EFFECT OF DIETARY ENERGY LEVEL ON EGG PRODUCTION, FATTY LIVER, PLASMA AND YOLK LIPIDS, YOLK CHOLESTEROL AND FATTY ACIDS OF S. C. W. LEGHORN PULLETS RAISED UNDER SUB-TROPICAL CONDITIONS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
ANASTACIO LAIDA PALAFOX
1970

This is to certify that the

thesis entitled

EFFECT OF DIETARY ENERGY LEVEL ON EGG PRODUCTION, FATTY LIVER, PLASMA AND YOLK LIPIDS, YOLK CHOLESTEROL AND FATTY ACIDS OF S. C. W. LEGHORN PULLETS RAISED UNDER SUB-TROPICAL CONDITIONS

presented by

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has been accepted towards fulfillment
of the requirements for

PH.D. degree in POULTRY SCIENCE

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Major professor

Date April 30, 1970

O-169

ABSTRACT

EFFECT OF DIETARY ENERGY LEVEL ON EGG PRODUCTION, FATTY LIVER, PLASMA AND YOLK LIPIDS, YOLK CHOLESTEROL AND FATTY ACIDS OF S. C. W. LEGHORN PULLETS RAISED UNDER SUB-TROPICAL CONDITIONS

By

Anastacio Laida Palafox

An experiment was conducted with S. C. White Leghorn pullets to study the effect of 16 percent protein layer rations containing 2576, 2682, 2788, 2894 and 3000 kcal M.E./kg on efficiency of egg production, tissue lipids and yolk fatty acids. The birds were raised under sub-tropical conditions.

Increase in dietary energy level significantly increased body weight, egg weight, egg production and daily energy consumption. However, increasing dietary energy concentration significantly decreased daily feed and protein consumption per egg produced.

Daily feed consumption per egg produced decreased with increase in dietary energy level. The concentration of energy in the diet significantly affected liver fat scores. Liver fat scores increased linearly with increase in dietary energy level.

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The number of eggs produced from 70 through 74 weeks of age and liver fat score of pullets fed the control diet were significantly and negatively correlated.

Yolk oleic acid level increased linearly, whereas yolk linoleic acid decreased linearly with increase in dietary energy concentration.

For optimum overall efficiency, pullets fed the diet which contained 2894 kcal M.E./kg were superior to those fed the diets which contained 2576, 2682, 2788 and 3000 kcal. M.E./kg.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Poultry Science

1970

To my wife and children

ACKNOWLEDGMENTS

It is with pleasure that I express my sincere gratitude to Dr. Cal J. Flegal, Dr. Theo H. Coleman, Dr. Robert K. Ringer, Dr. Duane E. Ullrey and Dr. Howard C. Zindel for their inspiring guidance, unfailing interest and continued encouragement.

Grateful thanks are also due to the Community Scholarship Program and the Hawaii Veterans Memorial Scholarship Fund for the opportunity to continue my graduate studies with their scholarship grant.

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INTRODUCTION

Studies on the effect of different energy concentration in the nutrition of laying chickens have received considerable attention since the importance of higher energy rations for broilers was demonstrated by Scott, Matterson and Singsen (1947). Brown (1964) ably reviewed the use of high energy diets and the use of fats in such diets for poultry. His review revealed that limited information has been published on the use of high energy diets for laying chickens raised under conditions outside of the temperate zones.

The objectives of this experiment were to study the effects of 16 percent protein layer rations containing 2576, 2682, 2788, 2894 and 3000 kilocalories of metabolizable energy on efficiency of egg production, fatty liver, plasma and yolk lipids, yolk cholesterol and yolk fatty acids of pullets raised under sub-tropical conditions (18° C to 31° C).

REVIEW OF LITERATURE

As a background for the studies to be reported herewith, it is of interest to review first pertinent information on the use of energy in the diets of mature female chickens.

Calorie:Protein Ratio

Berg and Bearse (1957) reported that White Leghorn pullets fed diets with different calorie:protein ratios laid at different rates of egg production. They used 14 and 16 percent protein rations containing 1100 and 1450 kcal M.E./lb. The 1450 kcal M.E./lb diet containing 14 percent protein depressed egg production compared to the 14 percent protein diet containing 1100 kcal M.E./lb. The 16 percent protein diets containing 1100 and 1450 kcal M.E./lb were not significantly different. Hocreich *et al*. (1958) fed 17 percent protein diets with or without 6.6 percent yellow grease. The diet containing 6.6 percent yellow grease improved egg production and feed efficiency of White Leghorn pullets. Brown, Waring and Squance (1965) demonstrated that the optimum calorie:protein ratio for egg production, expressed as kcal M.E./lb, was realized at ratios of 76 and 78.

Effect of Dietary Energy Level

Body weight.--Skinner, Quisenberry and Couch (1951) reported that body weight of White Leghorn pullets fed high efficiency rations was superior to that of pullets fed the control ration. Hill, Anderson and Dansky (1956) observed that body weight of White Leghorn pullets in production was maintained at somewhat higher levels when fed high energy rations and that body weight tended to increase with increase in dietary energy. Lillie and Denton (1965) also reported that body weight of laying chickens increased with increase in dietary energy level. Waring, Addison and Brown (1968) found that there was no simple relationship between body weight and the energy content of the diet although, in general, body weight gain tended to increase with increase in dietary energy. Jackson, Kirkpatrick and Fulton (1969) reported that successive increase in energy in the diet up to 3550 kcal M.E./kg resulted in significant increases in body weight gain. There was a decrease in weight of hens fed the diet containing 4150 kcal M.E./kg.

Feed consumption.--Waring, Addison and Brown (1968) reported that White Leghorn laying pullets fed high energy diets consumed less than those fed low energy diets. Jackson, Kirkpatrick and Fulton (1969) and Scott, Nesheim and Young (1969) found that feed intake of mature White Leghorn pullets decreased with increase in dietary energy level.

Egg production.--Results of published experiments evaluating the effects of high energy diets on egg production are controversial. Research workers do not agree to the beneficial effect of high energy diets on egg production. Skinner, Quisenberry and Couch (1951) reported that egg production of White Leghorn pullets fed high energy diets was superior to that of pullets fed the control ration. Hill, Anderson and Dansky (1956) noted that high energy diets supported the highest egg production during the months of cold weather, whereas all energy levels were equally effective during the rest of the year. On the other hand, Lillie and Denton (1965) found that dietary energy level did not significantly affect egg production of S. C. W. Leghorn pullets. Waring, Addison and Brown (1968) reported that egg production of Brown Leghorn pullets fed the control diet was significantly superior to that of birds fed diets containing tallow. Jackson, Kirkpatrick and Fulton (1969) found that similar egg production can be obtained with diets containing varying metabolizable energy concentrations from as low as M.E 2150 kcal M.D./kg to as high as 3070 kcal M.E./kg.

Feed efficiency.--Many researchers are agreed that high energy diets improve feed efficiency. Skinner, Quisenberry and Couch (1951) reported that feed efficiency (feed/doz eggs) of pullets fed diets containing high energy was superior to that of the control ration. Lillie et al. (1952) found that the inclusion of 8 percent lard in the diet improved feed efficiency. Hill, Anderson and Dansky (1956) also observed that increasing dietary energy with the use of fat reduced feed requirements per dozen eggs. Waring, Addison and Brown (1968) noted that feed conversion (g feed/ g egg) was lower for Brown Leghorn pullets fed high energy diets than that of the control birds.

Egg weight.--Blamberg, Bossard and Combs (1964) studied the effect of various fats in the diets of pullets maintained in cages. They reported that those fed diets containing added fat laid eggs which were significantly heavier than pullets fed the control ration. The metabolizable energy intake appeared to influence egg weight more than linoleic acid intake. According to Jackson, Kirkpatrick and Fulton (1969) egg weight tended to increase linearly with dietary energy level. Mean egg weight significantly increased with dietary energy concentration. On the other hand, Lillie and Denton (1965) found that dietary energy did not significantly affect egg weight.

Mortality.--Weiss and Fisher (1957) reported that diets containing 5 to 10 percent animal fat increased mortality of White Leghorn pullets. Likewise Donaldson and Gordon (1960) found that the addition of 3 percent animal fat in the diet caused increased mortality. Jackson, Kirkpatrick and Fulton (1969) noted that in two experiments, there was a very high mortality of birds fed a diet containing 28.5 percent fat. However, there was no apparent effect of 0 to 15 percent added fat on mortality.

On the other hand, Waring, Addison and Brown (1968) fed up to 31.6 percent tallow to laying hens without any unusual high mortality. March and Bieley (1963) fed equicaloric diets (2800 kcal M.E./kg) containing 0, 5 and 10 percent fat to laying hens over a period of 11 months. Although the overall mortality was high, supplementation of the diets with fat had no effect on mortality nor did it influence the cause of mortality.

Fatty liver.--Fatty liver syndrome (FLS) is a serious problem. It occurs under stress conditions and may lower peak production in laying chickens. This syndrome was first reported in laying hens by Couch (1956). He reported that FLS is a classical example of fatty degeneration of the liver. He recommended the use of choline chloride, vitamin B_{12}, vitamin E and antibiotics to alleviate the depressing effect of fatty liver on egg production.

The importance of FLS has been recognized by many researchers since Couch published his observation in 1956. Barton, Flegal and Schaible (1966) reported that a low-energy, high fiber ration (14.8% protein, 2360 kcal M.E./kg) prevented fatty liver from developing while fatty livers developed in birds receiving the high energy diet (15% protein, 3000 kcal M.E./kg).

Quisenberry et al. (1967) observed that fatty liver symptoms in commercial layers were not caused nor prevented by dietary calorie level. Duke, Ringer and Wolford (1968) reported that plasma protein level was not a satisfactory index of developing fatty liver condition. They found no significant difference in plasma protein level between two dietary groups until a fatty liver condition was well-developed in one group.

Total plasma lipids.--Limited information has been published on the effect of dietary energy level on the concentration of total plasma lipids of mature female chickens. However, Greenberg et al. (1936) reported that the total plasma lipids of non-laying hens averaged 550 mg/100 ml. Lorenz et al. (1938) found that the concentration of total plasma lipids of laying chickens varied from 652 to 2308 mg/100 ml of plasma. A low-fat ration caused a slight decrease in total plasma lipids while a high-fat ration produced no change whatsoever.

Total yolk lipids.--The effect of dietary fat on yolk lipids has received much attention. Reiser et al. (1951) reported that the type and amount of dietary fat did not have any effect on total lipids of egg yolk. Marion and Edwards (1962) found that egg lipids of hens fed 10 percent corn oil remained unchanged from the control. Chung, Rogler and Stadelman (1965) observed that total yolk lipids of eggs from pullets fed corn oil, lard or hydrogenated coconut oil fed at a level of 10 percent were not significantly different to the control.

Total yolk cholesterol.--Fisher and Leveille (1957) reported that the cholesterol content of egg lipids was not significantly affected by feeding diets containing either 30 percent safflower oil or 20 percent linseed oil. Similarly, Wheeler et al. (1959) found that the cholesterol content of eggs from hens fed diets containing either safflower oil or linseed oil at levels as high as 30 percent did not differ significantly from values obtained with eggs from hens fed the control diet.

Daghir *et al*. (1960) found no change in yolk cholesterol content of eggs from pullets fed 12 percent of either soybean oil or white grease compared to the control. Combs and Helbacka (1960) observed no increase in the cholesterol content of eggs from hens fed 10 percent tallow compared to that of the control. They also reported that hens fed 10 percent corn oil produced eggs which contained increased amounts of cholesterol. Edwards *et al*. (1962) reported that yolk cholesterol content of eggs from White Leghorn chickens fed corn oil, tallow and lard was not significantly different from that of the control. Chung *et al*. (1965) noted that amount of egg yolk cholesterol of eggs from hens fed 10 percent of either corn oil, lard or hydrogenated corn oil was not sifnificantly different from that of the control.

Yolk fatty acids.--The fact that the distribution of fatty acids in egg yolk varies considerably with the dietary fat appears to be well established. Cruickshank (1934) reported variation in fatty acids of egg yolks from hens fed high levels (28 percent) of various fats. The total fatty acids of eggs from hens fed either a highly saturated fat, palm kernel oil, or a highly unsaturated fat, hemp oil, consisted of 16.1 and 47.7 percent linoleic acid respectively. Reiser (1951) found that either a fat-free diet or the presence in the diet of a highly saturated fat resulted in reduction of the dienoic acid (Cruickshank, 1934; Reiser, 1951; Fiegenbaum and Fisher, 1959) but oleic acid levels were not influenced by feeding high levels of unsaturated fats.

Fisher and Leveille (1957) studied the linoleic and linolenic acid levels in egg yolk of hens fed 20 percent fat in the diet. Yolk fatty acids were proportional to the level in the diet. A more recent study (Fiegenbaum and Fisher, 1959) indicated that when various oils were fed as 10 percent of the diet, the composition of the egg yolk fat was influenced only by the dietary polyunsaturated fatty acids, whereas the composition of the body fat was influenced by either saturated or unsaturated fatty acids in the diet.

Wheeler, Peterson and Michaels (1959) found, on all diets they tested, that the principal change accompanying an increase in linoleic acid was a corresponding decrease in oleic and palmitoleic acids. No change occurred in the level of stearic acid in the yolk. Botino (1965) reported a large increase in the level of linoleic acid was accompanied by a decrease in the level of oleic and palmitoleic acids in the egg yolk of pullets fed soybean oil meal.

Sell, Choo and Kondra (1968) found that feeding soybean oil altered the relative distribution of nearly all the fatty acids of egg yolk. There were significant decreases in the proportions of myristic, palmitic, palmitoleic and oleic acids. There was an appreciable increase in the linoleic acid concentration of egg yolk.

MATERIALS AND METHODS

Part I

Brooding and rearing.--Three hundred one-day-old commercial S. C. W. Leghorn (Hy-Line) pullets hatched and shipped air freight from California to Honolulu, Hawaii were brooded in electrically heated all-wire Oakes battery brooders from 3 through 8 weeks and then moved to all-wire developer pens from 8 through 18 weeks of age. The birds were fed a standard starter mash ration from 1 day through 6 weeks and a standard grower mash ration from 6 through 18 weeks of age. Feed and water were provided ad libitum. The chicks were vaccinated with an attenuated fowl pox vaccine at 3 weeks and a live virus New Castle Disease vaccine at 7 and 17 weeks.

Experimental design.--At 18 weeks, the birds were housed in a 731.52 cm x 1219.20 cm building with open sides enclosed with 1.27 cm x 1.27 cm wire mesh cloth. Electric lights in the building were turned on and off, with the use of a time switch at 5:00 A.M. and 8 A.M., respectively, during the duration of the experiment to provide the birds with a minimum of 14 hours of light daily.

Each bird occupied an all-wire 25.40 cm x 45.72 cm laying cage in the laying house during the adjustment period (18-20 weeks), pre-test period (20-22 weeks) and test period (22-74 weeks). The pullets were fed a standard mash layer ration during the adjustment period, whereas they were fed the test diets shown in Table 1 during the pre-test and test periods. At 20 weeks, 250 pullets were selected for general vigor and apparent sexual maturity as manifested by good comb development. The birds were distributed at random into 25 cage groups, each consisting of ten individually caged birds. At this time each bird was weighed. They were fed the test diets allocated in a 5 x 5 Latin square design shown in Table 2. Each diet was fed to only one group of birds in each column and in each row.

Data were obtained on body weight, feed consumption, egg production, egg weight, sexual maturity and mortality.

Part II

Total plasma lipids.--After the pullets were weighed at 74 weeks of age, at 4:00 o'clock P.M., feed was removed; however, the birds were allowed access to drinking water. On the following morning at 9:00 o'clock, four birds from each group, 20 birds per treatment, were killed by cutting the jugular vein, blood collected into heparinized centrifuge tubes, centrifuged for five minutes, 5 ml plasma pipetted into test tubes, stoppered, identified, refrigerated and then analyzed for total plasma lipids (TPL).

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Table 1. Composition of experimental diets

Diet No.	1	2	3	4	5
Unit	%	%	%	%	%
Cellulose	7.13	5.63	4.13	2.63	1.13
Prime tallow	....	1.50	3.00	4.50	6.00
Corn meal	59.93	59.93	59.93	59.93	59.93
Soybean meal (44%)	22.32	22.32	22.32	22.32	22.32
Alfalfa meal (17%)	3.00	3.00	3.00	3.00	3.00
Tricalcium phosphate	2.30	2.30	2.30	2.30	2.30
Limestone	4.25	4.25	4.25	4.25	4.25
Salt	0.50	0.50	0.50	0.50	0.50
Premix M5[1]	0.36	0.36	0.36	0.36	0.36
DL-Methionine	0.21	0.21	0.21	0.21	0.21
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis:					
Kcal M.E./kg[2]	2576	2682	2788	2894	3000
Protein, %	16	16	16	16	16
Energy:Protein ratio[3]	161	168	174	181	188

[1]Provided the following per kg diet: Vitamin A, 7900 I.U.; vitamin D_3, 2378 I.C.U.; vitamin E, 1.58 I.U.; riboflavin, 6.3 mg; d-calcium pantothenate, 8.7 mg; niacin, 29 mg; choline chloride, 317 mg; vitamin B12, 0.009 mg; ethoxyquin, 0.59 mg; B.H.T., 179 mg; menadione, 1.59 mg; manganese, 86 mg; iron, 29 mg; copper, 29 mg; cobalt, 0.28 mg; and zinc, 39.6 mg.

[2]Kilocalories of metabolizable energy per kilogram of diet.

[3]Based on kcal M.E./kg diet.

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Alfalfa meal (17%)	3.00	3.00	3.00	3.00	3.00
Tricalcium phosphate	2.30	2.30	2.30	2.30	2.30
Limestone	4.25	4.25	4.25	4.25	4.25
Salt	0.50	0.50	0.50	0.50	0.50
Premix M5[1]	0.36	0.36	0.36	0.36	0.36
DL-Methionine	0.21	0.21	0.21	0.21	0.21
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Table 2. Experimental design and diet allocation in the 5 x 5 Latin square

Experimental Design:

Source of Variation	Number	Degrees of Freedom
Total	25	24
Diets	5	4
Columns	5	4
Rows	5	4
Error		12

Diet Allocation:

A	B	C	D	E
1*	2	4	5	3
5	3	1	2	4
2	4	5	3	1
3	1	2	4	5
4	5	3	1	2

*Indicates experimental diets.

Extraction of TPL was conducted according to the method reported by Folch et al. (1957). Twenty ml of cold chloroform:methanol (2:1, v/v) were used for each ml of plasma, mixed thoroughly, let stand for two hours with occasional mixing and then filtered through a fat-free filter paper. The clear filtrate was washed with 0.2 of its volume with distilled water in a 250 ml separatory funnel, let stand for four hours at 5^{o} C during which time a biphasic system was obtained. The lower phase containing the lipids, was drawn off into a 200 ml volumetric flask and then made to volume with chloroform:methanol. To get the weight of the total lipids an aliquot was pipetted into a tared moisture dish and then dried to constant weight at 50^{o} C in a convection oven.

Liver fat scores.--After the birds were killed, they were scalded in hot water (77^{o} C), defeathered with the use of a feather picking machine, cooled in a tank of tap water and then examined for gross lesions of the liver and development of the ovary to identify laying and non-laying birds. Livers were excised, identified, placed in cellophane bags, refrigerated at 8^{o} C until the next morning when they were fat scored from one through five. A liver which looked normal and mahogany brown in color was assigned a score of 1, whereas a score of 5 denoted a yellow friable liver (Barton, 1967).

Part III

Total yolk lipids.--At 65 weeks of age, six eggs laid on the same day were collected from each of the 25 groups of pullets. The six yolks from each group were pooled, mixed and two 10-gram samples were weighed for extraction of total yolk lipids (TYL) according to the method reported by Folch *et al*. (1957). To get the weight of the total yolk lipids, an aliquot of the extract was pipetted into a tared moisture dish and then dried to constant weight at 50° C in a convection oven.

Total yolk cholesterol.--The total yolk cholesterol (TYC) content of the eggs was analyzed according to the direct method reported by Zlatkis *et al*. (1953) as modified by Weiss *et al*. (1964).

Yolk fatty acids.--Twenty ml of yolk lipid extract were pipetted into 120 ml boiling flasks, evaporated in a 50° C water bath under a stream of air under the hood. Esterification of the fatty acids was accomplished according to the method of Dronawat *et al*. (1966). The methyl esters of the fatty acids were pipetted into screw capped vials and kept under refrigeration at 0° C until analyzed.

One microliter of petrolem ether containing the methyl esters of the fatty acids was injected into an Aerograph Model W-600 C unit containing a five feet by one-eighth inch stainless steel column packed with 20 percent diethylene-glycol succinate, 5 percent isothalic acid on a 60/80 hexamethyl-disilazane-treated Chromosorb W. Peak areas for myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids were obtained using a disc chart integrator. Chromatographic runs were made using authentic samples of methyl esters of fatty acids. Retention on the column was the criterion used for identifying the fatty acid components of the yolk lipids.

The data were statistically analyzed according to Duncan's multiple range test (1955) and Snedecor and Cochran's analysis of variance, correlation and regression (1967).

RESULTS AND DISCUSSION

Part I

Body weight.--The data obtained on the effect of dietary energy level on mean body weight may be seen in Table 3. Initial weights at 20 weeks were not significantly different indicating that the pullets were effectively distributed at random into the 25 groups. At 22 weeks, two weeks after the test diets were fed, pullet weight tended to increase with increase in dietary energy concentration.

Dietary energy level significantly affected mean body weights at 26, 42, 58 and 74 weeks of age. At 26 weeks, pullets fed the 2894 kcal M.E./kg diet were significantly heavier than those fed the diet containing 2682 kcal M.E./kg. The mean body weights of the pullets fed diets containing 2576, 2682, 2788 and 3000 kcal M.E./kg were not significantly different.

At 42, 58 and 74 weeks of age, the pullets fed the 2894 kcal M.E./kg diet were significantly heavier than those fed diets containing 2576, 2682 and 2788 M.E./kg. The pullets fed the diets containing 2576, 2682, 2788 and 3000 kcal M.E./kg were similar in weight at these ages.

Table 3. Effect of dietary energy on body weight and mortality[1]

Diet	1	2	3	4	5	Regression Coefficient on Diet Energy[2]	
						L	D
Kcal M.E./kg	2576	2682	2788	2894	3000		
Protein, %	16	16	16	16	16		
Energy:Protein ratio	161	168	174	181	188		
Body Weight:							
20 weeks, g	1319	1309	1291	1318	1324		
22 weeks, g	1396	1405	1416	1451	1453		
26 weeks, g	1483[ab]	1477[a]	1486[ab]	1528[b]	1515[ab]		
42 weeks, g	1499[a]	1510[a]	1507[a]	1590[b]	1544[ab]		
58 weeks, g	1538[a]	1528[a]	1546[a]	1651[b]	1585[ab]		
74 weeks, g	1591[a]	1604[a]	1596[a]	1718[b]	1660[ab]	11.10	3.24
Gain, 20-74 weeks, g	272	295	304	400	335		
Relative gain, %	100.0	108.4	112.1	147.0	123.0		
Mortality due to all causes:							
22-30 weeks, %	4.0	0.0	0.0	2.0	2.0		
30-74 weeks, %	10.0	12.0	8.0	16.0	14.0		
22-74 weeks, %	14.0	14.0	8.0	18.0	16.0		
Mortality due to leucosis, %	10.0	8.0	2.0	4.0	4.0		

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant regression coefficient: Linear (L), 4.75 ($P < 0.05$); Deviation from regression (D), 3.49 ($P < 0.05$), d.f. -3.

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Body Weight:							
20 weeks, g	1319	1309	1291	1318	1324		
22 weeks, g	1396	1405	1416	1451	1453		
26 weeks, g	1483[ab]	1477[a]	1486[ab]	1528[b]	1515[ab]		
42 weeks, g	1499[a]	1510[a]	1507[a]	1590[b]	1544[ab]		
58 weeks, g	1538[a]	1528[a]	1546[a]	1651[b]	1585[ab]		
74 weeks, g	1591[a]	1604[a]	1596[a]	1718[b]	1660[ab]	11.10	3.24
Gain, 20-74 weeks, g	272	295	304	400	335		
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[2]Significant regression coefficient: Linear (L), 4.75 ($P < 0.05$); Deviation from regression (D), 3.49 ($P < 0.05$), d.f. -3.

The preceding observations are in agreement with those of Hill et al. (1956) and Jackson, Kirkpatrick and Fulton (1969). They found that the body weight of laying pullets was maintained at somewhat higher levels by rations high in energy concentration. Weight gain during the experiment tended to increase with an increase in energy in the diet. They attributed the increase in weight gain to a considerable excess of energy above the requirement for maintenance.

In the present experiment, mean body weight of pullets at 74 weeks of age increased as dietary energy level increased from 2576 to 2894 kcal M.E./kg. Mean body weight of pullets fed the 3000 kcal M.E./kg diet increased less than that of pullets fed the 2894 kcal M.E./kg diet. These observations are at variance with those of Jackson, Kirkpatrick and Fulton (1969) who reported that there was a decrease in weight of hens fed a diet containing 4150 kcal M.E./kg compared to the weight of hens fed a diet containing 3530 kcal M.E./kg. They reported successive increases in the body weight of laying White Leghorn chickens fed up to 3530 kcal M.E./kg diets.

Regression analysis was made to describe the relationship of pullet body weight at 74 weeks and dietary energy level. The regression coefficient of the two variates was significant indicating that pullet body weight increased linearly with increase in dietary energy level (Table 3). The F-ratio for deviation from the regression line (D) was not significant, indicating that the straight line was an adequate fit.

The preceding observations reveal that there is an upper limit of dietary energy level to which pullets continue to gain in body weight. Beyond the upper limit the birds decline in weight. The reason for the decline may be partly due to decreased feed consumption. Because of the high concentration of energy in the diet the birds consumed feed to meet their energy requirement but other nutrients are not necessarily met for optimum efficiency.

Also the preceding observations reveal that the upper limit at which dietary energy level in the diet that is conducive to increased gain in body weight is influenced by the weather conditions at which the birds are being raised. Under the sub-tropical conditions of this experiment, the upper limit of dietary energy level was 2894 kcal M.E./kg, whereas in the temperate weather conditions of Jackson et al. (1969), the upper limit at which body weight increased was 3550 kcal M.E./kg diet. Chickens require more dietary energy to maintain their body temperature in temperate than sub-tropical weather conditions.

Mortality.--The concentration of energy in the diet did not significantly affect mortality from 22 through 30, 30 through 74, and 22 through 74 weeks of age. Mortality from 22 through 74 weeks ranged from 8.0 to 18.0 percent. These data agree with Waring, Addison and Brown (1968) who reported that they found no unusual mortality with hens fed up to 3.16 percent tallow. In the present experiment mortality due to leucosis (2-10%) was not significantly affected by dietary energy level.

Egg weight.--Data on the effect of dietary energy level on egg weight is listed in Table 4. During the first six weeks of the test (22-28 weeks of age), dietary energy level did not significantly affect egg size. Eggs weighing from 35 through 42 grams (size 1) consisted of 5.00 to 7.45 percent whereas those weighing from 43 through 49 grams (size 2) consisted of 36.42 to 42.79 percent. Eggs weighing from 50 through 56 grams (size 3) consisted of 46.06 to 55.90 percent and those weighing from 57 grams and over (size 4) consisted of 2.54 to 5.75 percent of the total eggs produced.

Dietary energy level significantly affected weight of eggs laid during the 54th week, from 30 through 62 weeks and from 22 through 62 weeks of age. During the 54th week, pullets fed the 3000 kcal M.E./kg diet laid eggs which were significantly heavier than those from pullets fed the 2576 and 2682 kcal M.E./kg diets. Weight of eggs laid by pullets fed the diets containing 2788, 2894 and 3000 kcal M.E./kg were not significantly different. Likewise weight of eggs laid by pullets fed diets containing 2576 and 2894 kcal M.E./kg was similar.

From 30 through 62 weeks, pullets fed the diets containing 2894 and 3000 kcal M.E./kg laid eggs which were significantly heavier than those from pullets fed the 2682 kcal

Table 4. Effect of dietary energy on egg weight[1,2]

Diet No.	1	2	3	4	5
Kcal M.E./kg	2576	2682	2788	2894	3000
Egg size: 22-28 wks					
1. 35-42 g, %	5.75	7.45	5.40	5.00	5.38
2. 43-49 g, %	40.52	42.79	36.42	34.73	39.81
3. 50-56 g, %	49.23	46.06	52.43	55.90	52.27
4. 57 g and over	4.50	3.70	5.75	4.37	2.54
Total, %	100.00	100.00	100.00	100.00	100.00
Egg weight at age:					
23rd week, g	39.7	40.7	39.8	41.1	41.1
28th week, g	52.2	51.6	52.6	52.8	52.0
22-28 weeks, g	48.6	47.8	48.6	49.1	48.1
54th week, g	59.8[ab]	59.6[a]	61.9[c]	61.6[bc]	62.1[c]
62nd week, g	61.7	60.7	62.2	62.4	62.7
30-62 weeks, g[3]	57.9[ab]	57.6[a]	59.0[ab]	59.4[b]	59.4[b]
22-62 weeks, g[3]	55.6[ab]	55.3[a]	56.3[ab]	56.5[ab]	56.9[b]
Egg weight gain:					
22-62 weeks, g	21.0[ab]	19.5[a]	23.6[b]	21.3[ab]	21.5[ab]

[1]Means on the same horizontal line bearing the same postscript letter are not significantly different at the 5 percent level of probability.

[2]All eggs except crack and soft shell eggs were individually weighed.

[3]Egg weights every 4th week were averaged.

M.E./kg diet. Diets containing 2576, 2788, 2894 and 3000 kcal M.E./kg were not significantly different.

Egg weight increased with age of the pullets. A graphical representation of egg weights obtained every four weeks from 22 through 62 weeks of age may be seen in Figure 1. It may be seen that eggs from pullets fed the diets containing 2576 and 2682 kcal M.E./kg weighed less than those from pullets fed the diets containing 2788, 2894 and 3000 kcal M.E./kg at each of the respective dates.

It was noteworthy that the average egg weight from 22 through 62 weeks of age ranged from 55.3 to 56.9 grams and that egg weight increased with increase in dietary energy level. The regression analysis of egg weight on dietary energy level revealed a significant F-ratio indicating a linear relationship between the two variates (Table 4). The foregoing observations suggest an apparent advantage in the use of high energy diets for laying chickens to improve egg size.

The preceding data agree with those of Hocreich _et al_. (1958), Treat _et al_. (1960), Blamberg _et al_. (1964) and Jackson _et al_. (1969) who reported that the addition of fat to laying rations increased egg size but are at variance with MacIntyre and Aitken (1957) who found that high energy in the diet had no effect on egg weight. March and Bielay (1963) also found that the addition of 10 percent tallow significantly decreased egg weight compared with the effect of an isocaloric diet containing 0 to 5 percent tallow on

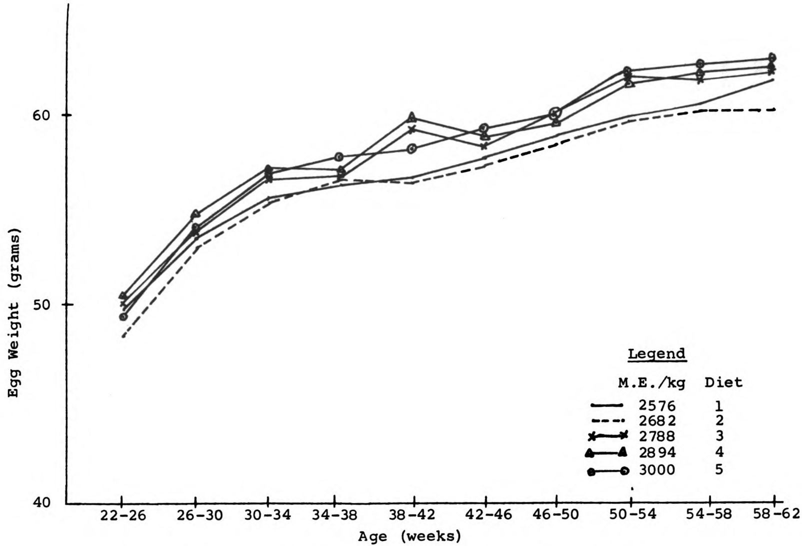

Figure 1. Egg weight every 4 weeks from 22 through 62 weeks.

this factor. They suggested that supplementary fat per se cannot be deduced to affect egg size but rather, feed intake appeared to be the causative factor. Birds fed the 10 percent tallow consumed less feed. It was not known if the reduced feed intake was due to poor palatability or induced satiety by the ration. Lillie and Denton (1965) also reported that dietary energy level did not significantly affect egg weight.

Egg production.--The data on the effect of dietary energy on egg production are presented in Table 5. Dietary energy level did not significantly affect age at 50 percent egg production. The ages ranged from 163.9 to 166.1 days. No data have been reported on the effect of dietary energy level on age at sexual maturity but it is generally agreed that restricted feeding (Milby and Sherwood, 1953), declining light during the growing period (Morris and Fox, 1960) and low dietary protein, provided protein was sufficiently low to avoid compensation by increasing appetite (Howes and Cottier, 1967), delay sexual maturity.

Dietary energy level significantly affected egg production from the 30th to the 74th week of age. However, it did not significantly affect egg production during the 23rd, 30th, from the 22nd to the 30th and from the 70th to the 74th week of age. These observations suggest that energy concentration in the diet is more critical for egg production from 30 through 70 weeks than from 22 through 30 weeks of age.

Table 5. Effect of dietary energy on hen-day production and feed consumption[1,2]

Diet Number	1	2	3	4	5	Regression Coefficient on Diet Energy[3] L	D
Kcal M.E./kg	2576	2682	2788	2894	3000		
Age at 50% production, days	164.3	166.1	165.7	163.9	165.1		
Egg Production at age:							
23rd week, %	33.7	27.4	23.7	32.9	30.3		
30th week, %	73.7	75.1	72.6	71.6	72.6		
22-30 weeks, %	61.5	60.7	58.5	63.8	61.3		
30-74 weeks, %	62.4[a]	66.4[ab]	64.1[ab]	67.4[b]	63.0[a]		
70-74 weeks, %	58.4	60.2	57.5	63.9	56.1		
22-74 weeks, %	61.5[a]	64.5[ab]	62.7[a]	66.5[b]	62.0[a]		
Feed Consumption at age:							
22-30 weeks, kg	4.12[a]	3.94[ab]	3.89[ab]	3.95[ab]	3.81[b]		
30-74 weeks, kg	32.60[a]	31.46[a]	30.60[a]	30.70[a]	28.35[b]		
22-74 weeks, kg	36.72[a]	35.40[b]	34.49[c]	34.65[bc]	32.16[d]	92.14	4.18

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Weeks indicate age.

[3]Significant regression coefficient: Linear (L), 4.75 OP < 0.05); Deviation (D), 3.49 ($P < 0.05$).

Weekly hen-day egg production from 22 through 30 weeks is graphically presented in Figure 2. Pullets fed diets containing 2576, 2682, 2894 and 3000 kcal M.E./kg reached their peak of egg production at 27 weeks of age, whereas peak production was reached at 28 weeks for those fed the diet containing 2788 kcal M.E./kg. All pullets fed the five diets peaked in egg production by the 28th week of age. Egg production of birds on the experimental diets ranged from 61.5 to 66.5 percent between treatments for the 52 weeks of egg production.

It may be seen from the graphic presentation of egg production from 22 through 74 weeks of age (Figure 3) that the pullets fed the 3000 kcal M.E./kg diet laid at the lowest rate from 46 to 50 weeks of age, the period of lowest production after the pullets reached their peak of egg production in six out of 13 four week periods during the laying year. These observations probably partly explains the decreased egg production of the pullets fed the 3000 kcal M.E./kg diet compared to those fed the diet containing 2894 kcal M.E./kg.

The foregoing data disagree with those of MacIntyre and Aitken (1957) and Anderson et al. (1957) who reported that increasing the metabolizable energy of layer diets had no effect on the rate of egg production. These workers did not report on the relationship of feed, energy and protein consumption on egg production.

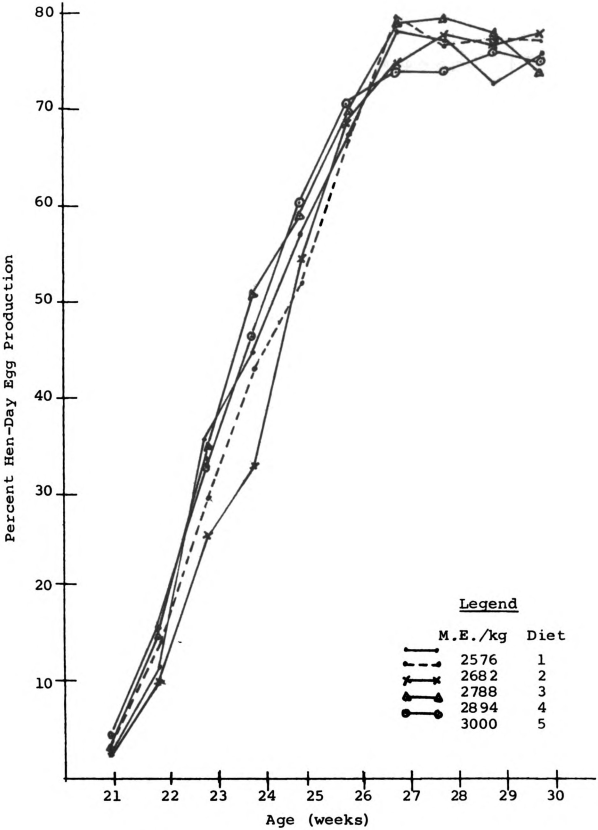

Figure 2. Weekly hen-day egg production from 22 through 30 weeks.

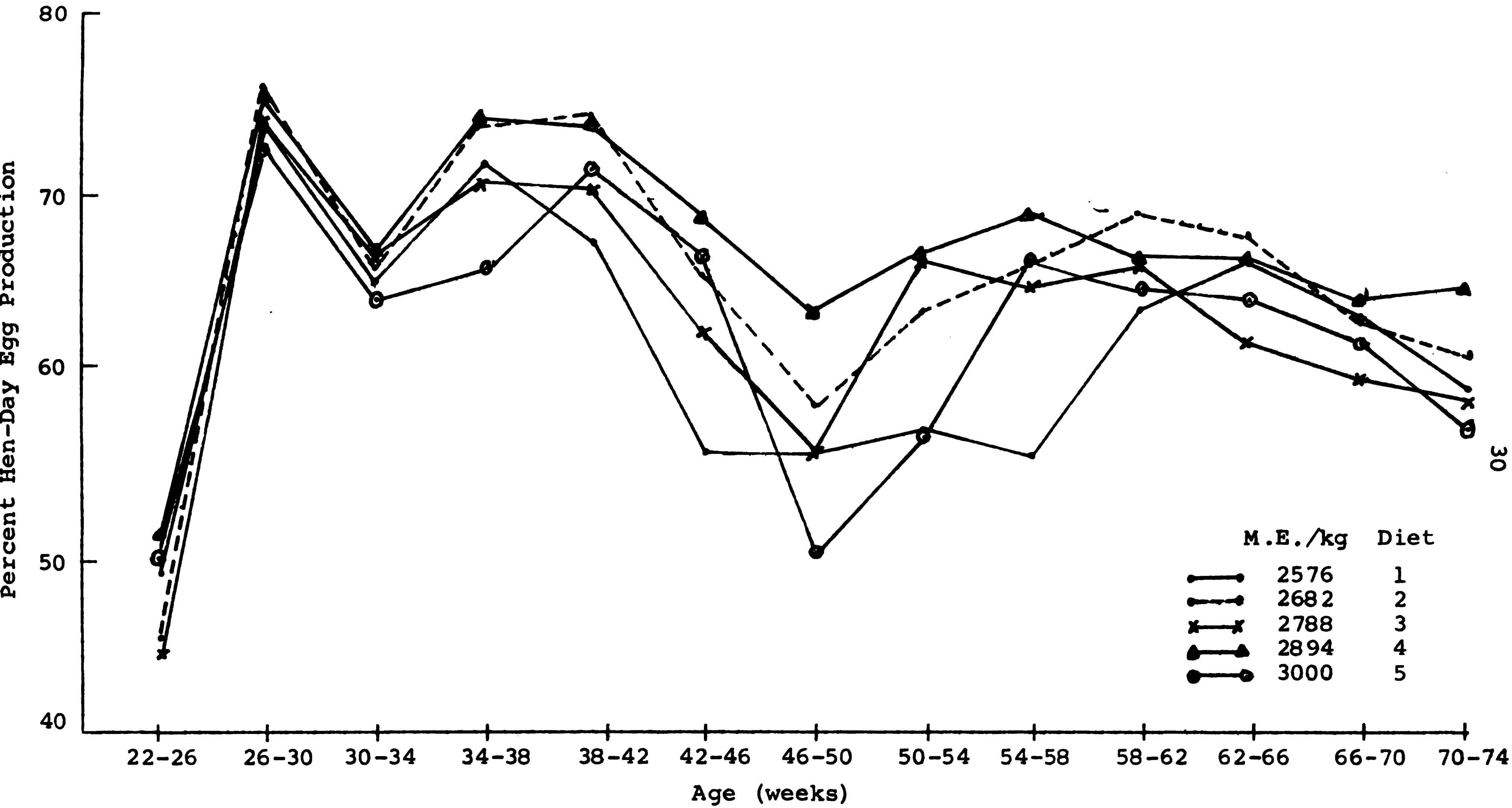

Figure 3. Hen-day egg production from 22 through 74 weeks.

The results presented in this study agree with those of Skinner *et al*. (1951), Singsen *et al*. (1952) and Hill *et al*. (1956). The latter workers reported that an increase in energy level in the diet increased egg weight only during the winter months. Jackson, Kirkpatrick and Fulton (1969) obtained the most satisfactory egg production from pullets fed 3.5 percent tallow in one experiment and 7.5 percent tallow in the second when compared with the control diet.

Total feed consumption.--Dietary energy level significantly affected total feed consumption from 22 through 30 weeks, 30 through 74 weeks and 22 through 74 weeks of age (Table 5). From 22 through 30 weeks, average feed consumption per bird ranged from 3.81 to 4.12 kilogram. Pullets fed the diets containing 2576 kcal M.E./kg consumed significantly more feed than those fed the diet containing 3000 kcal M.E./kg, whereas those fed diets containing 2576, 2682, 2788 and 2894 kcal M.E./kg consumed similar amounts of feed.

From 30 through 74 weeks, pullets fed the diets containing 3000 kcal M.E./kg consumed significantly less feed than those fed diets containing less metabolizable energy. Pullets fed all other diets consumed similar amounts of feed.

From 22 through 74 weeks of age, average total feed consumption per bird ranged from 32.16 to 36.72 kilograms. Pullets fed the 3000 kcal M.E./kg diet consumed significantly less feed than those fed diets containing less kilocalories of metabolizable energy per kilogram of feed.

Diets containing 2788 and 2894 kcal M.E./kg were not significantly different. Likewise diets which contained 2682 and 2894 kcal M.E./kg were similar for total feed consumption. Pullets fed the diet containing the least energy (2576 kcal M.E./kg) consumed significantly more feed than those fed diets that contained more energy.

Total feed consumption decreased with increase in dietary energy level. The regression analysis of total feed consumption from 22 through 74 weeks of age on dietary energy level revealed a significant F-ratio (Table 5) indicating a linear relationship between the two variates. The analysis also revealed a significant F-ratio for deviation due to regression indicating that the regression is curvilinear.

The preceding data agree with those of Peterson _et al_. (1960) who found that the most prominent effect of increasing dietary energy concentration on laying pullets was to decrease feed consumption.

Average daily feed consumption.--The data on average daily feed consumption are presented in Table 6. Dietary energy level significantly affected average daily feed consumption from 22 through 30 weeks, 30 through 74 weeks and 22 through 74 weeks of age. From 22 through 30 weeks, average daily feed consumption per bird ranged from 68.1 to 73.5 grams. Pullets fed the 2576 kcal M.E./kg diet consumed significantly more feed than those fed the 3000 kcal M.E./kg diet. Diets which contained 2576, 2682, 2788 and 2894 kcal

Table 6. Effect of dietary energy on daily consumption of feed, energy and protein and efficiency of feed, energy and protein utilization[1]

Diet Number	1	2	3	4	5	Reg. Coef. on Diet Energy	
Kcal M.E./kg	2576	2682	2788	2894	3000	L	D
Age, weeks:							
Feed/day:							
22-30 weeks, g	73.5[a]	70.4[ab]	69.5[ab]	70.5[ab]	68.1[b]		
30-74 weeks, g	105.9[a]	102.1[b]	99.3[b]	99.6[b]	92.7[c]		
22-74 weeks, g	100.9[a]	97.2[b]	94.8[b]	95.2[b]	88.4[c]	9.74	4.22
M.E./day:							
22-30 weeks, kcal	189.3[a]	188.8[a]	193.8[ab]	204.1[b]	204.2[b]		
30-74 weeks, kcal	272.9[a]	278.3[ab]	277.0[ab]	288.3[b]	278.1[ab]		
22-74 weeks, kcal	260.0[a]	260.7[a]	264.2[a]	275.5[b]	265.1[a]	9.32	4.50
Protein/day:							
22-30 weeks, g	11.8[a]	11.3[ab]	11.2[ab]	11.3[ab]	10.9[b]		
30-74 weeks, g	16.9[a]	16.3[b]	15.9[b]	15.9[b]	14.8[c]		
22-74 weeks, g	16.1[a]	15.5[b]	15.1[b]	15.2[b]	14.1[c]	87.38	4.99
Feed/egg:							
22-30 weeks, g	119.6	116.1	119.4	110.8	112.1		
30-74 weeks, g	171.2[a]	155.1[ab]	156.7[ab]	149.2[b]	147.8[b]		
22-74 weeks, g	168.5[a]	150.9[b]	151.2[b]	143.1[b]	143.0[b]	39.63	0.02
M.E./egg:							
22-30 weeks, kcal	308.0	311.4	332.8	320.5	336.4		
30-74 weeks, kcal	440.9[a]	415.9[b]	436.9[b]	423.4[ab]	443.3[a]		
22-74 weeks, kcal	423.6[ab]	406.0[b]	421.8[ab]	405.1[b]	428.9[a]	0.03	4.93
Protein/egg:							
22-30 weeks, g	19.1	18.6	19.1	17.7	17.7		
30-74 weeks, g	27.4[a]	24.8[b]	25.1[b]	23.9[b]	23.6[b]		
22-74 weeks, g	26.3[a]	24.1[b]	24.2[b]	22.9[c]	22.9[c]	39.46	8.33

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant coefficient: Linear (L), 4.75 ($P < 0.05$); Deviation (D), 3.49 ($P < 0.05$).

M.E./kg were consumed in similar amounts. Moreover, the birds fed the diets which contained 2682, 2788, 2894 and 3000 kcal M.E./kg consumed amounts of feed which were not significantly different.

From 30 through 74 weeks, average daily feed consumption of pullets fed the five diets ranged from 92.7 to 105.9 grams per bird. Pullets fed the diet which contained the least energy (2576 kcal M.E./kg) consumed significantly more feed than those fed diets which contained 2682, 2788, 2894 and 3000 kcal M.E./kg. Pullets fed diets which contained 2682, 2788 and 2894 kcal M.E./kg consumed similar amounts of feed, but significantly more than the feed consumed by pullets fed the 3000 kcal M.E./kg.

From 22 through 74 weeks, average daily feed consumption per bird ranged from 88.4 to 100.9 grams. Pullets fed the 2576 kcal M.E./kg diet consumed the most feed. Their feed consumption was significantly more than that of pullets fed diets containing 2682, 2788 and 2894 kcal M.E./kg which were not significantly different. The pullets which were fed the 3000 kcal M.E./kg diet consumed significantly less feed than those fed diets containing 2576 up to 2894 kcal M.E./kg during the 52-week test period.

Average daily feed consumption decreased with increase in dietary energy level. Regression analysis revealed significant F-ratios for regression and deviation from regression indicating a curvilinear relationship between feed comsumption and dietary energy level.

The foregoing data are in agreement with NRC (1966) and Jackson, Kirkpatrick and Fulton (1969) who reported that White Leghorn pullets laying approximately 200 eggs per year consumed from 94 to 105 grams of feed daily.

Average daily energy consumption.--Dietary energy concentration significantly affected average daily energy consumption per bird from 22 through 30 weeks, 30 through 74 weeks and from 22 through 74 weeks of age. Average daily metabolizable energy consumption from 22 through 30 weeks ranged from 188.8 to 204.2 kilocalories per bird. Pullets fed the 2576 and 2682 kcal M.E./kg diets consumed significantly less energy than those fed the diets containing 2894 and 3000 kcal M.E./kg which were similar. Pullets which were fed diets containing 2576, 2682 and 2788 kcal M.E./kg were not significantly different in the amount of feed consumed.

From 30 through 74 weeks, the average daily metabolizable energy consumption ranged from 272.9 to 288.3 kilocalories. Pullets fed the 2576 kcal M.E./kg diet consumed significantly less energy daily than those fed the 2894 kcal M.E./kg diet. Those fed diets which contained 2682, 2788, 2894 and 3000 kcal M.E./kg consumed similar amounts of feed.

From 22 through 74 weeks, the average daily metabolizable energy consumption ranged from 260.0 to 275.5 kilocalories. The pullets fed the diet containing 2894 kcal M.E./kg consumed significantly more energy daily than those fed the diets which contained 2576, 2682, 2788 and 3000 kcal M.E./kg. It was noteworthy that the pullets fed the 3000 kcal M.E./kg diet consumed significantly less energy daily than those fed the 2894 kcal M.E./kg diet.

Average daily energy consumption tended to increase with increase in dietary energy level. Regression analysis revealed significant F-ratios for regression and deviation from regression indicating that the relationship of daily energy consumption and dietary energy level is curvilinear.

The average daily energy consumption data presented in this study agree with Jackson, Kirkpatrick and Fulton (1969) who reported that daily metabolizable energy intake increased with increasing dietary energy concentration, but are contrary to the suggestion of Hill (1962) that the highly productive laying hen adjusts her level of intake on diets of widely varying energy concentrations, consuming approximately 350 kcal of metabolizable energy per day.

Average daily protein consumption.--The data on the effect of dietary energy level on dietary protein consumption are presented in Table 6. Dietary energy concentration significantly affected average daily protein consumption. From 22 through 30 weeks, average daily protein consumption ranged from 10.0 to 11.8 grams, whereas from 30 through 74 weeks the range was from 14.8 to 16.9 grams and from 22 through 74 weeks the range was from 14.1 to 16.1 grams.

From 22 through 30 weeks, pullets fed the diet containing 3000 kcal M.E./kg consumed significantly less protein daily than those fed the diet containing 2576 kcal M.E./kg. However, average daily protein consumption by birds fed diets which contained 2682, 2788, 2894 and 3000 kcal M.E./kg was not significantly different. This was also true for birds fed the diets which contained 2576, 2682, 2788 and 2894 kcal M.E./kg.

From 30 through 74 weeks, pullets fed the 3000 kcal M.E./kg diet consumed significantly less protein daily than those fed the other diets. On the other hand, pullets fed diets containing 2682, 2788 and 2894 kcal M.E./kg consumed similar amounts of protein daily, but consumed significantly less protein daily than those fed the diet which contained 2576 kcal M.E./kg.

From 22 through 74 weeks, pullets fed the 3000 kcal M.E./kg diet consumed significantly less protein daily than the pullets fed all other diets. Pullets fed diets which contained 2682, 2788 and 2894 kcal M.E./kg consumed similar amounts of protein daily, but consumed significantly less protein daily than those fed the diet which contained 2576 kcal M.E./kg.

Average daily protein consumption decreased with increase in dietary energy level. Regression analysis revealed significant F-ratios for regression and deviation from regression, indicating that the regression of daily protein consumption and dietary energy level is curvilinear.

The foregoing data are comparable with published information. NRC (1966) reported that S. C. W. Leghorn pullets laying at a rate of 60 percent and weighing 1800 grams require 16.0 grams of protein daily. The birds used in this study weighed from 1591 to 1718 grams at 74 weeks of age. Because of smaller body size and difference in egg production, they consumed only 14.8 to 16.9 grams of protein daily. Scott <u>et al</u>. (1969) found that the protein requirement of S. C. White Leghorn pullets raised under warm environmental conditions is 16.0 grams per day when the metabolizable energy of the diet is 2800 kcal M.E./kg.

<u>Average daily feed consumption per egg</u>.--The data on the effect of dietary energy level on average daily feed consumption per egg produced may be seen in Table 6. Dietary energy concentration did not significantly affect the amount of feed consumed daily per egg produced from 22 through 30 weeks. However, it significantly affected the daily feed consumption per egg from 30 through 74 weeks of age. The average daily feed consumption per egg from 22 through 30 weeks ranged from 110.8 to 119.6 grams, whereas the daily feed consumption per egg ranged from 147.8 to 171.2 grams from 30 through 74 weeks and the range was from 143.0 to 168.5 grams of feed per egg produced from 22 through 74 weeks of age.

From 30 through 74 weeks, pullets fed diets containing 2894 and 3000 kcal M.E./kg consumed significantly less feed daily per egg produced than did those fed the diet containing 2576 kcal M.E./kg. On the other hand, pullets fed the diets containing 2682, 2788, 2894 and 3000 kcal M.E./kg consumed daily amounts of feed per egg which were not significantly different. Those fed diets containing 2576, 2682 and 2788 kcal M.E./kg consumed daily amounts of feed per egg which were not significantly different.

From 22 through 74 weeks, pullets fed the 2682, 2788, 2894 and 3000 kcal M.E./kg diets consumed similar amounts of feed daily per egg produced but consumed significantly less feed daily per egg than those fed the 2576 kcal M.E./kg diet.

Average daily feed consumption per egg produced decreased with increase in dietary energy level. Regression analysis revealed a significant F-ratio for regression but a non-significant F-ratio for deviation from regression indicating that the regression of feed consumption per egg produced on dietary energy level is linear.

The foregoing observations agree with those of Singsen et al. (1952), Griminger and Scott (1954), Hill (1956) and Anderson et al. (1957). They reported that diets containing low concentrations of energy are less efficient than those which contain high concentrations of energy.

Average daily energy consumption per egg.--Dietary energy level did not significantly affect average daily metabolizable energy consumption per egg produced from 22 through 30 weeks. However, it did from 30 through 74 weeks and from 22 through 74 weeks. The average daily metabolizable energy consumption per egg produced from 22 through 30 weeks ranged from 308.0 to 336.4 kilocalories, whereas it ranged from 415.9 to 443.3 kcal from 30 through 74 weeks and from 405.1 to 428.9 kilocalories from 22 through 74 weeks. Those fed diets containing 2682, 2788 and 2894 kcal M.E./kg diet consumed similar amounts of energy per egg produced.

From 22 through 74 weeks, pullets fed the 3000 kcal M.E./kg diet consumed significantly more energy daily per egg produced than those fed the 2682 and 2894 kcal M.E./kg diets. Pullets fed the diets containing 2576, 2682, 2788 and 2894 kcal M.E./kg consumed similar amounts of energy daily per egg produced. Pullets fed the 2682 and 2894 kcal M.E./kg diets produced at the highest rate of egg production and consumed significantly less energy per egg produced than those fed the 3000 kcal M.E./kg diet.

Regression analysis of daily energy consumption per egg produced on dietary energy level revealed that the two variates were not linearly related.

Average daily protein consumption per egg.--Dietary energy level did not significantly affect average daily protein consumption per egg produced from 22 through 30 weeks. However, it significantly affected the daily protein consumption per egg from 30 through 74 weeks and from 22 through 74 weeks of age. The range of daily protein consumption per egg from 22 through 30 weeks was from 17.7 to 19.1 grams, whereas the range was from 23.6 to 27.4 grams from 30 through 74 weeks and from 22.9 to 26.3 grams from 22 through 74 weeks of age.

From 30 through 74 weeks, pullets fed the diets which contained 2682, 2788, 2894 or 3000 kcal M.E./kg consumed significantly less protein daily than those fed the diets which contained 2576 kcal M.E./kg. There were no significant differences in the amounts of protein consumed daily per egg produced by the pullets fed the diets which contained 2682, 2788, 2894 and 3000 kcal M.E./kg.

Average daily protein consumption per egg produced decreased with increase in dietary energy level. Regression analysis revealed significant F-ratios for regression and deviation from regression, indicating that the regression of daily protein consumption per egg produced and dietary energy level is curvilinear.

Part II

Liver fat scores.--The data on the effect of dietary energy level on liver fat scores are presented in Table 7. Dietary energy level significantly affected the fat scores of livers from 74-week old pullets. The range of liver fat scores within treatments (20 livers per treatment) was from one to five each of the five dietary energy levels. However, the range of liver fat scores among treatments was from 2.02 to 2.85. Livers of pullets fed the 2894 kcal M.E./kg diet had fat scores which were significantly higher than those of pullets fed the 2576 kcal M.E./kg diet. Livers from pullets

Table 7. Effect of dietary energy level on fatty liver and plasma lipids[1]

						Regression Coefficient on Diet Energy[2]	
Diet Number	1	2	3	4	5	L	D
Liver fat score	2.05[a]	2.15[ab]	2.50[ab]	2.85[b]	2.60[b]	4.90	0.67
Plasma total lipids, mg/100 ml	1272	1544	1214	1770	1216		

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant regression F-ratio: Linear (L), 4.75 ($P < 0.05$); Deviation (D), 3.49 ($P < 0.05$).

Table 7. Effect of dietary energy level on fatty liver and plasma lipids[1]

						Regression Coefficient on Diet Energy[2]	
Diet Number	1	2	3	4	5	L	D
Liver fat score	2.05^{a}	2.15^{ab}	2.50^{ab}	2.85^{b}	2.60^{b}	4.90	0.67
Plasma total lipids, mg/100 ml	1272	1544	1214	1770	1216		

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant regression F-ratio: Linear (L), 4.75 ($P < 0.05$); Deviation (D), 3.49 ($P < 0.05$).

Table 7. Effect of dietary energy level on fatty liver and plasma lipids[1]

Diet Number	1	2	3	4	5	Regression Coefficient on Diet Energy[2] L	D
Liver fat score	2.05[a]	2.15[ab]	2.50[ab]	2.85[b]	2.60[b]	4.90	0.67
Plasma total lipids, mg/100 ml	1272	1544	1214	1770	1216		

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant regression F-ratio: Linear (L), 4.75 ($P < 0.05$); Deviation (D), 3.49 ($P < 0.05$).

fed the 2682, 2788, 2894 and 3000 kcal M.E./kg diets had fat scores which were not significantly different.

Fat scores of livers from pullets fed diets containing 2576 up to 2894 kcal M.E./kg increased with dietary energy level. Those of livers from pullets fed the 3000 kcal M.E./kg diet scored slightly lower than those from pullets fed the 2894 kcal M.E./kg diet.

Regression analysis of liver fat score and dietary energy level revealed a significant F-ratio for regression but a non-significant F-ratio for deviation from regression indicating that the regression of liver fat score on dietary energy level is linear.

The preceding observations are in agreement with those of Barton, Flegal and Schaible (1966) who reported that in one of their experiments with laying chickens, a low-energy, high fiber ration prevented fatty liver from developing while fatty livers developed in birds on the high energy ration. The data reported by Quisenberry, Young and Murthy (1967) that dietary energy level had no effect on fatty liver of laying chickens and that fatty liver symptoms were neither caused nor prevented by dietary energy level are at variance with the data obtained in the present study. The dissimilarity of the results of Quisenberry et al. (1967) and those obtained in the present experiment may be explained in part by the energy to protein ratio used. Quisenberry and co-workers used diets with different dietary energy and protein levels, but maintained increasing concentrations of energy and protein so that the energy to protein ratio remained the same. Liver fat score increased with dietary energy level in the present study, whereas Quisenberry et al. (1967) did not find any increase in liver fat scores with an increase in dietary energy level. The energy to protein ratio may be an important factor in the assessment of fatty liver in laying chickens.

Total plasma lipids.--Dietary energy level did not significantly affect the concentration of total lipids in the plasma. The concentration of total plasma lipids (TPL) ranged from 1214 to 1770 mg/100 ml. These observations are comparable to those of Lorenz et al. (1938) and Walker et al. (1951) who reported that the level of dietary fat had little effect on the level of total plasma lipids. In the present experiment, there was no indication of increasing concentrations of total plasma lipids with increasing dietary energy concentrations.

Interrelationships: Correlation analysis was used to describe the relationship of liver fat score (LFS), total plasma lipids (TPL) and/or number of eggs (NE) produced during the last four weeks of production (70-74 weeks of age). The data may be seen in Table 8. Correlation coefficients of liver fat scores ranged from -0.762 to 0.479. The correlation coefficients of LFS on NE of birds fed diet 1 which contained 2576 kcal M.E./kg was negative and significant (-0.762) indicating that egg production decreased with increase in liver fat score.

Table 8. Correlation coefficients of certain traits[1]

Diet	Liver Fat Score	Total Plasma Lipids
1. Egg number (70-74 weeks)	-0.762	0.050
2. Egg number (70-74 weeks)	-0.579	-0.441
3. Egg number (70-74 weeks)	0.479	0.173
4. Egg number (70-74 weeks)	0.374	0.487
5. Egg number (70-74 weeks)	0.257	0.218

[1]Significant correlation coefficient is 0.632 at the 5 percent level of probability (10 pairs of observation, 8 degrees of freedom).

On the other hand, the coefficient of correlation of LFS on NE of birds fed the diet containing 2682 kcal M.E./kg (diet 2) was -0.579, whereas the coefficients of correlation of LFS on NE of pullets fed the 2788 (diet 3), 2894 (diet 4) and 3000 (diet 5) kcal M.E./kg diets were not significant. The correlation coefficients of egg number on liver fat score from the diets containing 0 and 1.5 percent tallow (diets 1 and 2) were negative, whereas the coefficients of correlation of the two variates were positive when egg numbers and liver fat score of the liver were correlated. The significance of this observation is not clearly evident.

The total plasma lipids and number of eggs laid from 70 to 74 weeks of age were also analyzed. The correlation coefficients ranged from -0.441 to 0.487. The tabular value for significance at the 5 percent level of probability (8 degrees of freedom) is 0.632 (Snedecor and Cochran, 1967).

Total plasma level and liver fat score were not significantly correlated.

Part III

Total yolk lipids.--Dietary energy level did not significantly affect the concentration of total lipids in egg yolk (Table 9). The treatment means of total yolk lipids ranged from 33.72 to 34.84 percent (wet basis). These observations agree with those of Reiser et al. (1951), Marion and Edwards (1962) and Chung et al. (1965) and Palafox (1968). They reported that the energy level in layer diets did not significantly affect total yolk lipid concentration.

Total yolk cholesterol.--Dietary energy level did not significantly affect total yolk cholesterol (Table 9). The treatment means ranged from 13.22 to 13.96 milligrams per gram of fresh yolk. Similar observations have been reported by Daghir et al. (1960) who reported that the cholesterol content of egg yolk from hens fed 12 percent white grease did not differ significantly from that of control. Combs and Helbacka (1960) and Edwards et al. (1962) reported that the total yolk cholesterol of eggs from White Leghorn pullets fed tallow was similar to that of eggs from birds fed the diets without tallow.

Yolk saturated fatty acids.--Dietary energy level significantly affected the concentration of myristic, palmitic and total saturated fatty acids (myristic, palmitic

Table 9. **Effect of dietary energy level on yolk lipids, cholesterol and fatty acids**[1]

Diet Number	1	2	3	4	5	Regression Coefficient on Diet Energy[2]	
Kcal M.E./kg	2576	2682	2788	2894	3000	L	D
Total yolk lipids, %	33.72	34.70	34.08	34.84	34.22		
Total yolk cholesterol, mg/g	13.86	13.40	13.96	13.88	13.22		
Saturated fatty acids:							
Myristic (C_{14}), %	0.60^{a}	0.62^{a}	0.82^{ab}	0.96^{b}	0.92^{b}		
Palmitic (C_{16}), %	27.86^{ab}	28.04^{a}	25.64^{bc}	26.88^{bc}	24.86^{c}		
Stearic (C_{18}), %	6.74	5.92	6.18	6.12	6.00		
Total, %	35.20^{a}	34.58^{ab}	32.64^{ab}	33.96^{ab}	31.80^{b}		
Unsaturated fatty acids:							
Palmitoleic ($C_{16}{}^{1=}$), %	6.28	6.58	6.60	6.24	7.00		
Oleic ($C_{18}{}^{1=}$), %	39.58^{a}	41.64^{ab}	43.66^{bc}	45.50^{cd}	47.70^{d}	61.08	0.01
Linoleic ($C_{18}{}^{2=}$), %	18.94^{a}	17.20^{a}	17.10^{a}	14.30^{b}	13.50^{b}	32.45	0.70
Total, %	64.80^{a}	65.42^{ab}	67.36^{ab}	66.04^{ab}	68.20^{b}		

[1]Means on the same horizontal line bearing a different postscript letter are significantly different at the 5 percent level of probability.

[2]Significant regression coefficient: Linear (L), 4.75 ($P < 0.05$); Deviation (D), 3.49 ($P < 0.05$).

and stearic) of egg yolk (Table 8). However, the level of energy in the diet did not significantly affect the concentration of stearic acid.

Yolk myristic acid concentration ranged from 0.60 to 0.96 percent. Yolk of eggs from pullets fed diets containing 2894 and 3000 kcal M.E./kg contained significantly more myristic acid than those from birds fed 2576 and 2682 kcal M.E./kg diets. Pullets fed diets containing 2576, 2682 and 2788 kcal M.E./kg were not significantly different in yolk myristic acid concentration. This was also true with egg yolks of pullets fed diets containing 2788, 2894, and 3000 kcal M.E./kg. The yolks contained similar amounts of myristic acid.

Palmitic acid concentration in egg yolk ranged from 24.86 to 28.04 percent. Egg yolks from pullets fed the 3000 kcal M.E./kg diet contained significantly less palmitic acid than those from pullets fed 2576 and 2682 kcal M.E./kg diets. Yolks from eggs of pullets fed diets containing 2788, 2894 and 3000 kcal M.E./kg were not significantly different in palmitic acid concentration.

Total saturated fatty acids in egg yolk ranged from 31.80 to 35.20 percent in this experiment. Egg yolks from pullets fed the 3000 kcal M.E./kg diet contained significantly less saturated fatty acids than those from pullets fed the diet containing 2576, 2682, 2788 and 2894 kcal M.E./kg. Egg yolks from pullets fed from 2576 to 2894 kcal

M.E./kg diets contained similar amounts of unsaturated fatty acids.

These observations are in agreement with those of Cruickshank (1934) who reported that when she fed high levels (28 percent) of various fats, she found variations in fatty acids of egg yolk. Wheeler, Peterson and Michaels (1959) found no change in the level of stearic acid in egg yolk of pullets fed different diets.

Yolk unsaturated fatty acids.--Dietary energy level significantly affected the concentration of oleic, linoleic and total unsaturated fatty acids (palmitoleic, oleic and linoleic) in egg yolk. However, the level of energy in the diet did not significantly affect the concentration of palmitoleic acid.

Yolk oleic acid concentration ranged from 39.58 to 47.70 percent. Egg yolks from pullets fed the 3000 kcal M.E./kg diet contained significantly more oleic acid than those from pullets fed the 2576, 2682 and 2788 kcal M.E./kg diets. Oleic acid content of egg yolks of pullets fed 2576 and 2682 kcal M.E./kg was not significantly different. Pullets fed 2782 and 2788 kcal M.E./kg laid eggs which contained similar concentrations of oleic acid in their egg yolks. Likewise pullets fed diets containing 2788 and 2894 kcal M.E./kg were not significantly different in their egg yolk oleic acid content.

Yolk linoleic acid level ranged from 13.5 to 18.94 percent. Egg yolks from pullets fed the 2894 and 3000 kcal M.E./kg diets contained significantly less linoleic acid than those from pullets fed diets containing 2576, 2682 and 2788 kcal M.E./kg. Pullets fed diets containing 2894 and 3000 kcal M.E./kg laid eggs which contained similar concentrations of linoleic acids in their yolk lipids.

Regression analysis revealed that the regression of yolk oleic acid concentration on dietary energy level was positive and significant indicating that yolk oleic acid concentration increased linearly with increase in dietary energy level. The F-ratio of deviation from regression was not significant indicating the linearity of the regression of the two variates.

Linoleic acid concentration decreased linearly with increase in dietary energy level as indicated by the significant F-ratio of the regression of the two variates and the non-significance of the F-ratio of the deviation from regression.

The observation that the linoleic acid content of egg yolk decreased with increasing concentration of metabolizable energy with the addition of tallow is in agreement with the data reported by Cruickshank (1934). When she fed 28 percent palm kernel oil, a highly saturated fat, to laying hens, their egg yolks contained 16.1 percnet of the total fatty acids as linoleic acid, but when she fed a highly unsaturated fat, hemp oil, the linoleic acid content of the yolk was 41.1 percent. Also Reiser (1951) reported that the presence of a highly saturated fat (bayberry tallow) in the diet of laying chickens resulted in the reduction of the dienoic acid content of the egg yolk to very low levels. Machlin _et_ _al_. (1962) found that fatty composition of egg yolks reflect the fatty acid composition of the diet.

SUMMARY

A 5 x 5 Latin Square experiment was conducted with 250 S. C. White Leghorn (Hy-Line) pullets raised under sub-tropical conditions to study the effect of 16 percent protein layer rations containing increasing concentrations of metabolizable energy (2576, 2682, 2788, 2894 and 3000 kcal M.E./kg) from 22 through 74 weeks of age with the addition of tallow. Criteria measured were efficiency of egg production, fatty liver, total lipids of plasma and egg yolk, yolk cholesterol and yolk fatty acids.

Dietary energy level did not significantly affect (1) mortality, (2) egg weight from 22 through 28 weeks, (3) age at 50 percent egg production, (4) egg production from 22 through 30 weeks and (5) daily feed, energy and protein consumption per egg produced from 22 through 30 weeks of age.

However, dietary energy level significantly affected (1) egg weight from 22 through 62 weeks and (2) egg production, total feed, daily feed, energy and protein consumption and daily feed, energy and protein consumption per egg produced from 22 through 74 weeks of age.

Pullet body weight increased and feed per egg produced decreased linearly with increase in dietary energy level. Daily feed consumption of feed, protein and protein consumption per egg produced decreased with increase in the concentration of energy in the diet.

Dietary energy level significantly affected liver fat score. Liver fat score increased linearly with increase in dietary energy level. Total plasma lipid concentration was not significantly affected by the level of energy in the diet.

The number of eggs produced from 70 through 74 weeks of age and liver fat score of pullets fed the control diet were significantly and negatively correlated.

Dietary energy level significantly affected the level of myristic acid in egg yolk. The concentration of myristic acid in egg yolk increased with increase in the level of dietary energy.

The concentration of energy in the diet significantly affected oleic and linoleic acid content of egg yolk. Yolk oleic acid level increased linearly, whereas yolk linoleic acid decreased linearly with increase in the level of energy in the diet.

The results of this experiment indicate that additional research is needed to determine optimum energy and protein concentrations in the diet of laying chickens housed in temperature controlled environmental conditions.

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