Effect of Calcium and Protein Source on the Plasma Cholesterol of Japanese Quail*

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Eighty adult male Japanese quail were randomly allotted to four dietary treatments consisting of two sources of protein and two levels of calcium in a 2 x 2 factorial designed experiment. Cholesterol was added at a level of 0.5% to all diets. The birds were sacrificed at the end of an eight-week feeding period. Blood was collected and analyzed for plasma total and HDL cholesterol. Plasma total cholesterol increased significantly in all groups, and casein was more hypercholesterolemic than was soy protein. Dietary calcium has a hypocholesterolemic effect; this effect was greater for birds feeding on diets with casein as the protein source. Plasma HDL cholesterol levels did not differ significantly between groups.

INTRODUCTION

Atherosclerotic cardiovascular diseases are the leading cause of death in the United States (1,2), accounting for over half of all deaths annually (1,4). A large body of epidemiological, clinical, and experimental evidence points to a positive correlation between total serum cholesterol, low-density lipoproteins (LDL), and atherosclerosis. A strong negative correlation exists between plasma high-density lipoprotein (HDL) atherosclerosis and mortality. The total cholesterol:HDL ratio is considered the best predictor of coronary heart disease risk within a population, especially for people over age 55 (1).

Animal proteins in the diet have been demonstrated to be more cholesterolemic than vegetable protein in laboratory rabbits, mice, and rats (6-8), but not in chickens, or quail (9,10). Studies with humans have found no change in total serum cholesterol levels with soy or casein as the source of dietary protein (11). Experiments with laboratory rats suggest that dietary calcium may be hypolipidemic (12) but increasing dietary calcium for rabbits results in elevated plasma cholesterol. There is need for additional determinations of the effect of dietary protein source and calcium quantity upon plasma cholesterol.

The Japanese quail is a useful animal model for atherosclerosis research (13), particularly in nutritional (14) and pharmacological (13) studies. This paper reports results of our determination of the effect of two levels of dietary calcium upon the plasma cholesterol of Japanese quail with casein or soy as the source of dietary protein.

MATERIALS AND METHODS

Male Japanese quail (*Coturnix coturnix japonica*) were fed from hatching to eight weeks of age with Ralston Purina Game Bird Startena. At six weeks, demineralized water was given in watering jars in place of tap water. At eight weeks of age twenty birds were sampled to serve as pre-diet controls. The remaining eighty birds were weighed, banded, and randomly assigned to one of four dietary treatments which varied by protein source and calcium level. The quail were allowed food and demineralized water ad libitum throughout the study. After eight weeks on experimental diets blood samples were collected from each quail and saved for analysis.

Quail were housed in metal batteries in a room with controlled temperature (18 °C) and constant light. The top four tiers of a 58 x 37.5-inch chick battery were utilized. Each tier of the battery was divided into four pens with dimensions of 36 x 18.75 x 9.5 inches. Five birds from each diet treatment were randomly assigned to one pen per tier, for a total of four pens per diet

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TABLE 1. Composition of experimental diets (percent).

	Soy + 0.8% Calcium	Soy + 1.6% Calcium	Casein + 0.8% Calcium	Casein + 1.6% Calcium
Cornstarch	57.43	53.51	59.82	55.86
Soy protein	28.43	28.48	0.0	0.0
Casein	0.0	0.0	25.26	25.26
Glista salts[a]	5.37	5.37	5.37	5.37
Polyolefin	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.15	5.62	3.35	8.86
Corn oil	2.0	2.0	2.0	2.0
Calcium carbonate	0.87	1.32	0.50	0.95
Cholesterol	0.50	0.50	0.50	0.50
Vitamin preMix[b]	0.20	0.20	0.20	0.20

[a]Glista salts = (5.6% $CaCO_3$; 52.2% $Ca_3(PO_4)_2$; 16.8% K_2HPO_4; 16.4% NaCl; 6.5% $MgSO_4$; 1.2% $MnSO_4$; 0.9% Fe(III) citrate; 0.2% $ZnCO_3$; 0.04% $CuSO_4$; 0.02% H_3BO_3; 0.02% Na_2MoO_4; 0.07% KI; 0.001% $CoSO_4$; and 0.0004% Na_2SeO_3)
[b]Vitamin preMix = OSU poultry vitamin premix 100

with one diet represented on each level of the battery.

Diet composition is given in Table 1. Diets varied by protein source (soy protein or casein) and calcium content. Diets supplied 24% crude protein regardless of source. The low calcium diets met the nutrient requirement. Calcium was added as calcium carbonate and dicalcium phosphate. All diets had cholesterol added at 0.5%.

Approximately five mL of blood was collected from each animal following decapitation. Blood was collected in 50-mL beakers containing 50 μL of 10% ethylenediaminetetraacetic acid (EDTA), pH 7.4. Samples were mixed by swirling, transferred to glass tubes, and kept on ice until centrifuged. Tubes were centrifuged at 100 x *g* for five min. The plasma was transferred to a plastic vial, capped, and stored at -28 °C until analyzed.

The standard fluorometric method utilizing the Liebermann-Burchard reaction (5) as adapted for serum cholesterol was used to determine total and HDL cholesterol. Total cholesterol was determined directly for aliquots of the plasma. The HDL cholesterol values were determined on aliquots of the plasma supernatant following treatment of the plasma with magnesium phosphotungstate solution and centrifugation to remove all but the HDL particles (3). Cholesterol standards were obtained from Sigma Chemical Co.

Significance of difference between means was determined by analysis of variance.

RESULTS AND DISCUSSION

Weight changes

The mean weight of the control group 1 was 136.0 ± 1.57 g. Pre-treatment weights were 147.6 ± 2.7, 141.3 ± 2.3, 143.6 ± 2.6 and 142.4 ± 2.8 g for diet groups listed in Table 1. Quail fed the low-calcium diets gained an average of 5.0 g when fed the soy protein diets and 8.73 g when fed the casein diets. Birds fed the high-calcium diet showed a slight weight loss. Average weight losses were 4.58 g on soy-high calcium and 2.15 g on casein-high calcium diets.

Plasma total and HDL cholesterol

Results for total plasma cholesterol and HDL cholesterol are summarized in Fig. 1.

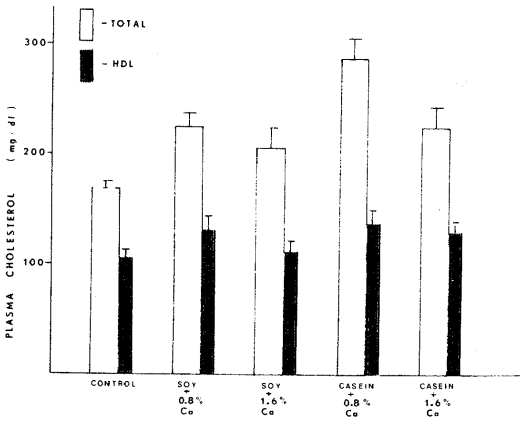

FIGURE 1. Effect of dietary calcium and protein source upon the plasma total and HDL cholesterol of quail. Bars represent the mean + S.E. n = 20.

The control represents the cholesterol level in eight-week-old birds before being placed on experimental diets with added cholesterol. Post-treatment total plasma cholesterol levels were significantly higher ($P < 0.01$) than pre-treatment levels and probably reflect the addition of 0.5% cholesterol to the diet. Similar increases in the plasma cholesterol of quail on high-cholesterol (0.5%) diets were reported by other investigators (13, 14). Plasma HDL cholesterol concentrations (Fig. 1) changed with change in diet and the changes correspond to change observed for the plasma total cholesterol. With HDL cholesterol, however, the difference between means is not significant.

Source of dietary protein and the concentration of calcium in the diet both influenced the plasma total cholesterol. Quail feeding on diets with casein and 0.8% calcium had plasma cholesterol values (296.20 ± 22.34 mg/dL) that are some 30 percent (64.5 mg/dL) higher than the values for quail feeding on diets with soy protein and 0.8% calcium. This difference is significant ($P < 0.05$) and agrees with reports in the literature that suggest that casein is hypercholesterolemic (6-8). Increasing the concentration of dietary calcium from 0.8 to 1.6% caused a significant ($P < 0.05$) lowering of plasma cholesterol with either casein or soy protein has the dietary protein. In fact, the diet with 1.6% dietary calcium was more hypocholesterolemic (21.0% vs 10.0%) when casein is the dietary protein. There is no significant difference between mean plasma cholesterol values for the quail feeding on diets with casein and 1.6 percent calcium and quail feeding on diets with soy protein and 1.6 percent calcium. Any conclusion about the hypo- or hyper-cholesterolemic effect of a single dietary component must be made in consideration of all other dietary components. This study suggests that in experiments that compare effects of diet upon plasma cholesterol, calcium levels must be maintained constant in the diets.

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