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Research Article

The Effect of Chromium Supplementation on Egg Production, Egg Quality and Some Serum Parameters in Laying Hens

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Abstract: The effects of chromium (Cr) on egg production, egg quality, egg yolk cholesterol level and selected serum parameters of laying hens were investigated. Sixty 16-wk-old Hyline White 77 strain were randomly assigned to two groups of 30 hens each and fed either a basal diet or basal diet supplemented with 20 ppm Cr ($CrCl_3.6H_2O$). Egg and blood samples were collected at monthly intervals after the egg production reached peak level. Sera were analysed for chromium, calcium, inorganic phosphorus, magnesium, triglycerides and total cholesterol. Eggs were examined for interior or exterior quality and for yolk cholesterol content. Chromium supplementation resulted in a 1.88% reduction in feed consumption and 4.28% improvement in the efficiency of feed utilisation. Chromium had no effect on live weight change, overall mean egg production, egg weight, specific gravity, shape index, shell thickness and Haugh unit, but increased shell breaking strength, albumen and egg yolk index values were noted. Supplemental chromium had no significant effect on serum phosphorus, while it resulted in increases in calcium and magnesium concentrations at first sampling. Serum total cholesterol concentrations slightly decreased while triglyceride levels significantly decreased. Significant reductions were observed in yolk cholesterol content in chromium supplemented group at weeks 36 and 40 ($p<0.001$). The results of the experiment indicated that chromium supplementation to the diet of layers may be of practical value due to the slight reduction in feed consumption and improvement in efficiency of feed utilisation and reduced egg cholesterol content without any adverse effect on egg quality.

Key Words: Chromium, serum parameters, egg quality, cholesterol, laying hen.

Yumurta Tavuğu Yemine Krom İlavesinin Yumurta Üretimi, Yumurta Kalitesi ve Bazı Serum Parametrelerine Etkisi

Özet: Bu çalışmada, kromun (Cr) yumurta tavuklarında, yumurta üretimi, yumurta kalitesi ve yumurta kolesterol düzeyi ve bazı serum parametrelerine etkisi incelendi. Altmış adet, 16 haftalık Hyline White 77 hattı yumurta tavuğu 30'ar hayvandan oluşan iki gruba ayrılarak bazal rasyonla veya 20 mg/kg krom ($CrCl_3.6H_2O$) ilave edilen bazal rasyonla beslendi. Yumurta üretimi pike ulaştığında birer ay arayla alınan kan örneklerinin serumlarında krom, kalsiyum, inorganik fosfor, magnezyum, trigliserit ve total kolesterol konsantrasyonları ölçüldü. Yumurtaların iç ve dış kalitesiyle kolesterol düzeyi belirlendi. Krom ilaveli yemle beslenen hayvanların yem tüketimi %1.88 azaldı, yemden yararlanma oranı % 4.28 arttı. Canlı ağırlık, yumurta üretimi, yumurta ağırlığı, yumurta özgül ağırlığı, şekil indeksi, kabuk kalınlığı ve Haugh birimi krom ilavesinden etkilenmedi ancak, yumurta kırılma mukavemeti, ak ve sarı indeksi değerleri yükseldi. Serum fosfor düzeyi etkilenmezken, kalsiyum ve magnezyum düzeyleri ilk örnekleme döneminde yükseldi. Serum total kolesterol konsantrasyonunda önemsiz, trigliserit düzeyinde önemli azalmalar saptandı. Krom ilaveli yemle beslenen hayvanların yumurta kolesterol düzeyi 36. ve 40. haftalarda düştü ($p<0.001$). Sonuç olarak, yumurta tavuğu yemine krom ilavesi, yem tüketimini azaltıp yemden yararlanma oranını iyileştirdiği ve yumurta iç ve dış kalitesi üzerine olumsuz bir etki yapmaksızın yumurta kolesterolünü düşürdüğünden uygulamada önem taşıyabilir.

Anahtar Sözcükler: Krom, serum parametreleri, yumurta kalitesi, kolesterol, yumurta tavuğu.

Introduction

Chromium (3^+) is an essential element that is required for the normal metabolism of carbohydrates, proteins and lipids in humans and laboratory animals (1). Chromium complexes with proteins and nucleic acids, and forms tight bonds with oxygen- and sulphur-containing ligands. Chromium stimulates the action of insulin, probably through a glutathione-like complex consisting of niacin, trivalent chromium and amino acids (glucose tolerance factor) (2).

Chromium, a low toxic element (1), improves immunity (3,4), carcass characteristics (5) and growth (6,7). The effect of chromium on lipid metabolism is variable. In some studies, chromium increased (8) or decreased (9-12) plasma cholesterol, whereas in some other studies chromium had no effect on cholesterol concentrations (7,13,14). Limited evidence suggests that chromium may interact with other minerals (4,15,16). This experiment was performed to determine the effects of inorganic chromium on selected serum parameters related to mineral and lipid metabolism, egg production, interior and exterior quality of eggs as well as egg yolk cholesterol content in laying hens.

Materials and Methods

Animals and Dietary Treatments

Sixty 16-wk-old, Hyline White 77 strain were randomly assigned to two groups of 30 hens each. The hens were individually housed in wire cages (25 by 46 cm) at 18-20°C, on a 16-hour light schedule. The hens received a typical layer diet containing 2700 ME kcal/kg and 17.67% CP to meet or slightly exceed the nutrient requirement by NRC (17) (Table 1). The hens were fed the basal diet (Control Group) or the basal diet supplemented with 20 ppm Cr ($CrCl_3.6H_2O$, Sigma)

Table 1. Ingredients and chemical composition of basal diet fed to laying hens

Ingredients	%	Chemical Analysis	%
Corn	32.00	Crude protein	17.67
Wheat	20.00	Crude ash	13.8
Sunflower meal	14.50	Crude cellulose	7.55
Soybean meal (44% CP)	8.50	Phosphorus	1.02
Barley	8.00	Calcium	3.6
Limestone	8.00	Chromium (mg/kg)	4.1
Full fat soya	3.00		
Vegetable oil	2.50		
Meat-bone meal	2.40		
Salt	0.35		
Dicalcium phosphate	0.30		
Vitamin-amino acid-mineral mix*	0.45		
Calculated Analyses			
ME (kcal/kg)	2700		
Sodium	0.18		
Lysine	0.75		
Methionine	0.35		
Linoleic acid	1.26		

**: Provided by per kg of diet: vitamin A, 12 000 IU; vitamin D3, 2000 IU, vitamin E, 35 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; niacin, 20 mg; vitamin B6, 5 mg; vitamin B12, 0.015 mg; folic acid, 1 mg; biotin, 0.045 mg; ascorbic acid, 50 mg; canthaxanthine, 1.5 mg; apocarotenoic acid ester 0.5 mg; choline chloride, 125 mg; manganese, 80 mg; iron, 60 mg; zinc, 60 mg; copper, 5 mg; cobalt, 0.2 mg; iodine, 1 mg; selenium, 0.15 mg .*

(Treatment Group). Feed and water were supplied ad libitum. The supplemental level of 20 ppm Cr was chosen in view of the literature which indicated no additional effect of higher levels of supplemental chromium (6).

Sample Collection and Analysis

Egg and blood samples were collected when the egg production reached peak levels at 27 weeks of age. The hens were fasted for 12 hours prior to blood sampling. Hens were weighed and blood samples were collected from the V. brachialis five times at monthly intervals. Sera were separated by centrifugation at 3000 rpm after one hour incubation at room temperature and stored at -20°C until analysis. Sera were analysed with a spectrophotometer (Schimadzu, Model 1208) using commercial kits for calcium, phosphorus, magnesium (Chema Diagnostica, Italy), triglycerides and total cholesterol (Valtek, Chile). A Schimadzu AA-660 Atomic Absorption Spectrophotometer equipped with GFA-4B Schimadzu Graphite Furnace Atomiser was used for determinations of serum Cr concentrations and feed Cr contents (18,19). Feed samples were analysed for crude protein, crude ash, crude cellulose, phosphorus and calcium (20).

Egg numbers and egg weight were recorded daily from the 22nd week throughout the experiment. The eggs were collected on the same day as the blood sampling to study the following egg characteristics. The specific gravity of a whole egg was measured by Archimedes' method with an instrument designed for the measurement of egg weight in air (Wa) and in water (Ww) which was at 15.6°C and specific gravity was calculated with the following formula [Specific gravity = Wa/(Wa-Ww)] on the same day of egg collection. The other egg quality parameters were measured 24 hours later. The shape index was measured by an instrument (BV. Apparatenfabreik Van Doorn, Holland). Shell breaking strength was measured by an instrument (Dr. ING. George Wazna Mess+Prüftechnik, Berlin) which assessed the resistance of the egg to crushing. Shell thickness was measured by a micrometer (Mitutoyo, 0.01 mm, Japan). Albumen height (H) was measured by a tripod micrometer (Mitutoyo, 0.01 mm, Japan), albumen length (L) and width (W) by a compass (Swordfish, 0.02 mm, China) and then the albumen index was calculated with the following formula [Albumen index = H/{(L+W)/2}x100]. Yolk height (H) was measured by a tripod micrometer (Mitutoyo, 0.01 mm, Japan) and yolk diameter (D) by a compass (Swordfish, 0.02 mm, China), then the yolk index was calculated with the following formula [Yolk index = (H/D)x100] (21).

Two eggs from each hen were collected one day before and after the blood sampling and the cholesterol content of the egg yolk was determined by using the methods of Hammad et al. (22) and Berrio and Hebert (23) with slight modifications. The eggs were hard-boiled for 15 min, cooled immediately, stored at 2-8°C and analysed within a week for cholesterol content. The yolks were separated and sample of 0.1 g of yolks, pooled from two eggs belonging to same hen, were weighed accurately. Yolk lipids were extracted with isopropanol (4 ml/ 0.1 g of yolk, Rieden-de Haen) then vortex mixed and centrifuged at 3000 rpm for 10 min. The yolk cholesterol concentration was determined in the filtered samples by UV spectrophotometer using a commercial kit (Valtek, Chile). The cholesterol concentration of the egg yolk (mg cholesterol / g of egg yolk) was calculated (24).

Statistical Analysis

Statistical analysis of data were performed by SPSS 9.0 version for Microsoft. Independent sample t-test was used for the differences between groups. The chi square test was performed to evaluate the differences between the groups for the egg production rate. All data were recorded on an individual basis except feed consumption, which was recorded on a group basis at weekly intervals. Therefore, statistical analysis was not performed for food consumption and for efficiency of food utilisation. Data were expressed as mean ±SEM.

Results

No significant differences in body weight change were observed between control and treatment groups (Table 2). Average daily feed consumption was 116.83 g in the treatment group and 119.07 g in the control group (Table 3) thus, a decrease of 1.88% was calculated. However, supplemental chromium did not affect overall mean egg production (Table 4). Feed efficiency was 1.87 and 1.79 for the control and treatment groups respectively; thus, Cr supplementation improved feed efficiency by 4.28% (Table 5) while it had no effect on egg weight (Table 6), specific gravity, shape index or shell thickness. Shell breaking strength increased ($p<0.05$) in the treatment group at week 27 and no differences were observed thereafter. In the treatment group, the albumen

Table 2. The effects of supplemental chromium on the body weight change (g)

Weeks	n	Control	n	Chromium	p
27	30	1554.43±29.12	30	1519.73±23.34	-
32	30	1616.90±29.84	30	1606.00±31.02	-
36	30	1669.10±33.92	30	1649.98±32.05	-
40	29	1774.00±38.12	29	1725.69±35.96	-
43	29	1771.38±39.90	29	1737.21±38.37	-

-:not significant

Table 3. The effects of supplemental chromium on feed consumption of laying hens by weeks (g/bird per day)

Weeks	Control	Chromium
22	95.23	100.95
23	96.19	92.38
24	113.33	110.00
25	114.28	109.04
26	116.19	117.61
27	117.61	112.86
28	111.90	114.76
29	117.38	115.48
30	121.66	117.85
31	124.76	120.95
32	117.86	114.76
33	128.80	124.28
34	135.24	130.00
35	132.38	126.19
36	116.66	110.00
37	126.19	115.27
38	133.00	128.00
39	122.60	114.28
40	105.41	116.25
41	133.49	120.68
42	124.18	121.67
43	115.27	136.94
Mean	119.07	116.83

Table 4. The effects of supplemental chromium on egg production of laying hens by weeks (%)

Weeks	Control	Chromium	X^2
22	14.7^a	17.6^b	0.63*
23	47.6^a	56.6^b	3.44*
24	88.0^a	90.0^b	0.39*
25	95.2	95.7	0.05
26	97.1^b	95.7^a	0.62*
27	97.1^a	98.5^b	1.02*
28	97.6^b	94.7^a	2.34*
29	97.1	97.1	0.00
30	95.2^a	96.6^b	0.55*
31	95.7^a	97.6^b	1.18*
32	97.1^b	95.7^a	0.62*
33	94.2^b	92.8^a	0.36*
34	95.7^a	96.1^b	0.06*
35	94.2	94.7	0.05
36	95.7^b	92.8^a	1.59*
37	90.9^a	92.1^b	0.18*
38	94.7^b	91.6^a	1.61*
39	87.6^a	89.6^b	0.39*
40	90.1	90.6	0.03
41	92.1^b	89.6^a	0.74*
42	91.6^a	93.1^b	0.31*
43	89.6^b	87.1^a	0.60*
Mean	88.13	88.45	0.22

a-b: Values within the same row with different superscripts differ significantly
*: p< 0.05

index value increased (p<0.05) at week 36 but there were no differences at the first two sampling times. Yolk index values increased at weeks 27 and 36. The reductions in yolk cholesterol content (p<0.001) were significant (Table 7).

Serum Cr concentrations slightly increased over the experimental period with a significant increase (p<0.05) at week 43, Ca (p<0.001) and Mg (p<0.001) concentrations were significantly elevated at week 27. Chromium caused slight but not significant increases in serum inorganic P levels. Chromium supplementation resulted in nonsignificant reductions in serum total cholesterol levels with reductions in serum triglycerides (p<0.001, 0.05) (Table 8).

Table 5. The effects of supplemental chromium on feed efficiency of laying hens by weeks (kg diet/a dozen eggs)

Weeks	Control	Chromium
22	7.76	6.88
23	2.42	1.96
24	1.54	1.47
25	1.44	1.37
26	1.44	1.47
27	1.45	1.37
28	1.38	1.45
29	1.45	1.43
30	1.53	1.46
31	1.56	1.47
32	1.46	1.44
33	1.64	1.61
34	1.70	1.62
35	1.68	1.60
36	1.46	1.42
37	1.66	1.50
38	1.68	1.68
39	1.68	1.53
40	1.40	1.54
41	1.73	1.62
42	1.63	1.57
43	1.54	1.87
Mean	1.87	1.79

Discussion

It has been reported that the supplementation of 20 ppm chromium chloride to the diet of broilers resulted in higher body weight (25). Lien et al. (26) found that chromium picolinate (Crpic) markedly enhanced weight gain due to increased feed consumption in broilers. Steele and Rosebrough (6) reported that 20 ppm Cr supplementation improved growth rate and feed efficiency. In the present experiment, 20 ppm Cr did not affect body weight as in the results of Cupo and Donaldson (7) in chicks and Şahin et al. (16) in rabbits, but reduced feed consumption and improved feed efficiency. Egg production and egg weight were not affected by chromium supplementation, consistent with the results of Lien et al. (27).

In the present study, serum Cr concentrations increased with supplemental chromium. Moonsie-Shageer and Mowat (4) found increases in serum Ca and Mg concentrations by supplemental chromium in calves. Chang and Mowat (3) reported a relationship between Cr

Table 6. The effects of supplemental chromium on egg weights of laying hens by weeks (g)

Weeks	n	Control	n	Chromium	p
22	10	46.22 ±1.38	11	46.96 ± 1.34	-
23	25	50.05 ± 0.81	26	50.85 ± 0.92	-
24	29	54.13 ± 0.81	30	53.58 ± 0.81	-
25	30	56.47 ± 0.73	30	56.16 ± 0.65	-
26	30	57.54 ± 0.75	30	57.48 ± 0.68	-
27	30	58.15 ± 0.65	30	57.89 ± 0.65	-
28	30	58.17 ± 0.65	30	58.93 ± 0.67	-
29	30	58.89 ± 0.63	30	58.87 ± 0.63	-
30	30	59.59 ± 0.69	30	59.05 ± 0.58	-
31	30	59.66 ± 0.69	30	59.37 ± 0.68	-
32	30	59.95 ± 0.63	30	59.17 ± 0.56	-
33	30	60.62 ± 0.73	30	59.86 ± 0.72	-
34	30	61.60 ± 0.71	30	60.94 ± 0.74	-
35	30	61.66 ± 0.68	30	61.45 ± 0.72	-
36	30	61.76 ± 0.73	30	61.18 ± 0.75	-
37	30	62.30 ± 0.76	29	62.13 ± 0.72	-
38	30	62.89 ± 0.73	29	62.32 ± 0.76	-
39	29	63.26 ± 0.79	29	62.83 ± 0.54	-
40	29	64.41 ± 0.85	29	64.42 ± 0.72	-
41	29	64.05 ± 0.86	29	63.73 ± 0.80	-
42	29	63.66 ± 0.89	29	63.86 ± 0.67	-
43	29	63.37 ± 0.91	29	63.59 ± 0.78	-

-: not significant

Table 7. The effects of supplemental chromium on egg quality parameters and egg yolk cholesterol content

Parameters	Weeks	n	Control	n	Treatment	p
Specific	27	28	1.0896 ± 0.0008	29	1.0879 ± 0.0011	
Gravity	32	24	1.0859 ± 0.0008	27	1.0857 ± 0.0009	
(g/cm3)	36	29	1.0862 ± 0.0008	29	1.0863 ± 0.0007	
Egg Shape	27	28	79.04 ± 0.35	25	78.80 ± 0.46	
Index	32	24	78.50 ± 0.61	26	78.25 ± 0.33	
	36	29	77.58 ± 0.51	29	77.67 ± 0.28	
Shell	27	28	2.64 ± 0.08	25	2.97 ± 0.11	*
Breaking	32	23	2.73 ± 0.09	26	2.56 ± 0.15	
Strength	36	29	2.84 ± 0.10	28	2.85 ± 0.13	
(kg/cm2)						
Shell	27	28	38.95 ± 0.59	25	40.20 ± 0.45	
Thickness	32	24	40.28 ± 0.41	26	40.61 ± 0.49	
(mm x102)	36	29	39.50 ± 0.35	29	39.41 ± 0.32	
Haugh Unit	27	27	94.37 ± 1.14	22	93.18 ± 1.30	
	32	22	88.00 ± 2.11	25	86.76 ± 1.76	
	36	29	87.24 ± 1.32	29	90.10 ± 1.51	
Albumen	27	26	12.40 ± 0.36	22	12.45 ± 0.42	
Index	32	22	10.85 ± 0.59	25	10.46 ± 0.47	
	36	29	9.69 ± 0.38	28	11.03 ± 0.48	*
Egg	27	26	48.81 ± 0.46	23	50.70 ± 0.77	*
Yolk	32	22	48.67 ± 0.52	25	47.60 ± 0.63	
Index	36	29	44.70 ± 0.38	28	47.44 ± 0.53	**
Egg Yolk	32	17	12.38±0.75	14	12.93±0.41	
Cholesterol	36	20	17.48±0.48	21	14.92±0.52	**
(mg/g)	40	27	17.25±0.44	27	13.42±0.51	**
	43	19	18.05±0.97	25	16.91±0.61	

*: $p<0.05$
**: $p< 0.001$

and Mg. In contrast, Kalaycıoğlu et al. (15) found no effect of Cr on serum Mg levels in broilers. Page et al. (11) and Şahin et al. (16) suggested that Cr had no effect on Ca and inorganic P levels. In the present study, serum inorganic P concentrations slightly increased in the treatment group. Shell thickness was not affected by Cr supplementation as indicated by Lien et al. (27). Although these authors found a decrease in shell strength, in the present study, an increase in egg resistance to crushing and elevations in serum calcium and magnesium levels were observed at the first sampling time. However, this effect does not seem to be a result of Cr supplementation, because similar results were not obtained at the following sampling times as well as no differences in other shell quality parameters. Thus, it is possible to say that chromium did not affect the parameters related to egg shell quality investigated in this study. However, chromium supplementation increased albumen and yolk quality.

Table 8. The effects of supplemental chromium on serum parameters

Parameters	Weeks	n	Control	n	Chromium	p
Chromium	27	8	1.88±1.93	8	2.04±1.56	
(μg/L)	32	8	1.74±1.77	8	1.93±1.29	
	36	8	1.91±2.19	8	1.95±1.45	
	40	8	1.92±1.57	8	2.03±1.67	
	43	8	1.55±1.30	8	2.19±2.38	*
Calcium	27	30	24.84±0.87	30	30.79±1.23	**
(mg/dl)	32	30	23.03±1.08	29	22.74±0.88	
	36	30	22.28±0.77	29	24.12±0.70	
	40	29	33.00±0.82	28	33.40±0.85	
	43	29	30.93±0.72	27	31.93±0.86	
Phosphorus	27	30	7.37±0.35	30	8.61±0.81	
(mg/dl)	32	30	8.75±0.99	29	8.24±0.74	
	36	30	6.62±0.65	29	7.51±0.36	
	40	29	10.45±0.50	28	11.54±0.65	
	43	29	10.67±0.79	27	10.51±0.80	
Magnesium	27	30	3.35±0.18	30	4.83±0.26	**
(mg/dl)	32	30	4.92±0.19	29	5.06±0.19	
	36	30	5.78±0.20	29	5.06±0.12	
	40	29	5.39±0.15	28	5.13±0.15	
	43	29	4.81±0.18	27	5.04±0.17	
Triglyceride	27	30	2853.19±172.00	30	2021.18±117.23	**
(mg/dl)	32	30	2341.69±156.92	29	2115.52±116.59	
	36	30	2445.76±104.74	29	1833.54±220.89	**
	40	29	2478.53±113.32	28	2125.56±115,91	*
	43	29	2277.90±128.81	28	1887.74±109.38	*
Total	27	30	179.23±8.28	30	165.91±9.92	
Cholesterol	32	30	149.95±9.29	29	138.40±6.80	
(mg/dl)	36	30	146.63±8.74	29	143.61±6.27	
	40	29	143.09±7.85	28	135.14±6.95	
	43	29	122.81±10.77	28	125.60±7.50	

*: $p<0.05$
**: $p< 0.001$

Studies in human and various animal species on the influence of Cr supplementation on lipid parameters are conflicting. Amoikon et al. (8) reported that fasting plasma cholesterol concentrations in pigs were increased by chromium picolinate. Different forms of Cr reduced total cholesterol in humans (9,10), pigs (11), lambs (12) and layers (27). Reductions in total cholesterol levels in the present study were not significant and agreed with the results of the studies reporting no effects of $CrCl_3$ on total cholesterol in humans (28), lambs (13) rabbits (14) and broilers (7,29). The reduced triglyceride concentrations in this study supported the results of Lefavi et al. (10) and Uyanık (13).

Most of the cholesterol found in egg yolk is derived from circulating plasma lipoproteins, which are synthesized in the liver in response to hormonal changes with the onset of lay (30). Marks and Washburn (31) found a negative correlation between yolk and plasma cholesterol. However, no correlation was found between yolk and plasma cholesterol in quail studies by Hammad et al. (22). Similarly, in the study presented herein, the lack of a clear relationship between blood and yolk cholesterol might be due to the rapid changes in the concentration of blood cholesterol available to the developing yolk over time (32).

Many studies have been done to reduce yolk cholesterol by supplementing diet with different substances (33-35). Reports on chicken egg cholesterol values show considerable variation. However, the egg yolk cholesterol content of the control group determined in this study was within the range of yolk cholesterol levels of 12.1±0.9 to 23.4±1.7 mg/g yolk (33,36,37). Lower yolk cholesterol content in the eggs collected from chromium supplemented diet fed hens than that of the controls is in agreement with the results of Lien et al. (27), who found a dose-dependent reduction in the yolk cholesterol of hens fed chromium picolinate supplemented diet. Egg yolk cholesterol levels of domestic fowl are known to be influenced by genetic and environmental factors (36). The dietary intake of a hen has been shown to influence the hepatic lipogenesis (38), and egg cholesterol content (34). Reduced serum lipid and egg yolk cholesterol concentrations may result from the reduced feed intake due to chromium supplementation.

The results of the present study indicated that chromium supplementation to the layer diet had no effect on body weight but resulted in a slight reduction in food consumption and an improvement in the efficiency of feed utilisation. The cholesterol content of eggs may be reduced by chromium with a slight improvement in the interior quality of the eggs without alterations in shell quality.

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