Influence of Dietary Fat and Protein on Serum Cholesterol of Cholesterol-Fed Chicks

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Dietary protein has been shown to have a significant effect upon serum cholesterol levels in chicks. A high protein diet tends to protect chickens against hypercholesterolemic and atherogenic effects of cholesterol-fat ingestion (10, 12). When the level of dietary protein is suboptimal (7, 10), serum cholesterol is significantly increased.

There have been a number of indications that the kinds and amounts of dietary fat are in some way related to the quantity of cholesterol in the blood. The feeding of oils containing large amounts of "essential" fatty acids (1, 2, 3) has been found to cause a lowering of elevated serum cholesterol levels produced by the feeding of saturated fats. Under other conditions dietary fats of varying degrees of saturation differed slightly (9) or markedly (6, 8) in their influence on serum cholesterol.

This experiment was conducted to provide more information regarding the effects of dietary fats of varying degrees of saturation, and of protein levels upon serum cholesterol levels in growing chicks.

Experimental

One-hundred-and-two-day-old White Leghorn cockerels were placed on a basal diet (11) for a period of 21 days. During this time the diet consisted of ground barley 28, ground corn 22, ground wheat 10, wheat bran 10, soybean oil meal 10, fish meal 7.5, dried whey 2.5, dried skim milk 2.5, liver meal 2, alfalfa meal 2, ground limestone 1.5, steamed bone meal 1.2, sodium chloride 0.5, fortified fish oil (2,250 A-300 D per gram) 0.25, $MnSO_4 \cdot H_2O$ 0.025, and riboflavin 2 mg. per kilogram of diet. At the conclusion of a preliminary feeding period of 21 days the birds were individually weighed, and blood samples were taken by heart puncture. On the basis of the 21-day weights the cockerels were divided into groups of high, intermediate, and low. These groups were then subdivided into above-average and below-average groups with respect to serum cholesterol levels. One bird was randomly selected from each group for each of the experimental treatments. The design of the experiment was a completely balanced $3 \times 2 \times 2$ factorial.

The 12 treatments consisted of the feeding of three oils (cottonseed, corn, and partially hardened cottonseed) at two levels of fat (4% and 10%) and at two levels of protein (19% and 25%). The three supplemental dietary fats were added to the basal diet at the expense of ground barley. The 25% protein level in the high protein basal diet (II) was obtained by the equal substitution of vitamin-free casein for ground barley. Cholesterol was added to all diets at a level of 0.5%. Inasmuch as the method of adding cholesterol (14) was not critical, it was dissolved in the oil; then both were mixed with the respective basal diets in a Hobart mixer for 20 min.

The two levels of supplemental dietary fat were selected to provide diets which supply the recommended calorie:protein ratio (5) for chicks, or contain an excessive amount of calories in regard to the amount of protein present. In Table I the calorie-protein ratio of the diet is expressed as the number of calories (productive energy) per gram of crude protein. The combination of Basal I (19% protein) and 4% fat approximates a balanced calorie:protein ratio (10.7 to 1) for growing chicks whereas the combination of the same basal diet and 10% fat produces an imbalanced ratio (13.1 to 1) in which there is an excessive amount of calories per unit of protein. With Basal II (25% protein) 10% of fat can be added without producing an imbalance; the ratio is 10.8 to 1.

The respective analytical data for the cottonseed oil, corn oil, and partially hardened cottonseed oil are as follows: iodine values 109.9, 124.8, and 62; linoleic acid 46.9, 54.1, and <1.0; and linolenic acid 0.16, 0.49, and 0.0.

The chicks were housed in an electrically-heated battery brooder for the first four weeks (three-week preliminary and one-week test period), then transferred to growing batteries kept in an air-conditioned room at 76°F. Water and test diets were offered *ad libitum*. Body weights and one-milliliter blood samples (by heart puncture) were obtained after 14, 24, and 38 days on test. The diet combinations and related data are shown in Table I. The serum cholesterol values, obtained by the method of Carr (4) and expressed as milligram percentage were subjected to an analysis of variance. For testing the significance of the experimental treatment effect, the error of variance was found to be 5,876.

Results

The data indicate that the largest differences in serum cholesterol levels were those caused by protein level in the diet. Serum cholesterol values, irrespective of the level or type of fat, were significantly higher in those groups fed Basal I (19% protein) than in those fed Basal II (25% protein). These differences were statistically significant ($P<0.01$). This effect of additional dietary protein on serum cholesterol is in agreement with the results obtained by other workers (10).

The group of chicks fed the diet containing 10% cottonseed oil and 19% protein (Basal I) for 38 days produced an average serum cholesterol level, which was significantly higher than that of any other group, *i.e.*, 639 mg. % (range 500–919). The higher serum cholesterol level of this group as compared with that

TABLE I

Average Serum Cholesterol Levels of Chicks Fed Diets Differing in Kinds and Amounts of Fat and Levels of Protein

	Calories per 100-g. diet	C.P. ratio[b]	Total avg. protein consumed	Total avg. fat consumed	Avg. body wt.	Avg. serum cholesterol Days			
						0	14	24	38
			g.	g.	g.	mg. %	mg. %	mg. %	mg. %
Corn oil[a]									
Basal I (19%) + 4% oil	204	10.7	362	76	917	125	343	450	407
Basal II (25%) + 4% oil	222	8.9	553	88	938	128	330	369	321
Basal I (19%) + 10% oil	249	13.1	337	183	808	123	316	361	390
Basal II (25%) + 10% oil	270	10.8	440	173	999	123	296	298	308
Cottonseed oil[a]									
Basal I (19%) + 4% oil	204	10.7	389	81	952	123	343	371	392
Basal II (25%) + 4% oil	222	8.9	474	75	934	124	317	368	353
Basal I (19%) + 10% oil	249	13.1	310	168	822	122	347	483	639
Basal II (25%) + 10% oil	270	10.8	392	154	883	127	286	327	329
Hydrogenated cottonseed oil[a]									
Basal I (19%) + 4% oil	204	10.7	385	81	857	129	274	457	344
Basal II (25%) + 4% oil	222	8.9	455	72	920	121	330	306	273
Basal I (19%) + 10% oil	249	13.1	353	192	903	124	279	399	382
Basal II (25%) + 10% oil	270	10.8	452	171	939	122	246	312	358

[a] All diets include 0.5% cholesterol. [b] Represents the calculated number of calories (productive energy) provided by the diet for each gram of dietary protein. Each diet was fed to six cockerels.

of the group fed the same basal diet plus 10% corn oil is in agreement with the findings of others (8). However the difference in the effects of corn oil and cottonseed oil on levels of serum cholesterol disappears when the protein level of the diet is increased to give a more nearly optimal calorie:protein ratio.

With the exception of the combination of Basal I (19% protein) and 10% cottonseed oil, there was no significant effect on serum cholesterol when the level of added dietary fat was increased from 4% to 10%. Excluding the above-mentioned diet, the degree of saturation did not have any great effect upon the level of serum cholesterol since the mean differences among the oils and between the fat levels were not statistically significant.

Discussion

The addition of enough protein in the diet to maintain a balanced calorie-protein ratio appears to be an important factor in controlling the elevation in serum cholesterol which results when this ratio is disturbed by the consumption of additional fat. In other tests the inclusion of sufficient dietary protein to provide a reasonable calorie:protein ratio also has prevented an increase in serum cholesterol although fat was fed at high levels in the diet. Only when protein quantity is marginal or low does the amount or type of fat appear to influence serum cholesterol levels. Other workers (15) have reported the importance of maintaining a balanced calorie:protein ratio for the prevention of hypercholesterolemia.

It may be noted that the influence of partially hydrogenated cottonseed oil on serum cholesterol in these experiments does not indicate a relationship between the level of the unsaturated fatty acids and hypercholesterolemia (6). This partially hardened fat with an iodine value of 62 contained approximately 70% oleic acid and less than 1% essential fatty acids. Serum cholesterol levels of birds fed this fat were not significantly different from the corresponding dietary group fed cottonseed or corn oils except for the 19% protein–10% cottonseed oil group which had a definitely higher value. Thus, at a marginal calorie:protein ratio, the increase in saturation decreased rather than increased serum cholesterol. That the partially hydrogenated cottonseed oil was utilized is indicated by the body-weight figures and also by a calorie-utilization value of 86% compared with 95% for the nonhydrogenated cottonseed oil as determined by a method devised for measuring the available energy of foodstuffs (13).

Summary

Cottonseed oil, partially hydrogenated cottonseed oil and corn oil, were fed at 4% and 10% of the diets with two levels of protein, 19% and 25%, and with 0.5% cholesterol to cockerels 21 days of age for a period of 38 days. Blood samples were obtained at 0, 14, 24, and 38 days *via* heart puncture.

The data indicate that the serum cholesterol value, irrespective of the level or type of fat, was significantly lower in those groups of birds which were fed the higher level of protein. Excluding the combination of 19% protein and 10% cottonseed oil, the degree of saturation did not have any apparent effect upon serum cholesterol because the mean differences among the oils and between the fat levels were not statistically significant.

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