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Nutritional Factors Modifying Fatty Liver Hemorrhagic Syndrome in Caged Laying Hens

Chandi C. Rakshit

NUTRITIONAL FACTORS MODIFYING FATTY LIVER HEMORRHAGIC SYNDROME IN CAGED LAYING HENS

By

Chandi C. Rakshit

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, 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.

C. W. Carlson — Date
Thesis Advisor

John Romans — Date
Head, Animal and Range Sciences Department

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ABSTRACT

Chandi C. Rakshit

Under the guidance of Professor C. W. Carlson

Three experiments each with two phases were conducted to study the effect of different levels of distillers dried grain (DDG), fat, oats and a mixture of oats and DDG on Fatty Liver Hemorrhagic Syndrome (FLHS) in caged laying hens. The second phase of each experiment was conducted by force-feeding the same diets used in the first phase at 120% of their normal intakes. This produces FLHS and thereby permits studying the effect of diets. During the force-feeding period of the third experiment, serum estradiol levels of hens from each treatment and each type of feeding were measured to study the effect of endogenous estradiol level on FLHS.

The 30% DDG in the diet was more effective against FLHS than when present at the 20% level. Addition of 5 to 10% grease in the diet decreased feed consumption significantly ($P < 0.05$). Only oats or the mixture of oats and DDG in the diet reduced FLHS significantly as compared to when DDG alone was included in the diet or the regular corn-soy ration was used.

The serum estradiol level and liver hemorrhage incidences of hens on the DDG diet were significantly ($P < 0.05$) higher suggesting that endogenous estradiol level is also involved in FLHS.

Depending on the amount of fat present in the liver varying sizes of fat droplets inside the liver cells were revealed by photomicrographs. In some instances the larger lipid droplets made for some morphological changes inside the cell, obviously due to pressure, which could also be one of the causes of hemorrhage.

Force-feeding at 120% of the normal intake has continued to be effective in producing FLHS.

INTRODUCTION

The main site for lipid synthesis in birds is the liver (Leveille et al. 1975). In addition to other factors, synthesis and degradation of lipids are dependent upon energy balance of the body. In normal conditions this dynamic process is very routine. However if the lipid synthesis is in great excess or its degradation and mobilization from liver is slowed down, lipid droplets accumulate in the liver cells in varying degrees. The accumulation of liver lipid is mainly due to fatty acid synthesis (Ivy and Nesheim, 1973). They observed that as liver lipid increased, oleic acid content was higher than linoleic acid which was dietary in origin. Extreme cases of lipid accumulation in the liver predisposes for multiple hemorrhages leading even to death. This was first identified by Couch, (1956). He reported it as Fatty Liver Syndrome which was afterwards called Fatty Liver Hemorrhagic Syndrome (FLHS) by Wolford and Polin, (1972a). Since then the later name has been accepted and followed. In this present thesis the condition also will be refered to as Fatty Liver Hemorrhagic Syndrome (FLHS).

In this disease, affected birds usually do not show any external symptoms like going off feed or ceasing production. This makes it difficult to diagnose in vivo.

Though several research studies have been conducted during the last three decades to elucidate the etilogical agent, nothing definite has been demonstrated. Many of the earlier studies suggested that

there was no clear cut nutritional influence on the level of fat obtained (Price et al. 1957; Weiss and Fisher, 1961; McDaniel et al. 1959). However later studies have shown that by manipulating dietary ingredients, certain dietary components contain unknown factors that lessen the disease. Among these are fish meal, distiller dried grain (DDG), oats, choline, methionine, inositol, vitamin B_{12} etc. (Maurice et al. 1979; Jensen et al. 1974; Nelson, 1978; Rakshit, 1981; Griffith et al. 1969; Roberson et al. 1970; Schexnailder and Griffith, 1973; Akiba et al. 1983). On the other hand various studies showed that a regular corn-soy type of ration could cause a higher incidence of the problem than other diets when compared isocalorically. Canola meal and rape seed meal when included in the layer ration may result in a similar problem (Jensen et al. 1976; Maurice et al. 1979; Grandhi et al. 1977). Other factors such as temperature, environment, strain, sex, age, exercise, toxins and hormones etc., have been studied with variable results (Lee et al. 1975; Pearce, 1971; Balnave, 1969; Polin and Hossein, 1982; Jensen et al. 1976; Nelson, 1978; Hartfiel et al. 1970; Smith and Hamilton, 1970).

Three different experiments were conducted each with two phases to study the effects of different levels of fat, DDG, oats and the combination of oats and DDG on FLHS. During the second phase of each experiment force-feeding at 120% of the normal intake was incorporated to induce FLHS artificially and thereby study the influence of nutritional factors on FLHS. In the second phase of the last experiment blood samples were assayed to measure the endogenous

estradiol level and ascertain if there was any correlation between treatment, type of feeding, hemorrhage and endogenous estrogen level.

LITERATURE REVIEW

Since the identification of FLHS, research has been conducted on different factors affecting egg production including nutrition, environmental temperature, confinement system, age, sex, strain of birds, both exogenous and endogenous hormonal effects, effects of toxins and chemicals, and the inducing of FLHS artificially by force-feeding a higher quantity of feed. The review of literature will cover all the above areas with most emphasis on nutritional factors.

Effect of Fat

Addition of fat to a poultry ration has shown variable results on liver lipid, depending on age, breed, quality and quantity of fat, etc. The presence of fat has shown a beneficial effect on growth rate and feed efficiency (Sunde, 1956; Waibel, 1955). Cullen and Marion *et al.* (1962) observed a similar effect from tallow fatty acids. In addition to growth increases, fat at 12% of the diet with 21 and 24% protein produced a reduced liver lipid content (Akinwande, 1981). As reviewed by Pearce (1968) dietary fat is part of a homeostatic control mechanism to prevent over-production of fat. Goodridge (1968) studied lipogenesis in both chick embryos and growing chicks and suggested that the rate of fatty acid synthesis is very low in chick embryos. A high fat and low carbohydrate diet suppressed fatty acid synthesis. Age has some effect on fat absorption as observed by Polin and Hussein

(1982). They mentioned that the absorptive mechanism for lipids and proteins is not fully developed in the very young chicks and that dietary bile salts tend to improve lipid but not nitrogen absorption. During the first week of life, absorption is limited, but improves subsequently. Renner and Hill (1960) showed that age affects the utilization of tallow by growing chicks up to eight weeks of age after which they utilize tallow at a rate equal to adult birds. As reviewed by Carew et al. (1972) chicks were very efficient in absorbing corn oil and lard without any change between two to eight weeks of age. However, their absorption of tallow increased with age. They observed a similar effect of age on any kind of fat absorption as mentioned by Polin and Hussein (1982). Fedde, (1960) found that absorption rate of fat in diets containing 10 to 20% dietary fat were similar. However, vegetable fat and lard were absorbed at a higher rate than tallow. They also suggested that absorption of tallow increases with age. Haghighi-Rad and Polin (1981) tried to determine the effect of supplementing a corn-soy diet with starch and corn oil. Corn oil produced significantly lower liver lipid than starch. They indicated that lipid at the proper level in the diet acts through a feed back mechanism to prevent excessive hepatic lipid accumulation. Starch on the other hand enhances lipid accumulation. Bragg et al. (1973) compared the effect of animal fat and vegetable oil on the fatty liver condition and suggested that 8% dietary animal fat or rape seed oil produced fatty livers, whereas soybean or sunflower oil showed protection against fat accumulation. Addition of 8.6% corn oil, 10%

yellow grease, choline (1500 mg/kg) and biotin (1.1 mg/kg) to a 14% protein basal diet showed a reduction in liver lipid by corn oil and yellow grease only but not with choline or biotin. (Chah et al. 1975) Nelson, (1978) showed that the addition of 2 to 10% animal fat could reduce liver lipid and hemorrhages. Katongole, and March (1979) studied fatty acid binding protein in the intestine of both newly hatched chicks and adult birds. These authors suggested that fatty acid binding protein is breed dependent. New Hampshire chicks utilized fat more efficiently than broiler type or White Leghorn breeds.

Type and Source of Carbohydrate

It is well established that lipid synthesis in the liver is dependent on dietary regime of the animal (Pearce, 1968). As reviewed by him, a high carbohydrate diet increased lipogensis whereas the activity decreased when animals are starved. Jensen et al. (1976) tried to determine the influence of different sources of carbohydrate by mixing different proportions of corn and wheat. As the corn portion of the diet was increased, liver lipid content was found to be higher. They also compared other grains in their study and found that liver lipid was highest in hens fed sorghum, corn or triticale and lowest for those fed barley, oats or rye. Wheat resulted in an intermediate level of fat. When wheat was replaced by corn oil or animal fat there was no difference. When corn was partially replaced by glucose monohydrate there was no difference in liver lipid. Grains

from different geographical locations had similar effects. However, in experiments conducted at different locations hens fed similar diets had different liver lipid contents showing the existence of an unidentified environmental factor. Maurice and Jensen (1979) suggested that hepatic and plasma lipid concentrations are dependent on the amount and type of dietary carbohydrate. They (1977a) studied both in vitro and in vivo lipogenesis in Japanese quail. They used isocaloric and isonitrogenous corn-soy and wheat-soy diets. Lipogensis was greatly increased with the corn-soy diet. The lipids from livers of birds fed the corn-soy diet were significantly higher in the 14:0, 16:0, 16:1 and 18:1 fatty acids and lower in 18:0, 18:2 and 20:4 fatty acids. Their data suggest that corn-soy diets enhance lipogenesis and alter hepatic fatty acid composition. They also (1977b) studied the effect of dietary cereal source on lipid concentration with respect to time and they evaluated the effect of a water extract of wheat bran on hepatic and plasma lipid. Again the diets used were the corn-soy and wheat-soy types made isocaloric. After two weeks birds fed the corn-soy type of ration had significantly higher liver lipid content (% dry matter basis). Liver lipids of birds fed the corn-soy diet over a period of 1, 2, and 3 weeks totaled 17, 20.8 and 20.9%. Corresponding figures for birds on the wheat-soy ration were 16.5, 16.6 and 15.9% respectively. Plasma lipids (mg/dl) of birds on the corn-soy diet were 2250, 2130 and 2240 as compared to 223, 1690 and 1630 respectively for birds fed the wheat-soy diet. Replacing 10% of the cereal with a water extract of

wheat bran significantly reduced hepatic and plasma lipid. They concluded that a 14-day period is enough to demonstrate hepatic and plasma lipid concentration differences due to diet and that a water extract of wheat bran contains an antilipidic factor. Similar results were shown using wheat and oats as the primary sources of carbohydrate in that they reduced liver lipid accumulation and hemorrhage (Nelson, 1978; Rakshit 1981). These workers suggested the existence of an unknown factor in wheat or oats that prevents FLHS. To determine whether the factor is present in the bran or hull portion or in the grain itself Nelson (1978) utilized wheat bran and oat hulls and reported that the fiber portions of the grains are not the source of the factor(s) preventing FLHS.

Akiba, and Matsumoto (1978) supplemented a purified starch casein diet with cellulose in the form of powdered filter paper involving both ad libitum and force-feeding of 14-day old Single Comb White Leghorn male chicks. Their findings were that force-feeding improved growth rate and feed efficiency and that feeding cellulose did not effect body weight gain or feed efficiency. Liver weight and liver and plasma lipid content were markedly elevated by force-feeding. However, these factors were depressed by dietary cellulose in force-fed chicks. As they mentioned in their previous studies feeding of dietary fiber, cellulose, pectin and mannan depressed liver lipid accumulation. The same authors (1980) experimented with different fiber sources, i.e., cellulose A and B in the form of powdered and finely ground filter paper, rice hulls,

alfalfa meal, polyamide and peanut hulls. They observed that plasma lipid concentrations were significantly reduced by cellulose, alfalfa meal and polyamide containing diets, but not by rice hulls or peanut hulls. They suggested therefore, that certain dietary fibers are effective in reducing liver lipid content. Cherry and Jones (1982) found that supplemental fish meal and wheat bran did not affect liver lipid or liver weight but that fish meal reduced serum lipid. Cellulose at a 10% level however, reduced liver weight, serum lipid and liver lipid.

Effect of Fermentation By-Product

Successful use of distillers dried grain (DDG) in poultry rations was made as early as 1940 (Sloan, 1940). Similar results were reported by Matterson et al. (1966). Harms et al. (1969) suggested that DDG at a level of 10% can be used in the poultry diet provided the diet is formulated on the basis of amino acid content of DDG. Dried steep liquor concentrate (DSLC) is a mixture of different fractions obtained in the wet milling of corn. One of the ingredients in it is condensed fermented corn extractives. DSLC was used in poultry rations to improve egg quality (Waldroup et al. 1971; and Hazen and Waldroup, 1972). Damron et al. (1976) reported that brewers dried grain could be used to improve internal egg quality. This was accomplished by increasing protein content of the diet.

Jensen et al. (1974) conducted two experiments to determine the nutritional value of distillers dried grain with solubles (DDGS).

In one experiment, they found that adding 5% DDGS to a wheat-based ration improved egg production, but in the other experiment addition of the same level of (DDGS), either to a wheat or corn-based ration did not change production but improved egg weight. Addition at a 10% level reduced egg production as compared to the 5% level. The depressing effect of the higher level was corrected by adding 0.025% L-lysine. In both experiments the addition of DDGS tended to reduce liver fat accumulation. Maurice and Jensen, (1978) evaluated different levels of (DDG) and fat. Inclusion of the fermentation by-product reduced liver fat, liver weight, plasma lipid and cholesterol without affecting reproductive performance. In the experiment conducted during the summer, birds fed the diet without the fermentation by-product showed liver hemorrhages. DDGS at 20% of diet reduced FLHS more than when added at the 10% level. In two other trials Jensen et al. (1976a) found that 20% brewers dried grain reduced liver lipid in the summer but not in the winter. Ten percent brewers dried grain in the diet gave an intermediate result. The higher percentage of fermentation by-product reduced production significantly in winter but not in summer. Maurice and Jensen (1978a) observed that hens with equivalent metabolizable energy intake and egg production exhibit differences in liver and plasma lipid due to variations in dietary composition. They concluded that the fermentation by-product contains some essential factor for control of lipid metabolism in caged layers. Maurice and Jensen (1979) observed that a corn-torula yeast diet containing added selenium (0.1 mg/g)

with or without supplemental chromium (10 mg/g) reduced liver lipid and hemorrhage as compared to a corn-soy diet. They suggested that the hepatic lipid response to selenium resulted from an interaction with an unidentified factor in torula yeast. Inclusion of 5% brewers yeast in the corn-soy diet or vitamin E (50 IU/kg) added to a corn-torula yeast diet reduced liver lipids similar to that from corn-torula yeast and selenium. Akiba et al. (1982) observed that liver lipid content was significantly reduced in hens fed DDGS or fish meal. Thornton et al. (1962) substituted 20% brewers grain for corn, milo and sorghum meal. Body weight gain and liver fat accumulation in birds in the brewers grain diet were minimum during the last 2/3 days of the experiment. Control birds on the grain-soy diet required less energy intake per unit gain of body weight than birds in the experimental brewers grain diets. There was a high correlation between liver fat and body weight gain among experimental birds but not among control birds. The presence of an unknown factor in brewers dried grain was suggested by the authors. Keinholz et al. (1963) observed a similar effect of reducing liver lipids and body weight by using 40% brewers dried grain. Keinholz et al. (1967) mentioned improved fertility as one further result of using brewers dried grains in poultry rations. In 1972 however, the same authors noticed reduced egg size and quality which they thought was due to deterioration of the grain by storage. Rakshit (1981) did not find any significant effect of lowering liver fat or incidence of hemorrhage by using 10% (DDGS) in the layer ration.

Effect of Energy Intake and Force-feeding

Nelson's review (1978) indicated that the amount of energy consumed is a primary factor in producing FLHS. Wolford and Murphy (1972) reduced liver lipid content by feeding a low energy diet. On the other hand, the level of fat in the livers of laying hens seems to be under some metabolic control independent of energy intake (Ivy and Nesheim, 1973). They observed that within the same dietary treatment the level of fat in the liver was not always correlated with energy intake of the hens. However, it was also true in their findings that liver fat content could be markedly influenced by changing the energy content of the diet or by force-feeding. Wolford and Polin (1972a) had reported that there was a positive correlation between the amount of energy intake and liver lipid content. But, higher liver lipid content does not necessarily mean hemorrhage in the liver. However, high liver lipid content in some manner is related to greater incidence of hemorrhage. They tried to induce fatty liver by a restricted and refeeding program. They observed liver hemorrhage only in birds with high liver fat content. Wolford and Murphy (1972) suggested liver lipid content and liver size are not the only cause of hemorrhage because in their study they found that birds without hemorrhage had liver lipid equal to or higher than birds with hemorrhage. Wolford and Polin (1972b) described a technique of force-feeding in which FLHS can be induced artificially. Several others also have followed the same procedures (Ivy and Nesheim, 1973; Nelson, 1978; Rakshit, 1981; and many others). Nelson (1978) could

produce increased liver lipid and hemorrhages three to five fold by force-feeding graded levels of diet for three weeks. However the response varied between experiments indicating environmental factors also play a role in the incidence of FLHS.

Effect of Source and Amount of Protein

Most of the research on Fatty Liver Hemorrhagic Syndrome has been devoted to the effect of dietary energy. But quality and quantity of protein might be involved in producing the hemorrhagic lesions. McDaniel et al. (1957) found that dietary protein, 15 to 20% in equicaloric diets had no effect on liver lipid content. The same workers (1959) later observed higher liver lipid content in birds fed higher percentages of protein in the diet, i.e., 15, 20, and 25%. Wolford and Murphy (1972) mentioned that increasing dietary protein level did not prevent the occurence of liver hemorrhage. Wolford, (1971) however, observed that feeding iodinated casein at 0.33% of the diet reduced liver lipid significantly. Maurice et al. (1979) studied the source of protein and its effect on hepatic and plasma lipid content. They used corn-fish meal and corn-soybean meal diets. The corn-fish meal diet could reduce liver lipid, plasma lipid and hemorrhage. When selenium and other trace minerals like As, Br, Cr, F, Mo, Ni and V were added to the corn-soy diet there was no effect on liver or plasma lipids. Selenium addition to the corn-soy diet could reduce hemorrhage, however.

Effect of Hormones

Both normal endogenous production of estrogen in the hen and administration of estrogen to sexually immature fowl result in an increase in total liver lipid content and its composition is altered (Balnave, 1968; Lorenz, 1954). Balnave (1969) also mentioned that estrogen was the only hormone to significantly increase liver weight and total liver lipid content (dry matter basis). He suggested that both estrogens and androgen can influence liver lipid but not progesterone. The androgen effect was in the opposite direction as that of estrogen and when administered together with estrogen was effective only to a limited extent. The effect of estrogen in both intact and functional hepatectomized birds was studied by Ranney and Chaikoff (1951). They observed that concentration of blood lipids increased six hours after the injection of diethyl stilbesterol. No increase of liver lipid was observed in birds having functional hepatectomy. As reviewed by Takahasi et al. (1984) estrogen administration markedly enhances hepatic lipid deposition in chicks. From this study they also suggested that a range for estrogen administration exists wherein dietary effects may be expressed. Also they showed an interraction between environment, temperature, dietary composition and estrogen. Akiba et al. (1983) described a laboratory model with chicks that is suggested as an assay for dietary factors affecting liver lipid accumulation in laying hens. They injected estrogen to growing male chicks. Diets were fed ad libitum following two days starvation. Liver lipid was increased with increased levels

of estrogen. Fishmeal, alfalfa meal and torula yeast significantly reduced liver lipid of estrogenized chicks. This correlates with a similar response in laying hens. Kudzma *et al.* (1973) observed with chicks that livers from Di Ethyl Stilbestrol (DES) treated birds incorporate 4.5 times more acetate into triglyceride than livers from untreated birds. This suggests that estrogen induced hyperlipidimia is due at least in part to enhanced hepatic lipogenesis. Polin and Wolford (1977) tried to induce fatty livers in immature male and female chicks. Force-feeding at 125% and 150% of *ad libitum* intake produced higher levels of liver fat but not hemorrhage. Intramuscular injection of B-estradiol-17-dipropionate at 2 mg/kg body weight three times weekly for 21 days produced a gradient response to hemorrhage score and an increase in *ad libitum* feed intake. Testosterone dipropionate at 25 mg/kg of body weight injected three times per week in immature females force-fed at a 150% level increased liver fat without hemorrhage. Therefore, they also suggested estrogen along with positive energy balance to be factors in the production of FLHS. Pearce (1971) investigated the enzyme activity related to lipid synthesis in laying hens, non-laying pullets and cockerels. He showed that the activities of ATP citrate lyase and malic synthetase enzymes were lower in non-laying pullets and cockerels than in laying hens and that there was a parallel correlation with liver lipid content. As reviewed by Haghighi-Rad and Polin (1981) fatty livers are often observed in layers but since most non-layers do not have this problem that hormonal balance may be involved in this condition. They

examined the relationship of plasma estradiol and progesterone levels with FLHS. They used both the ad libitum type of feeding and force-feeding at 120% and 135% of the normal intake. Force-feeding for three weeks produced FLHS. The average liver lipid content was 31.3%, 75.1% and 76.8% respectively on a dry matter basis. Plasma estradiol averaged 165 pg/ml, 194 pg/ml and 245 pg/ml respectively. The differences were significant at the 1% level. There was no difference in plasma progesterone levels. They too suggested that high endogenous estrogen levels are associated with FLHS. Akiba et al. (1982) conducted experiments using distillers dried grain with solubles (DDGS) and fish meal. They maintained hens in different temperatures, 13^{o} - $24^{o}C$ and 24^{o} - $35^{o}C$. The higher temperatures reduced plasma estradiol levels. Plasma estradiol and thyroxine levels were significantly lower in birds fed DDGS or fishmeal. There were positive correlations between liver lipid content and plasma thyroxine and between liver lipid and estradiol level. They concluded that plasma estradiol and thyroxine levels are influenced by diet composition and that these hormones have a strong relationship in inducing fatty livers in laying hens. Maurice et al. (1979) also suggested a similar relationship between liver lipid and estrogen level. In addition to the gonadotropic hormones, thyroxine and glucagon also effect fatty acid synthesis. Insulin appears to play a lesser role (Leveille et al. 1975).

Effect of Minerals Vitamins and Lipotropic Agents

Since lipid metabolism is enzyme-related, several of the minerals, vitamins and lipotropic agents have been studied by various workers to find out if there is any effect of these in FLHS. Abbott and Demasters (1940) mentioned that a low choline diet can reduce egg production and increase liver fat. Jensen et al. (1953) studied the effects of selenium, choline, inositol, vitamin B_{12} and vitamin E on liver fat accumulation. Selenium at 1 ppm in the corn-soy diet with or without fat could reduce liver fat. Addition of vitamins E and B_{12} and inositol to the corn-soy diet without fat reduced liver fat but not when fat was added to the diet. A wheat-peas-fat basal diet with 0.5 ppm selenium showed low liver fat levels. Griffith et al. (1969) studied the effect of choline methionine and vitamin B_{12} and observed that all three could increase egg production but only choline supplementation reduced liver fat. Schexnailder and Griffith (1973) concluded after that choline and/or vitamin B_{12} supplementation improved production and reduced liver lipid content. Supplementing the diets with inositol, riboflavin, pyridoxine, calcium pantothenate, vitamin E, folic acid, biotin or Terramycin had no effects on liver lipid. Quisenberry et al. (1967) could not demonstrate an effect from supplementing a layer diet with choline, vitamin E, vitamin B_{12} or Protamone on FLHS. Wolford and Murphy (1972) reported that incorporation of lipotropic vitamins (B_{12}, E, choline and inositol) into the diet of laying hens did not reduce liver lipid content. Nelson and Carlson (1978) conducted two

experiments. In the first one they compared a corn-soy diet with or without choline and a methionine supplement. They used both ad libitum and force-feeding methods. In the second experiment both corn-soy and oat-soy diets with and without choline and methionine supplementation were compared. In the first experiment, choline reduced liver lipid by 30% to 40% in birds fed ad libitum but not in force-fed birds. Methionine had no effect. In the second experiment birds fed the oat-soy diet ad libitum showed reduced egg production which was corrected during force-feeding. None of the birds fed the oats-soy diet had FLHS, irrespective of feeding method.

ENVIRONMENTAL FACTORS

Effect of Confinement System

Factors other than dietary are also involved in Fatty Liver Hemorrhagic Syndrome. A few among them are temperature, confinement system, exercise, etc. (FLHS) is mostly observed in cage layers. Price et al. (1957), suggested that development of fatty livers in birds may be either due to some defficiency in cage layer diets or to restriction of movement in cages. Garlich et al. (1974) observed that birds maintained two per cage had more liver lipid than birds maintained seven per cage or in floor pens. Similar results of confinement, i.e., cages producing more liver lipid than floor pens were observed by Griffith et al. (1969) and McDaniel et al. (1957). Lack of exercise in the caged birds was proposed by Hartfiel et al. (1970) as the cause of higher liver lipid levels. Cage density is often increased by commercial producers to reduce housing costs. As reviewed by Jensen et al. (1976b), Wilson et al. (1967); Grover et al. (1972) and Foss and Carew (1974) observed that body weight gain was reduced when cage density was increased. On the other hand Tower et al. (1967) and Loe and Heywang (1964) found that body weight gain was greater in multiple cages. Jensen et al. (1976b) observed that liver weight was greater for hens housed individually than for those housed three per cage.

Effect of Temperature

A relationship between environmental temperature and liver lipid content was observed by Griffith et al. (1969). Wolford (1971) studied the effect of temperature and iodinated casein on FLHS. Placing hens in a 1.7°C environment for 28 days reduced liver lipid significantly in comparison to the liver lipid content of hens maintained at 26.7°C. However Lee et al. (1975) reported that changing temperature from 22.2°C to 30.6°C or from 30.6°C to 22.2°C did not change liver fat level significantly. They observed the greatest incidence of fatty livers in birds on a restricted feed intake and exposed to a temperature of 30.6°C.

Effect of Toxins and Chemicals

There are certain natural compounds such as aflatoxins produced by fungi which may contaminate poultry diets and cause damage to internal organs when ingested. Smith and Hamilton (1970) studied the effect of aflatoxin in broiler rations. They fed graded levels of the toxin and observed decreased growth rate and enlarged livers, spleens and pancreases but regressed bursae of fabricus. Lipids often accounted for about 60% of the dry weight of affected livers. There was a linear correlation between dose levels and their effects on body and organ weights. Hamilton and Garlich (1971) studied Fatty Liver Syndrome as induced by dietary inclusion of aflatoxin. Aflatoxin reduced egg production and liver weights were increased but there was

no effects on spleen and pancreas. A graded increase in liver lipid content was revealed with increased levels of the toxins.

Detrimental effects from rape seed meal on chick growth due to the presence of toxic material have been cited (MacGregor and Blakely, 1964). In their study with adult birds however, they did not show any deleterious effect of substituting rape seed meal for soybean meal on egg production, egg size, body weight or fertility of hatching eggs. Summers et al. (1971) reported reduced production and egg size from the addition of rape seed meal to a broiler breeder ration. They concluded that amino acid balance rather than toxic factors was the explanation for poor performance. Grandhi et al. (1977) showed that with 10% rape seed meal in a layer diet, egg production was not affected while at 20% there was a decrease in production with a higher incidence of liver hemorrhage and mortality. They compared two varieties of rape seed meal and showed that a higher level of glucosinolate in one variety was the cause of increased toxicity of that variety over the other.

The use of a complex chemical in the chick diet was shown to prevent experimentally produced fatty livers (Akiba et al. 1984). They used Diisopropyl-1, 3-dithiolan - 2-ylidenemalonate (NKK-100 at 250, 500, 1000, 1500 mg/kg of diet. Liver weight was significantly decreased at the 1500 mg/kg level.

MATERIALS AND METHODS

Three experiments, each with two phases, were conducted with White Leghorn layers. The first phase involved ad libitum type of feeding. During the second phase, a part of the birds from each treatment were force-fed at 120% of their normal intake using much of the same procedure as Wolford and Polin (1972).

Experiment One (First Phase)

Eighty-four Hy-line hens were used in this experiment. They had been fed a starter mash (Table 1) until eight weeks of age, a grower diet until twenty weeks of age and then a layer diet until starting the experiment, which was when the birds were fifty-one weeks of age. The hens were maintained in single cages. Four diets were used as shown in Table 2 with twenty-one birds alloted at random for each diet. Diet one, a regular corn-soy type of ration, was used as the control. In diet two, oats was used as the major grain. Diets three and four, respectively, contained 20 and 30 percent Solulac (a commercial product containing distillers dried grain with solubles Table 3 and 4). This phase of the experiment was continued for three 28-day periods. Data were collected for egg production, feed consumption, egg weight, Haugh unit, body weight and analyzed statistically by least-squares analysis of variance (Steel and Torrie, 1960).

TABLE 1

COMPOSITION OF STARTER, GROWER AND LAYER DIETS USED BEFORE STARTING THE EXPERIMENTS

	Starter	Grower	Layer
Ingredients	%	%	%
Corn	64.5	80.0	72.0
Soybean meal (46%)	27.0	8.0	14.4
Alfalfa meal (17%)	2.0	6.0	2.0
Fishmeal	2.0	----	----
Limestone	1.0	1.0	6.0
Dicalcium phosphate	1.5	2.0	2.0
Grease	1.0	2.0	2.0
Salt mix (a)	0.5	0.5	0.5
Vitamin mix (b)	0.5	0.5	0.5
Methionine	----	----	0.1
Calculated Analysis			
Crude Protein (%)	20.33	12.0	13.77
ME (K cal/Kg)	3030	3172	3036
Lysine (%)	1.12	0.49	0.65
Methionine (%)	0.36	0.23	0.35
Cystine (%)	0.31	0.19	0.22
Arginine (%)	1.41	0.74	0.90
Tryptophan (%)	0.26	0.14	0.18

(a) Salt mix contains in percent, Mn not less than .250; Cu, 0.33; Co, .0025; Zn, .005; NaCl, 97; S, .1; Fe, .2; I, .007.

(b) Vitamin mix contains per Kg, Vitaming A, 1,056,000 IU; Vitamin 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 EXPERIMENT 1 (BOTH PHASES)

INGREDIENT	DIET 1	DIET 2	DIET 3	DIET 4
	%	%	%	%
Corn	66.45	10.36	55.7	50.13
Soybean Meal	21.42	19.17	11.9	7.34
Oats	—	55.44	—	—
Distillers Dried Grain (Solulac)	—	—	20	30.37
Alfalfa	2.02	2.02	2.02	2.02
Dicalcium Phosphate	2.02	2.02	2.02	2.02
Limestone	7.07	7.07	7.07	7.07
Salt Mix (a)	0.5	0.5	0.5	0.5
Vitamin Mix (b)	0.5	0.5	0.5	0.5
Grease	—	2.92	—	—
Calculated Analysis				
Crude Protein (%)	16.4	16.3	16.1	16
ME (K cal/Kg)	2800	2606	2802	2801
Met	0.28	0.25	0.29	0.3
Cystine	0.26	0.30	0.26	0.25
Lys	0.83	0.89	0.69	0.60
Arg	1.13	1.16	0.93	0.84

(a) See Table 1

(b) See Table 1

TABLE 3

COMPOSITION OF SOLULAC USED IN THE DIET 3 AND 4
(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 (mgm/Kg)	5.5
Niacin (mgm/Kg)	68.97
Riboflavin (mgm/Kg)	7.4
Pantothanic acid (mgm/Kg)	11.9
Vitamin B_{12}	4.91
Pyridoxine (mgm/Kg)	10.2
Folacin (mgm/Kg)	1.5
Choline (mgm/Kg)	4915
Biotin (mgm/Kg)	.37
Inositol (mgm/Kg)	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 Nutrient (%)	80
Digestible Protein (%)	22

TABLE 4

AMINO ACID COMPOSITION OF SOLULAC USED IN DIET 3 AND 4

COMPOSITION	PERCENT
Arginine	1.04
Lysine	.80
Methionine	.50
Methionine and Cystine	1.00
Tryptophan	.25
Histidine	.53
Leucine	2.88
Iso Leucine	1
Phenyl alanine	1.21
Phenylalanine and Tyrosine	2.15
Threonine	.91
Valine	1.28
Glycine	.98

Experiment One (Second Phase)

At the end of the first phase, one third of the birds from each diet were selected at random and force-fed at 120% of their normal intake for three weeks. Force-feeding involved grinding the feed in a Wiley mill and then mixing it with water in a blender for two minutes. The proportion of feed to water was approximately 1:1.5 (weight:volume). The blended feed showed a semi-liquid slurry type of consistency which was forced in to the stomach of each bird by a plastic tube and a 60 ml syringe. Force-feeding was performed twice a day, both morning and evening. The rest of the birds continued to receive feed ad libitum for the three weeks period.

Production and feed consumption data were recorded. At the end of the second phase all the birds were sacrificed by cervical dislocation. Livers were removed, weighed and scored for hemorrhage. Scoring involved counting the number of hemorrhage points. A score of one was given if there was no hemorrhage, a two was given for 1 to 10 hemorrhages, three for 10 to 25 hemorrhages and a score of four was given to a liver sample with more than 25 hemorrhages. The liver samples were then frozen and at a later date analyzed for lipid content.

Experiment Two (First Phase)

One hundred forty-four White Leghorn hens of the Shaver-288 strain were used in the experiment. The birds were nine months old at the beginning of the experiment. They had been fed starter, grower

and layer rations (Table 1) until the initiation of the experiment. The birds were housed in groups of four per (61 x 41 cm) multiple cage. Four diets were again used in this experiment with 36 birds being alloted at random per diet. As shown in Table 5, diet one had corn as the major source of energy and was used as the control. Diets two and three contained 5 and 10% grease, respectively with proportionate amounts of corn being reduced in these two diets. Diet four contained oats as the major source of energy. Production and feed consumption data were recorded daily. Data for eight 28-day periods were analyzed statistically by least-squares analysis of variance (Steel and Torrie, 1960).

Experiment Two (Second Phase)

At the end of the first phase one sixth of the birds from each treatment were selected at random and force-fed for three weeks their same diets using 120% of normal intake. Force-feeding was done as described in Experiment One. The rest of the birds were fed ad libitum. The birds in this phase of the study were maintained in individual cages. At the end of three weeks all the birds were sacrificed and livers were collected, weighed and scored for hemorrhages. Scoring was done as described in Experiment One. Three samples from each liver weighing approximately two grams were used for analyzing lipid content. The samples were dried in an oven for 24 hours at 100^{o}C then refluxed with ethyl ether in a soxhlet extraction apparatus. Data for both liver and production parameters were

TABLE 5

COMPOSITION OF DIETS USED IN EXPERIMENT TWO (BOTH PHASES)

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIET 4
	%	%	%	%
Corn	71.0	65.0	58.5	—
Oats	—	—	—	79.1
Soybean Meal	17.0	18.0	19.5	8.9
Alfalfa	2.0	2.0	2.0	2.0
Limestone	7.0	7.0	7.0	5.0
Dicalcium Phosphate	2.0	2.0	2.0	2.0
Salt Mix (a)	0.5	0.5	0.5	0.5
Vitamin Mix (b)	0.5	0.5	0.5	0.5
Grease	—	5.0	10.0	2.0
Methionine	—	—	—	0.1
Calculated Analysis				
Crude Protein (%)	14.8	14.7	14.9	14
ME (K cal/Kg)	2877	3072	3262	2412
Lys	0.73	0.74	0.77	0.69
Met	0.28	0.26	0.26	0.31
Cystine	0.23	0.23	0.23	0.24
Arg	0.99	1.00	1.02	0.97

(a) See Table 1

(b) See Table 1

analyzed statistically by the method of Waller-Duncan K-ratio (SAS, 1982).

Experiment Three (First Phase)

This study was conducted with 360 White Leghorn birds of the Shaver-288 strain. The birds were maintained on starter mash (Table 1) until eight weeks of age, the grower diet up to twenty weeks and the layer ration until starting the experiment. The birds were six months old when they were put on four experimental treatments (Table 6). They were randomly alloted at 90 birds/treatment.

They were maintained ten per cage in multiple cages (61 x 41 cm). Treatment one was the usual corn-soy type of ration. Treatment two contained 20% DDG replacing corn and soybean meal. For treatment three a mixture of DDG and oats was incorporated and treatment four contained oats as the major source of energy. Feed consumption and egg production were recorded daily and data for the twelve 28-day periods were analyzed statistically by the least-squares analysis of variance (Steel and Torrie, 1960).

Experiment Three (Second Phase)

At the end of the first phase six birds from each treatment were selected at random and placed in individual cages for force-feeding. The rest of the birds were also individually caged and continued to receive feed ad libitum. Force-feeding at 120% of normal was utilized for three weeks, following the same procedure as described in the first experiment. During this three-week period, six

TABLE 6

COMPOSITION OF DIETS USED IN EXPERIMENT THREE (BOTH PHASES)

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIET 4
	%	%	%	%
Corn	66.0	50.0	—	—
Oats	—	—	49.5	61.0
Distillers Dried Grain	—	30.0	20.0	—
Soybean Meal	20.0	7.0	10.0	18.0
Alfalfa	4.0	3.0	2.0	2.0
Limestone	7.0	7.0	7.0	7.0
Dicalcium Phosphate	2.0	2.0	2.0	2.0
Salt Mix (a)	0.5	0.5	0.5	0.5
Vitamin Mix (b)	0.5	0.5	0.5	0.5
Grease	—	—	8.5	9.0
Calculated Analysis				
Crude Protein (%)	16.2	16.1	16.0	16.0
ME (K cal/Kg)	2806	2722	2706	2702
Lys	0.82	0.61	0.74	0.89
Met	0.28	0.30	0.27	0.25
Cystine	0.25	0.26	0.27	0.27
Arg	1.09	0.84	0.98	1.2

(a) See Table 1

(b) See Table 1

birds from each treatment (three from each type of feeding) were selected at random and 5 ml blood samples in 10 ml tubes were collected. They were then centrifuged, the serum was decanted off and kept frozen at -20°C for later assay of serum estradiol. At the end of three weeks all the birds were sacrificed. Then livers were collected, weighed and scored as before for hemorrhages. Triplicate samples of livers were analyzed for lipid content. Data for the production and liver parameters were analyzed statistically by Waller-Duncan K-ratio procedure (SAS, 1982).

Radio-Immuno Assay (RIA)

The Radio-Immuno Assay (RIA) for estradiol was that described in the manual supplied by the Diagonistic Product Corporation. Before starting the analysis, the serum samples were thawed and 100 ul of serum were put into antibody coated tubes. Duplicates were prepared for each sample. One ml of solution containing 35000 cpm labeled estradiol 17 B I^{125} was added to each tube. In addition to the unknowns, six standard tubes were prepared containing 100 ul of different concentrations of estradiol and 1 ml of the labeled estradiol standard. Two tubes for total count were also made up containing 1 ml of the labeled estradiol standard. Non-specific binding was determined by putting 100 ul of buffered normal rabbit serum into non-antibody coated tubes. Maximum binding tubes were prepared using antibody coated tubes and adding 100 ul buffer and 1 ml of the labeled estradiol solution. All the tubes were incubated for

24 hours at room temperature. Then all the tubes were decanted and the pellets counted for radioactivity in a gamma counter. The results were analyzed statistically by Waller-Duncan multiple range test (Steel and Torrie, 1960).

Precision was demonstrated in that the standard deviation was 3.5 with a co-efficient of variability of 7% at a sample size of 50. Sensitivity was 8 pg/ml approximately. The assay was found to be parallel between standard cow serum and sterile serum. Recovery was checked by adding a known amount of estradiol and found to be 104%.

RESULTS AND DISCUSSION

Experiment One (First Phase)

Average data for egg production, feed consumption, egg weight, body weight, Haugh units, feed efficiency and the analyses of variance for these data are given in Tables 7 and 8. Hens on diet two with oats produced significantly fewer eggs and consumed significantly less feed than those on the control diet ($P < 0.01$). Also, the feed efficiency on diet two was significantly less ($P < 0.05$). Hens on diet three and four with 20% and 30% distillers dried grain respectively showed intermediate values for egg production, feed consumption and feed efficiency. However, the differences were not significant. There were no significant differences in egg weight, Haugh units and body weights for the two different levels (20% and 30%) of DDG. Though not significant, hens on 20% DDG appeared to perform better than those on the 30% DDG diet. This is similar to the findings of Jensen *et al.* (1974).

Experiment One (Second Phase)

Data for liver weight, liver score, total liver lipid, percentage liver lipid (wet basis) and liver as a part of body weight are shown in Table 9. Table 10 shows the AOV for these data. Hens on diet two with oats and diet four with 30% DDG had significantly less hemorrhage on their livers and less total liver lipid ($P < 0.05$) than for birds on diet three with 20% DDG which agrees with our previous

TABLE 7

EFFECTS OF DIFFERENT LEVELS OF DISTILLERS DRIED GRAIN AND OATS ON PRODUCTION PARAMETERS IN EXPERIMENT 1 PHASE 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 Corn-soy (b)	72.74	114.64	65.34	67.38	1.89	1.87
Diet 2 Oat-soy (b)	53.86**	100.39**	66.05	72.02	1.80	2.38*
Diet 3 Solulac (20%) (b)	67.23	115.41	65.58	68.71	1.82	2.01
Diet 4 Solulac (30%) (b)	65.44	116.10	65.14	66.47	1.84	2.15

(a) Average for 3, 28-day periods

(b) See Table 2 for diet composition

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

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

TABLE 8

ANALYSES OF VARIANCE FOR PRODUCTION PARAMETERS
IN EXPERIMENT 1 (PHASE 1)

		MEAN SQUARES					
SOURCE OF VARIATION	d.f.	HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	EGG WEIGHT	HAUGH UNIT	BODY WEIGHT	KG OF FEED PER DOZEN OF EGGS
Repticate	6	408.4	63.3	40.5	94.6	0.03	0.98
Treatment	3	1324.2	1187.7	3.2	124.2	0.02	1.0
R X T	18	121.6**	48.5**	17.1	113.8	0.02	0.3*
Month	2	406.8	7387.9	8.0	54.2	0.00	5.1
R X M	12	64.9	184.1	7.8	17.9	0.00	0.3
T X M	6	96.9	156.6	12.2	19.3	0.00	0.2
R X T X M	36	41.4	41.3	6.4	21.2	0.00	0.2

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

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

TABLE 9

EFFECTS OF DIFFERENT LEVELS OF DISTILLERS DRIED GRAIN AND OATS ON LIVER PARAMETERS IN EXPERIMENT 1 PHASE 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)	37.36	1.79 AB	3.28 AB	8.03 A	1.93
Diet 2 Oats-soy (b)	32.71	1.14 B	0.86 B	2.55 B	1.83
Diet 3 20% Solulac (b)	37.93	1.85 A	4.13 A	9.21 A	1.97
Diet 4 30% Solulac (b)	31.86	1.14 B	1.20 B	3.61 B	1.77
Force-feeding (c)	35.96	1.71*	2.93*	7.56*	1.79*
Ad libitum	33.96	1.25	1.80	4.15	1.96

(a) 21 Days

(b) See Table 2 for diet composition

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

Means with different letters are significantly different at ($P < 0.05$)

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

TABLE 10

ANALYSES OF VARIANCE FOR LIVER PARAMETERS
IN EXPERIMENT 1 PHASE-2

		MEAN SQUARES				
SOURCES OF VARIATION	d.f.	LIVER WEIGHT	LIVER SCORE	TOTAL LIVER LIPID	LIVER LIPID (WET BASIS)	LIVER AS PART OF (BODY WEIGHT)
Replicates	6	9.28	0.56	5.72	18.49	0.061
Treatment	3	136.40	2.16	35.41	150.42	0.124
T X R	18	53.45	0.59*	9.01*	26.59*	0.144
Type	1	56.00	3.02	17.81	162.99	0.378
Type X R	6	16.12	0.06*	0.14*	7.66*	0.035*
Type X T	3	26.71	1.11	4.27	30.52	0.049
Type X T X R	18	33.34	0.27	3.28	9.57	0.064

*Significant at ($P < 0.05$) level

work (Rakshit, 1981). In this work 20% DDG in the diet did not reduce liver lipid or liver hemorrhages significantly. However, Jensen et al. (1976a) observed that 20% brewers dried grain in the diet reduced liver lipid in the summer. Lipid percents for livers from hens fed diet two with oats and diet four with 30% DDG were significantly lower ($P < 0.05$) than birds on diet one with corn or diet three with 20% DDG. The reduction of liver lipid with a higher percentage of DDG in the diet was quite similar to the findings of Maurice and Jensen (1978a). They had observed that 20% DDG in diet was more efficient in reducing liver lipid than 10% DDG. There were no differences in liver weight as related to body weight or total liver weight values. Force-feeding at 120% increased liver score, total liver lipid and liver weight signfiicantly ($P < 0.05$), but decreased liver as part of body weight ($P < 0.05$). Hen-day feed consumption, egg production, final body weight (Table 11) and the analyses of variance (Table 12) are shown. There was a significant difference ($P < 0.05$) in final body weight. Hens on diet two fed oats were lighter than birds on diet one fed the corn-soy control. Though not significant, hens on diet four with 30% DDG apparently were lighter than birds on the 20% DDG diet. There were no differences in feed consumption or egg production of birds on these different diets. Feed consumption and final body weight were significantly higher ($P < 0.05$) for birds force-fed. Force-feeding did not change the egg production parameter significantly. However, ad libitum fed birds may have had higher rates of production probably due to less stress.

TABLE 11

EFFECTS OF DIFFERENT LEVELS OF DISTILLERS DRIED GRAIN AND OATS ON PRODUCTION PARAMETERS IN EXPERIMENT 1 PHASE-2 (a)

DIETS	HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	FINAL BODY WEIGHT
	%	gm	Kg
Diet 1 Corn-soy (b)	51.85	138.0	1.90 A
Diet 2 Oat-soy (b)	63.17	145.7	1.78 B
Diet 3 20% Solulac (b)	54.31	148.6	1.91 AB
Diet 4 30% Solulac (b)	62.05	151.4	1.81 AB
Ad libitum	64.00	110.9*	1.7*
Force-feeding (c)	51.68	180.9	2.0

(a) 21 days

(b) See Table 2 for composition of diets

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

Means with different letters are significantly different at ($P < 0.5$) level

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

TABLE 12

ANALYSES OF VARIANCE ON PRODUCTION PARAMETERS
IN EXPERIMENT 1 - PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARES		
		HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	FINAL BODY WEIGHT
Replicate	6	263.90	0.603	0.029
Treatment	3	440.81	0.204	0.075
T X R	18	342.99	0.121	0.024*
Type	1	2122.98	30.018	1.086
Type X R	6	514.41	0.131*	0.031*
Type X T	3	646.78	0.022	0.029
Type X T X R	18	407.63	0.078	0.021

*Significant at ($P < 0.05$) level

Experiment Two (First Phase)

Egg production, feed consumption, egg weight, body weight and feed efficiency are shown in Table 13. The analyses of variance are shown in Table 14. Hens on diet three containing 10% yellow grease produced significantly fewer eggs than birds on the control diet or diet two with 5% yellow grease ($P < 0.05$). Hens on diet four with oats produced significantly heavier eggs ($P < 0.05$). There were no other significant production parameters. The apparent improvement in feed efficiency from 5% and 10% grease would agree with the findings of Sunde (1956) and Waibel (1955).

Experiment Two (Second Phase)

Data for liver weight, liver score, liver lipid and liver as part of body weight are given in Table 15 with the analyses of variance shown in Table 16. Hens on diet three with 10% grease had significantly higher liver lipid content ($P < 0.05$) than birds on diet four with oats. There were no other differences in either liver weight or liver score which was based on hemorrhage incidence. The addition of yellow grease increased liver lipid content which agrees with Bragg *et al.* (1973). They found that animal fat additions produced fatty liver whereas vegetable fat did not. However, Nelson (1978) suggested that the addition of animal fat to a layer diet could reduce liver lipids and hemorrhages. In this experiment however, we observed that addition of animal fat increased liver lipid significantly ($P < 0.05$) but there was no significant difference in

TABLE 13

EFFECTS OF DIFFERENT LEVELS OF FAT AND OATS
ON PRODUCTION PARAMETERS IN EXPERIMENT 2 PHASE-1 (a)

DIETS	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 Corn-soy (b)	81.27	120.96	65.42	77.59	1.77	1.76
Diet 2 5% Grease (b)	83.53	123.54	63.94	78.15	1.79	1.75
Diet 3 10% Grease (b)	73.05*	104.95	63.56	78.97	1.79	1.71
Diet 4 Oats-soy (b)	75.38	138.37	67.89*	77.12	1.80	2.16

(a) Average for 8, 28-day periods

(b) See Table 5 for diet composition

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

TABLE 14

ANALYSES OF VARIANCE FOR PRODUCTION PARAMETERS
IN EXPERIMENT 2 PHASE-1

		MEAN SQUARES					
SOURCES OF VARIATION	d.f.	HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	EGG WEIGHT	HAUGH UNIT	BODY WEIGHT	KG OF FEED PER DOZEN OF EGGS
Replicate	8	359.007	3840.46	19.984	35.82	0.033	0.849
Treatment	3	1735.87	13489.25	277.55	45.14	0.019	3.260
R X T	24	329.79*	4064.93	34.369*	74.02	0.035	1.247
Month	7	1609.27	4802.08	62.955	391.56	0.143	1.700
R X M	56	26.136	3738.71	4.091	12.82	0.016	0.927
T X M	21	97.195	2768.86	3.718	9.22	0.011	0.926
R X T X M	168	25.609	3539.08	2.998	11.86	0.014	0.900

* Significant at ($P < 0.05$) level

TABLE 15

EFFECTS OF DIFFERENT LEVELS OF FAT AND OATS ON LIVER PARAMETER IN EXPERIMENT 2 PHASE-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)	42.64	1.33	2.57 AB	5.86 AB	2.26
Diet 2 5% Grease (b)	42.45	1.29	3.63 AB	7.54 AB	2.23
Diet 3 10% Grease (b)	45.58	1.33	4.87 A	9.37 A	2.35
Diet 4 Oats-soy (b)	38.29	1.00	1.18 B	2.96 B	2.07
Ad libitum	37.86*	1.06*	1.88*	4.71*	2.19
Force-feeding (c)	46.62	1.41	4.24	8.16	2.27

(a) 21 days

(b) See Table 5 for diet composition

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

Means with different letters are significantly different at ($P < 0.05$) level

* Significant at ($P < 0.05$) level

TABLE 16

ANALYSES OF VARIANCE ON LIVER PARAMETERS
IN EXPERIMENT 2 PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARES				
		LIVER WEIGHT	LIVER SCORE	TOTAL LIVER LIPID	LIVER LIPID WET BASIS)	LIVER AS PART OF BODY WEIGHT
Replicate	5	72.21	0.180	6.03	19.39	0.0008
Treatment	3	107.90	0.311	29.58	89.01	0.0017
T X R	15	51.75	0.402	9.81*	26.06*	0.0010
Type	1	921.37	1.505	67.05	142.28	0.0007
Type X R	5	86.27*	0.230*	7.55*	29.35*	0.0016
Type X T	3	12.17	0.227	13.19	30.17	0.0014
Type X T X R	15	39.36	0.352	7.82	20.78	0.0070

*Significant at ($P < 0.05$) level

hemorrhage incidence. Especially was this true for the liver lipid content of hens on diet three with 10% grease in that they had the highest liver lipid content but hemorrhage incidence was quite similar to that of birds on diet one without any grease. Hens on diet two with 5% grease appeared to have a higher liver lipid content than the control birds with fewer hemorrhages, but the differences however were not significant. The reason for significantly more liver lipid content in birds on diet three with 10% grease without any significant increase in hemorrhage was not apparent.

Hen day consumption, egg production and body weight data are given in Table 17 with the analyses of variance as shown in Table 18. Hens on diet four with oats consumed significantly more feed ($P < 0.05$) than birds on diet three with 10% grease or diet two with 5% grease. Hens on diet two with 5% grease consumed significantly less feed ($P < 0.05$) than birds on the control feed. Feed consumption was significantly higher ($P < 0.05$) for force-fed birds. There were no other production or body weight differences observed in the experiment.

Experiment Three (First Phase)

Data for egg production, feed consumption, egg weight, and other production parameters are shown in Table 19 with their analyses of variance shown in Table 20. Hens on diet three with a mixture of DDG and oats and those on diet four with oats as the only grain source

TABLE 17

EFFECTS OF DIFFERENT LEVELS OF FAT AND OATS ON PRODUCTION PARAMETER IN EXPERIMENT 2 PHASE-2 (A)

DIETS	HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	FINAL BODY WEIGHT
	%	gms	Kg
Diet 1 Corn-soy (b)	62.29	148.20 AB	1.9
Diet 2 5% Fat (b)	61.68	131.37 C	1.9
Diet 3 10% Fat (b)	49.52	136.70 BC	1.9
Diet 4 Oats-soy (b)	59.20	157.93 A	1.8
Ad libitum	63.07	127.61*	2.04
Force-feeding (c)	53.35	162.49	2.05

(a) 21 days

(b) See Table 5 for diet composition

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

Means with different letters are significantly different at ($P < 0.05$) level

* Significantly different at ($P < 0.05$) level

TABLE 18

ANALYSES OF VARIANCE ON PRODUCTION PARAMETERS
IN EXPERIMENT 2 PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARES		
		HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	FINAL BODY WEIGHT
Replicate	5	454.56	193.55	0.0120
Treatment	3	425.21	1694.62	0.0121
R X T	15	353.26	338.46*	0.0123
Type	1	1131.99	17217.97	0.000008
Type X R	5	409.63	44.60	0.0129
Type X T	3	591.63	349.91*	0.0120
Type X T X R	15	512.11	303.66	0.0146

*Significant at ($P < 0.05$) level

TABLE 19

EFFECTS OF DIFFERENT GRAINS ON PRODUCTION PARAMETERS IN EXPERIMENT 3 PHASE-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 Corn-soy (b)	74	127	62.7	79	1.69	2.11
Diet 2 DDG (b)	74.8	132	62.3	82	1.68	2.16
Diet 3 DDG + Oats (b)	71.5	118*	62.7	80	1.70	2.05
Diet 4 Oats-soy (b)	71.6	116**	62.0	79	1.69	1.98

(a) Averages for 12 28-day period

(b) See Table 6 for diet composition

* Significant at ($P < 0.05$) level

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

TABLE 20

ANALYSES OF VARIANCE ON PRODUCTION PARAMETERS IN EXPERIMENT 3 PHASE-1

SOURCES OF VARIATIONS	d.f.	MEAN SQUARES					
		HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	EGG WEIGHT	HAUGH UNIT	BODY WEIGHT	KG OF FEED PER DOZEN OF EGGS
Replicate	8	263.83	474.98	18.80	42.68	0.052	0.217
Treatment	3	316.11	4787.31	11.46	149.79	0.013	0.698
R X T	24	131.27	622.13*	50.49	60.88	0.066	0.270
Month	11	5348.13	2013.25	440.20	244.29	0.096	9.725
R X M	88	52.74	73.18	15.03	15.26	0.004	0.089
T X M	33	61.25	85.45	16.85	9.79	0.009	0.123
R X T X M	264	43.51	80.93	16.86	16.37	0.006	0.091

* Significant at ($P < 0.05$) level

consumed significantly less feed ($P < 0.05$). There were no other significant differences observed in this phase of the experiment.

Experiment Three (Second Phase)

Data for liver weight, liver score, total liver lipid and liver as part of body weight are shown in Table 21 with the analyses of variance presented in Table 22. Hens on diet three with DDG plus oats and diet four with oats had significantly lower hemorrhage scores ($P < 0.05$) than birds on the other two diets. This agrees with the findings of Maurice and Jensen (1978a) in that they observed that birds fed fermentation by-products had less liver hemorrhage. Oats as a major source of grain in the diet was found to be effective in reducing liver lipid and hemorrhage by Nelson (1978) and Rakshit (1981). In the present study, oats reduced hemorrhage significantly. The apparent reduction in liver lipid content was not significant. Oats plus DDG also reduced hemorrhage significantly but not liver lipids. Therefore, it is assumed that oats and DDG may contain some factor which tends to protect against hemorrhage even with higher liver lipid content. This agrees with the suggestion of Maurice and Jensen (1978), Thornton *et al.* (1962) and Nelson (1978). In this experiment oats alone reduced both fat content and hemorrhage incidence of livers while the mixture of oats and DDG reduced hemorrhage incidence despite the fact that fat content of the liver was higher. As hemorhage is more important in causing death than a higher liver lipid content, the mixture of oats plus DDG would seem to

TABLE 21

EFFECT OF DIFFERENT GRAINS ON LIVER PARAMETERS IN EXPERIMENT 3 PHASE-2 (a)

TREATMENT	LIVER WEIGHT	LIVER SCORE	TOTAL LIVER LIPID	LIVER LIPID (WET BASIS)	LIVER AS PART OF BODY WEIGHT
	gm		gm	%	%
Diet 1 Corn-soy (b)	37.50	1.92A	3.06	7.68	1.83
Diet 2 DDG (b)	46.91	1.83A	4.02	7.07	2.42
Diet 3 DDG + Oats (b)	43.16	1.33B	6.33	10.04	2.14
Diet 4 Oats-soy (b)	36.58	1.00B	1.82	4.82	1.87
Force-feeding (c)	45.42	1.92*	6.34	11.57*	2.05
Ad libitum	36.67	1.12	1.27	3.2	2.08

(a) Average for 21 days

(b) See Table 6 for diet composition

(c) Force-feeding @ 120% of normal intake

Means with different letters are significantly different at ($P < 0.05$) level

* Significantly different at ($P < 0.05$) level

TABLE 22

ANALYSES OF VARIANCE ON LIVER PARAMETERS
IN EXPERIMENT 3 PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARES				
		LIVER WEIGHT	LIVER SCORE	TOTAL LIVER LIPID	LIVER LIPID (WET BASIS)	LIVER AS PART OF BODY WEIGHT
Replicate	5	105.83	0.57	50.61	83.06	0.182
Treatment	3	285.80	2.24	43.79	44.29	0.900
R X T	15	130.22	0.39*	29.64	42.26	0.373
Type	1	918.75	7.52	308.002	831.75	0.018
R X Type	5	180.60	0.77*	50.14	71.61*	0.209
T X Type	3	265.14	1.19	52.65	72.02	0.203
T X R X Type	15	91.65	0.37	26.35	37.23	0.195

*Significant at ($P < 0.05$) level

have an advantage over oats or DDG alone. Force-feeding again increased liver score and liver lipid percent significantly ($P < 0.05$).

Data for production, feed consumption, body weight change and serum estradiol 17-B level are shown in Table 23. The analyses of variance are shown in Table 24 for the production parameters and that for the estradiol in Table 25. Hens on diet two with DDG consumed significantly more feed ($P < 0.05$) than birds on the other treatments, but they had significantly less body weight gain ($P < 0.05$). Hens on diet four with oats showed the highest body weight change ($P < 0.05$).

Serum estradiol levels of hens on diet two with DDG were significantly higher than birds on the other diets ($P < 0.05$). These hens also had higher hemorrhage incidence than birds on diets with the oats additions. On the other hand birds on diet four with all oats showed apparently the minimum serum estradiol value, liver lipid content, and hemorrhage incidence. Serum estradiol contents of force-fed birds were not significantly higher than those fed ad libitum. On the whole, it is apparent that with very little exception serum estradiol level is found to have a positive correlation with liver lipid content and hemorrhage incidence which agrees with the suggestion of Balnave (1968); Lorenz (1954); Haghighi-Rad and Polin (1981); Akiba et al. (1982) and Maurice et al. (1979). Akiba et al. (1982) observed an interrelationship between plasma estradiol, diet composition and liver lipid content. Birds fed fermentation by-products in their study had a lower plasma estradiol

TABLE 23

EFFECT OF GRAINS ON PRODUCTION PARAMETERS
IN EXPERIMENT 3 PHASE-2 (a)

TREATMENT	HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	BODY WEIGHT CHANGE	SERUM ESTRADIOL
Diet 1 Corn-soy (b)	34.9	129A	111A	103.51B
Diet 2 DDG (b)	40.5	152B	107B	166.67A
Diet 3 DDG + Oats (b)	34.1	137A	111A	99.95B
Diet 4 Oats-soy (b)	35.3	136A	115C	64.72B
Force-feeding (c)	32.5	165*	120*	112.01
Ad libitum	39.9	112	102	105.41

(a) Averages for 21 days

(b) See Table 6 for diet composition

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

Means with different letters are significantly different at ($P < 0.05$) level

* Significantly different at ($P < 0.05$) level

TABLE 24

ANALYSES OF VARIANCE ON PRODUCTION PARAMETERS
IN EXPERIMENT 3 PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARE		
		HEN-DAY PRODUCTION	HEN-DAY FEED CONSUMPTION	BODY WEIGHT CHANGE
Replicate	5	1000.04	1131.93	136.92
Treatment	3	647.02	34186.15	4052.97
R X T	15	672.05*	151.61*	240.99
Type	1	143.30	11.05	130.79
R X Type	5	269.37*	273.85*	77.79
T X Type	3	152.43	150.07	83.94*
T X R X Type	15	289.56	297.50	48.08

*Significant at ($P < 0.05$) level

TABLE 25

ANALYSES OF VARIANCE ON SERUM ESTRADIOL LEVELS
IN EXPERIMENT 3 PHASE-2

SOURCES OF VARIATION	d.f.	MEAN SQUARE SERUM ESTRADIOL
Replicate	2	3809.83
Treatment	3	10796.37
R X T	6	1813.64*
Type	1	261.69
R X Type	2	3528.00
T X Type	3	858.25
T X R X Type	6	2866.71

*Significant at ($P < 0.05$) level

level. Existance of a similar interrelationship was also mentioned by Takahashi et al. (1984) with exogenous estradiol administration.

Gross Examination of Livers

At the end of this experiment whole livers were examined to study the differences in gross appearance as related to fat content. Figures One and Two show pictures of liver numbers 5, 3, 2, 44, 43, 31, 11, 17 and 21, which contained 22.31, 11.86, 12.42, 1.86, 1.07, 13.68, 13.86, 4.5 and 3.64 percent of fat respectively. Usually livers containing higher amounts of fat appear to be paler, yellowish and bulging. Figure Three shows a composite picture of liver numbers 21, 19, 11, 15, 18 and 17, which contained 3.64, 4.46, 13.86, 5.34, 6.21, and 45 percent of fat and weighed 23, 31, 65, 50, 40 and 90 grams respectively. Figure Four shows the enlarged picture of sample number 11 which weighed 65 grams and contained 9.01 grams of total liver lipid. In addition to multiple hemorrhages few white spots were marked on the surface. They were hard to the hand and appeared to be calcified.

Histological Observations

Livers from different hens from the second phase of this third experiment were preserved for electron micrographic examination. Figures Five and Six show the electron micrographs of a liver considered to be normal weighing 39 grams and containing 4.5 grams of total liver lipid. Both the figures show major parts of four adjacent

cells with prominent nuclei almost centrally located. There were very few fat droplets found inside the cells.

Figures Seven and Eight are sections from a liver weighing 45 grams and containing 10.4 grams of total liver lipid. This had been given a hemorrhage score of 4. In Figure Seven the fat droplets are comparatively larger than in Figures Five and Six. There was a trend for the nuclei to be on one side due to pressure of the lipid droplets. In Figure Eight the lipid droplets were very prominent, occupying the major area in the cell and pushing the nucleus to a corner.

Figure One:

Livers from hens on Diet One of Experiment Three during force-feeding containing 10.4, 5.34 and 2.97 grams of fat respectively (wet basis).

Diet one - Corn-soy

Figure Two:

Livers from hens numbered 44, 43, 31, 11, 17, and 21, on Diet Four, Four, Two, Two, Three, and Four respectively. Sample numbers 44, 43, and 31 were from ad libitum fed hens and sample numbers 11, 17, and 21 were from hens force-fed. They contained 0.31, 0.53, 6.70, 9.01, 40.5 and 1.20 grams of lipids, respectively. Sample number 21, 11, and 17 were lighter in color due to increased amount of fat.

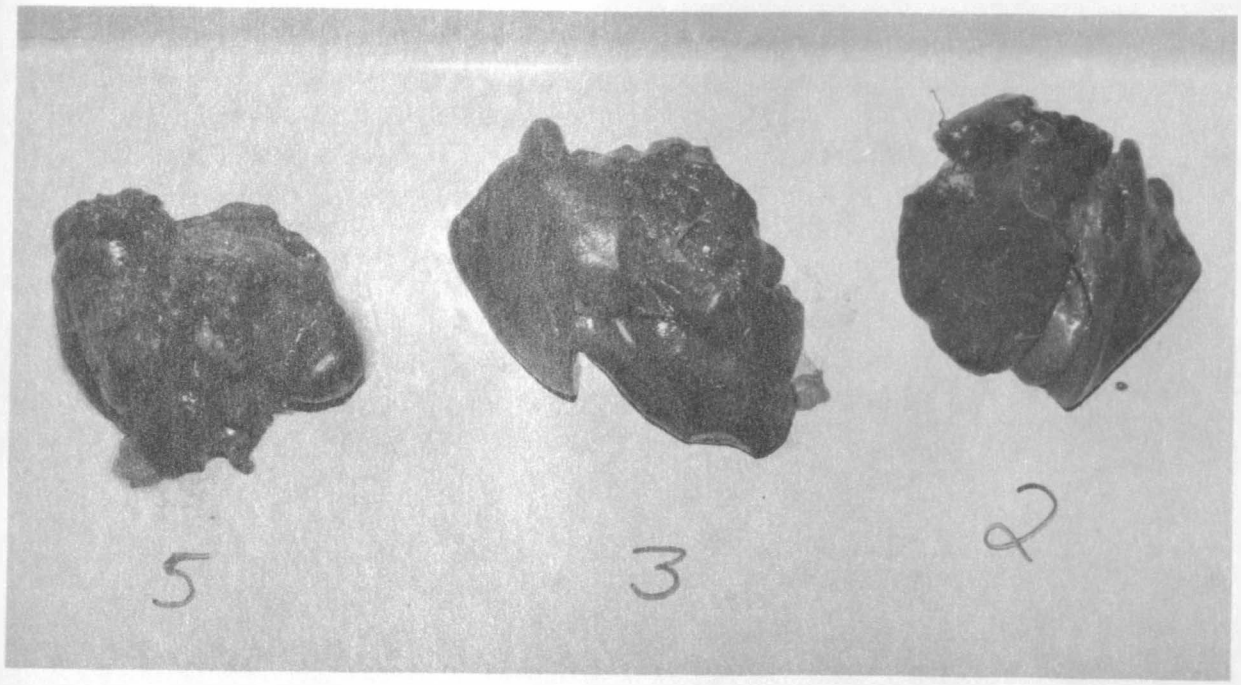
Diet one - Corn-soy

Diet two - 30% DDG

Diet three - DDG + oats

Diet four - Oats-soy

N:13: Second sample in top row is number 43 which is not printed and sample number 49 to be read as 31.


5
3
2

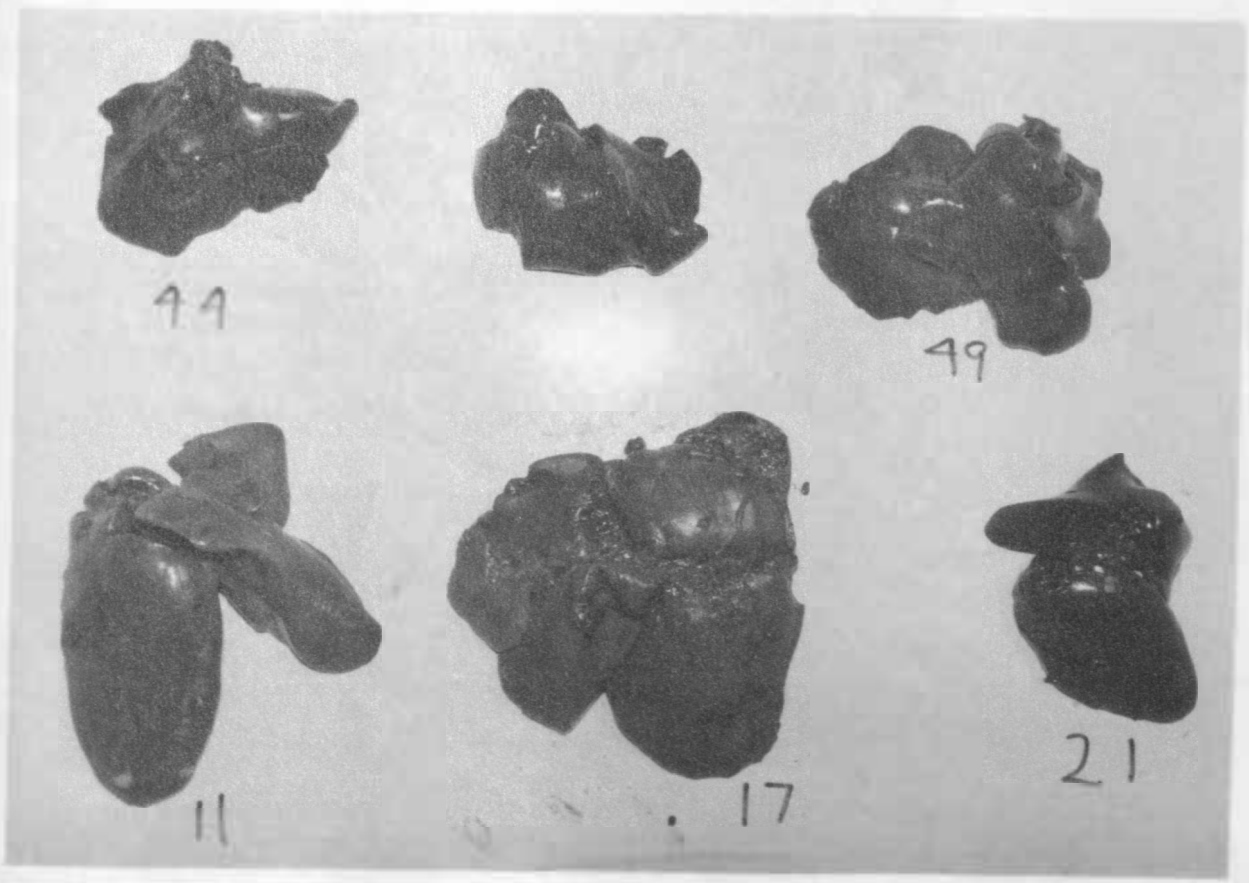

44
49
11
17
21

Figure Three:

Liver numbers 21, 19, 11, 15, 18, and 17 are from hens on Diets Four, Four, Two, Three, Three and Three respectively containing 1.20, 1.38, 9.01, 2.67 2.48 and 40.5 grams of lipids. The color differences corresponded to the fat content.

Diet two - 30% DDG

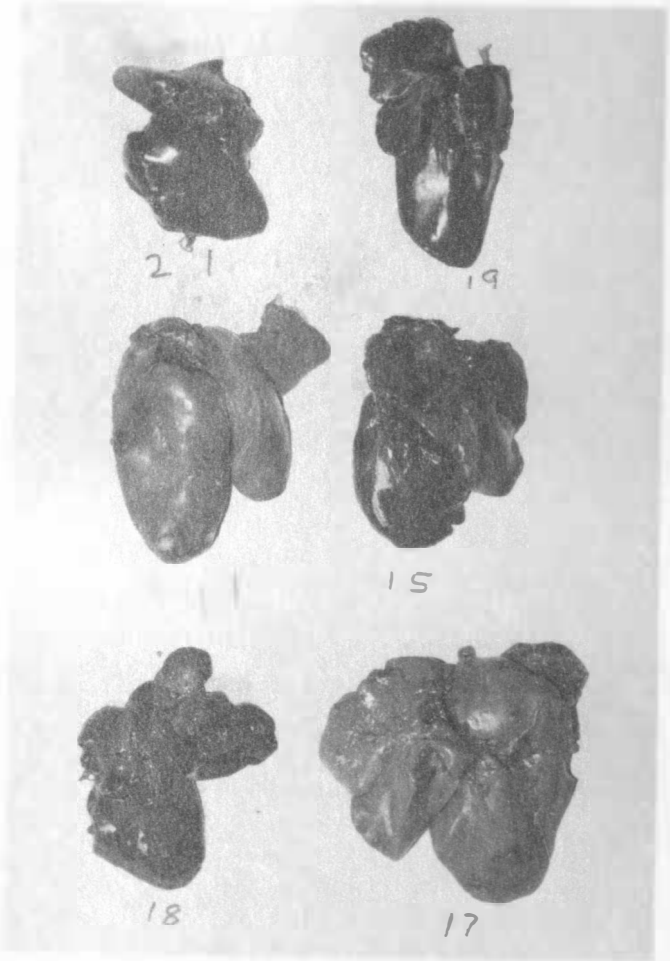
Diet three - DDG + oats

Diet four - Oats-soy

Figure Four:

Liver sample from a hen which was force-fed Diet Two. The liver weighed 65 grams and contained 9.01 grams of fat. In addition to the large amount of fat it had a bulged appearance.

Diet two - 30% DDG


21
19
15
18
17

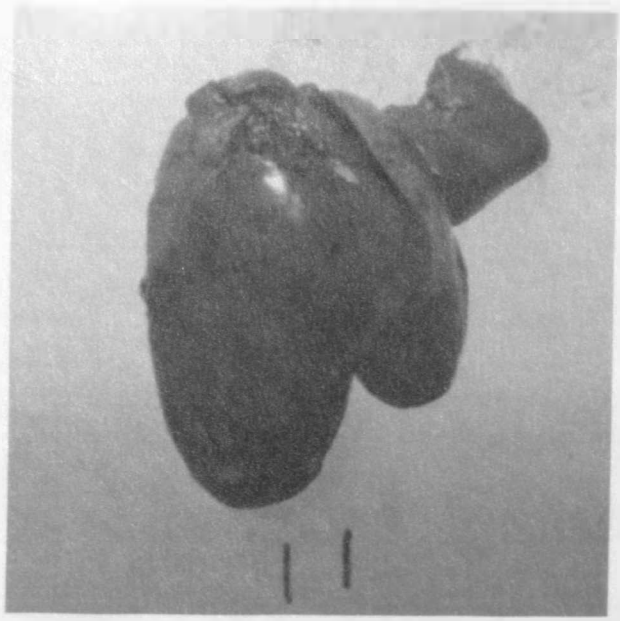

11

Figure Five:

Electronmicrograph of a hen's liver force-fed Diet Two containing 11.54% of fat. It appears to be quite normal (x 12000). L, N, M and BD stands for Lipid, Nucleus, Mitochondria and Bileduct respectively.

Diet two - 30% DDG

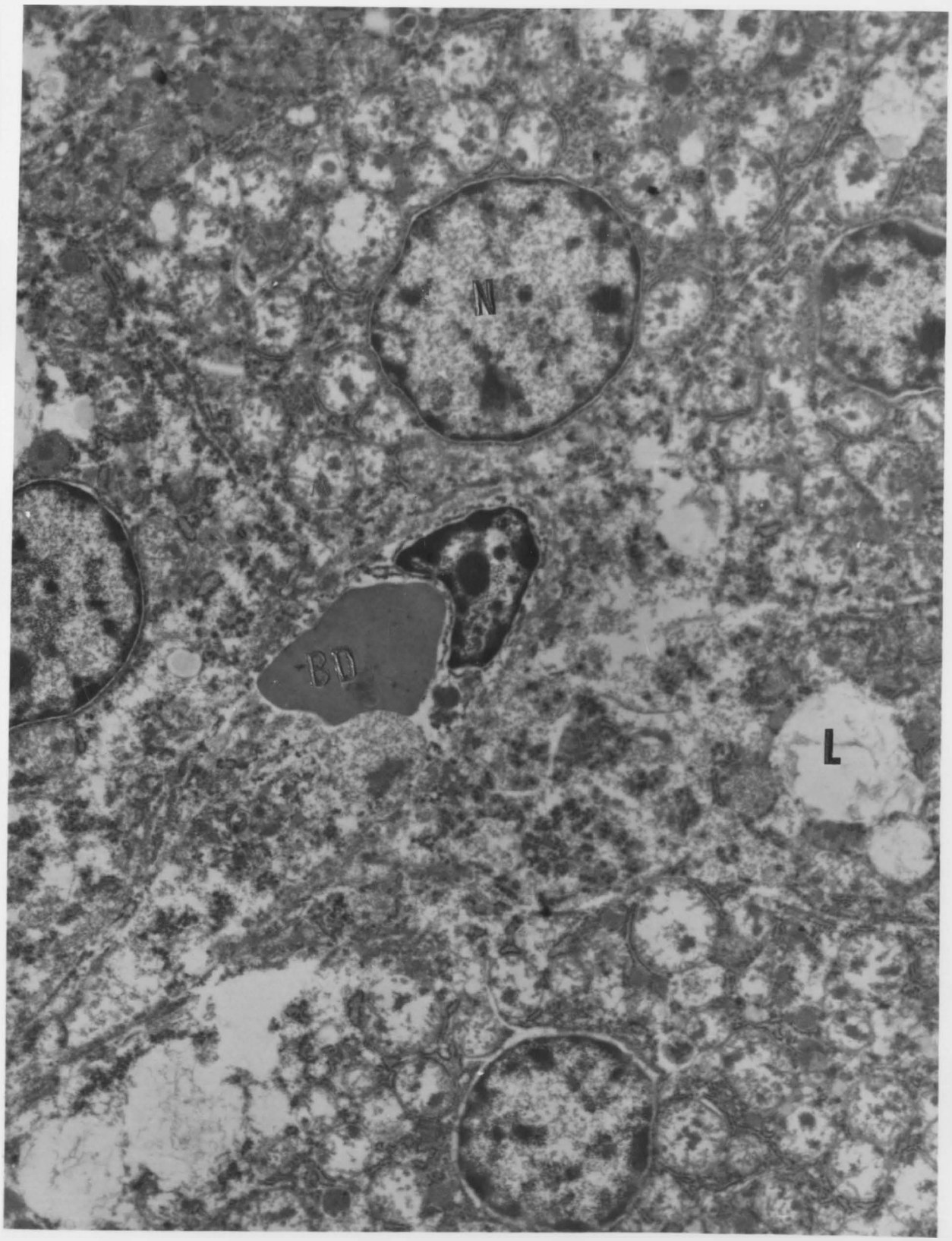

N
BD
L

Figure Six:

Section from the same liver sample as in Figure Five, but from a different area showing quite a few adjacent cells with very little droplets in the cytoplasm (x 12000). L, N and M stands for Lipid, Nucleus and Mitochondria.

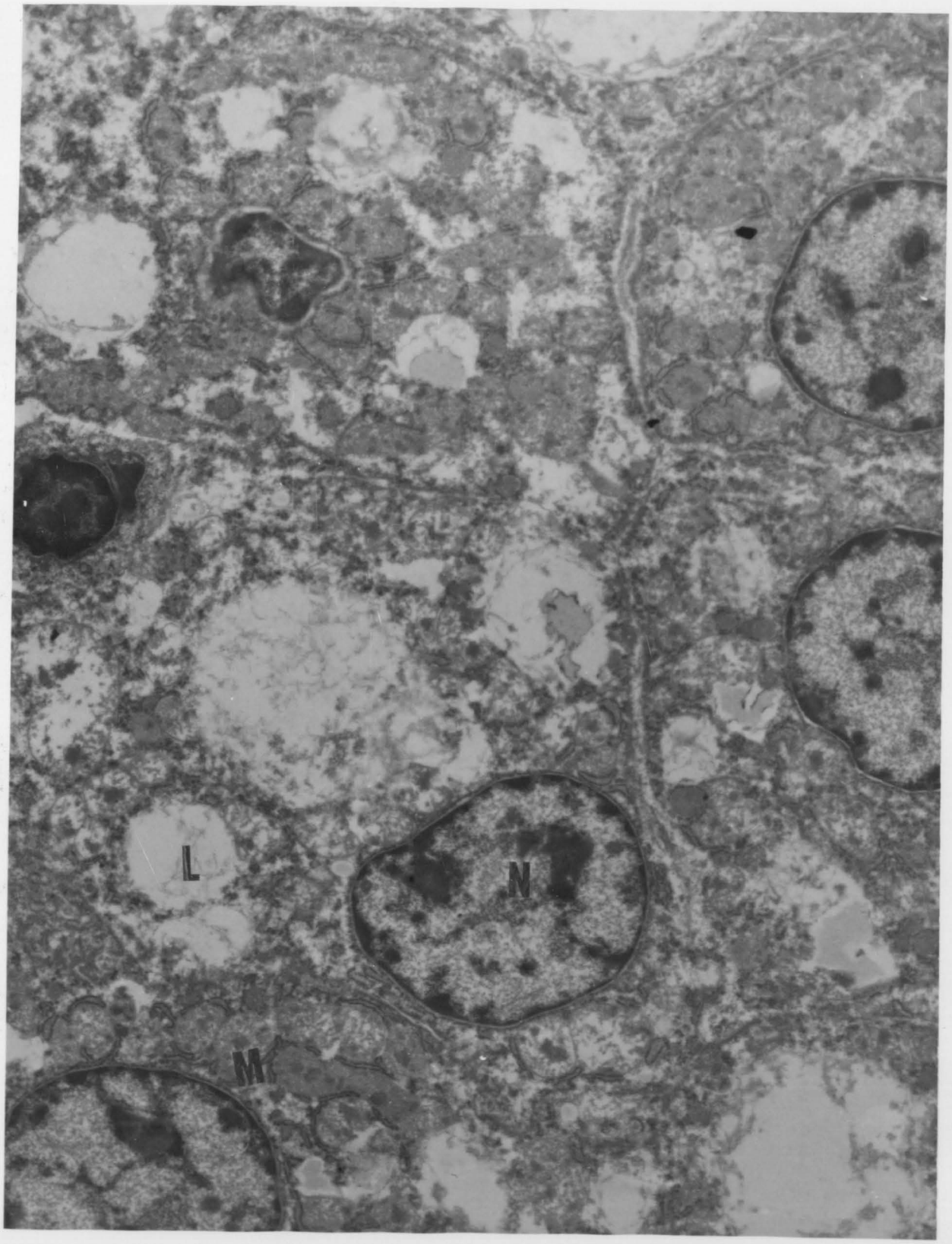

L
N
M

Figure Seven:

Section of liver sample from a hen force-fed Diet One containing 22.31% of lipid. This liver was scored 4 for hemorrhage (x 12000). L and N stands for Lipid and Nucleus respectively.

Diet one - Corn-soy

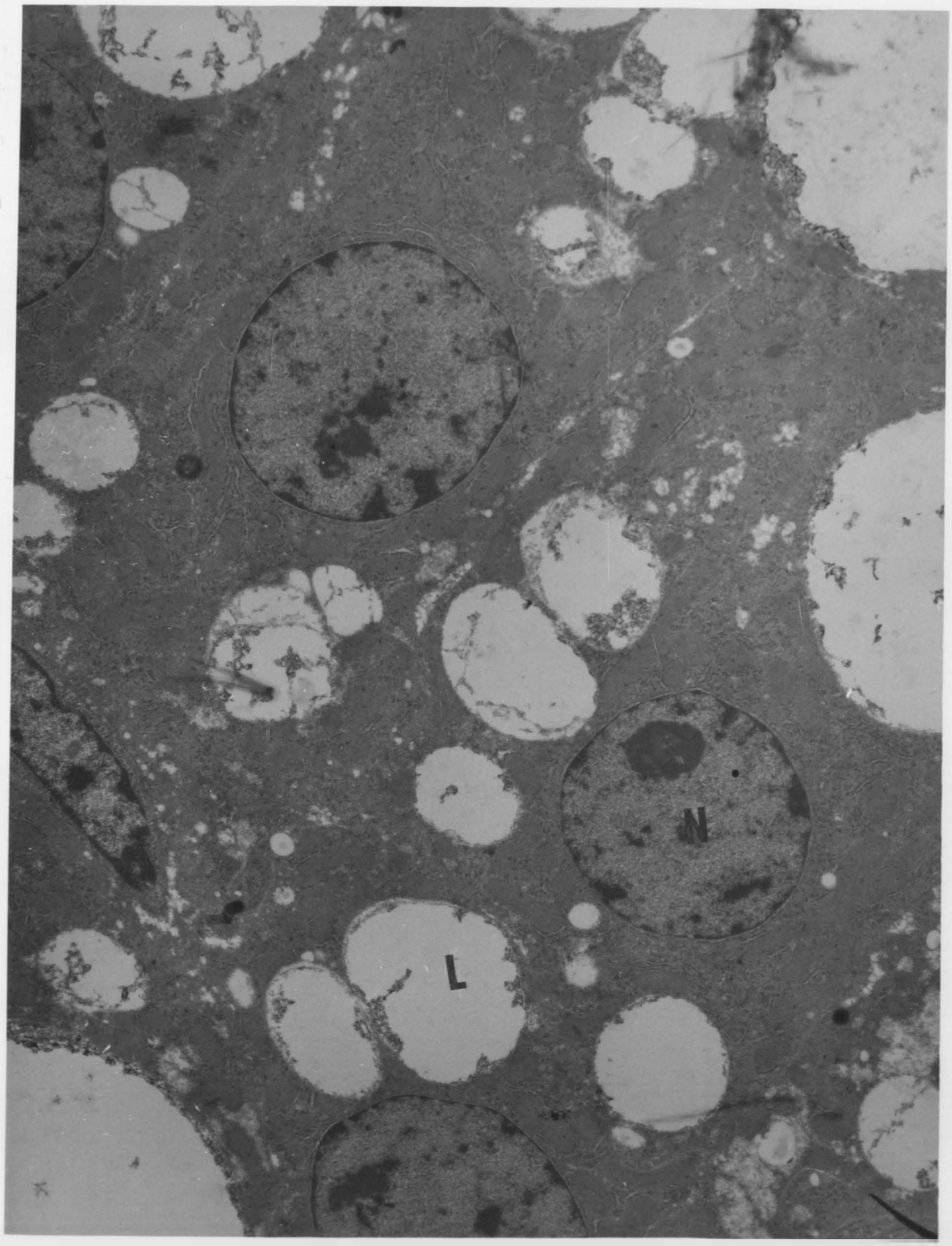
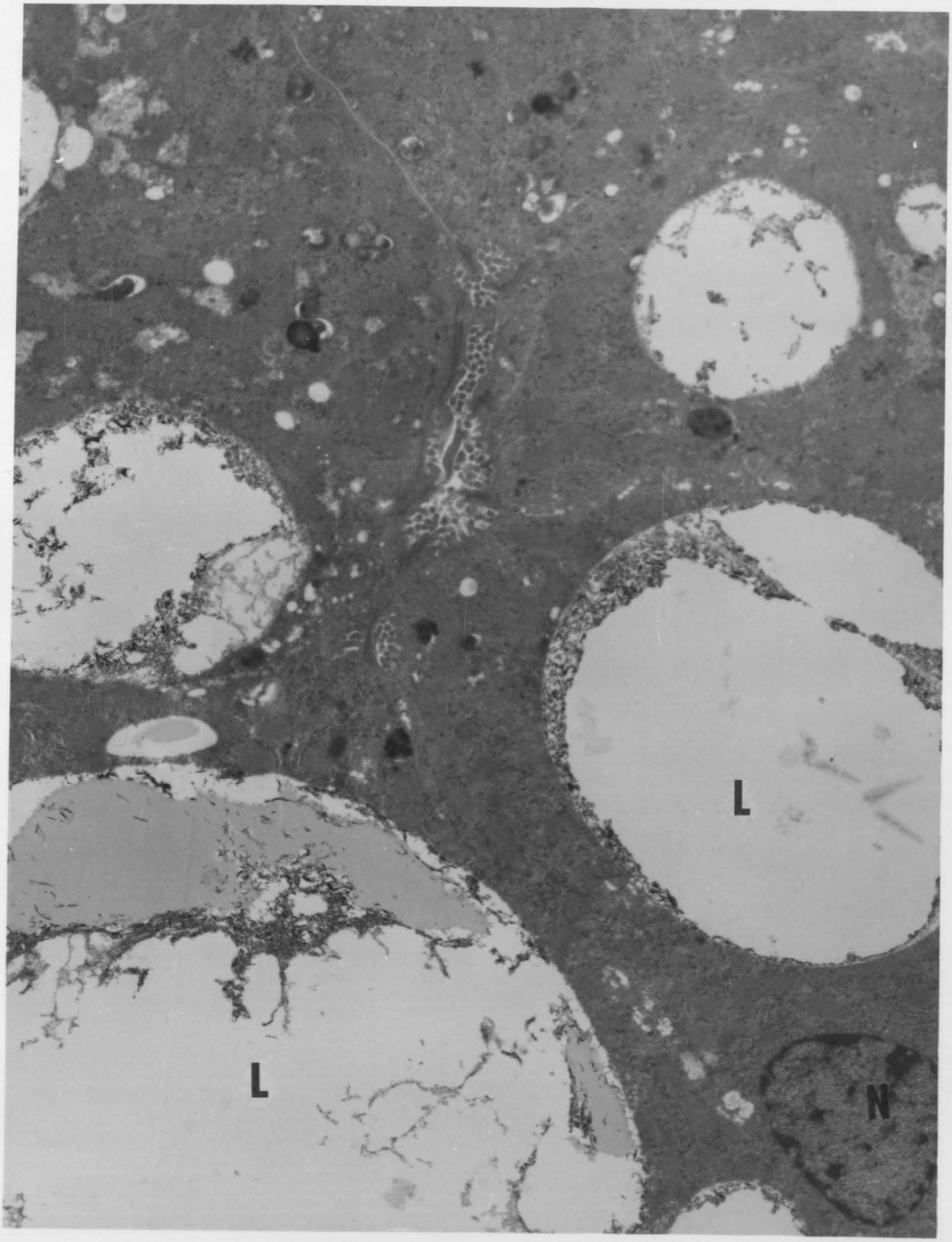

N
L

Figure Eight:

Section of the same liver sample as in Figure Seven, but from a different area showing a major portion of the cytoplasm to be occupied by the lipid droplets (x 12000). L and N stands for Lipid and Nucleus respectively.


L
L
N

GENERAL DISCUSSION

In comparing the different levels of distillers dried grain in both phases of Experiment One, it is noted that there was no significant difference in production or feed efficiency. In phase one, the performance with the diet with 20% DDG was slightly higher than with 30% whereas in phase two it was just the opposite. The drop in production by the higher levels of DDG was quite similar to the work of Jensen et al. (1974) where a higher percentage of DDG depressed production. This was corrected by adding 0.025% L-Lysine. In our experiment however, we did not make any correction for amino acids. Probably 30% DDG was too high a level for maximum production in that Harms et al. (1969) suggested no more than a 10% level of DDG in the diet. Other reports also show that the fermentation by-product should be used at a level 20% or lower. During the second phase of our experiment the hens on the diet with 30% DDG showed somewhat higher production than those on the diet with the 20% level. That is probably due to the factor present in DDG which helped resist the production of FLHS. The lower level did not reduce hemorrhage and liver lipid content significantly.

Improved efficiency by the addition of animal fat as might be expected from the literature was not observed during the first phase of Experiment Two. The reason for this could not be ascertained. However, in the second phase feed efficiency improved. Addition of grease increased liver fat content significantly especially when added

at the 10% level, but the hemorrhage score was same as for birds on the other diets. That is probably due to some other factor involved in causing hemorrhage in addition to the amount of lipid present.

Feed consumptions of hens on the DDG plus oats diet or the oats diet in Experiment Three were significantly less than the controls. Production rates were similar. This was not observed in Experiment One with either level of DDG, possibly due to the shorter duration of Experiment One. Though both the DDG plus oats and the oats diet were isocaloric and feed consumptions were similar, hens on the diet with DDG plus oats had higher liver lipids than hens on only the oats diet. On the other hand both groups had similar incidences of hemorrhages. Combining oats with DDG had a greater effect in preventing hemorrhage than when either ingredient was used alone. This occurred despite higher fat contents in livers. Production data from Experiment One showed that when oats alone is used, production was significantly reduced. This was not observed in Experiment Two and appeared to be corrected by adding DDG with oats.

The serum estradiol level was significantly higher for hens fed the diet with DDG without oats than for hens on the other dietary groups. The hemorrhage score for this group was also higher than for hens fed diets with oats or the mixture of DDG and oats. This suggests that oats or the mixture of oats and DDG in the diet were more effective against FLHS than DDG alone.

Force-feeding a higher percentage of diet has always increased liver lipid and hemorrhage, thus showing that positive energy balance

appears to be the primary factor in producing FLHS. This can be corrected to some extent by unknown factors present in dietary ingredients like oats or DDG.

SUMMARY

Three different experiments were conducted each involving two types of feeding to study the effects of different dietary ingredients on FLHS, production parameters and serum estradiol levels in laying hens. The conclusions are:

1. Birds on diets containing 30% DDG or mostly oats had significantly lower liver lipid percent than birds on the control corn-soy diet or the diet with 20% DDG.

2. Total liver lipid and liver hemorrhage incidence were significantly higher in birds on the 20% DDG diet than for hens on other diets ($P < 0.05$).

3. The diet with 30% DDG was comparatively better than the diet with 20% DDG so far as liver lipid content of hens receiving them was concerned but birds on the 30% DDG diet had a significantly lower feed conversion rate ($P < 0.05$).

4. Hens on diets with 10% grease had significantly higher liver lipid content than birds on diets with oats and produced significantly fewer eggs than birds on other diets ($P < 0.05$).

5. Feed consumption was reduced significantly by the addition of 5 to 10% of grease to the diet ($P < 0.05$) during the force-feeding phase of the study whereas during phase one there was no difference.

6. Hens on diets with oats plus DDG or oats alone showed significantly less hemorrhage incidence than those on other diets ($P < 0.05$).

7. During the ad libitum phase of Experiments One and Three hens on the mostly oats diet consumed significantly less feed ($P < 0.01$) and hens on the oats plus DDG diet consumed significantly less feed ($P < 0.05$) than control hens.

8. In Experiment Three hens on diets with DDG had significantly less body weight gain than hens on other diets ($P < 0.05$).

9. Serum estradiol levels of hens on the DDG diet were significantly higher than for hens on the other diet ($P < 0.05$).

10. In all the experiments force-feeding at 120% of normal intake increased liver lipid content and hemorrhage significantly ($P < 0.05$). In addition to this, liver weights and body weight gains were also significantly higher ($P < 0.05$) in Experiment Two and Three due to force-feeding.

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