laying hen progresses in maturity, cholesterol biosynthesis increases rapidly due to increased demand for cholesterol in egg formation [7].

Egg cholesterol is crucial for embryonic development during the final stages of incubation [8]. As such, young chick lacks the ability to synthesize cholesterol [9]. Nevertheless, in the post-hatching period, cholesterol becomes a major structural component of cell membrane and a precursor for many steroid hormones [10]. Evidence has shown an elevation of serum cholesterol increases the risk of cardiovascular diseases [2]. Hence, the strong negative publicity on the harmful effect of cholesterol has led to decreased egg consumption in many countries worldwide [11]. The cholesterol content of the chicken eggs is influenced by some factors, including age of the laying hens [12]. Research progress made on the effect of oviposition time on egg cholesterol content is imperative for deciding which eggs are appropriate for human consumption. Accordingly, it was estimated that the yolk cholesterol contents (mg/g yolk) of eggs laid early in the morning compared to eggs laid in the afternoon had slightly higher yolk cholesterol content but the difference was non-significant [13]. Additionally, the effect of oviposition time on egg cholesterol content in cages and litter system had shown no significant difference [14]. The mean rate of eggs laying in a flock at a particular age is determined by a sequence of clutch length [15] that in turn affects oviposition time [16]. The aim of this study was to investigate the effect of laying hen's age and oviposition time on yolk cholesterol content and total yolk cholesterol in ISA-Brown hens. This could provide an impetus for the

consumer demand from a human health perspective.

Materials and Methods

Experimental birds and management

A total of three hundred (300), twenty-two (22) week old ISA-Brown laying hens were raised in layer battery cages under confined system slated for a 16-hour lighting and 8-hour darkness up to 38 weeks. A typical layer diet was fed to the birds as shown in Table 1. Feed and water were provided *ad libitum*. The temperature was maintained between 20-23 °C and the relative humidity did not exceed 60%.

Table 1 Ingredients and chemical composition of the layer diet.

Ingredients	%	Chemical composition	%
Sorghum	35.00	Dry matter	96.0
Wheat	23.00	Crude protein	17.0
Barely	10.00	Ether extracts	4.45
Soya bean meal	15.00	Crude fiber	3.80
Plant protein meal	10.00	Ash	13.50
Limestone	5.00	Calcium	3.42
Oil	1.00	Magnesium	1.76
Salt	0.25	Phosphorus	0.33
Vitamins	0.25		

Experimental design

Two experimental treatments were designed, including the age of the laying hens as of 22 to 38 weeks old, and the oviposition time twice daily slated for 8:00 AM (early oviposition) and 2:00 PM (late oviposition).

Collection of egg samples

Egg samples were randomly collected nine times at one-week interval based on age. At each time, fifteen eggs laid in the early morning and another fifteen-laid in the afternoon were collected and stored at 20-23°C.

Estimation of yolk cholesterol content

Eggs were first hard cooked, allowed to cool, shells peeled off, yolks weighed and recorded (Table 2). Saponification and extraction of lipids from the cooked yolk were carried out as described by Folch [17]. Yolk cholesterol content was estimated by an enzymatic method using a spectrophotometer at the wavelength of 500 nm [18]. Total yolk cholesterol (mg/egg) was calculated as follows:

Total yolk cholesterol = mg cholesterol/g yolk × yolk weight (g)

Table 2 Average yolk weight used to calculate total yolk cholesterol.

Age (weeks)	Yolk weight (g)		Mean (age)
	Early oviposition	Late oviposition	
22	12.37	12.30	12.33
24	12.92	12.69	12.81
26	13.38	13.11	13.25
28	13.87	13.27	13.57
30	14.20	13.70	13.95
32	14.67	14.09	14.38
34	14.91	14.24	14.57
36	15.20	14.49	14.85
38	15.33	14.73	15.03
Mean (oviposition)	14.09	13.62	-

Table 3 Effect of laying hen's age and oviposition time on yolk cholesterol concentration.

Age (weeks)	Yolk cholesterol concentration (mg/g)		Mean (age)
	Early oviposition	Late oviposition	
22	12.59±0.03	16.29±0.03	14.44 ±1.93 ^a
24	12.57±0.03	16.27±0.02	14.42±1.93 ^b
26	12.56±0.03	16.26±0.02	14.41±1.93 ^{bc}
28	12.54±0.03	16.26±0.01	14.40±1.94 ^{cd}
30	12.54±0.02	16.24±0.01	14.39±1.93 ^{de}
32	12.53± 0.02	16.22±0.02	14.38±1.92 ^{ef}
34	12.51±0.02	16.20±0.02	14.36±1.92 ^g
36	12.49±0.01	16.19±0.01	14.34±1.93 ^h
38	12.48±0.02	16.17±0.02	14.32±1.92 ⁱ
Mean*(oviposition)	12.53±0.04 ^x	16.23±0.04 ^y	-

* = Significant ($P<0.01$) difference; x, y = means within a row; a-i = means within a column

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS version 16 [19]. The means were compared with Fisher's Least Significant Difference test at the 1 % level. All results were presented as the mean \pm standard deviation (m \pm SD).

Results and Discussion

Egg cholesterol content was expressed as yolk cholesterol content and total yolk cholesterol. The results of the effect of the laying hen's age and oviposition time on yolk cholesterol content and total yolk cholesterol are exclusively shown in Tables 3 and Table 4, respectively. In this study, the effect of laying hen's age on egg cholesterol values has shown a significant ($P<0.01$) treatment effect. Consequently, as the age of the laying hens progressed, yolk cholesterol content was decreased (14.44-14.32 mg/g yolk); whereas, total yolk cholesterol was increased (178.04-214.75 mg/egg). These findings are in line with the results obtained by Jiang and Sim [20] and Zhirong and Jeong [21]. However, these results are in partial agreement with the findings of Pandely et al. [22] who reported decreased total yolk cholesterol (mg/egg) with age.

Similarly, Zemkova et al. [23] and AL-Zubaidy and AL-Taha [24] documented increased yolk cholesterol content (mg/g yolk) with age. Unsurprisingly, our results contradicted with the findings of Li and Lee [25]. This disparity could be due to some physiological factors because of poultry breed differences and seasonal variation. Oloyo [26] documented significantly higher yolk cholesterol content in younger birds while Li and Lee [25] concluded that yolk cholesterol content showed no significant correlation with the hen's age. The two expressions of egg cholesterol values showed the opposite trend; milligram cholesterol per gram of yolk, and the value decreased with advancing age. On the contrary, milligram cholesterol per egg had shown the value increased with advancing age. The increase in the latter value despite the decrease in the former indicates that the increase in yolk weight was greater than the reduction in yolk cholesterol content. Hence, heavier yolk eggs are likely to contain more cholesterol, which provides strong evidence to the above explanation [27-29]. Thus, it seems that the weight of the yolk was the determining factor of total egg cholesterol. The effect of oviposition time on yolk cholesterol content and total yolk cholesterol showed a significant ($P<0.01$) treatment effect. The data

Table 4 Effect of laying hen's age and oviposition time on total yolk cholesterol.

Age (weeks)	Total yolk cholesterol (mg/egg)		
	Early oviposition	Late oviposition	Mean (age)
22	155.72±1.09	200.37±0.52	178.04±23.33 ⁱ
24	162.43±0.63	206.49±0.41	184.46±23.01 ^h
26	168.10±0.39	213.24±0.58	190.67±23.58 ^e
28	173.89±0.57	215.77±0.85	194.83±21.88 ^f
30	178.02±1.21	222.57±0.63	200.29±23.28 ^c
32	183.88±1.04	228.32±0.52	206.10±23.22 ^d
34	186.46±0.30	230.69±0.55	208.57±23.10 ^e
36	189.85±0.63	234.62±0.43	212.24±23.39 ^b
38	191.32±0.22	238.19±0.63	214.75± 23.48 ^a
Mean*(oviposition)	176.63±11.97 *	221.14±12.39 ^y	-

* = Significant ($P < 0.01$) difference; x, y = means within a row; a-i = means within a column

distinctly showed significantly lower yolk cholesterol content of morning egg collection (12.53 mg/g yolk) compared to that of afternoon eggs (16.23 mg/g yolk). Furthermore, this study showed that the morning eggs had significantly lower total yolk cholesterol (176.63 mg/egg) than afternoon eggs (221.14 mg/egg). Apparently, our results with regard to the effect of oviposition time on yolk cholesterol content were not in line with those of Tumova et al. [13], who reported the non-significant difference of yolk cholesterol content between morning and afternoon eggs. However, they pointed out that afternoon egg collection had shown slightly higher yolk cholesterol contents than morning eggs that may be partial evidence for the findings of this study.

Conclusions

The effect of oviposition time on yolk cholesterol content and total yolk cholesterol showed a significant treatment effect in ISA-Brown hens. Morning eggs are superior to afternoon eggs in lower cholesterol content. Therefore, morning eggs are considered the better to meet the consumer demand as safe and healthy food, and the interest of table egg producers.

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Conflict of Interest

The authors have no conflict of interest.

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