EFFECT OF DIETARY LIPIDS ON LIVER, SERUM AND EGG YOLK CHOLESTEROL CONTENTS OF LAYING HENS

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Summary

The effect of dietary lipid factors (plant and animal oil, cholesterol and β-sitosterol) on the liver, serum, and egg yolk cholesterol levels of the laying hen was studied. Single Comb White Leghorn laying hens, at 28 weeks of age, were fed two basal diets containing 8.0% soybean oil or 8.0% fish oil, with or without supplemental cholesterol (1.0%), β-sitosterol (2.0%) or combinations of both. Restricting caloric intake resulted in significantly ($p < .05$) decreased egg production and the total amount of cholesterol excreted via the egg was significantly ($p < .05$) different among treatment groups. Cholesterol supplementation to the two basal diets resulted in a significant elevation of liver, serum and egg yolk cholesterol levels. The addition of β-sitosterol lowered the cholesterol levels in liver and serum, while increased in the egg yolk (SO + ST, FO + ST). The anticholesterogenic effect of dietary β-sitosterol was not clearly exhibited in this study.
(**Key Words**: Dietary Lipids, Cholesterol, β-sitosterol, Laying Hen)

Introduction

The laying hen is a highly dynamic model for studies of cholesterol metabolism. Cholesterol biosynthesis takes place primarilly in the liver where it is regulated by various dietary factors, mainly oils and sterols (Weiss et al., 1964, 1967a,b; Naber, 1983).

Ovarian cholesterol biosynthesis follows a pattern different from that in liver and is not influenced by dietary fat or cholesterol (Naber, 1983), whereas dietary cholesterol is one of the major factors influencing cholesterol level of the egg yolk (Sim and Bragg, 1977). However, the ability of the laying hen to absorb dietary cholesterol is highly dependent upon the nature of dietary oil (March and Biely, 1959; Hullett et al., 1964; Chung et al., 1965).

It is known that as laying birds progress in maturity, cholesterol biosynthesis increases rapidly due to an increased demand for egg formation (Husbands and Brown, 1965) and endogenous origin of cholesterol is more significant than dietary origin in meeting this requirement (Weiss et al., 1967a; Naber, 1983). Thus, it is obvious that almost all tissue and ovarian cholesterol levels are regulated largely by synthetic origin and not by cholesterol absorption of dietary origin to meet the requirement of egg yolk formation in the laying bird (Sim et al., 1984). Sitosterol interferes with intestinal absorption of dietary and enterohepatically circulating cholesterol (Gerson et al., 1965); thus, it lowers cholesterol levels by promoting fecal excretion of sterols and their degradation products (Haust and Beveridge, 1963). This effect of phytosterols is greater when they are incorporated simultaneously with cholesterol into the diet.

The mechanism of the anticholesterolemic action was postulated to involve the formation of a non-absorbable complex of plant sterols and cholesterol at the intestinal absorption site (Davis, 1955; Sim et al., 1980). However, the evidence (Clarenburg et al., 1971) that β-sitosterol lowers egg yolk cholesterol levels when added with or without dietary cholesterol, and is found in the egg yolk, suggests that the absorptive site is not the sole point of plant sterol action. The present study was carried out to investigate the effect

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of dietary lipids and interactions of these dietary factors on the serum, liver and egg yolk cholesterol levels of the laying hen.

Materials and Methods

Fourty-eight Single Comb White Leghorn laying hens (28 weeks of age) were placed in cages (2 hens/cage) equipped with an automatic water system. During a 2-week pre-experimental period, daily egg production was recorded. Two basal diets containing either 8.0% soybean oil (SO) or 8.0% fish oil (FO) were prepared (table 1). These two refined oils were obtained from a market. These basal diets were supplemented with 1.0% of cholesterol (CH), 2.0% of β-sitosterol (ST) or a combination of both (CH + ST). Feed and water were provided *ad libitum* for the entire 3 week experimental period. The chicks received an 17-h light: 7-h dark photoregimen. Blood samples were collected from the heart puncture of individual hen and sera were harvested for the cholesterol determination. Eggs were collected at the intervals of three days and yolks were pooled for the determination of cholesterol content. Livers were carefully removed and weighed. Livers were homogenized in a ultra-turrax blender for 3 min. Feed consumption per 2 hens was recorded weekly with corrections made for feed spillage by measuring the amount of spilled feed in collection pens below the cages.

The egg yolks from each treatment were pooled and analyzed for cholesterol content by the colorimetric assay of Bair and Marion (1978) on the fat extract obtained by a modification of the procedure of Folch et al. (1957). Serum cholesterol was determined by the colorimetric method developed by Zlatkis et al. (1953). Liver cholesterol was determined by extracting 1 g samples twice with acetone-ethanol (1 : 1, v/v) in a ultraturrax blender. After centrifugation of the mixture, the amount of cholesterol in the extract was determined by the same procedure used with serum. Chemical analysis of experiment basal ration was carried out by following A.O.A.C. (1980) procedures. All samples were run in triplicate. A comparison of the cholesterol levels was made to determine if significant differences existed among treatments. Analysis of variance was used for identifying the variation in cholesterol contents among treatments. Means were compared by a multiple range test (Duncan, 1955).

TABLE 1. COMPOSITION (%) OF BASAL RATION

Ingredient	Soybean oil	Fish oil
Ground wheat	70.0	70.0
Soy protein	8.0	8.0
Alfalfa meal	1.0	1.0
Ground limestone	5.0	5.0
Phosphate	2.5	2.5
Salt	0.5	0.5
Vit. & mineral mix[2]	1.0	1.0
Corn starch	4.0	4.0
Soybean oil	8.0	–
Fish oil	–	8.0

[1] Chemical analysis of two basal diet: Crude protein 14.1%, 14.1%; Ether extract 9.7%, 9.3%; Calcium 3.47 %, 2.40%; Phosphorus 0.71%, 0.64%; Cholesterol 0.0084 mg %, 0.0043 mg %.

[2] Vitamin-mineral premix supplies the following per kilo-gram of ration: Vit. A, 6,430 I.U.; Vit. D_3, 1,140 I.U.; Vit. E, 2.3 I.U.; Vit B_1, 1.40 mg; Vit. B_2, 570 mg; Vit. B_6, 0.90 mg; Vit. B_{12}, 2.90 μg; Ca. D. pantothenic acid, 7.10 mg; Niacin, 28.6 mg; Folic acid, 0.15 mg; Vit. C, 1.45 mg; Vit. K_3, 1.45 mg; Choline bitatrate, 14.30 mg; D.L. methionine, 14.30 mg; Mn, 17.0 mg; Zn, 11.5 mg; Fe, 21.0 mg; Cu, 1.40 mg; I, 0.60 mg; Co, 0.60 mg.

Results and Discussion

All response variables for the different treatments are shown in table 2. The difference in metabolizable energy (ME) intake between the basal diets appeared nonsignificant and only the sitosterol treatment have affected significantly on the ME intake between the two dietary lipid groups (SO vs. FO). Caloric intake has been shown to influence cholesterol metabolism in many species (Okey et al., 1960; Ide et al., 1980). The egg hen-day was calculated by dividing the total numbers of egg laid during the experimental period by cumulative hen numbers of one day unit. The basal diet hens had a better overall rate of laying than that of the dietary lipid group hens ($0.699 \pm .067$ EHD vs. $0.576 \pm .008$ EHD); this difference was significant ($p < .05$). Restricting feed and energy intake may be resulted in decreased egg production (Swanson and Bell, 1974; Lowe and Garwood, 1982). Dietary cholesterol produced a significant increase ($p < .05$) in egg cholesterol concentration for

TABLE 2. EFFECT OF DIETARY LIPIDS AND CALORIC INTAKE ON CHOLESTEROL EXCRETED VIA EGG THROUGHOUT THE EXPERIMENTAL PERIOD

Variables	Treat. Diet	Basal		CH		ST		CH+ST	
		SO	FO	SO	FO	SO	FO	SO	FO
ME, kcal/g		3.56	3.54	3.71	3.68	3.76	3.77	3.81	3.83
Feed intake[1]		101.7	112.7	103.2	100.0	100.8	110.3	93.7	104.8
ME intake[2]		362.1^{b}	399.0^{ab}	382.9^{b}	368.0^{b}	379.0^{b}	415.8^{b}	357.0^{b}	401.4^{ab}
Eggs hen-day		0.746^{a}	0.651^{ab}	0.580^{b}	0.579^{b}	0.572^{b}	0.572^{b}	0.587^{b}	0.564^{b}
Yolk cholesterol[3]		16.06^{ab}	17.93^{b}	23.41^{c}	24.33^{c}	15.68^{a}	18.82^{b}	16.58^{ab}	19.27^{b}
Yolk weight, g		15.02	14.02	14.01	13.79	15.14	13.05	15.34	13.98
Chol. excreted[4]		180.0^{ab}	163.6^{b}	190.2^{a}	194.3^{a}	135.8^{c}	140.5^{c}	149.3^{bc}	151.1^{bc}

[1] g/hen/day. [2] kcal/hen/day. [3] mg/g. fresh yolk. [4] mg/hen/day.
[a,b,c] Mean values with the same superscripts in a row are not significantly different at $p < .05$.

cholesterol supplemented groups. Several studies have indicated that cholesterol in the hen's diet increases cholesterol concentration of the egg (Budowski et al., 1961; Wood et al., 1961; Harris and Wilcox, 1963; Sutton et al., 1984). This increase in excretion of cholesterol via the egg probably enables the hen to prevent hypercholesterolemia when ingesting large levels of dietary cholesterol. Thus, variables that were able to change egg production (feed and energy intakes) had little effect on egg cholesterol content.

Supplementary sitosterol (SO + ST) reduced the amount of yolk cholesterol compared to the two basal diets, whereas the FO + ST has higher yolk cholesterol than either of the basal diets. These data are not in agreement with other study where cholesterol lowering effect of soysterol was clearly demonstrated in egg yolk by feeding soysterols alone as well as by feeding soysterols in combination with cholesterol (Sim and Bragg, 1977). These differences were mainly due to changes in egg production and yolk cholesterol content. The correlation coefficients of yolk cholesterol with yolk weight and egg production were negative and nonsignificant. These results are in agreement with other reports (Bartov et al., 1969; Cunningham et al., 1974; Ali, 1977). In the study of Ansah et al. (1985), the correlation coefficients of yolk cholesterol with yolk weight, egg weight, and egg production were inconsistent and nonsignificant until the third generation of selection. Data pertaining to the overall cholesterol concentration of liver, serum, and egg yolk among treatment groups are presented in table 3. Liver cholesterol concentrations were significantly increased ($p < .05$) in cholesterol supplemented diets. Livers from hens fed the diet containing soybean oil was generally heavier with a lower cholesterol contents of liver, serum and yolk than those from hens fed the diet containing fish oil irrespective of dietary cholesterol and/or sitosterol supplementation. The addition of cholesterol to the basal diets produced an increase in liver weights, but this difference was not significant. In terms of liver weight, the heavier livers (FO + ST) had a relatively low liver cholesterol levels and the lighter livers (SO + CH) had a higher cholesterol levels. However, the difference in the liver cholesterol concentrations represented actual changes in the total amount of cholesterol being stored in the liver. These results are in agreement with previous reports that the degree of cholesterol accumulation is closely related to liver weight (Bragg et al., 1973; Sim and Bragg, 1978).

Serum cholesterol levels showed the lowest in hens receiving the soybean oil basal diet (SO); however, the changes in serum cholesterol by supplementation of dietary cholesterol produced a significant increase the level in SO + CH diet group.

The anticholesterogenic effect of dietary β-sitosterol was somewhat demonstrated in FO + ST diet group compared to that of fish oil basal diet (FO); however, this difference was not statistically significant. The reduction in serum cholesterol levels was greater in the hens supplemented with ST than did in those supplemented with CH + ST. Cholesterol lowering effect of β-sitosterol was not clearly demonstrated in both

TABLE 3. EFFECT OF DIETARY LIPIDS ON LIVER WEIGHT AND CHOLESTEROL CONCENTRATIONS OF LIVER, SERUM AND EGG YOLK IN LAYING HENS

Treatment	Liver wt. (g)	Total-cholesterol		
		Liver (mg/g wet tissue)	Serum (mg/dl)	Yolk (mg/g wet tissue)
SO	47.34	2.46^{a}	90.43^{A}	15.18^{a}
FO	38.75	3.79^{ab}	169.76^{B}	18.70^{b}
SO + CH	50.03	12.48^{c}	387.69^{C}	30.72^{d}
FO + CH	56.25	7.06^{b}	188.50^{B}	29.73^{d}
SO + ST	39.42	3.93^{ab}	108.34^{AB}	17.41^{b}
FO + ST	55.29	3.72^{ab}	145.01^{B}	18.43^{b}
SO + CH + ST	41.86	3.84^{ab}	162.08^{B}	17.40^{b}
FO + CH + ST	41.19	4.18^{ab}	172.74^{B}	21.44^{c}

^{A,B,C,a,b,c,d} Means with the same capital and small letter superscripts within a column are not significantly different at $p < .01$ and $p < .05$, respectively.

serum and egg yolk by feeding β-sitosterol alone as well as by feeding β-sitosterol in combination with cholesterol.

Although most of the cholesterol found in the yolk is synthesized in the liver of the hen, transported by the blood in the form of lipoproteins, and deposited in developing follicles, the concentration of plasma cholesterol is not closely associated with the concentration of yolk cholesterol (Constantin and Neague, 1983; Sutton et al., 1984). However, the correlation between serum and yolk cholesterol levels was 0.679. Sim and Bragg (1977) reported that changes in egg yolk cholesterol levels were generally preceded by changes in serum levels. As expected, cholesterol addition to the two basal diets increased the cholesterol levels significantly in egg yolk ($p < .05$). For the combination groups of supplemental CH and ST, the cholesterol level of egg yolk in hens fed SO + CH + ST diet was significantly lower than those of hens fed FO + CH + ST diet ($p < .05$). Weiss et al. (1967b) reported that the addition of 1.0% β-sitosterol to a low-fat basal diet had no effect on the cholesterol except that it retards the rise of cholesterol in blood and egg yolk when the dietary cholesterol was added. The action of plant sterols in cholesterol metabolism has been attributed to the inhibition of cholesterol absorption in the gut because the fecal excretion of sterol metabolites was enhanced when soysterol and cholesterol were fed (Sim et al., 1980).

The weekly pattern of hens responses in egg yolk cholesterol level to the dietary lipid supplementation during the experimental period of 21 days was presented in figure 1. The initial level at day 0 (17.34 ± 0.31 mg/g of egg yolk) was estimated while birds were maintained on a low-fat commerical diet. For the two basal diets, FO diet produced an additional increase in egg yolk cholesterol levels during the entire

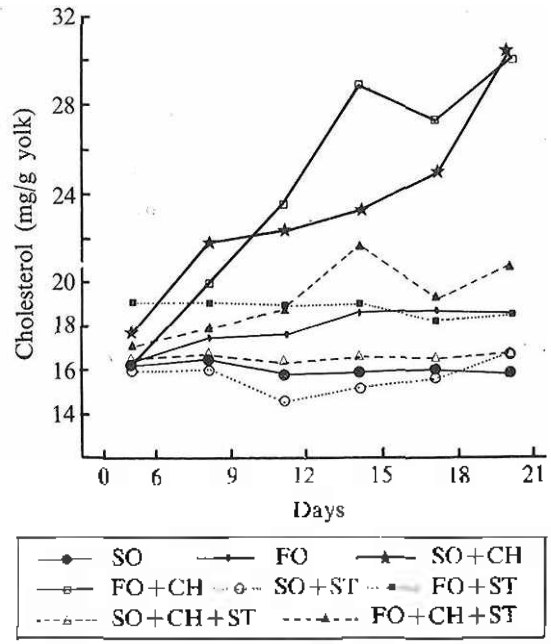

Figure 1. Effect of dietary lipids on the changes in egg yolk cholesterol levels during the experimental period 21 days.

3 week experimental period. The other SO diet appeared to have similar cholesterol levels. Hirata et al. (1986) found that the cholesterol content in egg yolk did not differ between hens fed diets containing soybean oil, coconut oil, lard or beef tallow, although fatty acid composition of egg yolk was markedly influenced by the type of lipid in the diets. The egg yolk level in cholesterol-supplemented diets (SO + CH, FO + CH) had a gradually increasing from the first week of experiment.

Supplementation of cholesterol increased egg cholesterol by as much as 70%. The anticholesterogenic effect of dietary β-sitosterol was not clearly exhibited in the cholesterol-fed diets (SO + CH + ST, FO + CH + ST) as well as the cholesterol free diets (SO + ST, FO + ST). However, Sim and Bragg (1977) found a significant reduction in cholesterol levels in egg yolk (ranging from 16 to 33% depending upon the lipid type or content in the diet) when 2.0% soysterols were added to the diets containing saturated or unsaturated oil with or without cholesterol.

The rise of the cholesterol level was greater in groups fed FO + CH + ST diet than in those fed SO + CH + ST diet. The simultaneous presence of β-sitosterol and cholesterol in the diet did not present the anticholesterogenic effect in this study. This indicates that the simultaneous presence of soysterol and cholesterol in the diet is not mandatory in order to achieve the anticholesterogenic effect in laying hens. Sim and Bragg (1977) has shown that the anticholesterogenic function of plant sterols was not sufficient enough to mask the properties of dietary oils in regulating cholesterol metabolism. Feeding diets to hen containing 0, 1, 2 or 4% β-sitosterol reduced egg cholesterol by as much as 35% (Clarenburg et al., 1971), whereas the addition of β-sitosterol to a diet containing safflower oil increased the cholesterol in the egg yolk, approximately 29% and lowered the cholesterol in blood, 22% (Weiss et al., 1967b).

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