

Effects of cholestyramine and lovastatin upon plasma lipids and egg yolk cholesterol levels of laying hens

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Efeitos da colestiramina e da lovastatina sobre os lípidos plasmáticos e teores de colesterol da gema do ovo de galinhas poedeiras

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SUMMARY

The purpose of this study was to evaluate the effect of cholestyramine and lovastatin, lipid-lowering agents, upon egg quality, reproductive performance, plasma lipids and egg yolk cholesterol levels of Shaver laying hens. Twenty-six-weeks-old hens were fed basal diet without animal products containing 0.2% cholestyramine (COL1), 0.3% cholestyramine (COL2) or 0.005% lovastatin (LOV) for 6 weeks. It was observed that the supplementation of the drugs did not impair albumen and shell quality. Hen performance was not adversely affected, with the exception of the significant reduction ($p < 0.05$) in egg weights. No significant changes were observed on plasma lipids, and egg yolk cholesterol remained unchanged with the addition of the drugs.

UNITERMS: Cholesterol; Cholestyramine; Lovastatin; Egg Yolk; Chickens.

INTRODUCTION

Concern about the relationship between dietary fat and the development of atherosclerosis has led to many attempts to change egg lipid composition. The quantity of saturated dietary fat has been pointed out as being greatly responsible for the increase in plasma cholesterol concentration, which is related to the incidence of coronary heart disease⁹. Although eggs contain low proportion of saturated fat³ and the contribution of dietary cholesterol to the incidence of coronary heart disease is subject to considerable debate, the high concentration of cholesterol in the egg yolk has been highlighted in the last decades and has caused restrictions to its consumption. Thus, any feasible means of reducing cholesterol content of eggs would be of interest to egg producers and consumers.

Many studies have dealt with the effects of genetic selection and various dietary factors in layers feeds on egg yolk cholesterol. However, reductions in egg cholesterol levels have not reached significant values. Therefore, much attention has been focused on the use of numerous pharmacological agents in an attempt to lower the cholesterol content of eggs^{5,6,13,19,30}.

Cholestyramine, a bile acid sequestrant, is a hypocholesterolemic drug that binds bile acids in the lumen of the gut and enhances their excretion^{8,24}. The effects of

cholestyramine on plasma lipids were studied in mice¹⁴, rats²⁴ and chicks²⁸. Singh²⁶ verified a decrease in egg cholesterol content in laying hens by dietary cholestyramine.

Lovastatin is included in the group of the most potent hypocholesterolemic agents. It acts as competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in the cholesterol biosynthetic pathway^{8,27}. In laying hens, Elkin; Rogler⁵ found reduction in yolk cholesterol concentration by using 0.0059% to 0.0265% dietary lovastatin, with a reported maximum reduction of 15.3% in egg cholesterol level. More recently, Mori¹⁸ reported a decrease of 12.1% in yolk cholesterol by feeding 0.0015% lovastatin to hens. On the other hand, Luhman *et al.*¹⁷ did not report effects on the yolk cholesterol concentration by the addition of 0.0035% lovastatin to laying hen diet.

The objectives of the present study were to evaluate the effects of cholestyramine and lovastatin added to animal products-free diets upon egg quality, laying performance, plasma lipids and egg yolk cholesterol levels of laying hens.

MATERIAL AND METHOD

Birds, Diets and Management

A total of 128 Shaver layers at 26 weeks of age were

distributed into 16 replicates (four cages per replicate with two birds per cage) and randomly assigned to each of the four experimental diets (four replicates per treatment) for a period of 6 weeks (spring and summer). Hens fed basal diet without animal products (Tab. 1) were the control group (CON). This basal diet was supplemented with 0.2% cholestyramine (COL1), 0.3% cholestyramine (COL2) or 0.005% lovastatin (LOV).

Feed and water were provided *ad libitum* and a photoperiod of 16 hours per day was given. Basal diet met all nutrient requirements for laying hens²². During the experiment, egg production and egg weight data were recorded daily and feed consumption weekly. Average egg production, egg weight, feed intake and feed conversion (kilograms of feed consumed per dozen eggs and per kilogram of eggs) were calculated for each replicate group.

Egg Quality

For the evaluation of the shell quality, the specific gravity of 16 eggs per treatment (four per replicate) was determined by the saline solutions method¹². Albumen quality (Haugh units) was evaluated by a S-8400 Ames® micrometer. Egg shells were individually weighed and egg shell thickness was measured by a 25M-5 Ames® micrometer.

Plasma Lipids Analysis

At the end of the experimental period, 5 mL of blood was drawn from the brachial vein from 10 birds per treatment, and collected in heparinized tubes. The birds were bled during the morning period, immediately after oviposition, and each sample was provided by blood from two birds. Plasma was immediately separated by centrifugation for 10 min at 1,400 x g. Plasma triglyceride* and total cholesterol** were determined by enzymatic-colorimetric methods (according to the manufacturer's directions). Plasma samples were processed by an autoanalyzer Technicon® model RA-100.

Sample Preparation and Yolk Cholesterol Analysis

At the termination of the experiment, four eggs were randomly collected from each replicate. Eggs were weighed and hard-cooked by immersion in boiling water for 5 min. Yolks were individually weighed and were prepared by pooling and blending four yolks per sample and then were stored in freezer at -20°C.

About 0.1 g of pooled yolk samples was subjected to direct saponification followed by extraction of the unsaponifiable fraction, according to Hamill; Soliman¹¹. The organic phase was redissolved with ethanol before injection into a HPLC¹⁵. The HPLC apparatus consisted of two Model

* Sera-Pak® kit 6684/6687, Bayer Co., NY.

** Monotest® kit 1 497 456, Boehringer Mannheim Argentina S.A.

*** Shimadzu Co., Kyoto, Japan.

Table 1

Composition of the basal diet (São Paulo, 1999).

Ingredients	Percentage
Corn	58.20
Soybean meal	21.00
Rice bran	5.00
Gluten meal	3.00
Wheat middling	3.00
DL-Methionine	0.07
Choline chloride (50%)	0.03
Salt	0.30
Limestone	7.50
Dicalcium phosphate	1.50
Vitamin premix (*)	0.25
Trace mineral premix (**)	0.25
Calculated composition	
ME, kcal/kg	2,710.00
CP	17.80
Methionine	0.33
Methionine and cystine	0.62
Calcium	3.35
Phosphorus, total (%)	0.62
Phosphorus, available (%)	0.41

(*) Vitamin premix provided the following per kilogram of feed: vitamin A, 10,000 UI; vitamin D3, 1,500 UI; vitamin E, 10 UI; vitamin K3, 2 mg; riboflavin, 4.0 mg; vitamin B12, 0.08 mg; nicotinic acid, 20 mg; pantothenic acid, 10 mg; antioxidant, 65 mg;

(**) Trace mineral premix provided in milligrams per kilogram of feed: Fe, 60 mg; Cu, 4 mg; Zn, 40 mg; Mn, 65 mg; I, 0.8 mg; Co, 0.2 mg; selenium, 0.1 mg.

LC-10AD pumps (Shimadzu®)*** and a Model Class-LC 10 version 1.40 modular system (Shimadzu®) equipped with a Model SPD-10A ultraviolet-visible spectrophotometric detector (Shimadzu®). A 5 mm CLC-ODS (250 X 4,6 mm) Shim-Pack® column was used with a 5 mm LC G-ODS (10 X 4 mm) Shim-pack® guard column. The solvent was an isocratic mixture of acetonitrile and 2-propanol (3:1) and flowed at a rate of 1.0 mL/min. Samples were injected with a 20 mL loop. The ultraviolet detector was set at 215 nm¹⁵.

Statistical Analysis

Statistical analysis was performed using the one-way ANOVA procedure of the SAS Institute²⁵ and Duncan's multiple range test was used to compare treatment means.

RESULTS AND DISCUSSION

Hen Performance

Both levels of cholestyramine (0.2% and 0.3%) used

Table 2

Performance of laying hens fed diets supplemented with cholestyramine and lovastatin (São Paulo, 1995).

Treatments	Egg Weight (g)	Egg production (%)	Egg consumption (g/hen/d)	Feed Conversion	
				Kg of consumed feed per dozen eggs	kg of eggs
Con	53.8 ± 0.2 ^{a*}	88.0 ± 0.8 ^a	87.5 ± 1.2 ^a	1.20 ± 0.02 ^a	1.85 ± 0.03 ^a
Col1	53.1 ± 0.3 ^b	90.4 ± 1.0 ^a	90.7 ± 1.4 ^{ab}	1.21 ± 0.02 ^a	1.90 ± 0.04 ^{ab}
Col2	52.8 ± 0.2 ^b	89.5 ± 1.1 ^a	92.7 ± 1.5 ^b	1.25 ± 0.03 ^a	1.97 ± 0.04 ^b
Lov	52.6 ± 0.2 ^b	90.9 ± 1.1 ^a	89.1 ± 1.5 ^{ab}	1.18 ± 0.03 ^a	1.87 ± 0.04 ^{ab}

* Means ± SEM within columns with no common superscript differ significantly (p < 0.05).

Table 3

Egg quality from hens fed diets supplemented with cholestyramine and lovastatin (São Paulo, 1995).

Treatments	Specific gravity	Shell Weight		Shell Thickness (mm)	Haugh Unit (%)
		(g)	(%)		
Con	1.0893 ± 0.0013 ^{a*}	5.20 ± 0.14 ^a	9.36 ± 0.1	0.380 ± 0.006 ^a	97.5 ± 1.3 ^{ab}
Col1	1.0888 ± 0.0012 ^a	5.01 ± 0.12 ^a	9.30 ± 0.2	0.374 ± 0.007 ^a	96.0 ± 1.2 ^b
Col2	1.0903 ± 0.0012 ^a	5.10 ± 0.11 ^a	9.46 ± 0.1	0.383 ± 0.007 ^a	96.5 ± 1.0 ^{ab}
Lov	1.0910 ± 0.0009 ^a	5.13 ± 0.11 ^a	9.51 ± 0.11 ^a	0.385 ± 0.006 ^a	99.6 ± 1.0 ^a

* Means ± SEM within columns with no common superscript differ significantly (p < 0.05).

Table 4

Plasma triglyceride and total cholesterol concentrations of laying hens fed diets supplemented with cholestyramine and lovastatin (São Paulo, 1995).

Treatments	Triglyceride		Total cholesterol	
	(mg/dl)	Percentage change**	(mg/dL)	Percentage change**
Con	1076 ± 103 ^{a*}	—	86.2 ± 6.7 ^a	—
Col1	1130 ± 110 ^a	+5.1	85.5 ± 6.7 ^a	-0.8
Col2	1057 ± 157 ^a	-1.7	84.6 ± 10.0 ^a	-1.9
Lov	915 ± 98 ^a	-14.9	77.5 ± 5.0 ^a	-10.1

* Means ± SEM within columns with no common superscript differ significantly (p < 0.05);

** Percentage change in comparison to control group (CON).

in this trial caused a significant reduction in egg weight, but there was no alteration in egg production (Tab. 2). As noted in Tab. 2, cholestyramine at 0.2% (COL1) did not affect feed consumption, agreeing with Ueda *et al.*²⁸. However, 0.3% cholestyramine (COL2) resulted in a significant increase in feed intake, resulting, consequently, in an adverse effect on feed conversion expressed as kg of feed consumed per kg of eggs (Tab. 2). Feed conversion, expressed as kg of feed consumed per dozen eggs, was not different among treatments.

The reduction in egg weight (Tab. 2) observed by the supplementation of 0.005% lovastatin (LOV) agrees with Elkin; Rogler⁵, who reported a decline in the egg and yolk weights by 0.0124% and 0.0265% dietary lovastatin.

Table 5

Egg yolk cholesterol content, percentage change in comparison to control groups, yolk weight (g) and egg weight (g). (São Paulo, 1995).

Treatments	Egg yolk cholesterol content				Yolk weight (g)	Egg weight (g)
	(mg/yolk)	Percentage change**	(mg/g)	Percentage change**		
Con	164.2 ± 4.4 ^{a*}	—	12.3 ± 0.2 ^a	—	13.3 ± 0.2 ^a	53.5 ± 0.5 ^a
Col1	184.3 ± 5.9 ^b	+12.2	12.8 ± 0.3 ^a	+4.3	14.4 ± 0.2 ^b	54.7 ± 0.5 ^a
Col2	181.5 ± 1.3 ^b	+10.5	12.9 ± 0.1 ^a	+4.5	14.1 ± 0.1 ^b	54.6 ± 0.3 ^a
Lov	168.1 ± 5.3 ^a	+2.3	12.1 ± 0.4 ^a	-1.8	13.9 ± 0.2 ^b	54.3 ± 0.4 ^a

* Means ± SEM within columns with no common superscript differ significantly (p < 0.05).

** Percentage change in comparison to control group (CON).

However, Luhman *et al.*¹⁷, who used 0.0035% lovastatin in the diet of White Leghorn hens did not observe influence of the drug on egg weights. According to Naber *et al.*²¹, drugs that limit hepatic lipogenesis would not affect egg production due to the great lipogenic capacity of the liver to support lipid synthesis for egg yolk formation.

Egg Quality

Specific gravity, shell weight, shell thickness and albumen quality of eggs obtained from hens fed diets containing cholestyramine or lovastatin did not differ from those laid by hens fed the basal diet (Tab. 3). The birds fed LOV produced eggs with albumen quality (Haugh unit) significantly higher than COL1 (Tab. 3). The absence of undesirable effects on the quality of the egg shell by the addition of lovastatin, agrees with the studies of Luhman *et al.*¹⁷

Plasma Lipids Concentrations

Plasma triglyceride (1,076 mg/dL) and cholesterol concentrations (86.2 mg/dL) from CON (Tab. 4) were similar to those reported by Elkin; Rogler⁵, 1,231 mg/dL and 89.5 mg/dL, respectively, in hens fed control diet. However, Weiss *et al.*³⁰, Luhman *et al.*¹⁷ and Mori¹⁸ reported higher values for hen plasma lipids. Plasma triglyceride and total cholesterol levels were not affected by COL1 and COL2 in hens fed animal products free diets (Tab. 4). Ueda *et al.*²⁸ noted that the addition of 2 to 4% cholestyramine to chicks fed a cholesterol-free diet did not affect serum cholesterol concentration. The absence of effects observed with the use of cholestyramine can be attributed to the compensatory increase in cholesterol synthesis from acetate or mevalonate⁸. According to Naber²⁰ another factor responsible for the inefficiency of cholestyramine would be the fact that bile excretion does not represent a significant amount of cholesterol elimination in this species, where the main excretion route would be represented by the egg.

Lovastatin at 0.005% (LOV) showed a tendency to reduce the average values for triglyceride (14.9%) and total cholesterol (10.1%) relative to CON, but this reduction was not statistically significant. However, Elkin; Rogler⁵ reported significant decreases in plasma lipids of hens fed diets supplemented with 0.029% and 0.241% lovastatin, and Mori¹⁸ found great reduction in triglycerides (38.5%) and cholesterol (36.0%) by the addition of 0.001% lovastatin in the diet. The lack of effect observed in the present experiment, by the addition of 0.005% lovastatin, could be explained mainly by the lower plasma lipid levels of these hens. Probably, the animal products-free diet collaborated to these reduced values¹³. Besides, such differences could be also explained by the age of the birds^{7,17}, and environmental conditions.

Egg Cholesterol Content

The control group presented an average cholesterol concentration of 164.2 mg/yolk, and when it was expressed as milligrams per gram of yolk, 12.3 mg/g of yolk. In spite of the fact that egg cholesterol content is usually expressed as milligrams per yolk, or milligrams per egg, the use of the unit mg/g yolk eliminates the influence of both yolk and egg weight, being therefore more recommended. Thus, the average cholesterol concentration from the CON group approaches that found by Elkin; Rogler⁵, 12.1 mg/g of yolk for the control group, by using the HPLC method. Beyer; Jensen² and Jiang *et al.*¹⁵, also using HPLC, reported average cholesterol values of 11.0 mg/g of yolk and 11.7 mg/g of yolk, respectively, lower than those found in the present work. Luhman *et al.*¹⁷ reported an average cholesterol content of 15.55 mg/g of yolk for the control group using the enzymatic method. Usually published egg cholesterol

data present many divergences, due to different methods used in its determination. Besides, such differences could be due to diet composition^{13,19}, egg production^{4,19}, and age of the birds^{10,17}. The HPLC method is the most recommended to determine egg cholesterol content¹¹.

Hens fed cholestyramine (COL1 and COL2) did not differ from CON in yolk cholesterol concentration, expressed as mg/g of yolk. These findings do not support the observations of Singh²⁶, who observed reduction in the cholesterol content by supplementing with 0.41%, 0.83% and 1.64% cholestyramine in laying hens diet. The inefficacy of cholestyramine in reducing yolk cholesterol can be explained by the capacity of the hens to synthesize cholesterol surplus to the necessary for the deposition in the egg³⁰. Just a small amount of this cholesterol is secreted as bile acid²⁰.

The reduced intestinal bile acid reabsorption decreases the hepatic cholesterol, which, by regulatory feedback mechanism, provides the elevation in endogenous cholesterol⁸. This could determine an increased cholesterol excretion. Thus, it was observed a significant increase in yolk cholesterol from hens fed 0.2% or 0.3% cholestyramine (COL1 and COL3) when it was expressed as mg/yolk, due to the elevation in yolk weight (Tab. 5). Unfortunately, the egg comprises the major pathway for such excretion²³, increasing egg cholesterol content, as observed in the present study. As a result of these factors, bile acid sequestrants are not effective in the reduction of egg cholesterol content.

The supplementation of 0.005% lovastatin (LOV) did not affect yolk cholesterol concentration, either expressed as mg/g of yolk or mg/yolk (Tab. 5). On the other hand, Elkin; Rogler⁵ and Mori¹⁸ reported reductions of 11.6% and 12.1%, respectively, in the cholesterol content of the yolk (mg/g) with the addition of 0.0265% and 0.0015% lovastatin in the diet.

According to Khan *et al.*¹⁶ and Arad *et al.*¹, reduction in liver cholesterol and VLDL cholesterol contents determined by lovastatin, would be just a consequence of a considerable reduction in the esterified cholesterol, without any effect on free cholesterol levels. Thus, the decrease of the yolk cholesterol attained in the present work and in the experiments of Naber *et al.*²¹, Waldroup *et al.*²⁹ and Elkin; Rogler⁵, could probably be attributed only to reductions in esterified cholesterol content. On the other hand, it is known that about 21% of the yolk cholesterol is under the form of esterified cholesterol²³. Therefore, the possibilities of egg cholesterol reduction beyond this limit are, probably, very remote.

Egg cholesterol remained unchanged with the addition of cholesterol and lovastatin, at the dosage and period of this study. The available studies suggest that only small

reductions in yolk cholesterol content are possible. Campaigns advised by scientific and public health communities should be driven towards a better comprehension of the population with regard to the real nutritional value of eggs and the true role of dietary cholesterol in the incidence of the cardiovascular diseases. Eggs, being considered one of the most complete food, can and should be included in the daily diet, as long as they take part of a saturated and unsaturated fat well balanced diet.

ACKNOWLEDGMENTS

The authors are grateful to Bristol-Myers Squibb Brasil S.A. and Merck Sharp & Dohme Química Farmacêutica Ltda. for the generous donation of pharmacological agents. To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing financial support of this study.

RESUMO

O presente trabalho teve por objetivo avaliar os efeitos da colestiramina e da lovastatina, drogas hipolipemizantes, sobre a qualidade do ovo, desempenho das aves, teores de lípidos plasmáticos e de colesterol na gema do ovo de galinhas poedeiras Shaver. Aves com 26 semanas de idade receberam como alimentação dieta formulada sem ingredientes de origem animal (COM) acrescida de colestiramina a 0,2% (COL1) e 0,3% (COL2) e lovastatina a 0,005% (LOV) durante seis semanas. A adição das drogas não prejudicou a qualidade da casca e do albúmen dos ovos. De um modo geral, o desempenho produtivo das aves não foi afetado, com exceção da redução observada no peso médio dos ovos. Não foram observadas mudanças nos teores de lípidos plasmáticos das aves e a concentração de colesterol na gema permaneceu inalterada mediante a adição das drogas.

UNITERMOS: Colesterol; Colestiramina; Lovastatina; Gema de Ovo; Galinhas.

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Received: 23/04/1999

Accepted: 05/08/1999