HEPATIC LIPID LEVEL IN THE LAYING HEN AS INFLUENCED BY DIETARY FAT SOURCE AND DIETARY FAT LEVEL

By

JOHN ROBERT MILLER

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Thesis Approved:

Collin H. Thayer
Thesis Adviser

Charles L. Maxwell

Ronald B. Johnson

N. N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

In recent years, the problem of abnormal lipid accumulation in livers of laying hens has become of major importance. This condition, known as "Fatty Liver Syndrome" (FLS), was first observed in the southwestern United States in 1954 (Couch, 1956), and in later years appeared in the north central region (Ringer and Sheppard, 1963), and east coast (Nesheim et al. 1969). Couch (1956) first described the problem as an increase in liver fat, sometimes amounting to as much as 70% of the liver dry matter. It was accompanied by an increase in body weight, decreased egg production, and increased mortality within the flock. Liver capillary hemorrhages and hematomas were also seen on occasion.

Reedy (1968) reported that the combs of affected birds may be pale with dark or cyanotic tips. A scaliness or dandruff may also be seen on the comb. Large deposits of fat were observed in the intestinal mesenteries, and abdominal cavities of the hens. The livers were enlarged, contained a higher than normal fat content and were often extremely friable. Liver color in birds affected with the syndrome varied from tan to pale yellow.

Several workers have observed that the condition is limited mainly to high producing, caged layers; whereas, a much lower incidence is found in floor birds (Barton, 1967; Deacon, 1968; Bicknell et al. 1969). The Fatty Liver Syndrome does not appear to be contagious, although there

tends to be a high incidence in affected flocks.

Couch (1956) and Reedy (1968) both observed that stress or elevated environmental temperature may precipitate mortality in the flock. Nesheim and others (1969) also found this to be true with the incidence of hen mortality confined mainly to the months of April, May, and June. Due to the relationship of the occurrence of FLS and increasing environmental temperature, it is thought that positive energy balance in the hens may be part of the problem. Nesheim and others (1969) suggest that a reduction in energy consumption during the spring months to minimize the accumulation of fat in the liver and adipose may prevent the high incidence of the condition. It has been difficult to predict the onset of FLS in the flock due to the fact that external symptoms in the hen do not necessarily precede a spontaneous occurrence of mortality.

The etiology of this problen is still very unclear. Nutritional factors have been implicated although it is possible that other causitive agents are involved. Due to the lack of understanding of FLS in addition to its economic effects on the flock, (which include decreased egg production and a high mortality rate) this study was undertaken to examine the effects of the type and level of dietary fat on liver fat in the laying hen.

CHAPTER II

REVIEW OF LITERATURE

Effects of Dietary Lipid on Hepatic Lipid Levels

Liver fat in the avian is under the influence of many different stimuli, both of dietary and environmental origin. It has been found in numerous studies that the fatty acid composition of an animal generally reflects the fatty acid pattern of the diet (Hegsted et al. 1960). This has been found to be true in the avian, especially in the fatty acid pattern of the liver (Machlin et al. 1962; Marion and Edwards 1964).

Sim et al. (1973) designed an experiment to study the relationship among the fatty acids in the livers of laying hens. It was shown that a low-fat diet or a diet containing animal tallow resulted in a low level of linoleic acid in the liver, while this fatty acid was increased by the addition of soybean oil or sunflower oil. The results of this study indicated that there is a positive relationship between the amount of linoleic acid in the diet, and the amount deposited in hepatic tissue.

The situation was changed when the level of oleic acid was studied. Soybean and sunflower oils tended to depress the hepatic oleic acid level; whereas, the level was much higher in hens fed a low-fat or animal tallow-supplemented diet. From these observations, it was hypothesized that the composition of fat deposited in tissues may be influenced directly by dietary fat or possibly by the effect of some nutrient on fatty acid synthesis in the liver.

In addition, high levels of linoleic acid in sunflower oil altered the hepatic fatty acid composition. There was an inhibition of de novo synthesis of oleic and palmitoleic acids, and an increase in the synthesis of saturated fatty acids. The liver of the hen ostensibly possesses a homeostatic mechanism to regulate fatty acid biosynthesis relative to dietary fatty acids; thus maintaining a specific ratio of saturated to unsaturated fatty acids.

The composition of dietary fat has been shown to be of some importance in laying hens. Bragg and others (1973) suggested that at least 1% linoleic acid was needed in the diet for maintaining optimum egg production, egg size, and reproductive performance. This is generally in agreement with previous work, although some studies have indicated that 2% dietary linoleic acid is required for maximum performance (Menge et al. 1965; Menge, 1968).

By increasing the quantity of fat in the diet using animal tallow or rapeseed oil, Bragg et al. (1973) showed that feed efficiency and egg weight can be increased. In addition, this also increases both liver size (expressed as liver weight/body weight) and the lipid content of the liver. These two fat sources increased the incidence of Fatty Liver Syndrome. Besides the increased fat content, livers were enlarged and yellowish in color, and some signs of hemorrhages were seen. When comparable levels of sunflower or soybean oils were fed, liver lipids were lowered. The results of this study indicate that the fatty acid composition of the diet may be more important than energy per se, and that the dietary level of linoleic acid from sunflower or soybean oils was inversely related to hepatic size and hepatic lipid level. This is in agreement with several other studies which have shown that linoleic acid

may prevent or retard lipid accumulation in the livers of rats and laying hens (Donaldson and Gordon, 1960; Menge, 1967; Morton and Horner, 1961).

High levels of animal tallow in layer diets have been implicated as a cause of Fatty Liver Syndrome (Sunde, 1966). Similar observations have been made with high energy diets in which carbohydrates contributed the bulk of the energy (Barton et al. 1966; Duke et al. 1968). Leveille and Fisher (1958) found this to be the case in comparing a diet high in unsaturated fat (corn oil) with a diet high in saturated fat (animal tallow). Utilization of the two fat sources appeared to be different, in that hens fed the animal tallow had abnormally high fat deposits in the liver and adipose tissue, similar to that seen in FLS.

Effect of Feed Restriction and Force-Feeding on FLS

Examining the problem in another manner, Wolford and Polin (1972) attempted to reduce the severity of FLS by restricted feeding. During a six-week period, the restricted hens lost 4.3% of their body weight, as would be expected, but there was also a significant decrease in liver weight. This included a reduction in both liver lipids and non-lipid dry components. In addition, there was a loss of weight in terms of abdominal fat. After the restricted feeding period, the hens were put on an ad libitum feeding program for eight weeks (recovery period). At the end of this time, the liver components and abdominal fat had increased to a weight comparable to that of the hens fed ad libitum for the entire fourteen-week period. During the recovery period, the previously-restricted hens deposited liver and abdominal fat at a greater rate than the non-restricted hens.

In describing Fatty Liver Syndrome, Wolford and Polin have referred to the condition as Fatty Liver Hemorrhagic Syndrome (FLHS), in which they categorized the birds on two considerations: liver fat and liver hemorrhages. At the end of the six week restricted feeding period, 25% of the control group had hemorrhages, but none were seen in the restricted hens. At the end of the recovery period, FLHS was still present in approximately 25% of the control group and only 10% of the formally restricted hens. High fat levels did not necessarily reflect the presence of hemorrhages, yet hemorrhages were seen only in those livers with a high lipid content. From this study, it appeared that restricted feeding did not increase the incidence of FLHS above the norm.

Later work with force-feeding (Polin and Wolford, 1973) was in agreement with the previous study. Hens on control diets often had liver lipid values similar to hens afflicted with FLHS, except that the latter group had liver hemorrhages. It was suggested that due to this difference, there must be some sort of resistance factor against the rupture of hepatic vessels. It is not clear, what this factor is and why it is lost in FLHS.

Ivy and Nesheim (1973) observed a wide range of liver lipid values (20-78%) among hens in good production on identical rations. Hepatic lipid level could be changed markedly by varying the energy density of the diet or by force-feeding. The level of dietary fat did not seem to be correlated with energy intake, and due to this observation, it was suggested that liver fat levels were under a metabolic control process independent of energy intake. This process may be linked to <u>de novo</u> fatty acid synthesis, as there was an increase in oleic acid levels, and a decrease in linoleic acid levels in liver triglycerides.

Effect of Management on FLS

Besides the effects of quantity and quality of dietary fat on Fatty Liver Syndrome, certain management practices appear to have some influence in producing the condition. Several studies have shown rather conclusively that layers kept on the floor have a significantly lower incidence of FLS, and sometimes even a complete absence of the condition as compared to hens in individual cages (Barton, 1967; Deacon, 1968; Bicknell et al., 1969). Griffith et al. (1969) found that hens housed on the floor had 30-50% less liver fat than caged layers. It has been suggested that increased stress due to confinement in cages or increased bird density in floor operations may be responsible in part for the onset of FLS.

Wolford (1971) found that by reducing environmental temperature from 26.7 degrees C. to 1.7 degrees C., the severity of FLS was not as great as in birds held at warmer temperatures. In addition, there was a significant decrease in hepatic lipid content at lower temperatures. This was also found to be the case by Schexnailder and Griffith (1973). As environmental temperature was increased, liver lipids increased.

Effect of Dietary Protein on Hepatic Lipogenesis

Variations in dietary fat, protein, and carbohydrates have been used in studying other lipogenic responses besides changes in liver lipid levels. Yeh and Leveille (1969) found that fatty acid biosynthesis was depressed in the chick by increasing the crude protein content of the diet. This was illustrated by a decrease in acetate-1-^{14}C incorporation into fatty acids. Varying levels of lysine were used to study the effects of protein quality on fatty acid synthesis, but this did not sig-

nificantly alter acetate-1-^{14}C incorporation. It was suggested that fatty acid synthesis is under the influence of the level of dietary protein, but is not affected by protein quality. In further experiments, it was observed that by increasing the level of dietary protein or fat, and thereby decreasing the number of calories from carbohydrates, hepatic lipogenesis was decreased. Greater decreases in lipogenesis resulted from high dietary protein level than high dietary fat level. It is implied that different mechanisms may be involved in the depression of hepatic lipogenesis.

Plasma Proteins as an Indicator of FLS

Since plasma proteins associated with lipid transport are synthesized in the liver, Duke et al. (1968) conducted a study to determine if these would be an accurate indicator of the development of a fatty liver. Comparisons were made between two groups of hens; one group received a high energy diet to induce FLS, and the other was given a low energy ration to alleviate FLS. Liver lipid levels were monitored from 20 to 45 weeks of age. It was found that from 35 to 45 weeks of age, hepatic lipid contents between the two groups were significantly different. Plasma protein levels were significantly different from 36 to 39 weeks of age, although no differences were seen from 21 to 33 weeks or 42 to 45 weeks. Due to the inconsistent differences in plasma protein levels, it was concluded that this is not a satisfactory index of the development of a fatty liver.

The Role of the Liver in Lipogenesis

Lipogenesis appears to be a highly flexible process in avian species

depending on the physiological state of the bird. The primary organ involved in lipogenesis is the liver. Husbands and Brown (1965) observed higher liver triglycerides in layers than non-layers. It was observed that layers incorporate acetate-1-^{14}C into fatty acids faster than cockerels, and this is most likely due to the additional fat requirement for egg production.

The relative roles of liver and adipose tissue in lipogenesis have been thoroughly studied by Goodridge and Ball (1966). *In vitro* studies in the pigeon showed that adipose tissue had a very poor capacity for *de novo* fatty acid synthesis. From this it was postulated that the liver was the chief site of lipid biosynthesis, and the adipose tissue served mainly as a depository for fat synthesized elsewhere in the body. Later work (Goodridge and Ball, 1967) showed that this was in fact the case. *In vivo* studies with the pigeon showed that liver converts glucose-U-^{14}C to fatty acids 25 times faster than does adipose tissue. The liver was estimated to account for 96% of the entire capacity to synthesize fatty acids and adipose tissue is responsible for no more than 4%.

It has been found in other studies that a similar situation is true in chickens. Leveille *et al.* (1968) conducted experiments to determine the relative importance of liver and adipose tissue in fatty acid synthesis. *In vivo*, the liver accounted for 64% to 75% of the total fatty acids synthesized, with the remaining 25% to 36% coming from adipose tissue. It was pointed out that these latter values may be an overestimation due to translocation of lipids out of the liver into adipose tissue. The young chick and the hen appear to be similar in lipogenic capability. This high lipogenic activity remains high when the hen begins egg production, but in cockerels, decreases with age.

Leveille (1969) also conducted *in vitro* studies comparing fatty acid synthesis of both the growing chick and laying hen. As was seen in the previous *in vivo* experiment, fatty acid synthesis was of the same magnitude in both the chick and hen. It was noted also that in the hen, adipose tissue may make more of a contribution in lipid biosynthesis than is realized due to the fact that its mass is large, but biosynthetic rate is slow. Later studies (O'Hea and Leveille, 1969) were in agreement with the results of this study, and estimated that the liver was responsible for 70% to 95% of the *de novo* fatty acid synthesis in the chick.

Effects of Fasting and Refeeding on Hepatic Fatty Acid Synthesis

Yeh and Leveille (1970) reported data on a study designed to examine the effects of short-term fasting and refeeding on hepatic fatty acid synthesis, on the activities of related enzymes, and on plasma free fatty acid levels. Using male, crossbred chicks, it was found that hepatic fatty acid synthesis was depressed 30 minutes after withdrawal of feed, and was 90% abolished within 2 hours. Upon refeeding, biosynthesis was restored within 30 to 60 minutes. During the fasting and refeeding periods, no alterations were seen in the activities of malic enzyme or citrate cleavage enzyme. It has been thought that fatty acid synthesis was dependent on enzymatic control, possibly with these two enzymes involved. This did not appear to be the case here, and it was suggested that free fatty acids themselves may exert some influence in the control of hepatic fatty acid synthesis. Alterations in plasma free fatty acids always preceded any change in fatty acid synthesis in the

liver. It was suggested that there is a competition for coenzyme A by both citrate cleavage enzyme and free fatty acids. Utilization of coenzyme A by fatty acids may be favored, thus reducing its availability for citrate cleavage enzyme. When plasma free fatty acids are put back into triglycerides by the liver, coenzyme A can be used again for hepatic fatty acid synthesis.

Dietary Fat and Hepatic Lipogenesis

The effect of dietary fat on hepatic lipogenesis and enzyme activity has been studied in the chick. Yeh, Leveille, and Wiley (1970), using male, crossbred chicks, force-fed corn oil (10 ml/kg body weight), and fatty acid synthesis was reduced within 30 to 60 minutes after feeding. Other work showed that incorporation of acetate-1-^{14}C and glucose-U-^{14}C into fatty acids was depressed as the level of dietary corn oil increased, and hepatic lipogenesis was decreased significantly. This was accompanied by reduced activities of malic enzyme and citrate cleavage enzyme. Although the control process over liver lipid biosynthesis is not well understood, the participation of circulating free fatty acids is again implicated. Malic and citrate cleavage enzyme activities have been correlated with hepatic lipogenesis, but appear to respond to alterations in free fatty acid levels rather than bring about the change in enzyme activities.

The Relationship of Vitamins and Minerals to Fatty Liver Syndrome

In addition to studies on dietary fat and environmental conditions and their effects on liver lipids, there has also been a great interest

in vitamins and minerals as lipotropic agents in the reduction of the severity of FLS. Couch et al. (1972) designed an experiment to study choline, inositol, and selenium as lipotropic factors in a corn-soy diet. During the 308-day test period, no cases of FLS were seen in the hens. When selenium was added to the diet, there was a consistent reduction in liver fat levels. Choline and inositol had no apparent effect on the level of liver fat. The lowest level of hepatic lipid seen in this study was in hens fed a starch diet, which indicated that a high carbohydrate diet did not promote FLS, although this is not in agreement with other studies (Bragg et al. 1973).

Jensen and others (1970) discovered that combinations of choline chloride, inositol, vitamin E, and vitamin B_{12} could significantly reduce liver weight and total liver fat accumulation. It was also found that selenium had the same effect, and it was suggested that the mineral may be involved in the etiology of the condition.

By feeding a choline-free diet to hens during the first 12 weeks of lay, Nesheim et al. (1967) observed liver lipid values comparable to control hens fed choline during the first 12 weeks of lay. Fatty livers were found in hens whether choline was supplemented or not, suggesting that choline does not prevent or reduce the severity of FLS.

Studies with rats (Engel, 1942) have shown that abnormal lipid accumulation in the liver may be associated with vitamin imbalance. When inositol was added to a purified diet, liver fat was reduced to a level comparable to that in rats on a stock diet. Prolonged feeding of a diet deficient in pyridoxine or essential fatty acids resulted in a fatty liver, even though choline levels were adequate. This suggests that either pyridoxine or essential fatty acids, or both, may be necessary

for choline to function properly as a lipotropic agent.

Griffith et al. (1969) fed different protein levels (16% and 19% crude protein) with graded levels of choline to determine if this had any effect on the level of fat in the livers of hens. Fatty livers were still seen, and there were no significant differences in liver weight between treatments. Although protein was ineffective in controlling the development of fatty livers, another trial showed that the addition of choline or a combination of choline, methionine, and vitamin B_{12} significantly lowered hepatic lipid content. The combination treatment had a greater effect than choline alone, while methionine and vitamin B_{12} together had no effect on liver lipids. A practical farm diet was fed as a control, and this reduced liver fat levels to a greater extent than did any of the treatments.

Schexnailder and others (1973) also compared different vitamins as lipotropic agents. These included riboflavin, pantothenic acid, folic acid, pyridoxine, vitamin B_{12}, choline, biotin, inositol, and vitamin E. The only combinations which appeared to have any significant effect in depressing liver lipid level were choline and vitamin B_{12}; methionine and vitamin B_{12}; and choline, methionine, and vitamin B_{12}.

Wolford and Murphy (1972) conducted a study on the effects of lipotropic agents on liver hemorrhages in the laying hen. It was found that the incidence of hemorrhages, liver weight, final body weight, and total liver lipids were not reduced by the addition of vitamin B_{12}, vitamin E, choline, inositol, selenium, or cobalt. However, by simply reducing the energy density of the ration from 2.9 kcal ME/g to 2.4 kcal ME/g, the occurrence of hemorrhages was completely eliminated, and total liver lipids and liver weight were significantly lowered. It was

concluded that dietary energy plays an influential role in the occurrence of Fatty Liver Syndrome.

Leveille and Bray (1970) designed an experiment to study the effect of inositol on hepatic lipid content. During a 5 month production period, the addition of inositol to layer diets increased body weight, but had no effect on liver lipids. Other work has shown that inositol does not act universally as a lipotropic agent (Pearce, 1972).

Aflatoxin and FLS

In addition to nutritional factors being responsible for FLS in layers, it has also been suggested that aflatoxin may be a contributing factor. Smith (1972) reviewed some of the problems associated with aflatoxicosis in broiler flocks. These include depressed growth and feed efficiency; increased mortality; enlargement of the liver, heart, spleen, and pancreas; and an increase in liver fat. These effects are not universal as there is a wide variation within a species for aflatoxin resistance. In layer flocks, the occurrence of aflatoxicosis causes reduced egg production and hatchability as well as fatty livers. Newberne and Butler (1969) pointed out that the liver is usually the first tissue affected by aflatoxin. The first sign of damage is fatty infiltration, followed by enlargement of the organ. In the advanced stages, the incidence of hemorrhage increases, and hepatic cell necrosis is seen.

Hamilton and Garlich (1971) specifically looked at the effect of high aflatoxin levels on hepatic lipid content in layers. The livers were yellowish in color, enlarged, and extremely friable. A significant increase in liver fat was seen, increasing from a control value of 36% lipid to above 55% lipid in the aflatoxin-treated hens. No differences

were observed in the spleen or pancreas. These symptoms associated with aflatoxicosis appeared to be identical to those seen in field cases of FLS. The liver apparently is the target organ involved when aflatoxin is present in the feed. It has been suggested that the toxin causes some sort of malfunction in hepatic lipid transport, resulting in a fatty liver. The results of this study are generally in agreement with those obtained by other workers (Kratzer et al. 1969; Nesheim and Ivy, 1971).

Studies have been conducted to determine the effects of inositol, choline, vitamin B_{12}, and vitamin E on aflatoxin-induced fatty livers in layers (Hamilton and Garlich, 1972). It was found that these vitamins clearly had no effect on decreasing or retarding development of fatty livers due to aflatoxicosis.

Hepatic lipogenesis and hepatic lipid levels of the laying hen have been shown to be under the influence of many different stimuli. As it has been shown in the literature, these stimuli include dietary fat, protein, and carbohydrates, vitamins, trace minerals, environmental conditions, and toxic materials. There have been many attempts to link these factors to Fatty Liver Syndrome, but no conclusive evidence involving these elements has been found. The recurring idea throughout the studies conducted seems to be that alterations in hepatic fatty acid synthesis, hepatic lipogenic enzyme activities, and hepatic lipid content are affected quite strongly by the dietary lipid which is presented to the hen in the diet. In view of this observation and the lack of understanding of the relationship between dietary lipids and hepatic lipids, this experiment was conducted in an effort to understand more clearly the relationships between levels and types of dietary fat and levels of fat in the liver of the laying hen.

CHAPTER III

MATERIALS AND METHODS

Introduction

This experiment was conducted on the Oklahoma State University poultry Farm in a caged layer laboratory, in which the environmental conditions were partially controlled. The experimental animals, 288 Heisdorf-Nelson pullets, approximately 20 weeks of age, were obtained from a commercial hatchery. These hens were placed in individual wire cages which were equipped with automatic waterers, individual feeders, and individual feed storage cans to measure feed consumption of individual hens.

Temperature in the house was regulated by a gas furnace, air ducts, and fans. During the experimental period, temperatures ranged from 13 degrees C. to 24 degrees C. Incandescent lamps which were regulated by an automatic time clock, were set to give 16 hours of light per day.

The experiment, which was broken down into six 28-day periods, was begun November 8, 1972 and terminated on April 24, 1973. Each hen was randomly assigned to one of six dietary treatments (which will be explained in more detail later), with a total of 48 hens per treatment. The experimental design within the house was completely random. Previous uniformity trials within this house had indicated that there were no significant differences in the performance of hens due to location. It was therefore possible to utilize a completely randomized design.

At the beginning and end of each period, feed weights and body

weights were recorded for individual hens. From these data, daily feed consumption and daily body weight change could be calculated. Egg production for individual hens was recorded on separate cards on every cage. Egg weights were recorded for individual hens from periods 2 through 6. Two days out of each week, eggs were numbered, collected, and weighed. If a hen had already laid an egg on the first day of collection, an egg weight from the second collection day would be discarded to give only one egg weight per hen per week. If no eggs were collected on either collection day, a zero value was recorded for egg weight for that week. However a zero value was not averaged into the mean.

In addition to the performance data which were collected, two selections of 90 hens each (15 hens per treatment) were made for the sampling of livers. The two selections were at random, and took place at the end of period 5 and at the end of period 6.

Dietary Treatments

One of six different rations was fed ad libitum to each group of hens for each of the six periods. The compositions of the completed rations are presented in Table I. A basal ration was made up and combined with corn, soybean meal, ground polyethylene, and the appropriate fat source to give the experimental ration desired. All rations were calculated to supply 16 grams of protein per 100 grams of diet, and 300 kcal ME/100 grams of diet. The variable in the ration was one of the following fat level-fat source combinations: low, intermediate, or high levels of either animal tallow or soybean oil. The composition of the vitamin-mineral mix (VMC-60) used in the diets is shown in Table II.

TABLE I

COMPOSITION OF RATIONS FOR 6 TREATMENTS

Ingredient (given in parts)	Treatment 1	2	3	4	5	6
Ground Corn	55.2	44.3	33.8	55.2	44.4	33.8
Soybean Meal (44%)	13.3	15.4	17.5	13.3	15.4	17.5
Corn Gluten Meal	2.4	2.4	2.4	2.4	2.4	2.4
Alfalfa Meal (17%)	2.6	2.6	2.6	2.6	2.6	2.6
Fishmeal (60%)	2.3	2.3	2.3	2.3	2.3	2.3
Meat and Bone Scrap (45%)	2.9	2.9	2.9	2.9	2.9	2.9
Live Yeast Culture	1.7	1.7	1.7	1.7	1.7	1.7
Dried Whey	1.7	1.7	1.7	1.7	1.7	1.7
Distiller's Solubles	1.7	1.7	1.7	1.7	1.7	1.7
dl-Methionine	0.001	0.001	0.001	0.001	0.001	0.001
Dicalcium Phosphate	1.5	1.5	1.5	1.5	1.5	1.5
Calcium Carbonate	7.2	7.2	7.2	7.2	7.2	7.2
Salt	0.5	0.5	0.5	0.5	0.5	0.5
VMC-60[1]	0.5	0.5	0.5	0.5	0.5	0.5
Animal Tallow	6.5	11.1	15.5	0.0	0.0	0.0
Soybean Oil	0.0	0.0	0.0	5.5	9.4	13.2
Ground Polyethylene Fluff	0.0	4.2	8.2	1.0	5.8	10.5

[1]See Table II for composition of VMC-60.

TABLE II

COMPOSITION OF VITAMIN-MINERAL CONCENTRATE (VMC-60)

Vitamins and Minerals	Units	10 kg. of Conc.	Adds Per kg. of Finished Ration
Vitamin A	U.S.P.	35,273,600	17,637.0
Vitamin D_3	I.C.U.	5,291,040	2,646.0
Vitamin E	I.U.	26,455	13.2
Vitamin K	mg.	13,228	6.6
Vitamin B_{12}	mg.	35	0.018
Riboflavin	mg.	17,637	8.8
Niacin	mg.	41,095	20.5
Pantothenic Acid	mg.	35,274	17.6
Choline Chloride	mg.	2,204,600	1,102.0
Manganese	mg.	122,135	61.1
Iodine	mg.	3,792	1.9
Cobalt	mg.	2,601	1.3
Iron	mg.	96,121	48.1
Copper	mg.	7,275	3.6
Zinc	mg.	100,089	50.0

Sampling of Livers

A random sampling of 179 hens was carried out for the sampling of livers. At the end of period 5 (April 2, 1973), 90 hens were sacrificed (15 hens from each of the six treatments). At the end of period 6, (May 10, 1973), 89 hens were sacrificed (15 hens from each of the six treatments excluding ration 3 where 14 hens were sacrificed due to the loss of one experimental unit). The hens were killed by cervical dislocation, and the livers were removed, and immediately weighed. They were scored on a scale of 1 through 4 on the basis of color to predict total liver lipid content. Livers were then quick frozen in the presence of dry ice, and placed in plastic bags under nitrogen at -24 degrees C. until further preparation could be carried out.

Preparation of Livers

Preparation and analysis of livers was done by a modification of the method of Folch et al. (1956). The livers were removed from the freezer and thawed at room temperature (approximately 22 degrees C.). Hepatic tissue was separated from large blood vessels and dehydrated tissue by scraping with a scalpel. Approximately 1 gram of liver tissue was weighed and homogenized in 20 ml of 2:1 chloroform-methanol in a Potter-Elvehjem mixer with a teflon pestle. The homogenized liver in solution was then filtered under vacuum through a Buchner funnel to almost complete dryness. Exactly 15.0 ml of filtrate were recovered and placed in a glass-stoppered centrifuge tube with 3.0 ml water. This was then refrigerated for 1 hour to aid in the separation of the water and chloroform-methanol phases. The solution was then centrifuged for 10 minutes at 2000 x G after which the aqueous upper layer was removed and

the remaining chloroform-methanol solution was placed in a glass vial. The volume level of the vial was marked so that it could be brought back up to the original volume upon any evaporation of chloroform-methanol. Nitrogen was flushed into the vials, they were stoppered with screw caps and were placed in a freezer at approximately -26 degrees C. until further analysis.

Colorimetric Determination of Total Lipids

Colorimetric determination of total lipids was done by the method of Bragdon (1951). Vials were removed from the freezer and allowed to reach room temperature (approximately 22 degrees C.). A 1.0 ml aliquot of sample was placed in a test tube and diluted with 10.0 ml of 2:1 chloroform-methanol. The solution was mixed with a vortex mixer, and two 1 ml samples of the diluted aliquot were placed in separate test tubes. The tubes were then placed in a water bath and evaporated to dryness in a nitrogen atmosphere. To the dried sample, 4.0 ml of 2% potassium dichromate in sulfuric acid was added. The tube was then heated for 30 minutes in a 120 degrees C. sand bath. At the end of this period, the tubes were cooled in an ice bath and 6.0 ml of distilled water were added to each tube and the solution was mixed with a vortex mixer. The samples were allowed to reach room temperature (approximately 22 degrees C.) and the optical density of the sample solution was then read in a galvanometer. Percent total lipids on an as-is basis were calculated from the percent transmittance of the sample, which was determined from a standard optical density table.

Determination of Total Lipids by Goldfisch Extraction

Approximately 1.5 grams of sample were taken from the frozen liver tissue remaining after the modified Folch extraction. The sample was weighed and dried in a vacuum oven. The drying process included a 6 hour period at 25 degrees C. followed by a 12 hour period at 55 degrees C. The dried sample was weighed again and dry matter percent was calculated. The dried sample was extracted by the Goldfisch method (A.O.A.D., 1960) for 18 hours. Samples were extracted at random on two 6-burner extraction apparatuses. At the end of 18 hours, the ether was evaporated and the beakers plus extracts were dried for 45 minutes at 100 degrees C. These were weighed and calculations were made for percent total lipids on both an as-is and dry matter basis.

Statistical Analysis

Analyses of variance, as outlined by Snedecor (1956), were used in the analysis of all variables in all periods. These analyses were carried out using a factorial arrangement with all six rations for the following response variables: feed consumption on a daily basis, body weight change on a daily basis, total egg production for each 28-day period, average egg weight per 28-day period (from a sample of 1, 2, 3, or 4 eggs collected during the period), liver weight at the time of sacrifice, percent liver dry matter, percent liver lipids on both a dry matter and as-is basis as determined by Goldfisch extraction, percent liver lipids on an as-is basis as determined by Folch extraction, and liver score.

Feed Analysis

All experimental rations were analyzed for crude protein and ether extract. The average values for these are found in the Appendix.

CHAPTER IV

RESULTS AND DISCUSSION

Feed Consumption

In periods 1 through 6 of this experiment, no statistically significant differences ($P > .05$) were observed in feed consumption among fat sources (Tables III-VIII). However, throughout the six periods (Table IX), there appears to be a trend where the animal tallow-fed groups consistently consumed less feed than the soybean oil-fed groups (with the exception of period 4). The reason for the difference in feed consumption between fat sources could be explained by one of two things. It is possible that during the preparation of the experimental rations (which took place approximately every five weeks during the experiment), an incorrect amount of either soybean oil or animal tallow was used. This could result in a change in the energy density of the rations which in turn could alter feed consumption within fat source treatments. This does not appear to be a likely possibility due to the rather consistent differences in feed consumption between fat sources. All rations were formulated to be isocaloric (300 kcal ME/100 gm feed), and although the amounts of fat in each ration varied, energy density was kept constant with the use of ground polyethylene. Although palatability can often affect feed intake, it was not thought to be a factor in this experiment. A more plausible explanation for the differences would be that the metabolizable energy value for one or both fat sources was estimated incor-

TABLE III

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 1

Source of Variation	df	MS	F Value
Corrected Total	287	162.57	--
Fat Source	1	1.43	.009
Fat Level	2	241.52	1.48
Fat Source x Fat Level	2	101.18	0.62
Cage (Fat Source x Fat Level)	282	163.01	--
Coefficient of Variation = 13.56%			

TABLE IV

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 2

Source of Variation	df	MS	F Value
Corrected Total	281	172.28	--
Fat Source	1	11.65	0.07
Fat Level	2	5.43	0.03
Fat Source x Fat Level	2	22.15	0.13
Cage (Fat Source x Fat Level)	276	175.16	--
Coefficient of Variation = 5.60%			

TABLE V

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 3

Source of Variation	df	MS	F Value
Corrected Total	275	380.58	--
Fat Source	1	7.99	0.02
Fat Level	2	1,017.67	2.68
Fat Source x Fat Level	2	16.46	0.04
Cage (Fat Source x Fat Level)	270	379.94	--

Coefficient of Variation = 4.51%

TABLE VI

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 4

Source of Variation	df	MS	F Value
Corrected Total	269	234.21	--
Fat Source	1	92.71	0.40
Fat Level	2	747.59	3.22*
Fat Source x Fat Level	2	31.95	0.14
Cage (Fat Source x Fat Level)	264	232.39	--
Coefficient of Variation = 5.91%			

*Significant at .05 level of probability.

TABLE VII

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 5

Source of Variation	df	MS	F Value
Corrected Total	269	301.13	--
Fat Source	1	834.39	2.79
Fat Level	2	638.43	2.14
Fat Source x Fat Level	2	24.20	0.08
Cage (Fat Source x Fat Level)	264	298.65	--
Coefficient of Variation = 5.31%			

TABLE VIII

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION IN PERIOD 6

Source of Variation	df	MS	F Value
Corrected Total	173	542.76	--
Fat Source	1	218.53	0.43
Fat Level	2	3,703.95	7.27**
Fat Source x Fat Level	2	312.50	0.61
Cage (Fat Source x Fat Level)	168	509.80	--

**Significant at .01 level of probability.

TABLE IX

MEANS FOR DAILY FEED CONSUMPTION (GRAMS) IN PERIODS 1-6

Period	Ration						Overall
	1	2	3	4	5	6	
1	74.17	75.22	72.89	76.68	74.16	71.87	74.17
2	83.64	83.89	82.67	84.04	83.32	84.05	83.60
3	92.59	89.88	86.73	93.89	89.90	86.44	89.90
4	100.09	95.02	93.44	97.63	94.06	93.35	95.60
5	93.61	91.24	88.08	97.48	93.59	92.41	92.73
6	101.34	95.85	81.38	99.78	96.72	88.79	93.98

TABLE IX (Continued)

Period	Fat Source: Soybean Oil Rations (4,5,6)	Fat Source: Animal Tallow Rations (1,2,3)	Period	Fat Level: Low Rations (1,4)	Fat Level: Intermediate Rations (2,5)	Fat Level: High Rations (3,6)
1	74.24	74.09	1	74.52	74.69	72.38
2	83.81	83.40	2	83.84	83.61	83.36
3	90.07	89.73	3	93.24	89.89	86.58
4	95.01	96.19	4	98.86	94.54	93.40
5	94.49	90.98	5	95.54	92.41	90.25
6	95.10	92.86	6	100.56	96.29	85.09

rectly. If animal tallow actually contained more energy than the assumed value (7.12 kcal ME/gm.), then the rations containing animal tallow would be energetically more dense than 300 kcal ME/100 gm. feed; therefore, the hens receiving these diets would tend to eat less. If, on the other hand, the energy value of the soybean oil had been overestimated (8.40 kcal ME/100 gm. feed), then the soybean oil rations would contain less than 300 kcal ME/100 gm. feed; therefore, the hens receiving these diets would tend to eat more to reach their proper energy intake level.

Among fat levels (low, intermediate, and high), there were no statistically significant differences ($P > .05$) in feed consumption in periods 1, 2, 3, and 5 (Tables III, IV, V, and VII). However, statistically significant differences were observed in periods 4 and 6 (Tables VI, VIII). There was an obvious trend in feed consumption among fat levels for all six periods, as seen in Table IX. As the amount of fat in the diet is increased, daily feed consumption tends to decrease. This observation may serve as further evidence to support the thinking that there is some error in ration formulation or an incorrect estimation of the metabolizable energy values of the fat sources. If an estimation of one or both energy values was not correct, then this error would be further magnified as fat levels were increased in the diet. This situation would lead to a greater change in feed consumption as dietary fat levels rose.

Another factor which may have some effect on feed consumption is increased nutrient digestibility as dietary fat levels are increased. Bigbee et al. (1957), and Kelley and Potter (1971) found that increased dietary fat resulted in increased feed efficiency. This could possibly be the case in this experiment. As dietary fat levels are raised, more

nutrients are made available to the hen. If more energy is made available, then this would increase energy density, in effect, and the hen would not have to eat as much feed to meet its energy requirement.

No statistically significant differences ($P > .05$) in interaction between fat source and fat level were found for daily feed consumption (Tables III-VIII). Although some statistically significant differences ($P < .05$) were observed among fat levels, it is thought that these differences are not critical to the outcome of the experiment due to their inconsistent appearance in feed consumption among fat levels. It is also concluded that any differences in feed consumption between treatments due to errors in ration formulation, had no serious effect on other response variables studied.

Daily Body Weight Change

The data for daily body weight change are summarized in Tables X-XVI. No statistically significant differences ($P > .05$) were observed between fat sources for periods 1, 2, 3, 4, and 6 (Tables X-XIII, XV). A statistically significant difference was found between fat sources for period 5 (Table XIV). However, the reason for the difference between fat sources for daily body weight change is not known due to the presence of a statistically significant interaction ($P < .05$) in the same period.

A trend was seen which was similar to the one found in daily feed consumption between fat sources (Table XVI). Changes in body weight tended to be more positive in hens fed soybean oil than in hens fed animal tallow, with the exception of period 2. The cause of these differences in daily body weight change may be due to the differences observed in daily feed consumption. As previously stated, the soybean oil treat-

TABLE X

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 1

Source of Variation	df	MS	F Value
Corrected Total	287	21.53	--
Fat Source	1	1.49	0.07
Fat Level	2	1.63	0.07
Fat Source x Fat Level	2	11.84	0.54
Cage (Fat Source x Fat Level)	282	21.81	--

Coefficient of Variation = 29.57%

TABLE XI

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 2

Source of Variation	df	MS	F Value
Corrected Total	281	9.66	--
Fat Source	1	7.18	0.76
Fat Level	2	31.75	3.37*
Fat Source x Fat Level	2	20.41	2.16
Cage (Fat Source x Fat Level)	276	9.43	--
Coefficient of Variation = 180.00%			

*Significant at .05 level of probability.

TABLE XII

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 3

Source of Variation	df	MS	F Value
Corrected Total	275	6.60	--
Fat Source	1	3.87	0.59
Fat Level	2	5.05	0.77
Fat Source x Fat Level	2	13.73	2.09
Cage (Fat Source x Fat Level)	270	6.57	--

Coefficient of Variation = 223.22%

TABLE XIII

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 4

Source of Variation	df	MS	F Value
Corrected Total	269	4.01	--
Fat Source	1	0.06	0.01
Fat Level	2	2.52	0.63
Fat Source x Fat Level	2	7.39	1.84
Cage (Fat Source x Fat Level)	264	4.01	--
Coefficient of Variation = 167.81%			

TABLE XIV

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 5

Source of Variation	df	MS	F Value
Corrected Total	269	6.02	--
Fat Source	1	100.40	18.06*
Fat Level	2	0.17	0.03
Fat Source x Fat Level	2	24.82	4.46*
Cage (Fat Source x Fat Level)	264	5.56	--
Coefficient of Variation = 816.12%			

*Significant at .05 level of probability.

TABLE XV

ANALYSIS OF VARIANCE FOR DAILY BODY WEIGHT CHANGE IN PERIOD 6

Source of Variation	df	MS	F Value
Corrected Total	173	7.27	--
Fat Source	1	0.95	0.13
Fat Level	2	9.55	1.30
Fat Source x Fat Level	2	7.29	1.00
Cage (Fat Source x Fat Level)	168	7.28	--
Coefficient of Variation = 270.00%			

TABLE XVI

MEANS FOR DAILY BODY WEIGHT CHANGE (GRAMS) IN PERIODS 1-6

Period	Ration						
	1	2	3	4	5	6	Overall
1	+12.66	+12.01	+11.89	+12.01	+12.66	+12.33	+12.26
2	-2.22	-1.89	-2.93	-3.60	-1.79	-2.60	-2.51
3	+2.23	+1.07	+1.32	+1.63	+1.99	+1.71	+1.66
4	+2.06	+1.59	+1.17	+1.46	+1.74	+1.70	+1.62
5	-1.13	-0.72	-1.81	-0.14	-0.42	+0.56	-0.61
6	+0.69	+0.49	+1.60	+1.56	+0.63	+1.06	+1.00

TABLE XVI (Continued)

Period	Fat Source: Soybean Oil Rations (4,5,6)	Fat Source: Animal Tallow Rations (1,2,3)	Period	Fat Level: Low Rations (1,4)	Fat Level: Intermediate Rations (2,5)	Fat Level: High Rations (3,6)
1	+12.33	+12.19	1	+12.33	+12.33	+12.11
2	-2.66	-2.35	2	-2.91	-1.84	-2.77
3	+1.78	+1.54	3	+1.92	+1.53	+1.52
4	+1.63	+1.60	4	+1.76	+1.66	+1.43
5	-0.01	-1.22	5	-0.64	-0.57	-0.63
6	+1.08	+0.93	6	+1.13	+0.55	+1.33

ments consumed more feed on the average than the animal tallow treatments. If both groups were taking in the same amount of energy, then intakes of protein, calcium, phosphorous, vitamins, and trace minerals would have been slightly greater in the soybean oil treatments. However, the only nutrient which would probably account for the difference in body weight change would be protein since the differences in vitamin and mineral intakes between fat source groups would be very small. Although differences in body weight change exist, possibly due to differing protein intakes between groups, these differences are very small and most likely have no significant impact on the experiment.

Among fat levels, only one statistically significant difference ($P < .05$) was observed. This was in period 2 (Table XI). Due to the appearance of only one statistically significant difference and the absence of any trends among fat levels (Table XVI), it is concluded that no significant differences exist for daily body weight change among fat levels.

Only one statistically significant interaction ($P < .05$) was observed between fat source and fat level. This was in period 5 (Table XIV). Due to the absence of interaction in all other periods, it is suggested that the significant response observed is merely a chance occurrence and that there is no interaction between fat source and fat level for body weight change in this experiment.

Another point which should be mentioned concerning daily body weight change is the coefficient of variation. In periods 2 through 6 (Tables XI-XV), the coefficients of variation appear abnormally high. However, due to the manner in which these values were calculated, a very high coefficient of variation is expected. This value was obtained by divid-

ing the square root of the error mean square by the mean for daily body weight change. As shown in Table XVI, most of these means are quite small, approaching zero. When the mean is very small, the coefficient of variation will tend to be quite large. In this experiment, the rations were formulated to keep body weight change to a minimum. This has been achieved in periods 2 through 6 due to the fact that there is a large coefficient of variation, and therefore, a very small mean. As the coefficient of variation increases in magnitude, it indicates that daily body weight change among all birds in that period has been kept small.

Table X shows that there is a small coefficient of variation in period 1. This is not surprising because the hens had not reached their mature size at this point in the experiment. Table XVI indicates that daily body weight change was much greater in period 1 than in the other periods. This higher value for mean daily body weight change resulted in a lower coefficient of variation, indicating the body weight change was not held close to zero.

Average Egg Production

There were no statistically significant differences ($P > .05$) in egg production between fat sources (Tables XVII-XXII). A trend appeared to be present, similar to the one seen in the previous two response variables (Table XXIII). Hens fed soybean oil tended to lay more eggs per period than the hens fed animal tallow. This may be the result of a higher nutrient intake (protein, vitamins, and minerals) in the soybean oil diets due to an overestimation of the metabolizable energy value of soybean oil or a lower nutrient intake due to an underestimation of the

TABLE XVII

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 1

Source of Variation	df	MS	F Value
Corrected Total	287	36.30	—
Fat Source	1	14.22	0.39
Fat Level	2	34.17	0.94
Fat Source x Fat Level	2	34.13	0.94
Cage (Fat Source x Fat Level)	282	36.41	—

Coefficient of Variation = 9.44%

TABLE XVIII

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 2

Source of Variation	df	MS	F Value
Corrected Total	281	16.36	—
Fat Source	1	12.77	0.78
Fat Level	2	30.64	1.87
Fat Source x Fat Level	2	4.24	0.26
Cage (Fat Source x Fat Level)	276	16.36	—
Coefficient of Variation = 8.88%			

TABLE XIX

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 3

Source of Variation	df	MS	F Value
Corrected Total	275	15.67	--
Fat Source	1	4.70	0.30
Fat Level	2	42.01	2.71
Fat Source x Fat Level	2	15.70	1.01
Cage (Fat Source x Fat Level)	270	15.52	--
Coefficient of Variation = 16.80%			

TABLE XX

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 4

Source of Variation	df	MS	F Value
Corrected Total	269	14.23	--
Fat Source	1	4.28	0.31
Fat Level	2	72.23	5.27*
Fat Source x Fat Level	2	30.74	2.24
Cage (Fat Source x Fat Level)	264	13.71	--
Coefficient of Variation = 23.65%			

*Significant at .05 level of probability.

TABLE XXI

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 5

Source of Variation	df	MS	F Value
Corrected Total	269	16.96	—
Fat Source	1	15.64	0.97
Fat Level	2	125.81	7.80**
Fat Source x Fat Level	2	18.51	1.15
Cage (Fat Source x Fat Level)	264	16.13	—
Coefficient of Variation = 18.94%			

**Significant at .01 level of probability.

TABLE XXII

ANALYSIS OF VARIANCE FOR EGG PRODUCTION IN PERIOD 6

Source of Variation	df	MS	F Value
Corrected Total	173	31.57	--
Fat Source	1	13.80	0.53
Fat Level	2	195.33	6.75**
Fat Source x Fat Level	2	97.56	3.37*
Cage (Fat Source x Fat Level)	168	28.95	--
Coefficient of Variation = 46.77%			

*Significant at .05 level of probability.

**Significant at .01 level of probability.

TABLE XXIII

MEANS FOR AVERAGE EGG PRODUCTION (NUMBER OF EGGS PER 28 DAYS) IN PERIODS 1-6

Period	Ration						Overall
	1	2	3	4	5	6	
1	10.15	10.27	10.08	11.95	10.17	9.71	10.39
2	23.57	23.23	22.09	23.72	23.45	23.00	23.18
3	24.02	24.11	22.22	24.07	23.67	23.39	23.58
4	24.53	23.73	21.67	23.78	23.71	23.20	23.44
5	23.78	23.02	20.65	23.58	23.16	22.16	22.72
6	23.10	22.14	17.28	22.00	21.38	20.83	21.12

TABLE XXIII (Continued)

Period	Fat Source: Soybean Oil Rations (4,5,6)	Fat Source: Animal Tallow Rations (1,2,3)	Period	Fat Level: Low Rations (1,4)	Fat Level: Intermediate Rations (2,5)	Fat Level: High Rations (3,6)
1	10.61	10.17	1	11.05	10.22	9.90
2	23.39	22.96	2	23.64	23.34	22.54
3	23.71	23.45	3	24.04	23.89	22.80
4	23.56	23.31	4	24.16	23.72	22.43
5	22.96	22.48	5	23.68	23.09	21.40
6	21.40	20.84	6	22.55	21.76	19.05

metabolizable energy value of animal tallow.

A more plausible explanation may be that differences in egg production were the result of different fatty acid compositions of the fat sources. Menge (1968) found that egg production could be increased by raising the amount of linoleic acid in the diet. Higher egg production values were observed in the soybean oil treatments which is not surprising. Soybean oil contains approximately 51% linoleic acid whereas animal tallow contains only 2% linoleic acid (Caster et al. 1966). This difference in linoleic acid contents between fat sources may possibly be the reason for the consistent differences in egg production.

Among fat levels, statistically significant differences ($P < .05$) were observed in egg production in periods 4, 5, and 6 (Tables XX, XXI, XXII). Another trend was observed which again appeared to be linked to nutrient intake (Table XXIII). As the level of fat in the diet increased, egg production consistently decreased. It is possible that if the metabolizable energy value of soybean oil was overestimated or the value of animal tallow was underestimated, then one fat source would be calorically more dense as the dietary fat level increased. Low fat diets would have a lower energy density per unit feed than higher fat diets. More nutrients could be ingested on the low fat diets and may account for the slightly higher egg production values.

Statistically significant interaction ($P < .05$) between fat source and fat level was limited only to period 6. Due to the fact that this was the only sign of interaction, it is thought that this occurred by chance and a true interaction does not exist between fat source and fat level for average egg production.

Average Egg Weight

Data for average egg weight is summarized in Tables XXIV - XXVIII). In all periods where egg weights were recorded (periods 2-6), heavier egg weights were found from hens fed soybean oil than from hens fed animal tallow (Table XXIX). This could have been the result of several factors. Since hens fed soybean oil tended to eat slightly more feed (due to a possible error in calculation of nutrient density), the additional protein, vitamins, and minerals may have had a positive effect on egg weight. Several studies (Menge et al. 1965; Menge, 1970) have shown that increased levels of dietary linoleic acid may increase egg weight. As pointed out previously, soybean oil contains high levels of linoleic acid whereas the levels in animal tallow are quite low. The differences observed in egg weights between fat sources may actually be due to the differences in linoleic acid levels between fat sources.

Statistically significant differences ($P < .05$) were observed for average egg weights among fat levels for periods 2 and 3 (Tables XXIV, XXV), although differences between fat levels in the other periods were not statistically significant (XXVI-XXVIII). There appears to be a trend in average egg weight among fat levels. As dietary fat changes from low to high, egg weight increases. This was true only in periods 3, 4, and 6, and not in periods 2 and 5. As fat levels are increased, this would make more fat available to the hen for direct transport to the egg. Increased fat deposition could in turn lead to heavier egg weights. It should be noted, however that the changes in egg weight among fat levels were not large and the trend was not consistent. It is suggested that these differences have no special importance, although it is not known whether other response variables were affected. In all

TABLE XXIV

ANALYSIS OF VARIANCE FOR AVERAGE EGG WEIGHT IN PERIOD 2

Source of Variation	df	MS	F Value
Corrected Total	275	7.49	—
Fat Source	1	13.55	1.84
Fat Level	2	26.69	3.64*
Fat Source x Fat Level	2	3.70	0.50
Cage (Fat Source x Fat Level)	270	7.36	—
Coefficient of Variation = 3.70%			

*Significant at .05 level of probability.

TABLE XXV

ANALYSIS OF VARIANCE FOR AVERAGE EGG WEIGHT IN PERIOD 3

Source of Variation	df	MS	F Value
Corrected Total	263	7.99	--
Fat Source	1	15.71	2.02
Fat Level	2	31.59	4.06*
Fat Source x Fat Level	2	7.63	0.98
Cage (Fat Source x Fat Level)	258	7.78	--
Coefficient of Variation = 5.12%			

*Significant at .05 level of probability.

TABLE XXVI

ANALYSIS OF VARIANCE FOR AVERAGE EGG WEIGHT IN PERIOD 4

Source of Variation	df	MS	F Value
Corrected Total	263	8.35	--
Fat Source	1	46.07	5.65**
Fat Level	2	6.06	0.74
Fat Source x Fat Level	2	16.48	2.02
Cage (Fat Source x Fat Level)	258	8.16	--
Coefficient of Variation = 7.33%			

**Significant at .01 level of probability.

TABLE XXVII

ANALYSIS OF VARIANCE FOR AVERAGE EGG WEIGHT IN PERIOD 5

Source of Variation	df	MS	F Value
Corrected Total	263	9.78	--
Fat Source	1	85.68	9.18**
Fat Level	2	18.04	1.93
Fat Source x Fat Level	2	21.30	2.28
Cage (Fat Source x Fat Level)	258	9.33	--
Coefficient of Variation = 8.18%			

**Significant at .01 level of probability.

TABLE XXVIII

ANALYSIS OF VARIANCE FOR AVERAGE EGG WEIGHT IN PERIOD 6

Source of Variation	df	MS	F Value
Corrected Total	167	9.15	—
Fat Source	1	45.45	5.00*
Fat Level	2	0.84	0.09
Fat Source x Fat Level	2	3.88	0.43
Cage (Fat Source x Fat Level)	162	9.09	—
Coefficient of Variation = 3.40%			

*Significant at .05 level of probability.

TABLE XXIX

MEANS FOR AVERAGE EGG WEIGHT (GRAMS) IN PERIODS 2-6

Period	Ration						Overall
	1	2	3	4	5	6	
2	50.94	52.32	51.97	51.75	52.34	52.46	51.96
3	53.07	54.19	53.82	53.44	54.16	54.94	53.96
4	54.72	55.46	54.69	55.43	55.50	56.45	55.38
5	55.13	56.68	55.73	56.66	56.70	57.60	56.42
6	57.18	57.70	57.24	58.34	58.17	58.74	57.89

TABLE XXIX (Continued)

Period	Fat Source: Soybean Oil Rations (4,5,6)	Fat Source: Animal Tallow Rations (1,2,3)	Period	Fat Level: Low Rations (1,4)	Fat Level: Intermediate Rations (2,5)	Fat Level: High Rations (3,6)
2	52.18	51.74	2	51.34	52.33	52.21
3	54.18	53.69	3	53.25	54.17	54.38
4	55.79	54.96	4	55.08	55.48	55.57
5	56.99	55.85	5	55.89	56.69	56.66
6	58.41	57.37	6	57.76	57.93	57.99

periods where average egg weight was measured (periods 2-6), no significant interactions ($P > .05$) were seen between fat sources and fat levels (Tables XXIV-XXVIII).

Summary of Production Response Variables

In the production response variables studied, many trends have been found with differing dietary fat sources and dietary fat levels. In the feed consumption data, hens fed soybean oil tended to eat more than hens fed animal tallow. In addition, feed consumption tended to decrease as dietary fat level increased. Body weight change also responded to both dietary fat source and level. Hens fed soybean oil tended to have more positive body weight changes than hens fed animal tallow. The response to fat level was somewhat erratic and no trend appeared to be present.

Soybean oil in the diet resulted in higher egg production than when animal tallow was the fat source. Hens fed lower fat levels tended to lay more eggs than hens receiving a higher level of dietary fat. In reference to egg weight, hens receiving soybean oil layed heavier eggs on the average than hens fed animal tallow. In addition, higher dietary fat levels resulted in heavier egg weights than lower dietary fat levels, although the trend here was not as consistent as seen in other response variables.

In this experiment, rations were formulated to supply the same amount of nutrient to each hen. However, as it appears in the previous production response variables, some groups received more nutrients (i.e. protein, vitamins, and minerals) than others. As mentioned previously, it is thought that this is due to an incorrect estimation of the metabolizable energy values of one or both dietary fat sources.

In addition to certain nutrient imbalances causing differences in the response variables, linoleic acid levels in the soybean oil diets seemed to have an effect on both egg production and average egg weight. Although statistically significant differences have been observed between fat sources and among fat levels, the numerical differences in means were usually small and from this, it is thought that the differences found in the production response variables did not affect the liver response variables appreciably.

Liver Weight

The analysis of variance revealed no statistically significant differences ($P > .05$) in liver weight between fat sources for either of the two sampling periods (April 2, 1973 and May 10, 1973), as seen in Tables XXX and XXXI. However, the table of means for fat sources (Table XXXII) does indicate that there was a similar difference in means found in both sampling periods. Hens fed soybean oil tended to have lighter liver weights than hens fed animal tallow. In the first group of samples (April 2, 1973) when the hens were approximately 40 weeks of age (20 weeks into the laying period), the difference in liver weights between the two fat sources was very slight. In the second sampling period (May 10, 1973) when the hens were approximately 45 weeks of age (25 weeks into lay), the difference in liver weights between the two fat sources was greater. This indicates that animal tallow has a greater effect on increasing liver weight as the hens become older. It should be remembered that these differences in liver weight were not statistically significant ($P > .05$) and it is possible that these differences are due merely to chance.

TABLE XXX

ANALYSIS OF VARIANCE FOR LIVER WEIGHT IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	20.85	--
Fat Source	1	2.38	0.12
Fat Level	2	5.44	0.27
Fat Source x Fat Level	2	89.93	4.54*
Cage (Fat Source x Fat Level)	84	19.80	--
Coefficient of Variation = 34.42%			

*Significant at .05 level of probability.

TABLE XXXI

ANALYSIS OF VARIANCE FOR LIVER WEIGHT IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	43.44	--
Fat Source	1	140.20	3.29
Fat Level	2	59.55	1.40
Fat Source x Fat Level	2	37.32	0.88
Cage (Fat Source x Fat Level)	83	42.56	--
Coefficient of Variation = 18.12%			

TABLE XXXII

MEANS FOR LIVER WEIGHT

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	28.94	36.97
2	28.97	34.89
3	25.23	33.04
4	26.50	32.00
5	26.76	34.26
6	28.90	31.14
Fat Source		
Soybean Oil (Rations 4,5,6)	27.39	32.47
Animal Tallow (Rations 1,2,3)	27.71	34.97
Fat Levels		
Low (Rations 1,4)	27.72	34.48
Intermediate (Rations 2,5)	27.86	34.58
High (Rations 3,6)	27.06	32.09
Overall	27.55	33.72

Among fat levels, no statistically significant differences ($P > .05$) for liver weight were seen in either sampling period (Table XXX and XXXI), and it can be concluded that in this experiment, dietary fat level had no effect on liver weight.

A statistically significant interaction ($P < .05$) was observed between fat source and fat level for the first sampling period (April 2, 1973), but not for the second sampling period (May 10, 1973). Since the interaction is inconsistent through both periods, it is not known whether a true interaction exists between fat source and fat level in reference to liver weight.

Percent Liver Dry Matter

As shown in Table XXXIII, differences between fat sources in percent liver dry matter in sample period 1 (April 2, 1973) were not statistically significant ($P > .05$). However, in sample period 2 (May 10, 1973), differences between fat sources were statistically significant ($P < .01$) as seen in Table XXXIV. In both sampling periods, the hens fed soybean oil had a lower percent liver dry matter than hens fed animal tallow, indicating that dietary animal tallow results in a higher amount of liver dry matter than does dietary soybean oil (Table XXXV).

Among fat levels, differences in percent liver dry matter were not statistically significant in sample period 1, (Table XXXIII) but were statistically significant in sample period 2, (Table XXXIV). It appeared that in both sample periods increasing fat levels resulted in lower liver dry matters (Table XXXV).

The importance of this observation will be discussed later in relation to other response variables. No statistically significant interac-

TABLE XXXIII

ANALYSIS OF VARIANCE FOR PERCENT LIVER DRY MATTER IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	7.38	--
Fat Source	1	1.52	0.20
Fat Level	2	10.05	1.34
Fat Source x Fat Level	2	2.90	0.39
Cage (Fat Source x Fat Level)	84	7.49	--

Coefficient of Variation = 5.55%

TABLE XXXIV

ANALYSIS OF VARIANCE FOR PERCENT LIVER DRY MATTER IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	13.15	--
Fat Source	1	82.48	7.14**
Fat Level	2	50.21	4.35*
Fat Source x Fat Level	2	7.68	0.66
Cage (Fat Source x Fat Level)	83	11.55	--
Coefficient of Variation = 8.37%			

*Significant at .05 level of probability.

**Significant at .01 level of probability.

TABLE XXXV

MEANS FOR PERCENT LIVER DRY MATTER IN SAMPLE PERIODS 1 AND 2

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	31.07	35.10
2	31.23	33.25
3	30.21	33.82
4	31.42	34.08
5	30.34	31.54
6	29.98	30.80
Fat Source		
Soybean Oil (Rations 4,5,6)	30.58	32.14
Animal Tallow (Rations 1,2,3)	30.89	34.06
Fat Level		
Low (Rations 1,4)	31.24	34.59
Intermediate (Rations 2,5)	30.79	32.40
High (Rations 3,6)	30.09	32.31
Overall	30.71	33.10

tion ($P > .05$) was observed between fat source and fat level for percent liver dry matter in either sampling period.

Percent Total Liver Lipids by Goldfisch Method on a Dry Matter Basis

Between fat sources, there appeared to be a trend in percent total liver lipids similar to that observed in the previous response variables. In each of the sampling periods, hens fed soybean oil had lower percent total liver lipids than hens fed animal tallow. In sample period 1 (Table XXXVI), the difference between fat sources was not statistically significant ($P > .05$). However, in sample period 2 (Table XXXVII), the difference was statistically significant ($P < .01$). This indicates that dietary fat source has some effect on the amount of lipid which has accumulated in the liver. Table XXXVIII shows that the difference in lipid levels between fat sources in the first sampling period is quite small. In the second sampling period, the differences are much more clear cut, and the trend is evident.

Among fat levels, there was a decrease in the percent total liver lipids as dietary fat level increased. The differences were not statistically significant in sample period 1 (Table XXXVI) but in sample period 2 (Table XXXVII), these were statistically significant ($P < .025$). From this, it appears that dietary fat level also affects percent total liver lipids. No statistically significant interactions ($P > .05$) were observed between fat source and fat level for either period.

Another important observation seen in this analysis was the high coefficient of variation seen in both sampling periods for percent total liver lipids (Tables XXXVI and XXXVII). These values indicate that there

TABLE XXXVI

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON A DRY MATTER BASIS IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	82.05	--
Fat Source	1	9.05	0.11
Fat Level	2	155.32	1.92
Fat Source x Fat Level	2	99.05	1.23
Cage (Fat Source x Fat Level)	84	80.77	--
Coefficient of Variation = 50.88%			

TABLE XXXVII

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON A DRY MATTER BASIS IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	124.70	--
Fat Source	1	773.87	7.20**
Fat Level	2	508.81	4.73*
Fat Source x Fat Level	2	130.44	1.21
Cage (Fat Source x Fat Level)	83	107.49	--
Coefficient of Variation = 44.03%			

*Significant at .05 level of probability.

**Significant at .01 level of probability.

TABLE XXXVIII

MEANS FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON A DRY MATTER BASIS FOR SAMPLE PERIODS 1 AND 2

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	20.35	34.44
2	22.45	24.32
3	16.84	27.86
4	22.68	26.94
5	17.77	23.19
6	17.29	18.88
Fat Source		
Soybean Oil (Rations 4,5,6)	19.25	23.01
Animal Tallow (Rations 1,2,3)	19.88	28.87
Fat Levels		
Low (Rations 1,4)	21.52	30.69
Intermediate (Rations 2,5)	20.11	23.75
High (Rations 3,6)	17.06	23.37
Overall	19.56	25.94

is a great deal of variation among total lipid levels in hens within a treatment. It is not known whether this variation is due to methods of laboratory analysis or whether total lipid levels naturally have a wide variation within a population of hens.

Percent Total Liver Lipids by Goldfisch Method on an As-Is Basis

No statistically significant differences ($P > .05$) were observed between fat sources or among fat levels for sample period 1 (Table XXXIX). However, differences between fat sources and among fat levels were statistically significant ($P < .05$) for sample period 2 (Table XL). Interaction in either sampling period was not statistically significant ($P > .05$). The trend for mean values for percent total liver lipids by the Goldfisch method on an as-is basis (Table XLI) was very similar to the trend seen for percent total liver lipids by the Goldfisch method on a dry matter basis (Table XXXVIII). This was expected due to the fact that the same amount of lipid measured by the Goldfisch method on a dry matter basis was also being used to calculate the as-is percent total lipid values. The difference between the two calculations is in the inclusion of the percent moisture (approximately 70%) in the as-is percent total lipid values. This tends to lower the numerical value of the percent total lipids on an as-is basis. However, the relative magnitude of the percent total lipids among livers remains about the same since percent moisture values among livers are all approximately 70%.

TABLE XXXIX

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON AN AS-IS BASIS IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	11.41	--
Fat Source	1	0.79	0.07
Fat Level	2	22.73	2.03
Fat Source x Fat Level	2	14.26	1.27
Cage (Fat Source x Fat Level)	84	11.19	--
Coefficient of Variation = 61.70%			

TABLE XL

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON AN AS-IS BASIS IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	25.11	—
Fat Source	1	162.36	7.64**
Fat Level	2	119.05	5.60**
Fat Source x Fat Level	2	22.96	1.09
Cage (Fat Source x Fat Level)	83	21.24	—
Coefficient of Variation = 53.54%			

**Significant at .01 level of probability.

TABLE XLI

MEANS FOR PERCENT TOTAL LIVER LIPIDS BY GOLDFISCH METHOD ON AN AS-IS BASIS FOR SAMPLE PERIODS 1 AND 2

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	6.34	13.06
2	7.15	8.16
3	5.14	9.67
4	7.40	9.45
5	5.48	7.49
6	5.19	5.89
Fat Source		
Soybean Oil (Rations 4,5,6)	6.02	7.61
Animal Tallow (Rations 1,2,3)	6.21	10.30
Fat Level		
Low (Rations 1,4)	6.87	11.25
Intermediate (Rations 2,5)	6.31	7.82
High (Rations 3,6)	5.17	7.78
Overall	6.12	8.95

Percent Total Liver Lipids by the Modified Folch Method on an As-Is Basis

In sample period 1 (Table XLII), no statistically significant differences ($P > .05$) were observed between fat sources for percent total liver lipids by the modified Folch method on an as-is basis. In sample period 2 (Table XLIII), the difference between fat sources was statistically significant ($P < .005$). The diets containing animal tallow resulted in a higher percent total liver lipids than the soybean oil diets (Table XLIV). This relationship was present in sample period 1 also, but the difference between fat sources was very small.

Among fat levels, differences in sample period 1 (Table XLII) were not statistically significant ($P > .05$), but statistical significance ($P < .005$) was observed in sample period 2 (Table XLIII). In both periods, percent total liver lipids tended to decrease as the level of dietary fat increased. There was no statistically significant interaction ($P > .05$) between fat source and fat level in either of the two sampling periods.

The results of this analysis tend to agree with trends which have been observed in the previous response variables. Between fat sources, diets containing soybean oil tend to result in lower percent total liver lipid levels than diets containing animal tallow. Among fat levels, percent total liver lipid levels tend to decrease as dietary fat increases. These results show that both fat source and fat level have some effect on total liver lipid levels.

Liver Score

The response of the liver score appeared to be quite similar to that

TABLE XLII

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY FOLCH METHOD ON AN AS-IS BASIS IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	6.64	--
Fat Source	1	0.43	0.07
Fat Level	2	12.36	1.89
Fat Source x Fat Level	2	8.56	1.31
Cage (Fat Source x Fat Level)	84	6.54	--
Coefficient of Variation = 47.26%			

TABLE XLIII

ANALYSIS OF VARIANCE FOR PERCENT TOTAL LIVER LIPIDS BY FOLCH METHOD ON AN AS-IS BASIS IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	12.41	--
Fat Source	1	113.50	11.24**
Fat Level	2	68.13	6.75**
Fat Source x Fat Level	2	1.88	0.19
Cage (Fat Source x Fat Level)	83	10.10	--
Coefficient of Variation = 16.72%			

**Significant at .01 level of probability.

TABLE XLIV

MEANS FOR PERCENT TOTAL LIVER LIPIDS BY FOLCH METHOD ON AN AS-IS BASIS FOR SAMPLE PERIODS 1 AND 2

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	6.40	11.03
2	6.68	8.40
3	5.68	8.55
4	7.34	8.83
5	5.49	6.63
6	5.52	5.78
Fat Sources		
Soybean Oil (Rations 4,5,6)	6.12	7.08
Animal Tallow (Rations 1,2,3)	6.25	9.33
Fat Levels		
Low (Rations 1,4)	6.87	9.93
Intermediate (Rations 2,5)	6.09	7.51
High (Rations 3,6)	5.60	7.16
Overall	6.19	8.20

of percent total liver lipids, as would be expected, since liver score is a visual estimation of the liver lipid on the basis of liver color. No statistically significant differences ($P > .05$) were seen between fat sources for either of the two periods (Tables XLV, XLVI). As seen in Table XLVII, liver score was lower in the soybean oil diets in the first sampling period. In the second sampling period, liver score for soybean oil treatments was higher than for animal tallow treatments. One would expect that the score for soybean oil treatments would be less than the score for animal tallow since percent total liver lipid levels were lower for diets containing soybean oil than for diets containing animal tallow in the second sampling period. This unexpected difference is not critical due to the fact that liver score is a subjective, visual observation to estimate the amount of total liver lipid. The correlation coefficients between percent total liver lipid on a dry matter basis and liver score were quite high (Table XLVIII). These values indicate that there is a strong relationship between the two variables. Liver score (as a means quantifying total liver lipids on the basis of liver color) is highly dependent upon the amount of total liver lipids on a dry matter basis.

Among fat levels, statistically significant differences ($P < .05$) were observed in both sampling periods. As seen in Table XXXVI, there were no statistically significant differences among fat levels for percent total liver lipids on a dry matter basis for the first sampling period. One would expect that if liver scores were significantly different, then percent total liver lipid values would be different. The reason this is not true may lie in the fact that the coefficient of variation for percent total liver lipids for this period was quite high

TABLE XLV

ANALYSIS OF VARIANCE FOR LIVER SCORE IN SAMPLE PERIOD 1 (APRIL 2, 1973)

Source of Variation	df	MS	F Value
Corrected Total	89	0.59	--
Fat Source	1	0.29	0.50
Fat Level	2	2.70	4.82*
Fat Source x Fat Level	2	0.08	0.14
Cage (Fat Source x Fat Level)	84	0.56	--
Coefficient of Variation = 9.89%			

*Significant at .05 level of probability.

TABLE XLVI

ANALYSIS OF VARIANCE FOR LIVER SCORE IN SAMPLE PERIOD 2 (MAY 10, 1973)

Source of Variation	df	MS	F Value
Corrected Total	88	0.46	--
Fat Source	1	0.04	0.09
Fat Level	2	2.31	5.37*
Fat Source x Fat Level	2	0.18	0.42
Cage (Fat Source x Fat Level)	83	0.43	--
Coefficient of Variation = 12.03%			

*Significant at .01 level of probability.

TABLE XLVII

MEANS FOR LIVER SCORE FOR SAMPLE PERIODS 1 AND 2

Ration	Sample Period 1 (April 2, 1973)	Sample Period 2 (May 10, 1973)
1	3.20	3.73
2	2.93	3.33
3	2.53	3.33
4	3.07	3.87
5	2.73	3.47
6	2.53	3.20
Fat Source		
Soybean Oil (Rations 4,5,6)	2.78	3.51
Animal Tallow (Rations 1,2,3)	2.89	3.47
Fat Level		
Low (Rations 1,4)	3.13	3.80
Intermediate (Rations 2,5)	2.83	3.40
High (Rations 3,6)	2.53	3.27
Overall	2.83	3.49

TABLE XLVIII

CORRELATION COEFFICIENTS FOR PERCENT TOTAL LIVER LIPIDS (DRY MATTER BASIS) AND LIVER SCORE

	April 2, 1973	May 10, 1973
r	0.938	0.936

(50.88%). The coefficient of variation for liver score was quite low (9.89%) and possibly this made it easier to detect statistically significant differences. Between fat source and fat level, no statistically significant differences ($P > .05$) were observed for interaction for either sampling period for liver score.

CHAPTER V

CONCLUSIONS

Several interesting contrasts were observed between diets containing soybean oil and diets containing animal tallow. It was apparent that throughout the experiment, the level of fat accumulation in the liver was quite high. From the 179 livers which were sampled, 176 showed symptoms of fat accumulation to some degree. This indicates that neither fat source completely eliminated the incidence of hepatic lipid accumulation in this experiment. However, soybean oil tended to slightly reduce the severity as seen in the consistent differences in liver response variables between diets containing soybean oil and diets containing animal tallow. Both liver weights and liver dry matter values were lower in hens fed soybean oil. This reduction took place mostly as a reduction in liver lipids. Table XLIX shows that moisture in the liver responded inversely to the amount of lipid deposited in the liver. This was expected since this relationship has been understood for a long time. The non-lipid fraction did not appear to undergo any specific changes in response to dietary fat source on an as-is basis. Table L indicates that the percent non-lipid fraction (which is mainly protein) increases to some extent on a dry matter basis with soybean oil as opposed to animal tallow. If, in fact, the response of the non-lipid fraction to dietary fat source is real and not due simply to chance, it may be possible that lower non-lipid fraction values associated with animal

TABLE XLIX

MEANS FOR LIVER COMPONENTS (PERCENT) ON AS-IS BASIS

Ration	April 2, 1973			May 10, 1973		
	Moisture	Total Lipids	Non-Lipid Fraction	Moisture	Total Lipids	Non-Lipid Fraction
1	68.93	6.34	24.73	64.90	13.06	22.04
2	68.77	7.15	24.08	66.75	8.16	25.09
3	69.79	5.14	25.07	66.18	9.67	24.15
4	68.58	7.40	24.02	65.92	9.45	24.63
5	69.66	5.48	24.86	68.46	7.49	24.05
6	70.08	5.19	24.79	69.20	5.89	24.91
Fat Source						
Soybean Oil (Rations 4,5,6)	69.42	6.02	24.56	67.86	7.61	24.53
Animal Tallow (Rations 1,2,3)	69.11	6.21	24.68	65.94	10.30	23.76
Fat Levels						
Low (Rations 1,4)	68.76	6.87	24.37	65.41	11.25	23.34
Intermediate (Rations 2,5)	69.21	6.31	24.48	67.60	7.82	24.58
High (Rations 3,6)	69.91	5.17	24.92	67.69	7.78	24.53
Overall	69.29	6.12	24.59	66.90	8.95	24.15

TABLE L

MEANS FOR LIVER COMPONENTS (PERCENT) ON DRY MATTER BASIS

Ration	April 2, 1973		May 10, 1973	
	Total Lipids	Non-lipid Fraction	Total Lipids	Non-lipid Fraction
1	20.35	79.65	34.44	65.56
2	22.45	77.55	24.32	75.68
3	16.34	83.16	27.86	72.14
4	22.68	77.32	26.94	73.06
5	17.77	82.23	23.19	76.81
6	17.29	82.71	18.88	81.12
Fat Source				
Soybean Oil (Rations 4,5,6)	19.25	80.75	23.01	76.99
Animal Tallow (Rations 1,2,3)	19.88	80.12	28.87	71.13
Fat Levels				
Low (Rations 1,4)	21.52	78.48	30.69	69.31
Intermediate (Rations 2,5)	20.11	79.89	23.75	76.25
High (Rations 3,6)	17.06	82.94	23.37	76.63
Overall	19.56	80.44	25.94	74.06

tallow are related to the amount of liver lipid accumulation. If the decrease in non-lipid fraction is due to decreased liver protein synthesis, such as a decrease in the rate of synthesis of lipoproteins (triglyceride carrier molecules), then lipids would tend to accumulate in the hepatic cell. However, the decrease in the non-lipid fraction of the liver may not relate at all to liver lipid levels or may be due to chance in this case. Table L indicates that as percent liver lipid levels increase from soybean oil to animal tallow, the percent non-lipid fraction decreases, but the weight of the non-lipid fraction actually increases with the animal tallow (Table LI). From this, it is thought that the changes in non-lipid fractions are not directly related to the amount of lipid in the liver. The overall conclusion which can be drawn is that dietary fat source does have some effect on the amount of fat in the liver. Diets with soybean oil result in less lipid accumulation than diets with animal tallow. This is characterized by lower values for liver weight, liver dry matter, and most important lower liver lipid levels, both on an as-is and dry matter basis.

This brings up the question of what component of the dietary fat source is causing the differences in liver lipid levels. The major difference between fat sources is the fatty acid composition. The high linoleic acid level in soybean oil could have a protective action against liver lipid accumulation. This could be accomplished in several ways. Linoleic acid may be esterified into triglyceride more effectively than saturated fatty acids and oleic acid, which compose almost 98% of the fatty acids found in animal tallow. This could result in an increased rate of transport of linoleic acid out of the liver, thus reducing the hepatic accumulation of this fatty acid. It is possible that linoleic

TABLE LI

MEANS FOR WEIGHT (GRAMS) OF LIVER COMPONENTS ON DRY MATTER BASIS

Ration	April 2, 1973			May 10, 1973		
	Dry Matter	Total Lipids	Non-lipid Fraction	Dry Matter	Total Lipids	Non-lipid Fraction
1	8.99	1.83	7.16	12.97	4.47	8.50
2	9.05	2.03	7.02	11.60	2.82	8.78
3	7.62	1.28	6.34	11.17	3.11	8.06
4	8.33	1.89	6.44	10.91	2.94	7.97
5	8.12	1.44	6.68	10.81	2.51	8.30
6	8.66	1.50	7.16	9.59	1.81	7.78
Fat Source						
Soybean Oil (Rations 4,5,6)	8.38	1.61	6.77	10.44	2.40	8.04
Animal Tallow (Rations 1,2,3)	8.56	1.70	6.86	11.91	3.44	8.47
Fat Level						
Low (Rations 1,4)	8.66	1.86	6.80	11.93	3.66	8.27
Intermediate (Rations 2,5)	8.58	1.73	6.85	11.20	2.66	8.54
High (Rations 3,6)	8.14	1.39	6.75	10.37	2.42	7.95
Overall	8.31	1.63	6.68	11.16	2.89	8.27

acid is oxidized more efficiently than saturated fatty acids or oleic acid. Linoleic acid may have a greater depressing effect on hepatic fatty acid synthesis than saturated fatty acids or oleic acid found in animal tallow. From all indications in this experiment, it can be concluded that the source (or composition) of dietary fat affects the amount of fat in the liver. A dietary fat high in polyunsaturated fatty acids (soybean oil) resulted in lower liver lipid levels than a dietary fat low in polyunsaturated fatty acids (animal tallow).

Throughout the liver response variable studied, it was apparent that the level of dietary fat influenced liver weight, liver dry matter, and total liver lipid levels. There appeared to be an inverse relationship between these response variables and dietary fat level. Table XLIX shows that percent total lipids on an as-is basis was the liver component which decreased as dietary fat level increased. This change was accompanied by an increase in moisture. The non-lipid fraction did not appear to change significantly. Table L shows that on a dry matter basis, the percent non-lipid fraction increased as liver fat decreased. On a weight basis, there was no definite relationship between dietary fat level and the non-lipid fraction in the liver. It was assumed that any differences in the non-lipid fraction among fat levels were insignificant.

The inverse relationship between dietary fat level and total liver lipids is not unexpected. This observation may possibly be the result of a depression of fatty acid synthesis. Fatty acid synthetase, an enzyme complex in the liver (and other fatty acid-synthesizing tissues) is less active when high fat levels are supplied to the hen in the diet. As dietary fat levels are increased the activity of fatty acid synthetase

is depressed, resulting in fewer fatty acids being synthesized and less fat accumulating in the liver.

Although it has been shown that both dietary fat source and dietary fat level have an effect on the amount of fat in the hen's liver, the experiment was not designed to determine the exact cause of hepatic lipid accumulation. It has not been determined whether the liver fat accumulation observed in this experiment is actually Fatty Liver Syndrome. Due to the lack of symptoms of FLS (high mortality, liver hemorrhages), it is possible that this is not the case. In addition, high liver lipid values were observed in these hens, but there is still some question as to whether this lipid accumulation is abnormal.

Wolford and Polin (1972) found that high liver fat values were not necessarily indicative of Fatty Liver Syndrome. Due to this observation, it is entirely possible that liver lipid accumulation and FLS are not necessarily related. However, high liver lipid levels may be a predisposing factor for the symptoms of the condition (liver hemorrhage, mortality) to appear.

It is possible that the liver lipid accumulation is abnormal, resulting from some biochemical disorder. Many studies have shown that liver lipid accumulation and fatty livers in rats can be caused by a variety of factors. Lombardi (1966) categorized the causes of metabolic disorder into four groups: 1) synthesis of liver triglycerides is normal but their utilization is blocked; 2) utilization of hepatic triglycerides is normal but the rate of synthesis is increased; 3) a situation may exist where both utilization is blocked and synthesis is increased, 4) synthesis of hepatic triglyceride takes place in a compartment of the cell other than the endoplasmic reticulum, where synthesis normally

occurs. Under experimental induction of fatty livers in rats, it is thought that in most cases, impaired transport of triglycerides out of the liver is the problem. This is based on work done with chemicals such as ethionine, orotic acid, and carbontetrachloride (Villa-Trevino et al. 1963; Windmueller, 1964; Smuckler et al. 1962). It is not known which, if any, of these biochemical disorders is taking place in the livers of the hens in this experiment.

It has been shown that many dietary factors can affect the hepatic lipid levels; dietary fat source, dietary fat level, vitamins, dietary energy level, minerals, and environmental conditions. Due to the many variables involved, it is probable that liver lipid accumulation in the hens in this experiment is a result of a combination of factors and the solution to the problem will not be found easily. The conclusions that dietary fat source and dietary fat level do influence liver fat levels may provide some insight into understanding and controlling liver lipid accumulation in the laying hen.

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RATION ANALYSIS

Ration	Ether Extract (%)	Crude Protein (%)
1	9.48	15.65
2	13.65	16.29
3	17.89	15.21
4	9.48	15.51
5	12.62	15.80
6	16.14	16.80

VITA

John Robert Miller

Candidate for the Degree of

Master of Science

Thesis: HEPATIC LIPID LEVEL IN THE LAYING HEN AS INFLUENCED BY DIETARY FAT SOURCE AND DIETARY FAT LEVEL

Major Field: Animal Science

Biographical:

Personal Data: Born in Los Angeles, California, August 26, 1950, the son of Robert and Bernadette Miller.

Education: Graduated from La Salle High School, Pasadena, California, June, 1968. Received the Bachelor of Science degree from the University of California. Davis, California, with a major in Animal Science, in June, 1972; completed the requirements for the Master of Science degree in July, 1974.

Professional Experience: Worked as student technician for Department of Instructional Media (Office of Health Science Television) while attending the University of California at Davis, 1968-72; Research and teaching assistant in the Department of Animal Science and Industry, Oklahoma State University, Stillwater, Oklahoma, 1973-74.

Organizations: Member of the American Society of Animal Science.