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PHYSIOLOGICAL RESPONSES OF COCKERELS TO DIETARY

PROTEIN AND ENERGY RESTRICTION

by

Ronald Joseph Rose

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Major Subject: Poultry Nutrition

Approved:

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INTRODUCTION

Atherosclerosis, a disease of the arteries, has been the leading cause of death in the United States for at least two decades. The understandable limitations surrounding human research have promoted extensive use of experimental animals for atherosclerosis research to obtain a better understanding of the underlying causes and the nature of this serious disease. Medical researchers have found the chicken to be an excellent experimental animal for studying the atherosclerotic process. The review by Katz and Pick (1961) emphasizes this viewpoint.

Most investigators agree with the theory that atherosclerosis is a disease of multiple causation. In fact, so many factors have been implicated as determinants of this disease, it is difficult to pinpoint and stress any one factor without being aware of the influence and possible interactions of other predisposing factors. A list of the most often mentioned "risk factors" or conditions frequently associated with atherosclerosis in humans would include the following: diet, physical inactivity, hypercholesterolemia, hyperbetalipoproteinemia, hypertension, age and sex, ethnic background, obesity, predisposing diseases, psychological factors and genetic factors. The first three items are mentioned as the most important factors in the majority of human population studies.

One of the more popular approaches to the atherosclerosis problem has been the investigation of disturbed lipid metabolism. By dietary manipulation it is possible to induce changes in the distribution of various blood and tissue lipids as well as other important metabolites. Much research has been conducted in attempts to determine the effects of dietary cholesterol and saturated or unsaturated fats on the various blood lipid components and the development of atherosclerotic lesions. In comparison, relatively little is known about the influence of dietary protein or energy intake upon similar parameters. The present study was initiated to determine more clearly the effects of dietary protein or energy restriction, with and without cholesterol, in the growing cockerel. Observations were made on the incidence of atherosclerosis in the thoracic and abdominal segments of the aorta, systolic blood pressure, serum cholesterol, total serum lipids, liver lipids and body weight gains.

REVIEW OF LITERATURE

Atherosclerosis has so many interrelationships and ramifications it would be a monumental task to present a thorough and comprehensive review of literature here. For introductory background information, several excellent reviews are available. A good general review of the overall atherosclerosis problem has been presented by Page (1954). More recently, a volume edited by Sandler and Bourne (1963) presents an up-to-date discussion on atherosclerosis, with emphasis on lesions and their derivation. Poole and French (1961) specifically reviewed the relationship of thrombosis to atherosclerosis, both as a terminal complication and also as a causative process. The 1953 monograph by Katz and Stamler gives a comprehensive review of experimental atherosclerosis, with special reference to investigations utilizing chickens. The importance of avian species as experimental animals in the study of experimental atherosclerosis was further stressed in a later review by Katz and Pick (1961).

Since studies with dietary lipids dominated the earlier investigations, reviews by Jolliffe (1959) and Klein (1960) stress the importance of dietary fats and cholesterol. Jolliffe's review relates the effects of these factors on coronary heart disease, which is often caused by atherosclerosis. Olson and Vester (1960) have presented an excellent discussion on nutrition-endocrine interrelationships in the control of fat transport and possible relevance to atherogenesis.

Many studies have been conducted concerning the metabolism of cholesterol because of its prominent association with the atherosclerosis problem. Representative reports or reviews concerning cholesterol metabolism include the following: Siperstein and Fagan (1958a,b); Portman and Stare (1959); Siperstein and Guest (1960); Nishida _et al._ (1960); Kritchevsky (1960); Bloomfield (1963); Hess (1964); Bloch (1965) and Gould and Swyryd (1966). It should be pointed out that the rat was used as the experimental animal in many of the investigations.

High blood pressure is often designated as one of the "risk factors" associated with atherosclerosis. Nichols (1963) and Speers (1965) have reviewed many of the factors that affect blood pressure in chickens and other experimental animals. In addition, they have reported their findings of the effects of diet on blood pressure of the growing chicken and laying hen, respectively.

Restriction of Food Intake

Although dietary lipid levels and types have received much attention from researchers other nutrients have also been scrutinized. A more general approach has been to restrict the intake of a normal diet by an allotted amount, thus restricting all of the nutrients in the diet. Rodbard _et al._ (1951a) demonstrated with chicks that limitation of dietary intake to two-thirds of that normally eaten did not protect against the development of hypercholesterolemia and atherosclerosis. Even though the restricted chicks received less cholesterol than the birds fed similar diets _ad lib._, the restricted birds initially exhibited higher serum cholesterol levels and had an equal or greater degree of atherosclerosis at the end of the experiment. Chick growth and development were curtailed by feed restriction.

Griminger *et al.* (1963) conducted a moderate food restriction study with male chicks from one day of age to three years of age. One group of birds was fed *ad lib.* while the restricted birds were given an amount of food equal to 80% of that consumed by the full-fed group at a similar body weight. Throughout the three-year period the restricted birds had lighter body weights than the full-fed group. Mortality was about three times greater in the full-fed group as compared to the restricted group for the first six months. Plasma cholesterol and blood pressure did not differ significantly between the two groups. However, abdominal aortas from food-restricted birds showed less atherosclerosis than those from the full-fed birds.

Tissue composition data from this same experiment have been reported by Fisher and Griminger (1963). Even though the restricted birds were smaller, feed restriction did not alter the percentage composition of their carcasses at the end of the three-year period. Cholesterol concentration in the abdominal aortas of the full-fed birds averaged nearly twice that of the restricted birds. The restricted birds exhibited a higher hydroxyproline content (collagen index) in the abdominal aortas, but this difference was apparent only at the six- and 12-month sampling periods.

Nichols and Balloun (1963) have reported on the effects of feed restriction where birds were fed 70% of the amount consumed by a group receiving feed *ad lib.* from one to 16 weeks of age. Weight gains were reduced but blood pressure and serum cholesterol were not affected by this degree of feed restriction.

Another form of feed restriction is to merely withhold feed for a determined period of time. Rodbard et al. (1951b) fed chicks a diet containing 2% cholesterol every other day and compared these chicks with a group fed similarly, except on the alternate days the second group of chicks received a similar diet without added cholesterol. Although cholesterol ingestion for both was approximately the same, the birds receiving no feed on alternate days had higher plasma cholesterol levels and aorta scores.

Cohn et al. (1961) have compared chicks fed ad lib. (nibblers) with those fed only two one-hour periods each day (meal-eaters). They used cockerels that were eight to 10 weeks old in experiments of five to seven weeks' duration. On an atherosclerosis-inducing diet, the "meal-eaters" exhibited twice the serum cholesterol levels and seven times the severity of coronary atherosclerosis as the "nibblers." When regular type diets were fed for studying the regression of experimental atherosclerosis, "meal-eaters" had a slower decline of cholesterol levels to normal and a marked decrease in the rate of healing of the coronary lesions as compared to the "nibblers."

Using a similar meal-eating versus nibbling feeding regimen, Feigenbaum et al. (1962) have obtained body composition data for chickens. Meal-eating resulted in reduced body fat and increased body water while the protein content of the carcass was not altered. These authors mentioned that these effects of meal-eating versus nibbling on body composition are the reverse of those observed in the rat.

Nichols and Balloun (1963) fed cockerels a diet without added cholesterol for two one-hour periods each day. Limited feeding time did not affect serum cholesterol or blood pressure. After an adjustment period, the smaller birds on limited eating were able to adapt and consume a normal amount of food and regain a normal growth rate.

Limiting the feeding time to one three-hour or two one-hour periods per day was studied with the young rat by Okey et al. (1960). Rat weight gains were limited to about the extent that food intakes were limited. Serum lipids and cholesterol were never greatly lowered and, in the case of females fed twice daily, were increased by limiting feeding time. Total liver lipids and, to a greater extent, cholesterol, were especially decreased in older cholesterol-fed animals.

Cohn et al. (1964) have indicated that feeding frequency is a factor in the utilization of dietary protein. They measured urinary excretion of urea N^{15} and the activities of hepatic urea cycle enzymes in rats fed ad lib. and rats pair-force-fed. Their results indicated that there is a limit to the magnitude of the load of dietary protein that an organism can utilize per unit of time period for protein synthetic purposes. When the limit is exceeded, "excess" nitrogen is excreted in the urine as enzymatic reactions adapt themselves to disposing of the non-utilized nitrogen.

Cohn (1964) has recently reviewed the data which suggest that feeding frequency has an influence on experimental atherosclerosis and on serum cholesterol levels of man. Data concerning experimental atherosclerosis for the bird, rat, rabbit, dog and monkey were included.

Restriction of Protein Intake

As was the case with restriction of total food intake, the preponderance of the experimental atherosclerosis studies dealing with protein intake restriction of chickens have originated from just two or three research laboratories. Johnson *et al.* (1958) studied arginine, lysine, methionine and tryptophan deficiencies at different levels of protein intake in relationship to growth rate and plasma cholesterol in the young chick. It was shown that at suboptimal protein intakes, a hypercholesterolemia resulted which could be modified by supplementing the deficient protein with amino acids such that more protein would become available to the bird. Methionine produced a cholesterol-lowering effect which was not related to any improvement in protein quality or growth rate. Although the feeding of 2% cholesterol increased the hypercholesterolemia of birds fed low-protein diets, essentially normal plasma cholesterol levels were observed when the protein deficiency was alleviated.

Stamler *et al.* (1958a) likewise observed that inadequate methionine intake led to increased hypercholesterolemia in chicks fed purified diets containing cholesterol. Such a diet also led to increased coronary and aortic atherosclerosis.

It was shown by Nishida *et al.* (1958) that serum cholesterol, β-lipoprotein levels and incidence of experimental atherosclerosis in chicks were more dependent on dietary protein than on dietary fat or cholesterol. A methionine deficiency at low protein levels significantly elevated the serum cholesterol, β-lipoprotein, total serum lipid and liver cholesterol levels of chicks, indicating that the imbalance of dietary cholesterol, fat and methionine was particularly atherogenic at the low protein level.

Experiments featuring the feeding of cholesterol along with various protein and fat levels were conducted with young male chicks by Leveille and Fisher (1958) and March and Biely (1959). Kokatnur *et al.* (1958)

conducted a similar experiment using 12- to 18-month-old male chickens. In each instance the birds fed low-protein diets had increased serum cholesterol levels which were not affected by the type or level of dietary fat.

Fisher et al. (1959) also noted that low-protein diets were associated with higher levels of plasma and aorta cholesterol in the growing cockerel, irrespective of dietary fat level.

In an investigation with young chicks, Nikkila and Ollila (1958) found that a low-protein diet did not raise serum cholesterol levels significantly unless cholesterol was added to the diet. Then, aortic atheromatosis was also marked. Birds on the low-protein diet had a lowered phospholipid concentration and cholesterol-fed birds on the same diet showed evidence of bilirubin retention, said to be a destructive effect of dietary cholesterol overload.

Stamler et al. (1958b) reported that cholesterolemia and atherogenesis were markedly aggravated in cockerels nine to 14 weeks of age when the protein intake was reduced by sucrose addition to a diet containing fat and cholesterol.

Pick et al. (1959) found that low protein intake intensifies, and adequate protein intake suppresses, hypercholesterolemia and experimental atherosclerosis in mature roosters and hens as well as in young growing cockerels. However, inhibition of coronary atherogenesis induced in by endogenous estrogen secretion continued to supervene despite low protein intake.

In an effort to determine if dietary protein level affects regression of experimental atherosclerosis, Pick and Katz (1959) fed chicks for two weeks diets ranging in protein levels from 10 to 36%. These birds had been previously fed an atherogenic diet for five weeks. At the end of the two-week period mean plasma cholesterol levels showed a stepwise decline proportional to the increasing dietary protein levels. On the other hand, coronary lesions regressed more rapidly on the low-protein diets, whereas thoracic aorta lesions showed no appreciable change.

Leveille et al. (1960) investigated the effects of dietary protein, fat and cholesterol on plasma cholesterol and serum protein components of the growing chick. Feeding a low-protein diet resulted in an elevated plasma cholesterol level and decreased total serum protein and serum albumin levels. The decreased serum albumin level was accompanied by a decreased albumin to globulin ratio. The amount of lipid bound to β-lipoprotein was significantly increased by feeding cholesterol and to an even greater extent by feeding a low-protein and cholesterol combination, regardless of the type of fat. Another report by Leveille and Sauberlich (1961) gives additional details of the influence of dietary protein levels on serum protein components for chicks. Total serum protein increased from 2.33 to 3.06 g per 100 ml for the 10 and 25% dietary protein levels, respectively. The changes in serum proteins were attributed to variations in the albumin level. The level of globulins remained constant at all protein levels fed. Serum cholesterol levels again were decreased as dietary protein level increased. The plasma volume remained constant as a percent of body weight, except for the lowest protein level.

Chaikoff et al. (1961) investigated the effect of dietary protein level on the development of naturally-occurring aortic atherosclerosis in cockerels for the period between four and 10 months of age. Throughout the study the plasma cholesterol levels were the highest for birds fed the low-protein diet. A higher score of non-lipid intimal thickening was observed in the abdominal aortas of birds fed the low-protein diet. However, no difference was noted in the gross size of the atheromata or of microscopic lipid deposition in the thoracic and abdominal portions of the aortas of the birds fed either level of protein.

Nishida et al. (1960) showed that the level of dietary protein influenced the rate of cholesterol-4-C^{14} catabolism in bile duct-cannulated chicks and rats. Animals on a high-protein diet for five weeks excreted cholesterol-4-C^{14} as bile acids at a faster rate than those on a low-protein diet. The presence of dietary cholesterol had no influence on the rate of cholesterol-4-C^{14} catabolism at either high or low dietary protein levels. A species difference in the rate of cholesterol catabolism was also noted. Bile duct-cannulated rats excreted a much greater volume of injected cholesterol-4-C^{14} than did their chick counterparts in a 72-hour period. Serum cholesterol, especially in the form of low Sf β-lipoproteins, appeared to be used rapidly for the conversion of cholesterol to bile acids.

Six-week-old male White Carneau pigeons were used in a nine month study by Prichard et al. (1962) to determine the interactions among dietary fat, protein and cholesterol. In the absence of a cholesterol supplement, serum and aortic cholesterol, total lipids, atherosclerotic index and prevalence of coronary artery atherosclerosis were not affected by the dietary variations which included protein levels of five, 15 and 30%. In cholesterol-fed birds, a lowered incidence of coronary atherosclerosis was demonstrated on the low-protein regimen.

Experiments with both adult and young swine were conducted by Barnes et al. (1959a,b) to evaluate the effect of dietary fat and protein on serum cholesterol. A hypercholesterolemic effect was observed in large sows (300-500 lbs) only when the protein content of the diet was reduced below 5%. Eight-week-old male weanling pigs fed a low-protein diet for 36 weeks also exhibited increased serum cholesterol levels. The cholesterol values reached a peak between the fourth and eighth week and then declined slowly towards the levels found in adult swine. The low-protein, high-fat group did not return toward the minimal level as rapidly as the other groups.

More recently, Greer et al. (1966) reported on three swine trials each of which involved 45 pigs. Initial average weights and ages were 32.7, 34.7 and 35.0 Kg and 85, 101 and 98 days, respectively. The three trials were for 140, 257 and 96 days, respectively. These experiments were conducted to study the effects of high and low levels of energy intake from two fat sources, protein level and the addition of 1% cholesterol to the above fat sources on serum cholesterol levels and the incidence of atherosclerosis in the thoracic and abdominal arteries and coronary arteries. Pigs fed the diets containing 12% protein had slightly elevated serum cholesterol levels as opposed to those fed the 18% protein diets. The effect of protein level on the incidence of lesions was inconsistent.

Seidel et al. (1960) studied the interrelationships among the effects of dietary protein, sulfur-containing amino acids and choline on rat serum cholesterol concentration. Their data suggested that the serum cholesterol-lowering effect of protein supplements was due largely to the sulfur amino acids provided.

By increasing the protein content of the diet the thrombotic syndrome induced by hyperlipemic diets was prevented in rat experiments by Renaud and Allard (1964). Kim and Lee (1966), using rats, found that thrombosis and infarcts produced by diets containing thiouracil, cholesterol, bile salts and 40% butter could also be obtained without thiouracil if dietary protein was reduced to low levels.

Engen and Swenson (1966) measured blood pressure of rats fed purified atherogenic diets deficient in protein, vitamin B_{12} and choline. Although interactions prevented statistical analysis, numerical blood pressure readings were higher for male and female rats on a low-protein diet than they were on a high-protein diet.

Contrary to the findings with experimental animals, Furman et al. (1958) showed that, in humans, serum cholesterol levels are lowered when dietary protein intake is decreased. Replacement of sufficient dietary protein to establish nitrogen equilibrium increases serum cholesterol and phospholipid levels to normal.

Restriction of Energy Intake

Not many investigations have been designed specifically to test the effects of energy restriction on serum cholesterol levels or the incidence of atherosclerosis. Some caloric restriction is no doubt achieved in studies where animals are fed 70 to 80% of the amount of feed consumed by a control group. However, the determination of any caloric effect would be complicated by the effect of the other restricted nutrients as well.

Although a great number of studies have included various levels of dietary fat, many of these utilized isocaloric diets or else no mention is made of actual caloric intakes. When studies of different fat levels are conducted in such a manner that variable caloric intake are obtained, interpretation of the results is difficult because Bloomfield (1963) and others have shown that fat is involved with cholesterol absorption. Bloomfield also has found with rat balance studies that total animal sterol synthesis was proportional to caloric intake. He defines a cholesterol-accumulating or atherogenic diet as one which is high in calories, well refined and low in fecal residue. Such a diet would contain a high fat content to provide plentiful calories with minimal residue and optimum conditions for absorption and reabsorption.

Dupont and Lewis (1963) reported a rat study where cholesterol synthesis was enhanced and noncholesterol lipid depressed by higher amounts of dietary fat. In a recent study Dupont (1966) noted a linear relationship between the rate of oxidation of total dietary lipid and acetate incorporation into cholesterol in male and female rats.

Most reducing diets for humans contain lower caloric levels. Jolliffe et al. (1962) reported that human serum cholesterol levels were lowered and maintained at the lower levels by the use of "the prudent reducing diet."

EXPERIMENTAL PROCEDURE

Management

Commercial broiler-strain, vent-sexed, male chickens obtained from a commercial hatchery were used for all experiments. The day-old chicks were reared to four weeks of age in wire-floored, electrically-heated battery brooders and then transferred to non-heated, intermediate or finisher grower batteries until the birds were put on experiment. Both types of batteries were located in a building equipped with supplemental heating units. Water and a standard chick starter ration were available _ad lib._ until experiments were started at which time feed was administered according to ration formulation as will be discussed later.

Extremely heavy or light birds were not used for the experiments. Selected birds were wing-banded and for the first three experiments were caged individually in a triple-decked 24-cage unit. Individual feeders were provided and each waterer was shared by two birds in adjoining cages. For the fourth experiment, birds were caged in triple-decked individual units located in two environmental chambers where temperatures were maintained at $70^{o} \pm 5^{o}$ F. Individual feeders and waterers were used.

Feeding

Experimental diets were formulated and fed in such a manner that it was not necessary to include large amounts of inert filler material to obtain the desired energy and protein restrictions. Practical corn-soybean diets were fed in experiment I while in experiments II, III and IV semi-purified diets based on glucose or sucrose and isolated soybean protein were fed. These diets are presented in Tables 1, 2 and 3.

Table 1. Diet composition for experiment I

Ingredient	Diet (%) 1 & 2	3 & 4	5 & 6
Corn, grd. yellow	71.5	42.0	80.0
Soybean meal (50%)	18.0	23.0	6.5
Fish solubles, cond.	2.0	2.0	2.0
Alfalfa meal, dehy. (20%)	2.0	2.0	2.0
Dicalcium phosphate	2.0	2.0	2.0
Oystershell flour	1.0	1.0	1.0
Vitamin mix, C-20[a]	1.0	1.0	1.0
Salt mix[b]	0.5	0.5	0.5
Cellulose or cholesterol[c]	2.0	2.0	2.0
Total	100.0	75.5	97.0
Calculated to provide:[d,e]			
Protein (%)	17.1	17.0	12.0
Energy (M.E. Cal/lb)	1347	943	1352

[a]Vitamin mix provides per kg of diet: Vitamin A, 7,000 IU; Vitamin D_3, 1,000 ICU; Vitamin E, 10 IU; Vitamin K, 1 mg; Vitamin B_{12}, 10 mcg; Riboflavin, 5 mg; Pantothenic acid, 10 mg; Niacin, 25 mg; Choline, 450 mg; Santoquin, 0.0125%; Penicillin, 6.6 mg; Streptomycin, 33 mg.

[b]Salt mix provides per kg of diet: NaCl, 0.425%; Mn, 125 mg; Zn, 3.3 mg; Fe, 50 mg; Cu, 5 mg.

[c]Two percent cholesterol replaced cellulose in diets 2, 4 and 6.

[d]Calculated from values in Titus (1961).

[e]When mixed on a 100 lb basis with diets 3 and 4 and diets 5 and 6 being fed 75.5 and 97.0%, respectively, by weight, of the amount fed to birds receiving diets 1 and 2.

Table 2. Diet composition for experiment II

Ingredient	Diet (%)			
	1	2	3	4
Glucose	56.22	61.47	66.47	71.72
Isolated soybean protein	27.00	21.75	16.75	11.50
Soybean oil	5.00	5.00	5.00	5.00
Cellulose	3.00	3.00	3.00	3.00
Cholesterol	2.00	2.00	2.00	2.00
Mineral mix[a]	4.78	4.78	4.78	4.78
Vitamin mix[b]	1.00	1.00	1.00	1.00
Methionine	0.67	0.67	0.67	0.67
Glycine	0.33	0.33	0.33	0.33
Total	100.00	100.00	100.00	100.00
Calculated to provide:[c]				
Protein (%)	22.1	18.0	14.1	10.0
Energy (M.E. Cal/lb)	1608	1604	1600	1595

[a]Mineral mix provides per kg of diet: Ca, 1.09%; P, 0.6%; NaCl, 0.425%; K, 0.2%; Mn, 125 mg; Zn, 51 mg; Fe, 51 mg; Cu, 5 mg; Mg, 660 mg.

[b]Vitamin mix provides per kg of diet: Vitamin A, 7920 IU; Vitamin D_3, 594 ICU; Vitamin E, 31 IU; Vitamin K, 2.2 mg; Thiamine, 6.6 mg; Riboflavin, 4.4 mg; Pantothenic acid, 13.9 mg; Niacin, 39.6 mg; Pyridoxine, 4.4 mg; Inositol, 1.1 gm; Biotin, 0.13 mg; Choline chloride, 1.87 gm; Folic acid, 0.88 mg; P-amino-benzoic-acid, 110 mg; Vitamin B_{12}, 13 mcg; Santoquin, 0.0125%.

[c]Calculated from values in Titus (1961).

Table 3. Diet composition for experiments III and IV

Ingredient	Experiment III 1 & 2	Experiment III 3 & 4	Experiment IV 1 & 2	Experiment IV 3 & 4	Experiment IV 5 & 6
	Diet (%)				
Glucose	72.72	47.72	---	---	---
Sucrose	---	---	50.44	25.44	58.44
Isolated soybean protein	11.50	11.50	20.00	20.00	12.50
Soybean oil	5.00	5.00	5.00	5.00	5.00
Cellulose[a]	4.00	4.00	4.00	4.00	4.00
Mineral mix[b]	4.78	4.78	4.56	4.56	4.56
Vitamin mix[c]	1.00	1.00	1.00	1.00	1.00
Methionine	0.67	0.67	0.67	0.67	0.67
Glycine	0.33	0.33	0.33	0.33	0.33
Total	100.00	75.00	86.00	61.00	86.50
Calculated to provide:[d,e]					
Protein (%)	9.97	9.97	17.00	17.00	11.00
Energy (M.E. Cal/lb)	1611	1198	1400	980	1400

[a]Cholesterol replaced 1% cellulose in diets 1 and 3 in experiment III, and in diets 2, 4 and 6 in experiment IV.

[b]Mineral mix provides per kg of diet - - same as listed for experiment II.

[c]Vitamin mix provides per kg of diet - - same as listed for experiment II.

[d]Calculated from values by Matterson et al. (1965).

[e]When mixed on a 100 lb basis and then fed at the appropriate level, the non-sugar components are equalized and the above calculated values are obtained.

For experiment II, the isocaloric diets were fed ad lib. However, for experiments I and III, the birds receiving the restricted diets were fed an amount of feed determined by the quantity of feed consumed by the basal birds, as a percentage of body weight, and by the subsequent use of the appropriate formulation totals. In experiment IV it was desirable to keep soybean oil and cellulose levels similar to those of previous experiments and still obtain the desired caloric content for the basal diet. This necessitated feeding all the birds a scheduled amount of feed as a percentage of body weight according to a schedule worked out from feed consumption data for similar-aged birds in three previous experiments.

Feeding schedules were calculated for each bird weekly and each bird's daily feed allotment was weighed-out individually for experiments I, II and IV. Body weights and feed weighbacks were taken weekly for all experiments.

Chemical Determinations

Blood samples

Birds were fasted 12 hours before blood samples were taken from a pricked brachial wing vein. About 10-12 ml of blood were collected into a centrifuge tube, centrifuged and the serum frozen and stored in vials until the chemical determinations could be made.

Serum cholesterol

Total serum cholesterol was measured with a Technicon Autoanalyzer by the method described by Technicon Instruments Corp. (1964). This quantitative procedure is based on the reaction of concentrated sulfuric acid and ferric chloride in acetic acid with steroids having the 5-ene, 3-beta-ol grouping.

Serum lipids

A simple turbidimetric method described by Huerga et al. (1953) was used for the determination of total serum lipids. Lipids were extracted with Bloor's mixture and p-dioxan and then were emulsified with a sulfuric acid solution. Readings were taken with a Spectronic-20 colorimeter.

Liver lipids

Livers were placed in a drying oven at $100^{o}F$ for 12 hours and then dried under 28 inches Hg vacuum at $100^{o}F$ for four more hours. Duplicate dried liver samples were extracted with a solvent mixture containing ethyl alcohol, ethyl ether and Skelly B (5:5:1) on the Goldfisch extraction apparatus.

Aorta Tissue Scores

Aortas were dissected from the point where the aorta enters the heart to just below the iliac bifurcation, stripped of adhering tissues and separated into thoracic and abdominal segments. They were then subjected to a modification of the fixing and staining procedure of Geer et al. (1958). This procedure consisted of fixing for 24 hours in a 10% neutral formalin solution, staining for 15 minutes with a saturated solution of Sudan IV in equal parts of acetone and 70% alcohol, differentiating for 20 minutes in 80% ethanol and washing in cold tap water for one hour.

Each segment was then visually scored 1 through 4 by three individuals according to the amount of lipid stain and severity of lesions present. A score of 1 represented very little or none, whereas a score of 4 indicated the most intense lipid staining and/or severe lesions.

Blood Pressure

Systolic blood pressure was measured indirectly by a method described by Nichols (1963) and Speers (1965). Basically, a specially designed condenser microphone picks up the pressure pulse and transmits it electrically to a battery-powered signal-receiving apparatus which transmits the signal output to an oscilloscope. The appearance and disappearance of the pulse wave can be seen on the oscilliscope screen and is monitored by an aneroid manometer which measures the pressure in mm Hg. The pulse pick-up and small modified pressure cuff are attached to the restrained bird over the inside of the shank and just above the hock. An average blood pressure value was obtained from three consecutive readings which were taken in rapid sequence on each bird.

Organ Weights

Testes and gall bladders were removed when birds were sacrificed at the end of experiments III and IV. These organs were frozen and stored until it was convenient to weigh them at a later date. Weights were taken on a wet-weight basis after removal of adhering tissues.

Statistical Analysis

A completely randomized design was used in experiments I, II, and III, whereas a randomized block design was used for experiment IV. The Iowa State Computation Center conducted the initial analyses for experiments I, II, and III.

A least-squares analysis of variance, designed for data with unequal subclass numbers by Harvey (1960), was used for the first three experiments. Comparisons among the treatments was subsequently made when needed, using inverse components for the multiple range test as outlined by Harvey.

A general analysis of variance was used on the data from experiment IV, followed by Duncan's multiple range test where appropriate. Orthogonal comparisons were made to detect linear or quadratic effects for the experimental periods. Orthogonal coefficients for unequal intervals were calculated according to Grandage (1958).

RESULTS

Experiment I

The influence of restricted energy or protein on various physiological responses of cockerels fed practical diets with and without added cholesterol is shown by the data in Table 4. The birds were nine weeks of age at the beginning of the 12-week experiment. Body weight gains were greatly reduced when protein or energy intake was restricted to approximately 70% of that in the basal diet.

Lower systolic blood pressures were obtained from birds fed any of the nutrient-restricted diets, however, differences were significant only for energy-restricted birds.

Total serum cholesterol values were increased whenever cholesterol was added to the diet. Restricted energy intake resulted in lowered ($P < 0.01$) serum cholesterol values whereas restricted protein intake increased ($P < 0.01$) serum cholesterol. Similar, but less pronounced and nonsignificant effects were apparent when cholesterol was not added. The magnitude of these differences can be seen in Figure 1.

Total liver lipids were markedly increased when any of the diets were supplemented with cholesterol. A comparison with the basal group shows that restricting protein intake increased liver lipids 37%, whether or not the diet contained added cholesterol. When restricted energy intake diets are compared with the basal diet liver lipid changes are quite small and fluctuate according to whether or not the diet contained added cholesterol. Reduced energy intake decreased liver lipids 8.7% when cholesterol was added, but increased lipids 9.3% without added cholesterol.

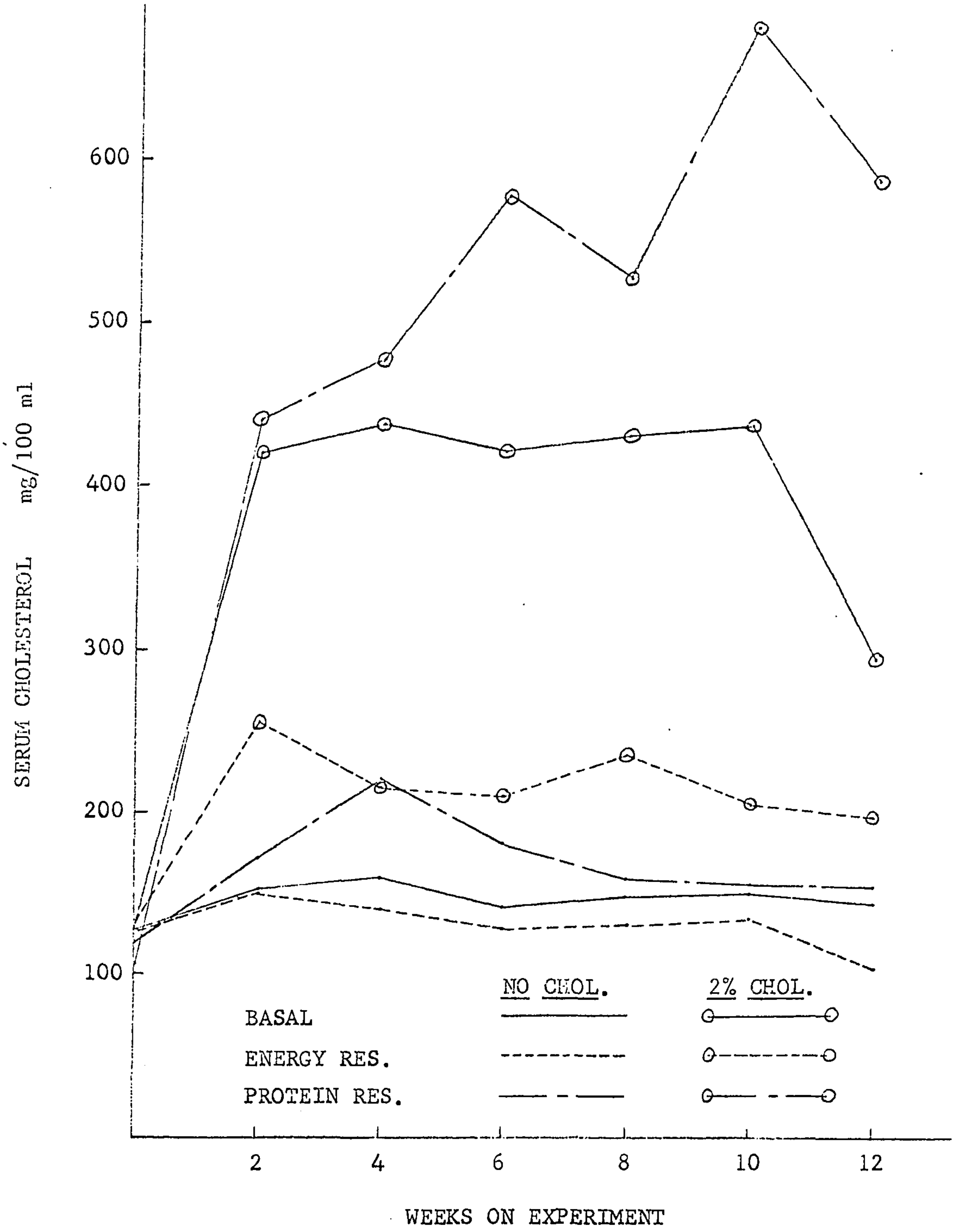


Figure 1. Effect of energy and protein restriction on serum cholesterol levels of cockerels fed practical diets with and without added cholesterol

Table 4. Effect of restricted protein or energy intake on cockerels fed practical diets with and without added cholesterol

Treatment	Body weight gain (kg)	Systolic blood pressure (mm Hg)	Serum cholesterol (mg/100 ml)	Liver lipid (%)[a]
	Average Data - - 12-Week Period			
Basal	2.31	146	146	15.0
Energy restricted[b]	0.96	126	131	16.4
Protein restricted	1.17	126	160	20.6
Basal + cholesterol[c]	2.20	147	366	26.3
Energy restr. + cholesterol	1.01	115	206	24.0
Protein restr. + cholesterol	1.11	133	483	36.0

[a]Dry weight basis.

[b]Approximately 30% restriction for specified nutrient.

[c]2% cholesterol.

Figure 2 illustrates the effect of these diets on the incidence of lipid infiltration and atherosclerotic lesions in the aortas as indicated by aorta scores. When the diets did not contain added cholesterol thoracic scores were not affected to any extent by restriction of either protein or energy intake. Abdominal aorta scores were slightly, but not significantly, increased by restriction of protein intake.

With the exception of scores of thoracic aorta from energy-restricted birds being only moderately increased, atherosclerosis was much more prevalent ($P < 0.05$) in both aorta segments from the remaining diets whenever supplemental cholesterol was fed. Among cholesterol-fed birds, thoracic aorta scores appeared to be decreased by restricted energy intake and

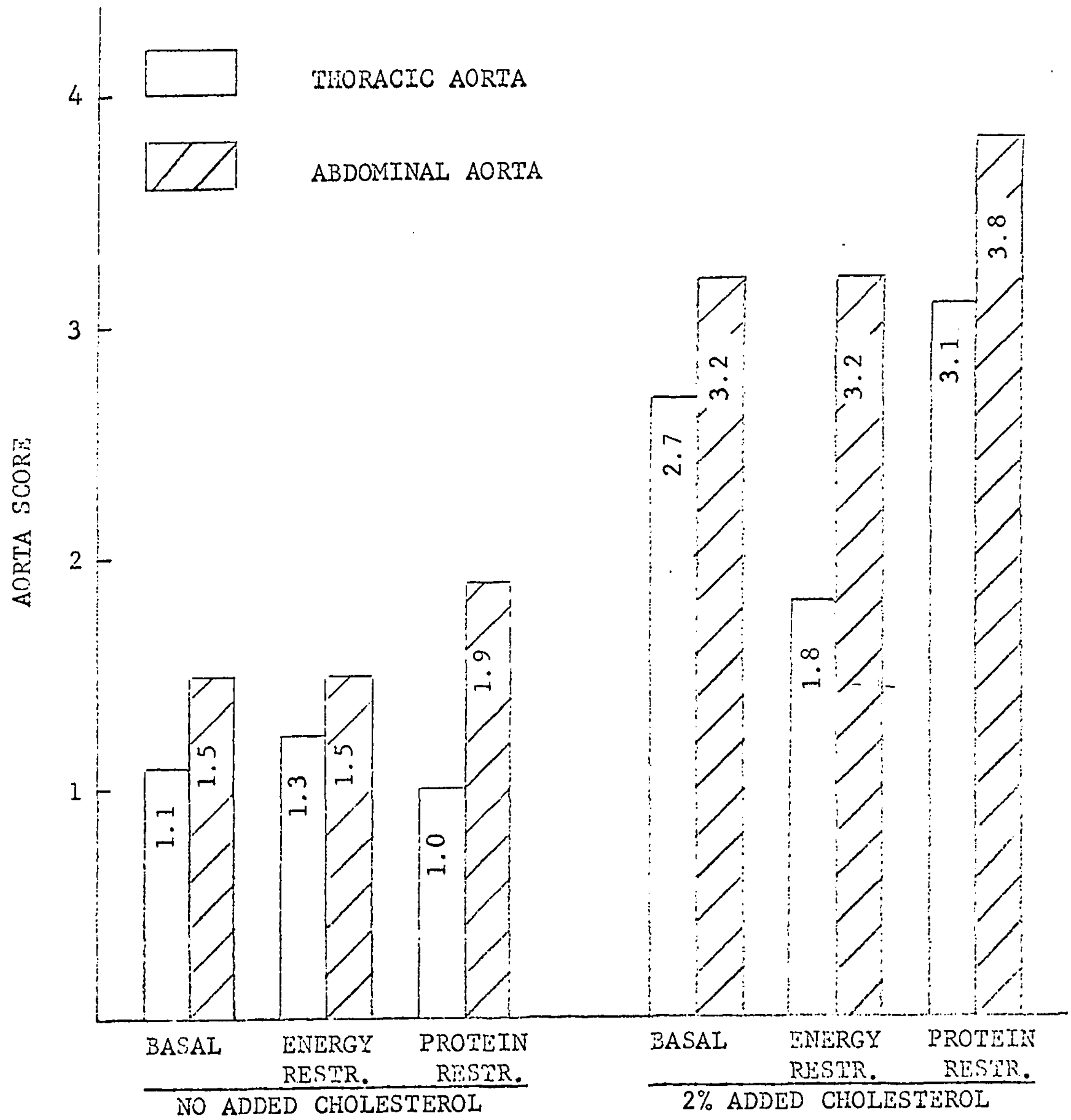


Figure 2. Effect of energy or protein restriction on aorta scores of cockerels fed practical diets with and without added cholesterol

slightly increased by restricted protein intake. Abdominal aorta scores were not affected by reduced energy intake, but were slightly increased by restricted protein intake. None of these differences were significant at the 5% or 1% probability level.

Experiment II

Isocaloric, semipurified diets containing 2% cholesterol were formulated to contain 22, 18, 14, or 10% protein and fed to 9-week-old cockerels. The variation in diet protein concentration resulted in actual average daily protein intakes ranging from 10.3 to 5.5 g/day/kg body weight over the 10-week experimental period.

Data obtained from the experiment are presented in Table 5. As was expected, body weight gains progressively decreased as protein intake was reduced. Blood pressure was elevated for birds fed the 14% protein diet as compared to those receiving the 22 and 18% protein diets.

Serum cholesterol levels of the birds in this experiment were relatively high because diets contained added cholesterol. However, cholesterol levels were increased to very high levels when protein intake was restricted to 6.5 or 5.5 g/day/kg body weight. This wide range of serum cholesterol levels is illustrated in Figure 3.

The range of protein levels used in this experiment did not produce a significant variation in aorta scores for either aorta segment, although a trend toward an increase in atherosclerotic involvement can be detected at low protein intakes.

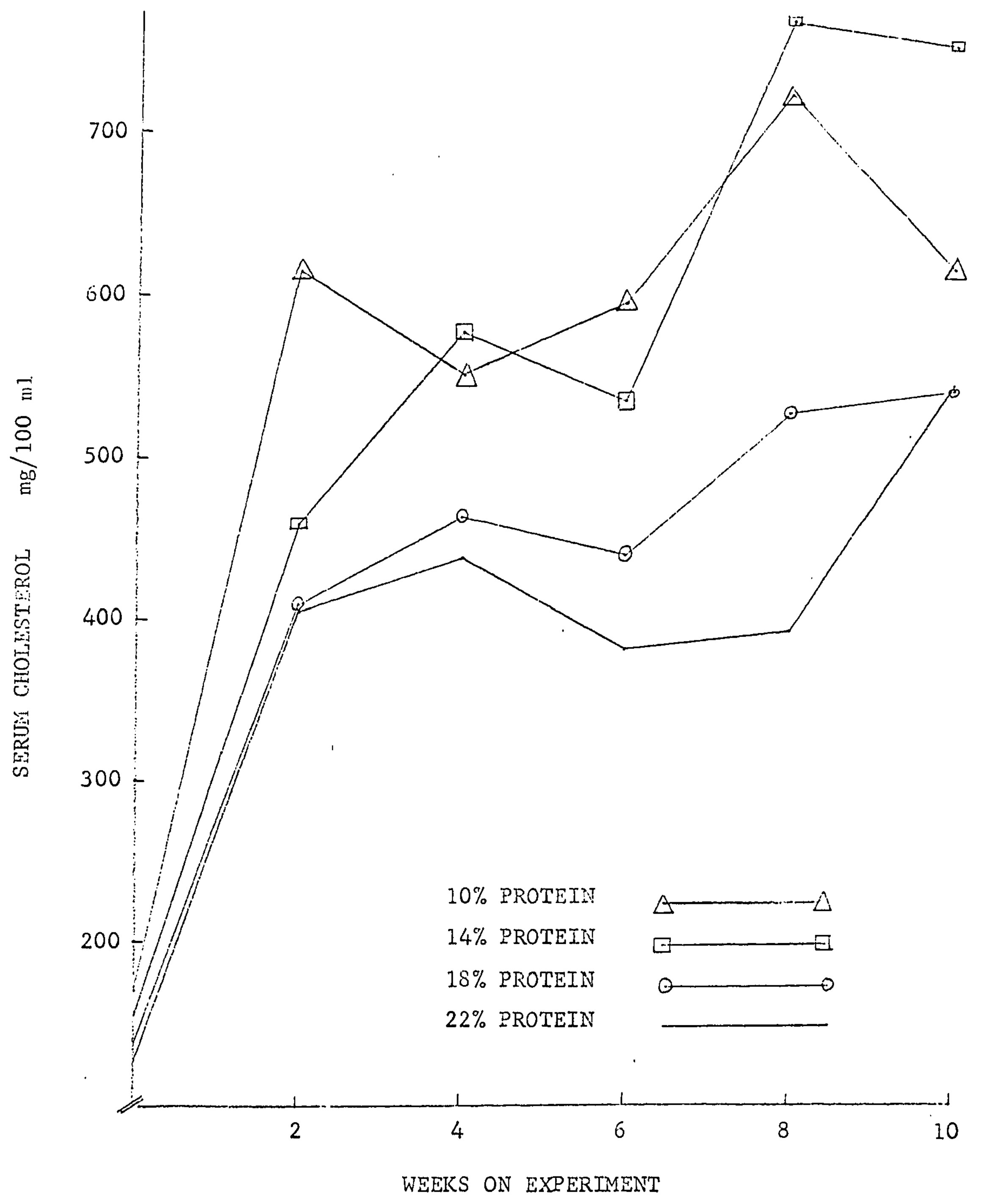


Figure 3. Effect of protein intake on serum cholesterol levels of cockerels fed isocaloric diets supplemented with 2% cholesterol

Table 5. Effect of varied protein intake on cockerels fed isocaloric, semi-purified diets[a]

Protein Level		Average Data - - 10-Week Period				
(%)	Daily intake (gm/kg body wt)	Body weight gain (kg)	Systolic blood pressure (mm Hg)	Serum cholesterol (mg/100 ml)	Aorta Score A[b]	Aorta Score T[c]
22	10.3	2.05	159	380	2.6	2.3
18	8.5	1.98	160	434	2.2	2.5
14	6.5	1.84	170	570	2.7	2.3
10	5.5	1.62	163	543	2.7	2.7

[a]All diets contained 2% cholesterol.

[b]Abdominal aorta segment.

[c]Thoracic aorta segment.

Experiment III

Isonitrogenous, 10% protein, semipurified diets containing 1600 or 1200 M.E. Calories/lb were fed with and without added cholesterol to 9-week-old cockerels. Data from this 6-month experiment are presented in Table 6. Weight gains were reduced when caloric intake was reduced and when the diets were supplemented with cholesterol. The birds fed the 1200-Calorie, plus-cholesterol diet gained very little primarily because of weight losses incurred during the last eight weeks of the experiment. Daily caloric intake was approximately the same for the two 1200-Calorie groups. The birds fed the 1200-Calorie, plus-cholesterol diet exhibited

Table 6. Influence of caloric intake on cockerels fed isonitrogenous, low-protein[a] diets with and without added cholesterol

Treatment			Average Data - - 6-Month Period				
M.E. Cal/lb	Added cholesterol[c]	Daily Caloric Intake[b] M.E./kg Body weight	Body weight gain (kg)	Systolic blood pressure (mm Hg)	Serum cholesterol (mg/100 ml)	Serum lipid (g/100 ml)	Liver lipid (%)[d]
1600	--	145	1.99	149	208	1.34	14.4
1200	--	119	1.31	150	204	1.33	13.5
1600	+	155	1.72	147	886	2.74	45.2
1200	+	117	0.61	135	658	2.54	26.0

[a]10% protein.

[b]Average daily intake values for 24-week experimental period.

[c]1% cholesterol.

[d]Dry weight basis.

the lowest blood pressure readings of any of the groups.

Serum cholesterol was markedly increased ($P < 0.01$) by dietary cholesterol supplementation at either energy level, although a somewhat lower ($P < 0.05$) value was obtained when caloric intake was reduced. Serum cholesterol levels were similar for both caloric intakes when dietary cholesterol was not added. The serum cholesterol values are shown in Figure 4.

Total serum lipids responded to the treatments in the same manner as did serum cholesterol values. Serum lipid levels were markedly elevated ($P < 0.01$) when supplemental cholesterol was used, but to a lesser extent on the lower caloric intake. The differences are shown in Figure 5.

Liver lipids were increased for both energy intakes by dietary cholesterol addition, but to a much greater extent on the high-energy diet. Reduced energy intake lowered liver lipids 58% when the diets contained added cholesterol, but only 6.3% when cholesterol was not added.

Figure 6 graphically illustrates the degree of atherosclerosis involvement obtained when birds were fed these low-protein diets at different energy levels. Aorta scores were much higher ($P < 0.01$) for cholesterol-fed birds, however, the thoracic score was significantly lower ($P < 0.05$) for birds consuming fewer calories. On the other hand, energy level had no significant effect on the aorta scores when diets did not contain added cholesterol. The thoracic aorta scores for the high-energy, cholesterol-fed birds were strikingly high and the aorta segments from this group were the most severely affected aortas we have observed to date.

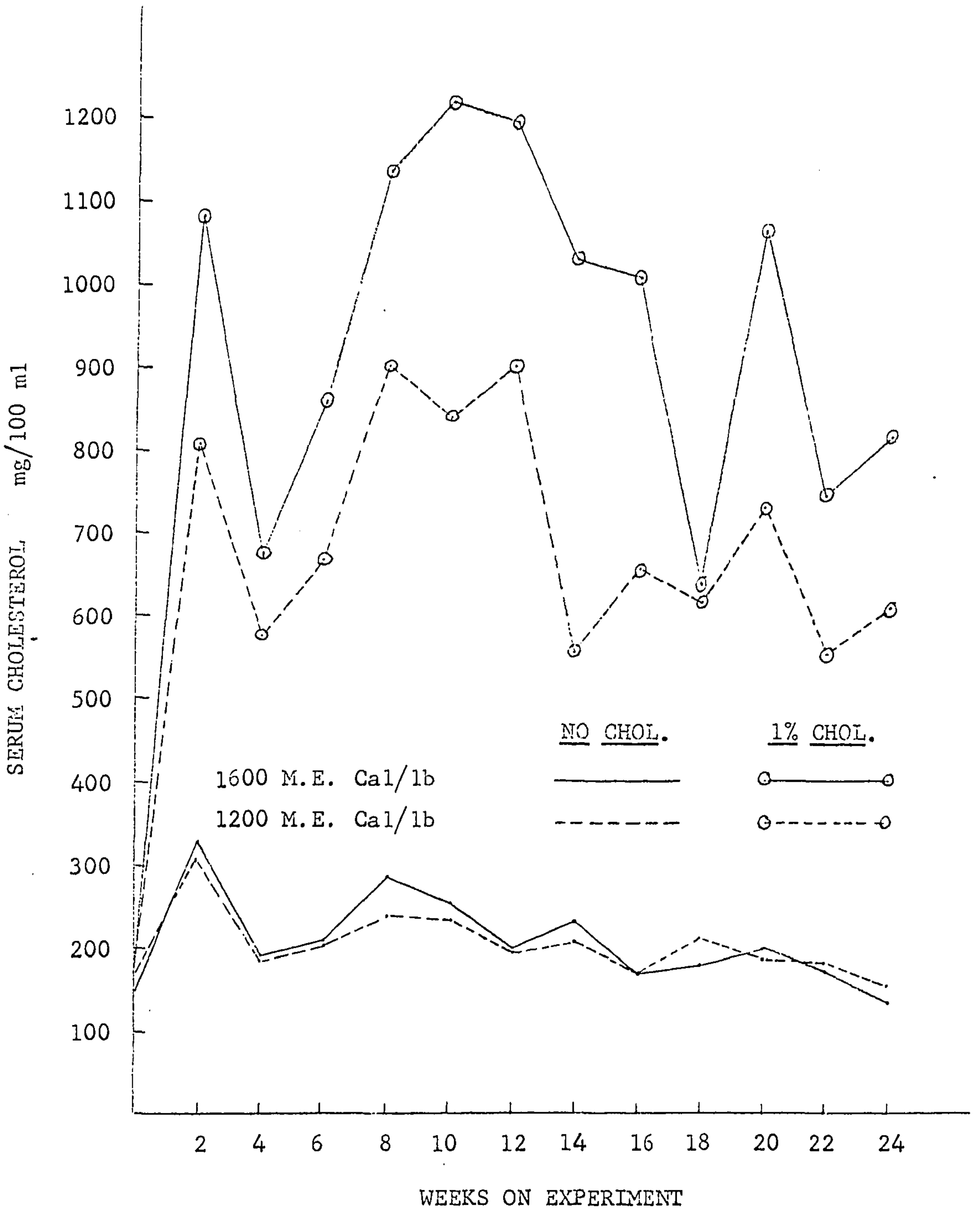


Figure 4. Effect of energy intake on serum cholesterol levels of cockerels fed isonitrogenous diets with and without added cholesterol

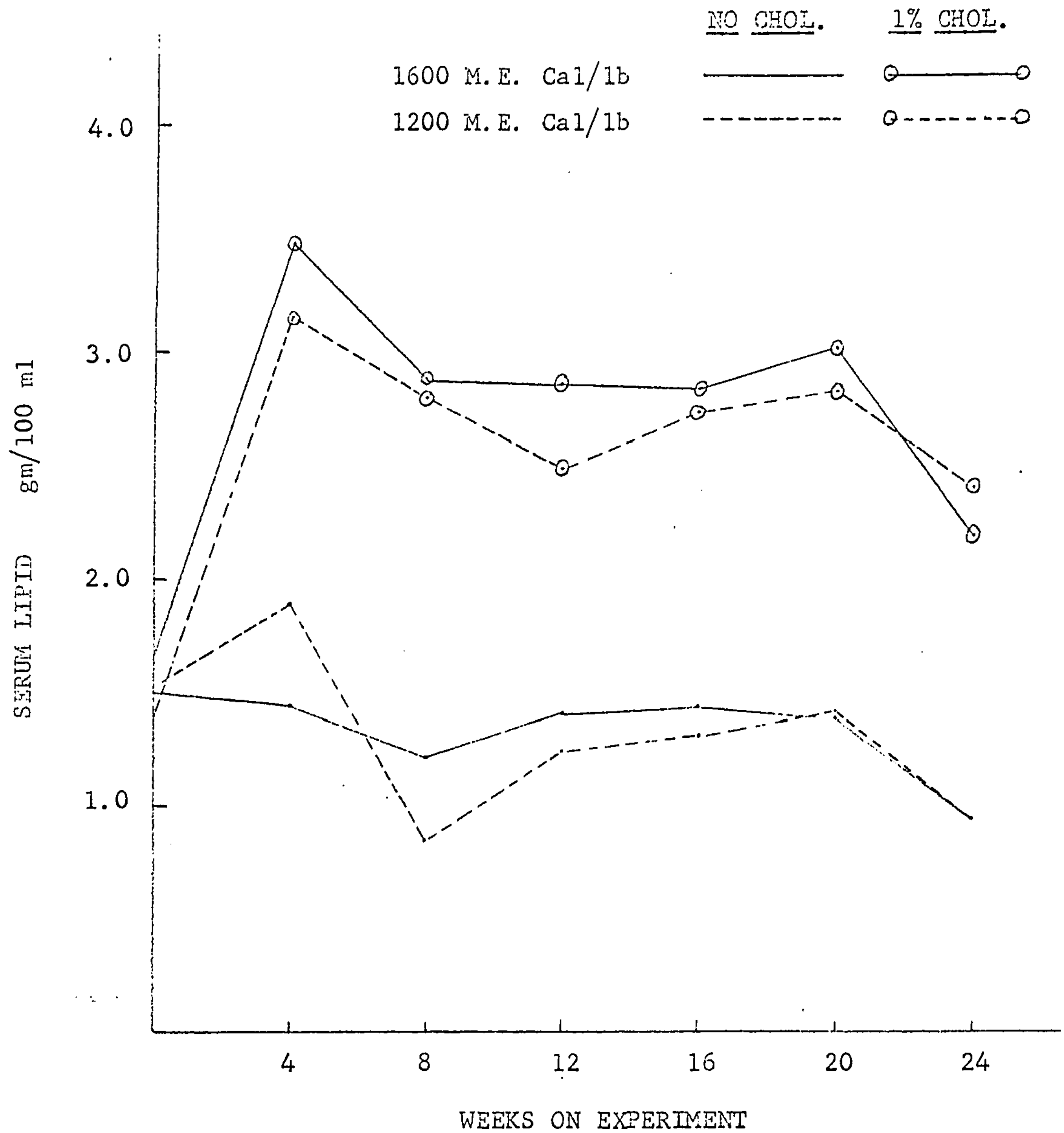


Figure 5. Effect of energy intake on serum total lipid levels of cockerels fed isonitrogenous (10% protein) diets with and without added cholesterol

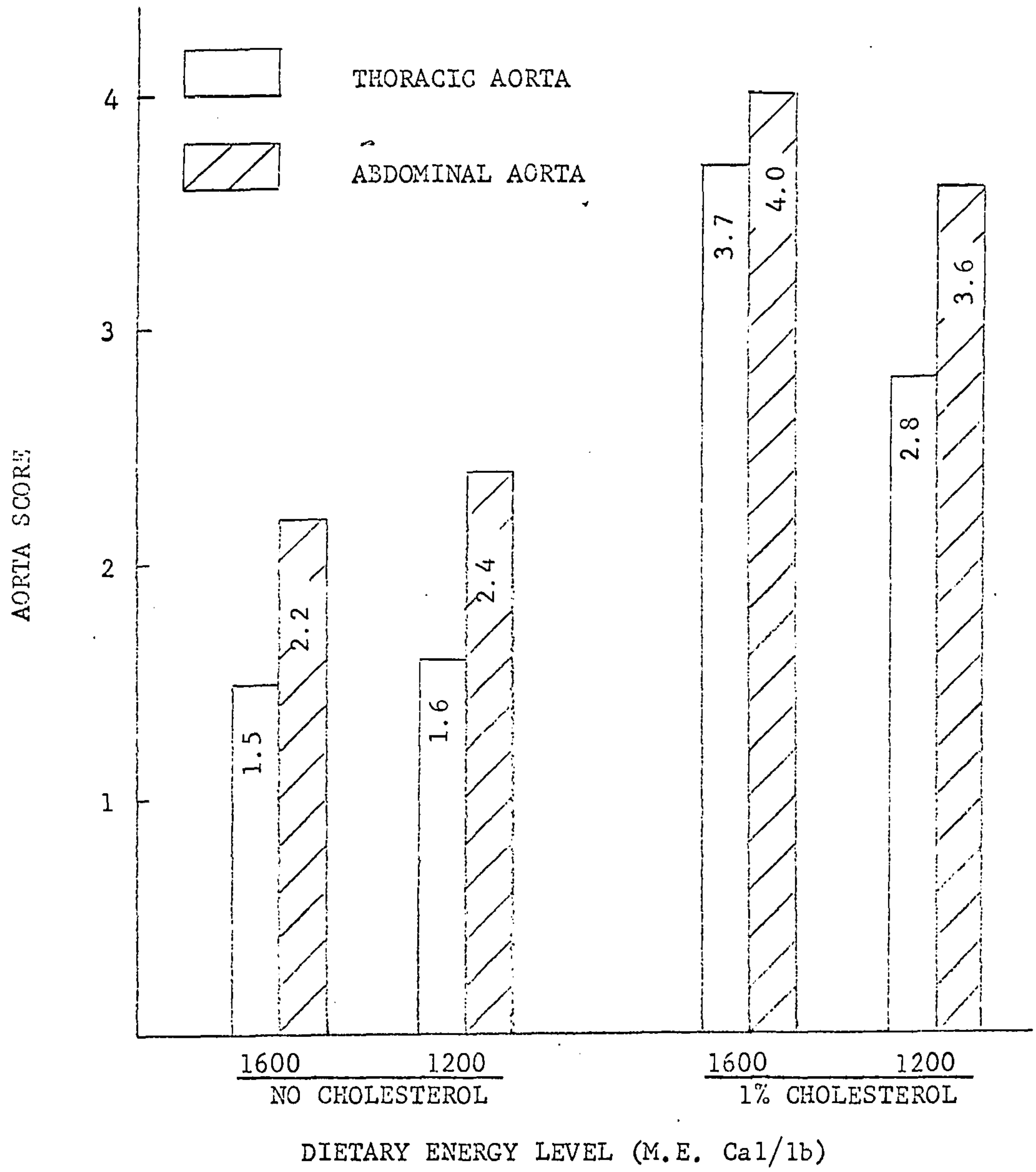


Figure 6. Influence of lowered caloric intake on aorta scores of cockerels fed isonitrogenous (10% protein) diets with and without added cholesterol

Figures 7 and 8 illustrate atherosclerotic involvement for thoracic and abdominal aorta segments of birds fed the two energy levels with added cholesterol.

At the end of the experiment, testes and gall bladder weights were taken because of their relationship to the metabolite cholesterol. These organ weights are listed in Table 7. Added dietary cholesterol had no influence on testes weight at either level of energy intake. However, when caloric intake was reduced, with or without added cholesterol, testes weights were lowered 40 and 36%, respectively.

On the other hand, gall bladder weights were not affected by level of energy intake, without added dietary cholesterol, but were increased about one and one-half and five-fold when cholesterol was added to 1600 and 1200-Calorie diets, respectively.

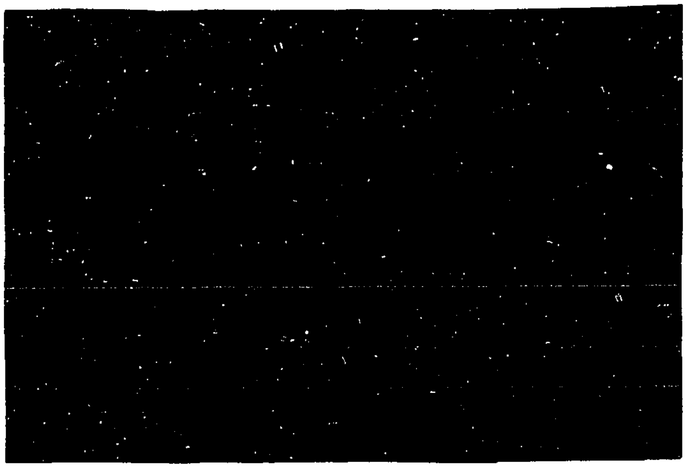
Table 7. Influence of energy restriction on testes and gall bladder weights of cockerels fed isonitrogenous diets with and without added cholesterol

Treatment	Organ Weight[a] (% of body wt.)	
	Testes	Gall bladder
1600 Cal.[b]	0.90	0.07
1200 Cal.	0.58	0.07
1600 Cal. + cholesterol[c]	0.95	0.17
1200 Cal. + cholesterol	0.57	0.42

[a]Wet weight basis.

[b]Metabolizable energy Calories per pound.

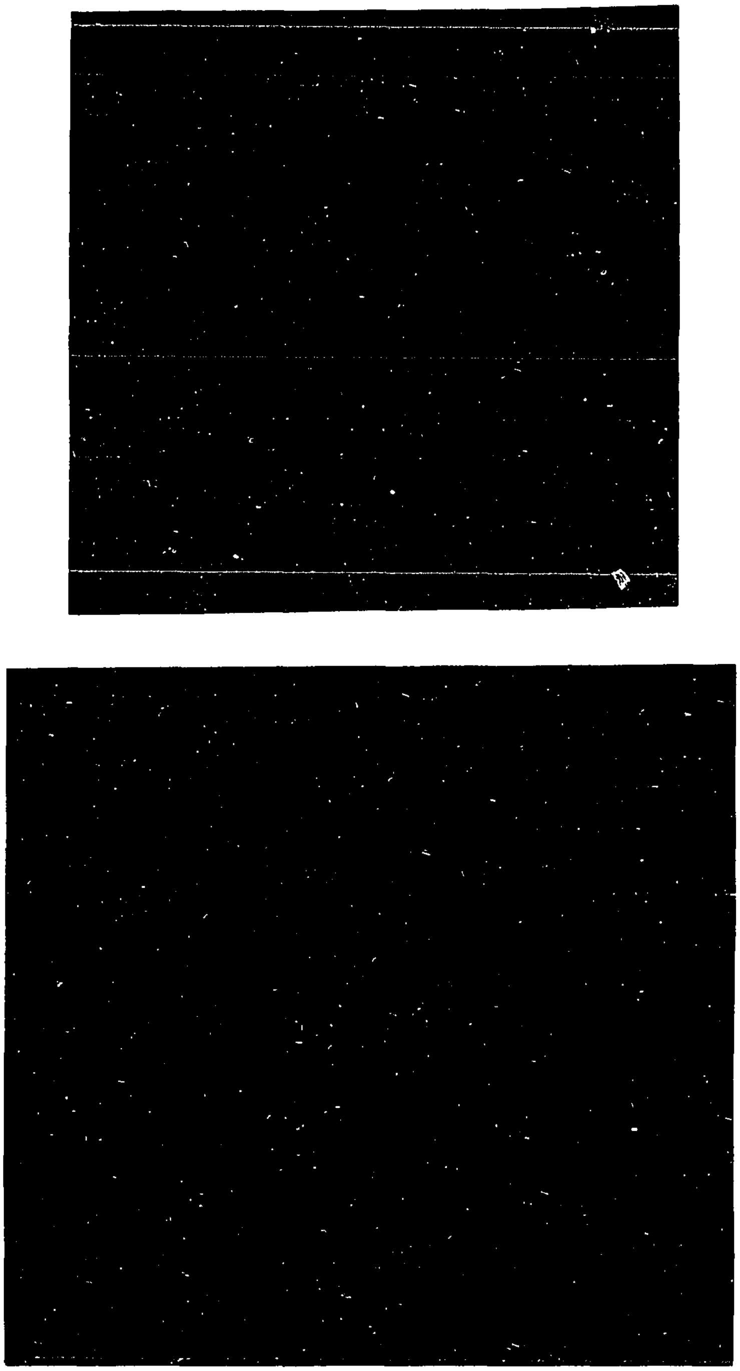
[c]1% cholesterol.



1200
M.E. Cal/lb

1600
M.E. Cal/lb

Figure 7. Lipid-stained (Sudan IV) thoracic aortas from cockerels fed isonitrogenous (10% protein) diets containing 1200 or 1600 M.E. Cal/lb and added cholesterol



1200
M.E. Cal/lb

1600
M.E. Cal/lb

Figure 8. Lipid-stained (Sudan IV) abdominal aortas from cockerels fed isonitrogenous (10% protein) diets containing 1200 or 1600 M.E. Cal/lb and added cholesterol

Experiment IV

Energy or protein intake was restricted for 11-week-old cockerels fed semipurified diets with and without added cholesterol. Restricted energy and protein intakes averaged 73.5 and 65.0%, respectively, of the energy and protein intakes of birds fed the basal diet. These percentages were derived from daily feed intake data expressed in terms of calories of energy or grams of protein consumed per kilogram of body weight.

Data from this 12-week experiment are given in Table 8. Body weight gains were reduced when any of the restricted diets were fed, but to a much lesser extent for the protein-restricted group.

Table 8. Effect of restricted energy or protein intake on cockerels fed semipurified diets with and without added cholesterol

	Average Data - - 12-Week Period				
Treatment	Body weight gain (kg)	Systolic blood pressure (mm Hg)	Serum cholesterol (mg/100 ml)	Serum lipid (g/100 ml)	Liver lipid (%)[a]
Basal	1.62	131	178	1.20	17.7
Energy restricted[b]	0.46	123	200	1.27	16.3
Protein restricted	1.43	139	200	1.43	20.9
Basal + chol.[c]	1.77	124	438	1.93	34.6
Energy restr. + chol.	0.45	125	305	1.56	24.0
Protein restr. + chol.	1.48	144	770	2.40	40.7

[a]Dry weight basis.

[b]26% energy and 35% protein restrictions were obtained for the energy and protein restricted diets, respectively.

[c]1% cholesterol.

Blood pressure values were higher when protein intake was restricted in conjunction with added cholesterol. Blood pressure was lowered ($P < 0.05$) by energy restriction when the diet did not contain added cholesterol.

Dietary cholesterol supplementation resulted in increased ($P < 0.01$) cholesterol levels for birds fed all diets. As noted in the previous experiments, these increased levels were lower when energy intake was restricted and higher when protein intake was reduced. These relationships were highly significant ($P < 0.01$) and are graphically illustrated in Figure 9.

The range of serum lipid values can be seen in Figure 10. As was the case for serum cholesterol, serum lipids were markedly increased ($P < 0.01$) by the addition of cholesterol to the diet. These cholesterol-altered levels were significantly lower ($P < 0.01$) for energy-restricted birds and significantly higher ($P < 0.01$) for protein-restricted birds. Serum lipid concentration was also increased by protein restriction when the diet did not contain added cholesterol.

Liver lipids were increased whenever cholesterol was present in the diet, however the increase was less for energy-restricted birds and greater for protein-restricted birds. In comparison to the appropriate basal diets, liver lipids of the energy-restricted birds were decreased 31 and 8%, respectively, when fed with and without cholesterol. On the other hand, liver lipids increased 18% for protein-restricted birds whether or not cholesterol was fed.

Figure 11 graphically shows the various degrees of atherosclerosis which resulted from the diets fed in this study. Aorta scores were

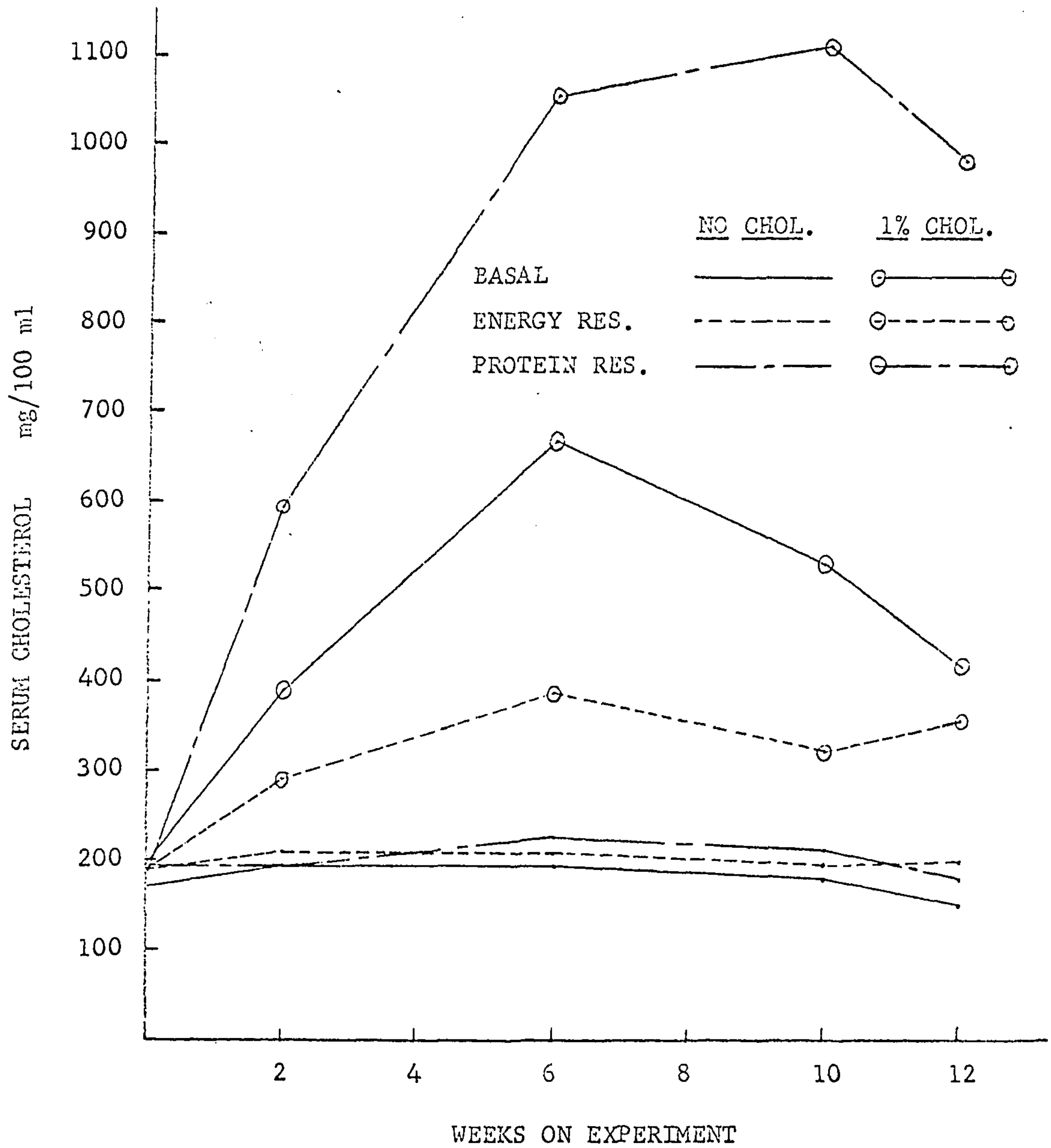


Figure 9. Effect of reduced energy or protein intake on serum cholesterol levels of cockerels fed diets with and without added cholesterol

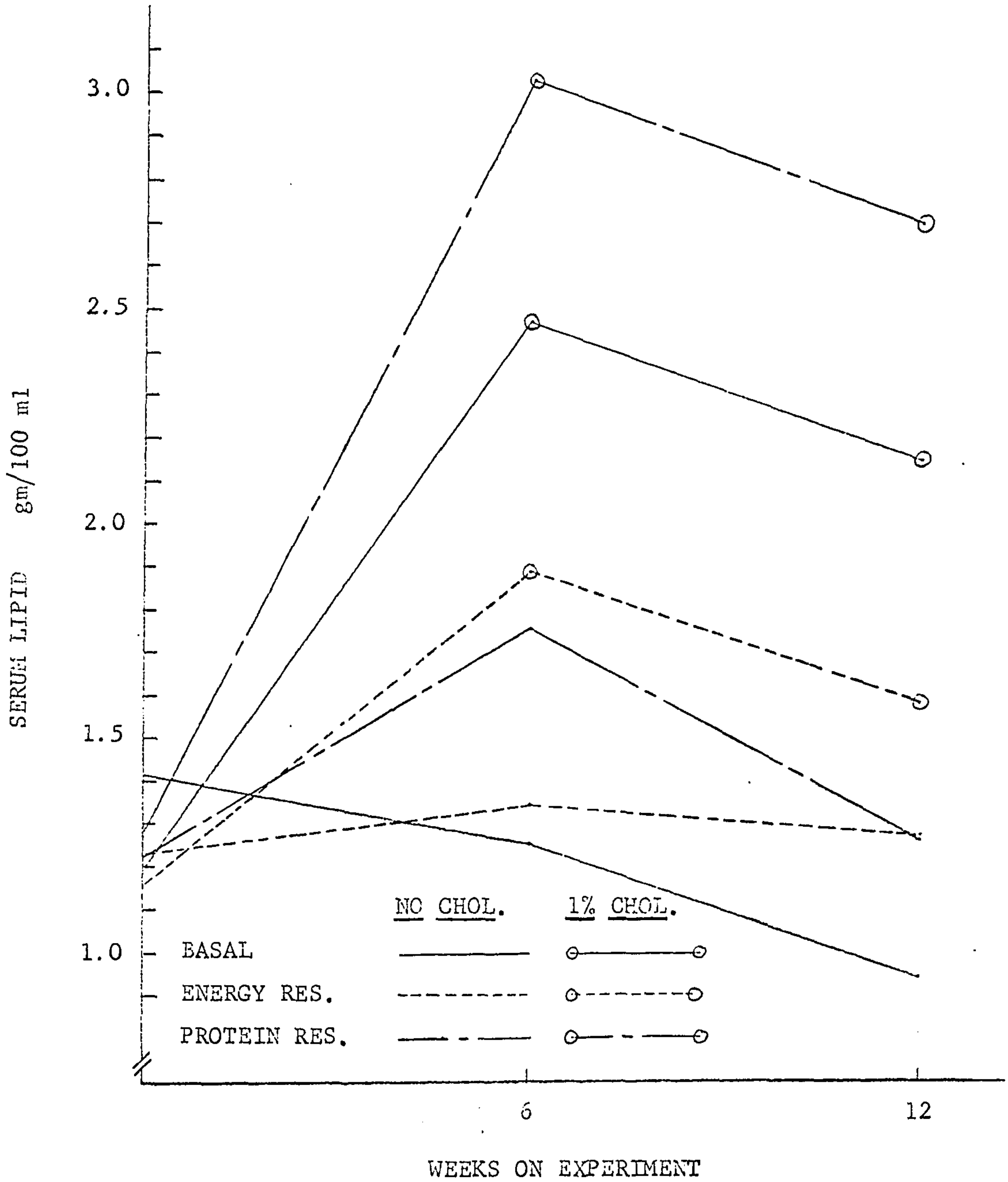


Figure 10. Effect of reduced energy or protein intake on serum lipid levels of cockerels fed diets with and without added cholesterol

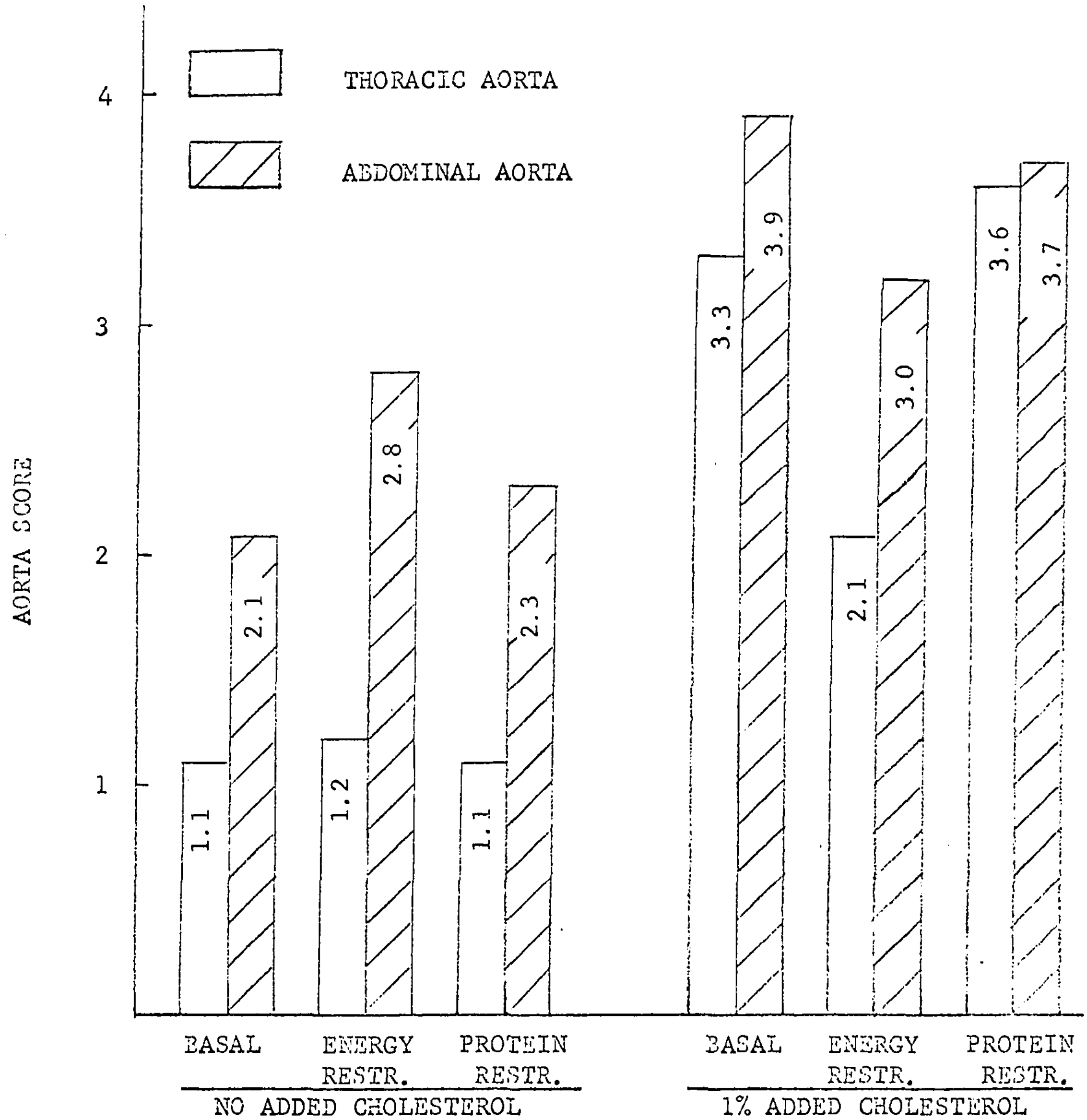


Figure 11. Aorta scores for cockerels receiving reduced energy or protein intakes with and without added cholesterol

comparable for the various diets not supplemented with cholesterol, except for the increased abdominal score for the energy-restricted birds. Other than the abdominal scores from energy-restricted birds, aorta scores were increased ($P < 0.01$) whenever cholesterol was added to the diet. In comparisons among cholesterol-fed birds, scores were lower for both aorta segments when energy intake was decreased. On the other hand, because of the extremely high aorta scores recorded for the basal birds, restricted protein intake had little effect.

Testes and gall bladder weights obtained at the end of the experiment are given in Table 9. Testes weights were markedly reduced for energy-restricted birds and only slightly reduced for the protein-restricted group. For cholesterol-fed birds, testes weights were similar to those from birds not fed supplemental cholesterol, except for a slight reduction for the basal-fed group.

Restricted energy or protein intake did not affect gall bladder weights when cholesterol was not added to the diets. However, gall bladder weights were doubled when cockerels were fed the reduced caloric-intake diet containing added cholesterol.

The orthogonal comparisons which were made indicate that linear or quadratic effects are sometimes present for the various physiological determinations over the length of the experimental period and are often dependent on particular treatments. With the blood pressure data the linear effect was highly significant ($P < 0.01$) for energy-restricted diets with and without added cholesterol. On the other hand, there were no significant quadratic effects.

Significant ($P < 0.01$) linear and quadratic effects were evident

Table 9. Influence of energy or protein restriction with and without added dietary cholesterol on testes and gall bladder weights of cockerels

Treatment	Organ Weight[a] (% of body wt.)	
	Testes	Gall bladder
Basal	0.81	0.09
Energy restricted[b]	0.11	0.12
Protein restricted	0.64	0.11
Basal + cholesterol[c]	0.65	0.15
Energy restr. + cholesterol	0.11	0.31
Protein restr. + cholesterol	0.70	0.16

[a]Wet weight basis.

[b]26% energy and 35% protein restriction was obtained for the energy and protein restricted diets, respectively.

[c]1% cholesterol.

for serum cholesterol data when protein or energy were restricted in conjunction with added cholesterol diets.

Linear and quadratic effects were also associated with cholesterol-supplemented diets for serum lipid data. All diets containing added cholesterol exhibited highly significant ($P < 0.01$) linear and quadratic effects. Without added cholesterol only the basal and protein-restricted diets showed highly significant ($P < 0.01$) linear and quadratic effects, respectively. It should be noted that the error term used for these calculations represented all treatments rather than birds for individual treatments.

DISCUSSION

As expected, restriction of dietary energy or protein intake affected weight gains of cockerels in these experiments. Energy restriction consistently resulted in lower weight gains than did a comparable degree of protein restriction. It is desirable for birds to maintain their body weight during an experiment of this nature. In experiment IV where actual daily caloric intake averaged 74% of that of basal-fed birds, body weights were barely maintained and further energy restriction would have resulted in constant weight loss. However, in the same experiment, body weights were readily maintained by birds whose protein intake amounted to 65% of the protein consumed by birds receiving the basal diet. Protein intake probably could have been restricted more and still maintained body weights since weight gains were only reduced about 12-16% for the protein-restricted birds.

Overall, the effect of dietary energy or protein restriction on systolic blood pressure of cockerels appears to be inconsistent. This agrees with the results reported by Nichols and Balloun (1963) that dietary restriction had no significant effect on systolic blood pressure of cockerels, although blood pressure was consistently lower for birds fed a restricted-energy diet. Although blood pressure was lower for restricted energy and restricted protein diets in experiment I, most of this difference was probably related to the light body weights of the birds fed the restricted diets. Nichols _et_ _al_. (1963a) found that systolic blood pressure increased 8.64 mm Hg per kilogram of increase in body weight for cockerels between 8 and 14 weeks of age.

In experiments I and III blood pressure was lower in birds fed energy-restricted, cholesterol-supplemented diets. This effect cannot be entirely explained by body weight differences. However, this effect did not occur in experiment IV.

Blood pressure was increased in birds fed the protein-restricted diets in experiment IV, but not in experiment I. In experiment II only one of two low protein diets resulted in increased blood pressure. Speers (1965) reported that different dietary protein or energy levels affected systolic blood pressure inconsistently in laying hens. However, the energy and protein effects which were detected were of the same nature as in the present study. It should be noted that birds used in experiment I were four to six weeks younger than those used in the other experiments and that responses may be different for this rapidly growing stage of the cockerel's life. The fact that practical rather than semipurified diets were fed in experiment I also complicates comparisons with the other three experiments.

Nichols et al. (1963b) concluded that dietary cholesterol consistently increased systolic blood pressure towards the end of an eight-week experiment which began when cockerels were five weeks of age. The present series of experiments does not substantiate this effect of dietary cholesterol supplementation on systolic blood pressure.

Serum cholesterol values were increased in cholesterol-fed birds regardless of the nature of the diet. Restriction of energy or protein intake resulted in opposite responses of serum cholesterol concentration for birds fed cholesterol-supplemented diets. As reported by several investigators, low protein intakes resulted in markedly increased serum cholesterol levels. The restricted-protein effect was greater for semi-purified diets than for practical diets. Nishida et al. (1958) reported that serum cholesterol levels were more dependent on dietary protein than on dietary cholesterol. This observation is not supported by the present study where much greater differences in serum cholesterol levels were obtained in response to dietary cholesterol than to reduced protein intake. On the other hand, low energy intake resulted in decreased serum cholesterol levels. The magnitude of the serum cholesterol response to energy restriction was not as great as that attributed to protein restriction.

The effect of restricted energy or protein intake on serum cholesterol concentration was not apparent unless the diets contained added cholesterol. In experiment I a small, but nonsignificant, decrease and increase in serum cholesterol was obtained from birds fed energy- and protein-restricted diets without added cholesterol, respectively.

Total serum lipid levels responded to dietary treatments in a manner similar to serum cholesterol levels. However, unlike serum cholesterol, significantly higher serum lipid levels were obtained from birds fed the protein-restricted diet without added cholesterol.

Liver lipids increased whenever cholesterol was added to the diets. With cholesterol-supplemented diets liver lipids increased due to protein restriction and decreased due to energy restriction, thus following a pattern of responses similar to those set by serum cholesterol and serum lipids for these same diets. Liver lipids were consistently slightly elevated for birds whose protein intakes were restricted in diets without added cholesterol.

It has long been known that added dietary cholesterol is atherogenic for cockerels, particularly when used in conjunction with dietary fats. Aorta scores, the product of subjective visual grading of gross lipid-stained aorta segments, were higher whenever cholesterol was added to the diets. As is normally the case, the incidence of aortic atherosclerosis was more severe in the abdominal segment than in the thoracic segment of the aorta regardless of dietary treatment.

With one exception, differences due to treatments were not significant for either aorta segment when diets did not contain added cholesterol. The single exception was a slightly increased abdominal aorta score due to restricted energy intake in experiment IV. These results are not in agreement with those of Griminger _et al_. (1963) who reported a lower incidence of spontaneous atherosclerosis for birds that were fed 80% of the food consumed by full-fed birds. However, their extended 37 1/2 month experimental period may explain some of the difference.

With added dietary cholesterol, abdominal aorta scores were not significantly different in experiment IV although the low caloric intake group had lower scores.

A more clearcut pattern was established for the thoracic aorta scores when cholesterol was added to the diet. Scores were reduced in energy-restricted and increased in protein-restricted birds. These differences were significant except for those for the protein-restricted groups of experiments II and IV.

From these results it appears that cholesterol-induced atherosclerosis of the thoracic aorta is more readily affected by restricted protein or energy intake than is the incidence of similarly induced atherosclerosis of the abdominal aorta in cockerels.

Testes weights were not affected by dietary cholesterol addition, but were markedly reduced when energy intake was restricted. The effect of energy restriction was slightly reduced in experiment III where energy restriction was imposed upon a low-protein diet. These effects can be explained by the fact that malnutrition has an adverse effect on reproductive organs and this effect is related to diminished pituitary gonadotrophins in the circulation. Turner (1966) mentions that inanition, vitamin deficiencies, caloric restriction, or insufficient quantities of specific food substances such as protein are capable of impairing testicular functions.

Comb size was reduced in cockerels fed the energy-restricted diets and occasionally was reduced in protein-restricted birds. Comb size is known to reflect the activity of the testes and, therefore, was another reflection of the malnutrition effect.

Throughout this study it was apparent from handling the birds that the feet and shanks of energy-restricted birds in particular, were cold to the touch. A check of rectal temperatures at the end of experiment IV revealed that the temperatures of the birds fed the basal and protein-restricted diets averaged 106.3° F, whereas temperatures of birds fed energy-restricted diets averaged 105° F. These reduced temperatures could have been another reflection of the effect of chronic malnutrition on pituitary gland secretion and altered metabolic rate.

Gall bladder weights were not affected by dietary treatment when the diets did not contain cholesterol. Supplemental cholesterol induced slightly increased gall bladder weights of birds fed basal and protein-restricted diets and doubled the gall bladder weights of birds receiving energy-restricted diets.

The pattern of response of serum cholesterol, serum and liver lipids, and thoracic aorta scores to restricted protein or energy intake was similar whenever cholesterol was added to the diet. The data indicate that dietary restriction of energy or protein has a bearing on lipid metabolism and that energy is more important in this respect.

SUMMARY

Practical or semipurified diets with and without added cholesterol were fed to growing cockerels to determine the effect of dietary energy or protein restriction on several physiological responses. Observations were made on systolic blood pressure, total serum cholesterol, total serum lipids, liver lipids, incidence of atherosclerosis in thoracic and abdominal segments of the aorta, testes and gall bladder weights and body weight gains. Individual feeding of individually caged birds permitted reasonably accurate control of protein and caloric intake.

Restriction of protein or energy intake reduced body weight gains, however, energy restriction had a greater effect on weight gains than did protein restriction.

The effect of dietary protein or energy restriction on systolic blood pressure was inconsistent. Variations in blood pressure seemed to be associated more with body weight than with restriction of protein or energy as such. However, relationship to body weight did not account for the entire reduction of blood pressure in birds fed an energy-restricted diet containing added cholesterol.

Serum cholesterol, serum lipids and liver lipids were markedly increased when cockerels were fed diets containing added cholesterol. Each of these were increased by protein restriction and decreased by energy restriction when diets contained added cholesterol. Without added dietary cholesterol, serum cholesterol was not significantly affected by protein or energy restriction. On the other hand, serum lipids were increased and liver lipids were slightly increased when birds were fed protein-restricted

diets without added cholesterol.

Aortic atherosclerosis was more prevalent when the diets contained added cholesterol and more severe in the abdominal segment than the thoracic segment of the aorta regardless of dietary treatment. Protein or energy restriction had its greatest effect on atherosclerosis when the diets contained added cholesterol, and also affected the thoracic segment in these instances. Incidence of atherosclerosis in thoracic aorta was reduced in energy-restricted and increased in protein-restricted birds.

Testes weights were not affected by dietary cholesterol addition, but were markedly reduced when energy intake was restricted. Gall bladder weights were not affected by dietary treatment unless the diets contained added cholesterol. Then gall bladder weights were slightly increased in birds fed basal and protein-restricted diets and were doubled in birds fed energy-restricted diets.

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APPENDIX

Table 10. Analysis of variance[a] and multiple range test[b] of blood pressure data, experiment I

Source of variation	d.f.	m.s.	F
Treatment	5	8066.48	5.28**
Birds within treatment	18	1528.77	
Period	12	972.86	3.99**
Error	266	243.97	

Treatment[c]	5	2	3	6	1	4
Avg. blood pressure	115	126	129	133	146	147
$P < 0.05$						
$P < 0.01$						

[a]** and * refer to $P < 0.01$ and $P < 0.05$, respectively, throughout the appendix.

[b]Means underscored by the same line are not significantly different at the levels indicated throughout the appendix.

[c]Treatments for experiments I and IV are coded throughout the appendix as follows: basal - 1, energy restriction - 2, protein restriction - 3, basal + cholesterol - 4, energy restriction + cholesterol - 5, protein restriction + cholesterol - 6.

Table 11. Analysis of variance[a] and multiple range test[b] of serum cholesterol data, experiment I

Source of variation	d.f.	m.s.	F
Treatment	5	565103.94	11.88**
Birds within treatment	17	47578.30	
Period	6	76761.33	19.33**
Treatment x period	30	24735.10	6.23**
Error	102	3971.09	

Treatment[c]	2	1	3	5	4	6
Avg. serum cholesterol conc.	131	146	160	206	366	483
$P < 0.05$						
$P < 0.01$						

Table 12. Analysis of variance[a] and multiple range[b] test of thoracic aorta scores, experiment I

Source of variation	d.f.	m.s.	F
Treatment	5	9.1652	3.96*
Birds within treatment	17	2.3137	
Grader	2	0.1184	0.78
Treatment x grader	10	0.1239	0.82
Error	34	0.1520	

Treatment[c]	3	1	2	5	4	6
Avg. thoracic aorta score	1.00	1.08	1.25	1.83	2.75	3.08
$P < 0.05$						
$P < 0.01$						

Table 13. Analysis of variance[a] and multiple range[b] test of abdominal aorta scores, experiment I

Source of variation	d.f.	m.s.	F
Treatment	5	11.8541	7.23**
Birds within treatment	17	1.6405	
Grader	2	0.3611	1.14
Treatment x grader	10	0.2411	0.76
Error	34	0.3170	

Treatment[c]	1	2	3	4	5	6
Avg. abdominal aorta score	1.50	1.50	1.89	3.17	3.17	3.83
$P < 0.05$						
$P < 0.01$						

Table 14. Analysis of variance[a] of blood pressure, experiment II

Source of variation	d.f.	m.s.	F
Treatment	3	1020.26	0.40
Birds within treatment	20	2529.5	
Regressed period	1	35331.06	121.19**
Error	230	291.54	

Table 15. Analysis of variance[a] and multiple range[b] test of serum cholesterol, experiment II

Source of variation	d.f.	m.s.	F
Treatment	3	263999.79	4.49*
Birds within treatment	20	58776.17	
Period	5	722311.38	21.56**
Treatment x period	15	35345.28	1.06
Error	95	33496.29	

Treatment	22%	18%	10%	14%
Avg. serum cholesterol conc.	380	454	543	570
$P < 0.05$				
$P < 0.01$				

Table 16. Analysis of variance of thoracic aorta scores, experiment II

Source of variation	d.f.	m.s.	F
Treatment	3	0.701288	0.19
Birds within treatment	19	3.739766	
Grader	2	0.082540	0.68
Treatment x grader	6	0.192110	1.57
Error	38	0.122222	

Table 17. Analysis of variance[a] of abdominal aorta scores, experiment II

Source of variation	d.f.	m.s.	F
Treatment	3	0.890177	0.25
Birds within treatment	19	3.534503	
Grader	2	1.130688	6.60**
Treatment x grader	6	0.103221	0.60
Error	38	0.171345	

Table 18. Analysis of variance[a] of blood pressure, experiment III

Source of variation	d.f.	m.s.	F
Treatment	3	3094.34	0.71
Birds within treatment	20	4370.4	
Period	11	721.57	3.00**
Treatment x period	33	314.11	1.31
Error	206	240.37	

Table 19. Analysis of variance[a] and multiple range[b] test of serum cholesterol, experiment III

Source of variation	d.f.	m.s.	F
Treatment	3	8511129.49	28.18**
Birds within treatment	20		
Period	12	395202.13	14.43**
Treatment x period	36	104021.86	3.80**
Error	222	27393.80	

Treatment	1200	1600	1200+	1600+
Avg. serum cholesterol conc.	204	208	674	913
P < 0.05	———	———		
P < 0.01	———	———	———	———

Table 20. Analysis of variance[a] and multiple range[b] test of serum lipid, experiment III

Source of variance	d.f.	m.s.	F
Treatment	3	23037066.03	60.00**
Birds within treatment	20	383927.56	
Period	6	2440987.28	21.22**
Treatment x period	18	822212.86	7.15**
Error	107	115008.95	

Treatment	1600	1200	1200+	1600+
Avg. serum lipid conc.	1335	1336	2628	2780
$P < 0.01$				

Table 21. Analysis of variance[a] and multiple range[b] test of thoracic aorta scores, experiment III

Source of variation	d.f.	m.s.	F
Treatment	3	17.769577	19.75**
Birds within treatment	17	0.899673	
Grader	2	0.074232	0.45
Treatment x grader	6	0.152910	0.92
Error	34	0.165359	

Treatment	1600	1200	1200+	1600+
Avg. thoracic aorta score	1.50	1.61	2.75	3.73
$P < 0.05$				
$P < 0.01$				

Table 22. Analysis of variance[a] and multiple range[b] test of abdominal aorta scores, experiment III

Source of variation	d.f.	m.s.	F
Treatment	3	11.821429	23.72**
Birds within treatment	17	0.498366	
Grader	2	0.425532	1.78
Treatment x grader	6	0.182540	0.77
Error	34	0.238562	

Treatment	1600	1200	1200+	1600+
Avg. abdominal aorta score	2.22	2.44	3.58	4.00
$P < 0.01$				

Table 23. Analysis of variance[a] and multiple range[b] test of blood pressure, experiment IV

Source of variation	d.f.	m.s.	F
Total (287-10)	277		
Among birds	71	947	
Among chambers, tiers and diets	35	1047	
Chamber (C)	1	1024	1.20
Tier (T)	2	142	0.17
Diet (D)	5	3733	4.39**
C x T	2	419	0.49
C x D	5	660	0.78
T x D	10	665	0.78
C x T x D	10	589	0.69
Among birds within C, T and D	36	850	
Periods (P)	3	2038	11.46**
Residual	203	255	1.44*
Px among C, T, and D	105	329	1.86*
P x C	3	92	0.52
P x T	6	172	0.97
P x D	15	1106	6.25**
3 and 4-way interactions	81	205	1.16
Px among birds within C, T and D	98	177	

Treatment[c]	2	4	5	1	3	6
Avg. blood pressure	123	124	125	131	138	144
$P < 0.05$						
$P < 0.01$						

Table 24. Analysis of variance[a] and multiple range[b] test of serum cholesterol, experiment IV

Source of variation	d.f.	m.s.	F
Total (359-12)	347		
Among birds	71	262699	
Among chambers, tiers and diets	35	490233	
Chamber (C)	1	162011	3.91
Tier (T)	2	142507	3.43*
Diet (D)	5	3132315	75.50**
C x T	2	101106	2.44
C x D	5	72219	1.74
T x D	10	38851	0.94
C x T x D	10	9774	0.24
Among birds within C, T and D	36	41485	
Periods (P)	4	787574	45.87**
Residual	272	37831	2.20**
Px among C, T and D	140	57313	3.34**
P x T	8	47349	2.76**
P x C	4	26384	1.54
P x D	20	276176	16.09**
3 and 4-way interactions	108	18666	1.09
Px among birds within C, T and D	132	17169	

Treatment[c]	1	3	2	5	4	6
Avg. serum cholesterol concentration	178	200	201	305	438	770
P < 0.01	————	————	————			

Table 25. Analysis of variance[a] and multiple range[b] test of serum lipid, experiment IV

Source of variation	d.f.	m.s.	F
Total (215-10)	205		
Among birds	71	768245	
Among chambers, tiers and diets	35	1372555	
Chamber (C)	1	2400549	13.28**
Tier (T)	2	375371	2.08
Diet (D)	5	7563859	41.85**
C x T	2	263220	1.46
C x D	5	103087	0.57
T x D	10	363159	2.01
C x D x T	10	239537	1.33
Among birds within C, T and D	36	180721	
Periods (P)	2	9033170	42.64**
Residual	132	337859	1.59*
Px among C, T and D	70	449450	2.12**
P x T	4	125253	0.59
P x C	2	16343	0.08
P x D	10	2167974	10.23**
3 and 4-way interactions	54	171260	0.81
Px among birds within C, T and D	62	211869	

Treatment[c]	1	2	3	5	4	6
Avg. serum lipid conc.	1196	1270	1429	1560	1934	2401
P < 0.05						
P < 0.01						

Table 26. Analysis of variance[a] and multiple range[b] test of thoracic aorta scores, experiment IV

Source of variation	d.f.	m.s.	F
Total	107	1.297	
Among birds	35	3.793	
Among chambers and diets	17	7.013	
Chamber (C)	1	0.750	1.00
Diet (D)	5	22.787	30.26**
C x D and remainder	11	0.412	0.55
Among birds within C and D	18	0.753	
Graders (G)	2	0.176	1.43
Gx among birds	70	0.081	0.66
Gx among C and D	34	0.035	0.28
Gx among birds within C and D	36	0.123	

Treatment[c]	1	3	2	5	4	6
Avg. thoracic aorta score	1.06	1.11	1.22	2.06	3.28	3.56
$P < 0.01$	————	————	————		————	————

Table 27. Analysis of variance[a] and multiple range[b] test of abdominal aorta scores, experiment IV

Source of variation	d.f.	m.s.	F
Total	107	1.297	
Among birds	35	3.793	
Among chambers and diets	17	7.013	
Chamber (C)	1	0.750	1.00
Diet (D)	5	22.787	30.26**
C x D and remainder	11	0.412	0.55
Among birds within C and D	18	0.753	
Graders (G)	2	0.176	1.43
Gx among birds	70	0.081	0.66
Gx among C and D	34	0.035	0.28
Gx among birds within C and D	36	0.123	

Treatment[c]	1	3	2	5	6	4
Avg. abdominal aorta score	2.06	2.33	2.83	3.00	3.72	3.94
P < 0.05						
P < 0.01						

Table 28. Regression on time within treatment, testing for linear (L) and quadratic (Q) effects over the length of the experimental period for certain physiological responses to various diets, experiment IV

Diet	Significance level[a]					
	Blood pressure		Serum cholesterol		Serum lipid	
	L	Q	L	Q	L	Q
No added cholesterol						
Basal	--	--	--	--	**	--
Energy restriction	**	--	--	--	--	--
Protein restriction	--	--	--	--	--	**
Added cholesterol						
Basal	--	--	**	**	**	**
Energy restriction	**	--	--	--	**	**
Protein restriction	--	--	**	**	**	**