1981

Effects of Diets on Fatty Liver Hemorrhagic Syndrome (FLHS)

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EFFECTS OF DIETS ON FATTY LIVER HEMORRHAGIC SYNDROME (FLHS)

BY

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A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Animal Science, South Dakota
State University

1981

EFFECTS OF DIETS ON FATTY LIVER

HEMORRHAGIC SYNDROME (FLHS)

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. C. W. Carlson, Professor of Animal Science, for his precious advice, guidance and encouragement throughout the course of the experiment and for his kind suggestions during the writing of this manuscript. Appreciation is extended to the Animal Science staff, field workers and fellow graduate students, particularly Dr. Ali B. Kashani, for their assistance and cooperation in various stages of this research.

The author is highly indebted to Ivan Stotz and Melva Mengan in the Veterinary Science Department for their help in preparing the samples for both light and electron microscopic analysis and photography.

The author is highly thankful to his loving wife Anuradha and daughter Ananya for their sacrifice during the study. Obligations are also due to his parents and other elders for their consistent encouragements for this work.

Finally, the author expresses appreciation to Shirley Schliessmann for her special care in correcting and typing the final copy of this thesis.

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EFFECTS OF DIETS ON FATTY LIVER HEMORRHAGIC SYNDROME (FLHS)

ABSTRACT

Chandi C. Rakshit
Under the Supervision of Professor C. W. Carlson

Two experiments were conducted to study the effect of fermentation by-products and oats on production parameters and Fatty Liver Hemorrhagic Syndrome in caged laying hens. Also tested in this study was the effect of force-feeding at 120% of normal intake for experimentally producing FLHS. The first experiment was conducted with sixty hens on three diets at twenty birds per diet. Corn and soybean meal were used as the chief energy and protein sources in diet 1. For diet 2, 10% of a product containing distillers dried grain (30%) with corn distillers solubles (70%) was added, and in diet 3, oats and soybean meal were used as the chief energy and protein sources. Data were recorded on production, mortality and feed consumption for each of seven periods of 28 days. The data from Experiment one showed that distillers dried grain can be used satisfactorily in layer diets.

In Experiment 2, 50% of the birds on each diet were force-fed at 120% of the normal intake for a period of three weeks by the method described by Wolford and Polin (1972c). The rest continued to be fed on an ad libitum basis. The experimental data showed that force-feeding at 120% of the normal intake was satisfactory for experimentally producing FLHS. The liver lipid contents of the force-fed birds were three times as high as their normal counterparts. Egg production was reduced significantly ($P<0.01$) in the force-fed birds. The fermentation by-product was found

to have little beneficial effect in preventing FLHS. However, oats showed a significant effect in reducing liver fat content and hemorrhage, thereby preventing the disease.

Gross pictures of the livers show different color variations which corresponded with their lipid content. The lesser the lipid content, the darker the color of the liver. Microscopically (both light and electron), the most discernible difference found between the fatty and normal livers was the size of the fat droplets, thereby indicating the amount of fat infiltration.

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INTRODUCTION

Fatty Liver Hemorrhagic Syndrome (FLHS) has been recognized as a disease of laying hens for more than two decades. It was first named Fatty Liver Syndrome (FLS) by Couch, 1956.

Lipid synthesis to produce high liver fat may be a normal physiological response. Hence, research workers have supposed that high liver fat content alone would not be sufficient to produce the typical field type of Fatty Liver Syndrome (Meigering, 1980). Therefore, it is necessary that there be hemorrhage along with increased fat; this has been called Fatty Liver Hemorrhagic Syndrome (FLHS) by Wolford and Polin (1972b). In the present paper, the term FLHS will be used.

This disease is characterized by marked accumulation of lipids in the liver of affected birds. The affected birds also have an enlarged liver with small multiple hemorrhages. Death is primarily due to hemorrhages. Egg production of survivors may not be affected; hence, the major economic loss is due to the loss of the birds.

The etiology of the disease was first thought to be due to a pathogenic organism, but without much supportive evidence. Present evidence indicates that the disease is a metabolic disorder caused by nutritional deficiencies (Jensen, 1979). It is also the opinion of some workers that the disease may be due to over-consumption of high energy diets. Short-term experiments as conducted by Jensen (1979) have demonstrated that by supplementing layer diets with common feedstuffs like brewer's dried grain, distillers dried grain with solubles, fishmeal, brewer's yeast, etc., liver fat accumulation could be reduced.

The main objectives of this experiment were: (1) To measure the effect of a fermentation by-product and oats on egg production and liver parameters; and (2) To measure the effect of superimposing FLHS on the above by force-feeding at the rate of 120% of the control intake for three weeks.

LITERATURE REVIEW

The liver is the chief site of lipid synthesis. Lipoprotein serves to transport these lipids to different parts of the body. In the sexually mature female, estrogen stimulates hepatic production of a special lipoprotein which is transported by the blood to the ovary and deposited in the yolk of the egg (Garlich, 1979).

If lipid synthesis is in excess due to some reason or other or the transport mechanism of lipid from the liver is defective, then there is accumulation of lipids in the liver. This gives rise to a condition sometimes called Fatty Liver, which also has been named Fatty Liver Syndrome.

Hens in good laying condition have an increased liver fat content which is physiologically normal. It is difficult to find the dividing line between physiologically normal and pathologically high fat contents of the liver (Meigering, 1980). This review covers factors that are environmental, genetic and nutritional (which are supposed to be related with the disease), as well as other aspects of the disease.

Characteristics of the Syndrome

It is hard to differentiate hens suffering from FLHS from healthy ones in a flock (Meigering, 1980). Couch (1956) observed an increase in body weight of about 25-30 percent. As the result of severe hemorrhages, the combs may be pale and cold to the touch (Couch, 1968). Paleness of comb, face and wattles prior to death was also observed by Ringer and Sheppard (1963) as mentioned by Wolford and Polin (1972a). On post-

mortem examination, in addition to an excess accumulation of lipid in the liver, abdominal cavity and intestinal mysentry, the liver may have small multiple hemorrhages (Jensen, 1979; Meigering, 1980; Thayer et al., 1973). The liver is fragile and its color varies from pale brown to yellow. After incision, the tissue shows a pasty consistency (Meigering, 1980). Wolford and Polin (1971) found that livers having less than 33.3% fat (dry basis) did not show hemorrhages. However, lipid content of liver was not a predisposing factor for hemorrhage. They found birds with 48.5% liver lipid (dry basis) without hemorrhage, whereas birds with a liver lipid content of 41.8% (dry basis) showed hemorrhages. In general, the gross signs of the disease were described by Couch (1956) to be: (1) An increase in body weight; (2) A decrease in the rate of egg production; (3) An excess of abdominal fat, fatty livers, capillary hemorrhages and hematoma in the liver; and (4) An increase in mortality.

Microscopically, there is accumulation of fat globules in the cytoplasm of the liver cells. This may be in the form of multiple small globules or all of them fused to form one big fat globule, thereby displacing the nucleus and changing the shape of the cell (Thayer, et al., 1973). As reported by Wolford and Polin (1972a), Tudor (1967) found that excess accumulation of fat within the liver cells results in a friable and soft liver that is uniformly pale yellow in color. As a result, the vascular tissue might break down and result in hemorrhages within the liver. This sometimes extends to the liver capsule.

Genetic Factors

There has not been much research done to determine the genetic effect on liver fat accumulation. Jensen et al. (1976a) worked with two commercial strains, Babcock-300 and Dekalb-171 pullets. Although there was a significant difference in egg production and feed efficiency, there was no difference in liver fat accumulation between the two strains. Garlich et al. (1974) determined the average liver lipid values for 20 different strains of laying hens managed in three different confinement systems. There was a highly significant difference in liver fat among the different strains. Four strains showed consistently high liver fat and four others showed low liver fat with all three systems. Nelson (1978) worked with different commercial strains and in one experiment found that the Hyline-W-36 strain showed more liver lipid mobilization but few hemorrhages. Whereas, in another experiment the same strain showed more hemorrhages but less liver lipid accumulation. In another experiment, he observed liver weight to be significantly lower for the Hylines than for the Babcock strain. In another experiment, a Dekalb strain showed a higher incidence of hemorrhages than other strains. In general, Nelson (1978) showed marked differences in genetic susceptibility to FLHS. Two hybrid strains showed lower hepatic lipid than the regional control, and Hyline strains were found to be more efficient in lipid mobilization; hence, they showed less accumulation of fat in the liver.

Environmental Factors

Several environmental factors such as water, housing and temperature have been associated with FLHS. It was observed by Jensen et al.

(1976b) that FLHS is prevalent in some areas, whereas in other localities the disease was rare. He thought that drinking water may be a factor associated with the disease and worked with 21 samples of water collected from different commercial farms. On analysis of the waters, it was found that water from FLHS-occurring farms contained significantly more calcium, magnesium, strontium, sodium, and barium than samples from farms where FLHS was not a problem. In a similar experiment, Jensen et al. (1977) worked with water samples from 15 Experiment Stations classified as producing liver lipid accumulation with ease or difficulty. The two classes of water samples were analyzed for mineral content. The water samples classified as easy to produce FLHS showed significantly more calcium and cobalt ($P<0.05$) and magnesium, nickel and manganese ($P<0.01$) than the other type. Four experiments were conducted by the same author to determine the direct effect of water sources on liver lipid accumulation. Water from a Georgia farm with a history of FLHS was given to laying hens housed in floor pens and cages for a period of eight weeks and compared to hens on the University of Georgia water. Little difference was observed in liver lipid content.

Effect of Housing

Confinement of laying birds in individual-type cages for one year resulted in a marked atrophy of heart, liver and gizzard (Jeffrey and Britt, 1941). Caged birds were found by the same authors to be fatter than those kept on the floor. Price et al. (1957) also found higher liver fat levels in caged birds than in birds kept on the floor. It

was noticed that during 1950 to 1960, FLHS cases increased when laying hens were kept more and more in battery systems and fed high-energy feed (Couch, 1956; and Deacon, 1968). Price et al. (1957) and Griffith et al. (1969) reported that liver lipid was higher in birds maintained in cages than in those on the floor. Several other workers such as Barton et al. (1966), Barton (1967) and Hartfiel et al. (1970a, b) as reviewed by Ivy and Nesheim (1973) also agreed with the observations of Price et al. (1957). Hartfiel et al. (1970a) showed that forced exercise and consumption of feces caused a reduction of fat in hens kept in cages. Price et al. (1957) suggested that development of the fatty liver condition may be due to a restriction of movements of birds kept in cages or to a nutritional deficiency of the cage layer diet. The reduction of liver fat content due to exercise and consumption of feces as shown by Hartfiel et al. (1970a) would suggest that higher liver fat in caged birds results from limited exercise and lack of access to their feces.

To minimize housing costs, commercial producers often increase bird density in cages. This was studied by Jensen et al. (1976a), who found that liver weight (both absolute and per unit of body weight) was significantly greater for hens housed individually than for those housed three per cage. As reviewed by Jensen et al. (1976a), Grover et al. (1972), Wilson et al. (1967), and Foss and Carew (1974) observed that body weight gain was reduced as bird density increased. Whereas, Tower et al. (1967), and Lowe and Heywang (1964) found that body

weight gain was greater in multiple caged birds. Still others, Cook and Dembnicki (1966), Champion and Zindel (1968) found no effect of bird density on body weight gain.

Effect of Temperature

Griffith et al. (1969) suggested that there is a close relation between liver fat and environmental temperature. As mentioned by Wolford (1971), Ringer and Sheppard (1963) observed that FLHS increased when the environmental temperature exceeded 30°C for three consecutive days. Lee et al. (1975) observed that changing the atmospheric temperature from 12.2°C to 27.8°C or 22.2°C to 30.6°C did not significantly ($P<0.05$) change the liver fat content. About 50% incidence of FLHS occurred in birds which were restricted in feed intake and exposed to an environmental temperature of 30.6°C for four weeks followed by ad libitum feeding at a temperature of 22.2°C for four weeks.

Nutritional Factor - Effect of Lipotropic Agents, Vitamins and Minerals

Assuming that fat accumulation in the liver is due to a defective mechanism of transport of lipids from liver cells to blood, this transport is stimulated by certain lipotropic agents as they are a part of phospholipids. A deficiency of such lipotropic agents as choline, inositol, methionine, etc. may lead to FLHS. Because of the many negative results experienced by adding such compounds to the diet, it is not very likely that a deficiency of such compounds is one of the major causes of FLHS (Meigering, 1980). As reviewed by Nelson (1978), fatty livers produced in rats by low protein, low methionine and high

fat diets were prevented by choline supplementation. In this instance the main concern was a methyl group deficiency, because if methionine was supplied, choline was not needed. As mentioned before, Nesheim et al. (1971) and Abbott et al. (1940) found that choline supplementation of the basal diet increased egg production, decreased mortality and decreased the percentage of fatty acids in livers. Nesheim et al. (1971) also found that hens which received supplemental choline had less liver fat than those which did not receive choline. Of course, this effect was not observed in all the experiments of the series (Nesheim et al., 1971). Different workers have found that the addition of choline and/or B_{12} and/or methionine had little or no effect on egg production, size or other quality of egg or hatchability (Balloun, 1956; Chah et al., 1975; Gish et al., 1949; Ringrose and Davis, 1946; Bossard and Combs, 1970; Johnson, 1954; Skinner et al., 1951; and Crawford et al., 1967). Some others have shown that choline supplementation increases egg production and egg quality (Lucas et al., 1946; Burn et al., 1955; Couch et al., 1955; Schexnailder and Griffith, 1973; and Griffith and Schexnailder, 1972). Nelson (1978) conducted four experiments with choline and found that excessive supplementation of choline increased egg production from 1-11%. Different opinions are also available regarding the effectiveness of choline supplementation on liver fat accumulation. Griffith et al. (1969), Abbott and Demasters (1940), and Jensen et al. (1974) found that there was a significant effect of choline on reducing liver fat, whereas others showed that there was no effect of choline supplementation (Nesheim

et al., 1971; Chah et al., 1975; Bossard and Combs, 1970; Wolford and Murphy, 1972). Nelson (1978) agreed with Jensen et al. (1974) in that choline supplementation decreased liver fat by 16% in his work.

Wolford and Murphy (1972) worked with a mixture of choline, vitamin B_{12}, inositol and vitamin E and found that it did not have any effect in preventing hemorrhages. They noted that some birds receiving high-energy diets had liver lipid values equal to or higher than birds having hemorrhages. Hence, liver lipid content is not the only factor involved. However, it was suggested that increases in liver size may cause higher tensions in the vascular system and thereby cause hemorrhages.

As noted by Jensen et al. (1974), Karpov (1967) suggested that selenium may be a factor which could influence liver fat content, because he found that selenium supplementation reduced dystrophy of the liver in laying hens. Jensen et al. (1974) observed that selenium supplementation reduced liver fat content in one out of two experiments.

Contrary to earlier reports, Leveille et al. (1970) observed that dietary inositol depressed liver fat accumulation. Pearce (1972), Ragland et al. (1970) and Bossard et al. (1970) observed that inositol had no significant effect in reducing liver fat in hens.

Assuming that other vitamins like folic acid, riboflavin, pyridoxin, biotin or pantothenic acid may effect FLHS, Schexnailder and Griffith (1973), Chah et al. (1975), and Jensen et al. (1976c) tested these factors and found no effect on egg production, liver fat or hemorrhages. However, the authors concluded that the birds may have

had sufficient storage of those vitamins during the experimental period.

Effect of Different Sources and Level of Fat

Fat additions to the diet of laying hens has given controversial results as to egg production and FLHS. Feeding 0-14% poultry fat and keeping the calories-to-protein ratio between 100-125 K cal/Kg: 1% protein had little effect on liver fat content (Price et al., 1957). McDaniel et al. (1959) also studied the same condition and found that the addition of 10% animal or vegetable fat had no effect on liver lipid content. Abdominal fat content was shown to be in close association with the incidence of FLHS and increased body weight. Adding up to 7% animal fat, Jensen et al. (1974) found that it had no effect on liver parameters. Chah et al. (1975) showed that the additions of 8.6% corn oil, 10% yellow grease, 1500 mgm/Kg choline, and 1.1 mgm/Kg biotin to a 14% protein basal diet had no significant effect on egg production. However, the fat addition increased final body weight and average egg size. Based on the score given, livers from the control group showed maximum values whereas livers from the corn oil group showed minimum values. Addition of 2-10% animal fat did not increase liver lipid, rather a decrease of fat accumulation was noted (Nelson, 1978). However, Weiss et al. (1957) found that supplementing a diet with 5-10% animal fat elevated the plasma lipid. They showed excess deposits of body fat, friable and fatty livers, fatty deposits in and around the kidneys and a greater severity of aortic atherosclerosis. These conditions depend on the age of the birds and duration

of feeding. Bragg et al. (1973) worked with five levels (0,1,2,4, and 8%) of dietary tallow, soybean oil, sunflower oil and rapeseed oil. Fatty livers were observed with the high level (8%) of dietary tallow and rapeseed oil, whereas soybean and sunflower oil showed protection against fatty livers at all levels.

Effect of Energy Intake

When energy intake is more than the energy required for maintenance and production, much of the excess energy is converted to fat (Meigering, 1980). Feeding a high-energy diet to layers may lead to an over-consumption of calories, which with the lack of sufficient exercise in caged hens may lead to a positive energy balance (Meigering, 1980; Wolford and Polin, 1974). Both in human nutrition and also in goose liver production, it is well known that over-consumption of calories leads to liver fattening (Nelson, 1978; Meigering, 1980). FLHS was produced in hens by force-feeding which was reported identical to that found in the field by Wolford and Polin in 1972. Since then, several FLHS studies have been conducted showing that excessive energy intake is a contributing factor to FLHS (Wolford and Polin, 1974 and 1975; Ivy and Nesheim, 1973; Nelson, 1978).

To ascertain the relationship between hemorrhage occurrence and liver lipid content, Wolford and Polin (1972b) used Single Comb White Leghorn laying hens which were subjected to a restricted feeding program. Restricted feeding resulted in significantly ($P<0.01$) lower liver and body fat. None of the chickens under restricted feeding showed liver hemorrhage. However, the control ad libitum group showed a 25%

level of incidence. The increase in weight of liver components (water, lipid and non-lipid dry weight) were proportional to the amount of feed force-fed. This was for 21 days at 50% more than ad libitum (Wolford and Polin, 1974 and 1975). Wolford and Polin (1974) tried force-feeding at rates ranging from 50 to 150 percent of control intake. In one experiment they tried 150% of control intake to determine if force-feeding five days a week instead of seven days could produce FLHS. At the force-feeding rate of 150% of the control intake, there was a tremendous hypertrophy of the liver. Lower force-feeding rates were characterized by liver sizes proportional to the rates of force-feeding. Birds force-fed at the same rate of 50% showed average liver weights at 113.1% of the control value. Those fed at the rate of 100% showed average liver weights of 118.4% of the control. When force-feeding at 100, 125 and 150% of the daily rate was stopped and the hens were allowed to recover, their feed intake averaged 83, 72.1 and 66%, respectively. Body weight gain was directly proportional to the total feed intake. The weight of the liver and its lipid and water contents were significantly increased over control values. At the end of the 21-day recovery period following force-feeding, the chickens had significantly lower lipid and water contents in their livers in contrast to the hens which were killed 21 days before. Liver scores were also significantly increased by force-feeding at the rate of 100% or more. Force-feeding followed by ad lib feeding for 21 days lowered the liver score for FLHS. Birds in good laying condition vary in their lipid content (Ivy and Nesheim, 1973). They also found that liver lipid content can

be altered by changing the energy level of the diet or by force-feeding. The latter could not be correlated with the level of fat in the liver. They thought that liver lipid was under metabolic control and not governed by energy intake which agrees with Hartfiel et al. (1970b). Barton et al. (1966) found that a low-energy high-fiber ration (14.8% protein, 2,360 cal ME/Kg) prevented fatty livers, while a high-energy ration stimulated it. Other workers also agree that energy consumption is a primary factor affecting the level of FLHS (Polin and Wolford, 1973; McDaniel et al., 1959; Nesheim et al., 1969).

In three experiments, Wolford and Polin (1975) worked with different lipotropic agents and also energy levels to ascertain their response in producing FLHS. The controls averaged 12.2 gm of lipid per liver as compared to 2.6-5 gm. They noted one interesting result, that as the average hepatic lipid level declined, the average weight gain of the controls increased. This could not be explained, but they suggested that a control should be included in any field study on liver lipids. The lipotropic agents failed to decrease the syndrome. In one experiment, by force-feeding at the rate of 125%, liver lipids increased from 5 gms per liver to 18.5 gms. Liver score also increased from 2 to 3.9 with the same birds.

Only one report on fatty livers by Quisenberry (1969) was available. In one study, the basal diet was supplemented with protamone at 100 and 200 gms/ton. At 200 gms/ton, egg production and size were higher and feed consumption lower. Body weight was increased by a high-energy diet but depressed by protamone. Visual liver fat score was higher for

the basal diet and reduced by protamone. Fatty liver symptoms were neither caused nor prevented by dietary caloric level or protamone.

Effect of Different Grains

To measure the effect of different sources of carbohydrate on fat accumulation in liver, Jensen et al. (1976d) conducted four experiments with laying hens and one with broiler chicks. When corn and wheat were fed in different proportions, liver fat increased when the proportion of corn increased. When fed isocalorically, hens fed sorghum, corn or triticale showed the highest liver fat content and those fed barley, oats or rye showed the minimum values. Wheat had an intermediate effect. Broilers showed no significant difference in liver fat between birds fed corn or wheat. When corn was replaced by different levels of glucose monohydrate, there was no significant difference in liver fat accumulation. Corn and wheat from different areas (Georgia, Midwest or Farwest) had no effect on liver fat content. Polin and Wolford (1976) tried to measure the effect of various carbohydrate sources and a positive energy balance. They force-fed a corn-soy basal diet at the rate of 150% of daily intake. Composition of the diet was such that 2/3 of the ME came from corn and soybean meal and 1/3 from corn oil or glucose. FLHS was produced in all force-fed birds. Diets with low energy and corn oil produced lower FLHS scores. But the liver lipid contents of the birds with lower scores were the same as the others. They concluded that various types of diets and sources of energy in excess can induce FLHS. According to them, all diets with either carbohydrate or lipids as the excess source of

energy produce FLHS. They proposed that FLHS in laying hens is the result of a positive energy balance for a long time, and that there is a change from the typical fatty livers of good laying hens to the fatty hemorrhagic and fragile liver of true FLHS hens. Kim et al. (1976) conducted two experiments with White Leghorn laying hens to study the effects of different cereal grains on production parameters and liver fat content. Experiment one indicated that wheat or triticale (Trail Blazer) were equal to corn as far as production parameters and body weights are concerned. Henry wheat (a hard red winter type) was significantly ($P<0.05$) inferior to corn. There was no significant difference in liver fat contents amongst the above treatments. In the second experiment, opaque-2 corn was found to be slightly superior to normal corn and Triticale was comparable to normal corn in production parameters. Hens fed normal corn or opaque-2 corn as the only grain source had higher liver lipid contents than Triticale-fed hens.

Oats as the primary source of carbohydrate reduced production significantly (Nelson, 1978). He also suggested that oats contains a factor necessary for lipid metabolism, as there was reduced fat and no hemorrhage in the livers of hens fed oats diets. Two more experiments were conducted by the same author to measure the effect of fiber sources of the diet, using cellulose, oat hulls and wheat bran. The results indicated that the bran and hulls of wheat and oats respectively were not the factors responsible for lowering the liver lipid content.

Effect of Different Fermentation By-products

Sloan (1940) reported that distillers dried grains with solubles, along with meat scrap and soybean meal, could satisfactorily contribute 1/4 of the protein in poultry rations. This was later confirmed by several other workers (Matterson et al., 1966). Two experiments were conducted by Harms et al. (1969) to evaluate distillers dried grains with solubles (DDG/S). They found that it can be added to diets at the level of 10%, provided the diets are formulated on the basis of the amino acid content of DDG/S. Two experiments were conducted by Jensen et al. (1974a) to measure the nutritional value of DDG/S with respect to production and liver parameters.

In one experiment, adding 5% DDG/S to a wheat-based ration improved production, but adding the same level of DDG/S either to the wheat or corn rations increased egg weight but not the rate of production. Feed intake, fertility or hatchability were not affected. When 10% DDG/S was added, egg production was significantly reduced and it was below that obtained with the 5% (DDG/S) level. Adding 0.025% L-lysine to the 10% (DDG/S) supplemented diet increased egg production to the same level as that found with 5% (DDG/S). Liver fat content appeared to be reduced in both experiments with DDG/S, but the difference was not significant. Jensen et al. (1976e) conducted two more experiments, one in the summer and the other in the winter, to measure the effect of different levels of distillers dried grains on liver lipid accumulation. By adding 20% brewers dried grain, liver fat per unit body weight was reduced significantly in summer experi-

ments but not in the winter studies. With 10% brewers dried grain, an intermediate response was obtained. Twenty percent brewers dried grain improved interior egg quality. However, egg production in the winter experiment was significantly lower though there was no difference in the summer. Eldred et al. (1975) tried 5% and 10% brewers grain and a 5% brewers grain-yeast mixture. Egg production and egg weight were improved by the addition of brewers grain or the brewers grain and yeast mixture. Distillers grains significantly reduced body weight. Maurice et al. (1978b) observed in two experiments that 20% brewers dried grain, 10-20% distillers dried grains and 10% distillers dried solubles in the diet were effective in reducing relative liver weight and liver fat ($P<0.05$) both in the control environment and in the summer. This response was not related to the level of dietary fat but appeared to be induced by an intrinsic nutritional factor in the by-product. In four additional experiments, Maurice et al. (1978a) found that hens with equivalent ME intakes and egg outputs exhibited differences in liver and plasma lipids due to diet composition. They suggested that mill feeds and fermentation by-products contained an essential factor for the control of lipid metabolism in caged birds. Thornton et al. (1962) tried a 20% brewers grain substitution for corn, milo and soybean meal. The gains in body weight and liver fat accumulation in experimental birds were least during the last 50 weeks of the 68-week experimental period. A calculation of the daily calorie intake indicated that the control birds required less energy intake per unit gain of body weight than the experimental birds. There was a

high correlation between liver fat and body weight gain in experimental birds which the control birds failed to reveal. The authors suggested that brewers grain may contain an unknown factor or that the biological availability of recognized nutrients is greater in the brewers grain. Keinholz et al. (1963) used 40% BDG for laying hens kept both in cages and on the floor and observed excellent performance. However, receiving BDG caused the hens to have significantly less body weight and liver fat content. The reason for this was not known. Keinholz et al. (1972) included 0, 20 and 40% brewers grain in an experiment and noticed that brewers dried grain reduced egg size and quality. A previous experiment, by the same author, did not show such reduction in egg quality, and they concluded that the reason for the change in egg quality in the last experiment was due to storage of BDG in summer, thereby reducing its nutrient value. Keinholz et al. (1967) found that BDG diets improved fertility in 15 of the 17 tests and improved hatchability of fertile eggs in 14 out of 17 tests. Average improvement was 5% and 9% for fertility and hatchability of fertile eggs, respectively. The authors suggested that there may be an unknown factor in BDG which improved hatchability of fertile eggs and fertility. Dried steep liquor concentrate (DSLC) is a blend of fractions from wet milling of corn and includes corn germ meal, corn, corn gluten meal and dried condensed fermented corn extractives. DSLC is an acceptable ingredient in a nutritionally balanced diet and substitution of (DSLC) in the poultry ration significantly improved egg quality (Waldroup et al., 1971; and Hazen and Waldroup, 1972).

Effect of Dietary Protein Level and Source

To study dietary protein level and source, Maurice et al. (1979) conducted two experiments of five weeks each. In Experiment one, equicalorie (3.1 K cal/g) and isonitrogenous (12.5%) corn-fishmeal and corn soybean meal diets were used. Also, an equicalorie 16.5% protein corn-soybean diet was included in the study to measure the effect of protein level. The corn-fishmeal diet reduced liver weight, hepatic and plasma lipid and liver hemorrhage significantly ($P<0.05$) and plasma estrogen level highly significantly ($P<0.01$). There was no marked difference in liver parameters due to different levels of protein. There was no change in production parameters due to different sources of protein. The second experiment also showed similar results.

Effect of Hormones

Roberson et al. (1970) worked with methionine, thiouracil, dienestrol diacetate and thyroprotein. They used the following levels of each to study the developments and prevention of fatty livers: D-L methionine 0 and .07%, thiouracil 0 and .05%, dienestrol diacetate 0 and .007% and thyroprotein 0 and .0125%. Thiouracil and dienestrol diacetate contributed to the development of fatty livers, whereas methionine and thyroprotein prevented fatty livers by lowering liver and abdominal fat. Polin et al. (1977) observed that force-feeding at the rate of 125% to 150% of the ad libitum diet produced fatty livers and FLHS. Intramuscular injection of B-estradiol-17 dipropionate at 2 mgm/Kg body weight injected three times weekly for 21 days produced a gradient response in terms of increased incidence of hemorrhage and

an increase in ad libitum feed intake. There was no significant difference in liver score between the sexes, but females accumulated more liver fat than males. Testosterone dipropionate at the rate of 25 mgm per Kg body weight was injected three times weekly to immature females force-fed at the rate of 150%. Increased liver fat and feed intake were noted but no hemorrhage. The results show that chickens with positive energy balance may produce FLHS if treated with estrogen. Commercial laying hens with FLHS had increased serum calcium and cholesterol levels (Harms et al., 1972 and 1979). They found that hens with increased serum calcium and cholesterol levels had enlarged combs and excess deposits of fat in the abdomen. Based on these observations of serum calcium and cholesterol levels, it was suggested that FLHS may be caused by an hormonal imbalance.

Effect of Toxic Materials

Aflatoxins are groups of toxic compounds produced by the Aspergillus flavus types of mold when growing on feedstuffs (Smith et al., 1969). They found that graded doses of aflatoxin incorporated in the diet of broiler chickens resulted in decreased growth, enlarged livers, spleen and pancreas and a regressed bursa of fabricus. Lipid accounted for 60% of the dry weight increase of the liver. Hamilton and Garlich (1971) designed an experiment to establish whether dietary aflatoxin would induce the fatty liver syndrome. The hens were fed graded levels of aflatoxin for three weeks. Aflatoxin decreased egg production, and liver weight was increased but spleen and pancreas weights were unaffected. Analysis of liver for total lipid revealed that the mean

lipid content as percent dry liver weight was 36, 33, 43, 59, 55 and 55 for hens consuming 0, 1.25, 2.5, 5, 10 and 20 mg of aflatoxin per gram of diet, respectively. They suggested that dietary aflatoxin can cause a fatty liver syndrome.

MATERIALS AND METHODS

Two experiments were conducted with the Shaver 288 strain of laying hens in cages. Experiment one was carried out to measure the effects of different diets on egg production parameters with a normal feeding procedure (ad libitum type). Experiment two was conducted in which 50% of the birds from each diet were force-fed at 120% of their normal intake, using the force-feeding technique devised by Wolford and Polin (1972c).

Experiment 1

Sixty birds were selected at random for this experiment. The birds had been raised on a starter mash (Table 1) for eight weeks in a cage system, then fed the grower diet shown to twenty weeks of age. They were then maintained on the layer diet shown until the beginning of the experiment. At forty-six weeks of age the birds were transferred to individual cages (20x41 cm) and randomly allotted to each diet (20/diet). Three diets (Table 2) were used in this experiment. In diet 1, corn and soybean meal were used as the chief source of energy and protein. In diet 2, 10% of the diet was replaced by a product containing corn distillers solubles (70%) with distillers dried grain (30%). (Commercially known as Solulac, its detailed composition is given in Tables 3 and 4). Corn and some of the soybean meal were replaced by oats for diet 3. Data on egg production were recorded for each bird daily and summarized by 28-day periods for seven periods. The production parameters were analyzed statistically (Steel and Torrie, 1960).

TABLE 1. Composition of Diets Used up to Starting of the Experiments

	0-8 weeks Starter	(12%) 8-20 weeks Grower	(16%) 20-46 weeks Layer
Ingredient	%	%	%
Ground corn	64.5	80	64.8
Soybean meal 50%	27	8	20.2
Ground alfalfa	2	6	2
Fishmeal	2	--	--
Limestone	1	1	6
Dicalcium phosphate	1.5	2	2
Yellow grease	1	2	4
Salt mix (a)	.5	.5	.5
Vitamin mix (b)	.5	.5	.5
Calculated Analysis			
Crude protein (%)	21.6	12	15.8
ME (K cal/Kg)	2955	3021	2743
Lysine (%)	1.12	.49	.81
Methionine (%)	.36	.23	.28
Arginine (%)	1.41	.74	1.1
Tryptophan (%)	.26	.14	.2

(a) Salt mix contains in percent, Mn not less than .250; Cu, .033; Co, .0025; Zn, .005; Nacl, 97; S, .1; Fe, .2; I, .007.

(b) Vitamin mix contains per Kg, vit A, 1,056,000 IU; vit D_3, 275,000 IU; vit E, 4,400 IU; B_{12}, 1.76 mgm; Riboflavin, 1.320 gm; Niacin, 8.8 gm; d-Pantothenic acid, 1.76 gm; Choline, 76.384 gm; Menadione, 217.8 mgm; Folic acid, 220 mgm; d-Biotin, 22 mgm.

TABLE 2. Composition of Diets Used in Experiments 1 and 2.

	Diet 1	Diet 2	Diet 3
Ingredient	%	%	%
Solulac 26%	--	10 (c)	--
Oats	--	--	78.17
Ground yellow corn	73.5	67.37	--
Soybean meal 48%	14.5	10.63	9.83
Alfalfa meal 17%	2	2	2
Yellow grease	2	2	2
Limestone	5	5	5
Dicalcium phosphate	2	2	2
Trace mineral	.5 (a)	.5 (a)	.5 (a)
Vit. Premix	.5 (b)	.5 (b)	.5 (b)
D-L methionine	.1	.1	.1
Selenium, ppm	.1	.1	.1
Calculated Analysis			
Crude protein (%)	14.7	14.8	15
ME (K cal/Kg)	3299	2990	2626
Lysine (%)	.59	.55	.53
Methionine (%)	.31	.31	.33
Arginine (%)	.81	.77	.84
Tryptophan (%)	.18	.17	.17

(a), (b) See Table 1.

Diet 2 - (c) Detailed composition of solulac is given in Tables 3 and 4.

TABLE 3. Composition of Solulac Used in Diet 2 (70% Corn Distillers Solubles, 30% Distillers Dried Grain).

Composition	Percent
Protein	26
Fat	3
Fiber	8
Ash	7
ME (K cal/Kg)	2640
Productive energy (K cal/Kg)	1960
Thiamine (mgs/Kg)	5.5
Niacin (mgs/Kg)	68.97
Riboflavin (mgs/Kg)	7.4
Pantothenic acid(mgs/Kg)	11.9
Vitamin B_{12} (mcgs/Kg)	4.91
Pyridoxine (mgs/Kg)	10.2
Folacin (mgs/Kg)	1.5
Choline (mgs/Kg)	4915
Biotin (mgs/Kg)	.37
Inositol (mgs/gm)	6.03
Vitamin E (IU/Kg)	74.8
Minerals	
Calcium (%)	.35
Phosphorus (%)	.88
Potassium (%)	1.12
Magnesium (%)	.37
Sodium (%)	.05
Lactic acid (%)	4
Total Digestible Nutrients (%)	80
Digestible protein (%)	22

TABLE 4. Amino Acid Composition of Solulac Used in Diet 2.

Composition	Percent
Arginine	1.04
Lysine	.80
Methionine	.50
Methionine and cystine	1
Tryptophan	.25
Histidine	.53
Leucine	2.88
Isoleucine	1
Phenylalanine	1.21
Phenylalanine and tyrosine	2.15
Threonine	.91
Valine	1.28
Glycine	.98

Experiment 2

Fifty percent of the birds from each diet were selected at random at the end of Experiment 1 and force-fed the same diets for three weeks at 120% of the normal intake. The remaining 50% of the birds were fed ad libitum as before for the same period. For force-feeding, a special preparation of the feed was made. The feeds were first ground with a Wiley mill. They were then blended for two minutes in a blender with 1.5 parts water to one of diet (volume to weight) to a consistency of "cake batter." The proportions of water were adjusted slightly as needed to produce the consistency desired. This blended mixture was forced directly into the crop by means of a 60 cc polypropylene syringe and a "tygon tube" (1 cm diameter). Force-feeding was conducted at intervals of approximately 12 hours. Each time about 150 cc of the blended mixture was fed to each bird on diets 1 and 2 and 180 cc of diet 3. Diet 3 with oats had been blended with a little more water. At the end of the experiment the hens were sacrificed by cervical dislocation and the livers collected, weighed, scored for hemorrhage and analyzed microscopically and for lipids. Scoring was done by visual observation with 1 = no hemorrhage points, 2 = 1-10 hemorrhages, 3 = 10-25 hemorrhages and 4 = more than 25 hemorrhages.

After individual livers were scored and weighed, two samples from each liver were preserved for histological examination. Three samples from each liver (approximately 2 gms apiece) were randomly selected and analyzed for lipid content. The samples were dried in an oven at 100°C for 20 hours and then refluxed with ethyl ether in a

Soxhlet extraction apparatus for 16 hours. The data for production and liver parameters were analyzed statistically by the least squares analysis of variance (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Experiment 1

Average data for production, feed consumption, egg weight, body weight, feed efficiency and analysis of variance are shown in Tables 5 and 6. Birds maintained on diet 3, in which oats and soy were used as the chief energy and protein sources, showed significantly reduced weight gain as compared to the controls. However, considering the data on egg production, there was no significant difference in performance of hens that were fed the three diets. Neither was there a difference in feed efficiency. Though not significant, more of diet 2 with Solulac was consumed than the other diets which may have been due to the attractive flavor of the fermentation by-product. No significant difference was noted in egg weight resulting from use of the three diets. The data show that the fermentation by-product of 70% corn distillers solubles with 30% distillers dried grains can be used satisfactorily in a poultry ration, which agrees with the suggestion of Harms et al. (1969). However, Jensen et al. (1974a) found that the addition of 5% distillers dried grain to a wheat-based ration increased production, whereas the addition of 10% distillers dried grains reduced egg production.

Experiment 2

Average data for liver weight, liver score, total liver lipid, liver lipid percent and analysis of variance are given in Tables 7 and 8, and production parameters and the analysis of variance in Tables 9 and 10. Birds on diet 3 with oats as compared to the birds with corn showed a significant reduction in liver weight and liver

TABLE 5. Effect of Feeds on Production Parameters in Experiment 1 (a).

Treatment	Hen-day production	Hen-day feed consumption	Egg weight	Haugh unit	Body weight	Kg of feed per dozen of eggs
	%	gm	gm		Kg	Kg
Diet 1 (b) corn-soy	80.6	124.5	66.0	81.5	1.93	1.87
Diet 2 (b) solulac (10%)	77.6	129.9	65.3	82.6	1.84	2.05
Diet 3 (b) oat-soy	72.3	127.1	67.2	79.4	1.77*	2.18

(a) Average for 7, 28-day periods.

(b) See Table 2 for diet compositions.

* Significant at the ($P<0.05$) level from the corresponding control - Dunnett's t was used to test for difference from diet 1.

TABLE 6. Analysis of Variance for Production Parameters in Experiment 1.

Source of variation	d.f.	Mean-Squares					
		Hen-day production	Hen-day feed consumption	Egg weight	Haugh unit	Body weight	Kg of feed per dozen eggs
Replicates	8	749.8	985.1	190.3	119.8	0.31	1.2
Level	1	406.3	5677.8	224.8	2.7	0.02	0.5
RxL	8	513.5	528.8	91.9	362.9	0.33	1.5
Treatment	2	2204.9	914.5	118.8	340.1	0.83	2.9
RxT	16	755.8	794.4	140.9	77.2	0.20*	1.2
LxT	2	10.4	153.7	32.1	390.1	0.16	0.04
RxLxT	16	266.7	616.6	90.8	179.2	0.25	0.5
Month	6	1386.6	15927.5	24.4	264.9	0.07	3.2
RxM	48	164.3	115.9	7.5	21.7	0.01	0.4
LxM	6	46.2	924.8	7.4	143.6	0.01	0.4
RxLxM	48	178.3	176.6	7.4	24.1	0.01	0.4
TxM	12	373.6	1574.5	4.6	18.6	0.01	0.4
RxTxM	96	141.8	187.4	5.9	21.4	0.01	0.4
LxTxM	12	139.1	147.1	5.1	22.3	0.01	0.2
RxLxTxM	96	112.2	221.4	5.8	20.9	0.01	0.4

* Significant at the ($P<0.05$) level.

TABLE 7. Effect of Feeds on Liver Parameters in Experiment 2 (a).

Diet	Liver weight	Liver score	Total liver lipid	Liver lipid (wet basis)	Liver as part of body weight
	gm		gm	%	%
Diet 1 corn-soy (b)	53.9	1.7	13.57	22.6	2.5
Diet 2 solulac(10%)(b)	51.9	1.7	10.48	18.3	2.6
Diet 3 oats-soy (b)	40.7*	1.05*	2.94**	6.4**	2.2
Ad lib	41.5	1.2	4.76	10.7	2.2
Force-feeding (c)	56.2**	1.8**	13.23**	20.8**	2.7*

(a) 21 days.

(b) See Table 2 for diet composition.

(c) Force-feeding at the rate of 120% of normal intake.

* Significant at ($P<0.05$) level from the corresponding control.

** Significant at ($P<0.01$) level from the corresponding control - Dunnett's t was used to test for difference from the corresponding control.

TABLE 8. Analysis of Variance for Liver Parameters in Experiment 2

Source of variation	d.f.	Mean Squares				
		Liver weight	Liver score	Total liver lipid	Liver lipid (wet basis)	Liver as part of body
Rep	8	138.36	.1579	46.076	91.48	.326
Diet	2	943.58	2.7384	546.9789	1024.507	1.2922
Diet x Rep	16	87.43*	.2873*	52.1038**	84.209**	.2208
TRT	1	2633.17	4.12009	962.261	1141.39	2.6208
TRT x Rep	8	159.31**	.1996**	22.789**	67.08**	.2367*
Diet x TRT	2	143.2690	1.14583	106.6085	194.3321	.5247
Residual	53	189.5141	.3527	64.9263	99.0172	.3900

* Significant at ($P<0.05$) level.

** Significant at ($P<0.01$) level.

TABLE 9. Effect of Feeds on Production Parameters in Experiment 2 (a).

Diets	Hen-day production	Hen-day feed consumption	Final body weight	Egg weight
	%	gm	Kg	gm
Diet 1 corn-soy (b)	50.3	124.6	2.10	66.0
Diet 2 solulac 10% (c)	59.5	129.0	1.99	65.0
Diet 3 oats-soy (d)	68.8*	145.0*	1.91	68.3
Ad libitum	66.5	104.0	1.88	66.1
Force-feeding (e)	52.6**	161.8**	2.12**	66.8

(a) 21 days.

(b) See Table 2 for composition of diet.

(c) See Table 2 for composition of diet.

(d) See Table 2 for composition of diet.

(e) Force-feeding at the rate of 120% of normal intake.

* Significant at ($P<0.05$) level from the corresponding control.

** Significant at ($P<0.01$) level from the corresponding control.

TABLE 10. Analysis of Variance for Production Parameters in Experiment 2.

Source of variation	d.f.	Mean Squares			
		Hen-day production	Feed consumption	Final body weight	Egg Weight
Rep	8	726.58	.00132	.0029	34.8066
Diet	2	1515.717	.00897	.1769	57.738
Diet x Rep	16	342.777*	.00081*	.0364	36.101
TRT	1	4152.679	.18381	.6946	9.001
TRT x Rep	8	468.099**	.00224**	.0554**	21.681
Diet x TRT	2	1982.9368	.0012	.0124	48.961
Residual	53	389.6532	.0014	.0463	24.5391

* Significant at ($P<0.05$) level.

** Significant at ($P<0.01$) level.

score ($P<0.05$). The difference in total liver lipid and liver lipid percent was highly significant ($P<0.01$), which may be due in part to the low-energy content of oats. Further parameters from hens on the oats diet showed decreased liver scores with concurrently less hemorrhage, which agrees with the findings of Nelson (1978).

Hens fed the fermentation by-product did not show any significant difference in liver parameters, which does not agree with Jensen (1979), who showed that the inclusion of distillers dried grain in the layer ration would reduce liver fat content. However, in observing individual data, there was a trend for comparatively lower liver fat content, liver weight, and liver lipid percent for the birds maintained on diet 2. Force-feeding at the rate of 120% of the normal intake again significantly ($P<0.01$) increased liver weight, liver score, liver lipid content and liver lipid percent. This agrees with the basic concept that FLHS is a consequence of laying hens generally overeating (Polin and Wolford, 1976). The force-fed birds had average liver lipid contents three times that of birds fed on the ad libitum basis. The high liver fat content corresponded with the higher incidence of hemorrhages as evidenced by liver score ($P<0.01$).

There were some interactions between feed and feeding regime in Experiment 2, which is shown in Tables 11, 12, 13 and 14. When the data between the two types of feeding regime and three feeds were compared, it was only during force-feeding that there were some significant differences. Livers from birds on diet 3 with oats showed

TABLE 11. Effect of Feeds and Feeding Regime on Liver Parameters in Experiment 2.

Diet	Treatment	Liver weight	Liver score	Total liver lipid	Liver lipid (wet basis)	Liver as part of body weight
		gm		gm	%	%
1 (a)	1 Ad lib	46.4	1.4	8.5	17.2	2.35
2 (a)	1 Ad lib	42.1	1.2	4.5	11.4	2.20
3 (a)	1 Ad lib	36	1.0	1.3	3.5	2.07
1 (a)	2 Force-fed	61.5	2.0	18.6	28.0	2.75
2 (a)	2 Force-fed	61.6	2.2	16.4	25.1	2.98
3 (a)	2 Force-fed	45.4	1.1*	4.6**	9.4**	2.20

(a) See Table 2 for composition of diet.

* Significant at ($P<0.05$) level from the corresponding control.

** Significant at ($P<0.01$) level from the corresponding control - Dunnett's t was used to test the difference from control.

TABLE 12. Analysis of Variance for Liver Parameters in Experiment 2.

Sources of Variation	d.f.	Mean Square				
		Liver weight	Liver score	Total liver lipid	Liver lipid (wet basis)	Liver as part of body weight
Diet	2	943.58	2.7384	546.9789	1024.507	1.2922
TRT	1	2633.17	4.12009*	962.261**	1141.39**	2.6208
Diet x TRT	2	143.2690	1.14583	106.6085	194.3321	.5247
Residual	53	189.5141	.3527	64.9263	99.0172	.3900

* Significant at ($P<0.05$) level.

** Significant at ($P<0.01$) level.

TABLE 13. Effect of Feeds and Feeding Regime on Production Parameters in Experiment 2.

Diet	Treatment	Hen-day production	Hen-day feed consumption	Final body weight	Egg weight
		%	gm	Kg	gm
1 (a)	1 Ad lib	66.7	98.6	1.96	65.7
2 (a)	1 Ad lib	62.4	99.5	1.89	66.5
3 (a)	1 Ad lib	70.4	113.8	1.78	66.1
1 (a)	2 Force-fed	33.9	150.6	2.24	66.3
2 (a)	2 Force-fed	56.7*	158.6	2.08	63.4
3 (a)	2 Force-fed	67.2**	176.2*	2.04	70.6

(a) See Table 2 for composition of diet.

* Significant at ($P<0.05$) level from the corresponding control.

** Significant at ($P<0.01$) level from the corresponding control - Dunnett's t was used to test the difference from control.

TABLE 14. Analysis of Variance for Production Parameters in Experiment 2.

Sources of Variance	d.f.	Mean Squares			
		Hen-day production	Hen-day feed consumption	Final body weight	Egg weight
Diet	2	1515.717	.000897	.1769	57.738
TRT	1	4152.679**	.18381*	.6946	9.001
Diet x TRT	2	1982.9368	.0012	.0124	48.961
Residual	53	389.6532	.0014	.0463	24.5391

* Significant at ($P<0.05$) level.

** Significant at ($P<0.01$) level.

hemorrhage to be significantly lower than the control ($P<0.05$). Total liver lipid and liver lipid percent were markedly reduced ($P<0.01$), and liver weight was less at the $P<0.06$ level of significance. Birds maintained on diet 3 with oats showed significantly higher production and feed consumption than the control at the $P<0.01$ and $P<0.05$ levels, respectively. Birds fed diet 2 showed significantly higher egg production than the control ($P<0.05$), but no other difference was evident either in production or in liver parameters. In observing individual data, 75% of the force-fed birds on diet 1, 90% on diet 2 and only 11% on diet 3 showed liver hemorrhages. This also reinforces the observation that the distillers dried grain which was used in diet 2 had no beneficial effect on reducing liver hemorrhages as suggested by Jensen et al. (1979). Rather, it is evident that fewer birds on the oats diet showed liver hemorrhages, with or without force-feeding.

In the second experiment, birds on the oats diet showed significantly higher hen-day production and feed consumption. There was no significant difference in body weight or egg weight, although birds on the corn diet appeared to be heavier. The higher production rate of hens on diet 3 may have been due to less incidence of FLHS in those birds. Force-feeding decreased egg production but increased body weight significantly ($P<0.01$). The decrease in egg production in Experiment 2 may have been due to the stress of force-feeding and artificially producing the disease in most of the birds. Hens with a liver score of 1 showed 11.11% liver lipid content on an average, whereas birds with liver scores of 2 and 3 contained 22.65% and 29.1% liver lipids,

respectively. From these values it can be predicted that a higher lipid content of the liver may be a predisposing factor for hemorrhages. There were, however, individual exceptions in that a bird having 33.5% liver lipid content showed no hemorrhage, whereas a bird with 25.57% liver fat showed many hemorrhages.

Mortality

Only limited mortality was observed in the experiments. The causes of mortality encountered during both experiments are shown in Table 15. Out of a total of four deaths during both experiments, three were due to FLHS during the ad libitum feeding regime.

TABLE 15. Causes of Mortality in Experiments 1 and 2 (a).

Experiment number	Disease	Number of birds	Maintained on diet
1	Fatty Liver Hemorrhagic Syndrome	2	3
1	Fatty Liver Hemorrhagic Syndrome	1	1
2	Asphyxia	1	1

(a) Diagnosis made by South Dakota State University Diagnostic Laboratory.

Histological Findings

Livers, sampled at the end of the experiments, were used for histological examination to see if any difference existed between normal and fatty livers. Figure 1 shows livers No. 25 and 23 from birds fed diets 2 and 3 and containing 40.8 and 0.68 gms of lipids/liver, respectively. Figure 2 shows liver No. 25 from a bird on diet 2, liver No. 78 from a bird on diet 1, and liver No. 31 from a bird on diet 2 each weighing 94, 32 and 68 gms, respectively. In Figure 2 the difference in color between liver No. 78 containing 2.76 gms. of fat and liver No. 25 containing 40.8 gms of fat can be readily observed. Figure 3 shows livers No. 50 and 23 from birds on diet 3, weighing 37 and 34 gms, respectively. The birds were in good laying condition, i. e., 81% and 71%, respectively. Figure 4 shows liver No. 33 weighing 78 gms. The bird was on diet 1 and had shown 61% production. The difference of color between Figures 3 and 4 is quite marked. Figure 5 is a close-up picture showing multiple hemorrhages in liver No. 31.

Figures 6 through 10 show sections of livers stained with hematoxylin and eosin containing 43.42, 30.73, 23.16 and 12.99 and 3.07% of lipids, respectively. Crowding of the nucleus in Figure 6 in which fat content is highest can be compared with Figure 10, where the cells appear to be normal with a cord-like appearance. Figures 11 through 13 show liver sections stained with oil red O. These livers contained 43.42, 30.73 and 3.07% of lipids, respectively. Figure 11 shows both intra- and extra-cellular fat infiltration due to excess fat content

Fig. 1. Livers from hens on diets 2 and 3. Number 25 is from a bird which was force-fed and contained 40.8 gms of fat, whereas Number 23 is from a bird which was fed on an ad libitum basis and contained .68 gms of fat. The dark color of Number 23 is due to the lower fat content.

Fig. 2. Livers from hens on diet 2, 1 and 2. Livers Number 25 and 31 are from force-fed hens, whereas liver Number 78 is from a hen fed on an ad libitum basis. The livers contained 40.8, 2.76 and 20.9 gms of lipids, respectively. The difference of color between livers Number 25 and 78 is quite marked.

Fig. 3. Normal livers from hens on diet 3. Liver Number 50 contained 1.56 gms and Number 23 contained .68 gms of fat. The birds were in good laying condition.

Fig. 4. Liver Number 33 is from a force-fed hen. The hen was in good laying condition and contained 29.7 gms of lipids.

Fig. 5. A close-up view of the multiple hemorrhages of liver Number 31.

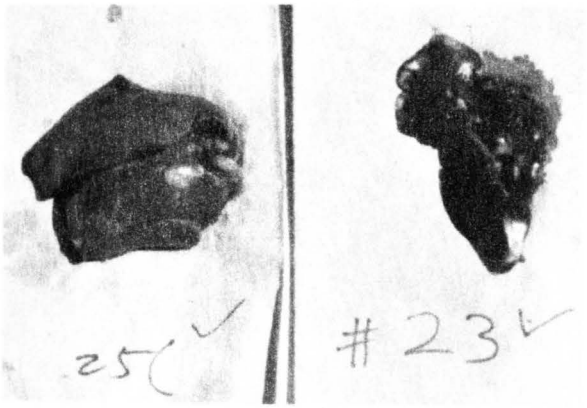


FIG. 1

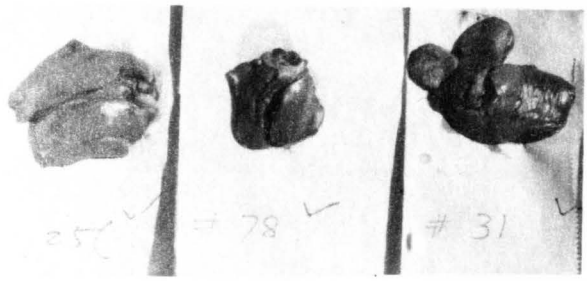


FIG. 2

FIG. 3

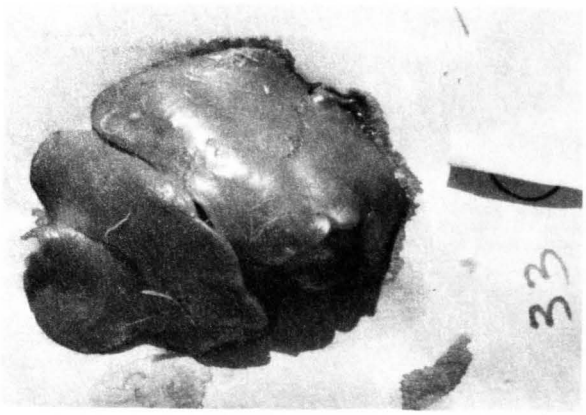


FIG. 4

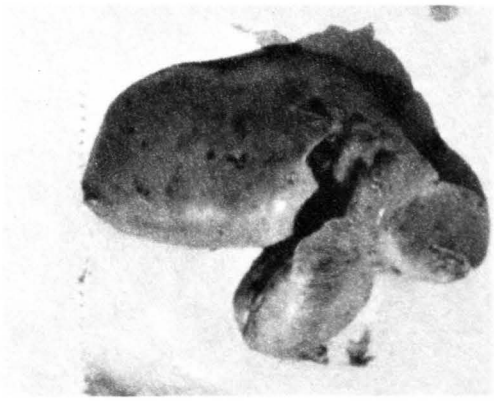


FIG. 5

Fig. 6-10. Photomicrographs of hen livers mounted in paraffin and stained with hematoxylin and eosin. The livers contained 43.42, 30.73, 23.16, 12.99 and 3.07% of lipids, respectively (wet basis). The crowding of the nuclei in Fig. 6 can be compared with the chord line arrangements and prominent sinusoid space in Fig. 10 (x800).

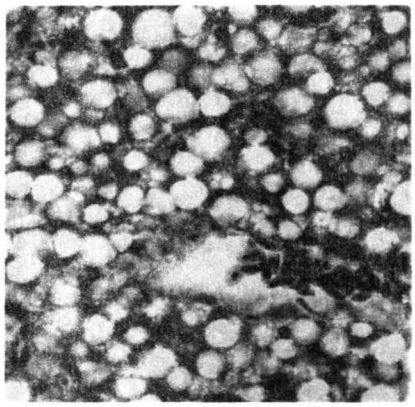


FIG. 6

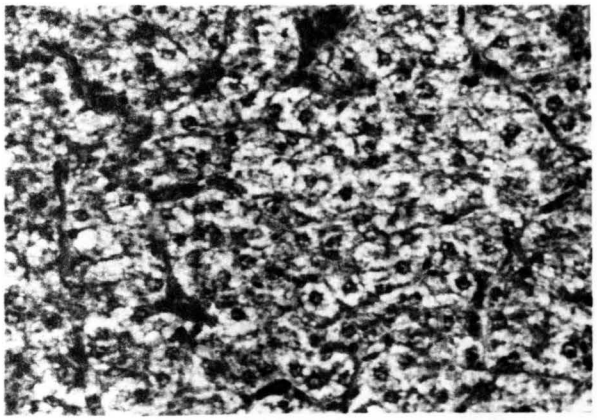


FIG. 7

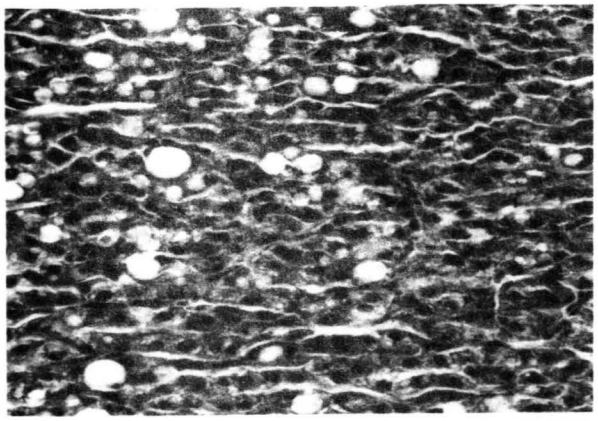


FIG. 8

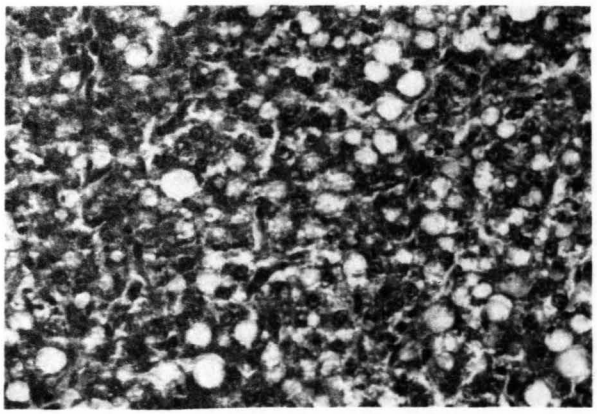


FIG. 9

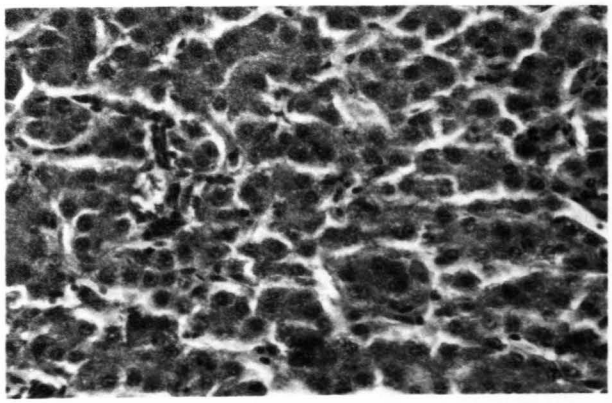


FIG. 10

Fig. 11-13. Photomicrographs of hen livers that were stained with oil red O. The livers contained 40.42, 30.73 and 3.07% of lipids, respectively (wet basis). The excessive infiltration, both intra- and extra-cellular of Fig. 11 can be compared with lesser fat infiltration in Fig. 13 (x800).

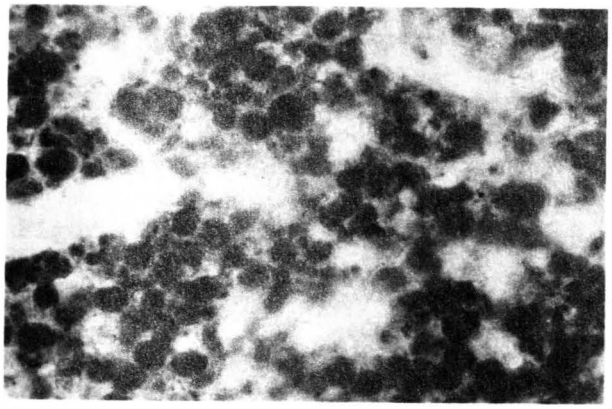


FIG. 11

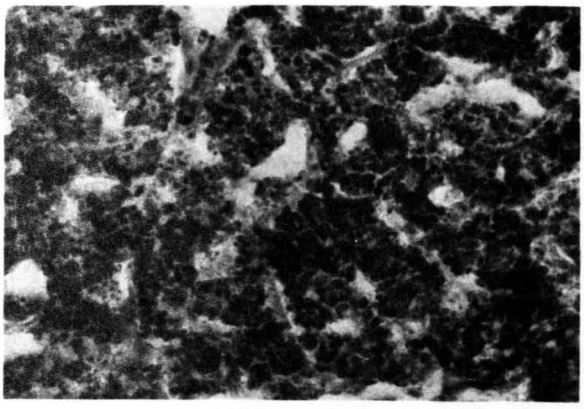


FIG. 12

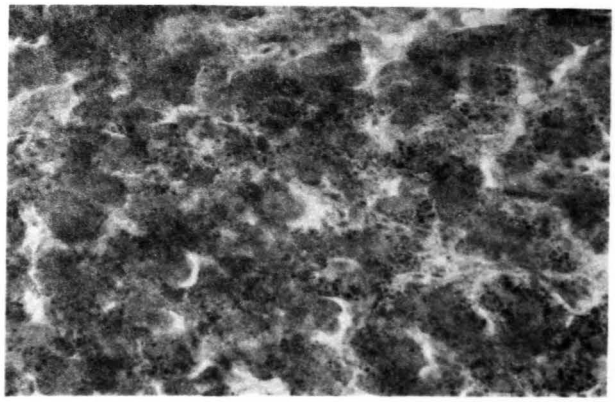


FIG. 13

while Figure 13 showed the liver to contain quite close to normal lipids with only intracellular fat infiltration. All the pictures are of x800 magnification.

Figure 14 shows an electron micrograph of a liver of magnification x12,000 containing 8.6% lipids, whereas Figures 15 and 16 are from fatty livers containing 46.9 and 43.4% fat content, respectively. In Figure 14 the limited fat contents are evident as compared with that shown by Figures 15 and 16. In Figures 15 and 16, the fat droplets are quite large and occupy the major portion of the cytoplasm. In Figure 15, it can be noted that the excess fat infiltration caused the displacement of the nucleus to one side from its normal and almost central location. The mitochondria in the fatty liver cells shown in Figure 15 appear to be swollen. However, apart from the greater fat infiltration and larger fat droplets, these photomicrographs do not show any other evident difference. Figures 15 and 16 are of magnifications x7,500 and x 16,800, respectively.

Fig. 14. Electron micrograph of a normal hen liver analyzed and shown to contain 8.6% lipids. The relative size of the lipid droplet can be compared with the size of the nucleus (x12,000).

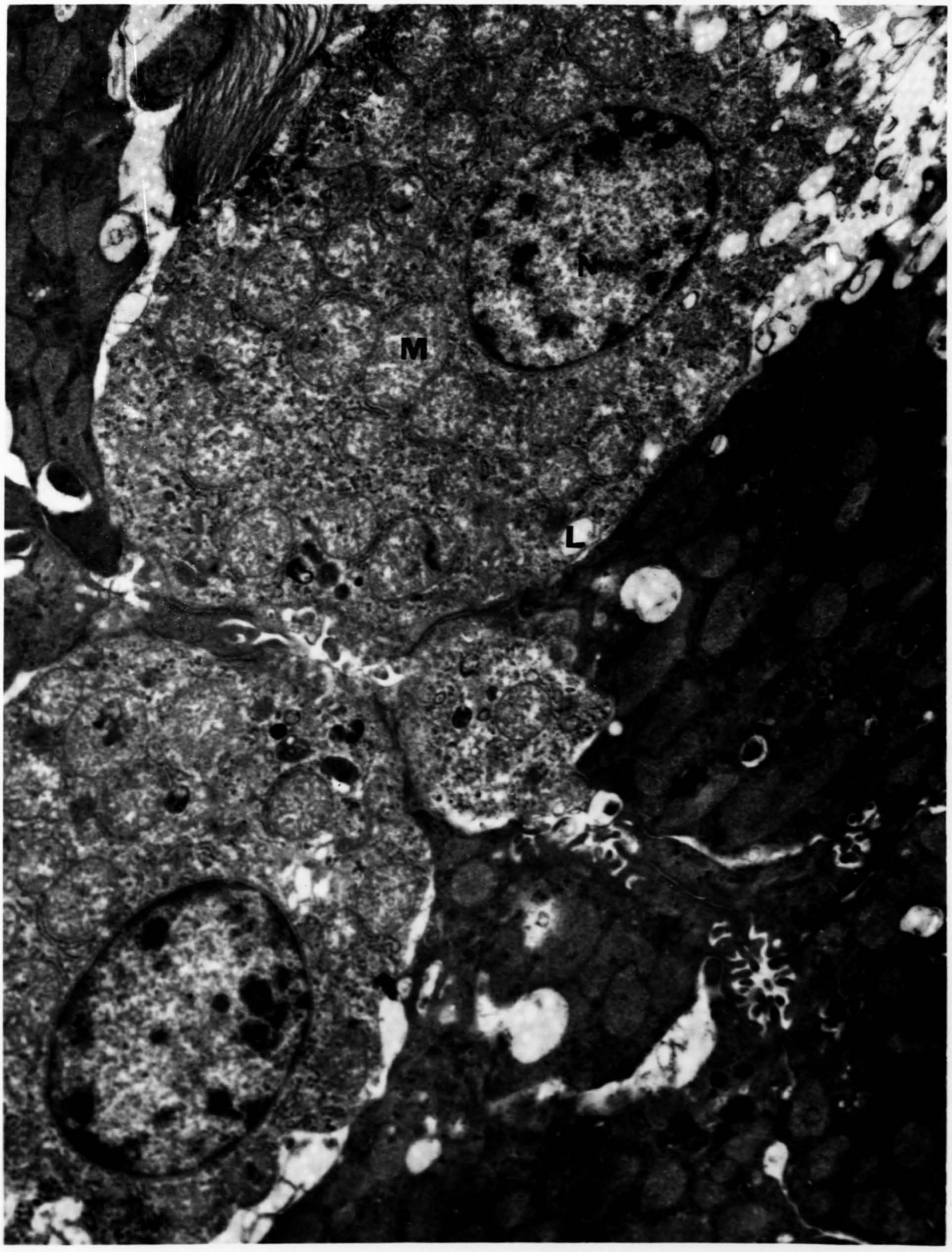
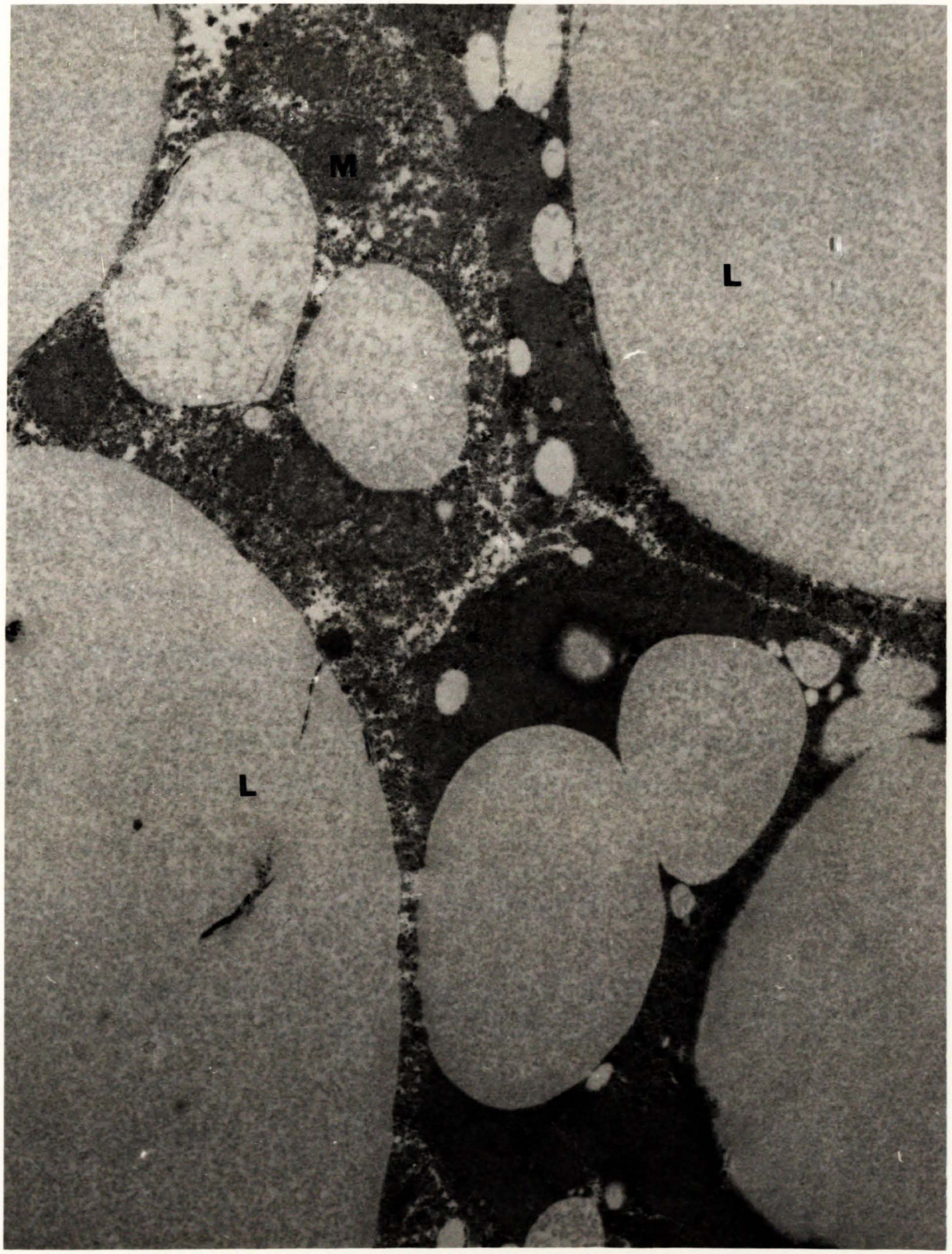

N
M
L

Fig. 15. Electron micrograph of hen liver affected with FLHS. The liver contained 46.9% lipids. The excess infiltration of lipids has caused the nucleus to be displaced to one side. The mitochondria seem to be swollen (x7,500).


L
L
N
M
N
L
L

Fig. 16. Electron micrograph of the fatty liver of a hen. The liver contained 43.4% of lipids. Lipids have occupied a major portion of the cytoplasm (x16,800).


M
L
L

SUMMARY

In this study two experiments were conducted with sixty laying hens to measure the effect of a corn-soybean diet, a diet containing 10% distillers by-products and an oats-soybean diet on production and FLHS. In Experiment one, the three diets were used with ad libitum feeding, and in Experiment 2, 50% of the birds on each diet were force-fed at 120% of the ad libitum intake of that diet to measure the effect of superimposing FLHS on the treatment. The conclusions are:

1. There was no significant difference in egg production among hens on the three diets fed ad libitum.
2. The oats-soybean meal diet reduced body weight significantly ($P<0.05$), due to its lower energy content, but it had no effect upon egg production.
3. The oats-soybean meal diet reduced the liver weight and liver score at the ($P<0.05$) level of significance and total liver lipid and liver lipid percent at the ($P<0.01$) level of significance, which confirms previous work showing that oats may contain some factor protective against the disease.
4. The corn distiller's fermentation by-product had no effect on performance or FLHS when fed at 10% of the diet for laying hens.
5. Force-feeding at 120% of the normal intake was effective

in producing FLHS. This technique resulted in significantly higher liver lipid parameters, which suggested that higher calorie consumption may be one of the causes of FLHS.

6. With but few exceptions, the liver scores were in agreement with the lipid content of the livers and with the appearance of photomicrographs. This suggests that the accumulation of fat may cause pressure and tension or fragility which results in rupture of the blood vessels.

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