Effect of Dietary Lipid-Lowering Drugs upon Plasma Lipids and Egg Yolk Cholesterol Levels of Laying Hens

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To evaluate the effect of lipid-lowering agents upon egg quality, reproductive performance, plasma lipids, and egg yolk cholesterol levels, 30-week-old Shaver laying hens were fed a basal diet (commercial ration) supplemented with 0.1% probucol (PROB), 0.025% gemfibrozil (GEMF), or lovastatin at 0.0005% (LOV1), 0.001% (LOV2), or 0.0015% (LOV3) for a 12-week experimental period. It was observed that the supplementation of the drugs did not impair albumen and shell quality. Hen performance was not adversely affected. The depression in triglyceride concentrations approached statistical significance only in LOV2 (38.5%), and total cholesterol was significantly depressed in LOV2 (36.0%), LOV3 (36.8%), PROB (29.6%), and GEMF (30.4%) treatments. Egg cholesterol content, expressed per gram of yolk, was significant lowered in LOV1 (7.5%) and LOV3 (12.7%).

Keywords: *Cholesterol; egg yolk; lipid-lowering drugs; lipids; hens*

INTRODUCTION

The lipid of the yolk is the major nutritional component of the egg, providing a significant source of energy in human diets. 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 disease (Grundy, 1997). In spite of its low proportion of saturated fat (Briz, 1997), the high concentration of cholesterol in the egg yolk has been highlighted in the last decades and has caused restrictions to its consumption.

Countless efforts to modify whole-egg cholesterol content have been accomplished in the past decades through genetic selection and manipulation of dietary components. However, reductions in egg cholesterol levels have unsuccessfully met the demands of health-conscious consumers. Therefore, much attention has been focused on the use of numerous pharmacological agents in an attempt to lower the cholesterol content of eggs (Weiss et al., 1967; Hargis, 1988; Elkin and Rogler, 1990; Naber, 1990; Griffin, 1992).

The hypocholesterolemic effects of probucol were studied in rats, mice, and monkeys by Barnhart et al. (1970). Probucol is indicated for the treatment of isolated hypercholesterolemia in humans for its lack of an effect on triglyceride levels (Grundy, 1990; Bertolami, 1992). Naber et al. (1982) and Waldroup et al. (1986) reported decreases in cholesterol concentrations in the egg yolk by feeding hens diets supplemented with 1.0% probucol.

Lovastatin is included in the group of the most potent hypocholesterolemic agents. It acts as a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in the cholesterol biosynthetic pathway (Tobert, 1982; Grundy, 1990). In laying hens, Elkin and Rogler (1990) found a reduction in the yolk cholesterol concentration by using 0.0059−0.0265% dietary lovastatin, with a reported maximum reduction of 15.3% in egg cholesterol level. On the other hand, Luhman et al. (1990) did not report effects on the yolk cholesterol concentration by the addition of 0.0035% lovastatin to the laying hen diet.

Gemfibrozil is a lipid-regulating agent that is particularly effective in reducing total plasma triglyceride (Todd and Ward, 1988; Bertolami, 1992). Its effects on cholesterol levels are variable, depending on initial levels of plasma triglyceride (Jones, 1996). To our knowledge, the effects of administering gemfibrozil to laying hens has not been reported.

The objectives of the present study were to evaluate the effects of the addition of probucol, gemfibrozil, and lovastatin, lipid-lowering agents, to a commercial-type diet on egg quality, laying performance, plasma lipids, and egg yolk cholesterol levels of laying hens.

MATERIALS AND METHODS

Birds, Diets, and Management. A total of 240, 30-week-old Shaver hens, a commercial white egg strain, were distributed into 30 replicates (four cages per replicate with two birds per cage) and randomly assigned to each of the six experimental diets (five replicates per treatment) for a period of 12 weeks (winter and spring). The control group (CON) was composed of hens fed a commercial corn−soy diet (CP = 18.0%, crude fat = 2.5%, calcium = 4.5%, phosphorus = 0.6%). The basal diet met all nutrient requirements for laying hens (National Research Council, 1994). This basal diet was supplemented with 0.1% probucol (PROB), 0.025% gemfibrozil (GEMF), or lovastatin at 0.0005% (LOV1), 0.001% (LOV2), or 0.0015% (LOV3).

Feed and water were provided ad libitum, and a photoperiod of 16 h per day was given. During the experiment, egg production and egg weight data were recorded daily and feed consumption weekly. The average egg production, egg weight,

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Table 1. Performance of Laying Hens Fed Diets Supplemented with Lipid-Lowering Drugs

treatments	egg weight (g)	egg production (%)	feed consumption (g/hen/d)	feed conversion, kg of consumed feed per	
				dozen eggs	kg of eggs
CON	59.5 ± 0.2[a]	85.9 ± 1.2[a]	121.4 ± 1.2[a]	1.71 ± 0.02[a]	2.40 ± 0.03[a]
PROB	59.1 ± 0.2[a]	87.2 ± 1.1[a]	121.4 ± 1.2[a]	1.68 ± 0.02[a,b]	2.37 ± 0.03[a]
GEMF	60.2 ± 0.1[b]	89.0 ± 0.7[a,b]	118.9 ± 1.0[a]	1.61 ± 0.02[b,c]	2.22 ± 0.02[b]
LOV1	59.1 ± 0.2[a]	91.5 ± 0.7[b]	120.4 ± 0.8[a]	1.58 ± 0.01[c]	2.23 ± 0.02[b]
LOV2	59.5 ± 0.2[a]	89.3 ± 0.5[a,b]	118.8 ± 1.2[a]	1.60 ± 0.02[b,c]	2.24 ± 0.02[b]
LOV3	59.3 ± 0.2[a]	86.9 ± 0.9[a]	134.0 ± 2.5[b]	1.86 ± 0.04[d]	2.61 ± 0.05s[c]

[a−c] Means ± SEM within columns with no common superscript differ significantly ($P < 0.05$) by Tukey test. CON = control; PROB = 0.1% probucol; GEMF = 0.025% gemfibrozil; LOV1 = 0.0005% lovastatin; LOV2 = 0.001% lovastatin; LOV3 = 0.0015% lovastatin.

Table 2. Egg Quality

treatments	specific gravity	shell index (I)	shell thickness (mm)	Haugh unit (%)
CON	1.0848 ± 0.0008[a]	7.52 ± 0.11[a]	0.374 ± 0.005[a]	89.5 ± 1.6[a]
PROB	1.0832 ± 0.0013[a]	7.28 ± 0.13[a]	0.365 ± 0.007[a]	93.5 ± 2.0[a]
GEMF	1.0820 ± 0.0012[a]	7.12 ± 0.18[a]	0.354 ± 0.010[a]	93.4 ± 1.0[a]
LOV1	1.0848 ± 0.0013[a]	7.53 ± 0.14[a]	0.370 ± 0.006[a]	90.5 ± 2.6[a]
LOV2	1.0840 ± 0.0015[a]	7.22 ± 0.19[a]	0.365 ± 0.010[a]	91.6 ± 1.7[a]
LOV3	1.0828 ± 0.0012[a]	7.29 ± 0.09[a]	0.366 ± 0.006[a]	91.6 ± 2.1[a]

[a,b] Means ± SEM within columns with common superscript do not differ significantly ($P < 0.05$) by Tukey test. Shell index (I) = SW/S × 100; where SW = shell weight in grams and S = surface in cm^2. CON = control; PROB = 0.1% probucol; GEMF = 0.025% gemfibrozil; LOV1 = 0.0005% lovastatin; LOV2 = 0.001% lovastatin; LOV3 = 0.0015% lovastatin.

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 evaluation of the shell quality, the specific gravity of 15 eggs per treatment (three per replicate) was determined by the saline solution method (Hamilton, 1982). Albumen quality (Haugh units) was evaluated by a S-8400 micrometer (Ames, Waltham, MA). Egg shells were individually weighed, and egg shell thickness was measured by a 25M-5 micrometer (Ames). Shell index (I) was calculated according to Sauver (1988) using the formula

$$I = SW/S \times 100$$

where SW is the shell weight (g) and S the surface (cm^2); S is calculated from the egg weight (EW) from the equation

$$S = K \cdot EW^{2/3}$$

where K has a value of 4.67, 4.68, or 4.69 depending on egg weight, being less than 60 g, between 60 g and 70 g, or greater than 70 g, respectively.

Plasma Lipids Analysis. At the termination of the experiment, 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 1400*g*. Plasma triglyceride (Sera-Pak kit 6684/6687, Bayer Co., NY) and total cholesterol (Sera-Pak kit 6684/6687, Bayer Co., NY) were determined by enzymatic−colorimetric methods (according to the manufacturer's directions). Plasma samples were processed by an autoanalyzer (Technicon, Saskatchewan, Canada) model RA-100.

Sample Preparation and Yolk Cholesterol Analysis. At the end of the experimental period, 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 prepared by pooling and blending four yolks per sample, and then they were stored in a freezer at −20 °C. About 0.1 g of pooled yolk samples was subjected to direct saponification followed by extraction of the unsaponificable fraction, according to Hamil and Soliman (1994). The organic phase was redissolved with ethanol before injection into a HPLC (Jiang et al., 1991). The HPLC apparatus consisted of two model LC-10AD pumps (Shimadzu, Shimadzu Co., Kyoto, Japan) 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 × 4.6 mm) Shim-Pack column was used with a 5 mm LC G-ODS (10 × 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 nm (Jiang et al., 1991).

Statistical Analysis. Statistical analysis was performed using the one-way ANOVA procedure of the SAS Institute (1985), and Tukey's test was used to compare treatment means. For egg cholesterol data, regression equations and correlation coefficients were calculated according to lovastatin level of the diets.

RESULTS AND DISCUSSION

Hen Performance. Diets containing 0.1% probucol (PROB) had no adverse effects on egg weight, egg production, feed consumption, and feed conversion (Table 1). These observations confirm the reports of Naber et al. (1982) and Waldroup et al. (1986). Egg weight and feed conversion were significantly improved by adding 0.025% gemfibrozil (GEMF) to the diet (Table 1). Egg weights were not influenced by treatments of LOV1, LOV2, and LOV3 during the experimental period (Table 1). These results are in agreement with Luhman et al. (1990), who used 0.0035% lovastatin in the diet of White Leghorn hens for 5 weeks. Feed conversion was statistically improved when lovastatin was administered at 0.0005% (LOV1) and 0.001% (LOV2) in the diet, and egg production was also increased by the former dose (Table 1). However, Elkin and Rogler (1990) reported a decline in the egg and yolk weights by 0.0124% and 0.265% dietary lovastatin, doses which are many times greater than those used in the present experiment. Therefore, only greater doses of drugs which limit the hepatic lipogenesis could have some negative effects on egg production, due to the great lipogenic capacity of the liver to support lipid synthesis for egg yolk formation (Naber et al., 1982).

Egg Quality. Specific gravity, shell index (I), shell thickness, and albumen quality of eggs obtained from hens fed diets containing probucol, gemfibrozil, or lovastatin did not differ from those laid by hens fed the basal diet (Table 2). The lack of effect observed by the addition of 0.1% probucol (PROB) corroborates the

Table 3. Plasma Triglyceride and Total Cholesterol Concentrations of Laying Hens Fed Diets Supplemented with Lipid-Lowering Drugs

	triglyceride		total cholesterol	
treatments	(mg/dL)	percentage change[c]	(mg/dL)	percentage change[c]
CON	2124 ± 236[a,b]		144.6 ± 16.8[a]	
PROB	1606 ± 217[a,b]	−24.4	101.8 ± 10.5[b]	−29.6
GEMF	1584 ± 360[a,b]	−25.4	100.6 ± 23.5[b]	−30.4
LOV1	1664 ± 137[a,b]	−21.7	114.2 ± 7.3[a,b]	−21.0
LOV2	1306 ± 195[b]	−38.5	92.6 ± 8.0[b]	−36.0
LOV3	1401 ± 141[a,b]	−34.0	91.4 ± 9.4[b]	−36.8

[a,b] Means ± SEM within columns with no common superscript differ significantly ($P < 0.05$) by Tukey Test. [c] Percentage change in comparison to control groups (CON). CON = control; PROB = 0.1% probucol; GEMF = 0.025% gemfibrozil; LOV1 = 0.0005% lovastatin; LOV2 = 0.001% lovastatin; LOV3 = 0.0015% lovastatin.

findings of Waldroup et al. (1986). The absence of undesirable effects on the quality of the egg shell by the addition of lovastatin (Table 2) agrees with the studies of Luhman et al. (1990).

Plasma Lipids Concentrations. There was a trend toward the decline (24.4%) in plasma triglyceride by the supplementation of 0.1% probucol (PROB), and the reduction in total cholesterol (29.6%) was statistically significant in comparison with CON (Table 3). There was a trend to reduction in plasma triglyceride concentration (25.4%) by dietary gemfibrozil (GEMF), but this decline was not statistically significant (Table 3). However, Todd and Ward (1988) reported that the main effect of gemfibrozil for human patients with dyslipidemia would be the decrease in plasma triglyceride. The results obtained herein showed a significant decrease (30.4%) in total plasma cholesterol by GEMF (Table 3). Krause and Newton (1985) showed that total plasma cholesterol was reduced by gemfibrozil in mice fed a high-cholesterol diet, but no effect was observed when a normal diet was provided. In laying hens, the effect of gemfibrozil on plasma cholesterol concentration could be due to the very high levels of lipids observed in the mature female during periods of egg formation (Griminger, 1986). The decline in the total cholesterol concentration observed in the present study by GEMF agrees with the results found in patients with severe hypercholesterolemia (Todd and Ward, 1988).

Plasma triglyceride levels were reduced by LOV1 (21.7%), LOV2 (38.5%), and LOV3 (34.0%) treatments, but only the LOV2 treatment was statistically reduced when compared to the CON group. With regard to plasma total cholesterol, significant reductions of 36.0% (LOV2) and 36.8% (LOV3) were observed in relation to CON (Table 3). These changes in percentage were greater than that reported by Elkin and Rogler (1990). The percentages of reduction observed in plasma triglyceride, due to the use of lovastatin, were very close to those verified in total cholesterol concentrations (Table 3). Elkin and Rogler (1990) have also consigned parallelism among those mentioned lipids, without reporting a relationship with the dosage of lovastatin. The average plasma triglyceride obtained in the CON group was 2124 mg/dL (Table 3), higher than values reported by Griminger (1986), 1521 mg/dL, and Elkin and Rogler (1990), 1231 mg/dL, in hens fed control diet. Plasma total cholesterol from CON (144.6 mg/dL) is in agreement with those data recorded by Weiss et al. (1967) and Luhman et al. (1990) but higher than those found by Elkin and Rogler (1990), 89.5 mg/dL. The greater percentages of reduction in plasma lipids verified in the present study could be explained in part by these enhanced values. Such differences could be explained by the age of the birds (Griminger, 1986; Luhman et al., 1990), diet composition (Hargis, 1988), and environmental conditions.

Egg Cholesterol Content. The control group (CON) had an average cholesterol concentration of 227.2 mg/yolk or 13.4 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 milligrams/gram of yolk eliminates the influence of both yolk and egg weight, therefore being highly recommended. This last value (13.4 mg/g of yolk) is higher than that reported by Elkin and Rogler (1990), who reported for the control group an average value of 12.1 mg/g of yolk using the HPLC method. Beyer and Jensen (1989) and Jiang et al. (1990), also using HPLC, reported cholesterol values of 11.0 and 11.7 mg/g of yolk, respectively, lower than those found in the present work. Luhman et al. (1990) 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 have many divergences, due to different methods used in its determination. Besides, such differences, also observed in plasma lipids, could be due to diet composition (Hargis, 1988; Naber, 1990), egg production (Bartov et al., 1971; Cunningham et al. 1974; Naber, 1990) and age of the birds (Turk and Barnet, 1971; Luhman et al., 1990; Hall and McKay, 1994). The HPLC method is recommended the most to determine egg cholesterol content (Hamill and Soliman, 1994).

There was no significant reduction in the yolk cholesterol concentration due to the addition of 0.1% probucol (PROB) in the diet (Table 4). Naber et al. (1982) and Waldroup et al. (1986), using the same dosage reported herein, found reductions of 4.6% and

Table 4. Egg Yolk Cholesterol Content, Percentage Change in Comparison to Control Groups, Yolk Weight (g) and Egg Weight (g)

	egg yolk cholesterol content					
treatments	(mg/yolk)	percentage change[d]	(mg/g)	percentage change[d]	yolk weight (g)	egg weight (g)
CON	227.2 ± 6.1[a]		13.4 ± 0.2[a]		17.0 ± 0.2[a,b]	61.5 ± 0.7[a]
PROB	230.9 ± 4.4[a]	+1.6	13.1 ± 0.3[a]	−2.2	17.6 ± 0.2[a]	62.2 ± 0.6[a]
GEMF	218.7 ± 5.9[a]	−3.7	12.7 ± 0.3[a,b]	−5.2	17.2 ± 0.1[a,b]	62.3 ± 0.2[a]
LOV1	213.0 ± 4.6[a]	−6.3	12.4 ± 0.2[a,b]	−7.5	17.2 ± 0.2[a,b]	60.5 ± 0.2[a]
LOV2	213.6 ± 5.1[a]	−6.0	12.7 ± 0.3[a,b]	−5.2	16.9 ± 0.1[b,c]	60.1 ± 1.0[a]
LOV3	190.7 ± 3.0[a]	−16.1	11.7 ± 0.1[b]	−12.7	16.2 ± 0.1[c]	59.5 ± 0.9[a]

[a−c] Means ± SEM within columns with no common superscript differ significantly ($P < 0.05$) by Tukey Test. [d] Percentage change in comparison to control group (CON). CON = control; PROB = 0.1% probucol; GEMF = 0.025% gemfibrozil; LOV1 = 0.0005% lovastatin; LOV2 = 0.001% lovastatin; LOV3 = 0.0015% lovastatin.

7.2% in the yolk cholesterol, respectively. In the present study, the PROB group showed a yolk weight significantly larger than the control group (CON). This fact makes the nonsignificant decrease (2.2%) in yolk cholesterol (mg/g of yolk) void when it is expressed as milligrams/yolk (Table 4).

Waldroup et al. (1986) reported the possibility of hen adaptation to probucol after 8 weeks, when a significant reduction in the cholesterol concentration in comparison to hens fed the control diet was no longer observed. Such observations could explain the absence of drug effects in this trial, where probucol was used for 12 weeks, the time required to have the birds adapted to the effects of the drug and the consequential return of egg cholesterol to normal levels.

Egg cholesterol content (mg/yolk) from hens fed lovastatin at the three levels (LOV1, LOV2, and LOV3) was significantly reduced in comparison to CON. This reduction was more evident (16.1%) with the addition of 0.0015% lovastatin (LOV3—Table 4). When the cholesterol concentration was expressed as milligrams/gram of yolk, a significant decrease was verified in the treatments LOV1 (7.5%) and LOV3 (12.7%) in relation to CON. However, in the LOV2 treatment, a trend to reduction (5.2%) was observed. The regression analysis clearly showed a significant negative correlation between dietary lovastatin and egg cholesterol content (mg/yolk, $r = -0.666$) or cholesterol concentration (mg/g of yolk, $r = -606$). The regression equations were $y = -21762x + 227.5$ and $y = -919.5x + 13.2$, respectively, indicating that egg cholesterol decreased linearly as dietary lovastatin increased.

Reductions in yolk cholesterol by lovastatin (LOV1, LOV2, and LOV3) agree closely with that previously reported by Elkin and Rogler (1990), who used higher dosages of the drug in 26-week-old hens for 5 weeks. These authors observed a reduction of 11.6% in the cholesterol content of the yolk (mg/g) with the addition of 0.0265% lovastatin in the diet, a value very close to that (12.7%) in the present study with 0.0015% lovastatin (LOV3). When cholesterol content was expressed as milligrams/yolk, the reduction was higher due to the reduced yolk weights studied in the LOV3 treatment, reaching a decrease of 16.1% (Table 4) in cholesterol if compared to CON.

According to Khan et al. (1989) and Arad et al. (1990), reductions in liver cholesterol and VLDL cholesterol contents determined by lovastatin would be 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. (1982), Waldroup et al. (1986), and Elkin and Rogler (1990) can 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 in the form of esterified cholesterol (Noble, 1987). Therefore, the possibilities of egg cholesterol reduction beyond this limit are, probably, very remote.

Griminger (1986) reported that plasma and yolk cholesterol do not change in the same direction, which is in agreement with the effects of 0.1% probucol (PROB), 0.025% gemfibrozil (GEMF), and lovastatin at 0.001% (LOV2). However, lovastatin at 0.0015% (LOV3) caused significant reductions both in the plasma and egg yolk, possibly because lovastatin inhibits cholesterol biosynthesis.

The available studies suggest that only small reductions in yolk cholesterol content are possible.

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ABBREVIATIONS USED

CON = control; PROB = 0.1% probucol; GEMF = 0.025% gemfibrozil; LOV1 = 0.0005% lovastatin; LOV2 = 0.001% lovastatin; LOV3 = 0.0015% lovastatin.

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