FACTORS AFFECTING LIVER FAT ACCUMULATION AND LIVER HEMORRHAGES ASSOCIATED WITH FATTY LIVER-HEMORRHAGIC SYNDROME IN LAYING CHICKENS

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ABSTRACT

FACTORS AFFECTING LIVER FAT ACCUMULATION AND LIVER HEMORRHAGES ASSOCIATED WITH FATTY LIVER-HEMORRHAGIC SYNDROME IN LAYING CHICKENS

By

Kwang Lee

Once a laying flock has been afflicted with the fatty liver-hemorrhagic syndrome (FLHS), the economic loss, primarily due to mortality resulting from liver hemorrhaging, may be large, yet the exact cause(s) of this syndrome has/have not been elucidated.

One of many difficulties in studying this problem has been that the syndrome is very difficult to reproduce in the laboratory at will since the exact causative agent(s) is/are unknown.

The objectives of this study were to investigate factors affecting liver fat accumulation, to attempt to produce fatty liver-hemorrhagic syndrome, and to study the relationship between liver fat infiltration and fatty liver hemorrhaging.

A series of four experiments were conducted with a commercial strain of Single Comb White Leghorn pullets.

When environmental temperature was "high" (27.8° C), more livers appeared to be fatty, and incidence of liver hemorrhages was greater than that obtained in "moderate" (12.2° C) temperature.

Percent liver fat as well as total liver fat obtained in the "high" environmental temperature was slightly higher among birds fed a high carbohydrate diet as compared with those fed a high fat diet. Statistical difference was not obtained, however.

Restricted feeding followed by ad libitum feeding resulted in a reduction of both total liver fat and the incidence of FLHS.

When a specific diet, Diet F, a commercial laying formula, was given to birds, amounts of liver fat increased markedly. Average feed consumption, final body weight and hen-day egg production, however, were not statistically different between birds fed Diet F and those fed the other diet.

The highest incidence of liver hemorrhages and FLHS resulted from one specific treatment where restricted feeding was practiced with Diet F for four weeks in 30.6° C environmental temperature then followed by an ad libitum feeding in 22.2° C.

FLHS hemorrhages occurred in livers with high fat content (high fat-liver) but high fat-livers were not always accompanied by severe hemorrhages. This showed that

high liver fat values may not necessarily be indicative of FLHS.

FLHS livers were significantly heavier than livers that did not have severe hemorrhages. Average feed consumption, final body weight and hen-day egg production of birds having FLHS hemorrhages did not differ from birds having livers with a low fat content. Thus, it appears that changes in feed consumption, body weight and egg production may not be used as satisfactory indications of FLHS in laying birds.

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I. INTRODUCTION

Fatty liver syndrome in mammals has been studied since 1932.

In poultry, however, fatty liver is a fairly new disease problem recognized during the development of a new management structure, viz., high bird-density cage system.

Several differences in fat metabolism between rats and chickens have been recognized. Rats synthesize most of their fatty acids in the adipose tissue. Chickens, however, synthesize most of their fatty acids in the liver. Causes of fatty liver in rats and chickens may not be the same. A lack of choline chloride in the diet results in fatty liver in rats but not necessarily in chickens.

The significance of liver hemorrhages has not been discussed until recently. It has been shown that mortality can result from severe liver hemorrhaging but not necessarily from the fatty liver *per se*.

Neither the exact causes of fatty liver (high fat-liver) nor liver hemorrhages in chickens have been elucidated.

One of the difficulties in studying fatty liver-hemorrhagic syndrome is the difficulty in reproducing

it in the laboratory, at will; accordingly, the objectives of this study were: (1) to investigate nutritional and environmental factors affecting liver fat accumulation; (2) to attempt to produce fatty liver-hemorrhagic syndrome in the laboratory and; (3) to study the relationship between liver fat infiltration and fatty liver hemorrhaging.

II. REVIEW OF LITERATURE

One of the classical examples of fatty liver syndrome can be found in choline deficiency in rats. As early as 1932, Best et al. (1932a) observed an accumulation of fat in hepatic cells of rats which were raised on a diet containing 2.5 grams of fairly saturated fat. This condition was cured by an oral administration of choline chloride (Best et al., 1932b). Chalvardjian (1969) noticed an increase in hepatic triglyceride pool in livers of rats fed a choline deficient diet. The C-16 (saturated and monoenoic) fatty acids were major components of triglyceride in liver lipids of rats fed a fat-free choline deficient diet. However, when fat was added to the diet, the fatty acid pattern of hepatic lipids was similar to that of dietary fat (Chalvardjian, 1966).

The lipotropic mechanism of choline is complicated. Due to the fact that the addition of choline resulted in an increase in body weight and deposition of fat in the body of rats, Maclean et al. (1937) suggested that the presence of choline in the diet favored the normal distribution of fat between the liver and body depots. Chalvardjian (1969) noticed a decrease in transport of triglycerides and phospholipids from the liver to serum in rats fed a

choline deficient diet. Perlman and Chaikoff (1939) reported that choline accelerated the rate of formation of, as well as a rapid removal of, phospholipids in the liver.

The lipotropic effect of methionine was reported by Tucker and Eckstein (1937). When methionine was added to the choline deficient diet, fatty liver incidence in rats was significantly reduced. The finding that cysteine and homocystein (sulfur containing amino acids) did not exert their effect in reducing fat content in the liver led them to the conclusion that the lipotropic action of methionine was due to the presence of a labile methyl group which was not present in the other two amino acids.

The metabolic interrelationship of methionine and choline in the rat was reported by Du Vigneaud et al. (1940). They demonstrated the biological transfer of the intact methyl group from methionine into the choline molecule (Transmethylation reaction). In the following year, they stated that the lipotropic action of methionine in a choline deficient situation might be due to the presence of the labile methyl group of methionine which was used for the synthesis of choline (Du Vigneaud et al., 1941). Similar observations were made in chickens. Leach et al. (1966) reported that when there were sufficient methyl groups in the diet, hens were able to synthesize choline. However, the addition of high levels of choline to the diet was not effective in preventing fatty liver in chickens (Fisher and Weiss, 1956).

Hegsted et al. (1941) reported that choline deficient chicks did not show fatty livers; however, choline was needed to prevent perosis.

Chickens have been recognized to be different from rats in several aspects of lipid metabolism. O'Hea and Leveille (1968) found that rats' adipose tissue was much more active in synthesizing fatty acids than chicks' adipose tissue. They also found that the incorporation of glucose-U-C^{14} into fatty acids was stimulated by insulin in rat adipose tissue but not in chick adipose tissue. Leveille (1967) reported that adipose tissue was the major site of fatty acid synthesis of rats. But due to the fact that the incorporation rates of both glucose-U-C^{14} and acetate-1-C^{14} into fatty acids were much higher in the liver than in the adipose tissue of chicks, Leveille et al. (1968) concluded that the liver was the major site of fatty acid synthesis in chicks.

The age and sex of chickens have been recognized to influence lipid metabolism in the liver. Leveille (1969) found that acetate-1-C^{14} incorporation into fatty acids was greater in the liver of chicks than that of hens. However, no statistical significance was noticed. Saloma et al. (1969) reported that the amount of linoleic acid in triglyceride was significantly higher in the liver of laying hens as compared to that of non-layers. They also reported that the major fatty material accumulated in the liver of

laying hens was triglyceride. Lorenz et al. (1937-1938) observed a marked increase in blood lipid concentrations, especially triglyceride, in actively laying hens as compared to that of immature female birds. Blood lipid concentrations of mature male, of immature male and of immature female chickens were not different from each other. These findings indicated that the rise in lipid level in the blood was related to the ovarian activity. However, once the female bird attained sexual maturity, increased blood lipid level was not related to the intensity of egg production. Shortly after, Lorenz et al. (1938) observed a marked increase in neutral fat in the liver at the onset of maturity of the female chicken. But phospholipid and cholesterol ester content were not changed.

O'Hea and Leveille (1969), when studying the origin of plasma fatty acids, found these originated from the liver since the pattern of distribution of glucose-U-C^{14} and acetate-1-C^{14} in plasma and in liver was similar after injection of these materials. Over 75 percent of the plasma lipid radioactivity was found in triglyceride. They also reported that the main transport form of lipid from liver to adipose tissue was β or low-density lipoprotein since these components contained over 66 percent of plasma radioactive lipid. Duke et al. (1968) observed no significant difference in plasma protein levels between two dietary groups of birds (the non-control group developed

fatty liver syndrome) which led them to conclude that plasma protein level was not a satisfactory index of developing fatty liver syndrome.

Oser (1965) pointed out the existence of different types of fatty liver in animals since the central vein area of the liver lobule was the first place fat appeared in choline deficiency, whereas periportal was the first place in starvation and in low-protein feeding. According to Wohl and Goodheart (1970), Peters and Van Slyke in 1946 classified fatty livers as physiologic and pathologic type. The physiologic type of fatty liver resulted from the mobilization of large amounts of fat from depots to liver to meet unusual requirements for lipid oxidation. This type of fatty liver occurred in fasting, carbohydrate starvation or in pancreatic diabetes. The pathologic type of fatty liver resulted from the impaired turnover of lipid in the liver by poisoning (chloroform, carbon tetrachloride, white phosphorous or a massive dose of alcohol) or by a dietary disorder (choline deficiency, amino acid imbalance or chronic alcoholism).

"Fatty liver syndrome" in chickens was first reported by Couch (1956) and was characterized by 1) increased body weight, 2) decreased egg production, 3) excessive amount of abdominal fat, 4) fatty livers, 5) capillary hemorrhages in the liver and hematoma. Reedy (1968) also characterized birds afflicted with fatty liver syndrome as: "very

healthy appearing birds suffering a gradual decrease in production," having a heavy body weight, having an excessive amount of abdominal fat, having an enlarged, fatty and friable liver and being sensitive to stress factors. Parker and Deacon (1968) stated that fatty liver syndrome occurred in all breeds of commercial hens fed all kinds of commercial diets.

Price et al. (1957) and Barton et al. (1966) observed a higher incidence of fatty liver problem in the caged layers than floor layers fed the same ration; however, bird density in a cage did not affect the total amount of lipid in the liver (Owings et al., 1967).

According to Couch (1964), Delaplane was unable to transmit fatty liver condition through feeding fecal materials, transplanting respiratory discharges and injecting blood of birds having fatty liver syndrome into normal birds.

Hamilton and Garlich (1970) reported that fatty liver syndrome could be produced by feeding aflatoxin. Later, Hamilton et al. (1972) demonstrated that feeding 1.0 ppm of aflatoxin to turkey poults resulted in a higher total lipid/liver as compared to the control group (0 ppm). However, the liver size was smaller in the aflatoxin fed group than the control fed group.

Dudley et al. (1961) reported that as the dietary cholesterol level was increased, liver cholesterol

content, total liver lipid and liver size also increased in young male chicks.

Turk et al. (1958) observed an increase in the amount of liver fat as the energy level of the diet was increased. Barton et al. (1966) made a similar observation that birds fed a high energy ration developed fatty liver syndrome which was prevented by feeding a low energy-high fiber diet. Morrison et al. (1970) also reported that a significantly higher content of liver fat was obtained in birds fed a diet containing 3080 Kcal. M.E./kg. diet in comparison to a group fed a 2640 Kcal. M.E./kg. diet.

Quisenberry et al. (1967) reported that even though body weight was increased as a result of feeding a high energy diet, fatty liver syndrome neither resulted nor was prevented by dietary calorie level. Donovan and Balloun (1955) also reported that energy level in the diet did not affect the amount of fat in the liver of chicks. In addition, they pointed out that the level of dietary protein and amount of lipid content in the liver of chicks was inversely related.

Howes and Fitzgerald (1966) found that as the dietary fat level was increased, liver fat also increased in coturnix quail. Weiss and Fisher (1957) also found an increased deposition of abdominal fat, as well as liver fat, when birds were fed a diet with 5 percent added fat in comparison to a no added fat group. On the contrary, Treat et al. (1960)

noticed the highest total liver weight and the percent liver fat from birds fed a diet containing no added fat in comparison to groups fed diets which contained 2.5 to 5 percent added fat. Due to the fact that the higher amount of fat was deposited in the livers of rats receiving a fat free diet, Harper et al. (1954) concluded that dietary fat per se did not accumulate to any greater extent in the liver and liver fat arose from the conversion of carbohydrate or protein to fat. McDaniel et al. (1959) also reported that added fat as an increased source of energy did not affect liver weight or liver fat of laying hens.

Nesheim et al. (1969) studied extensively fatty liver problems in laying hens. Some of the points of their report were:

1) Fatty liver mortality occurred almost entirely during the months of April, May and June which suggested that faulty adjustment of energy consumption during this period may be involved in causing this problem.
2) The best way to produce fatty livers might be to force feed diets in an amount above that normally consumed ad libitum.
3) High levels of liver fat per se may not be the problem in a laying flock as many hens that had high levels of liver fat were good performers and furthermore, hens did not die because of high

levels of liver fat but they died due to a massive liver hemorrhage.

In the following year, Nesheim and Ivy (1970) again stated that fatty liver might be developed due to an excess consumption of energy which might be the result of an inability of some hens to regulate energy intake. They also suggested to call fatty liver syndrome as "liver hemorrhage syndrome."

The term "fatty liver-hemorrhagic syndrome (FLHS)" was used by Wolford and Polin (1972a) who produced FLHS in laying hens by a force-feeding method. Groups of birds were force-fed feed in an amount of 50, 100, 112.5, 125 and 150 percent of control ad libitum fed group. Energy and protein content of the diet were 3095 Kcal. M.E./kg. and 17.5 percent, respectively. Within three weeks, birds that received the increased amount of feed showed an increased incidence and severity of hemorrhage in the liver. They concluded that overconsumption of feed (energy) was one of the probable causes of FLHS in laying hens.

Couch (1956) recommended a premix for the treatment of fatty liver syndrome which contained 5,000 to 10,000 IU of vitamin E, a minimum of 500 grams of choline chloride and 12 milligrams of vitamin B_{12} per ton of feed. The effect of this type of premix was not always satisfactory for preventing or alleviating fatty liver syndrome as reported by Barton (1967), Quisenberry et al. (1967), Deacon

(1968) and Parker and Deacon (1968). However, Bull (1968) noticed a more than 50 percent reduction of liver fat among flocks which had a typical fatty liver syndrome when vitamin B_{12} and vitamin E were added to the diet along with inositol. Reed et al. (1968) also found that adding inositol (½ lb./ton) to the diet decreased percentage of liver fat. Further decrease in percentage of liver fat was noticed when the combination of inositol (2.5 lbs./ton), choline, vitamin E and vitamin B_{12} was added to the diet.

Nelson (1968) reported that the addition of inositol (2 lbs./ton) caused a significant decrease in percentage of liver fat and a significant increase in liver moisture content of broiler breeder hens. On the other hand, Ragland et al. (1970) reported that addition of inositol (1,000 ppm) did not affect feed intake, egg production, body weight and percentage of liver fat of laying hens.

Jensen et al. (1970) introduced selenium as a new lipotropic agent. They observed a significant reduction in liver weight and total liver lipid when selenium was added to a diet of laying hens. On the contrary, Griffith and Schexnailder (1972) reported that addition of selenium, vitamin E or inositol to the laying ration did not significantly reduce liver fat while addition of choline and vitamin B_{12} was very effective.

III. GENERAL PROCEDURE

With the exception of part of experiment IV (treatments 2 and 4), all experiments were conducted in house 8-B (windowless) at the Michigan State University Poultry Science Research and Teaching Center, where two gas hovers were provided for the purpose of elevating environmental temperature whenever needed. A thermostat, an exhaust fan and an automatic light controlling device were also provided in the house.

A commercial strain of Single Comb White Leghorn (S.C.W.L.) pullets (De Kalb 131) were placed in triple deck laying cages (20.3 cm x 40.6 cm).

Body weights were measured at the beginning and at the end of each experiment. During all trials water was given ad libitum. Dead birds were sent to the Michigan State University diagnostic laboratory to determine the cause of death.

Birds were sacrificed by cervical dislocation, and their livers were collected for fat analysis. Liver samples were stored in a freezer (-15° C) until analyzed. Liver fat analysis was done according to the method described by Folch et al. (1957).

Those livers that contained 15 percent or more fat (wet basis) were considered as high fat-livers since the lowest percent liver fat with FLHS hemorrhages was found to be 15 percent, which was equivalent to 7.05 grams of fat per liver. When a liver contained 15 percent or more fat and also had more than 15 hemorrhages and/or hematoma (Wolford and Polin, 1972a), that liver was classified as FLHS liver in these experiments.

Data for percent hen-day egg production and percent liver fat were transformed to arcsin prior to statistical analysis. Analyses of variance and correlation coefficients were computed at the computer center of Michigan State University. Dunnett's "t" test (Kirk, 1968) was used whenever applicable for comparisons between the control and treatments.

IV. EXPERIMENTS

A. Experiment I

1. Procedure:

The primary purpose of conducting this experiment was to study the effect of different sources of energy (carbohydrate and fat) on the accumulation of fat in the liver of laying birds.

One hundred thirty-two, 22-week old S.C.W.L. pullets were randomly assigned to each of three replications in each treatment and were held in a thermocontrolled house (12.2^{o} C). Electric lights in the house were automatically turned on and off at 6:00 A.M. and 8:00 P.M., respectively, to provide the birds with a minimum of 14 hours of light daily. During the experimental period of 12 weeks (22 to 34 weeks of age), two pullets were kept in each of 66 cages and were fed diets ad libitum.

Based on different sources of carbohydrate or fat as a primary energy supplement, 11 dietary treatments were used (Table 1).

Diets used in treatments 1, 2 and 3 (Control) were corn-soy type formulae. Dietary levels of energy and/or protein of these three treatments were different from one another. Both the energy and protein level of the diet

Table 1. Treatment description, experiments I and II

Treatment	Energy Source				M.E. (Kcal/kg)	% Protein	C/P
	Primary	(%)*	Secondary	(%)*			
1	Corn	(84)	No oil	(0)	2899.2	15.0	193.2
2	Corn	(69)	Corn oil	(13)	3129.5	16.2	193.2
3 (Control)	Corn	(73)	Corn oil	(12)	3129.5	15.0	208.6
4	Wheat	(66)	Corn oil	(22)	3129.5	15.0	208.6
5	Milo	(72)	Corn oil	(14)	3129.5	15.0	208.6
6	Barley	(53)	Corn oil	(34)	3129.5	15.0	208.6
7	Oats	(53)	Corn oil	(34)	3129.5	15.0	208.6
8	Corn oil	(45)	Corn	(33)	3129.5	15.0	208.6
9	Safflower oil	(45)	Corn	(33)	3129.5	15.0	208.6
10	Herring oil	(45)	Corn	(33)	3129.5	15.0	208.6
11	Lard	(45)	Corn	(33)	3129.5	15.0	208.6

*Percentage of total calories in the diet.

in treatment 2 were higher than those of treatment 1, yet calorie-protein ratios were calculated to be identical. Treatment 3 was different from treatment 2 with respect to a lower dietary protein. Dietary energy levels of these two treatments were the same, however.

Diets having corn, wheat, milo, barley or oats as a primary energy source were designated to be treatments 3, 4, 5, 6 and 7, respectively. Corn oil, safflower oil, herring oil or lard were used as fat sources of energy, and corresponding diets were labelled as treatments 8, 9, 10 and 11, respectively.

Diets 3 to 11 were calculated to be isocaloric (3130 M.E. Kcal./kg.) and isonitrogenous (15 percent protein) (Table 1).

Composition of diets can be seen in Tables 2, 3 and 4.

Due to the fact that diets 6 to 11 were formulated to contain fairly high levels of fat, mixing feed for those diets was completed by two separate operations. First, all the dietary ingredients including 5 percent added fat were mixed in a feed mixer and then the feed was removed from the mixer and was placed on a concrete floor. In the second step, the remaining fat was added to the previously mixed feed, mixed with a shovel and then feed was forced through a wire mesh (0.7 cm x 0.7 cm). This hand-mixing was done at least three times per diet to

Table 2. Percentage composition of diets used in treatments 1, 2 and 3

Ingredients	Diet 1	Diet 2	Diet 3
Ground yellow corn	71.00	63.06	66.10
Soybean meal, 49% protein	15.00	18.71	15.90
Alfalfa meal, 17% protein	1.00	1.00	1.00
Distillers dried solubles, corn	1.00	1.00	1.00
Fishmeal, menhaden	1.00	1.00	1.00
Dried whey	0.50	0.50	0.50
Wheat bran	2.00	2.00	2.00
Ground limestone	6.04	5.80	5.91
Dicalcium phosphate	1.60	1.50	1.50
Corn oil		4.56	4.23
Salt, iodized	0.20	0.20	0.20
Vit.-min. premix*	0.58	0.58	0.58
Methionine	0.08	0.08	0.08
Total	100.00	100.00	100.00

*Refer to Appendix Table 1.

Table 3. Percentage composition of diets used in treatments 4, 5, 6 and 7

Ingredients	Diet 4	Diet 5	Diet 6	Diet 7
Wheat	67.30			
Milo		67.36		
Barley			62.58	
Oats				62.58
Soybean meal, 49% protein	9.82	12.56	10.98	10.98
Alfalfa meal, 17% protein	2.00	2.00	2.00	2.00
Distillers dried solubles, corn	2.00	2.00	2.00	2.00
Fishmeal, menhaden	2.00	2.00	2.00	2.00
Dried whey	0.10	0.10	0.10	0.10
Wheat bran	0.10	0.10	0.10	0.10
Ground limestone	6.28	6.20	5.91	5.97
Dicalcium phosphate	1.90	1.90	1.50	1.50
Corn oil	7.64	4.92	11.97	11.91
Salt, iodized	0.20	0.20	0.20	0.20
Vit.-min. premix*	0.58	0.58	0.58	0.58
Methionine	0.08	0.08	0.08	0.08
Total	100.00	100.00	100.00	100.00

*Refer to Appendix Table 1.

Table 4. Percentage composition of diets used in treatments 8, 9, 10 and 11

Ingredients	Diet 8	Diet 9	Diet 10	Diet 11
Ground yellow corn	30.00	30.00	30.00	30.00
Soybean meal, 49% protein	21.10	21.10	21.10	21.10
Alfalfa meal, 17% protein	2.00	2.00	2.00	2.00
Distillers dried solubles, corn	3.00	3.00	3.00	3.00
Fishmeal, menhaden	0.50	0.50	0.50	0.50
Dried whey	2.50	2.50	2.50	2.50
Wheat bran	3.00	3.00	3.00	3.00
Ground limestone	5.90	5.90	5.90	5.90
Dicalcium phosphate	1.50	1.50	1.50	1.50
Corn oil	15.89			
Safflower oil		15.89		
Herring oil			17.39	
Lard				16.50
Salt, iodized	0.20	0.20	0.20	0.20
Vit.-min. premix*	0.58	0.58	0.58	0.58
Methionine	0.08	0.08	0.08	0.08
Solkafloc	13.75	13.75	12.25	13.14
Total	100.00	100.00	100.00	100.00

*Refer to Appendix Table 1.

make sure that the feed ingredients were properly mixed with fat.

Feed consumption and body weight were measured bi-weekly and egg production was recorded daily.

In order to examine the degree of fat accumulation in the liver, one bird from each replication in each treatment was sacrificed at the end of both the sixth (28 weeks of age) and ninth (31 weeks of age) weeks of the experiment and were subjected to visual liver fat scoring. The scoring system is shown in Table 5.

At the end of the twelfth week of the experiment (34 weeks of age), all remaining birds were sacrificed and livers were collected for fat analysis.

2. Results and Discussion:

The effect of different sources of energy on the average visual liver fat score is presented in Table 6.

The data revealed that the average visual liver fat scores of the livers in all treatments were not high enough to indicate those livers as having high fat content. Livers with high fat content (high fat-liver) may be indicated by a liver score of 3.0 or above (Table 5). Also, there was no strong indication of further deposition of fat in the liver when liver samples were collected in two different periods.

None of the livers collected at the end of the sixth week (28 weeks of age) of the experiment were scored as

Table 5. Visual liver fat score*

Score	Liver	
	Texture	Color
0.5 and 1.0	Firm	Mahogany
1.5 and 2.0	Firm	Slight yellow in mahogany
2.5 and 3.0	Less firm	Yellow, little mahogany
3.5 and 4.0	Ruptured easily	Very yellow, no mahogany

*Wolford and Polin (1972b).

Table 6. The effect of different sources of energy on average liver fat score

Treatment	Ingredient	Avg. visual liver fat score	
		28 wks.*	31 wks.*
1	Corn	1.3	1.2
2	Corn	1.2	0.8
3 (Control)	Corn	1.2	1.7
4	Wheat	1.2	1.2
5	Milo	1.0	2.0
6	Barley	0.8	1.3
7	Oats	1.2	1.4
8	Corn oil	1.0	1.2
9	Safflower oil	1.3	1.0
10	Herring oil	1.2	1.3
11	Lard	1.0	1.3

*Age of birds.

3.0 or above, and only two livers were scored as 3.0 at the end of the ninth week (31 weeks of age).

The effect of different sources of energy on the performance and some of the liver characteristics are shown in Tables 7 and 8.

The largest average feed consumption and the heaviest average body weight resulted from the group of birds fed the corn-soy diet of treatment 1. The highest average egg production, however, was recorded from the birds fed the barley diet.

When birds were fed the diet that contained herring oil, the smallest average feed consumption and the lowest average egg production resulted.

It has been reported that birds eat to satisfy their energy requirement. Indirect evidence of this is that an increase in dietary energy from 2600 to 3350 calories (M.E.) per kilogram of diet will decrease feed consumption from 117 to 90 grams per hen per day during a 20 week laying period from 22 to 42 weeks of age (Scott et al., 1969).

In this experiment where isocaloric-isonitrogenous diets (treatments 3 to 11) were used, average feed consumption of birds in different dietary groups was different (56.3 to 107.1 gm./bird/day). This was particularly true when herring oil was included in the diet. This result implies that there are some factor(s) in the feed ingredients other than dietary energy which may influence feed consumption.

Table 7. The effect of different sources of energy on performance and some of the liver characteristics of laying birds

Treatment	Ingredient	Avg. feed/bird/day[1] 32-34 wks.[3]	Avg. initial body wt. 22 wks.[3]	Avg. final body wt. 34 wks.[3]	Avg. hen-day egg prod.[1] 32-34 wks.[3]	Liver Fat 34 wks.[3]	Liver Hemorrhage 34 wks.[3]	Liver FLHS hem.[2] 34 wks.[3]
		(gm)	(gm)	(gm)	(%)	(%)	(%)[4]	(%)[4]
1	Corn	107.1	1621	1817**	83.3	8.2(1.5)[5]	0	0
2	Corn	80.2	1480	1555	68.0	6.8(1.5)	0	0
3 (Control)	Corn	83.9	1476	1567	70.5	8.7(1.7)	16.6	0
4	Wheat	96.1	1579	1789**	81.4	10.8(1.6)	20.0	0
5	Milo	93.8	1514	1742**	82.1	9.8(2.2)	0	0
6	Barley	96.4	1497	1675*	89.2	7.0(1.5)	0	0
7	Oats	87.3	1519	1675*	71.8	6.6(1.5)	0	0
8	Corn oil	94.6	1583	1745**	83.1	5.9(1.5)	0	0
9	Safflower oil	86.6	1538	1699**	70.5	6.5(1.5)	0	0
10	Herring oil	56.3	1473	1649	53.9	10.9(1.7)	16.6	0
11	Lard	107.1	1560	1755**	86.2	9.2(1.7)	0	0

[1] Not computed for analysis of variance.

[2] More than 15 hemorrhages present in the liver.

[3] Age of birds.

[4] Percentage of birds within each treatment.

[5] Values in the parenthesis refer to average liver fat score.

* Significantly different ($P < 0.05$) from control according to Dunnett's "t" test.

** Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 8. Analyses of variance for initial body weight, final body weight and the percentage of liver fat in experiment I

Source of variation	Degrees of freedom	Mean square		
		Initial body wt. 22 wks.[1]	Final body wt. 34 wks.	% Liver fat[2]
Total	60			
Treatment	10	18642.1	51028.1*	14.93
Replication	2	13677.6	9190.7	14.37
T x R	20	19574.4	36719.9*	12.20
Error	28	13175.9	18434.4	7.34

[1]Initial body weights of those birds that were sacrificed at 28 and 31 weeks of age were not included.

[2]Arcsin transformed.

*Significant at $P < 0.05$.

Livers obtained on the last day of the experiment revealed that four livers (from treatments 1, 4, 5 and 10) contained 15 percent or more fat (wet basis).

Visual liver fat scoring system was also utilized on the terminal day of the experiment. A highly significant correlation ($r = +0.74$) between the visual liver fat score and the chemically determined percent liver fat was noted.

Average data for liver characteristics indicated that none of the diets produced high fat-liver or fatty liver-hemorrhagic syndrome (FLHS) equivalent to those observed in field cases. Some incidence of liver hemorrhages, however, was observed among birds fed diets containing corn (control), wheat and herring oil.

In this experiment 3.8 percent mortality was recorded but none of the birds died of FLHS.

B. Experiment II

1. Procedure:

Since most of the fatty liver mortality in field cases have been observed during spring-summer months as reported by Nesheim et al. (1969), a "high" environmental temperature (27.8° C) was employed in this experiment to see whether a "high" temperature along with different sources of energy affected liver fat accumulation.

Experiment II was a duplicate of the first experiment with the following exceptions: A high environmental temperature (27.8° C), older birds (29 weeks of age at the beginning of the experiment), less frequent intervals for measuring body weight and feed consumption (4 week interval) and no sacrifice of birds until the termination of the experiment.

Dietary treatments and the composition of diets used in this experiment were identical to those of the previous experiment (Tables 1, 2, 3 and 4).

Since the design of this experiment was split-plot, statistical analysis of the data for feed consumption and egg production was computed by analysis of variance for split-plot design (Kirk, 1968). Scheffe's "s" test (Kirk, 1968) was utilized to compare non-paired means (i.e.: high carbohydrate diet vs. high fat diet).

2. Results and Discussion:

The effect of the different sources of energy on the performance of laying birds kept in a "high" environmental temperature may be seen in Tables 9, 10 and 11.

The results showed that those birds fed the diet that contained wheat consumed the most feed and were the largest in average body weight. The highest average egg production, however, was obtained from the birds fed the diet that contained milo. As in the case of the previous experiment, birds fed the diet that contained herring oil consumed the least amount of feed and laid the least number of eggs.

The results also indicated that as long as dietary levels of energy and protein remained the same, the corn diet (control) could be replaced by all other grain diets without adversely affecting laying performance.

With the exception of the group of birds fed the diet that contained lard, all birds fed diets that contained fat as the primary energy source consumed significantly less feed and produced fewer eggs than the control group.

Liver samples collected on the terminal day of the experiment revealed that 16 livers (four from treatment 1, one from treatment 2, three from treatment 3, three from treatment 4, two from treatment 5, one from treatment 10 and two from treatment 11) contained 15 percent or more

Table 9. The effect of different sources of energy on the performance of laying birds kept in a "high" environmental temperature (27.8° C)

Treatment	Ingredient	Avg. feed/bird/day 29-41 wks.[1]	Avg. initial body wt. 29 wks.[1]	Avg. final body wt. 41 wks.[1]	Avg. hen-day egg prod. 29-41 wks.[1]
		(gm)	(gm)	(gm)	(%)
1	Corn	92.1	1608	1738	73.2
2	Corn	77.3**	1554	1531**	59.8**
3 (Control)	Corn	96.8	1644	1752	70.7
4	Wheat	106.3*	1627	1868**	78.5**
5	Milo	103.6	1658	1879**	79.1**
6	Barley	97.3	1548	1708	73.6
7	Oats	90.2	1613	1763	74.5
8	Corn oil	87.5*	1588	1693	63.4**
9	Safflower oil	81.0**	1610	1665*	60.1**
10	Herring oil	75.6**	1653	1656**	44.5**
11	Lard	90.7	1565	1710	71.6

[1]Age of birds.

*Significantly different ($P < 0.05$) from control according to Dunnett's "t" test.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 10. Analyses of variance for feed consumption and hen-day egg production in experiment II

Source of variation	Degrees of freedom	Mean square	
		Feed/bird/day	% Hen-day egg prod.[1]
Between subject			
Treatment	10	904.58**	349.47**
Subject/treatment	22	113.95	40.55
Within subject			
Period	2	1064.60**	785.59**
T x P	20	308.74**	943.65**
S x P	44	29.96	21.90

[1]Arcsin transformed.

**Significant at P < 0.01.

Table 11. Analyses of variance for initial and final body weights in experiment II

Source of variation	Degrees of freedom	Mean square	
		Initial body wt. 29 wks.	Final body wt. 41 wks.
Total	125		
Treatment	10	21720.7	110887.1*
Replication	2	47403.2	203306.2*
T x R	20	30420.5	44713.2
Error	93	23922.0	50837.0

*Significant at $P < 0.05$.

fat (wet basis) and four livers (two from treatment 1 and two from treatment 4) had hematomas.

The effect of different sources of energy on some of the liver characteristics of laying birds kept in a "high" environmental temperature can be seen in Tables 12 and 13.

The results showed that livers obtained from all of the treatments with the exception of treatment 1 were significantly lighter in average wet weight than those of the control group. Average percent liver fat and total liver fat values were also smaller than those of the control in most cases.

Eighteen percent of the birds that received the corn diet (control) and 9 percent of the birds that received the wheat diet were found to have FLHS hemorrhages. Considering the fact that average percentages of liver fat for those treatments (as well as in other treatments) were not very high, the occurrence of FLHS might not be due to those diets.

In this experiment, treatments 3, 4, 5, 6 and 7, and treatments 8, 9, 10 and 11 were collectively referred to as a high carbohydrate and a high fat diet, respectively. The effect of a high carbohydrate and a high fat diet on average feed consumption, body weight, egg production and liver fat accumulation can be seen in Table 14.

Under the conditions of this experiment, birds fed a high carbohydrate diet consumed significantly more feed and produced more eggs than the birds fed a high fat diet.

Table 12. The effect of different sources of energy on some of the liver characteristics of laying birds kept in a "high" environmental temperature (27.8° C)

Treatment	Ingredient	Liver				
		Avg. wet wt.	Avg. fat	Avg. total fat	Hemorrhage	FLHS hemorrhages[1]
		(gm)	(%)[2]	(gm)	(%)[3]	(%)[3]
1	Corn	33.6	13.4	4.7	36.3	18.1
2	Corn	29.4**	8.1**	2.5**	0	0
3 (Control)	Corn	34.5	13.0	4.5	18.1	0
4	Wheat	32.9*	11.1	3.8	18.1	9.1
5	Milo	32.7**	10.7	3.7*	8.3	0
6	Barley	30.8**	7.1**	2.2**	0	0
7	Oats	31.4**	6.5**	2.0**	0	0
8	Corn oil	29.0**	6.7**	1.9**	0	0
9	Safflower oil	29.1**	6.5**	2.0**	0	0
10	Herring oil	31.8**	7.8**	2.6**	0	0
11	Lard	30.2**	11.1	3.8*	16.6	0

[1]More than 15 hemorrhages present in the liver.

[2]On wet weight basis.

[3]Percentage of birds within each treatment.

*Significantly different ($P < 0.05$) from control according to Dunnett's "t" test.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 13. Analyses of variance for several liver characteristics in experiment II

Source of variation	Degrees of freedom	Mean square		
		Liver wet wt.	% Liver fat[1]	Total liver fat
Total	125			
Treatment	10	39.86**	64.62**	11.96**
Replication	2	88.24**	40.89	9.52
T x R	20	16.06	16.70	3.55
Error	93	13.82	20.13	4.12

[1]Arcsin transformed.

**Significant at P < 0.01.

Table 14. The effect of a high carbohydrate and a high fat diet on average feed consumption, final body weight, egg production and liver fat accumulation

Type of diet	Avg. feed/bird/day 29-41 wks.	Avg. final body wt. 41 wks.	Avg. hen-day egg prod. 29-41 wks.	Avg. liver fat 41 wks.	Avg. total liver fat 41 wks.
	(gm)	(gm)	(%)	(%)	(gm)
High carbohydrate[1]	98.8	1794	75.3	9.7	3.2
High fat[2]	83.7**(86.4*)[3]	1681 (1689)	60.0**(65.0*)	8.0 (8.1)	2.6 (2.6)

[1]Combined average of treatments 3, 4, 5, 6 and 7.

[2]Combined average of treatments 8, 9, 10 and 11.

[3]Values in the parenthesis refers to combined averages of treatments 8, 9 and 11.

*Significantly different ($P < 0.05$) from high carbohydrate diet according to Scheffe's "s" test.

**Significantly different ($P < 0.01$) from high carbohydrate diet according to Scheffe's "s" test.

The average values for final body weight and liver fat (percent and total) were also slightly higher for those birds fed a high carbohydrate diet as opposed to a high fat diet. Statistical difference due to diets was not obtained, however.

In this experiment 4.5 percent mortality was recorded. However, mortality due to FLHS was nil.

C. Experiment III

1. Procedure:

The purpose of conducting this experiment was to try to produce fatty liver-hemorrhagic syndrome in laying birds by taking advantage of the results of the previous experiment that showed a high carbohydrate diet in a "high" environmental temperature seemed to favor accumulation of liver fat. A restricted-ad libitum feeding system was employed to induce overeating during the ad libitum period, since Wolford and Polin (1972a) observed FLHS among force-fed hens which received an excess amount of feed.

This experiment was conducted with 60 S.C.W.L. pullets which were randomly assigned into three replications of five treatments. During the experimental period of 26 weeks, all birds were kept in individual cages.

The experimental period was divided into the following three phases:

First phase (phase 1): 14-20 weeks of age

Second phase (phase 2): 20-28 weeks of age

Third phase (phase 3): 28-40 weeks of age

Environmental temperatures during phases 1 and 2 were maintained at 15.9^{o} C and during phase 3 at 30.6^{o} C (actual average temperatures).

A 9-hour artificial light day (7:30 A.M. to 4:30 P.M.) was provided from 14 to 20 weeks of age; thereafter, 14 hours of light (6:00 A.M. to 8:00 P.M.) were provided daily.

During the first phase, birds in all treatments were fed a pullet developer diet (composition of pullet developer diet is listed in Appendix Table 2).

From 20 to 40 weeks of age (phases 2 and 3) birds in all treatments (with the exception of those in treatment 2) received the diet used in treatment 3 (control) of previous experiments. The diet used in treatment 2 was a commercial laying formula. (This ration is presently not available.) According to Barton (1967), birds that received this ration had experienced some problems with fatty liver in the field. For convenience, this diet will be referred to as Diet F. Diet F was made and was included in this experiment to determine if dietary factor(s) other than feed consumption (energy intake) is/are involved in producing FLHS.

The birds in treatments 1 and 2 received ad libitum feeding throughout the experiment, while a reduced amount of feed was given each day to the birds in treatments 3 and 4 during the first and second phases, respectively. The birds in treatment 5 received a reduced amount of feed daily during both phases 1 and 2, and then were allowed to eat ad libitum during the third phase.

Treatment description, the amount of feed allowed per day during the restricted feeding period, and the composition of Diet F can be seen in Tables 15, 16 and 17, respectively.

Table 15. Treatment description, experiment III

Treatment	Age of birds					
	14-20 wks.		20-38 wks.		28-40 wks.	
	Feeding system	°C*	Feeding system	°C*	Feeding system	°C*
1 (Control)	ad lib.	15.6	ad lib.	15.6	ad lib.	32.2
2	ad lib.	15.6	ad lib.**	15.6	ad lib.**	32.2
3	Restricted	15.6	ad lib.	15.6	ad lib.	32.2
4	ad lib.	15.6	Restricted	15.6	ad lib.	32.2
5	Restricted	15.6	Restricted	15.6	ad lib.	32.2

*Environmental temperature; Actual average temperature recorded for 15.6° C and 32.2° C were 15.9° C and 30.6° C, respectively.

**Diet F.

Table 16. Amount of feed allowed per day during restricted feeding period

Age of bird weeks	Feed restriction Pounds feed/100 pullets/day
14-16	13
16-18	14
18-19	15
19-20	16
20-28	19

Table 17. Composition of Diet F

Ingredient		Percent
Ground yellow corn		75.00
Soybean meal, 49% protein		11.90
Alfalfa meal, 17% protein		0.90
Meat and bone meal, 50% protein		2.20
Fishmeal, menhaden		0.40
Dried fish soluble		0.10
Feather meal		0.20
Blood meal		1.00
Ground limestone		5.85
Dicalcium phosphate		1.40
Salt, iodized		0.35
Animal tallow		0.50
Vit.-min. premix*		0.20
	Total	100.00
Calculated analysis		
Kcal. M.E./kg. diet		3004
Crude protein, %		15.2

*Supplied the following per kg of diet: Vitamin A, 3520 IU; Vitamin D_3, 1100 I.C.U.; Riboflavin, 3.1 mg; Pantothenic acid, 5.3 mg; Niacin, 11.0 mg; Choline chloride, 171.6 mg; Folic acid, 0.44 mg; Vitamin B_{12}, 5.28 µg; Vitamin E, 2.2 IU; Menadione sodium bisulfite, 0.7 mg; Manganese, 25.7 mg; Iodine, 0.40 mg; Copper, 0.16 mg; Cobalt, 102 µg; Zinc, 20 mg; Iron, 10.0 mg; Magnesium, 200 mg.

Body weight and feed consumption were measured at the end of 17 and 20 weeks of age for phase 1, 24 and 28 weeks for phase 2 and 34 and 40 weeks for phase 3. Egg production was recorded daily and egg weight was measured on three consecutive days of every week (1:30 P.M. on Tuesday, Wednesday and Thursday) during 20 to 40 weeks of age. At the end of 40 weeks of age, birds in all treatments were sacrificed for liver fat analysis.

The data for feed consumption and body weight obtained during the first and second phases were not subjected to the statistical analyses since a fixed amount of feed was given to birds during those periods.

Student's "t" test was used to compare FLHS livers and high fat-livers without having severe hemorrhages, and between FLHS livers and low fat-livers.

2. Results and Discussion:

The effect of feed restriction on average body weight and hen-day egg production is shown in Table 18.

As expected, the most obvious effect of feed restriction was a reduction of body weight during the feed restriction period (treatment 3 in phase 1 and treatment 4 in phase 2). During phase 2, birds that received a restricted amount of feed laid 4 percent less eggs than the control birds, which received ad libitum feeding.

When feed restriction was terminated, birds consumed 7 more grams of feed, on the average, per day and laid 4

Table 18. The effect of feed restriction on average body weight and hen-day egg production

Treatment	Avg. feed/bird/day		Avg. body weight			Avg. hen-day egg prod.
	14-20 wks.[1]	20-28 wks.[1]	initial[2]	20 wks.[1]	28 wks.[1]	20-28 wks.[1]
	(gm)	(gm)	(gm)	(gm)	(gm)	(%)
1 (Control)	85.8	117.0	1098	1456	1680	75.4
2	84.1	123.3	1094	1450	1692	79.4
3	63.4	124.3	1088	1254	1681	79.4
4	85.4	81.1	1077	1433	1525	71.2
5	63.3	86.2	1077	1269	1573	79.8

[1]Age of birds.

[2]Fourteen weeks of age.

percent more eggs than did the control birds during the next eight weeks of the ad libitum feeding period (treatments 3 vs. 1 in phase 2).

Birds fed Diet F consumed 6 more grams of feed (on the average) daily and produced 4 percent more eggs than did the birds in the control group during phase 2. This increased feed consumption might be due to lower energy content of Diet F in comparison to the control diet.

At the end of phase 2, birds in all treatments were allowed to eat ad libitum in the elevated environmental temperature (30.6^{o} C) for a period of 12 weeks.

The effect of restricted feeding followed by ad libitum feeding on the performance of laying birds is shown in Tables 19 and 20.

The results indicated that average feed consumption, final body weight and egg weight obtained during the final ad libitum feeding period (28 to 40 weeks of age) were not significantly affected by previous feed restrictions performed during either phases 1 or 2 or both. Thus, in the long run, the net effect of restricted feeding followed by ad libitum feeding may reside in the feed savings during the feed restriction period without adversely affecting the subsequent laying performance. Average egg weight, however, increased more rapidly in groups which had previously received a restricted amount of feed (treatments 3, 4 and 5) as compared with that of the control ad libitum fed group.

Table 19. The effect of restricted feeding followed by ad libitum feeding on the performance of laying birds

Treatment	Avg. feed/bird/day 28-40 wks.[1]	Avg. final body wt. 40 wks.[1]	Avg. hen-day egg prod. 28-40 wks.[1]	Avg. egg weight 28-34 wks.[1]	Avg. egg weight 34-40 wks.[1]
	(gm)	(gm)	(%)	(gm)	(gm)
1 (Control)	102.8	1773	74.0	52.8	56.6
2	104.1	1700	79.7	53.8	55.7
3	104.3	1720	80.6	51.9	57.1
4	99.9	1700	71.5	53.4	58.1
5	101.8	1788	80.3	52.2	58.0

[1]Age of birds.

Table 20. Analyses of variance for the performance of laying birds in experiment III

Source of variation	Degrees of freedom	Mean square				
		Feed/bird/day 28-40 wks.	Final body wt. 40 wks.	% Hen-day egg prod.[1] 28-40 wks.	Egg weight 28-34 wks.	Egg weight 34-40 wks.
Total	54					
Treatment	4	36.22	17946.8	107.06	6.81	11.70
Replication	2	370.41	3495.7	97.42	7.43	8.84
T x R	8	163.74	30463.1	16.56	14.60	11.56
Error	40	165.29	40950.5	45.80	14.70	18.49

[1]Arcsin transformed.

The results also indicated that birds fed Diet F (treatment 2) were not significantly different from the control as far as the average feed consumption, final body weight and hen-day egg production were concerned.

The effect of two diets and restricted feeding followed by ad libitum feeding on several liver characteristics are shown in Tables 21 and 22.

The results indicated that previous feed restriction during either the first (14 to 20 weeks of age) or the second phase (20 to 28 weeks of age) caused significant reductions in average liver wet weight, percent liver fat, total liver fat, liver fat per kilogram of body weight and incidence of liver hemorrhages at 40 weeks of age as compared with corresponding values obtained in the control ad libitum fed group.

When a group of birds which had previously received a restricted amount of feed during phases 1 plus 2 (treatment 5), those criteria mentioned above were not significantly different from the control (treatments 5 vs. 1 in phase 3). Furthermore, 16.7 percent of birds which had received this treatment showed FLHS whereas none of the FLHS occurred in the control or in other previously feed restricted groups. Considering the fact that the average percent liver fat of birds in treatment 5 was not very high, the occurrence of FLHS might have happened by chance alone.

Table 21. The effect of two diets and a restricted feeding followed by ad libitum feeding on several liver characteristics

Treatment	Liver						Energy intake/ hen/day	Eggs laid in last 7 days
	Wet wt.	Fat	Total fat	Fat/kg. body wt.	Hemorrhage	FLHS hem.[1]		
	(gm)	(%)[2]	(gm)	(gm)	(%)[3]	(%)[3]	(Kcal. M.E.)	(no.)
1 (Control)	42.5±3.0[4]	15.1±0.1	7.1±1.5	4.0±0.8	40.0	0.0	321.7	5.1
2	62.7±4.4**	28.0±0.2**	18.9±3.1**	11.2±1.9**	50.0	41.7	316.4	5.2
3	38.2±1.6**	10.0±0.1**	4.1±0.6**	2.3±0.3**	9.1	0.0	326.5	5.7
4	35.4±1.7**	9.8±0.1**	3.8±0.6**	2.2±0.6**	30.0	0.0	312.7	5.5
5	43.3±3.0	13.3±0.1	6.4±1.3	3.5±0.7	25.0	16.7	318.6	5.8

[1]More than 15 hemorrhages present in the liver.

[2]On wet basis.

[3]Percentage of hens within each treatment.

[4]Mean ± S.E.M.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 22. Analyses of variance for several liver characteristics in experiment III

Source of variation	Degrees of freedom	Mean square (Liver)				Mean square	
		Wet wt.	% Fat[1]	Total fat	Fat/kg. body wt.	Kcal. M.E./hen/day	Eggs in last 7 days
Total	54						
Treatment	4	1329.56**	370.57**	454.55**	163.98**	331.45	1.63
Replication	2	210.44	95.42*	60.07	20.15	3635.31	0.35
T x R	8	110.62	15.16	16.75	7.56	1596.18	0.40
Error	40	95.17	29.17	37.66	12.31	1599.93	0.52

[1]Arcsin transformed.

*Significant at P < 0.05.

**Significant at P < 0.01.

When birds were fed Diet F (treatment 2), average liver wet weight, percent liver fat, total liver fat and liver fat per kilogram of body weight were significantly higher than corresponding values obtained from all other treatments. The highest incidence of hemorrhages and fatty liver-hemorrhagic syndrome type of hemorrhages (FLHS hemorrhages) also occurred in birds that were fed Diet F.

In spite of the differences in liver characteristics, energy intake and egg production measured during the last seven days of the experiment were not significantly different between treatments (including Diet F). Thus, it appeared that the level of energy intake did not seem to be closely related to the production of high fat-liver or FLHS under the conditions of this experiment.

Average final body weight or hen-day egg production were not good indicators of FLHS of laying chickens, since correlations between those criteria and liver characteristics were very low (see Appendix Table 3).

High correlations between liver wet weight and the percentage of liver fat ($r = +0.85$) and between the percentage of liver fat and total amount of liver fat ($r = +0.93$) indicated that an increased liver wet weight was partly due to an increase in the degree of fat deposition which resulted in an increase in total fat in the whole liver.

In this experiment the lowest total liver fat (TLF) with FLHS hemorrhages was found to be 7.05 grams. Based

on the TLF of 7.05 grams, all birds used in this experiment were divided into the following three categories in order to compare the values of average feed consumption and liver fat content in relation to presence or absence of FLHS hemorrhages (Table 23).

Category A: Birds having more than 7.05 grams of TLF with FLHS hemorrhages

Category B: Birds having more than 7.05 grams of TLF without FLHS hemorrhages

Category C: Birds having equal to or less than 7.05 grams of TLF without FLHS hemorrhages

The data showed that out of 55 birds, 15 birds had high liver fat values without having severe hemorrhages. This indicated that although FLHS hemorrhages occurred in birds with high liver fat values (TLF and percent liver fat), a high level of fat in the liver was not always related to the incidence of FLHS hemorrhages (A vs. B).

The only statistical difference in liver characteristics measured between high fat-livers without having severe hemorrhages and FLHS livers was liver wet weight. FLHS livers were significantly heavier than high fat-livers without having severe hemorrhages. This weight difference could be due to the difference in water content, since liver fat content between those two types of livers was not significantly different.

During the course of this experiment, five birds died of various diseases, however, no birds died of FLHS.

Table 23. Comparison of average feed consumption and liver fat content in relation to presence or absence of FLHS hemorrhages

	FLHS hemorrhages			A vs. B	A vs. C
	A	B	C		
	Present	Absent	Absent		
	TLF > 7.05 gm[1]	TLF > 7.05 gm	TLF ≤ 7.05 gm	Significance	
Avg. feed/bird/day (gm)	106.0 ±1.4[2]	107.0 ±0.9	100.0 ±0.6		
Avg. liver wet wt. (gm)	67.4 ±1.4	53.1 ±0.8	36.5 ±0.4	*	**
Avg. % liver fat (arcsin)	31.86±1.00	29.22±0.53	17.74±0.31		**
Number of birds	7	15	33		

[1]FLHS hemorrhages did not occur in livers having less than 7.05 gm fat (total liver fat) in them.

[2]Mean ± S.E.M.

*Significant at $P < 0.05$ according to Student's "t" test.

**Significant at $P < 0.01$ according to Student's "t" test.

D. Experiment IV

1. Procedure:

Experiment IV was conducted with 32-week old S.C.W.L. pullets, which came from the same flock as the birds used in experiment III.

Before starting this experiment all birds received the same pullet developer diet and were also kept in the same environmental temperature (15.9° C) from 14 to 22 weeks of age as the birds utilized in experiment III. From 20 to 32 weeks of age, all birds were given a low-energy (Z-4) laying ration. (Composition of Z-4 diet is listed in Appendix Table 4.) Feed was given ad libitum from 14 to 32 weeks of age.

One of the two objectives of this experiment was to test the effect of Diet F on FLHS. The other objective was to study the effect of feeding regimes in combination with different environmental temperatures on liver fat accumulation and liver hemorrhage incidence.

During the experimental period of eight weeks, eight birds in each of four treatments were held in individual cages and were fed Diet F. The composition of Diet F may be seen in Table 17.

In addition to pen 8-B at the Poultry Science Research and Teaching Center, room 5-A (air conditioned) in Anthony

Hall was utilized to take advantage of different environmental temperatures. The temperature of room 5-A had been set to be 22.2° C, and electric lights in the room were automatically turned on and off at 7:00 A.M. and 12:00 midnight, respectively.

During the four-week feed restriction period (32 to 36 weeks of age), birds in all treatments except treatment 1 (control, ad libitum) received a cyclic feeding treatment (no feed every third day). From 36 to 40 weeks of age, all birds were fed ad libitum.

Birds in treatment 1 received ad libitum feeding at all times in 30.6° C (actual average temperature). Birds in treatment 2 received a restricted feeding treatment in 30.6° C and were then fed ad libitum in 22.2° C (actual average temperature). Restricted feeding followed by ad libitum feeding was performed in 30.6° C for treatment 3. Birds in treatment 4 received a restricted feeding treatment in 22.2° C and were then allowed to eat ad libitum in 30.6° C.

Treatment description which includes environmental temperatures during the feed restriction and the ad libitum feeding periods can be seen in Table 24.

Body weight was measured at the end of both the restricted and the ad libitum feeding periods. Feed consumption was measured every first and second day after feed restriction (no feed) during the first four weeks,

Table 24. Treatment description, experiment IV

Treatment	Age of birds			
	32-36 wks.		36-40 wks.	
	Feeding system	°C*	Feeding system	°C*
1 (Control)	ad lib.	32.2	ad lib.	32.2
2	Restricted**	32.2	ad lib.	22.2
3	Restricted**	32.2	ad lib.	32.2
4	Restricted**	22.2	ad lib.	32.2

*Environmental temperature; Actual average temperature recorded for 22.2° C and 32.2° C were 22.2° C and 30.6° C, respectively.

**No feed every third day.

and it was measured every week during the ad libitum feeding period of the second four weeks of experiment.

At the end of 40 weeks of age, birds in all treatments were sacrificed for liver fat analysis.

Student's "t" test was used to compare FLHS livers and high fat-livers without having severe hemorrhages, and between FLHS livers and low-fat livers.

2. Results and Discussion:

The effects of feed restriction in different environmental temperatures on average body weight and egg production are shown in Tables 25 and 26.

Regardless of environmental temperatures employed, birds in restricted feeding treatments were significantly smaller in average body weights as compared with the control ad libitum fed group, yet average hen-day egg production was not affected by feed restriction.

When average body weights were compared within feed restricted groups, birds kept in a lower environmental temperature were heavier than birds kept in a higher temperature. This was probably due to the increased feed consumption.

The effect of restricted feeding followed by ad libitum feeding in different environmental temperatures on the performance of laying birds can be seen in Tables 27 and 28.

Table 25. The effects of feed restriction in different environmental temperatures on average body weight and egg production

Treatment	Avg. feed/bird		Avg. body weight		Avg. hen-day egg prod.
	1st day[1]	2nd day[1]	Initial[2]	36 wks.[3]	32-36 wks.[3]
	(gm)	(gm)	(gm)	(gm)	(%)
1 (Control)	100.9	93.7	1650	1725	81.4
2	140.8**	108.3**	1666	1566**	74.1
3	147.4**	107.8**	1659	1516**	77.9
4	173.1**	130.4**	1686	1604**	81.0

[1]Following feed restriction (no feed).

[2]Thirty-two weeks of age.

[3]Age of birds.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 26. Analyses of variance for the performance of laying birds during the feed restriction period in experiment IV

Source of variation	Degrees of freedom	Mean square			
		Feed/bird		Final body weight	% Hen-day egg production[1]
		1st day[2]	2nd day	36 wks.	32-36 wks.
Total	29				
Treatment	3	6029.1**	1648.4**	53120.9*	40.61
Error	26	455.0	207.9	14017.2	57.26

[1]Arcsin transformed.

[2]Following feed restriction (no feed).

*Significant at $P < 0.05$.

**Significant at $P < 0.01$.

Table 27. The effect of restricted feeding followed by ad libitum feeding in different environmental temperatures on the performance of laying birds

Treatment	Avg. feed/bird/day 36-40 wks.	Avg. final body wt. 40 wks.	Avg. hen-day egg prod. 36-40 wks.
	(gm)	(gm)	(%)
1 (Control)	94.2	1742	79.4
2	119.5**	1791	71.3
3	95.0	1609**	82.1
4	99.4*	1766	83.3

*Significantly different ($P < 0.05$) from control according to Dunnett's "t" test.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 28. Analyses of variance for the performance of birds during the ad libitum feeding period in experiment IV

Source of variation	Degrees of freedom	Mean square		
		Feed/bird/day 36-40 wks.	Final body wt. 40 wks.	% Hen-day egg production[1] 36-40 wks.
Total	29			
Treatment	3	1080.73**	52171.6*	109.85
Error	26	97.65	16974.2	39.47

[1]Arcsin transformed.

*Significant at P < 0.05.

**Significant at P < 0.01.

At the same "high" environmental condition (30.6° C) average final body weight of birds that received a restricted feeding regime was significantly smaller than that of the control ad libitum fed group. Average feed consumption and hen-day egg production were not significantly different, however (treatments 3 vs. 1). These results along with those obtained during the feed restriction period indicate that body weight is more susceptible than the rate of lay in response to reduction of feed intake.

When environmental temperature of the ad libitum feeding period was decreased, average feed consumption increased significantly without affecting average final body weight and hen-day egg production (treatments 2 vs. 1). The same phenomenon was observed during the ad libitum feeding period from the group which was previously exposed to lower environmental temperature while feed restriction was performed (treatments 4 vs. 1). Therefore, changing environmental temperature in either the feed restriction or the ad libitum periods may affect feed consumption of birds during a later ad libitum period.

The effect of two environmental temperatures and restricted feeding followed by ad libitum feeding on some liver characteristics may be seen in Tables 29 and 30.

Average values for liver wet weight, percent liver fat and total liver fat obtained from a group of birds fed Z-4 diet were also included in Table 29 for reference.

Table 29. The effect of two environmental temperatures and restricted feeding followed by ad libitum feeding on several liver characteristics

Treatment	Liver						Energy intake/ hen/day	Eggs laid in last 7 days
	Wet wt.	Fat	Total fat	Fat/kg. body wt.	Hemorrhage	FLHS hemorrhage[1]		
	(gm)	(%)[2]	(gm)	(gm)	(%)[3]	(%)[3]	(Kcal. M.E.)	(no.)
1 (Control)	67.2±10.3[4]	28.9±0.3	21.3±4.6	12.3±2.6	66.7	33.3	286.4	5.2
2	69.0± 4.3	23.3±0.1	16.6±2.6	9.3±1.4	100.0	50.0	355.4**	5.1
3	55.5± 5.0	24.6±0.1	15.6±2.6	8.4±1.8	50.0	12.5	288.8	5.5
4	55.9± 2.8	30.3±0.1	17.3±2.0	9.9±1.1	62.5	12.5	302.2*	5.9
(2-4)	30.2	8.7	2.6	--	0.0	0.0	--	--

[1]More than 15 hemorrhages present in the liver.

[2]On wet basis.

[3]Percentage of hens within each treatment.

[4]Mean ± S.E.M.

*Significantly different ($P < 0.05$) from control according to Dunnett's "t" test.

**Significantly different ($P < 0.01$) from control according to Dunnett's "t" test.

Table 30. Analyses of variance for several liver characteristics in experiment IV

Source of variation	Degrees of freedom	Mean square (liver)				Mean square	
		Wet wt.	% Fat[1]	Total fat	Fat/kg. body wt.	Kcal. M.E./hen/day	Eggs in last 7 days
Total	29						
Treatment	3	392.60	36.73	40.13	18.76	7953.48**	0.93
Error	26	231.88	30.69	62.65	21.05	892.38	1.33

[1]Arcsin transformed.

**Significant at P < 0.01.

Although there were no significant differences in liver characteristics between treatments, high fat-livers and some of the FLHS livers were observed in all treatments.

A smaller total liver fat, and lesser incidence of liver hemorrhages and FLHS, in most cases were noted from groups of birds which had received restricted feeding regimes in comparison to the control ad libitum fed group.

The only exception can be seen in treatment 2 where birds received a restricted feeding treatment in 30.6° C and then were allowed to eat ad libitum in 22.2° C. More incidence of both liver hemorrhages and FLHS was observed from the birds in treatment 2 than birds in the control. This was probably due to a higher intake of energy of birds in treatment 2 as compared with that of birds in the control.

Higher incidence of liver hemorrhages was noted when the environmental temperature during the feed restriction and during the ad libitum period were different by 10° C (treatments 2 and 4 vs. 3). Thus, switching environmental temperature of the feed restriction and of the ad libitum period may affect incidence of liver hemorrhages.

As in the case of the previous experiment, FLHS hemorrhages occurred in livers with high fat but not all livers with high fat contained FLHS hemorrhages (Table 31).

Average feed consumption, final body weight and hen-day egg production between groups of birds having FLHS and

Table 31. Comparison of liver fat content and the performance of birds in relation to presence or absence of FLHS hemorrhages

	FLHS hemorrhages			A vs. B	A vs. C
	A	B	C		
	Present	Absent	Absent		
	TLF > 12.15 gm[1]	TLF > 12.15 gm	TLF ≤ 12.15 gm	Significance	
Avg. liver wet wt. (gm)	76.0 ±1.3[2]	62.3 ±0.8	43.5 ±1.0	*	**
Avg. % liver fat (arcsin)	30.78±0.74	34.43±0.47	25.43+0.74		*
Avg. feed/bird/day (gm)	107.3 ±1.5	99.8 ±0.8	102.3 ±1.5		
Avg. final body wt. (gm)	1744 ±4	1717 ±3	1724 ±5		
Avg. hen-day egg prod. (arcsin)	59.48±0.60	63.92±0.76	64.51±0.79		
Number of birds	8	15	7		

[1]FLHS hemorrhages did not occur in livers having less than 12.15 gm fat (total liver fat) in them.

[2]Mean ± S.E.M.

*Significant at P < 0.05 according to Student's "t" test.

**Significant at P < 0.01 according to Student's "t" test.

high liver fat content without massive hemorrhages, and between birds having FLHS and low liver fat content were not significantly different (Table 31).

Although the highest incidence of hemorrhages and FLHS occurred in the particular group of birds which consumed the highest energy (treatment 2), energy intake in general did not appear to be significantly related to liver characteristics as indicated by low correlations between these variables (Appendix Table 5) and by feed consumption comparison data of Table 31.

In this experiment, no mortality due to FLHS was recorded (2 birds died of other diseases).

Considering the fact that high fat-liver and FLHS liver occurred predominantly in the group of birds fed Diet F in experiment III, and that high fat-liver and some incidence of FLHS occurred in all treatments of experiment IV in which only Diet F was used, a possibility exists that some factor(s) in Diet F is/are involved in producing high fat-liver, if not FLHS.

V. GENERAL DISCUSSION

A review of the literature indicated that there are different opinions concerning the relationship between dietary energy level and occurrence of fatty liver in chickens. Several investigators have reported that an increased dietary energy level caused an increase in liver fat, while others did not observe this relationship. At any rate, it may not be reasonable to try to relate a low energy diet and high incidence of fatty liver problem unless some chemical substances which are toxic to the liver cell are present in the diet.

Little information was available on the performance of chickens and liver fat content as influenced by dietary energy sources. Thus, it seemed desirable to study the effect of high energy diets of different energy sources on the performance and liver fat accumulation of chickens for the first experiment.

The results of experiment I showed that when birds were fed the diet that contained herring oil as a primary energy source, those birds consumed the least amount of feed and produced fewer eggs than did the birds fed all other isocaloric-isonitrogenous diets. This phenomenon was also observed in experiment II, where environmental

temperature (27.8° C) was higher than that of experiment I (12.2° C). Thus, it appeared that although birds are known to have few taste buds, apparently the taste of herring oil diet (or some other factor(s) in the feed) was not desirable for them and as a consequence a reduction of feed consumption resulted. Decreased egg production appeared to be due to a marked decrease in feed intake unless some other factor in the herring oil diet influenced egg production.

The results also indicated that in either environmental temperature employed energy from carbohydrate sources was not statistically different from that of fat sources on their respective influence on liver fat content. Slightly higher percent liver fat, however, was obtained from birds fed a high carbohydrate diet in a "high" environmental temperature (27.8° C). In general, livers from laying birds fed the same diets but kept in a "high" environmental temperature had a higher percentage of liver fat and a higher incidence of liver hemorrhages than laying birds kept in a "moderate" (12.2° C) environmental temperature. Similar phenomenon has been previously observed by Nesheim et al. (1969). Direct comparison between the two experiments in these trials, however, cannot be made due to different experimental conditions.

The definition of FLHS liver is rather subjective. If FLHS livers were defined as those livers which were

"fatty" (15 percent or more fat on wet basis) and contained more than 15 hemorrhages and/or hematomas, none of the diets used in experiments I and II caused FLHS.

According to Masoro (1968) the amount of fat in the liver is influenced by the balance of the input processes of triglyceride precursors (from dietary carbohydrate and fat) into the liver for formation of triglyceride and the processes of removing triglyceride from the liver (output processes). There are two known output pathways. One is that the triglyceride is incorporated by the endoplasmic reticulum into the lipoproteins and secreted in this form into the plasma. The other pathway is that triglyceride undergoes lipolysis to free fatty acids and glycerol, which can be oxidized in the mitochondria.

In experiment III, when birds were fed a restricted amount of feed, average body weight was significantly smaller than the control ad libitum fed group. Average egg production, however, was not different from the control group. If one hypothesizes that the rate of hepatic lipid oxidation is constant and lipid going out into the yolk is much greater than that going out for the formation of body fat, one may expect to observe a smaller percentage of liver fat as well as a smaller liver wet weight of birds in a restricted feeding group.

When birds were allowed to eat ad libitum after termination of either the first (14 to 20 weeks of age) or the

second phase (20 to 28 weeks of age) of restricted feeding, their average liver wet weights and the percentages of liver fat were significantly smaller than those from birds that had no previous experience of receiving a restricted feeding regime. However, average feed consumption, final body weight and percent hen-day egg production during the final ad libitum period (28 to 40 weeks of age) were not significantly different from the birds that never had experienced restricted feeding. If feed intake for those previously feed restricted groups had been greater than the control group the liver fat content would be similar to that of the control, but since feed intake was not different from the control (along with body weight and egg production), liver fat content probably did not increase much after the time period when feed restriction was performed. Thus, percent liver fat remained at a low level.

While comparing a low (2359 Kcal. M.E./kg.) and a high energy diet (same ration as Diet F, 3004 Kcal. M.E./kg.), Barton (1967) observed fatty liver and liver hemorrhages (13 percent) only in the group of birds that were fed the high energy diet. Thus, he concluded that fatty liver incidence was higher among hens fed a diet containing high level of energy.

In experiment III, however, when a group of birds was given Diet F ad libitum, which was 130 kilocalories (M.E./kg.) lower than the control diet, a marked increase

in the average liver wet weight, liver fat (both the percentage and total), and FLHS resulted. This may indicate that a high level of dietary energy per se is not the major factor in producing high-fat liver or FLHS.

Wolford and Polin (1972a) reported that when hens were force-fed, the degree of liver fat accumulation and incidence of FLHS were linear to the amount of feed received. Their result was probably due to increased input, which led to increased formation of triglyceride in the liver, and little change in output of lipid in the form of egg production. However, it appears that an increase in feed (energy) intake is not the only way to produce FLHS because in experiment III, birds fed Diet F did not consume more calories than those in the control or in other treatments, yet the majority of birds having high fat-liver and FLHS were observed in this (Diet F) treatment group.

The relationship between high fat-liver and FLHS was also studied in experiments III and IV. The results showed that FLHS hemorrhages occurred in livers with high fat (high fat-liver), yet not all high fat-livers contained FLHS hemorrhages. This indicated that the cause of high fat-liver and FLHS may not necessarily be the same. A similar observation was made by Wolford and Polin (1972b).

In experiment IV, the highest incidence of FLHS hemorrhages resulted in birds that first received a restricted feeding regime in a "high" environmental temperature (30.6^{o} C) and then received an ad libitum feeding in a

reduced temperature (22.2° C). Thus, changing the environmental temperature coupled with a restricted-ad libitum feeding regime may affect the incidence of liver hemorrhages. However, further studies are needed to elucidate the exact cause(s) of liver hemorrhages.

Couch (1956) and Reedy (1968) characterized fatty liver syndrome as increased body weight and decreased egg production. In these experiments, however, birds having FLHS were neither heavier nor poorer egg producers than the control group. Ringer and Sheppard (1963) also reported that egg production rate was not affected by the fatty liver condition. Therefore, body weight and egg production may not be used as satisfactory indications of FLHS in laying birds.

VI. SUMMARY

A series of 11 diets were used in the first two experiments to study whether various sources of energy exhibited different effects on liver fat accumulation.

In isocaloric-isonitrogenous diets, different sources of carbohydrate and fat as primary energy supplements were not different in exerting their effect on liver fat accumulation in the "moderate" (12.2^{o} C) environmental temperature. When the environmental temperature was "high" (27.8^{o} C), more livers appeared to be fatty, and the incidence of liver hemorrhages was greater than those obtained in the "moderate" environmental temperature.

Average feed consumption of birds fed diets that were calculated to be isocaloric and isonitrogenous but formulated from different dietary ingredients was not the same. This implies that there are some factor(s) in the feed ingredients other than dietary energy which may influence feed consumption.

In one experiment, birds were given a restricted amount of feed for a certain period of time. Percentage of liver fat as well as total amount of liver fat of birds that received the restricted feeding regime was lower than the control ad libitum fed group.

When Diet F, a commercial laying formula, was fed ad libitum, liver wet weight, percent liver fat and total liver fat were markedly increased and some incidence of fatty liver-hemorrhagic syndrome was observed. However, average feed consumption, final body weight and percent hen-day egg production were not significantly different between treatments.

In another experiment feed restriction and ad libitum feeding were practiced consecutively under two different environmental temperatures to study their effect on liver fat accumulation and liver hemorrhaging. Once again fatty liver and some incidence of FLHS were observed among all treatments in which Diet F was used.

The highest amount of total liver fat was observed in the control group which received ad libitum feeding in a "high" (30.6° C) environmental temperature.

Total liver fat as well as the incidence of both the liver hemorrhages and FLHS were the lowest among birds that received a restricted feeding regime in the "high" environmental temperature. The highest incidence of liver hemorrhages and FLHS occurred among birds that first received a restricted feeding regime in 30.6° C and then were allowed to eat feed ad libitum in 22.2° C.

This indicated that feeding regime along with environmental temperature might affect liver hemorrhages.

FLHS hemorrhages occurred in livers with high fat values but not all livers with high fat exhibited FLHS

hemorrhages. FLHS livers were significantly heavier than livers that did not have severe hemorrhages.

Average feed consumption, final body weight and hen-day egg production between groups of birds having FLHS and low liver fat were not significantly different.

LITERATURE CITED

VII. LITERATURE CITED

Barton, T. L., C. J. Flegal and P. J. Schaible, 1966. "Fatty liver" syndrome in laying hens as influenced by protein-energy ratios. Poultry Sci. 45: 1068 (Abstract).

Barton, T. L., 1967. Fatty liver studies in laying hens. Ph.D. thesis. Michigan State University, East Lansing, Michigan.

Best, C. H., J. M. Hershey and M. E. Huntsman, 1932a. The effect of lecithin on fat deposition in the liver of the normal rat. J. Physiol. 75: 55-66.

Best, C. H. and M. E. Huntsman, 1932b. The effects of the components of lecithin upon deposition of fat in the liver. J. Physiol. 75: 405-412.

Bull, H. S., 1968. Fatty liver syndrome in laying hens. Papers covering fatty liver syndrome presented at the 23rd Annual Texas Nutrition Conference. pp. 55-61.

Chalvardjian, A., 1966. Mode of action of choline I. Fatty acid composition of liver, serum, and adipose tissue of choline deficient rats. Can. J. Biochem. 44: 713-724.

Chalvardjian, A., 1969. Mode of action of choline II. The fate of intravenously administered labelled fatty acids in choline-deficient rats. Can. J. Biochem. 47: 207-217.

Couch, J. R., 1956. Fatty livers in laying hens. A condition which may occur as a result of increased strain. Feedstuffs 28(47): 46-53.

Couch, J. R., 1964. The fatty liver syndrome in laying hens. Research Digest 11(5): 1-2.

Deacon, L. E., 1968. The fatty liver syndrome--History and early observations. Papers covering fatty liver syndrome presented at the 23rd Annual Texas Nutrition Conference. pp. 18-20.

Donovan, G. A. and S. L. Balloun, 1955. Effect of dietary protein and energy on chick liver fat accumulation. Proc. Soc. Exp. Biol. Med. 90: 692-694.

Dudley, W. A., L. J. Machlin and G. J. Marco, 1961. The effect of dietary vanadium and cholesterol on the storage of cholesterol in the liver of chicks. Poultry Sci. 40: 1396 (Abstract).

Duke, M. J., R. K. Ringer and J. H. Wolford, 1968. Failure of plasma protein level to indicate developing fatty liver in chickens. Poultry Sci. 47: 1098-1100.

Du Vigneaud, V., J. P. Chandler, M. Cohn and G. B. Brown, 1940. The transfer of the methyl group from methionine to choline and creatine. J. Biol. Chem. 134: 787-788.

Du Vigneaud, V., M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, 1941. The utilization of the methyl group of methionine in the biological synthesis of choline and creatine. J. Biol. Chem. 140: 625-641.

Fisher, H. and H. S. Weiss, 1956. Observation on the fatty liver syndrome of laying hens. Feedstuffs 28(51): 16.

Folch, J., M. Lees, G. H. Slone-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Griffith, M. and R. Schexnailder, 1972. Evaluation of choline and some other nutrients for effect on the liver fat of the laying hen. Poultry Sci. 51: 1813 (Abstract).

Hamilton, P. B. and J. D. Garlich, 1970. Aflatoxin as a cause of fatty liver syndrome in laying hens. Poultry Sci. 49: 1391 (Abstract).

Hamilton, P. B., H. T. Tung, J. R. Harris, J. H. Gainer and W. E. Donalelson, 1972. The effect of dietary fat on aflatoxicosis in turkeys. Poultry Sci. 51: 165-170.

Harper, A. E., W. J. Monson, D. A. Benton, M. E. Winje and C. A. Elvehjem, 1954. Factors other than choline which affect the deposition of liver fat. J. Biol. Chem. 206: 151-158.

Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart, 1941. Choline in the nutrition of chicks. J. Biol. Chem. 138: 459-466.

Howes, J. R. and T. C. Fitzgerald, 1966. Fatty livers in coturnix quail fed high dietary levels of fat. Poultry Sci. 45: 1903 (Abstract).

Jensen, L. S., G. W. Schumaier, A. D. Funk and T. C. Smith, 1970. A new lipotropic agent for the laying hen. Poultry Sci. 49: 1401 (Abstract).

Kirk, R. E., 1968. Experimental design: Procedures for behavioral sciences. Brooks/Cole Publishing Co., Belmont, California.

Leach, R. M., Jr., E. Ceballos and M. C. Nesheim, 1966. Recent studies on choline requirements of chicks and hens. Proc. Cornell Nutr. Conf. pp. 69-72.

Leveille, G. A., 1967. In vivo fatty acid synthesis in adipose tissue and liver of meal-fed rats. Proc. Soc. Exp. Biol. Med. 125: 85-88.

Leveille, G. A., E. K. O'Hea and K. Chakrabarty, 1968. In vivo lipogenesis in the domestic chicken (33022). Proc. Soc. Exp. Biol. Med. 128: 398-401.

Leveille, G. A., 1969. In vitro hepatic lipogenesis in the hen and chick. Comp. Biochem. Physiol. 28: 431-435.

Lorenz, F. W., C. Entenman and I. L. Chaikoff, 1937-1938. The influence of age, sex, and ovarian activity on the blood lipids of the domestic fowl. J. Biol. Chem. 122: 619-633.

Lorenz, F. W., I. L. Chaikoff and C. Entenman, 1938. Liver lipids of the laying and non-laying bird. J. Biol. Chem. 123: 577-585.

Maclean, D. L., J. H. Ridout and C. H. Best, 1937. The effects of diets low in choline upon liver function, growth, and distribution of fat in the white rat. Brit. J. Exp. Pathol. 18: 345-354.

Masoro, E. J., 1968. Physiological chemistry of lipids in mammals. W. B. Saunders Co., Philadelphia. London. Toronto.

McDaniel, A. H., J. H. Quisenberry, B. L. Reid and J. R. Couch, 1959. The effect of dietary fat, caloric intake and protein level on caged layers. Poultry Sci. 38: 213-219.

Morrison, W. D., A. W. Tremere and J. F. Standish, 1970. Response of caged layers to feed form and energy. Poultry Sci. 49: 1418 (Abstract).

Nelson, F. E., 1968. Effect of inositol on liver fat of Broiler breeders. Papers covering Fatty Liver Syndrome presented at the 23rd Annual Texas Nutrition Conference. pp. 1-5.

Nesheim, M. C., C. A. Ivy and M. J. Norvell, 1969. Some observations on fatty livers in laying hens. Proc. Cornell Nutr. Conf. pp. 36-41.

Nesheim, M. C. and C. A. Ivy, 1970. Factors influencing liver fat deposition in laying hens. Proc. Cornell Nutr. Conf. pp. 43-49.

O'Hea, E. K. and G. A. Leveille, 1968. Lipogenesis in isolated adipose tissue of the domestic chick (Gallus domesticus). Comp. Biochem. Physiol. 26: 111-120.

O'Hea, E. K. and G. A. Leveille, 1969. Lipid biosynthesis and transport in the domestic chick (Gallus domesticus). Comp. Biochem. Physiol. 30: 149-159.

Oser, B. L., 1965. Hawk's physiological chemistry, Fourteenth edition. McGraw-Hill Book Company, New York, New York.

Owings, W. J., S. L. Balloun, W. W. Marion and J. M. J. Ning, 1967. The influence of dietary protein level and bird density in cages on egg production and liver fatty acids. Poultry Sci. 46: 1303 (Abstract).

Parker, R. O. and L. E. Deacon, 1968. Field cases of the fatty liver syndrome. Papers covering Fatty Liver Syndrome presented at the 23rd Annual Texas Nutrition Conference. pp. 62-75.

Perlman, I. and I. L. Chaikoff, 1939. Radioactive phosphorus as an indicator of phospholipid metabolism. J. Biol. Chem. 127: 211-220.

Price, J. D., A. H. McDaniel, D. N. Smith, Jr., J. H. Quisenberry, B. L. Reid and J. R. Couch, 1957. The effect of energy and protein levels on egg production, feed efficiency and some lipid constituents of blood and liver of caged layer. Poultry Sci. 36: 1316-1321.

Quisenberry, J. H., L. A. Young and P. V. L. N. Murphy, 1967. The fatty liver syndrome in commercial layers. Poultry Sci. 46: 1308-1309 (Abstract).

Ragland, W. W., D. W. Cardin, D. C. Snetsinger, R. A. Zimmerman and D. E. Greene, 1970. Effect of inositol on layer performance and body characteristics. Poultry Sci. 49: 1430 (Abstract).

Reed, J. R., L. E. Deacon, F. Farr and J. R. Couch, 1968. Inositol and the fatty liver syndrome. Papers covering Fatty Liver Syndrome presented at the 23rd Annual Texas Nutrition Conference. pp. 40-54.

Reedy, L. M., 1968. Some clinical observations on the fatty liver syndrome (F.L.S.) in laying hens. Papers covering Fatty Liver Syndrome presented at the 23rd Annual Texas Nutrition Conference. p. 17.

Ringer, R. K. and C. C. Sheppard, 1963. Report of fatty-liver syndrome in a Michigan caged layer operation. Michigan Agr. Expt. Sta. Quart. Bull. 45: 424-427.

Saloma, A. E., F. Farr, J. Cooper, C. R. Creger and J. R. Couch, 1969. The fatty acid composition of hepatic lipids in hens. Poultry Sci. 48: 1865 (Abstract).

Scott, M. L., M. C. Nesheim and R. J. Young, 1969. Nutrition of the Chicken. M. L. Scott and Associates, Ithaca, New York.

Treat, C. M., B. L. Reid, R. E. Davies and J. R. Couch, 1960. Effect of animal fat and mixtures of animal and vegetable fats containing varying amounts of free fatty acids on performance of cage layers. Poultry Sci. 39: 1550-1555.

Tucker, H. F. and H. C. Eckstein, 1937. The effect of supplementary methionine and cystine on the production of fatty livers by diet. J. Biol. Chem. 121: 479-484.

Turk, D. E., H. R. Bird and M. L. Sunde, 1958. Effect of fats on replacement pullet and laying hens. Poultry Sci. 37: 1249 (Abstract).

Weiss, H. S. and H. Fisher, 1957. Plasma lipid and organ changes associated with the feeding of animal fat to laying chickens. J. Nutr. 61: 267-280.

Wohl, M. G. and R. S. Goodhart, 1970. Modern nutrition in health and disease. Fourth edition. Lea and Febiger, Philadelphia, Pennsylvania.

Wolford, J. H. and D. Polin, 1972a. Laboratory duplication of fatty liver-hemorrhagic syndrome: a photographic description. Feedstuffs 44(32): 41-42.

Wolford, J. H. and D. Polin, 1972b. Lipid accumulation and hemorrhage in livers of laying chickens. A study on fatty liver-hemorrhagic syndrome (FLHS). Poultry Sci. 51: 1707-1713.

APPENDIX

Appendix Table 1. Composition of vitamin-trace mineral premixture for laying ration (Diets 1 to 11)

Micronutrients	Per kilogram of diet
Vitamin A, I.U.	10208.00
Vitamin D_3, I.C.U.	3190.00
Riboflavin, mg.	8.93
Pantothenic acid, mg.	15.31
Niacin, mg.	31.90
Choline chloride, mg.	497.64
Folic acid, mg.	1.28
Vitamin B_{12}, µg.	15.31
Vitamin E, I.U.	6.38
Menadione sodium bisulfite, mg.	1.91
Manganese, mg.	71.98
Iodine, mg.	1.12
Copper, mg.	4.54
Cobalt, µg.	252.00
Zinc, mg.	56.00
Iron, mg.	28.10
Magnesium, mg.	580.00

Appendix Table 2. Composition of pullet developer diet

Ingredient		Percent
Ground yellow corn		50.00
Soybean, 49% protein		3.13
Ground oats		25.07
Wheat standard, middlings		10.00
Alfalfa meal, 17% protein		3.00
Fishmeal, 49% protein		2.00
Meat & bone meal, 50% protein		3.50
Dicalcium phosphate		1.00
Ground limestone		1.50
Salt, iodized		0.25
Manganese sulfate		0.01
Vit.-min. premix*		0.50
Methionine		0.04
	Total	100.00
Calculated analysis		
Kcal. M.E./kg. diet		2809.3
Crude protein, %		14.0

*Supplied the following per kg. of diet: Vitamin A, 6600 I.U.; Vitamin D_3, 2200 I.C.U.; Riboflavin, 4.08 mg; Pantothenic acid, 6.60 mg; Niacin, 19.80 mg; Choline chloride, 440 mg; Vitamin B_{12}, 9.92 µg; Menadione sodium bisulfite, 1.68 mg; Vitamin E, 1.68 I.U.; Manganese, 64.0 mg; Iodine, 1.00 mg; Copper, 4.0 mg; Cobalt, 249.6 µg; Zinc, 50.0 mg; Iron, 25.0 mg.

Appendix Table 3. Correlation coefficients of the variables obtained during the final ad libitum feeding period (28-40 weeks of age) of experiment III

Variables	Liver wet wt.	% Liver fat	Total liver fat
Feed consumption	0.165	0.109	0.092
Energy intake	0.099	0.045	0.025
Final body wt.	0.182	0.158	0.095
Hen-day egg prod.	0.130	0.113	0.124

Appendix Table 4. Composition of Z-4 laying formula

Ingredient	Percent
Ground yellow corn	34.5
Ground oats	20.0
Wheat bran	15.0
Flour middlings	10.0
Alfalfa meal, 17% protein	3.0
Meat scraps	3.0
Dried skim milk	2.0
White fish meal, 60% protein	2.5
Soybean meal, 44% protein	2.5
Ground oyster shell flour	5.0
Steamed bone meal	1.5
Salt, iodized	0.6
Cod liver oil (400 vitamin D, 2000 vitamin A)	0.4
Total	100.0
Calculated analysis	
Kcal. M.E./kg. diet	2359
Crude protein, %	14.8

Appendix Table 5. Correlation coefficients of the variables obtained during the ad libitum feeding period (36-40 weeks of age) of experiment IV

Variables	Liver wet wt.	% Liver fat	Total liver fat
Feed consumption	0.172	-0.211	-0.082
Energy intake	0.115	-0.180	-0.078
Final body wt.	0.305	0.100	0.263
Hen-day egg prod.	-0.104	0.110	0.073