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The Effects of High Fat Diets on Laying Hens

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MASTER OF SCIENCE THESIS

THE EFFECTS OF HIGH FAT DIETS

ON LAYING HENS

BY

SUSANA NGWENYI ALLO

Approved:

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

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Dean of the Graduate School

UNIVERSITY OF RHODE ISLAND

1966

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ABSTRACT

MASTER OF SCIENCE THESIS

OF

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1966

ABSTRACT

The objectives of this study were: (1) To determine the effects of feeding a corn-soybean type diet containing 25.0 percent stabilized yellow grease to Single Comb White Leghorn hens. (2) To determine the effects of such a diet when the protein, total vitamin and mineral contents of the diet were varied. In a six month study, four groups of 144 hens each were fed four experimental diets. Diet 1 represented a normal corn-soybean type laying mash. Diets 2, 3 and 4 each contained 25.0 percent stabilized yellow grease but varied in their protein, total vitamin and mineral contents. The experimental factors measured were: rate of egg production, feed consumption, feed conversion, egg weight, interior egg quality, egg fertility and hatchability, hen body weight, hen mortality, incidence of apparent fatty livers and blood serum and egg yolk cholesterol concentrations.

The addition of 25.0 percent stabilized yellow grease to the diet significantly lowered the rate of egg production and feed consumption. The feed conversion values were not significantly different between dietary treatment groups.

The addition of 25.0 percent stabilized yellow grease to the diet had no significant effect on either egg weight, interior egg quality, egg fertility, hatchability

or incidences of apparent fatty livers.

The largest body weight gains were observed with hens consuming a ration containing 25.0 percent stabilized yellow grease, 24.5 percent protein and an increased vitamin and mineral content. In comparison to the control group, the rate of hen mortality increased when fat was added to the diet without a corresponding increase in the protein, total vitamin and mineral content.

Egg yolk and blood serum cholesterol levels were not affected when a diet containing 25.0 percent stabilized yellow grease was fed to White Leghorn hens for a period of six months.

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The author wishes to acknowledge gratefully, the assistance given by Dr. R. S. Hinkson, Dr. L. T. Smith, Professor B. W. Henderson and Dr. W. K. Durfee in the preparation of this thesis.

Sincere appreciation also goes to Dean J. W. Cobble, Dean of College of Agriculture for his valuable suggestions made throughout the study.

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The author affectionately dedicates this thesis to

MR. and MRS. G. ALLO NJAH

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The use of added animal fat in high-energy type broiler rations has received considerable attention during the past several years. Although the advantages of fat supplementation to broiler diets have been amply demonstrated, the value of adding fat to the laying hen ration is still obscure. Relatively few experiments were conducted prior to 1940, pertaining to high energy diets for the laying hen. During the past sixteen years, the nutrient requirements of the laying hen, when fed diets containing relatively large quantities of animal fat or vegetable oils,

INTRODUCTION

It is generally accepted that chickens, as well as other animals, regulate their feed intake primarily to satisfy their energy requirements. Dietary energy studies with laying hens have indicated that feed conversion (pounds of feed required to produce a dozen eggs) may be improved by increasing the quantity of the energy-producing ingredients in their feed. It has been demonstrated that as much as 12.0 percent animal fat or vegetable oil when added to the hen's diet will improve her egg laying performance. Fats and oils are added to livestock feed by the animal and poultry nutritionists as high energy feed ingredients. Fats provide two and one-quarter times more physiological fuel value for animals than do carbohydrates and proteins.

The use of added animal fat in high-energy type broiler rations has received considerable attention during the past several years. Although the advantages of fat supplementation to broiler diets have been amply demonstrated, the value of adding fat to the laying hen ration is still obscure. Relatively few experiments were conducted prior to 1950, pertaining to high energy diets for the laying hen. During the past sixteen years, the nutrient requirements of the laying hen, when fed diets containing relatively large quantities of animal fat or vegetable oils,

has received increased attention.

The observations made in previous studies indicate that there is an increase in feed efficiency when laying hens consume diets containing up to 12.0 percent added animal fat. Controversial evidence concerning the deleterious effects of adding high levels of fat to the diet of the laying hen include its effect on the rate of egg production, fertility, hatchability and egg yolk and blood serum cholesterol concentrations.

The rate of egg production and the feed consumption may be reduced when more than 12.0 percent animal fat is added to the diet of the laying hen. Obesity, deranged fat metabolism and excessive mortality have also been associated with hens maintained on such high-fat diets. It has been observed that egg fertility and hatchability are reduced when breeding hens are fed diets containing relatively large amounts of animal fat. It is possible that egg yolk and blood serum cholesterol levels may be increased when a high level of animal fat is added to the diet of the laying hen.

The objectives of this experiment were two-fold:

- (1) To determine the effects on egg production, egg fertility, hatchability, egg weight, and blood serum and egg yolk cholesterol concentrations when feeding a diet containing 25.0 percent stabilized yellow grease (rendered

beef fat) to laying hens. (2) To study the effects of diets containing 25.0 percent stabilized yellow grease but varying in apparent crude protein, total vitamin and mineral contents, on the above experimental factors.

animal fats and vegetable oils as sources of energy for growing chickens. Numerous investigators have reported that animal fats and vegetable oils will improve the growth rate of chicks when added to their diet at levels as high as 33.8 percent (Gibler et al., 1957; Jacobson et al., 1957; Carey, 1963). However, the effects of adding fat to the laying hen's diet have not been completely elucidated.

Egg production and Feed Efficiency

Prior to 1960, very few studies were reported in the literature pertaining to the use of relatively high-fat diets for laying hens. During the past sixteen years, numerous research workers have reported their results on this subject.

Skinner et al. (1951) presented evidence that egg production and feed efficiency were enhanced by feeding a diet to laying hens, which was low in fiber and high in energy. Lillie et al. (1957) reported that feeding a diet containing eight percent added animal fat to laying hens had no deleterious effects on egg production. These results were in close agreement with those of Turk et al.,

II

REVIEW OF THE LITERATURE

Considerable research has been conducted using animal fats and vegetable oils as sources of energy for growing chickens. Numerous investigators have reported that animal fats and vegetable oils will improve the growth rate of chicks when added to their diet at levels as high as 33.8 percent (Siedler et al., 1953; Donaldson et al., 1957; Carew, 1963). However, the effects of adding fat to the laying hen's diet have not been completely elucidated.

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(1958).

Millar (1960) showed that significantly reduced egg production and increased body weight gains were obtained by feeding Rhode Island Red hens nutrient-balanced diets containing 30.4 percent added stabilized animal fat. The rate of egg production was not affected by the addition of either 8.2 or 18.2 percent fat to the diet of the laying hen. On the other hand, a significant decline in egg production was obtained when Fisher (1957) fed a diet containing 10.0 percent added animal fat to Single Comb White Leghorns. Fisher also showed that the addition of 10.0 percent animal fat to the diet resulted in obesity and deranged fat metabolism. The results obtained by Donaldson (1962) were in agreement with those of Fisher (1957). The results of Donaldson's study suggested that a reduction in egg production by White Leghorn hens was associated with an increase in body weight gains when the hens were fed a ration containing 30.4 percent added animal fat.

Treat et al. (1959) compared the effects of added animal fat and vegetable oils upon egg production, feed efficiency, egg weight and mortality. Their results revealed no significant differences in egg production by feeding a diet supplemented with either 2.5 or 5.0 percent added animal fat. These workers also observed no difference in egg production between laying hens maintained on diets

containing either 2.5 or 5.0 percent of a hydrolyzed mixture of animal fat and vegetable oils. The mixture of animal fat and vegetable oil improved feed efficiency as much as 6.09 percent. March and Biely (1963) observed that feeding laying hens a diet containing either 5.0 or 10.0 percent added animal tallow significantly decreased feed consumption, but had no effect on egg production.

Lillie et al. (1952) found that the addition of 10.0 percent lard to the laying ration enhanced body weight gains and improved feed efficiency in Rhode Island Red hens. However, it was observed that the lard had no marked effect on egg production. Singsen et al. (1952) and Gerry (1954) observed that feed efficiency was improved when laying hens were fed a diet containing a high concentration of energy producing ingredients as compared with a diet having a low energy value.

Hill et al. (1956) studied the effects of diets having a wide range of energy values on the rate and efficiency of egg production. They found that increasing the energy content of the hen's diet resulted in increased feed efficiency at a rate of approximately two percent for each one percent of added dietary fat.

Price et al. (1957) fed diets containing added animal fat to White Leghorn laying hens. They found that the amount of feed required to produce a dozen eggs decreased as the

energy level of the laying ration increased. The above findings suggest that feed utilization is enhanced when up to 10.0 percent animal fat is added to the laying hen's diet.

The interrelationship between the energy level and the protein content of poultry rations has attracted much attention among poultry nutrition researchers in recent years. March and Biely (1954) reported that feed efficiency was depressed when laying hens consumed a diet containing 19.0 percent protein and either 5.0 or 10.0 percent added animal fat. However, feed efficiency was enhanced by feeding laying hens a diet containing either 5.0 percent animal fat and 24.0 percent protein or 10.0 percent animal fat and 28.0 percent protein. Combs and Romoser (1955) confirmed the work of March and Biely (1954) and suggested the use of an adequate calorie:protein ratio when formulating a laying ration.

Miller et al. (1957) observed that both egg production and feed efficiency were increased when White Leghorn pullets were fed a diet containing 10.0 percent white grease and 20.9 percent protein. The research conducted by Hill and Anderson (1955) suggests that a diet containing five percent yellow grease and either 15.0, 16.5 or 18.0 percent protein increased the rate of egg production. However, the results of a preliminary study showed that the

addition of three percent yellow grease to a diet containing either 15.0 or 19.0 percent protein did not significantly affect the rate of egg production.

Weight and Interior Egg Quality

Miller et al. (1957) noted that when a diet containing 10.0 percent white grease was fed to laying hens, egg weight was not affected. There were no significant effects on interior egg quality when Orr et al. (1958) fed a diet containing either 2.5 or 5.0 percent animal fat to laying hens. The effect of adding moderate amounts of animal tallow to the diet of laying hens on egg size and interior egg quality was studied by March and Biely (1963). They found that egg size was significantly reduced when the hens were fed a diet containing either 5.0 or 10.0 percent added animal tallow. The addition of tallow to the diet had no significant influence on hen mortality and albumin quality. In 1959, Dagher et al. found no significant differences in egg production, egg size and exterior and interior egg quality when laying hens were fed diets containing either 12.0 percent soybean oil or 12.0 percent animal grease.

Fertility and Hatchability

Poultry nutrition research workers have reported that egg fertility and chick hatchability are adversely affected

when certain animal fats are incorporated in the diet of the breeding hen. Hatchability appears to be more seriously affected than fertility. Lillie et al. (1957) and Hochreich et al. (1958) reported no adverse effects on egg fertility and chick hatchability when diets containing either 8.0 percent stabilized lard or 6.6 percent stabilized yellow grease were fed to laying hens. These findings were in harmony with earlier work reported by Heywang (1942). Heywang compared the reproductive performance of four groups of White Leghorn pullets each of which was fed one of four dietary treatments. One group of birds was fed a basal diet, which had a total fat content of less than 1.0 percent. The remaining three groups of hens were fed diets containing either 2.0, 4.0 or 8.0 percent added corn oil. There was no significant difference in either fertility or hatchability between the treatment groups.

Hen Body Weight and Mortality

Hill and coworkers (1956) found that during the production year, the body weight of laying hens increased as the energy content of their diet increased. March and Biely (1962) conducted an experiment with caged layers to determine the effect of added dietary fat and high energy on hen mortality. The birds were fed the experimental diets from one day to three years of age. The birds were separated into

three dietary treatment groups. The experimental rations consisted of a control diet containing 2.5 percent and two diets containing 7.5 and 12.5 percent total dietary fat. The energy level of the above diets were maintained constant by adding equal amounts of ground oat hulls to the fat supplemented diets. Their results indicate that there were no deleterious effects on hen performance attributable to added dietary fat. March and Biely stated, "the lack of general adverse effects from the addition of fat to the diet, may be explained by the fact that the level of energy in the diets was not increased with the level of supplementary fat."

Incidence of Liver Derangements

Weiss and Fisher (1957) observed a disturbance in the lipid metabolism of hens consuming diets ranging from 5.0 to 10.0 percent added animal fat. They observed: (1) fatty deposits in and around the kidney; (2) friable and fatty livers; and (3) excessive fat deposits in the subcutaneous and abdominal tissue. Donaldson and Gordon (1960) observed a similar phenomenon when the laying diet contained only three percent added fat.

March and Biely (1962) found that the rate of mortality of White Leghorn hens which could be attributed to liver derangements, was approximately double when the hens were

fed diets containing either 5.0 or 10.0 percent added feed-grade animal fat. They reported that feeding such a diet to laying hens resulted in obesity and fatty livers. This led to a high rate of hen mortality.

An experiment conducted by Edwards et al. (1962) pointed out that liver fat deposits were significantly increased when 10.0 percent lard was added to the diet of Single Comb White Leghorn hens. The liver fat deposits were significantly decreased when 10.0 percent corn oil or beef tallow was added to the diet.

Egg Yolk Cholesterol

Cruickshank (1934) found that when either beef tallow, soybean oil, safflower oil or linseed oil was added to the diet of Light Sussex hens at the rate of 28.0 percent, the cholesterol content of the egg yolk remained essentially constant.

Edwards et al. (1962) maintained White Leghorn laying hens on a corn-soybean type basal diet supplemented with 10.0 percent of either corn oil, lard or beef tallow. The analyses of their data indicated no significant influence on the serum cholesterol level of the hens due to added dietary fat or oil. A significant difference was noted between the egg yolk cholesterol concentrations. Their data indicated that the eggs from laying hens maintained on a

diet containing 10.0 percent corn oil had an elevated egg yolk cholesterol. The average egg yolk cholesterol level ranged from 35.1 to 40.9 mg/gm egg yolk in the control and the fat treated birds, respectively. Egg production, egg weight, hen body weight, liver weight and liver cholesterol levels were not significantly influenced by the addition of 10.0 percent dietary animal fat or corn oil.

Blood Serum Cholesterol

Recent interest in the relationship between dietary ingredients and the metabolism of cholesterol and fatty acids in many animals has stimulated investigation in this area of poultry nutrition. Price et al. (1957) fed three diets differing in energy content to Single Comb White Leghorn laying hens. The addition of poultry fat to the diet of the laying hens at a level as high as 14.0 percent had little or no influence on serum cholesterol concentration. They also observed that the serum cholesterol content of caged layers was elevated above that of floor layers. It was noted also that the serum cholesterol content of floor housed layers was elevated above that of young pullets maintained under similar conditions. The average serum cholesterol level of the floor-housed layers and non-layers was 272 and 248 mg/100 ml. respectively.

Johnson et al. (1959) fed diets which ranged in

animal tallow content from 3.6 to 14.5 percent to various groups of New Hampshire pullets. A chemical analysis of the pullets' plasma cholesterol indicated no significant differences between treatment groups. The average plasma cholesterol level was 196, 210, 204 and 231 mg/100 ml. for the birds receiving 3.60, 5.80, 12.30 and 14.50 percent added dietary tallow, respectively. Weiss and Fisher (1957) reported an elevation in the plasma cholesterol level of laying hens maintained on a diet containing 10.0 percent added animal fat. The plasma cholesterol content was 172.9 and 231.2 mg./100 ml. for the control and the dietary treatment groups of birds, respectively.

Daghir et al. (1959) fed separate groups of White Leghorn hens individual diets containing no added fat, 12.0 percent soybean oil and 12.0 percent added animal grease. When the blood serum was quantitatively analyzed for cholesterol content, a significant decrease in the serum cholesterol content was noted only with hens receiving the diet containing the added soybean oil.

III

EXPERIMENTAL PROCEDURE

Birds, Feed and Management

The experimental birds, 576 hens and 48 males, were Single Comb White Leghorns. On the initial date of the experiment, the birds were 34 weeks of age. The birds were selected at random from the University of Rhode Island Agricultural Experiment Station's replacement flock. The hens were debeaked before housing.

The experimental flock was randomly divided into 16 floor pens which were located in two non-insulated laying-type houses. Building 1 had a removable plastic front whereas Building 2 had a permanent wooden front. There were four dietary treatment groups each of which was composed of four randomly assigned replicates consisting of 36 hens and 3 males. The males were rotated every seven days between replicates within each treatment group. Each pen had 150.0 square feet of floor space, allowing 3.85 square feet per bird. Each pen contained open front nests, a floor type feeder (five feet long and accessible from both sides), an automatic watering system and processed sugar cane litter. Natural day light was supplemented by artificial light to give a constant 14.0 hour light period.

Up to the day the experiment started, the birds were fed a practical corn-soybean type layer ration. The birds were fed the experimental diets from November 22, 1965 to May 10, 1966. All birds had free access to feed and water throughout the experimental period. The composition of the four experimental diets is presented in Table I. Diet 1 (no added fat) which was fed to the hens in Group 1, served as the control diet. As calculated, 25.0 percent stabilized yellow grease was added to diets 2, 3 and 4, which were fed to the hens in Groups 2, 3 and 4, respectively. The total vitamin and mineral content in diet 4 was increased by 20.0 percent in relation to diets 1, 2 and 3. The protein content of diets 1, 2, 3 and 4 was chemically analyzed to be 18.7, 16.9, 19.3 and 24.5 percent, respectively. The ingredients, which varied in quantity were added to the diets at the expense of ground yellow corn and soybean meal. The dietary protein content of each diet was maintained at the desired value throughout the experiment.

The feed ingredients used in the preparation of each of the diets were thoroughly mixed, in 500 pound quantities, in a horizontal-type feed mixer. As a precautionary measure, the diets were prepared every 14 to 15 days. This step was taken as a margin of safety to help minimize feed oxidation which may occur with a feed containing such a high level of fat.

TABLE I. Composition of the experimental diets.

<u>Ingredient</u>	<u>Diet</u>			
	<u>1</u>	<u>2</u> (per cent)	<u>3</u>	<u>4</u>
Corn, ground yellow	65.5	40.5	33.2	22.4
Soybean meal	23.0	23.0	30.3	39.3
Alfalfa meal	2.0	2.0	2.0	2.0
Stablized yellow grease	0.0	25.0	25.0	25.0
Limestone	6.0	6.0	6.0	7.2
Dicalcium phosphate	1.5	1.5	1.5	1.8
Vitamin and trace mineral premix*	1.5	1.5	1.5	1.8
Salt	0.5	0.5	0.5	0.5
TOTAL	100.0	100.0	100.0	100.0
Metabolizable Energy, calculated (Cal/lb.)	1253.0	1669.0	1629.0	1555.0
Protein, analyzed (%)**	18.7	16.9	19.3	24.5
Fat, analyzed (%)**	2.8	25.3	25.8	27.1

* Calculated to supply per pound of diet: riboflavin, 2.8 mg; niacin, 12.6 mg; d-pantothenic acid, 5.6 mg; vitamin A, 3,051 I.U.; vitamin D₃, 560 I.C.U.; vitamin B₁₂, 10 mcg; manganese, 30.72 mg; iron, 9.14 mg; copper, 0.91 mg; iodine, 0.61 mg; cobalt, 90 mcg.

**Average analysis taken from three separately prepared feeds.

Performance Data

Performance data was measured on the basis of six-28 day periods. Eggs were collected three times a day and daily egg production records were maintained for each pen. At the end of each 28 day period, percent egg production was determined on the hen-day basis. On one day of each week, throughout the experimental period, all eggs were identified as to pen number and weighed on a group basis. The average hen body weight in each pen was recorded initially, at the end of the third month and at the termination of the study. Feed consumption was recorded at the end of each 28 day period. Feed conversion was calculated on the basis of the pounds of feed required to produce a dozen eggs.

Mortality records were maintained on a per pen basis. All dead birds were identified as to dietary treatment group and sent to the Animal Pathology Diagnostic Laboratory at the University of Rhode Island for post mortem examinations. The causes of all deaths were recorded. When a male bird died, he was immediately replaced with an apparent healthy male.

Egg fertility and hatchability were determined in the following manner: The eggs were collected, identified as to pen number and held at a temperature of 55°F for two

successive days prior to the incubation period. The number of eggs set per pen was limited to the lowest number of eggs collected from any one pen during the two day collection period. Eggs were incubated once during the first 28 day period, twice during the fourth 28 day period and once during the final 28 day period.

The eggs were candled on the seventh day of incubation and those which showed no signs of chick development were broken out and examined for apparent infertility. The eggs which possessed a firm yolk and a clear germinal disc were recorded as apparent infertile eggs. The number of early dead embryos was also recorded. On the 21st day of incubation, the eggs which did not hatch were broken out, examined, and the results were recorded.

At the termination of the experiment, three eggs were taken at random from each replicate or pen of birds. These eggs were individually weighed, broken out and albumin height and shell thickness measured. The Haugh Unit Score (Haugh, 1937) for each egg was calculated. A micrometer, graduated in thousandths of an inch, was used to measure egg shell and membrane thickness.

Liver Examination and Cholesterol Analysis

At the termination of the experiment, twelve hens from each dietary treatment group (three from each repli-

cated pen) were selected at random and identified accordingly. The body weight of each hen was recorded. A 5.0 ml. sample of blood was taken from each hen via heart puncture. The blood samples were allowed to stand overnight in order to insure complete coagulation of the blood cells. After taking the blood samples, the hens were sacrificed, and the liver of each bird was visually examined for excessive fat deposits.

The serum was removed from the clotted blood samples and centrifuged in a Clinical Centrifuge. Two aliquots of 0.10 ml. each were taken from each serum sample and analyzed for cholesterol content by the method of Zlatkis et al. (1953).

Four eggs were collected at random from each experimental pen on the final day of the experiment. The intact eggs were individually weighed on a gram balance and then boiled for twelve minutes. After the eggs had been boiled, the yolk of each egg was removed and weighed. A 2.0 gram sample of each yolk was placed in a 125 ml. Erlenmeyer flask containing 50 mls. of a 2:1 chloroform-methanol solution. The flasks and their contents were placed on a vibrating platform and extracted for two hours at approximately 140 oscillations per minute (Harris and Wilcox, 1963). The contents of each flask were filtered through Whatman No. 1 filter paper and brought up to volume in a 50 ml. volu-

metric flask. Two-0.2 ml. aliquants of each diluted filtrate were analysed for cholesterol content by the method of Zlatkis et al. (1953).

Design and Method of Statistical Analyses

A completely randomized block design was used in this experiment. The analysis of variance (Snedecor, 1956) was applied to the egg production, feed consumption, feed efficiency, egg fertility, and hatchability and to the cholesterol data. All statistical differences between the means of each treatment group were determined by Duncan's Multiple Range Test (Duncan, 1955).

1. Prior to the onset of the experiment, egg production for all birds averaged 61.06 percent as calculated on a per-day basis.

A three way analysis of variance (Snedecor, 1956) showed that the diets fed to the hens significantly ($P < 0.01$) influenced the rate of egg production, Table III, page 20. The data in Table II, indicates that the rate of egg production is greatly reduced when the laying hens were fed a corn-soybean-type diet containing 25.2 percent stabilized yellow grease. The rate of egg production was significantly lower in Groups 2, 3 and 4 when compared to the rate of egg production in Group 1 in which the birds were fed the control diet containing no added fat. The rate of egg production

RESULTS AND DISCUSSION

Four groups of 144 White Leghorn hens were each fed one of four dietary regimes. The constituents in the diets which varied in quantity are presented in Table I (page 16).

The birds which were fed the four diets were designated as Groups 1, 2, 3 and 4, respectively.

Egg Production

The rate of egg production of the hens placed on the four experimental diets is presented in Table II and Figure 1. Prior to the onset of the experiment, egg production for all birds averaged 61.06 percent as calculated on a hen-day basis.

A three way analysis of variance (Snedecor, 1956) showed that the diets fed to the hens significantly ($P < 0.01$) influenced the rate of egg production, Table XII, page 60. The data in Table II indicates that the rate of egg production is greatly reduced when the laying hens were fed a corn-soy-bean-type diet containing 25.0 percent stabilized yellow grease. The rate of egg production was significantly lower in Groups 2, 3 and 4 when compared to the rate of egg production in Group 1 in which the birds were fed the control diet containing no added fat. The rate of egg production

TABLE II. Average hen-day egg production of the birds fed the four dietary regimes.

Period*	Diet			
	<u>1</u>	<u>2</u> (per cent)	<u>3</u>	<u>4</u>
Preliminary	65.47	62.38	60.54	55.41
1	64.29	41.78	43.12	44.03
2	62.48	46.44	54.36	47.14
3	61.87	48.02	51.16	51.69
4	65.79	45.15	54.34	55.85
5	64.78	44.44	55.57	57.46
6	60.30	49.53	51.96	52.62
Average**	63.25a	45.89b	51.74b	51.47b

* Each period represents 28 successive days.

**Means followed by different letters are significantly different at the .01 level of probability as determined by the Multiple Range Test (Duncan, 1955).

Figure 1. Egg production of the hens fed the four dietary regimes.

between Groups 2, 3 and 4 was not significantly different (Table II). The hens fed diet 2 which contained 25.0 percent stabilized yellow grease and 16.9 percent protein revealed the lowest rate of egg production of the four dietary treatment groups.

During the first 28 day period, a considerable drop in egg production was observed in Groups 2, 3 and 4. By comparison, during this same period, egg production declined only slightly in Group 1 where the birds were fed the control diet. The average rate of egg production for the birds fed diet 1 was 64.29 percent during the first 28 day experimental period. Egg production during this period for the hens fed diets 2, 3 and 4 was 41.78, 43.12 and 44.03 percent, respectively. With reference to the preliminary period, egg production of the birds fed diets 2, 3 and 4 declined at a rate between 18.0 and 33.0 percent during period one. The decline in egg production for the hens fed diet 4 during this period was less than that of the birds fed diets 2 and 3. In addition to containing 25.0 percent stabilized yellow grease, diet 4 also contained higher levels of protein, and total vitamins and minerals than did diets 2 and 3. The birds fed diet 4 consumed approximately 0.02 pounds (9.1 gms) more feed per day than did the birds in Groups 2 and 3. Therefore, these birds were not only consuming more protein, vitamins and minerals

but total feed as well. This may explain the smaller decline in the rate of egg production observed in Group 4 as compared to Groups 2 and 3 during period one.

A gradual and continuous decline in egg production was observed in Group 1 during the first three periods of the experiment. This trend may be attributed to the relatively lower energy level of this diet as compared to that of diets 2, 3 and 4. The rate of egg production by the hens in Groups 2, 3 and 4 increased during the second period and remained relatively high throughout the experiment when compared to their level of egg production during period one. This trend in egg production for Groups 2, 3 and 4 may be explained in terms of reduced palatability of the fat-supplemented diets. The birds in Groups 2, 3 and 4 consumed more feed after they became accustomed to it than they did during the first period. The observed increase in feed consumption (Table III, page 30) is to be expected since egg production followed a similar trend.

Fisher (1957) observed a significant decline in the rate of egg production when Single Comb White Leghorn pullets were fed diets containing 10.0 percent added animal fat. Later results obtained by Donaldson (1962) supported those of Fisher (1957). Donaldson studied the effect of restricting the intake of a diet high in animal fat on the performance of White Leghorn pullets. He observed that

restricting the intake of a 30.4 percent fat-containing diet to a level of nutrient intake comparable to that of the control group resulted in reduced egg production. The result of this experiment indicates that the reduction in the rate of egg production was due to the added dietary fat and not to the dietary energy level per se. Miller et al. (1957) observed that egg production was enhanced when White Leghorn pullets were fed a diet containing 10.0 percent added white grease.

During the sixth period, the rate of egg production declined by approximately five percent in all groups with the exception of Group 2. In this group, the rate of egg production increased by 5.09 percent over that observed during the fifth period. This result is difficult to explain since this group of birds was receiving the diet containing the lowest protein content and since feed consumption (Table III, page 30) by this group of birds did not change relatively when compared to Groups 3 and 4. It was also noted that the rate of egg production by the birds in Group 4 was significantly lower ($P < 0.01$) than that of the birds on the control diet during the entire six months period. The dietary constituent values in Table I, page 22, point out that diet 4, which was fed to the birds in Group 4, not only contained 25.0 percent stabilized yellow grease, it also supplied 31.0 percent more protein and 20.0 percent

more total vitamins and minerals than did the control diet. It was concluded that the reduction in egg production in Groups 2, 3 and 4 was due to the feeding of diets containing 25.0 percent stabilized yellow grease. However, the data in Table II does indicate that the increased protein level of diets 3 and 4, more so than the increased vitamin and mineral content of diet 4, was responsible for the average six percent higher egg production when compared to that noted in Group 2. A study conducted by Millar (1960) showed that there was no significant difference in egg production, attributable to dietary changes in fat level when either 8.2 or 18.2 percent animal fat was added to the diet of the laying hen. However, he reported that the rate of egg production was significantly reduced when laying hens were fed a diet containing 30.4 percent added animal fat. The diets formulated by Millar varied in animal fat content and energy level but were balanced with respect to other nutrients.

There was a significant ($P < 0.05$) interaction between building and period during the experiment (Table XII, page 60 and Figure 2). During the first four periods, the rate of egg production was significantly higher in Building 2 than in Building 1. The rate of egg production was lower in Building 2 than in Building 1 during the final two periods. The difference in egg production between buildings

Figure 2. Hen-day egg production for Buildings 1 and 2 over the test period.

may be attributed to the structure of these buildings and the resultant temperature changes in them during the experimental periods. Building 2 had a permanent wooden front whereas Building 1 had a removable plastic front. The higher rate of egg production in Building 2 may be an indication that this building might have more easily retained its heat during the winter; but was less readily heated by the sun than Building 1.

Feed Consumption and Feed Conversion

The addition of 25.0 percent stabilized yellow grease significantly ($P < 0.05$) lowered the quantity of feed consumed per one hundred hens per day (Table III and Figure 3). Feed consumption in Group 1 was significantly higher than in Groups 2, 3 and 4. The differences in feed consumption between the birds on dietary treatments 2, 3 and 4 was not significant. These observations indicate that the increase in protein, vitamin and mineral levels in diet 4 did not affect feed consumption. Apparently, the birds fed the high fat-supplemented feeds, diets 2, 3 and 4, were able to meet their energy requirements with much less feed than did the birds in Group 1.

There was a significant ($P < 0.05$) variation between periods and a significant interaction between building and period (Table XIII, page 61). The birds in Building 2 consumed less feed than the birds in Building 1 during the

TABLE III. The average pounds of feed consumed per 100 hens per day.

Period*	Diet			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1	28.1	17.9	19.5	21.5
2	30.9	22.8	24.3	22.5
3	30.5	23.1	23.4	25.5
4	32.7	21.1	22.4	24.7
5	34.5	23.3	23.9	23.2
6	31.7	25.7	27.0	25.7
Average**	31.4a	22.3b	23.4b	23.9b

*Each period represents 28 successive days.

**Means followed by different letters are significantly different at the 5.0 per cent level of probability.

Figure 3. Pounds of feed consumed per 100 hens per day.

Figure 4. Feed consumption per 100 hens for Buildings 1 and 2 over the test period.

first two and the final two periods of the experiment (Figure 4). The front of Building 2 was permanently enclosed with wood while Building 1 had a removable plastic covered front. Due to the difference in the structure of these buildings, Building 2 may have been warmer than Building 1 during the first three periods. This may account for the fact that the birds which were housed in Building 2 required less feed than the birds in Building 1 to maintain their body temperature.

The data in Table IV and Figure 5 indicate that feed conversion, the pounds of feed consumed for each dozen eggs produced, was not affected by feeding a diet containing 25.0 percent stabilized yellow grease to the laying hens. The analysis of variance (Snedecor, 1956), indicates that the feed-egg ratio remained essentially the same for all dietary treatment groups (Table XIV, page 62). As mentioned earlier there was a significant decrease in the rate of feed consumption by the birds which were fed diets 2, 3 and 4. However, there was no difference when the feed conversion in these groups was compared with the control. These results may be explained by the fact that egg production was also reduced in groups 2, 3 and 4 when compared to Group 1. These findings are in agreement with earlier observations by Millar (1960). He reported no significant difference in feed conversion between birds fed diets ranging in added

TABLE IV. Average pounds of feed consumed for each dozen eggs produced.

Period [*]	Diet			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1	5.25	5.22	5.49	5.96
2	5.94	5.94	5.28	5.72
3	6.01	5.90	5.93	6.05
4	6.01	5.64	5.07	5.31
5	6.42	6.33	5.51	4.90
6	6.39	6.30	6.38	5.90
Average	6.00	5.89	5.61	5.64

* Each period represents 28 successive days.

Figure 5. Pounds of feed consumed per dozen eggs produced.

Figure 6. Pounds of feed to produce a dozen eggs for Buildings 1 and 2 over the test period.

animal fat from zero to 30.4 percent. In contrast, Lillie et al. (1952), Price et al. (1957) and Orr et al. (1958), demonstrated that improved feed conversion resulted when fat was added to laying hen rations at levels up to 12.0 percent.

There was a significant ($P < 0.01$) interaction between building and period pertaining to feed conversion (Table XIV and Figure 6). The birds in Building 1 consumed significantly more feed per dozen eggs produced than did the birds in Building 2 during the third period. These birds also produced less eggs than the birds in Building 2 during this period. The above results may be an indication that, due to its structure, Building 1 with the removable plastic front may have been colder than Building 2 during this period which occurred during the month of January. If this was the case, the birds in this building would have required more feed to maintain their body temperature.

Egg Weight and Egg Quality

Egg weights, recorded throughout the study, are presented in Table V. The data pertaining to exterior and interior egg quality and Haugh unit score taken from each dietary treatment group is given in Table VI. An analysis of variance (Table XV, page 63) showed no significant differences between dietary treatment groups in egg weight,

TABLE V. Egg weight data collected throughout the experiment.

Period*	Diet			
	1	2	3	4
	(grams)			
1	53.25	52.13	52.05	53.40
2	55.84	54.88	54.94	55.28
3	56.30	56.30	58.30	56.45
4	56.83	57.03	57.47	58.37
5	57.70	57.00	57.65	58.63
6	58.03	58.30	58.80	59.53
Average	56.33	55.94	56.54	56.94

* Each period represents 28 successive days.

TABLE VI. The exterior and interior quality^{*,**} of eggs produced by hens on the various dietary regimes.

	Diet			
	1	2	3	4
Egg Weight (gms/egg)	62.41	59.71	59.43	60.28
Albumin height (0.1mm)	6.74	7.98	7.41	6.83
Shell and membrane thickness (0.001 inch)	.0165	.0164	.0163	.0178
Haugh Unit Score	80.79	88.67	85.88	81.46

*Twelve eggs were picked at random from each dietary treatment group.

**The determinations were conducted at the termination of the experiment.

However, there was significant variation ($P < 0.01$) in egg weight between periods. The average egg weight for six periods was:

Period	1	2	3	4	5	6
Egg Weight (grams)	52.71	55.24	56.84	57.43	57.75	58.67

No differences were observed between groups in albumen quality, egg shell and membrane thickness and Haugh unit score (Haugh, 1937). These findings were in accordance with earlier observations made by Orr *et al.* (1958). They reported that feeding diets containing as much as 12.0 percent added animal fat to laying hens had no deleterious effects on egg production, egg weight and interior egg quality.

Fertility and Hatchability

The fertility of eggs was not affected by the hen's diet (Table XVI, page 64). Table VII shows the fertility data obtained from four combined hatches. The results of this experiment indicate that feeding a diet containing 25.0 percent stabilized yellow grease to laying hens had no effect on subsequent egg fertility. This study also suggests that fertility was not affected when the hens were fed a 25.0 percent fat-supplemented diet containing relatively high levels of protein, vitamins and minerals, diet 4. These results are in agreement with the findings of

TABLE VII. The average egg fertility and hatchability for the four dietary treatment groups.

	Diet			
	1	2	3	4
Hatches, No.	4	4	4	4
Eggs incubated, No.*	303	304	304	304
Eggs, fertile, %	94.76	93.79	95.18	93.71
Embryonic mortality, %				
early dead (1-7 days)	1.65	7.57	3.95	3.62
late dead (8-21 days)	2.31	1.65	1.97	1.32
Hatchability, %				
of fertile eggs	94.76	89.83	92.58	92.34
of total eggs	89.78	84.24	88.07	86.59

* Eggs were incubated once during the first period, twice during the fourth period and once during the final period.

Lillie et al. (1957). In their study, the addition of 4.0 and 8.0 percent stabilized lard or 6.6 percent stabilized yellow grease to the diet of laying hens had no influence on either fertility or hatchability.

In this experiment, the hatchability of fertile eggs was not affected when 25.0 percent stabilized yellow grease was added to the laying hen's diet (Table XVII, page 65). Table VII shows the hatchability data obtained from four hatches. The analysis of variance (Table XVIII, page 66) indicates that there was no significant difference between dietary treatment groups in the hatchability of total eggs incubated. This indicates that supplementing the 25.0 percent fat-containing diet with relatively high levels of protein, total vitamins and minerals did not affect hatchability. These findings support earlier observations by Heywang (1942). His study suggests that diets low in fat or containing as much as 8.0 percent fat have no harmful effect on hatchability of eggs incubated.

In this experiment, the majority of the embryonic mortality occurred during the first seven days of the incubation period for Groups 2, 3 and 4. However, embryonic mortality for Group 1 was slightly higher during the last 14 days of incubation than it was during the first seven days of incubation.

Hen Body Weight and Mortality

The data in Table VIII shows the effect of feeding diets containing 25.0 percent stabilized yellow grease on the body weight of laying hens, during the six month experimental period. The hens in all treatment groups gained weight during the experiment. At the termination of the study, the birds in Groups 1, 2, 3 and 4 gained an average body weight of 0.26, 0.18, 0.06 and 0.54 pounds, respectively. Body weight gain in Group 4 was double the gain observed in Group 1. The birds in Group 4 also gained considerably more body weight than the birds in Groups 2 and 3. These results could be attributed to the relatively high level of protein, and total vitamins and minerals present in diet 4, which may have enabled the hens consuming this diet to utilize their feed more efficiently. Millar (1960) reported that significantly increased body weight gains were obtained when Rhode Island Red hens were fed nutrient-balanced diets containing 30.4 percent added stabilized animal fat. In the present study, the lower body weight gains for Groups 2 and 3 failed to substantiate earlier findings made by Fisher (1957). Fisher showed that obesity resulted when a diet containing 10.0 percent animal fat was fed to White Leghorn laying hens.

As shown in Table VIII, hen mortality during the six

TABLE VIII. Average body weight and mortality of hens fed the various dietary regimes.

Period	Diet			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1	4.07	4.11	4.10	4.03
3	4.22	4.09	4.32	4.33
6	4.33	4.29	4.16	4.57
Gain	.26	.18	.06	.54
Hen Mortality (per cent)	14.58	17.72	16.67	13.20

Incidence of Fatty Livers

Table IX shows the observations made when the livers of 12 hens from each dietary treatment group were analyzed at the termination of the experiment. The degree of fatty infiltration of the liver was determined by visual examination. Incidences of apparent fatty livers were observed in all experimental groups. On the basis of the observations made, it is concluded that feeding a diet containing 13.0 percent stabilized yellow grease to laying hens for a period of six months did not increase the fat content of their

month experimental period was slightly increased when the birds were fed diets containing 25.0 percent stabilized yellow grease. Hen mortality was 14.58, 17.72, 16.67 and 13.20 percent in Groups 1, 2, 3 and 4, respectively. Hen mortality was lower in Groups 1 and 4 than in Groups 2 and 3. The birds in Group 4 received 25.0 percent added dietary stabilized yellow grease. In addition to the added fat, diet 4 also contained 31.0 and 20.0 percent higher protein and total vitamin and mineral content, respectively, than did diets 1, 2 and 3. It might be concluded that the rate of hen mortality in Groups 1 and 4 were similar because diet 4 more closely approximated diet 1 with respect to total nutrient intake per hen than did diets 2 and 3.

Incidence of Fatty Livers

Table IX shows the observations made when the livers of 12 hens from each dietary treatment group were examined at the termination of the experiment. The degree of fatty infiltration of the liver was determined by visual observation. Incidences of apparent fatty livers were observed in all experimental groups. On the basis of the observations made, it is concluded that feeding a diet containing 25.0 percent stabilized yellow grease to laying hens for a period of six months did not increase the fat content of their

TABLE IX. The incidences of fatty livers observed in hens consuming the four dietary regimes.

Relative liver-fat content*		
Diet	Normal livers (no. of hens)	Apparent fatty livers (no. of hens)
1	8	4
2	7	5
3	11	1
4	11	1

*Livers of 12 hens were examined by visual observation at the termination of the six month experimental period.

livers.

Egg Yolk Cholesterol

The average egg yolk cholesterol content for Group 1 was 1600.8 mg of cholesterol per 100 grams (Table X). The average cholesterol content of egg yolks from Groups 2, 3 and 4 was 1642.2, 1571.1 and 1570.3 mg/100 grams, respectively (Table X). The analysis of variance (Snedecor, 1956) pertaining to the egg yolk cholesterol data is given in Table XIX, page 67. There were no differences in egg yolk cholesterol level between dietary treatment groups.

Edwards et al. (1960) observed significant differences in egg yolk cholesterol concentrations among eight strains of hens. They reported that the egg yolk cholesterol level of eggs produced by White Leghorn hens fed a normal laying mash ranged from 1500.0 to 1730.0 mg/100 grams. Harris and Wilcox (1963) experimenting with White Leghorn hens reported that the average egg yolk cholesterol content was 2,410 mg/100 grams.

The studies of Cruickshank (1934) suggest that the yolk cholesterol content of eggs from hens fed diets devoid of cholesterol was not altered by adding fat to the diet at the rate of 28.0 percent. In contrast, Edwards et al. (1962) found that the cholesterol content of the egg yolk from White Leghorn hens was elevated when the birds were

TABLE X. The egg yolk cholesterol data from the four dietary treatment groups.*

Replicate	Diet			
	1	2	3	4
	(mg cholesterol/100 gms)			
1	1428.13	1671.88	1653.13	1700.00
2	1596.88	1681.25	1637.50	1537.50
3	1709.38	1553.13	1475.00	1568.75
4	1668.75	1662.50	1518.75	1475.00
Average	1600.79	1642.19	1571.10	1570.31

*The yolks of four eggs from each replicate within a treatment group were analyzed for cholesterol content at the termination of the experiment.

fed a corn-soybean-type diet containing 10.0 percent corn oil.

Blood Serum Cholesterol

The data regarding the blood serum cholesterol determination and its analysis of variance (Snedecor, 1956) is presented in Table XI and Table XX (page 68). There were no significant differences in the serum cholesterol concentration of hens fed the various dietary regimes for the six month experimental period. These results suggest the blood serum cholesterol concentration of White Leghorn hens is not altered appreciably by feeding them a diet containing 25.0 percent stabilized yellow grease. The results also indicate that relatively high levels of protein, vitamins and minerals in a high-fat laying ration has little influence on the bird's blood serum cholesterol concentration. These observations are in agreement with an earlier report by Johnson et al. (1959). They demonstrated that the blood serum cholesterol level of New Hampshire pullets was not significantly influenced when 14.5 percent animal tallow was added to the laying ration. On the contrary, Weiss and Fisher (1957) observed that when laying hens were fed diets containing up to 10.0 percent animal fat, their blood plasma cholesterol level was significantly elevated as compared to the control group receiving no added dietary fat.

TABLE XI. The blood serum cholesterol content of hens receiving four dietary treatments.*

Birds	Diet			
	1	2	3	4
	(mg/100ml)			
1	132.0	118.0	181.0	174.0
2	161.0	145.0	135.0	118.0
3	137.0	164.0	150.0	119.0
4	170.0	139.0	156.0	140.0
5	155.5	180.0	146.0	141.0
6	151.0	146.0	138.0	144.0
7	174.0	153.0	141.0	113.0
8	148.0	135.0	141.0	155.5
9	130.0	135.0	138.0	127.0
10	122.0	145.0	135.0	81.0
11	93.0	121.0	138.0	135.0
12	130.5	74.5	139.0	139.0
Average	142.0	138.0	144.8	132.2

* The blood from twelve hens on each dietary regime was analyzed at the termination of the experiment.

With reference to the findings of earlier investigators, it was expected that feeding a 25.0 percent fat containing diet to laying hens may influence their serum cholesterol level. However, in this experiment a statistically significant difference ($P < 0.01$) was observed between the cholesterol levels of birds housed in different buildings. The average serum cholesterol content for hens housed in Building 1 was 147.52 mg/100 mls. as compared with 130.98 mg/100 mls. for the birds in Building 2. These buildings were different in structure. Building 1 had a removable plastic front whereas Building 2 had a permanent wooden front.

The difference between the average blood serum cholesterol concentrations for the birds housed in each building could not be explained. However, it may be significant to note that the difference was not due to the diet consumed by the birds.

SUMMARY

In a six month study to determine the effects of high fat diets on laying hens, four groups of 144 Single Comb White Leghorn hens each, were fed four dietary treatments. Diet 1 (control) was a normal laying ration containing 18.7 percent protein but devoid of stabilized yellow grease. Diets 2, 3 and 4 each contained 25.0 percent stabilized yellow grease and either 16.9, 19.3 or 24.5 percent protein, respectively. Diet 4 also contained 20.0 percent more vitamins and minerals than did diets 1, 2 and 3.

The following results were obtained:

1. The diets containing stabilized yellow grease significantly lowered egg production.
2. Significantly less feed was consumed by hens fed diets containing stabilized yellow grease.
3. There were no significant differences in feed conversion between dietary treatment groups.
4. The diets containing added fat had no effect on either egg weight, interior egg quality, egg fertility or hatchability.
5. The greatest body weight gains were obtained with hens fed diet 4.

6. Hen mortality was slightly higher with hens fed diets 2 and 3. Increasing the protein, vitamin and mineral content of the fat supplemented diet appeared to enhance livability.

7. Apparent fatty livers were observed in all groups of birds.

8. Egg yolk and blood serum cholesterol concentrations were not significantly influenced by feeding the hens the various dietary regimes.

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APPENDIX

TABLE XII. The analysis of variance of the egg production data.

Source of variation	df.	Mean squares	F
Total	95		
Diet	3	1264.02	125.77**
Period	5	109.96	2.07
Building	1	218.31	4.34
Diet x period	15	40.94	.76
Diet x building	3	24.30	.47
Period x building	5	156.73	3.08*
Diet x period x building	15	41.54	.77
Error	48	54.14	

APPENDIX

** Highly significant at the .01 level of probability.

* Significant at the .05 level of probability.

TABLE XII. The analysis of variance of the egg production data.

Source of variation	df.	mean squares	F
Total	95		
Diet	3	1284.02	23.72**
Period	5	109.96	2.03
Building	1	218.31	4.03
Diet x period	15	40.94	.76
Diet x building	3	24.30	.45
Period x building	5	166.73	3.08*
Diet x period x building	15	41.54	.77
Error	48	54.14	

** Highly significant at the .01 level of probability.

*Significant at the .05 level of probability.

TABLE XIII. The analysis of variance of the feed consumption data.

Source of variation	df	mean square	F
Total	95		
Diet	3	399.78	35.41**
Period	5	82.34	7.29**
Building	1	9.25	.82
Diet x period	15	10.38	.92
Diet x building	3	34.38	.30
Building x period	5	30.08	2.66*
Diet x building x period	15	14.35	1.27
Error	48	11.29	

* Significant at the .05 level of probability.

** Highly significant at the .01 level of probability.

TABLE XIV. The analysis of variance of the feed conversion data.

Source of variation	df	mean squares	F
Total	95		
Diet	3	.88	1.49
Period	5	1.34	2.27
Building	1	.21	.36
Diet x period	15	.59	1.00
Diet x building	3	.16	.27
Building x period	5	2.40	4.07**
Diet x building x period	15	.85	1.44
Error	48	.59	

** Highly significant at the .01 level of probability.

TABLE XV. The analysis of variance of the egg weight data.

Source of variation	df	mean square	F
Total	95		
Diet	3	5.62	1.61
Period	5	69.30	19.89**
Building	1	5.32	1.53
Diet x period	15	2.19	.63
Diet x building	3	5.66	1.63
Building x period	5	3.07	.88
Diet x building x period	15	2.71	.78
Error	48	3.48	

** Highly significant at the .01 level of probability.

TABLE XVI. The analysis of variance of the data pertaining to percent fertility of eggs incubated.

Source of variation	df	mean square	F
Total	63		
Diet	3	8.46	.28
Hatch	3	63.10	2.06
Building	1	3.44	.11
Diet x hatch	9	18.12	.59
Diet x building	3	31.59	1.03
Building x hatch	3	12.52	.41
Diet x building x hatch	9	47.67	1.56
Error	32	30.62	

TABLE XVII. The analysis of variance of the data pertaining to the hatchability of fertile eggs.

Source of variation	df	mean square	F
Total	63		
Diet	3	65.14	1.17
Hatch	3	55.81	1.00
Building	1	103.61	1.86
Diet x hatch	9	12.79	.23
Diet x building	3	104.22	1.87
Building x hatch	3	12.96	.23
Diet x building x hatch	9	23.55	.42
Error	32	55.60	

TABLE XVIII. The analysis of variance of the data pertaining to hatchability of total eggs incubated.

Source of variation	df	mean square	F
Total	63		
Diet	3	88.53	1.13
Hatch	3	126.46	1.61
Building	1	121.89	1.55
Diet x hatch	9	22.72	.29
Diet x building	3	61.88	.79
Building x hatch	3	1.16	.01
Diet x building x hatch	9	71.11	.91
Error	32	78.46	

TABLE XIX. The analysis of variance of the egg yolk cholesterol data.

Source of variation	df	mean square	F
Total	63		
Diet	3	18,313.33	.55
Building	1	18,890.00	.57
Diet x building	3	83,443.33	2.51
Error	56	33,205.30	

Highly significant at the .01 level of probability.

TABLE XX. The analysis of variance of the blood serum cholesterol data.

Source of variation	df	mean square	F
Total	47		
Diet	3	359.94	.81
Building	1	3,283.50	7.43**
Diet x building	3	49.38	.11
Error	40	441.96	

** Highly significant at the .01 level of probability.