Reduction of the Cholesterol Content of Eggs by the Oral Administration of Lovastatin to Laying Hens[†]

Robert G. Elkin* and John C. Rogler

Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907

Two experiments were conducted in which laying hens were administered lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme of the cholesterol biosynthetic pathway. In the first study, 26-week-old White Leghorn hens were fed corn–soybean meal diets containing either 0, 0.0059, 0.0124, or 0.0265% lovastatin (analyzed values) for a period of 35 days. Cholesterol content per gram of yolk was significantly lowered with each successive level of lovastatin, whereas egg cholesterol levels plateaued at approximately 151 mg, a 15% reduction from the basal value. In experiment 2, 44-week-old White Leghorn hens were fed corn–soybean meal diets containing either 0, 0.0290, 0.1198, or 0.2407% lovastatin (analyzed values) for a period of 9 days. In comparison to day 0 values, egg cholesterol contents on day 9 were reduced only at the two highest drug levels, with maximum observed decreases of approximately 8 and 13% when expressed as milligrams of cholesterol per gram of yolk and milligrams of cholesterol per egg, respectively. No drug residues were detected in acetonitrile extracts of eggs from hens fed the highest dietary levels of lovastatin in each experiment.

INTRODUCTION

In laying hens, cholesterol is primarily biosynthesized in the liver (Andrews et al., 1968) and secreted into the plasma in the form of very low density lipoproteins (VLDL) (Burley et al., 1984; George et al., 1987). VLDL are then transported to the ovary where they are bound and taken up by growing chicken oocytes via receptor-mediated endocytosis (Nimpf et al., 1988).

Egg yolk cholesterol levels of domestic fowl are influenced by species (Turk and Barnett, 1971; Bair and Marion, 1978), breed (Cunningham, 1977; Somes et al., 1977), strain (Washburn, 1979), and age (Turk and Barnett, 1971). In addition, a number of studies have shown that an inverse relationship exists between egg yolk cholesterol concentration and both rate of egg production (Bartov et al., 1971; Washburn and Marks, 1977; Washburn, 1979) and egg size (Nichols et al., 1963). Nevertheless, genetic selection programs have resulted in only modest changes in egg cholesterol levels, with a reported maximum reduction of approximately 7% (Hargis, 1988).

Nutritional factors, such as the amount and type of dietary fat and dietary fiber, can affect egg yolk lipid composition (Naber, 1976, 1979, 1983; Hargis, 1988). However, it is extremely difficult to reduce egg cholesterol levels to any great extent by dietary means. Thus, much attention has been focused on the evaluation of numerous pharmacological agents in an attempt to lower the cholesterol content of eggs. Unfortunately, no compound has proven effective in greatly reducing egg yolk cholesterol content without causing either a cessation of egg production or an accumulation of undesirable metabolites in the egg (Naber, 1976, 1979, 1983; Hargis, 1988).

Recent success in the treatment of hypercholesterolemia in humans with a unique class of cholesterol-lowering drugs offers a potentially new approach to the reduction of avian egg yolk cholesterol content. This new class of compounds includes lovastatin, which is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA reductase; mevalonate:NADP+ oxidoreductase (CoA-acylating), EC 1.1.1.34], the rate-limiting enzyme in the cholesterol biosynthetic pathway (Rodwell et al., 1976). In humans with heterozygous familial or polygenic (nonfamilial) hypercholesterolemia, oral lovastatin usually reduces plasma total cholesterol and low density lipoprotein–cholesterol concentrations by 25–40% over a period of several weeks (Henwood and Heel, 1988).

To our knowledge, the effects of orally administering lovastatin to laying hens has not been reported. Therefore, the objectives of the present work were to investigate the effects of lovastatin on egg cholesterol content and various egg production parameters and to determine if lovastatin or any of its metabolites accumulate in the egg.

MATERIALS AND METHODS

Birds, Management, and Diets. *Experiment 1.* A total of 64 26-week-old White Leghorn hens were placed in individual 30 cm × 35 cm × 45 cm slant-back cages in an environmentally controlled room [22 °C, 60% relative humidity, and 16 h of light (10 lx) daily] in the Layer Research Unit of the Baker–Purdue Animal Sciences Poultry Center. Hens were assigned to one of four dietary treatments based on two parameters: (1) egg production during the 14-day period prior to initiation of the study and (2) total egg production prior to initiation of the study. The average numbers of eggs laid 14 days prior to the initiation of experiment 1 were 13.7, 13.7, 13.7, and 13.6 for hens in treatment groups 1–4, respectively. The average total numbers of eggs laid prior to the initiation of experiment 1 were 26.3, 26.0, 27.1, and 26.9 for hens in treatment groups 1–4, respectively. Feed and water were provided ad libitum throughout the experiment.

The dietary amounts of lovastatin employed were based on the hen's cholesterol synthetic rate, rather than on body weight. A laying hen weighing about 1.7 kg synthesizes approximately

Table I. Diet Compositions (Experiment 1)

ingredient	diet			
	1,[e] %	2, %	3, %	4, %
corn	65.15	65.15	65.15	65.15
soybean meal	21.29	21.29	21.29	21.29
alfalfa meal	1.75	1.75	1.75	1.75
dicalcium phosphate	1.50	1.50	1.50	1.50
calcium carbonate	5.38	5.38	5.38	5.38
oyster shell	2.74	2.74	2.74	2.74
sodium chloride	0.45	0.45	0.45	0.45
vitamin and trace mineral mix[a]	0.25	0.25	0.25	0.25
methionine hydroxy analogue (Ca)	0.06	0.06	0.06	0.06
soybean oil	1.00	1.00	1.00	1.00
antioxidant[b]	0.10	0.10	0.10	0.10
mold inhibitor[c]	0.05	0.05	0.05	0.05
Mevacor[d]		0.07	0.14	0.28
cellulose:starch (1:1)	0.28	0.21	0.14	

[a] DI-Egg (Dawe's Laboratories, Chicago, IL). [b] Dry Polyanox (Agrimerica, Inc., Northbrook, IL). [c] Dry Mold-Chek Plus 5762 (Agrimerica). [d] Mevacor (Merck Sharp and Dohme, West Point, PA) contained 9.72% lovastatin by weight. [e] Calculated analysis: crude protein, 16.28%; methionine, 0.34%; cystine, 0.26%; lysine, 0.85%; calcium, 3.51%; phosphorus (total), 0.60%; phosphorus (available), 0.39%; linoleic acid, 1.82%; metabolizable energy, 2870 kcal/kg.

Table II. Diet Compositions (Experiment 2)

ingredient	diet			
	1,[e] %	2, %	3, %	4, %
corn	63.87	63.87	63.87	63.87
soybean meal	20.87	20.87	20.87	20.87
alfalfa meal	1.71	1.71	1.71	1.71
dicalcium phosphate	1.47	1.47	1.47	1.47
calcium carbonate	5.28	5.28	5.28	5.28
oyster shell	2.69	2.69	2.69	2.69
sodium chloride	0.44	0.44	0.44	0.44
vitamin and trace mineral mix[a]	0.24	0.24	0.24	0.24
methionine hydroxy analogue (Ca)	0.06	0.06	0.06	0.06
soybean oil	0.98	0.98	0.98	0.98
antioxidant[b]	0.10	0.10	0.10	0.10
mold inhibitor[c]	0.05	0.05	0.05	0.05
Mevacor[d]		0.28	1.12	2.24
cellulose:starch (1:1)	2.24	1.96	1.12	

[a] DI-Egg (Dawe's Laboratories, Chicago, IL). [b] Dry Polyanox (Agrimerica, Inc., Northbrook, IL). [c] Dry Mold-Chek Plus 5762 (Agrimerica). [d] Mevacor (Merck Sharp and Dohme, West Point, PA) contained 9.68% lovastatin by weight. [e] Calculated analysis: crude protein, 15.96%; methionine, 0.33%; cystine, 0.25%; lysine, 0.83%; calcium, 3.44%; phosphorus (total), 0.59%; phosphorus (available), 0.38%; linoleic acid, 1.78%; metabolizable energy, 2870 kcal/kg.

300 mg of cholesterol/day (Naber, 1983), whereas a 70-kg man synthesizes approximately 800 mg of cholesterol/day (McNamara et al., 1987). Thus, 37.5% (300/800) of the normal daily human dose of 40 mg calculates out to 15 mg of lovastatin per hen per day. Since a 30-week-old laying hen consumes approximately 110 g of feed/day (National Research Council, 1984), then a daily "human equivalent dose" would be 15 mg of lovastatin/110 g of feed, or a dietary level of 0.0136%.

Four replicate groups of four hens each were fed either a corn–soybean meal basal layer ration, or the basal ration supplemented with 0.07, 0.14, or 0.28% ground Mevacor tablets (Table I). Mevacor, which is Merck Sharp and Dohme's trade name for lovastatin, was purchased at the Purdue University Pharmacy, ground in a Thomas-Wiley intermediate mill at 1725 rpm without a screen, and incorporated into diets 2–4 at the expense of a 1:1 mixture of cellulose and starch. Mevacor contained only 9.7% lovastatin by weight, so the calculated dietary levels of the drug were 0.00675, 0.0135, and 0.027% for diets 2–4, respectively.

Individual egg production and egg weight data were obtained. Feed consumption, yolk cholesterol, plasma cholesterol, and plasma triglyceride values were obtained on a dietary replicate basis. Samples of each diet, as well as eggs from both basal-fed hens and birds fed 0.027% lovastatin, were analyzed for lovastatin content. At the conclusion of the 35-day experiment, all hens were fasted for 15 h and individual blood samples were obtained by cardiac puncture using heparinized needles and syringes. Plasma was prepared as previously described (Sell et al., 1980). Following procurement of the blood samples, all hens were fed the basal layer ration until the initiation of experiment 2.

Experiment 2. Approximately 13 weeks following the conclusion of experiment 1, 48 of the 63 remaining hens (now 44 weeks old) were assigned to one of four dietary treatments. Each new treatment group consisted of 12 individuals, 3 from each of the previous four dietary groups. In addition, hens also were assigned to dietary treatments on the basis of 14-day egg production and 7-day egg weights prior to the initiation of experiment 2. The average numbers of eggs laid during a 14-day period prior to the initiation of experiment 2 were 13.2, 13.3, 13.4, and 13.5 for hens in treatment groups 1–4, respectively. The average weights of eggs laid during a 7-day period prior to the initiation of experiment 2 were 59.7, 59.4, 60.3, and 59.7 g for hens in treatment groups 1–4, respectively.

Three replicate groups of four hens each were fed either a corn–soybean meal basal layer ration or the basal ration supplemented with 0.28, 1.12, or 2.24% ground Mevacor tablets (Table II). Mevacor was incorporated into diets 2–4 at the expense of a 1:1 mixture of cellulose and starch. Thus, the calcu-

lated dietary levels of lovastatin were 0.027, 0.108, and 0.216% for diets 2–4, respectively. Feed and water were provided ad libitum throughout the experiment.

Individual egg production and egg weight data were obtained. Feed consumption, yolk cholesterol, plasma cholesterol, and plasma triglyceride values were obtained on a dietary replicate basis. Samples of each diet, as well as eggs from both basal-fed hens and birds fed 0.216% lovastatin, were analyzed for lovastatin content. At the conclusion of the 9-day experiment, all hens were fasted for 15 h and individual blood samples were obtained by cardiac puncture using heparinized needles and syringes. Plasma was prepared as previously described (Sell et al., 1980). Following procurement of the blood samples, all hens were fed the basal layer ration.

Egg Preparation for Cholesterol Analyses. Each week (experiment 1), once during the 9-day study (experiment 2), and at various times after lovastatin had been removed from the feed (both experiments) one egg from each hen was hard-cooked and the yolks were separated, weighed, and crumbled. Exactly 0.25 g of yolk from each of the four hens per dietary replicate was combined (thus 1 g total) and placed into a 50-mL stainless steel homogenizing tube, and 15 mL of chloroform/methanol (2:1 v/v) was added. The tube was attached to a Model OM-115/0 Servall High-Speed Omnimixer (Ivan Sorvall, Norwalk, CT) equipped with a small six-bladed stainless steel knife (2.38-cm diameter). The Omnimixer was connected to a Type 1168 Powerstat variable transformer (Superior Electric Co., Bristol, CT). With the homogenizing tube in an ice bath and the transformer set at 50, the Omnimixer was turned on for 30 s. The contents of the tube were poured into a glass vial, which then was capped and shaken. After standing for 30 min, the vial was reshaken and sonicated for 30 s. The vial was allowed to stand for 10 min, and approximately 9 mL of the yolk homogenate solution was filtered by using a 12-cm³ luer-lock syringe with an attached Millex-HV 25-mm filter unit (0.45-μm pore size; Millipore Corp., Bedford, MA).

Egg and Plasma Cholesterol Analyses. Egg homogenate filtrates or plasma samples were analyzed for cholesterol content by the spectrophotometric method of Rudel and Morris (1973). Briefly, samples were saponified with KOH and ethanol, and cholesterol was extracted with hexane prior to reaction with orthophthalaldehyde. Unlike other reagents used in colorimetric procedures, orthophthalaldehyde specifically reacts with sterols possessing a 3β-hydroxyl group and a Δ^5-double bond (L. L. Rudel, personal communication). The reliability of the Rudel and Morris (1973) procedure for the determination of cholesterol in the hard-cooked egg samples was evaluated by analyzing duplicate samples of whole egg powder (Standard Reference Material 1845) obtained from the Nation-

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Table III. Chromatographic Gradient Conditions for the HPLC Analysis of Lovastatin[a]

time, min	% buffer A (acetonitrile:0.5 mM acetic acid, 35:65)	% buffer B (acetonitrile:0.5 mM acetic acid, 80:20)	gradient
0	100	0	
2	100	0	linear
12	0	100	linear
16	0	100	linear
21	100	0	linear

[a] The flow rate was 1.6 mL/min with the column temperature maintained at 30 °C.

al Institute of Standards and Technology (Gaithersburg, MD). The reliability of the Rudel and Morris (1973) procedure for the determination of cholesterol in plasma was assessed by comparing values from hens in experiment 1 with data obtained by using an enzymatic method (Eastman Kodak Co., 1985) conducted on a Ektachem 700 analyzer (Eastman Kodak Co., Rochester, NY).

Plasma Triglyceride Analyses. Free glycerol corrected total plasma triglyceride concentrations were determined by using a commercial kit (Triglycerides-GB, Boehringer Mannheim Diagnostics, Indianapolis, IN). Briefly, free glycerol was eliminated prior to hydrolysis of plasma triglycerides. Following this preliminary reaction, triglycerides were enzymatically hydrolyzed and the amount of liberated glycerol was indirectly determined by a series of linked enzymatic reactions.

Lovastatin Analyses. *Standard Preparation.* One Mevacor tablet (containing 20 mg of lovastatin) was placed in a 100-mL volumetric flask and diluted to volume with high-performance liquid chromatography (HPLC) grade acetonitrile. The flask was sonicated for 60 min, with the contents being mixed every 15 min. A 2-mL aliquot of the lovastatin solution was diluted 1:1 (v/v) with acetonitrile and filtered by using a 12-cm³ luer-lock syringe with an attached Millex-HV 25 mm filter unit (0.45-μm pore size; Millipore Corp., Bedford, MA). Ten microliters of the filtered solution, which contained 1 μg of lovastatin, was then subjected to HPLC analysis as described later.

Egg Preparation. Uncooked eggs from both basal-fed hens and hens fed the highest dietary levels of lovastatin in each experiment were individually prepared as follows. Each egg was broken into a 250-mL beaker, homogenized by hand with a wisk, transferred into a 125-mL Erlenmeyer flask, and mixed with 50 mL of acetonitrile. Although the eggs immediately coagulated upon the addition of acetonitrile, it was assumed that lovastatin or any of its metabolites (if present) would be extracted because the drug is soluble in acetonitrile (Merck and Co., 1983). The flasks were then sonicated for 60 min, with the contents being mixed every 15 min. The egg extracts were initially filtered with a 15-cm Whatman (Hilsboro, OR) No. 1 filter to remove large particulates. A 4-mL aliquot of each egg extract was then filtered as described above for the lovastatin standard solution. Ten microliters of the filtered extracts was then subjected to HPLC analysis as subsequently described.

Feed Preparation. Samples were obtained from every batch of diets mixed during each experiment. Twenty-five grams of each feed sample was placed into a 100-mL volumetric flask. HPLC grade acetonitrile was then added to the 100-mL mark. The flasks were then mixed, sonicated, and filtered as described previously for the eggs. Ten microliters of each feed extract was then analyzed for lovastatin content by HPLC as described below.

HPLC Equipment and Chromatographic Procedure. The HPLC system (Waters Associates, Milford, MA) consisted of two Model 6000A pumps, a Model 710B automatic sample injector, a Model 720 system controller, a Model 730 data module, a Model 481 variable-wavelength detector (238 nm, 0.2 AUFS), and a Guard-Pak precolumn module containing a μBondapak C18 insert. The analytical column used was a Beckman (Fullerton, CA) 4.6 mm × 250 mm Ultrasphere ODS (5 μm) maintained at 30 °C by using a Bioanalytical Systems (West Lafayette, IN) Model LC 22/23 electronic heater. The chromatographic procedure employed was based on that of Greenspan et al. (1988) and is shown in Table III.

Statistics. Analysis of variance was used to statistically analyze all data (Steel and Torrie, 1980). Individual treatment differences were tested by the Newman–Keuls multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Experiment 1. *Diet Analyses.* The analyzed lovastatin contents of diets 2–4 were 87.4, 91.9, and 98.1%, respectively, of the calculated values (Table IV). With regard to the validity of the HPLC procedure, peak areas were directly proportional to the amount of lovastatin injected over the range 0.01–1 μg (data not shown). The within and between assay coefficients of variation (CV) for 1-μg standard injections were 0.32 ($n = 10$) and 0.94% ($n = 4$), respectively. Recovery of 200 μg of lovastatin added to an extract of diet 1 was 99.2% ($n = 2$).

Hen Performance. Hens fed diets 1–4 gained an average of 74, 129, 97, and 94 g, respectively, during the 35-day experiment. Egg production, expressed as percent hen-day production [(100 × number of eggs laid)/(number of hens × days)], was extremely high, averaging 96.1% across all four diets during the 35-day study (Table IV). Lovastatin did not significantly affect egg production, although two hens fed diet 3 temporarily ceased laying for either 10 or 21 days during the study. This accounted for the high standard deviation (SD) for hen–day production noted in the birds fed 0.0124% lovastatin. Since all of the other hens, including those fed the highest dietary level of lovastatin (0.0265%), laid at least 31 eggs in 35 days, it was concluded that the temporary cessation of egg production in the two birds fed diet 3 was a random event unrelated to the lovastatin treatment. However, egg and yolk weights were significantly reduced by dietary lovastatin, particularly at the two highest drug dosages (Table IV).

Egg Cholesterol Content. Cholesterol content per gram of yolk was significantly lowered with each successive level of lovastatin, whereas egg cholesterol values plateaued at approximately 151 mg, a 15% reduction from the basal value (Table IV). Relative to the new USDA figure of approximately 208 mg/large egg (Holden et al., 1989), the low cholesterol content of the eggs from basal-fed hens (178 mg) was most likely due to their small yolk size (14.8 g; 26% of egg weight) and the hens' extremely high rate of lay (97.9%).

The average cholesterol contents of pooled egg samples obtained 4 weeks after all of the hens had been fed the basal diet (week 9 of experiment 1) were as follows (expressed as both milligrams per gram of yolk and milligrams per egg): 12.1 and 195.3, 11.7 and 189.1, 11.6 and 178.8, and 11.3 and 174.0 for hens originally fed diets 1–4, respectively. This indicated that, in comparison with the 5-week data in Table IV, the increased cholesterol content of eggs from hens originally fed diets 3 and 4 was proportionally greater than that of hens originally fed diets 1 and 2. Nevertheless, there were still differences among the four groups of hens, which suggested the possibility of a carry-over effect of lovastatin. However, since eggs were not analyzed for cholesterol content immediately prior to the initiation of experiment 1, it is not known if there were any group differences prior to the dietary administration of lovastatin.

The respective 9-week egg and yolk weights (in grams) for hens originally fed diets 1–4 were 59.1 and 16.1, 59.5 and 16.1, 57.5 and 15.5, and 57.2 and 15.4. Although group differences were still evident, hens originally fed the two highest dietary amounts of lovastatin exhibited a proportionately greater increase in both egg and yolk weights during the 4 weeks following removal of lovastatin from the feed than did birds originally fed diet 1 or diet 2.

Table IV. Effect of Lovastatin on Laying Hen Performance, Egg Cholesterol (Chol) Content, and Plasma Chol and Triglyceride Concentrations (Experiment 1)[a]

diet	analyzed lovastatin content,[b] %	hen-day production,[c] %	egg weight,[d] g	yolk weight,[e] g	mg of Chol/g of yolk[f]	mg of Chol/egg[f]	g of feed consumed per hen per day[g]	kg of feed consumed/ kg of eggs[g]	kg of feed consumed/ dozen eggs[g]	mg of Chol/100 mL of plasma[h]	mg of triglycerides/ 100 mL of plasma[h]
1	0	97.9 ± 3.0[a]	56.8 ± 4.8[a]	14.8 ± 0.6[a]	12.1 ± 0.9[a]	178.2 ± 14.5[a]	115.1 ± 5.3[a]	2.07 ± 0.06[a]	1.41 ± 0.05[ab]	89.5 ± 6.5[c]	1231.2 ± 105.9[c]
2	0.0059 ± 0.0004	96.1 ± 2.9[a]	56.5 ± 5.0[a]	14.5 ± 0.6[b]	11.8 ± 0.6[b]	170.9 ± 11.0[b]	116.4 ± 5.8[a]	2.15 ± 0.02[a]	1.45 ± 0.06[a]	92.9 ± 25.9[b]	1412.5 ± 476.4[b]
3	0.0124 ± 0.0006	93.8 ± 15.3[a]	53.3 ± 3.4[b]	13.8 ± 0.6[d]	11.1 ± 0.6[c]	153.5 ± 8.4[c]	105.5 ± 5.0[b]	2.12 ± 0.13[a]	1.35 ± 0.08[b]	103.3 ± 17.4[a]	1513.5 ± 341.7[a]
4	0.0265 ± 0.0019	96.6 ± 4.1[a]	54.5 ± 4.2[ab]	14.1 ± 0.6[c]	10.7 ± 0.7[d]	151.1 ± 8.1[c]	108.4 ± 2.9[ab]	2.06 ± 0.02[a]	1.35 ± 0.03[b]	79.1 ± 6.5[d]	1117.2 ± 128.1[d]

[a] The duration of the experiment was 35 days. Data are presented as means ± SD. Mean values within each column with different superscripts are significantly different ($P \leq 0.05$). [b] Mean values of duplicate analyses of three different batches of diets ($n = 6$). [c] Mean values of 16 hens/diet. [d] Mean values of 548, 538, 525, and 541 eggs for diets 1–4, respectively. [e] Mean values of 80 eggs/diet. [f] Mean values of 20 pooled samples per diet analyzed in duplicate ($n = 40$). [g] Mean values of four replicate groups of 4 hens/diet. [h] Mean values of four pooled samples per diet analyzed in duplicate ($n = 8$).

With regard to the validation of the Rudel and Morris (1973) procedure for measuring egg cholesterol content, a linear absorbance response was observed for 10–30 μg of cholesterol. In addition, the within and between assay CV were 2.45 ($n = 216$) and 2.40% ($n = 36$), respectively. Recovery of cholesterol (7.5 mg), added prior to the homogenization step to each of six different yolk samples, averaged 93.4%. Lastly, the National Institute of Standards and Technology Standard Reference Material 1845 (whole egg powder) was found to contain 18.9 ± 0.6 mg of cholesterol/g as compared to the certified concentration of 19.0 ± 0.2 mg cholesterol/g.

Feed Consumption and Efficiency of Feed Utilization. Daily feed consumption was lowest in hens fed the two highest levels of lovastatin (Table IV). There were no significant differences among treatments in feed efficiency expressed as kilograms of feed per kilogram of eggs. However, hens fed diets 3 and 4 required significantly less feed per dozen eggs than hens fed diet 2, which was probably a reflection of their laying smaller eggs.

Plasma Cholesterol and Triglyceride Concentrations. Although there were significant differences due to the level of dietary lovastatin, no discernible trend was evident in the plasma data (Table IV). However, cholesterol and triglyceride values paralleled each other, which suggested a possible effect of lovastatin on circulating lipoproteins, as the lipid portions of LDL and VLDL are composed primarily of cholesterol and triglycerides.

The accuracy of the Rudel and Morris (1973) procedure was assessed by analyzing the same plasma samples on a Kodak Ektachem 700 analyzer. The data obtained by the former method (Table IV) compared very closely with the following values (in milligrams of cholesterol/100 mL of plasma) determined by the Ektachem 700 analyzer: 88.6 ± 7.5 (diet 1); 92.3 ± 23.5 (diet 2); 99.0 ± 12.4 (diet 3); and 76.6 ± 6.4 (diet 4).

HPLC Analyses of Eggs for Possible Lovastatin Residues. Three eggs from hens fed the highest dietary level of lovastatin (0.0265%) were collected on day 35 of the experiment and were individually extracted and analyzed by HPLC for possible drug residues. Three eggs from hens fed the basal diet were also analyzed to provide background chromatograms. Chromatograms of acetonitrile extracts of eggs from hens fed 0% lovastatin were virtually identical with those of hens fed 0.0265% lovastatin. Representative chromatograms of each group appear in parts B and C of Figure 1, respectively. Since the detection limit of the HPLC procedure was approximately 10 ng/injection, it was concluded that if lovastatin or any of its metabolites [see Greenspan et al. (1988)] were transferred to the egg, they were present at concentrations of less than approximately 1 ppm.

Experiment 2. *Diet Analyses.* The analyzed lovastatin contents of diets 2–4 were 107.4, 110.9, and 111.4%, respectively, of the calculated values (Table V).

Hen Performance. Hens fed diets 1–4 gained an average of 13, 15, 6, and 13 g, respectively, during the 9-day experiment. Hen–day production was significantly greater in diets 3 and 4 versus diet 2 and averaged 90.1% across all four treatments (Table V). There was no significant effect of lovastatin on either egg or yolk weights.

Egg Cholesterol Content. Egg cholesterol contents, expressed either on a per gram of yolk or per egg basis, were significantly lowered with each successive dietary level of lovastatin (Table V). However, it was subsequently determined that a difference among treatment groups existed on day 0 (immediately prior to the administration of the experimental diets), as the basal-fed hens laid larger
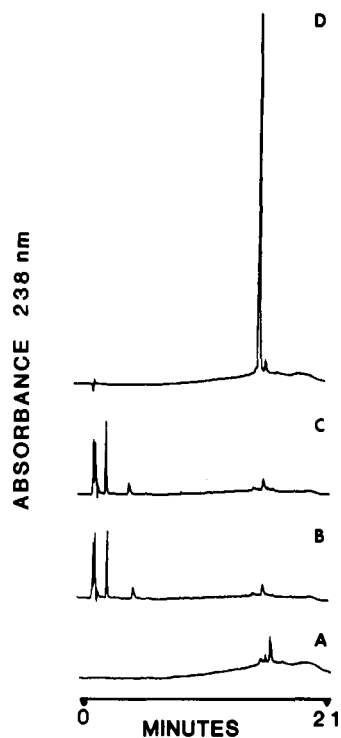
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Figure 1. Reversed-phase HPLC elution profiles: (A) mobile phase (blank) injection; (B) acetonitrile extract of an egg from a hen fed the basal diet (0% lovastatin); (C) acetonitrile extract of an egg from a hen fed the basal diet supplemented with 0.027% lovastatin; (D) lovastatin standard injection (1 μg/10 μL injection volume). The column used was a 4.6 mm × 250 mm Beckman Ultrasphere ODS (5 μm). See Table III for chromatographic conditions.

eggs (Figure 2) with larger yolks (Figure 3), more cholesterol per gram of yolk (Figure 4), and more cholesterol per egg (Figure 5) as compared with the three lovastatin-fed groups. This occurred despite the following facts: (1) Equal numbers of hens from each of the previous dietary treatments in experiment 1 were assigned to new treatment groups in experiment 2. (2) The four treatment groups laid virtually the same number of eggs during the 14-day period immediately prior to the start of experiment 2. (3) The average weights of all eggs laid 7 days prior to the study were similar for each treatment group (see Birds, Management, and Diets, Experiment 2). Thus, when compared with day 0 values, only hens fed diets 3 and 4 actually had reduced yolk and egg cholesterol contents as a result of the lovastatin treatments. Twenty-seven days following withdrawal of the drug from the diet (day 36 of the study), the average cholesterol contents of eggs from all four treatment groups had increased (Figures 4 and 5). However, the increase in cholesterol content of eggs from hens originally fed the two highest levels of lovastatin were proportionately greater than that of hens originally fed either the basal diet (diet 1) or the basal diet supplemented with 0.029% lovastatin (diet 2). In addition, the former two groups laid larger eggs (Figure 2) with almost the same amount of yolk (Figure 3) on day 36 as compared with the birds originally fed diet 1.

Feed Consumption and Efficiency of Feed Utilization. In contrast to the results of experiment 1, there were no significant effects of lovastatin on feed consumption, kilograms of feed consumed per kilogram of eggs, or kilograms of feed consumed per dozen eggs (Table V).

Plasma Cholesterol and Triglyceride Concentrations. Although there were significant differences due to dietary lovastatin level, no dietary trend was evident in the plasma data (Table V). As in experiment 1, cholesterol and tri-

Table V. Effect of Lovastatin on Laying Hen Performance, Egg Cholesterol (Chol) Content, and Plasma Chol and Triglyceride Concentrations (Experiment 2)[a]

diet	analyzed lovastatin content,[b] %	hen-day production,[c] %	egg weight,[d] g	yolk weight,[e] g	mg of Chol/g of yolk[f]	mg of Chol/egg[f]	g of feed consumed per hen per day[g]	kg of feed consumed/ kg of eggs[g]	kg of feed consumed/ dozen eggs[g]	mg of Chol/100 mL of plasma[h]	mg of triglycerides/ 100 mL of plasma[h]
1	0	88.0 ± 8.8[xy]	60.6 ± 4.2[a]	17.9 ± 0.7[a]	12.1 ± 0.7[a]	216.2 ± 15.2[a]	114.2 ± 4.1[a]	2.14 ± 0.05[a]	1.56 ± 0.07[a]	83.3 ± 15.9[a]	1220.4 ± 198.7[b]
2	0.0290 ± 0.0000	85.2 ± 12.8[x]	60.0 ± 4.6[a]	17.3 ± 1.5[a]	11.5 ± 0.3[b]	197.8 ± 13.1[b]	111.7 ± 2.2[a]	2.20 ± 0.18[a]	1.58 ± 0.07[a]	62.3 ± 4.4[b]	909.3 ± 158.7[d]
3	0.1198 ± 0.0001	93.5 ± 11.1[y]	59.4 ± 4.6[a]	16.8 ± 1.0[a]	10.6 ± 0.2[c]	177.4 ± 13.0[c]	115.7 ± 5.2[a]	2.10 ± 0.12[a]	1.49 ± 0.11[a]	83.7 ± 28.9[a]	1353.8 ± 562.7[a]
4	0.2407 ± 0.0007	93.5 ± 5.7[y]	59.3 ± 3.3[a]	17.0 ± 0.6[a]	9.9 ± 0.3[d]	167.7 ± 6.9[d]	113.8 ± 4.7[a]	2.05 ± 0.11[a]	1.46 ± 0.07[a]	60.2 ± 18.2[b]	999.8 ± 327.2[c]

[a] The duration of the experiment was 9 days. Data are presented as means ± SD. Mean values within each column (except for hen-day production) with different superscripts are significantly different ($P \leq 0.05$). Hen-day production values are different at $P \leq 0.06$. [b] Mean values of duplicate analyses of each diet. [c] Mean values of 12 hens/diet. [d] Mean values of 95, 92, 101, and 101 eggs for diets 1–4, respectively. [e] Mean values of 12 eggs/diet. [f] Mean values of three pooled samples per diet analyzed in duplicate ($n = 6$). [g] Mean values of three replicate groups of 4 hens/diet. [h] Mean values of 3 pooled samples per diet analyzed in duplicate ($n = 6$).
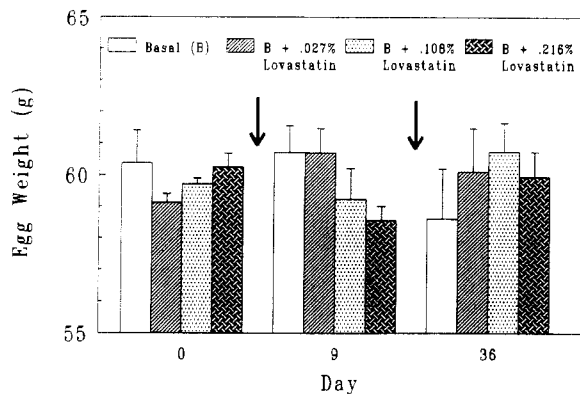
Figure 2. Mean egg weights (with standard errors) of hens fed various (calculated) dietary amounts of lovastatin (experiment 2). Arrows indicate dietary inclusion (day 1) and withdrawal (day 10) of the drug.
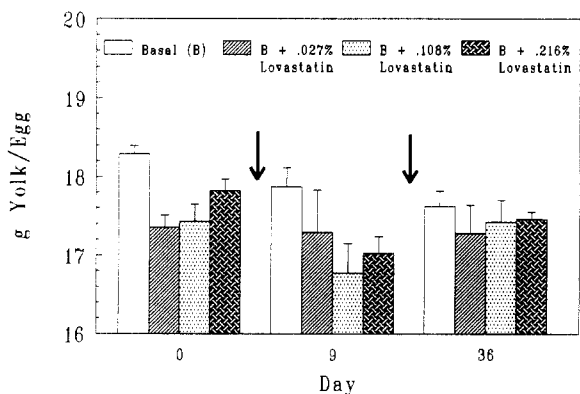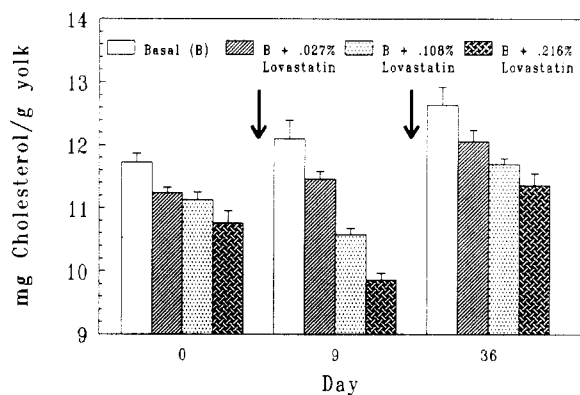

Figure 3. Mean yolk weights (with standard errors) of eggs from hens fed various (calculated) dietary amounts of lovastatin (experiment 2). Arrows indicate dietary inclusion (day 1) and withdrawal (day 10) of the drug.

Figure 4. Mean cholesterol content per gram of yolk (with standard errors) of eggs from hens fed various (calculated) dietary amounts of lovastatin (experiment 2). Arrows indicate dietary inclusion (day 1) and withdrawal (day 10) of the drug.

glyceride values paralleled each other, once again suggesting a possible effect of lovastatin on circulating lipoproteins.

HPLC Analyses of Eggs for Possible Lovastatin Residues. Eight eggs from hens fed the highest dietary level of lovastatin (0.2407%; analyzed value) were collected on day 9 of the study and were individually extracted and analyzed by HPLC for possible drug residues. Four eggs from hens fed the basal diet were also analyzed to provide background chromatograms. As in experiment 1, neither lovastatin nor any suspect metabolites were observed in any of the eggs from the lovastatin-fed hens.
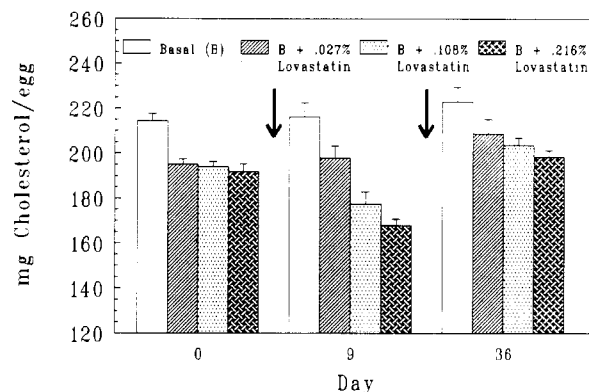

Figure 5. Mean cholesterol content per egg (with standard errors) of eggs from hens fed various (calculated) dietary amounts of lovastatin (experiment 2). Arrows indicate dietary inclusion (day 1) and withdrawal (day 10) of the drug.

SUMMARY AND CONCLUSIONS

The effect of dietary lovastatin on hen performance and egg cholesterol content has not been previously described. However, Endo (1980) reported that another HMG-CoA reductase inhibitor, compactin, administered to laying hens for 4 weeks at a dietary level of 0.06%, reduced egg cholesterol contents by 20%. However, no data were provided in his paper, and the effects of compactin on various parameters such as egg production, egg weights, yolk weights, hen weights, and efficiency of feed utilization were not reported. In addition, compactin has not been developed for human use, most likely because of preclinical toxicity concerns (Walker, 1988), whereas lovastatin was approved for human use in 1987 and has remarkably few adverse effects (Walker, 1988; Henwood and Heel, 1988).

The results of the present study indicated that the cholesterol content of eggs can be reduced by lovastatin without the apparent transfer of the drug to the egg. However, it appeared that the hens became somewhat refractory to lovastatin as they grew older. This was based on the fact that 44-week-old hens in experiment 2 required approximately 19 times more drug (0.2407 versus 0.0124%) than did 26-week-old hens in experiment 1 to lower egg cholesterol contents to approximately 160 mg/egg. However, the requirement for higher drug levels in older hens may have also been a reflection of their laying larger eggs containing a greater percentage of yolk than did younger hens (59 g with 29% yolk by weight versus 54 g with 26% yolk by weight, respectively). In addition, Hargis (1988) suggested that there may be some "critical level" of cholesterol that must be maintained to ensure adequate embryo survival and that, if this level is not reached, egg production would cease. Thus, the cholesterol content of the chicken eggs may be somewhat refractory to pharmacological manipulation. However, the critical level hypothesis remains to be proven (Hargis, 1988).

At the present time, the cost of lovastatin would preclude its use by egg producers. In addition, patent considerations and FDA approval would need to be obtained to use this type of compound. Providing that FDA-approved, less expensive "feed-grade" analogues could be made available to egg producers, HMG-CoA reductase inhibitors hold promise as effective egg cholesterol lowering agents.

Reduction of Egg Cholesterol by Lovastatin

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