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EFFECT OF DIETARY CARBOHYDRATES, CORN OIL, AND SELENIUM ON FATTY LIVER HEMORRHAGIC SYNDROME AND PLASMA ESTRADIOL IN WHITE LEGHORN HENS

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By

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ABSTRACT

EFFECT OF DIETARY CARBOHYDRATES, CORN OIL, AND SELENIUM ON FATTY LIVER HEMORRHAGIC SYNDROME AND PLASMA ESTRADIOL IN WHITE LEGHORN HENS

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Five experiments were conducted to study the relation of plasma steroid hormones (estradiol and progesterone) and dietary sources of energy with intensity of fatty liver hemorrhagic syndrome (FLHS) in Single Comb White Leghorn (SCWL) hens.

In experiments I to IV the experimental birds were on force-feeding schedules for almost three weeks, while in the last experiment (experiment V) all the birds were fed ad libitum.

The results obtained from experiments I to III indicated that force-feeding at a level of 135% of the bird's pre-experimental daily feed intake produced FLHS and increased the concentration of plasma estradiol. Force-feeding did not have any effect on plasma progesterone concentration. The correlation between plasma estradiol concentration and FLHS score ($r_1 = 0.70$, $r_2 = 0.72$ and $r_3 = 0.64$, obtained from experiments I, II and III, respectively), the correlation for hepatic fat levels and FLHS score ($r_1 = 0.74$, $r_2 = 0.81$, and $r_3 = 0.84$), and the correlation of plasma estradiol vs hepatic fat ($r_1 = 0.58$, $r_2 = 0.76$, and $r_3 = 0.61$) indicated that high plasma estradiol and large fat accumulation in the liver are required for FLHS to occur.

In the experiments II to V, either corn oil, corn starch, sucrose, or wheat starch was used to make wheat-soy diets isocaloric to corn-soy diets. Supplementation of wheat-soy diets with corn oil or sucrose produced lower values for FLHS, liver fat and plasma estradiol than using corn starch or wheat starch as an energy supplement in wheat-soy diets. The results revealed that either corn starch or wheat starch used in wheat-soy diets produced almost the same intensity of FLHS as the corn-soy diet.

Experiment V (ad libitum feeding) was conducted to find out if the results obtained by force-feeding could be duplicated by ad libitum feeding. No extensive FLHS was obtained in the experimental birds fed ad libitum for 25 weeks (21-46 weeks of age). The data obtained by ad libitum feeding revealed that using corn oil in the wheat-soy diet had a beneficial effect to reduce liver fat content.

The results from experiment V indicated that dietary selenium at a level of 0.45 mg/kg did not have an effect on hepatic lipid values. Selenium at a level of 0.38 mg/kg of wheat-soy diets used in the force-feeding experiments (experiments II, III and IV) did not reduce FLHS or fatty liver syndrome (FLS) in laying hens.

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I. INTRODUCTION

Fatty liver syndrome (FLS) in laying hens was first reported by Couch in 1956, describing field cases. Fatty liver syndrome is characterized by a high liver fat content. Wolford and Polin (1972a) reported that a high liver fat content alone would not be sufficient for a FLS diagnosis in laying hens. They called the syndrome Fatty Liver Hemorrhagic Syndrome (FLHS), postulating that FLHS was defined by a high liver fat content and the presence of hemorrhages in the liver. These authors held the opinion, that the presence of hemorrhages was of decisive importance, while the increased fat content was a predisposing factor that does not lead to death, whereas hemorrhaging could.

There is not a single factor directly responsible for the development of FLS or FLHS. A number of interrelated, possibly predisposing, factors have emerged. These factors can be classified as (a) genetic factors, (b) factors connected with the housing system, (c) factors in the field of nutrition and (d) hormonal (estradiol) factor.

Differences in FLS or FLHS susceptibility among various breeds or strains of birds in field tests suggest a genetic influence (Nesheim and Ivy, 1970; Garlich et al., 1974; and Campbell, 1979). There may also be an indirect genetic influence. Couch (1956) suggested that breeding for a high egg production level might be one of the factors responsible for the origin of FLS. In contrast to these reports, a number of investigators could not show any genetical effect on FLS or

FLHS (Hartfiel et al., 1973; Jensen et al., 1976a; and Polin and Wolford, 1977).

In the years 1950 to 1960 the number of FLS or FLHS cases in the U.S.A. sharply increased. During that period (more and more) laying hens were kept in batteries and high-energy feeds were introduced (Couch, 1956; Deacon, 1968). As considerably more cases of FLS occurred among flocks housed in batteries (Deacon, 1968), the battery system seemed to be an important predisposing factor.

Temperature is another factor related to housing which may contribute to the development of FLS or FLHS (Griffith et al., 1969; Wolford, 1971; and Pearson and Butler, 1978a). Couch (1956) observed that mortality due to FLS mainly occurs on hot days. Wolford (1971) observed that the liver fat content in birds housed at 1.7°C for 28 days was significantly lower than in hens housed at 26.7°C for the same period.

Factors in the field of nutrition which affect FLS or FLHS can be classified as (a) lipotropic compounds, (b) energy content of the diet, and (c) source of energy used in the diet.

Based on the hypothesis that liver fattening is caused by a defect in, or an overloading of the capacity of the mechanism transporting lipids from the liver cell to the blood, a variety of lipotropic compounds (vitamin E, vitamin B_{12}, choline, methionine, betaine and inositol) in various concentrations have been tested. However, because of the inconsistent, but often negative, results obtained by adding an extra amount of these lipotropic compounds to the diet (Bull, 1968; Parker and Deacon, 1968; Reed et al., 1968; Griffith et al., 1969; Nesheim et al., 1969; Leveille and Bray, 1970; Nesheim et al., 1971;

Wolford and Murphy, 1972; Schexnailder and Griffith, 1973; and Wolford and Polin, 1975), it is not very likely that a deficiency of these compounds will be one of the major causes of FLHS.

Barton (1967), and Ivy and Nesheim (1973) were indeed able to show a positive correlation between the fat content of the liver and the energy level of the diet. This finding agrees with the hypothesis by Polin and Wolford (1976), that FLHS may be caused by a prolonged positive energy balance. At the moment the only way to induce FLHS experimentally is by force-feeding as employed by Wolford and Polin (1972b). This method, based on caloric overconsumption, is a first step towards FLHS research under well-controlled laboratory circumstances.

A number of authors (Griffith et al., 1969; Jensen et al., 1974a, 1976b; Lee et al., 1975; Kim et al., 1976 and Maurice and Jensen, 1977a, 1977b, 1978a, 1978b, 1978c) suggest that the type of grain used in the feed may influence the liver fat content. The results are sometimes conflicting and may be influenced by other factors like the metabolizable energy (M.E.) content of the diet or the percentage or kind of fat used to make isocaloric diets. The importance of the type of feed grain fed in connection with FLHS is therefore not clear at the moment.

Since most non-laying hens do not have FLHS, hormonal balance may be involved in this condition (Barton, 1967). Polin and Wolford (1973) suggested that estradiol was a factor that caused FLHS. This was confirmed (Polin and Wolford, 1977) and then substantiated (Pearson and Butler, 1978b) when 17-β-estradiol-dipropionate caused FLHS in immature chickens. However, the dosages of estrogen used in these experiments were pharmacological rather than physiological. If estrogen were

involved in FLHS of force-fed, then plasma estrogen should probably be elevated in hens with FLHS.

The objectives of this study were: (1) to study the relationship of endogenous plasma estradiol and progesterone level with the FLHS in laying hens; (2) to compare wheat-soy diet with corn-soy diet on the induction of FLHS by force-feeding; and (3) to study the effect of different sources of energy (used to make wheat-soy diet isocaloric with corn-soy diet) on FLHS.

II. REVIEW OF LITERATURE

A. Lipid Metabolism

Dietary lipids ingested by birds apparently are digested and absorbed into the mucosal cells of the small intestine in the same manner as in mammals. The transport of lipids in birds differs from that in mammals in that the portal rather than the lymphatic system plays a dominant role. Fatty acids enter as low density lipoproteins rather than as chylomicrons (Noyan et al., 1964; Bensadoun and Rothfeld, 1972). Bensadoun and Rothfeld (1972) have proposed the name portomicrons for these lipoproteins.

Acetyl-CoA carboxylase, which is activated by biotin, catalyzes the formation of fatty acids. This enzyme is controlled by citrate, which enhances the enzyme activity (Lane et al., 1970), and by long chain acyl-CoA derivatives, produced partly from dietary fatty acids which behave as enzyme inhibitors (Goodridge, 1972 and 1973). Fatty acyl-CoA may inhibit fatty acid synthesis by inhibiting acetyl-CoA carboxylase or by inhibiting the mitochondrial citrate carrier, which in turn reduces the activation of acetyl-CoA carboxylase caused by citrate (Goodridge, 1973).

Adipose tissue is relatively unimportant as a site of fatty acid biosynthesis in the fowl, although it does have the ability to esterify fatty acid to triglycerides (Leveille et al., 1975). The available evidence (Goodridge, 1968; O'Hea and Leveille, 1969; Leveille et al.,

1975), therefore, suggests that in the chickens, and presumably other avian species, fatty acids are synthesized mostly in the liver. These fatty acids are transported as triglycerides in the plasma low-density lipoproteins to the adipose tissue for storage, or, in the case of the hen, to the ovary where they are deposited in the egg yolk (Nesheim and Ivy, 1970). The metabolic activity of the liver is, therefore, extremely high in the fowl, especially during egg production when lipogenesis is high. About half to two-thirds of all lipids found in hen's liver (6g) is required for the production of each egg and the amount synthesized per year is almost equal to the hen's body weight of 1500-2000 grams (Nesheim and Ivy, 1970). The scheme of pathway for synthesis of fatty acids is shown by Figure 1.

Because glycolysis is a main supplier of acetyl-CoA for fatty acid synthesis, a high dietary intake of carbohydrate may be expected to cause a marked increase in the fat content of the liver. This has been demonstrated experimentally on several occasions by feeding a high energy diet ad libitum or by force-feeding (Barton, 1967; Ivy and Nesheim, 1973; Wolford and Polin, 1974).

The rate of hepatic lipogenesis is reduced in mature female chickens (Balnave and Pearce, 1969) by dietary fat. This has been related to a reduction in the specific activities of two of the lipogenic enzymes, the citrate cleavage enzyme, and the malic enzyme. It may also be due to an increase in the levels of long-chain acyl-CoA derivatives which are believed to control the pathway by inhibiting the activity of acetyl-CoA carboxylase and the citrate translocation system (Weiss et al., 1967; Pearce, 1968 and 1971a; Balnave and Pearce, 1969; Leveille et al., 1975).

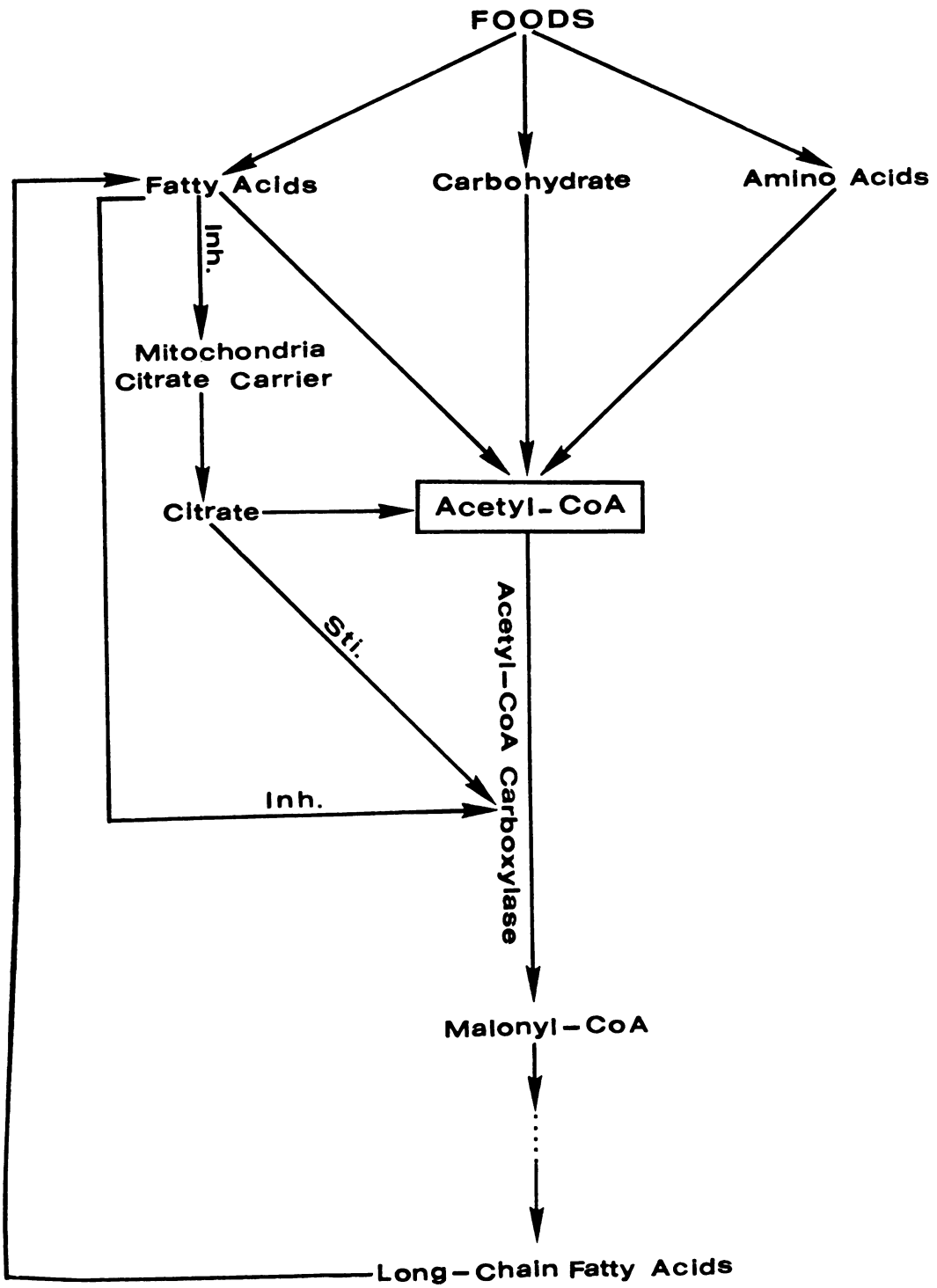

FIG.1. Scheme of pathway for synthesis of fatty acids which is inhibited (inh.) or stimulated (sti.) by fatty acids and citrate respectively.

B. Fatty Liver Syndrome

During the past decades fatty liver syndrome (FLS) has been observed in commercial laying hen flocks as a common disease. This disease which is characterized by an extremely fatty liver and liver hemorrhages, was first described by Couch (1956) in Texas. Since then it has been recognized in other parts of the USA (Ringer and Sheppard, 1963; Nesheim et al., 1969; Wolford, 1971) and in several other countries, including Britain (Anon., 1965), Canada (Bragg et al., 1973), and Australia (Neill et al., 1975, 1977). The term "fatty liver-hemorrhagic syndrome" (FLHS) was first used by Wolford and Polin (1972a) to differentiate it from non-hemorrhagic livers.

During the past years, many factors affecting liver fat content and FLHS have been studied and investigated, including heredity, environment, nutrition, and hormones.

C. Effect of Strain and Breed on Fatty Liver and FLHS

Nesheim and Ivy (1970) reported that the regulation of lipid biosynthesis, transport, energy intake, or even propensity to liver hemorrhage, is likely to be under genetic control. Later, the possibility that liver fat accumulation in laying hens could be affected by strain of bird was investigated by Hartfiel et al. (1973). They found no differences in liver fat among strains of Leghorn hens fed the same diet, but a significantly reduced content in heavy breed hens. Jensen et al. (1976a) also failed to show any differences in liver weight or liver fat between two strains of Single Comb White Leghorns.

In constrast to the above reports, measurement of liver lipid values in 20 strains of laying hens used in the North Carolina Random Sample Test showed a range of 25-49% lipid on a dry weight basis (Garlich et al., 1974). In this experiment four strains had consistently high and four consistently low liver lipid values in the three confinement systems used in the test. There was a marked strain difference in the incidence of hepatic hemorrhage on White Leghorn hens receiving dietary rapeseed meal (Campbell, 1979).

The effect of breed on lipogenesis was studied by Shapira et al. (1978) using heavy and light breed chickens. On ad libitum feeding heavy breed chicks consumed more feed, gained more weight, and deposited more fat than the corresponding light breed chicks. Their lipogenic enzymes were more active than in the light breed chicks in both adipose tissue and liver. Force-feeding increased the weight and fat content of liver and adipose tissue in both breeds. In the experiments conducted by Polin and Wolford (1977), FLHS was induced in immature (11 weeks of age) male and female chickens of broiler and egg-laying breeds by force-feeding and estradiol injection. The results revealed no significant difference between two breeds in the score values used to evaluate FLHS.

D. Effect of Environmental Temperature and Birds Density in the Cage

Over a period of several years, FLHS has been observed during spring and summer months in hens housed in cages or reared in floor pens (Nesheim and Ivy, 1970). Barton (1967) reported that most of the mortality from fatty liver in his experiment occurred during periods of hot weather. One of his experiments on which birds were artificially heated

to 32°C for 12 weeks, showed that mortality was higher from FLHS in a high temperature environment than in a cool environment (10-15.5°C). Griffith et al. (1969) obtained the same results from the experiment in which he observed a correlation of mean liver fat with ambient temperature during the hot summer months. Placing laying chickens in a 1.7°C temperature control room for 28 days significantly reduced total liver lipids in comparison to the values observed when layers were housed at 26.7°C (Wolford, 1971). Similarly in the experiment conducted by Schexnailder and Griffith (1973), liver fat was significantly higher in hens kept at a high temperature (25.5-29.5°C) than for those kept cool (13.0-15.5°C).

Pearson and Butler (1978a) designed an experiment which was carried out to compare lipid levels and hepatic hemorrhages in groups of laying hens when they were housed in isolators at temperatures above and below the optimum (29°C and 13°C). Their diet was formulated to provide 2655 kcal M.E./kg and 142 g crude protein/kg. A temperature of 29°C compared to 13°C caused the total lipid content of the liver to increase by about 60 percent on a fresh weight basis and by over 80 percent on a dry weight basis despite a reduction in feed intake. They concluded that an interaction between environmental temperature and the energy balance is not the only factor involved in the etiology of FLHS and may be of secondary importance.

Barton (1967) reported that fatty livers were produced in birds in the cage more than those kept in floor pens. No difference in total liver lipid was observed between birds housed two or three per 25 X 41 cm (512.5 vs 341.7 cm^2/bird) cage (Owings et al., 1967). Garlich et al.

(1974) observed significantly more liver fat accumulation in hens housed two per cage (563 cm^2 / bird) than those housed seven per cage (440 cm^2/ bird) or in floor pens, the latter supporting the data by Barton (1967).

The effect of cage density was also studied by Jensen et al. (1976c). In this experiment liver fat content and liver weight were significantly greater for hens housed individually (1150 cm^2/bird) than for those housed three per cage (383.3 cm^2/bird). The results revealed that feed intake decreased as cage density increased. They concluded that hens housed individually in cages get more energy and apparently use more calories for non-egg production purposes than do birds housed in multiple birds cages, and these non-egg production calories were reflected in a significant increase in liver fat.

E. Nutritional Effect

Vitamin treatment has been used as a therapeutic approach toward FLS or FLHS by several investigators. The fatty livers produced by feeding a beef liver fraction to rats was only slightly affected by giving choline, but was completely prevented by the simultaneous administration of lipocaic by rats (Gavin and McHenry, 1941). In this experiment inositol prevented the accumulation of both fat and cholesterol in the liver.

In the course of studies to determine the effect of manganese and choline on bone formation in the rat, Amdur et al. (1949), found that manganese as well as choline prevented the deposition of excess fat in the liver. The lipotropic action of manganese was much greater when the choline content of the diet was low.

While studying the nicotinic acid-tryptophan relationship in rats on a 9% casein ration containing 5% fat and 0.2% cystine and choline, yellowish livers with 15% fat were observed at autopsy (Singal et al., 1949). The addition of threonine reduced the liver lipid to 5.1%.

Griffith et al. (1969) investigated the problem of FLS in the laboratory by evaluating the effect of choline and other lipotropic agents on liver fat and performance of laying hens. They observed that a dietary supplement of choline (850 mg per kg) significantly reduced liver fat in hens fed a basal diet calculated to contain about 850 mg choline per kg, while vitamin B_{12} and methionine supplements failed to reduce significantly the liver's fat.

The choline deficiency in rats is primarily a methyl group deficiency since if methionine is supplied in sufficient amounts, supplemental choline is not necessary to prevent fatty livers in rats fed these diets (Nesheim et al., 1969). Since vitamin B_{12} and folic acid are concerned with the synthesis and transfer of methyl groups in the body, a deficiency of these nutrients will affect choline biosynthesis in rats (Nesheim et al., 1969). Therefore, under choline-deficient conditions, these nutrients can affect liver fat content. In addition, choline-deficient hens did not have liver fat values approaching those of hens dying from fatty liver hemorrhages, and no hens died from liver hemorrhages when they were fed choline-deficient diets. Nesheim et al. (1971) were unable to show any effect on liver fat content by choline supplementation of practical diets. They did, however, observe some effects of choline to decrease liver fat content when the vitamin was added to purified diets, but even this effect was not observed in all experiments. Wolford and

Polin (1975) did not detect any significant decrease in liver fat of hens fed practical diets supplemented with choline, vitamin E, vitamin B_{12}, lecithin, inositol or iodinated casein either as individual nutrient additions, or in various combinations. Their studies confirmed the observations of Bossard and Combs (1970) on choline and inositol supplementation, of Wolford and Murphy (1972) on inositol or a combination of choline, vitamin E, and vitamin B_{12}, and Jensen et al. (1974b) on inositol, vitamin B_{12}, choline and vitamin E.

In contrast to the above negative results on FLS or FLHS by choline and B_{12} in practical-type rations, Schexnailder and Griffith (1973) confirmed their previous observations that choline, choline and B_{12}, or methionine and B_{12} had the positive effect of lowering significantly the levels of liver fat in adult hens (Griffith et al., 1969; Griffith and Schexnailder, 1972). Successful treatment of FLHS in field cases by treatment with choline, vitamin E, vitamin B_{12}, and/or inositol were reported by Bull (1968), Reed et al. (1968), Parker and Deacon (1968), and partial success by Deacon (1968). On the other hand, Ragland et al. (1970), Leveille and Bray (1970), and Griffith and Schexnailder (1972) have found that inositol had no effect on liver fat of hens fed ad libitum in controlled laboratory experiments.

Wolford and Polin (1975) observed that FLHS was not prevented by lecithin, iodinated casein alone, or with inositol in force-feeding experiments. However, the mixture of choline, vitamin B_{12} and vitamin E without inositol appeared to reduce slightly (non-significantly) the lipid and hemorrhagic score of livers in some of the force-fed hens.

Griffith and Schexnailder (1972) noted that added quantities of vitamin E as well as other water-soluble vitamins (Schexnailder and Griffith, 1973) had no effect on reducing the liver fat of hens.

The possibility that selenium may be a factor which could influence liver fat content was suggested by the work of Karpov (1967), who indicated that supplementation of practical rations with selenium (0.9 mg per kg) reduced the incidence of lipid dystrophy of the liver in laying hens whose requirement for selenium according to N.R.C. (1977) is 0.1 mg per kg of diet or 0.1 PPM. Adding 1 PPM selenium to corn-soybean meal rations, with and without added fat, significantly reduced total fat accumulation per liver (Jensen et al., 1974b).

In the experiments conducted by Jensen et al. (1974a), hens fed the basal corn ration had significantly more liver fat than hens fed diets containing wheat replacing corn. Liver fat accumulation was not significantly increased by feeding a wheat diet made isocaloric with the corn diet by adding 3.8% animal grease.

Fatty livers were observed in laying hens fed a diet with a high level (8%) of dietary animal tallow or rapeseed oil, while soybean and sunflower oil showed protection against fatty liver at levels of 1, 2, 4 and 8% (Bragg et al., 1973). Barton (1967) included a high level of fat in a low energy diet and observed no increase in liver fat accumulation.

Liver fat accumulation was significantly lower for hens fed a wheat-pea-fat (6% animal grease) diet than for hens fed a corn-soy diet with (4% animal grease) and without added fat (Jensen et al., 1974b).

No difference in liver weight, liver fat content, and total fat per liver was observed among hens fed diets containing either zero, one-half,

or all glucose monohydrate substituted for corn (Jensen et al., 1976b). Hens fed diets containing corn had significantly more liver dry matter, liver fat, and total fat per liver than hens fed the isocaloric diets containing wheat.

Experiments on the effect of a dietary cereal source on hepatic and plasma lipids (Barton, 1976; Jensen et al., 1974a, 1974b; Maurice and Jensen, 1977a) indicate that corn accelerates or wheat depresses lipid accumulation in the hen's liver. The study conducted by Maurice and Jensen (1977b) assessed hepatic in vivo and in vitro lipogenic activity and fatty acid composition in Japanese quail fed isocaloric and isonitrogenous corn-soy and wheat-soy diets. In vivo lipogenesis was significantly increased in females fed the corn-soy diet. Incorporation of [1-C^{14}]-acetate (d.p.m./liver.100 gm body weight./0.5 hr.) into liver lipid was 153 x 10^3 and 5.2 x 10^3 in females and males fed the corn-soy diet compared to 87 x 10^3 and 4.1 x 10^3 in females and males fed the wheat-soy diet. They indicated that feeding the corn-soy diet results in enhanced lipogenesis and altered hepatic fatty acid composition in laying females. The liver weight relative to body weight, liver lipid content, and plasma lipid concentration were greater in Japanese quail fed on corn-containing diets compared to those fed on wheat-containing diets (Maurice and Jensen, 1978a).

Maurice and Jensen (1977b) demonstrated that differences in hepatic and plasma lipid concentration due to dietary cereal source are significant 14 days post-treatment and that a water extract of wheat bran contains an antihyperlipidemic factor. Factors other than energy intake appear to be important determinants of hepatic and plasma lipids under

normal feeding conditions in laying birds (Barton, 1967; Jensen et al., 1974a, 1974b, 1976a, 1976b; Maurice and Jensen, 1978a).

Maurice and Jensen (1978b) showed the effectiveness of brewers dried grains and mill by-products in reducing hepatic and plasma lipids and the incidence of liver hemorrhage in cage layers fed equicaloric and isonitrogenous corn-soy diets. They conducted two more experiments (Maurice and Jensen, 1978c) to study the effect of 20% brewers dried grains, 10 to 20% distillers dried grains, and 10% distillers dried solubles in equicaloric-equifat and isonitrogenous corn-soy diets on hepatic lipid accumulation in caged layers. The results demonstrated the effectiveness of brewers and distillers fermentation by-products in controlling liver lipid accumulation in caged layers fed equicaloric-equifat and isonitrogenous corn-soy diets. They concluded that the response is not related to the level of dietary fat and appears to be induced by an intrinsic nutritional property of these by-products.

Pearson et al. (1978) conducted an experiment in which isocaloric diets supplying about 2900 kcal/kg and based on corn, wheat, and barley respectively, were fed to pullets for 13 weeks from point of lay. The number of cases of subclinical FLHS and the mean total lipid, triglyceride and monoglyceride content of the liver decreased in the above order of diets. But the final body weight, weight gain, and abdominal fat score followed the order wheat greater than corn and corn greater than barley.

Maurice et al. (1979) designed two experiments to investigate the influence of dietary protein source and level on hepatic and plasma lipids and incidence of liver hemorrhage in cage layers fed equicaloric, equifat, and, where appropriate, isonitrogenous diets. FLHS was induced

under ad libitum feeding in birds fed the corn-soybean diet. The corn-fish meal diet reduced liver weight, hepatic and plasma lipids, and incidence of liver hemorrhage.

Experiments designed by Nesheim and Ivy (1970) were to evaluate if excessive deposition of fat in the liver and body of the laying hen may be a result of an inability of some hens to regulate energy intake as well as others. Three diets with M.E. values of 2970, 2530, and 3410 kcal/kg were fed to hens for varying periods of time. The results revealed that over a two-month period the feed intake of hens adjusted to the three diets; however, energy intake of hens fed the lowest energy diet was somewhat below that of hens fed the diet with 2970 kcal/kg; whereas, those fed the high energy diet consumed somewhat more calories. When the diets were fed for 16 days, the adjustment to the diet with 2530 kcal/kg was evident, but the average feed intake over the 16 days was about the same with the other diets. The liver fat content was least in hens fed the low energy diet even after only 16 days. Birds fed high energy (3000-3600 kcal/kg) diets usually "over-consume" calories and gain more in weight than birds fed lower energy (2200-2800 kcal/kg) diets (Morris, 1968).

Wolford and Polin (1972a) revealed that adult female chickens released from restricted feeding regimens overate during a withdrawal period of two months and exhibited fatty livers with a low incidence of FLHS. This observation called attention to the possible involvement of over-consumption of feed (energy) as a possible cause of FLHS. With this in mind, Wolford and Polin (1972b) used the technique of force-feeding to produce, in the laboratory, liver hemorrhages characteristic

of those seen in commercial layers afflicted with FLHS. The data obtained implicate over-consumption of feed as one cause of FLHS. The chickens exhibited the disease in 3 weeks as compared to the 8-15 weeks under field condition of feeding high energy diets (Polin and Wolford, 1973).

When laying hens were fed diets that were either low or high in caloric density, the liver's fat level changed as a positive correlation to the energy level of the diet (Barton, 1967). In the experiment conducted by Wolford and Polin (1974) the weights of liver components (water, lipid, and non-lipid dry weight) were increased in adult female chickens proportional to the amounts of feed force-fed for 21 days at daily amounts of up to 50 percent more than ad libitum fed control birds. Hepatic hemorrhages were induced in these force-fed birds and the hemorrhagic score, in particular, as well as the hemorrhagic incidence were directly related to the total daily feed intake and to hepatic fatty metamorphosis.

The effect of sources of energy and positive energy balance on the FLHS induction were studied by Polin and Wolford (1976). Adult female chickens were force-fed a corn-soy diet at 150% of the daily amount consumed by those allowed the same diet ad libitum. Other hens were force-fed diets isocaloric to the 150% group just mentioned, but diet composition was adjusted so that 2/3 of the M.E. came from the corn-soy diet and 1/3 from either corn oil or glucose; or force-fed a low energy diet accounting for 2/3 of the M.E. and corn oil 1/3 of M.E., or a purified diet accounting for all M.E. Liver hemorrhages were induced in all force-fed groups with only the low energy diet plus corn oil having produced a significantly lower score for FLHS.

F. Hormonal Effect

The first suggestion that birds might be fattened for market by estrogen administration was made by Lorenz (1943) with data on the effect of subcutaneous implantation of diethylstilbestrol pellets in cockerels. Heller and Thayer (1948) produced lipemia with estrogens in both chickens and turkeys. Endogenous production of estrogens in the female fowl and the administration of estrogen to sexually immature fowl result in an increase in the total lipid content of blood and liver (Lorenz, 1954). Ranney and Chaikoff (1951) have established that the liver is the essential organ involved in the lipaemia induced by estrogens.

Husbands and Brown (1965) have shown that more [C^{14}]acetate is taken up into the liver lipids of the laying hen than of the mature cockerel. Aftergood and Alfin-Slater (1965) indicated that "both androgen and estrogen exert an influence on lipid metabolism in the rat."

The data obtained by Hill et al. (1958) on body composition and tissue gains indicated that the increase in fat synthesis by the estrogen-treated chick under full-feeding conditions was accompanied by reduced nitrogen retention.

Experiments were carried out by Balnave (1968a) to investigate the changes in the lipid composition of the blood and liver of the male chicken in response to the gonadal hormones, estrogen, androgen, and progesterone. Estrogen was the only hormone to affect liver weight, liver dry matter percentage, or total liver lipid content significantly.

Although combined treatment with estrogen and androgen or progesterone induced increased oviduct growth (Common et al., 1947; Brant and Nalbandov, 1956) neither combination had any effect on the lipaemic

response to estrogen alone (Lorenz, 1954; Common et al., 1948; and Balnave, 1968b).

When the pullet comes into lay there is an increase in hepatic lipogenesis and this is reflected in the specific activities of the lipogenic enzymes, ATP-citrate lyase and NADP-malate dehydrogenase, which are greater in the laying hen than in the non-laying pullet (Pearce, 1971b), suggesting that endogenous estrogen may have a controlling influence.

Experiments were undertaken by Balnave and Pearce (1974) in an attempt to clarify the roles of estrogen, androgens, and progesterone in lipid metabolism in the liver of the domestic fowl. The results revealed that estradiol injection increased liver weight as early as one day after hormone administration. However, increases in the liver lipid content after estradiol or mixed hormone (estradiol and testosterone) treatment were not manifested so rapidly and it was only on day 4 of hormone treatment that the liver lipid content of birds receiving either of these treatments was significantly increased compared with the other treatment groups. The results also indicated that the build-up of lipid in the livers of birds given estradiol was much greater and more rapid than in birds given the mixed hormone treatment although by day 8 or 9 of hormone administration the differences between these treatments were greatly reduced. These results confirm the observations of Balnave (1968b) who found that of the three hormones (estradiol, progesterone, and testosterone) studied, estradiol was the only one to show any effect on the weight or lipid content of the livers after 10 days of hormone treatment.

Barton (1967) stated that "since most non-layers do not have fatty livers, hormonal balance may be involved in this condition." Polin and Wolford (1973) suggested that estrogen is a factor inducing FLHS along with the necessity for the chicken to be in a positive energy balance creating sufficient hepatic fat as a prime for FLHS to occur.

The above concept of the hormone-energy interrelationship in the induction of FLHS was explored in experiments (Polin and Wolford, 1977) using immature female and male chickens of broiler-type and egg laying strains. Force-feeding three times a day for 21 days, amounts of feed equal to 125% and 150% of ad libitum intake, produced a gradient response in hepatic steatosis (measured by liver fat content and the ratio of fat to the fat-free dry weight), but not FLHS. Intramuscular injection of 17-β-estradiol dipropionate at 2 mg/kg body weight, three times weekly for 21 days, produced a gradient response in hemorrhagic 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 150% level, produced lesser increases in feed intake and liver fat as did estrogen, but no hepatic hemorrhaging. Thus, estrogen is a factor in the production of FLHS along with the necessity for the chicken to be in a positive energy balance creating sufficient hepatic fat for FLHS to occur. Similarly, Pearson and Butler (1978b) reproduced FLHS in the immature fowl by also giving large doses of 17-β-estradiol dipropionate, and reported that the effect is related to the dose. When feed was withdrawn after the last injection of the estradiol, the FLHS score was decreased dramatically, but withdrawal of feed between injections resulted in no appreciable alteration in the

hemorrhage score. The results also illustrated the well-known effect of estrogens on the lipid content of the liver which was increased by over two-fold by the highest dose.

The dosages of estrogen used in the above experiments (Polin and Wolford, 1977; Pearson and Butler, 1978b) were pharmacological rather than physiological. If estrogen is involved in FLHS induction, then plasma estrogen should be elevated in hens with FLHS. Plasma estrogen was higher in birds with FLHS and liver lipid was positively correlated with plasma estrogen (Maurice et al., 1979).

III. EXPERIMENTAL PROCEDURES

A. Introduction and Concepts

Experiment #1

This experiment was conducted to determine the relationship of plasma estradiol and progesterone levels to FLHS in laying hens. FLHS was induced by force-feeding (Wolford and Polin, 1972b) and then the Plasma concentrations of estradiol and progesterone were measured by radioimmunoassay (RIA).

Experiment #2

Corn-soybean meal diets enhance lipogenesis. The liver weight and liver fat content of hens fed a corn-soybean meal diet are higher than those of hens fed wheat-soybean meal diets (Barton, 1967; Jensen et al., 1974a, 1976a; Maurice and Jensen, 1977a, 1977b). The wheat-soybean meal diets used in all of these experiments were made isocaloric to the corn-soybean diet by using fat. On the other hand, dietary fats reduce the rate of hepatic lipogenesis in mature female chickens due to its long-chain fatty acids content (Weiss et al., 1967; Pearce, 1968 and 1971a; Balnave and Pearce, 1969; Leveille et al., 1975). Thus, fat added to a wheat-soybean meal diet to raise its energy value to be isocaloric to a corn-soybean meal diet could be the agent responsible for the hepatic fat content being lower in hens fed wheat-soybean meal diet. To evaluate this concept experiment #2 was conducted to compare

corn oil and corn starch in two different wheat-soybean meal diets isocaloric to corn-soybean meal diet.

Experiment #3

This experiment was designed for further investigation of the effect of dietary energy sources on FLHS.

Experiment #4

Experiments #2 and #3 revealed that corn starch had increased FLHS. To resolve its action as a derived from grain or generally attributable from carbohydrates, experiment #4 was conducted using corn oil, corn starch and wheat starch to make wheat-soybean meal diets isocaloric to a corn-soybean meal diet.

Experiment #5

Force-feeding had been used to induce FLHS in all of the above experiments. Experiment #5 was conducted to find out if the same results obtained by force-feeding could be duplicated by ad libitum feeding.

Maurice et al. (1979) indicated that selenium at 0.3 mg/kg diet, supplemented as a salt in a corn-soybean meal diet, or at 0.17 mg/kg diet, mostly from fish meal in a corn-fish meal diet, possesses an anti-hepatohemorrhagic property in caged hens. Thus, experiment #5 was also conducted to study further the anti-hepatohemorrhagic effect of selenium.

B. General Procedure

All experiments were conducted in an environmental room at 23±1°C. The lighting period for all experiments was identical (14 hours a day).

Mature SCWL hens housed individually in 20.3 X 40.6 cm wire cages, were used in all experiments. All the experiments lasted 3 weeks except for experiment #5 which lasted 25 weeks. Feed intake and egg production were recorded for all experiments. Feed intake was measured for individual hens over a period of ten days before force-feeding was started. The amounts of feed force-fed were calculated on the basis of each hen's pre-experimental feed intake. The force-fed birds were also allowed feed ad libitum. To prepare homogenized liquid feed for force-feeding, one part diet to 1.5 parts of water were homogenized in a blender for 1.5 minutes and then the mixture was forced by a metal syringe through a 1 cm diameter stainless steel tube directly into the crop (Wolford and Polin 1972b). The liver vascular damage was evaluated (Wolford and Polin 1972b) on a graded score of one to five (1 = no hemorrhages, 2 = 1 to 5 hemorrhages, 3 = 6 to 15 hemorrhages, 4 = 16 to 25 hemorrhages, 5 = greater than 25 hemorrhages, as well as massive hemorrhages). Then the livers were ground, dried in a vacuum oven at 500-600 mm Hg and 50°C., and analyzed for fat content by hexane extraction (AOAC 1975).

The diet's M.E. values were measured in the last week of all experiments except in the first experiment. The amount of feed consumed and the quantity of excreta produced by four birds of each group of treatments were measured for a period of four days. The excreta was air-dried at room temperature and then ground. The gross energy of excreta and feed were measured using an adiabatic calorimeter (Parr Instrument Company). From the total gross energy consumed during four

days was subtracted total gross energy of excreta collected during the same period to arrive at the metabolizable energy (not corrected for retained nitrogen) of diet.

C. The Experiments

1. Experiment #1

Twenty-seven SCWL adult chickens at 32 weeks of age in cages were randomly divided into 3 groups of nine birds. The controls were fed ad libitum while the two other groups were force-fed three times each day a total amount of feed equal to either 120% or 135% of each hen's pre-experimental feed intake. A practical type (corn-soybean meal) mash diet was used, containing 16.9% protein and 3 kcal of M.E. per gram (Table 1). The experiment lasted 21 days during which feed intake and egg production were recorded. At the end of the experiment, all birds were weighed and blood (5 ml) withdrawn by cardiac puncture. Then the hens were sacrificed by cervical dislocation. Upon death, the liver was removed, its vascular damage evaluated, and then analyzed for fat (AOAC 1975).

2. Experiment #2

Thirty-six laying hens (SCWL), 36 weeks of age, were weighed and randomly divided into 4 groups of nine birds and assigned to receive one of four treatments. The controls were fed the corn-soybean meal diet (Table 2) ad libitum, and the other groups were either force-fed a corn-soybean meal diet or one of the wheat-soybean meal diets at a level of 135% of each bird's pre-experimental daily feed intake. Soybean meal was the major source of protein in all diets. Ground yellow corn

Table 1. Composition of experimental diet (Experiment #1).

Ingredients	Parts per 1,000
Corn, #2 yellow	618.00
Soybean meal, 48%	200.0
Meat meal, 55%	30.0
Alfalfa leaf meal, 17%	20.0
Corn oil, stable[1]	35.2
Limestone	75.0
Dicalcium phosphate	12.0
Salt	3.0
Vitamin mix[2]	5.0
Mineral mix[3]	0.5
DL-Methionine	1.3
Protein, %	16.9
Metabolizable energy, Kcal/g	3.1

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet: retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3] Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron, 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

Table 2. Composition of diets in the second experiment.

Ingredients	Parts per 1,000		
	Corn-soy diet	Wheat-soy-corn oil diet	Wheat-soy-corn starch diet
Yellow Corn	677.0	-----	-----
Wheat	-----	679.2	572.2
Soybean meal (48%)	208.0	188.0	208.0
Corn oil, stable[1]	17.3	31.0	17.3
Corn starch	-----	-----	113.7
Salt	3.0	3.0	3.0
Solka-floc	6.5	13.0	-----
Limestone	64.6	64.6	64.6
Defluorinated phosphate	17.6	14.2	14.2
Vitamin mix[2]	5.0	5.0	5.0
Mineral mix[3]	0.5	0.5	0.5
DL-methionine	0.5	1.5	1.5
Protein (%)	15.90	15.90	15.90
M. E. (Kcal/kg)	2960	2960	2960

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet; retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3] Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

was the major source of energy in corn-soybean meal diet. In other experimental diets (wheat-soybean meal diets) wheat replaced all of the yellow corn as the major source of energy. Either corn oil or corn starch was then used in the wheat-soybean meal diets to make them isocaloric to the corn-soybean meal diet (Table 2). The experiment lasted 21 days and all the birds were individually weighed at the termination of the experiment. Blood was drawn by cardiac puncture for hormone (estradiol and progesterone) analysis by radioimmunoassay (RIA). All the birds were then killed by cervical dislocation. The livers were removed, weighed, scored for hemorrhages, and then analyzed for fat.

3. Experiment #3

Forty, 38-week old hens (SCWL) were weighed and divided into 5 groups of 8 birds each. Each group of birds was then assigned to one of 5 treatments. The control birds were fed a corn-soybean meal diet (Table 2) ad libitum, while the other 4 groups were force-fed one of the three dietary treatments in Table 2 or the diet in Table 3. The latter diet of wheat-soybean meal was sucrose-supplemented to make the diet isocaloric to the corn-soybean meal diet. Force-feeding was conducted at the level of 135% of an individual's daily energy intake established during the pre-experimental period (10 days). The experiment lasted 24 days during which egg production, feed intake and energy balance were measured. At the termination, all birds were weighed, blood (5 ml) was withdrawn by cardiac puncture for estradiol and progesterone analysis, and then the birds were sacrificed by CO_2. The liver was removed to obtain the FLHS score and then analyzed for fat.

Table 3. Composition of wheat-soybean-sucrose diet (Experiment #3).

Ingredients	Parts per 1,000
Wheat	599.2
Soybean meal (48%)	200.0
Corn oil, stable[1]	17.3
Sucrose	94.7
Salt	3.0
Limestone	64.6
Defluorinated phosphate	14.2
Vitamin mix[2]	5.0
Mineral mix[3]	0.5
DL-methionine	1.5
Protein (%)	15.90
M.E. (Kcal/kg)	2960

[1]Ethoxyquin added to supply 125 mg/kg diet.

[2]Supplies/kg diet; retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3]Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron, 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

4. Experiment #4

The same treatments used in experiment 3 were duplicated in this experiment except that a wheat-soybean-sucrose diet was replaced by a wheat-soybean-wheat starch diet (Table 4). All the experimental procedures used in experiment 3 except for plasma hormone analyses, were conducted in experiment 4.

5. Experiment #5

This experiment was carried out in 3 stages with different practical-type diets. In the first stage (7 weeks) the birds were fed ad libitum either the corn-soybean diet or one of 3 different wheat-soybean diets (Table 5). Soybean meal and fish meal were the major sources of dietary protein in all diets (18.7% protein). Ground yellow corn or ground wheat were the major source of energy in corn-soybean or wheat-soybean diets, respectively. Wheat-soybean diets were calculated to be isocaloric (2950 kcal/kg) to the corn-soybean diet, using corn oil, corn starch, or wheat starch as explained in experiment #3.

Ninety-six SCWL pullets, 21 weeks old, were allocated into 12 groups with 8 birds per replication and 3 replications per treatment. The birds were fed ad libitum the experimental diets composed of 18.7% protein and 2950 kcal M.E./kg (Table 5) for 7 weeks (from 3rd of July to 21st of August, 1980) during which feed intake and egg production were measured. At the end of week 7 all birds were weighed and then the second stage of the experiment was implemented for 8 more weeks. Two of the three groups of hens from each treatment were randomly selected to be fed ad libitum the corresponding experimental diet with 16.3% protein

Table 4. Composition of wheat-soybean-wheat starch diet (Experiment #4).

Ingredients	Parts per 1,000
Wheat	572.2
Soybean meal (48%)	208.0
Corn oil, stable[1]	17.3
Wheat starch	113.7
Salt	3.0
Limestone	64.6
Defluorinated phosphate	14.2
Vitamin mix[2]	5.0
Mineral mix[3]	0.5
DL-methionine	1.5
Protein (%)	15.90
M.E. (Kcal/kg)	2960

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet; retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg;riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3] Supplies/kg diet; manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron, 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

Table 5. Composition of experimental diets used in the first stage of Experiment #5.

Ingredients	Parts per 1,000			
	Corn-Soy Diet	Wheat-Soy Corn Oil Diet	Wheat-Soy Corn Starch Diet	Wheat-Soy Wheat Starch Diet
Corn	632.0	-----	-----	-----
Wheat	-----	635.0	534.0	534.0
Soybean meal (48%)	194.0	175.0	194.0	194.0
Fish meal (61%)	65.0	65.0	65.0	65.0
Corn oil, stable[1]	16.0	29.0	16.0	16.0
Corn starch	-----	-----	106.0	-----
Wheat starch	-----	-----	-----	106.0
Solka-floc	6.0	11.0	-----	-----
Limestone	62.0	62.0	62.0	62.0
Defluorinated phosphate	16.0	13.1	13.1	13.1
Salt	3.0	3.0	3.0	3.0
Vitamin mix[2]	5.0	5.0	5.0	5.0
Mineral mix[3]	0.5	0.5	0,5	0.5
DL-methionine	0.5	1.4	1.4	1.4
Protein (%)	18.7	18.7	18.7	18.7
M.E. (Kcal/kg)	2950	2950	2950	2950

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet: retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3] Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

and 3014 kcal M.E./kg (Table 6). The third group from each treatment was used to study the effect of selenium on FLHS. Thus, 8 more treatments were added to the experiment during the second period. Eight birds of the third group in each treatment were divided randomly into two groups of 4 birds each. One group was fed a diet not supplemented with selenium while the second group received a diet supplemented with 0.3 mg selenium per kg (Table 7).

The period for the second stage of the experiment was 8 weeks during which feed intake, egg production and caloric balance were measured. At the end of week 8 the birds of the first group of each treatment, T_1, T_2, T_3, and T_4 (Table 6) were weighed and then sacrificed by CO_2. The liver was removed, scored for hemorrhages and then analyzed for fat. The FLHS score values obtained for these birds were very low (lower than 2) indicating that none of the experimental diets was causing FLHS at this early stage. The birds were only 36 weeks of age at this time and egg production was excellent (90%). Thus, the experiment was continued using the remainder of the birds, which received the same diets they had been fed, for 10 more weeks (third stage of the experiment). During the third stage feed intake and egg production were recorded. At the termination day, all the birds were weighed and then killed by CO_2. Liver hemorrhages were scored and then all the livers were analyzed for fat determination.

D. Radioimmunoassay Procedures

1. Estradiol Radioimmunoassay

The radioimmunoassay (RIA) used for estradiol determination was a modification of that by Oxender et al., 1977. The procedure was

Table 6. Composition of experimental diets (Experiment #5, stages 2 and 3).

Ingredients	Parts per 1,000			
	Corn-Soy Diet	Wheat-Soy Corn Oil Diet	Wheat-Soy Corn Starch Diet	Wheat-Soy Wheat Starch Diet
	T_1	T_2	T_3	T_4
Corn	629.0	-----	-----	-----
Wheat	-----	635.0	534.0	534.0
Soybean meal (48%)	134.0	115.0	144.0	144.0
Fish meal (61%)	65.0	65.0	65.0	65.0
Corn starch	-----	-----	166.0	-----
Wheat starch	-----	-----	-----	166.0
Corn oil, stable[1]	16.0	31.5	16.0	16.0
Solka-floc	6.0	10.5	-----	-----
Limestone	62.0	62.0	62.0	62.0
Defluorinated phosphate	16.0	16.0	16.0	16.0
Salt	3.0	3.0	3.0	3.0
Vitamin mix[2]	5.0	5.0	5.0	5.0
Mineral mix[3]	0.5	0.5	0.5	0.5
DL-methionine	0.5	0.5	0.5	0.5
Protein (%)	16.3	16.3	16.3	16.3
M.E. (Kcal/kg)	3014	3014	3014	3014

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet: retinylpalmitate 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U ; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts per 1,000 of diet.

[3] Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron 20 mg; magnesium, 490 mg; selenium, 0.10 mg.

Table 7. Composition of experimental diets (Experiment #5, stages 2 and 3).

Ingredients	Parts per 1,000							
	Corn-Soy Diet	Corn-Soy Diet+Se	Wheat-Soy-Corn Oil Diet	Wheat-Soy-Corn Oil Diet+Se	Wheat-Soy-Corn Starch Diet	Wheat-Soy-Corn Starch Diet+Se	Wheat-Soy-Wheat Starch Diet	Wheat-Soy-Wheat Starch Diet+Se
	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Corn	674.0	673.0	-----	-----	-----	-----	-----	-----
Wheat	-----	-----	655.5	654.5	375.0	374.0	375.0	374.0
Soybean meal (48%)	217.0	216.5	206.0	205.5	256.0	255.5	256.0	255.5
Corn starch	-----	-----	-----	-----	260.0	260.0	-----	-----
Wheat starch	-----	-----	-----	-----	-----	-----	260.0	260.0
Corn oil stable[1]	22.0	22.0	41.0	41.0	22.0	22.0	22.0	22.0
Solka-floc	-----	-----	10.5	10.5	-----	-----	-----	-----
Limestone	62.0	62.0	62.0	62.0	62.0	62.0	62.0	62.0
Defluorinated phosphate	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix[2]	5.0	5.0	5.0	5.0	5,0	5.0	5.0	5.0
Mineral mix[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Selenium permix (0.02%)	-----	1.5	-----	1.5	-----	1.5	-----	1.5
TOTAL	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Protein (%)	16.3	16.3	16.3	16.3	16.3	16.3	16.3	16.3
M.E. (Kcal/kg)	3015	3015	3015	3015	3015	3015	3015	3015

[1] Ethoxyquin added to supply 125 mg/kg diet.

[2] Supplies/kg diet: retinylpalmitate, 10,000 I.U.; cholecalciferol, 1,000 I.C.U.; dl-α-tocopheryl acetate, 10 I.U.; menadione sodium bisulfite, 2.0 mg; thiamine HCl, 3.0 mg; riboflavin, 10.0 mg; pantothenic acid, 15.0 mg; niacin, 100 mg; pyridoxine, 6.0 mg; biotin, 150 mcg; folic acid, 3.0 mg; vitamin B_{12}, 15 mcg; choline chloride, 500 mg; ground corn as carrier to 3 parts for 1,000 of diet.

[3] Supplies/kg diet: manganese, 40 mg; zinc, 20 mg; copper, 9.9 mg; iron, 20 mg; magnesium, 490 mg.

performed as follows: A phosphate buffered saline gelatine (PBSG) was prepared by adding 1.0 g of Knox Gelatin (Knox Gelatin, Inc., Englewood Cliffs, N.J.) to 1 liter of phosphate buffer (0.1 M, pH 7.0, containing 0.14 M NaCl and 0.1% ethylmercurithiosalicylic acid). The assay tracers consisted of aliquots of 2,4,6,7^{3}H- 17-β-estradiol with specific activity of 96.0 Ci/mmol, and 1,2,6,7^{3}H-progesterone with specific activity of 114 Ci/mmol (New England Nuclear Corporation). Each was purified on a mini-column (2.5 ml tuberculin syringe) passing it through 0.4 g Sephadex LH-20 with benzene:methanol (90:10) as a solvent. The antisera used was rabbit anti-17-β-estradiol and antiprogesterone (M.S.U. #74), obtained from the Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University. Anti-estradiol and anti-progesterone were diluted to a concentration of 1:5000 and 1:1250, respectively, using PBSG. The 17-β-estradiol and progesterone standards were obtained from Sigma Chemical Co. The estradiol was dissolved in ethanol to give a stock concentration of 50 ng/ml and the progesterone was dissolved in methanol to give a stock concentration of 10 ng/ml. The standard solutions were stored in a freezer at -20°C. A dextran-coated charcoal suspension was prepared using a concentration of 0.5% Norit-neutral charcoal (Fisher Scientific Co.) and 1% Dextran T-70 (Pharmacia Fine Chemicals) in redistilled water, and stored at 4°C until used.

The estradiol assay involved duplicate aliquots of 0.5 ml blood plasma. These were placed in disposable culture tubes (16 x 125 mm). To account for procedural losses, 5000 dpm of labelled estradiol was added to five aliquots of blood plasma. The tubes with ^{3}H-estradiol were mixed, and allowed to equilibrate at room temperature for at least one

hour. Four ml of diethyl ether was added to each tube and mixed on a vortex mixer for one minute. The tubes were allowed to stand until the aqueous and ether layers separated. Then the aqueous layer was frozen by placing the tubes in a dry ice-acetone bath for 5 minutes, and ether extract decanted. The pooled extract was then dried in a vacuum oven at 500-600 mmHg and 45-50°C. Column chromatograph was used to purify the extracted estradiol. Columns were prepared by allowing Sephadex LH-20 to expand overnight in butanol. Then a 2.5 ml tuberculin syringe was filled with this Sephadex LH-20 up to 2.0 ml mark and washed with at least 10 ml of the same solvent. The dried extracts from the extraction of plasma with ether (unknown samples and recoveries) were solubilized in 0.250 ml butanol and applied to the column. The tube was then washed by a further addition of eluting solvent (0.250 ml) and the rinsing transferred to the column. Butanol, 1.5 ml, was added to the column and the eluate was discarded. More solvent (2.0 ml) was added and the eluate was collected as a purified estradiol. The collected aliquots from columns were dried in a vacuum oven (at 500-600 mmHg and 45-50°C) and then the purified estradiol was taken up in a 0.5 ml of PBSG, mixed vigorously, and stored overnight at 4°C to allow the hormone to come into solution. To determine the recovery of estradiol from plasma, two 0.2 ml aliquots from the tubes containing tritiated tracer were each placed into 5 ml of scintillation fluid (Scintiverse, Fisher Scientific Company) and mixed. Recoveries were calculated by taking the average of counts of ten 0.2 ml aliquots dividing by the total counts (5000 dpm) and multiplying by 2.5 to obtain the value for 0.5 ml of sample. Duplicate samples of 0.2 ml aliquots of each stored unknown sample were set up into disposable

culture tubes (12 x 75 mm). In each assay, standard curves were established using 17-β-estradiol at levels of 5, 10, 20, 30, 40 and 50 pg by taking appropriate volumes of the standard 17-β-estradiol stock solution (50 ng/ml) and diluting them to 0.2 ml with PBSG. Each point on the standard curve was run in triplicate. One-tenth ml of the antisera was added to each of the assay tubes (unknowns and standards), vortexed and stored at 4°C for one hour. One-tenth ml of PBSG with 2,4,6,7 ^{3}H-17-β-estradiol (5000 dpm) was then added to each tube, vortexed, and incubated at 5°C overnight. Dextran-coated charcoal (0.5 ml) was added to each tube, vortexed and centrifuged at 1000 g and 4-5°C for 15 minutes. The supernatants (0.5 ml) were decanted into 8 ml scintillation vials (Sargent-Welch Scientific Company) into which 5 ml of scintillation fluid was added. In addition to the tubes for standard curve and the processed plasma samples, there were duplicated tubes containing antiserum spiked with stock ^{3}H-estradiol. The latter was treated in the same manner as tubes containing standards or unknowns and served as a reference to which all other tubes were compared. The relationship between percent bound values vs. the natural log of hormone concentration was determined using regression analysis (Snedecor and Cochran, 1968). The starting percent of labelled hormone bound to antibody in the absence of unlabelled hormone was 38.8% (S.E. = ± 0.02). A linear standard curve (Figure 2) with a regression of -0.98 was obtained with estradiol at doses ranging from 5-50 pg.

2. Progesterone Radioimmunoassay

The progesterone assay was conducted in the same manner as described for estradiol assay. In order to purify the extracted

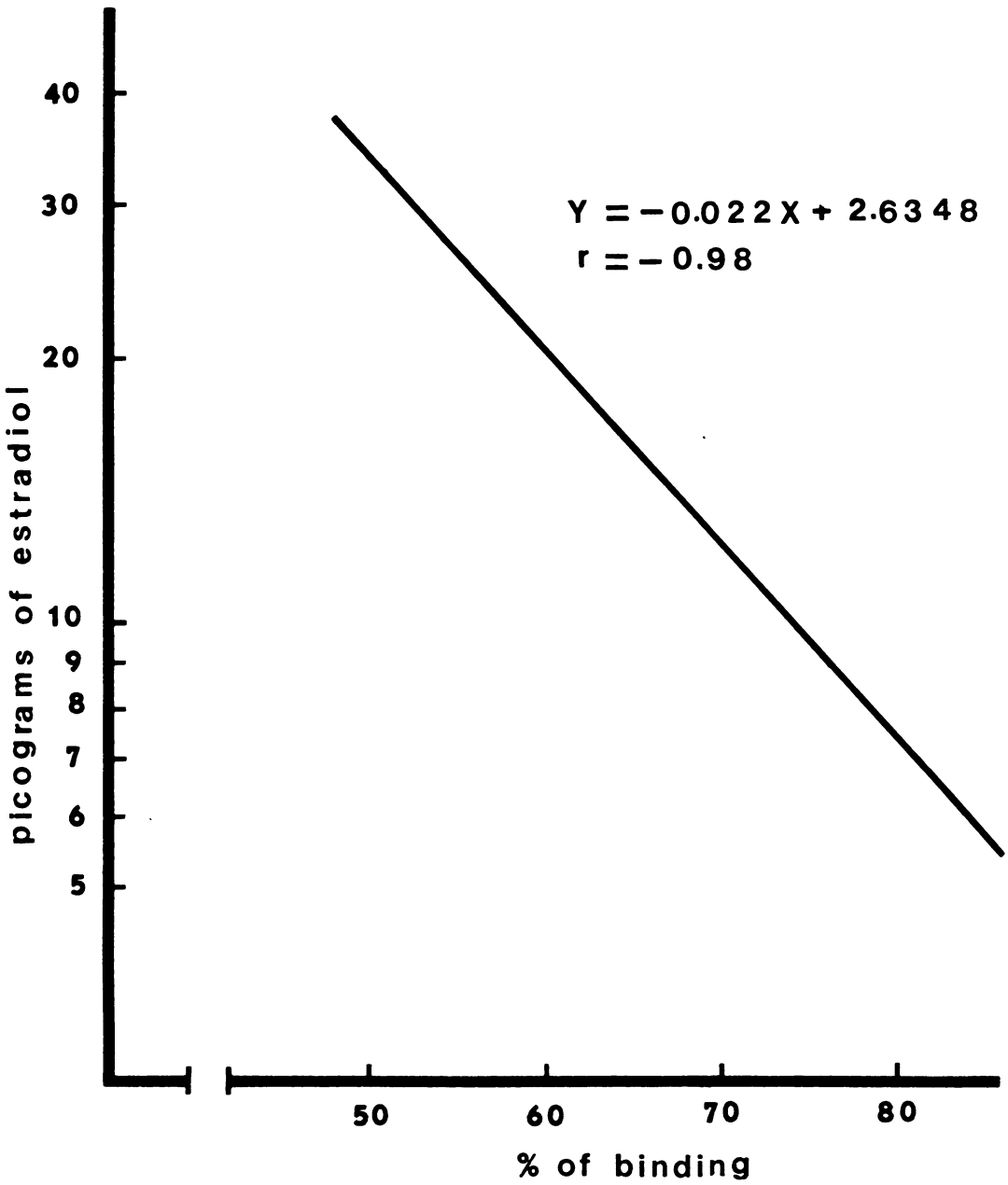

FIG.2. Standard curve for estradiol RIA.

progesterone, the dried extracted progesterone from the plasma was solubilized in two successive 0.250 ml butanol and applied to the column. Eluting solvent (1.0 ml) was added and the eluate discarded. More solvent (1.5 ml) was added and the eluate was collected as purified progesterone. The purified progesterone was taken up in a 0.5 ml of PBSG in the same way described for estradiol. The standard curves were established using stock solution of progesterone (0.1 ng/10 µl) at levels of 0.2, 0.3, 0.5, and 1.0 ng. Each standard had been dried and then resolubilized in 0.2 ml PBSG. The percent of labelled hormone bound to antibody in the absence of unlabelled hormone was 28.4% (S.E. = ± 0.41). The relationship between log of percent bound values vs. the log of hormone concentrations was determined using regression analysis (Snedecor and Cochran, 1968). A linear standard curve (Figure 3) with a mean regression of -0.99 (S.E. = 0.33) was obtained with progesterone from 0.2-1.0 ng.

Validation of the Estradiol Assay:

1. Recovery of known concentration of hormone

Spiking experiments were conducted by adding 25, 35, 50 and 100 pg of unlabelled 17-β-estradiol to assay buffer solution and plasma from adult female chickens. These were then extracted, chromatographed, and assayed. A recovery of 98.3% (± S.E. = 0.85) and 92.7% (± S.E. = 1.68) were obtained for each of the respective samples.

2. Blank values

Blanks, were obtained by extracting, and chromatographing PBSG (phosphate buffered saline gelatine). It gave nondetectable levels of estradiol.

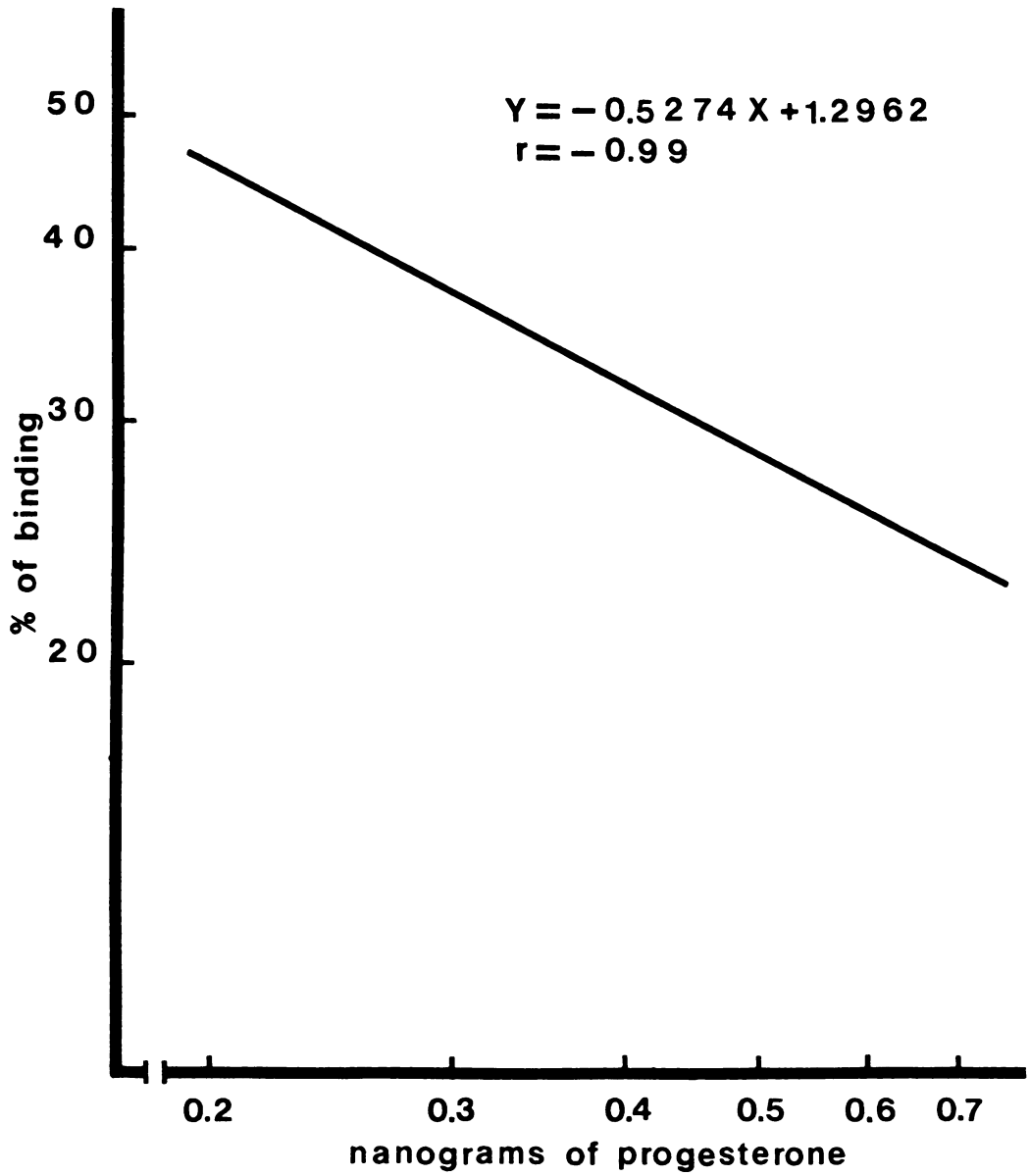

FIG.3. Standard curve for progesterone RIA.

3. Within and between assay variation

A pool of plasma from adult female chickens was obtained and aliquots of this pool were run in each assay. Coefficients of variation were determined for each assay to measure the amount of variation in the potency estimates of the hormone within an assay and between assays. The coefficient of variation (C.V.) was calculated by the equation $C.V. = s/\overline{x}$, where s equals the standard deviation of a sample and $\overline{x}$ equals the sample mean (Snedecor and Cochran, 1968). The coefficient of variation within an assay was determined by calculating the mean estradiol value and the standard deviation for 8 samples from the adult female chickens plasma pool. The coefficient of variation between assays was determined by calculating the overall mean estradiol value for the samples in each assay (Table 8).

Validation of the Progesterone Assay:

1. Recovery of known concentrations of hormone

Adult female chickens plasma pool samples and buffer solution were spiked with 0, 0.2, 0.5, and 1.0 ng of unlabelled progesterone, extracted, chromatographed, and assayed. A recovery of 96.3% (± S.E. = 0.6) and 98.7% (± S.E. = 0.8) were obtained for respective samples of plasma and buffer.

2. Blank values

Blanks were obtained by extracting, chromatographing, and assaying PBSG. It had nondetectable levels of progesterone.

Table 8. Coefficients of variation in the estradiol and progesterone radioimmunoassay.

Assay	n	Plasma Conc.	C.V. Within Assay (%)	C.V. Between Assay (%)
Estradiol	8	147.5 (pg/ml)	9.2	11.25
Progesterone	8	2.84(ng/ml)	6.8	10.10

3. Within and between assay variation

A pool of plasma from adult female chickens was obtained and aliquots of this pool were run in each assay. The coefficients of variation within assay and between assays were determined in the same manner as described for estradiol assay validation (Table 8).

E. Statistical Analysis

All experimental data were analyzed statistically by the analysis of variance (Gill, 1978), and the means were compared by a multiple range test (Duncan, 1955). Regression analysis (Snedecor and Cochran, 1968) was used whenever applicable to obtain the correlation between different measurements.

IV. RESULTS

A. Experiment I

The control group, fed ad libitum during the three weeks of the experiment, averaged 101g feed/bird/day. The total feed intakes for the force-fed hens given the corn-soy diet at 120% and 135% of their pre-experimental ad libitum feed intake averaged 127 and 140 g/bird/day, respectively. This included small amounts of diet consumed ad libitum in addition to amounts force-fed. Force-feeding the corn-soy diet markedly elevated the liver fat content, the plasma estradiol level and increased the incidence of FLHS (Table 9). A detailed examination of the stage of production for each hen at the time of sampling revealed that 5 of 8 hens fed ad libitum were expected to lay an egg over the next 24 hours while none of the hens force-fed at 135% levels and with the highest plasma estrogen levels were expected to lay an egg (Table 9). The concentration of plasma progesterone in force-fed birds was lower than those fed ad libitum but the difference was not significant ($P > 0.05$). Egg production was decreased by force-feeding ($P < 0.05$).

Regression analysis (Snedecor and Cochran, 1968) indicated (Figure 4) that plasma estradiol and the FLHS scores were significantly ($P < 0.01$) correlated ($r = 0.70$). The correlations between liver fat values vs FLHS score ($r = 0.74$) and plasma estradiol vs liver fat ($r = 0.58$) were significant (Figures 5 and 6, respectively) at $P < 0.05$.

Table 9. The effect from force-feeding a corn-soy type diet (Table 1) to SCWL hens on their production during the 3rd week, and on hepatic lipid, hemorrhagic score, plasma estradiol, plasma progesterone, and the stage of egg formation on the 21st day of force-feeding (Experiment I).

Levels of force-feeding	No. of hens[1]	Egg prod. days 15-21 %	Liver fat, % of dry matter	FLHS score	Plasma Steroids		Yolk in magnum	Egg in uterus
					estradiol pg/ml	progesterone ng/ml		
None	8	52	31.3_a[2] (± 13)[3]	1.6_a (± .74)	163_a (± 25)	3.01_a (± .82)	3 of 8	2 of 8
120%	8	25.3	75.1_b (± 9)	3.7_b (±1.58)	$195_{a,b}$ (± 74)	2.47_a (± .31)	2 of 8	None
135%	6	0	76.8_b (± 5)	4.5_b (± .84)	247_b (± 46)	2.37_a (± .27)	None	None

[1]Includes only those on which plasma steroid values were obtained.

[2]Values with the same subscript letter are not significantly different by Duncan's range test.

[3]Mean (± std. dev.).

Table 10. Analysis of variance of data on plasma estradiol, FLHS score and liver fat content (Experiment I).

Source of variation	d.f.	Mean Square		
		Plasma Estradiol	FLHS Score	Liver Fat Content
Total	21			
Treatment	2	10110*	15.91**	4095**
Error	19	2807	1.30	128

*P < 0.05

**P < 0.001

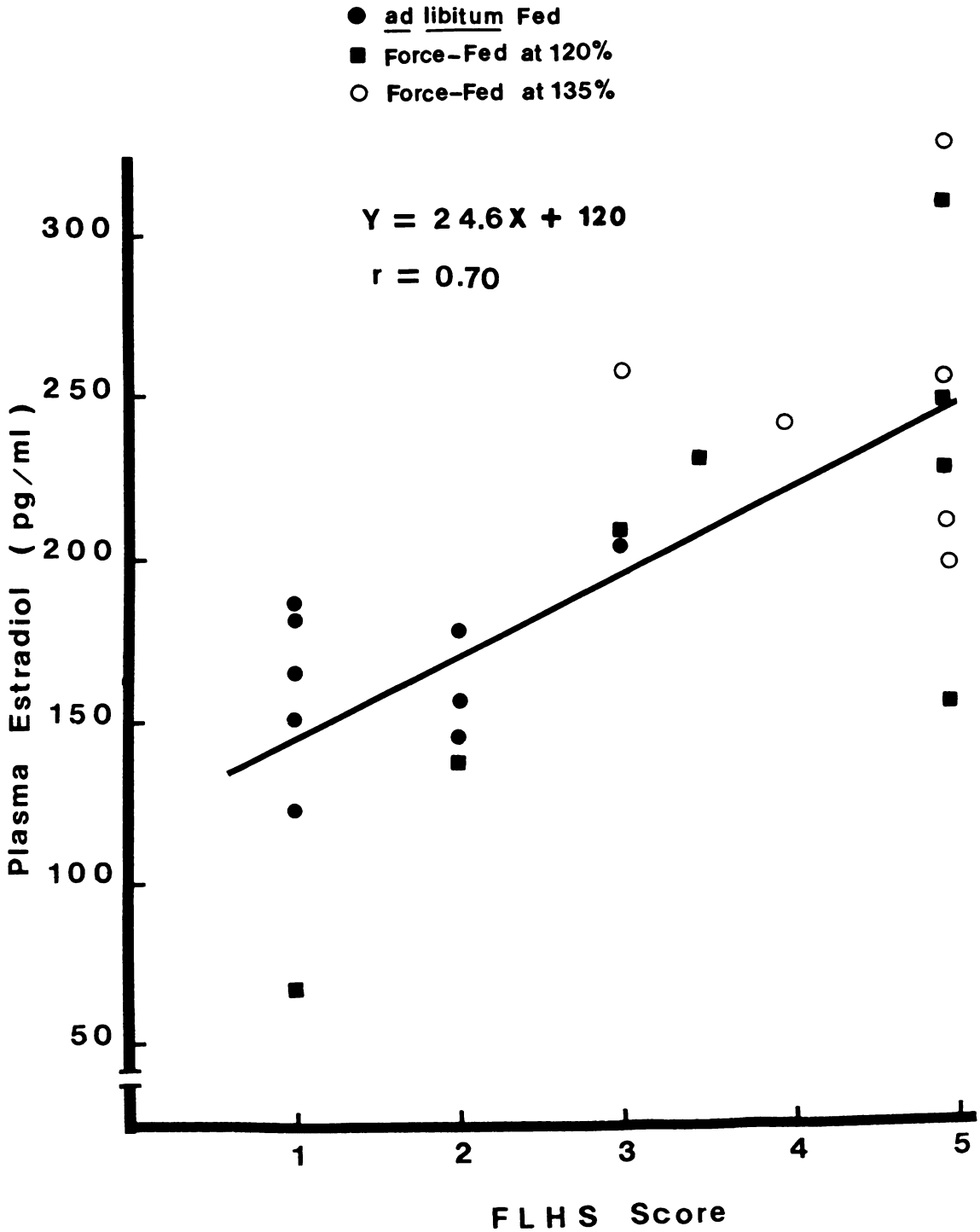

Fig. 4. Relation between FLHS and plasma estradiol concentration of SCWL hens when force-fed 21 days (Experiment #1), where X = FLHS score and Y = concentration of plasma estradiol (pg/ml).

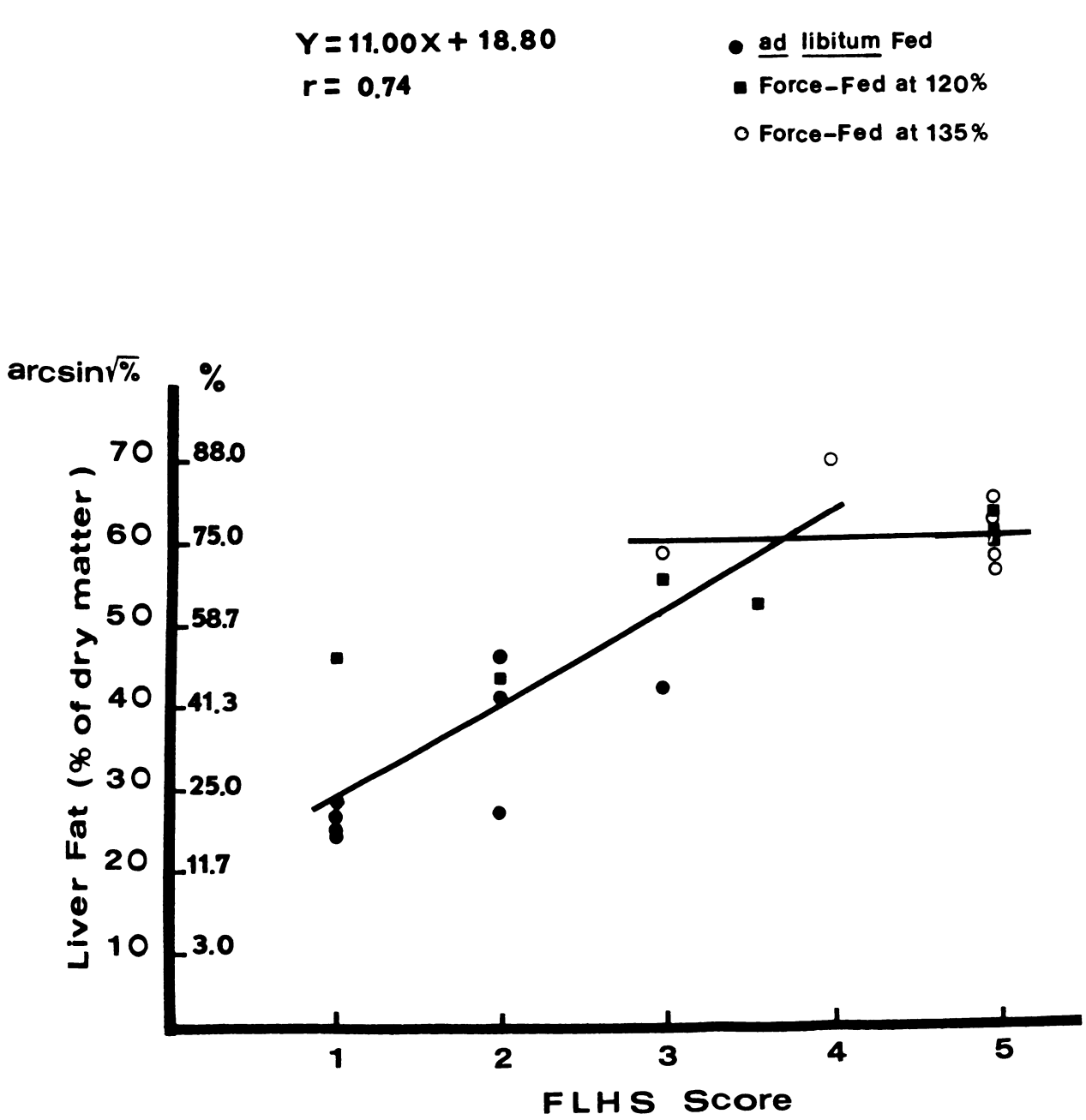

Fig. 5. Relation between FLHS and liver fat content in SCWL hens force-fed 21 days a corn-soy diet (Experiment #1), where X = FLHS score and Y = liver fat content (arcsin $\sqrt{\%}$).

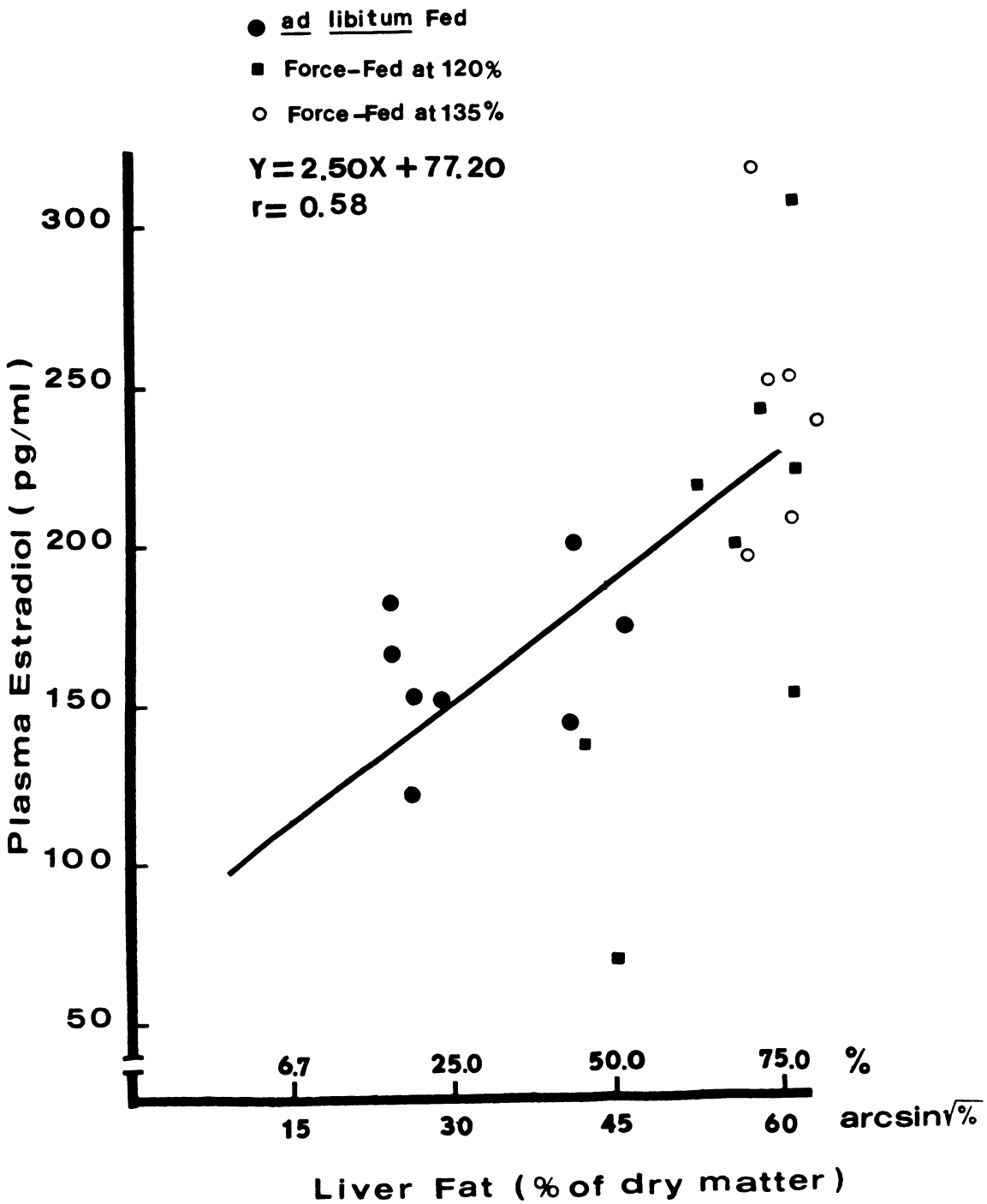

Fig. 6. Relation between plasma estradiol and liver fat content of SCWL hens force-fed 21 days a corn-soy diet (Experiment #1) where X = liver fat content (arcsin$\sqrt{\%}$) and Y = concentration of plasma estradiol (pg/ml).

B. Experiment II

The results for experiment II are presented in Table 11. The birds force-fed the corn-soy diet gained significantly ($P < 0.01$) more than those force-fed wheat-soy diets which were isocaloric and isonitrogenous with the corn-soy diet. In this experiment corn oil or corn starch were used in wheat-soy diets to make them isocaloric with the corn-soy diet. The gain of birds force-fed wheat-soy-corn oil (WSCO) diet was not significantly different from those force-fed wheat-soy-corn starch (WSCS) diet.

Force-feeding significantly ($P < 0.01$) increased the liver weight. Among the different diets (Table 2) used in force-feeding, the corn-soy diet caused the heaviest liver. Liver weight was significantly ($P < 0.01$) lower in the birds force-fed WSCO diet than those force-fed a corn-soy diet. The percentage of liver fat content, the FLHS score, and the plasma estradiol level were increased by force-feeding the experimental diets (Table 2). The WSCO diet had the lowest numerical values for the criteria that were evaluated, except for progesterone levels in plasma. Significant differences among force-fed groups were detected for weight gain, liver weight, FLHS and plasma estradiol. The score for FLHS caused by force-feeding the WSCS diet was almost the same as those caused by force-feeding the corn-soy diet. The use of corn oil in the wheat-soy diet tended to produce lower FLHS than the use of corn starch to make wheat-soy diet isocaloric with the corn-soy diets; however, the difference was not statistically significant at $P \geq 0.05$.

The plasma estradiol was elevated by force-feeding, but elevated the least by force-feeding the WSCO diet. The other diets (corn-soy and

Table 11. The effect of various types of diets on the weight gain, liver weight, FLHS, plasma estradiol and progesterone concentration of SCWL hens (Experiment II).

Experimental Dietary Treatments	No. of birds per group	Weight gain (g./bird/day)	Liver weight g.	Liver fat content (% of dry matter)	FLHS Score	Plasma estra-diol (pg/ml)	Plasma proges-terone (ng/ml)
Corn-Soybean (ad libitum)	9	4.3 (±0.89)[1] [2] $_a$	35.2 (±2.86)$_a$	27.34 (±2.92)$_a$	1.38 (±0.19)$_a$	105.0 (±3.0)$_a$	3.37 (±0.35)$_a$
Corn-soybean (force-fed)	9	26.3 (±1.06)$_b$	147.6 (±15.80)$_b$	76.68 (±2.37)$_b$	3.61 (0.36)$_b$	236.0 (±25.5)$_b$	2.36 (±0.44)$_a$
Wheat-soybean-corn oil (force-fed)	9	17.4 (±0.75)$_c$	89.1 (±9.87)$_c$	64.85 (±3.16)$_b$	2.50 (±0.38)$_{ab}$	165.0 (±19.6)$_{ab}$	2.74 (±0.38)$_a$
Wheat-soybean-corn starch (force-fed)	9	16.3 (±1.29)$_c$	110.2 (±12.06)$_{bc}$	71.65 (±2.69)$_b$	3.33 (±0.38)b	225.0 (±18.8)$_b$	2.24 (±0.41)$_a$

[1]Mean (± S.E.)

[2]Values with the same common letter are not significantly different by Duncan's range test.

Table 12. Analysis of variance of data on weight gain, liver weight, liver fat content, FLHS score, and plasma estradiol level (Experiment II).

Source of variation	d.f.	Mean Square				
		Weight gain	Liver weight	Liver fat content	FLHS score	Plasma estradiol
Total	35					
Treatment	3	731**	19816**	4714**	8.97**	31930**
Error	32	10.6	1280	122	1.25	3547

**$P < 0.001$

WSCS) had comparable capability to raise plasma estradiol levels.

The results obtained in this experiment as well as experiment #1 indicated that force-feeding has no significant effect on the plasma progesterone levels.

Significant ($P < 0.01$) correlations of $r = 0.76$, $r = 0.72$, and $r = 0.81$ were obtained for plasma estradiol levels vs liver fat values (Figure 7), plasma estradiol levels vs FLHS scores (Figure 8), and liver fat values vs FLHS scores (Figure 9), respectively. These results confirmed the data obtained in experiment I.

C. Experiment III

In this experiment in addition to those diets used in experiment II a wheat-soy diet was used in which sucrose was added to make it isocaloric with the other diets. Although all diets were formulated to be isocaloric and intakes for force-fed chickens were comparable, actual M.E., retained differed (Table 13). Energy balance determination revealed a higher M.E. value for the corn-soy diet (3.1 kcal/g) than for wheat-soy diets (2.45-2.70 kcal/g). The promoting effect of force-feeding on weight gain was significantly ($P < 0.05$) higher for corn-soy diet than wheat-soy diest (Table 13). This effect of the corn-soy diet appeared to be from a higher retained energy (Table 13). No significant differences were obtained for weight gain of the birds force-fed the other different kinds of wheat-soy diets at $P < 0.05$.

The birds force-fed corn-soy diet had significantly ($P < 0.05$) heavier livers than those force-fed the other experimental diets. The results obtained in this experiment and in experiment II clearly revealed that

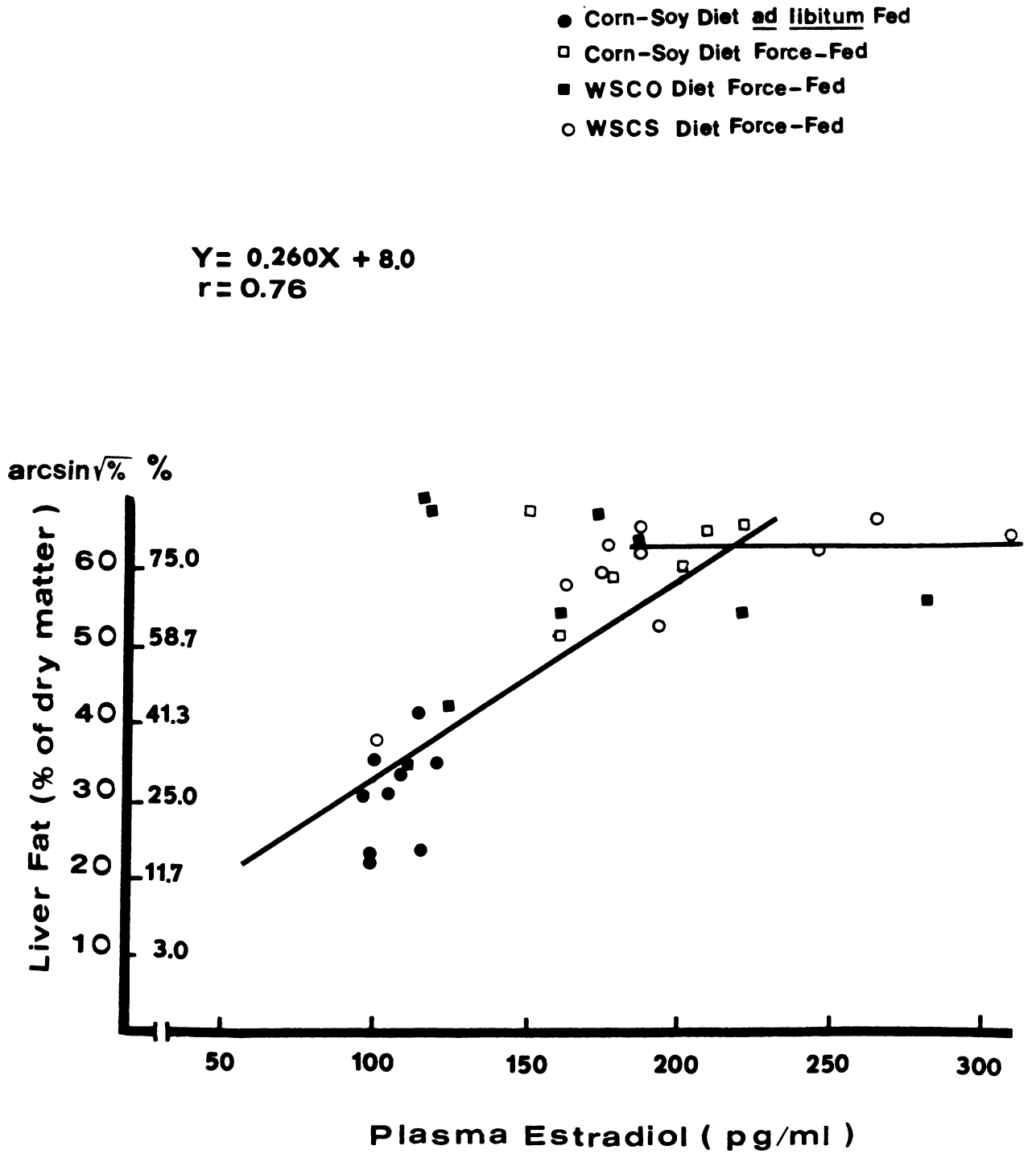

Fig. 7. Relation between estradiol and liver fat content in SCWL hens force-fed 21 days (Experiment #2), where X = concentration of plasma estradiol (pg/ml) and Y = liver fat content (arcsin$\sqrt{\%}$).

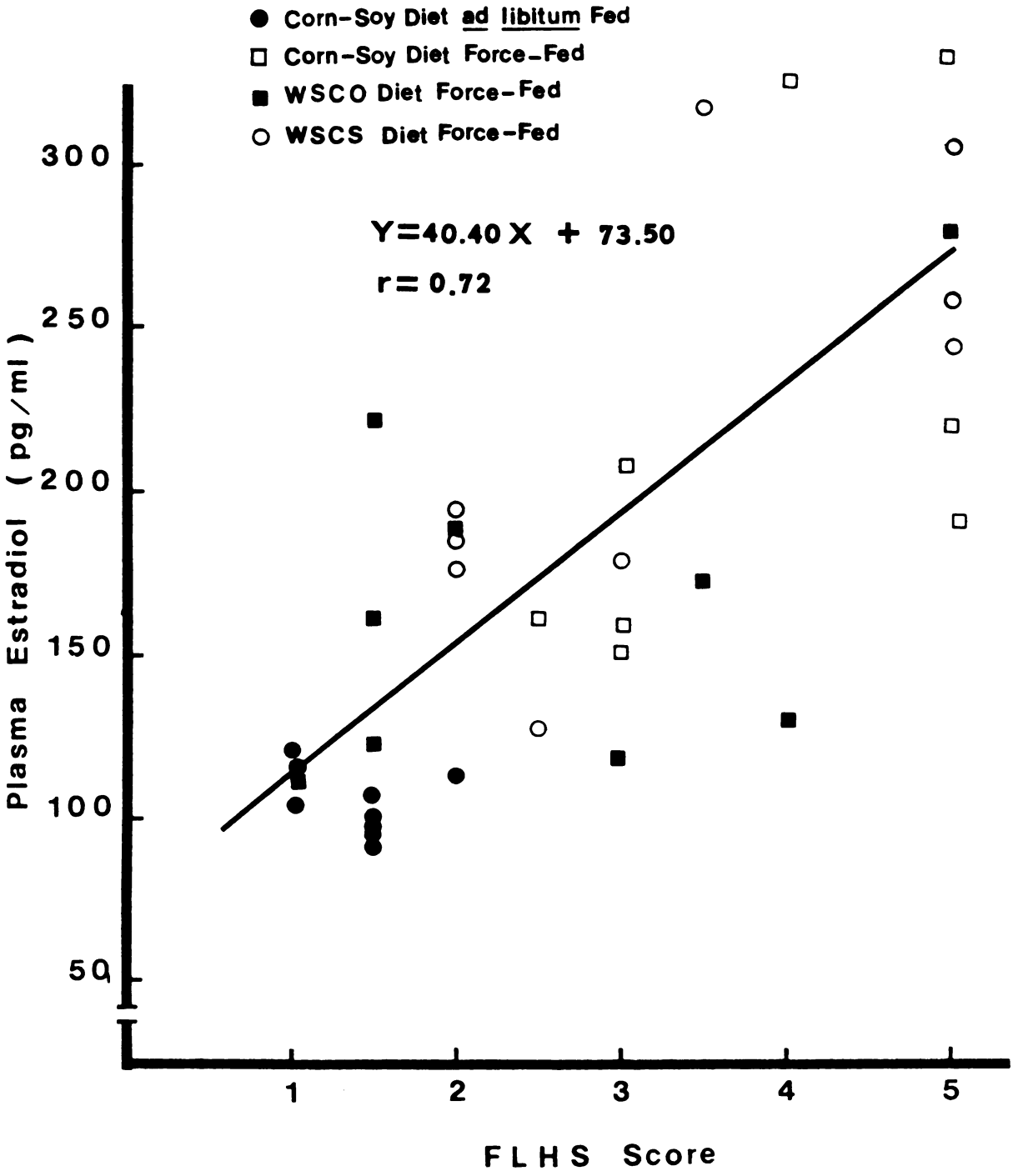

Fig. 8. Relation between FLHS and plasma estradiol concentration in SCWL hens force-fed 21 days (Experiment #2), where X = FLHS score and Y = concentration of plasma estradiol (pg/ml).

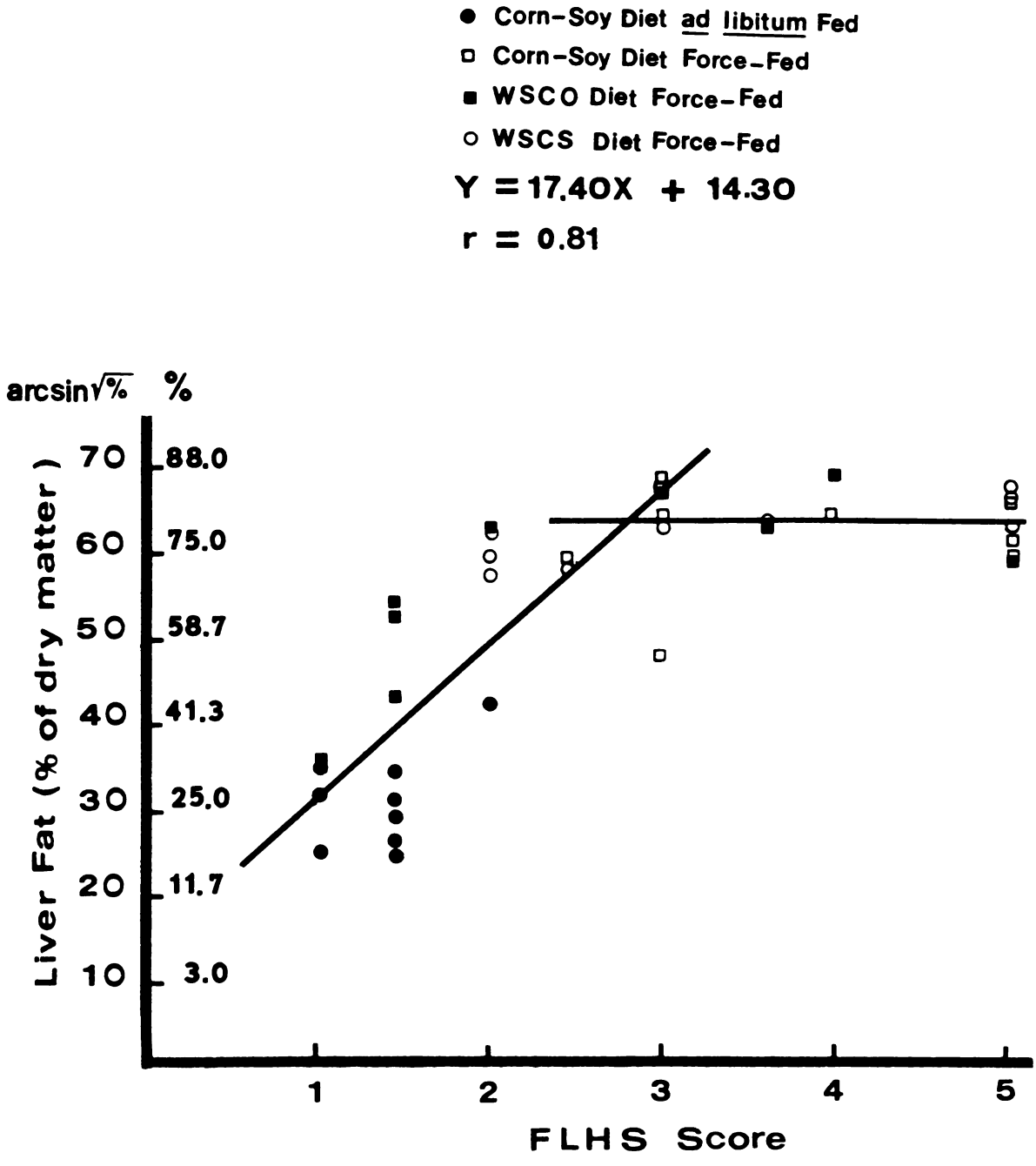

Fig. 9. Relation between FLHS and liver fat content in SCWL hens force-fed 21 days (Experiment #2), where X = FLHS score and Y = liver fat content (arcsin$\sqrt{\%}$).

Table 13. The effect of experimental diets on weight gain, liver weight, liver fat, FLHS score, plasma estradiol and plasma progesterone level of SCWL hens (Experiment III)

Experimental Dietary Treatments	No. of birds per group	Determined retained energy(kcal/ bird/day)	Weight gain (g/bird/ day)	Liver weight (g)	Liver fat (% of dry matter)	FLHS Score	Plasma estra-diol (pg/ml)	Plasma proges-terone (ng/ml)
Corn-soybean (ad libitum)	8	435 (100%)	4.15_a[1] (±1.38)[2]	52.0_a (±2.5)	30.67_a (±4.27)	1.00_a (± 0)	117_a (±7.7)	3.27_a (±0.31)
Corn-soybean (force-fed)	8	580 (133%)[3]	27.60_b (±1.52)	162.3_b (±6.8)	76.52_b (±1.71)	2.53_{bc} (±0.34)	214_{bc} (±14.6)	2.48_a (±0.21)
Wheat-soybean-corn oil (force-fed)	8	491 (113%)	16.67_c (±1.95)	82.1_{ac} (±10.2)	62.26_c (±3.1)	1.93_{abc} (±0.44)	184_{abc} (±30.7)	2.60_a (±0.25)
Wheat-soybean-corn starch (force-fed)	8	498 (114%)	17.90_c (±2.2)	118.5_{bc} (±18.5)	64.80_c (±4.02)	3.00_c (±0.53)	241_c (±28.4)	2.99_a (±0.32)
Wheat-soybean-sucrose (force-fed)	8	504 (116%)	16.33_c (±1.42)	83.8_{ac} (±12.2)	56.97_c (±6.12)	1.62_{ab} (±0.23)	164_{abc} (±27.0)	2.98_a (±0.52)

[1]Values with the same common letter are not significantly different by Duncan's range test.

[2]Mean (± S.E.)

[3]Represents % of ad libitum group.

force-feeding the laying hens a wheat-soy diet supplemented with corn starch caused high liver weight which was not significantly ($P > 0.05$) different from those obtained by force-feeding the corn-soy diet (Tables 11 and 13). The data obtained (Table 13) from the use of sucrose or corn oil in wheat-soy diets to make them isocaloric with the corn-soy diet, revealed that using these two sources of energy in the wheat-soy diets, to balance the dietary energy, produced lighter liver weight than did the use of corn starch.

Those birds force-fed the corn-soybean diet had the highest liver fat percentage. Force-feeding of wheat-soy diets supplemented with corn oil, corn starch or sucrose produced fatty livers which were intermediate between those of the ad libitum fed group and those of the group force-fed corn-soy diet. The liver fat values obtained from the experimental groups force-fed the different kinds of wheat-soy diets were not significantly different at $P > 0.05$. However, since the liver weight of the birds force-fed the WSCS diet was higher than those from the birds force-fed WSCO or wheat-soy-sucrose (WSS) diets the total liver fat content was lower for the two latter groups.

Although in this experiment there was not a high incidence of FLHS in any of the groups, the results presented in Table 13, revealed that using corn oil in the wheat-soy diet resulted in less liver hemorrhages than those produced by force-feeding the corn-soy diet. But when corn starch was used in the wheat-soy diet FLHS was as high as when the birds were force-fed the corn-soy diet. No significant difference was obtained between FLHS scores from two groups of birds force-fed WSCO and WSS diets.

Table 14. Analysis of variance of data on weight gain, liver weight, liver fat content, FLHS, and plasma estradiol level (Experiment III).

Source of variation	d.f.	Mean Square				
		Weight gain	Liver weight	Liver fat content	FLHS score	Plasma estradiol
Total	39					
Treatment	4	548**	14246**	1250**	4.85*	18088*
Error	35	28	1195	110	1.21	4943

**$P < 0.001$

*$P < 0.025$

Force-feeding the experimental diets increased the chicken's plasma estradiol level, but not comparably. Corn-soy and WSCS diets elevated the plasma estradiol levels more than the other two wheat-soy diets (Table 13).

Neither force-feeding nor diet composition had any significant effect on plasma progesterone level.

The regression analysis (Snedecor and Cochran, 1968) revealed that plasma estradiol levels were significantly ($P < 0.01$) correlated to liver fat content and FLHS score at $r = 0.61$ and $r = 0.64$, respectively (Figures 10 and 11). The correlation between liver fat content and FLHS was also significant ($P < 0.05$) at $r = 0.84$ (Figure 12).

The numerical values (weight gain, liver weight, liver fat percentage, FLHS score and plasma estradiol) were higher for the birds force-fed corn-soy or WSCS diets than for those force-fed the two other experimental diets. The same trend of results was obtained in experiment II in which corn-soy, WSCO and WSCS diets were used to produce FLHS by force-feeding.

D. Experiment IV

All treatments from experiment 3 were duplicated in this experiment except for the WSS diet, which was replaced by a wheat-soy-wheat starch (WSWS) diet. The comparison was done to find out whether the effect of corn starch on FLHS is related to its original ingredient.

The same trend of results was observed in this experiment as in the third experiment (Table 15). The weight gain of the birds force-fed the corn-soy diet was higher ($P < 0.05$) than those of the groups force-fed diets with wheat although energy retained (M.E.) was comparable (Table 15). No significant difference ($P > 0.05$) in weight gain was noted among

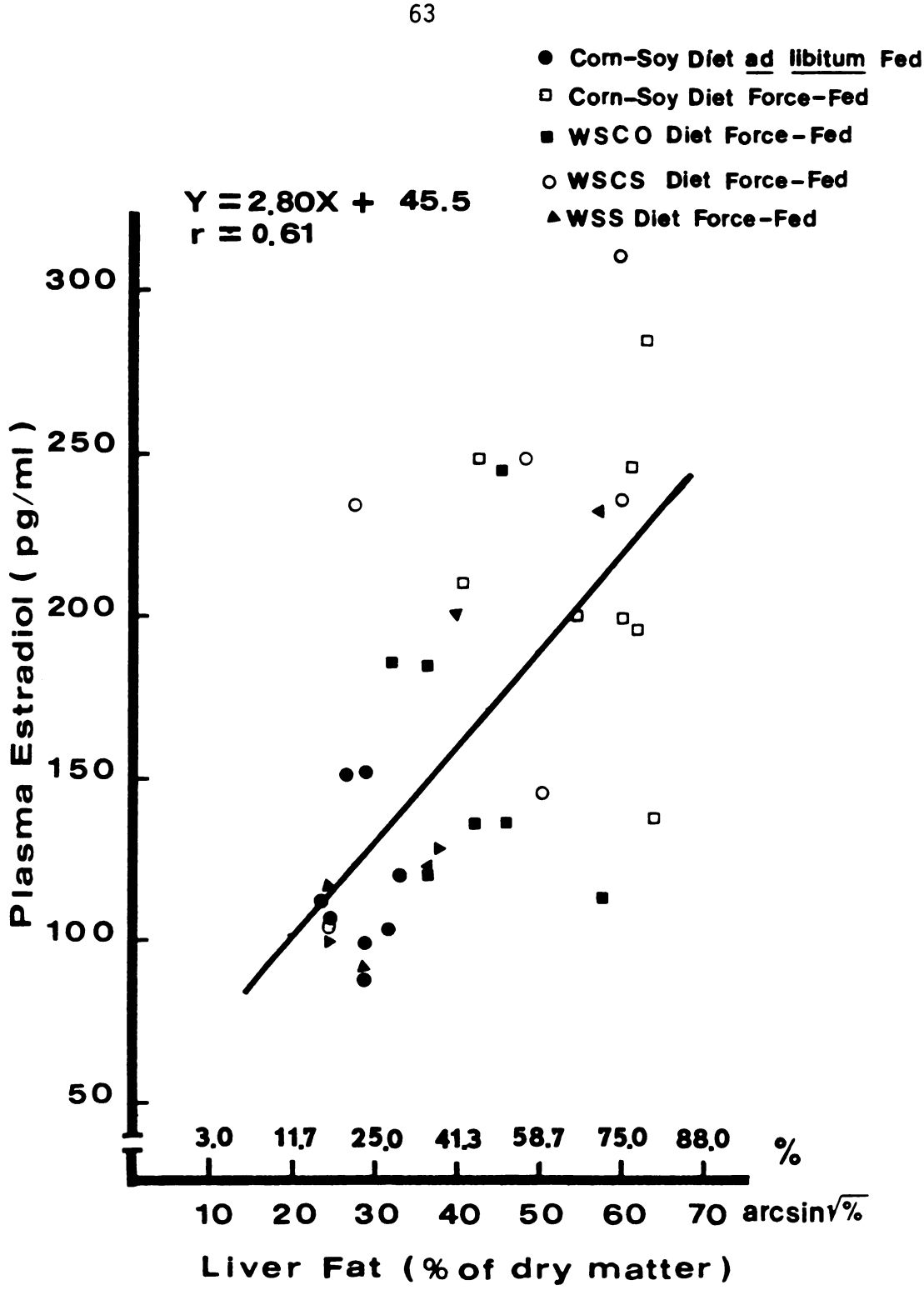

Fig. 10. Relation between plasma estradiol and liver fat content of SCWL hens force-fed 24 days (Experiment #3), where X = liver fat content (arcsin$\sqrt{\%}$), and Y = concentration of plasma estradiol (pg/ml).

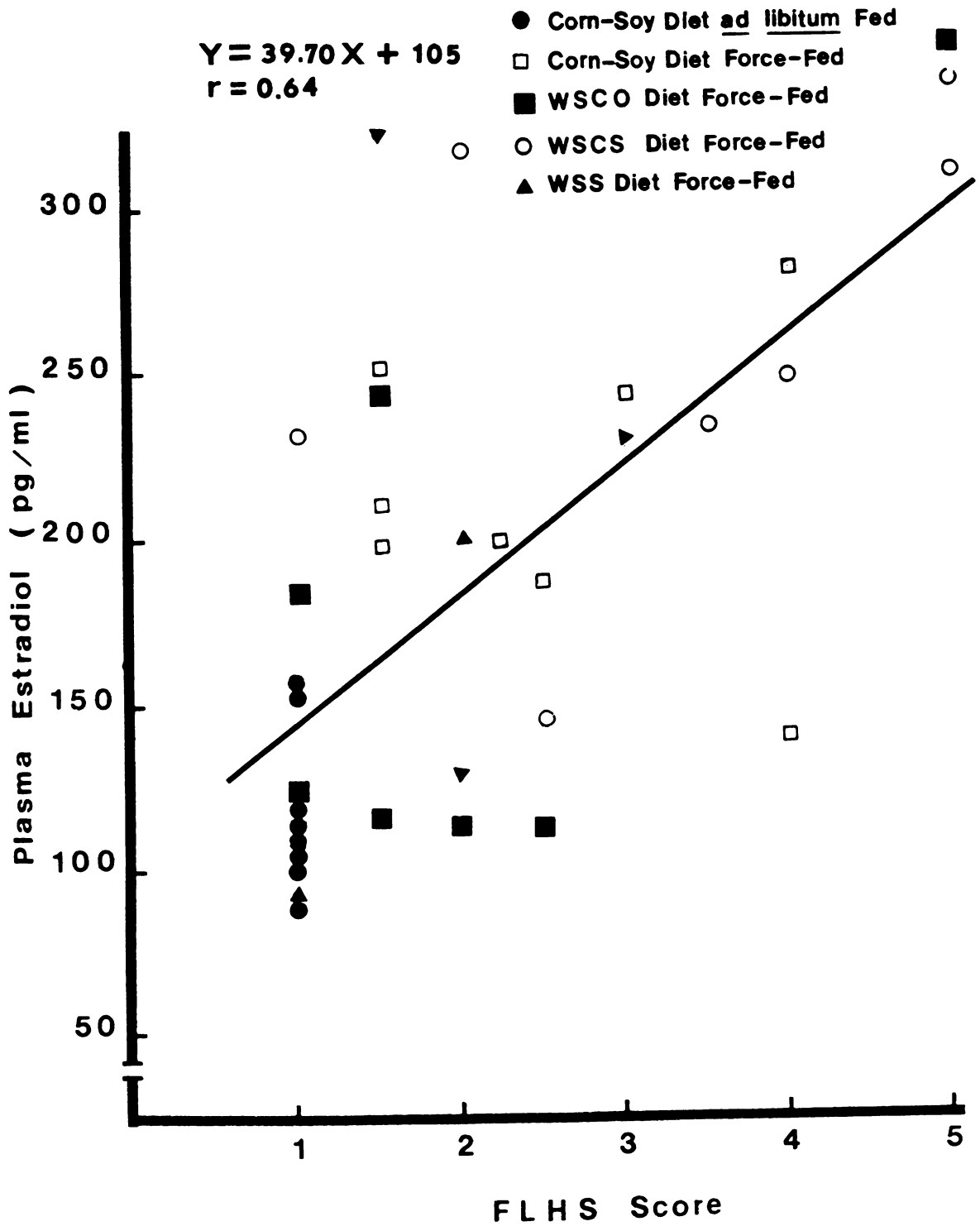

Figure 11. Relation between FLHS and plasma estradiol concentration in SCWL hens force-fed 24 days (Experiment #3), where X = FLHS score and Y = concentration of plasma estradiol (pg/ml).

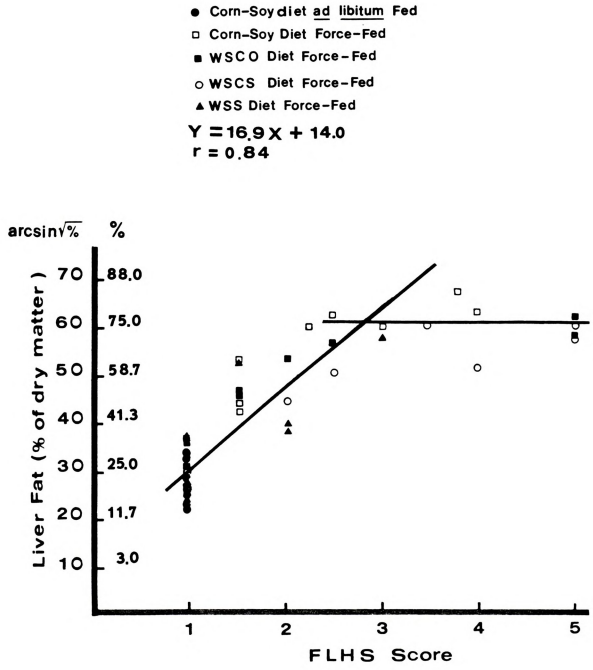

Figure 12. Relation between FLHS and liver fat percentage in SCWL hens force-fed 24 days (Experiment #3), where X = FLHS score and Y = liver fat concentration ($\arcsin\sqrt{\%}$).

Table 15. The effect of experimental diets on weight gain, liver weight, liver fat content, and FLHS of SCWL hens (Experiment IV).

Experimental Dietary Treatments	No. of birds per group	Determined retained energy (kcal/bird/day)	Weight gain (g/bird/day)	Liver weight (g)	Liver fat (% of dry matter)	FLHS Score
Corn-soybean (ad libitum)	8	320 (100%) (±0)	4.66_a[1] (± 1.50)[2]	66.0_a (± 6.8)	36.6_a (± 6.8)	1.250_a (± 0.125)
Corn-soybean (force-fed)	8	529 (165%)[3] (± 6.9)	24.47_b (± 1.74)	150.0_b (± 10.1)	64.0_b (± 3.60)	3.125_b (± 0.498)
Wheat-soybean-corn oil (force-fed)	8	542 (169%) (± 16.4)	14.14_c (± 1.72)	97.0_{ac} (± 5.9)	49.6_c (± 3.1)	1.25_a ($\pm 0.0\%$)
Wheat-soybean-corn starch (force-fed)	8	519 (162%) (± 10.9)	17.04_c (± 1.28)	148.0_b (± 10.5)	68.9_b (± 2.5)	2.940_b (± 0.495)
Wheat-soybean-wheat starch (force-fed)	8	515 (160%) (± 10.30)	17.80_c (± 1.15)	120.8_{bc} (± 12.6)	59.5_{bc} (± 4.5)	2.68_{ab} (± 0.595)

[1]Values with the same common letter are not significantly different by Duncan's range test.

[2]Mean (± S.E.)

[3]Represents % of ad libitum group.

experimental groups force-fed wheat-soy diets. However, hens force-fed the wheat-soy diets gained more than those fed the corn-soy diet ad libitum ($P < 0.05$). Although the attempt was made to keep dietary energy intake isocaloric, hens force-fed the WSCO diet received slightly more energy (169% of ad libitum group) than the other birds (160-165% of ad libitum group) but gained the least of the force-fed groups.

The livers of the hens force-fed diets were larger and more heavily laden with fat than those of the ad libitum fed hens (Table 15). The livers from the birds force-fed corn-soy diet were heavier than those from the birds force-fed wheat-soy diets. Despite higher energy retained (M.E.) in the birds force-fed WSCO diet, their liver weight was lower than other force-fed groups. Liver weight appeared to be somewhat lighter in birds force-fed a WSWS diet than those force-fed the WSCS diet.

The data in Table 15 revealed that the values for the percentage of fat in the liver followed the same trend as that obtained for liver weight.

The data obtained in this experiment (Table 15) indicated that corn oil has a moderating influence on FLHS when used in the wheat-soy diet to make it isocaloric with other experimental diets. The birds force-fed WSCO diet had FLHS score values the same as the ad libitum fed group. Dietary energy supplied in excess by corn starch in the wheat-soy diet induced FLHS at the same level as the excess of corn-soy diet. There was no significant ($P > 0.05$) difference between corn starch and wheat starch in producing FLHS (Table 15). However, the numerical values of FLHS score were lower in the birds force-fed WSWS diet than in those force-fed WSCS diet. This result revealed the same trend as that obtained for liver weight or liver fat percentage.

Table 16. Analysis of variance of data on weight gain, liver weight, liver fat content, and FLHS Score (Experiment IV)

Source of Variation	d.f.	Mean Square			
		Weight gain	Liver weight	Liver fat content	FLHS score
Total	39				
Treatment	4	414***	10188***	681**	4.35*
Error	35	20	828	104	1.44

* $P < 0.05$

** $P < 0.005$

*** $P < 0.001$

The results of this experiment indicated that if starch derived from wheat or corn is used in a wheat-soy diet to supply extra energy, there will be no significant difference between wheat and corn in producing FLHS. The implication is that corn oil in the wheat-soy diet appears to have a beneficial effect in reducing the hemorrhagic score as well as liver fat and liver weight values.

E. Experiment V (ad libitum feeding)

Chickens fed various kinds of diets (Tables 6 and 7) starting at the time they were 28 weeks of age, did not have FLHS or FLS when examined after 8 weeks of feeding these diets ad libitum. Data revealing liver weight, percentage of fat in the liver, FLHS scores and the rate of egg production are shown in Table 17. No significant differences were obtained among different experimental groups regarding these criteria. The livers appeared to be of excellent color and condition, and their weights and fat content reflected that appearance.

The results obtained on sisters of these hens at 46 weeks of age and fed various types of diets ad libitum (Tables 6 and 7) are given in Table 18. No significant liver hemorrhagic syndromes were observed in any experimental groups (Table 18). Livers obtained from hens fed corn-soy or wheat-soy diets were not statistically different ($P > 0.05$) in weight, but livers from hens fed wheat-soy diets with either corn starch or wheat starch tended to be heavier in weight (Table 18). These livers also had lower percentages of fat. The data revealed that using corn oil in the wheat-soy bean diet had a beneficial effect of reducing liver fat content. This confirmed the results obtained in the force-feeding experiments 2, 3 and 4

Table 17. The effect from ad libitum feeding of experimental diets for 8 weeks on egg production, hepatic weight, liver fat percentage and FLHS of 28-weeks old SCWL hens (Experiment V, Stage 2).

Experimental Dietary Treatments	Egg production (%)	Liver weight (g)	Liver lipid (% of dry matter)	FLHS
Corn-soybean	$86(\pm 1.7)_a$[1,2]	43.2 $(\pm 1.35)_a$	13.5 $(\pm 0.96)_a$	1.0
Wheat-soy-corn oil	$87(\pm 2.1)_a$	44.8 $(\pm 1.67)_a$	13.7 $(\pm 2.1)_a$	1.0
Wheat-soy-corn starch	$90(\pm 1.0)_a$	47.3 $(\pm 2.9)_a$	16.3 $(\pm 4.7)_a$	1.0
Wheat-soy-wheat starch	$90(\pm 1.0)_a$	42.3 $(\pm 2.7)_a$	12.9 $(\pm 2.9)_a$	1.0

[1]Values with the same common letter are not significantly different by Duncan's range test.

[2]Mean (± S.E.)

Table 18. Effect of sources of energy and selenium on feed intake, egg production, net retained energy, liver weight, liver fat percentage and FLHS score of SCWL hens in the 18 weeks ad libitum feeding experiment (Experiment V, Stage 3).

Experimental dietary treatments	Feed intake (g/bird/day)	Determined M.E. (kcal/g)	Egg Production		Net* retained energy (kcal/bird/day)	Liver weight (g)	Liver fat percentage (% of dry matter)	FLHS Score
			Percent	g/bird/day				
Corn-soybean								
1. With fish meal (0.17 mg Se/kg diet)	107	3.13	82±3.2	46.1±2.2	259±10	44±1.9 (a) [1,2]	34.8±7.6 (dc)	1.56±0.2
2. Without fish meal (0.04 mg Se/kg diet)	96	3.28	80±3.3	44.6±2.1	240± 8	41±3.7 (a)	31.8±12.1(c)	1.12±0.10
3. As 2 + Se (0.34 mg Se/kg diet)	98	3.28	75±6.0	42.1±3.3	251±12	44±2.4 (a)	30.0±7.4(bc)	2.0±0.5
Mean	100	3.23			250	43	32.2	1.56
Wheat-soybean-corn oil								
4. With fish meal (0.28 mg Se/kg diet)	106	3.13	81±2.1	45.0±1.0	257± 5	44±2.2 (a)	23.1±4.8 (ab)	1.25±0.1
5. Without fish meal (0.15 mg Se/kg diet)	98	3.17	95±2.5	41.4±1.0	242± 4	44±1.9 (a)	19.0±6.7 (a)	1.00±0.0
6. As 5 + Se (0.45 mg Se/kg diet)	96	3.17	74±7.6	44.8±4.9	243± 4	41±1.3 (a)	20.7±5.5 (a)	1.37±0.32
Mean	100	3.16			247	43	21.0	1.21
Wheat-soybean-corn starch								
7. With fish meal (0.25 mg Se/kg diet)	106	3.00	78±3.3	42.1±1.7	248± 8	45±2.0 (a)	37.5±11.0 (dc)	1.62±0.4
8. Without fish meal (0.10 mg Se/kg diet)	101	3.07	73±5.6	41.6±3.2	240±12	48±2.7 (a)	29.4±3.7 (bc)	1.75±0.6
9. As 8 + Se (0.40 mg Se/kg diet)	99	3.07	76±7.7	41.3±4.4	241±17	48±3.3 (a)	36.7±13.0 (dc)	1.25±0.23
Mean	102	3.05			243	47	34.5	1.54
Wheat-soybean-wheat starch								
10. With fish meal (0.25 mg Se/kg diet)	108	3.11	75±4.9	41.3±2.5	261±24	48±2.3 (a)	34.0±10.3 (dc)	1.31±0.18
11. Without fish meal (0.1 mg Se/kg diet)	105	3.13	80±1.1	45.0±0.7	229± 3	50±1.6 (a)	35.7±11.6 (dc)	1.0±0.
12. As 11 + Se (0.4 mg Se/kg diet)	96	3.13	72±2.5	38.3±1.5	236± 6	50±6.3 (a)	40.1±9.2 (d)	2.12±0.3
Mean	103	3.12			240	49	36.6	1.47

*The consumed M.E. values (determined) were corrected for energy going into egg production.

[1]Values with the same common letter are not significantly different by Duncan's range test.

[2]Mean ± S.E.

regarding the effect of corn oil on liver fat metabolism. In order to understand if the positive effect of wheat-soy-corn oil diet on reducing fatty liver was due to the birds having a lower energy retention, or the effect was due to corn oil, per se, the retained energy values were corrected for energy going into egg production which is deposited as eggs outside of the body and therefore, not retained. The energy (caloric) value of egg production was calculated from data on egg production, egg weight and the energy value of each gram of egg (1.66 kcal/g egg) according to report made by Bolton (1958). Net retained energy values were calculated using determined M.E. value of the experimental diets as follows:

$$\text{Net retained energy (kcal/bird/day)} = \left[\text{M.E. Consumed (kcal/bird/day)}\right] - \left[\text{1.66 (Egg Mass) g/bird/day}\right]$$

The results (Table 18) revealed that the lower liver fat percentage of the birds fed WSCO diet is not due to less energy being retained.

Selenium had no significant effect on reducing the liver fat value (Figure 13). The intensity of FLHS was not high enough (Table 18) to evaluate any beneficial effect on it by selenium.

The results from force-feeding experiments 1, 2, and 3 (Tables 9, 11, and 13) clearly indicated that plasma estradiol levels were significantly correlated to the intensity of FLHS. In all these experiments the plasma estradiol values in the birds with FLHS were significantly higher than values from normal birds, indicating that estradiol is a factor involved in FLHS induction. Correlations between FLHS vs liver fat values and plasma estradiol vs liver fat concentration, indicated that besides plasma estradiol, high liver fat content is required as a prime for FLHS to occur.

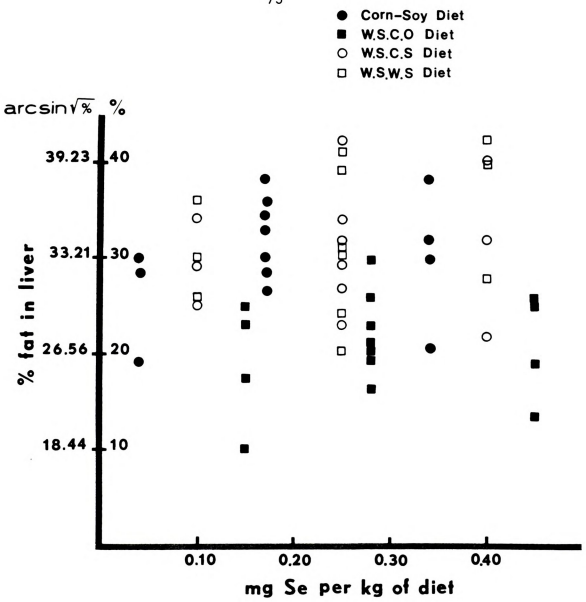

Fig. 13. Relation between dietary selenium levels and liver fat percentage of adult female SCWL chickens in the 18 weeks of imposed ad libitum feeding schedules, X = mg Se per kg of diet, and Y = liver fat percentage.

Table 19. Analysis of variance of liver fat percentage (Experiment V, Stage 3).

Source of variation	d.f.	S.S.	M.S.
Total	63	6786	
Treatment	11	2574	234*
Error	52	4212	81

* $P < 0.005$

The data obtained from force-feeding and ad libitum experiments (Tables 11, 13, 15 and 18) revealed that wheat-soy diet supplemented with corn oil (2.9-3.1%) compared to corn-soy diet (1.6-1.73%) had a beneficial effect on FLHS as well as on liver weight or percentage of liver fat. But when wheat-soy diets were supplemented with either corn starch or wheat starch no significant differences were obtained between corn-soy and wheat-soy diets in producing FLHS or liver fat percentage. The same trend of results was obtained from different experiments concerning the effect of energy sources on FLHS, fatty liver, liver weight and plasma estradiol levels.

V. DISCUSSION

The Relation of Plasma Estradiol and Progesterone Levels with the Fatty Liver Hemorrhagic Syndrome in Laying Hens

Estrogen has a controlling influence on hepatic lipogenesis in laying hens (Lorenz, 1943, 1954; Ranney and Chaikoff, 1951; Husbands and Brown, 1965; Hill et al., 1958; Balnave, 1968a, 1968b; Pearce, 1971b; Balnave and Pearce, 1974). Since most non-layers do not have fatty livers, hormonal balance may be involved in this condition (Barton, 1967). Polin and Wolford (1977) produced FLHS in immature female and male chickens by force-feeding and estradiol injection. Pearson and Butler (1978b) obtained the same results in experiments in which estradiol and ad libitum feeding were given to immature chickens. In the present studies FLHS was induced in mature female chickens, by force-feeding and then plasma estradiol and progesterone were measured. In all experiments in which plasma was analyzed for these two hormones, plasma estradiol content was significantly increased by force-feeding. The rise in plasma estradiol occurred despite a marked reduction in egg production. The rise in estrogen level does not appear to be related to a particular phase of the cycle for forming an egg. Furthermore, any influence of a diurnal variation on plasma estradiol levels would not have an influence due to the procedure to obtain samples in a relatively narrow span of time, with all treatments equally represented during that time span. Another interesting aspect of the estradiol level is that it persisted at the

higher levels, although feed or force-feeding was not allowed to any of the treatments during the last 18 hours prior to sampling. Stress from force-feeding was not a factor to account for the higher plasma estradiol levels in force-fed chickens with FLHS. Force-feeding at 50% or ad libitum which mimics all of the handling and intubation procedures did not produce FLHS (Wolford and Polin, 1974), and chickens fed ad libitum the same diets force-fed have normal estradiol levels.

The results substantiated that estradiol is associated with the cause of liver hemorrhages, in agreement with conclusions by Polin and Wolford (1977), Pearson and Butler (1978b), and Maurice et al. (1979). The role of estradiol in influencing hepatic hemorrhages to occur remains unknown, but Polin and Wolford (1977) suggested consideration be given to the catabolic and anabolic pathways of estrogens.

Substantial evidence has accumulated indicating that hydroxylation is the first step of estradiol degradation in the liver of animals (Aldercreutz, 1970). Two important components of the hydroxylase system in liver microsomes are NADPH- cytochrome c - oxidoreductase and cytochrome P-450 (Briggs and Brotherton, 1970) which were diminished in minimal deviation hepatomas and were almost completely absent in some fast-growing hepatomas (Sugimura et al., 1966). Therefore, the deficient hepatic degradation of estradiol may be the main reason for the high estradiol level in the plasma of the birds with FLHS. This hypothesis should be further studied in the future by measuring the liver microsomes hydroxylation enzyme activity in the hens with FLHS.

Plasma progesterone levels in force-fed hens were lower than those fed ad libitum (Table 8), but not significantly ($P > 0.05$). This presumably

reflected the decline in egg production. Progesterone does not have any role in liver lipogenesis activity (Balnave, 1968b; Balnave and Pearce, 1974). No correlations between plasma progesterone vs FLHS or liver fat content was observed in these experiments. Thus, progesterone does not appear to be involved in the induction of FLHS in laying hens.

Influence of Carbohydrate Source on FLHS

Factors other than energy intake appear to be important determinants of hepatic and plasma lipids under normal feeding conditions in laying hens (Barton, 1967; Jensen et al., 1974a, 1974b, 1976a, 1976b; Polin and Wolford, 1976; Maurice and Jensen, 1977a; and Pearson et al., 1978). In the experiment conducted by Jensen et al. (1976b) corn and wheat, in various proportions, were fed to laying hens. The percent fat and total fat per liver increased as the proportion of corn increased. They reported that, in a comparison of different cereal grains and wheat samples in isocaloric diets, the total liver fat accumulated was the highest for hens fed grain sorghum, corn, or triticale and the lowest for those fed barley, oats, rye or wheat. These results were confirmed by Maurice and Jensen (1978a), on Japanese quail. In the experiments conducted by Maurice and Jensen (1978b) a higher liver fat and FLHS were associated with the feeding of the corn-soy diets, and substitution of wheat bran and corn oil for corn reduced the liver hemorrhages as well as liver fat content. In the experiments by Pearson et al. (1978) wheat-soy and barley-soy diets had a reducing effect on hepatic fat and hemorrhages compared to corn-soy diet.

In all the experiments conducted to determine the effect of cereals on FLS or FLHS, corn oil or animal fats have been used in making the

diets isocaloric. Dietary fat depresses hepatic lipogenesis in mature female chickens (Balnave and Pearce 1969). In an in vitro experiment by Leveille et al. (1975), the fatty acid synthesis (acetate-1-^{14}C incorporation) in livers from growing chicks declined when an increasing percentage of feed energy was derived from fat.

It seems paradoxical to try to prevent or oppose the excessive accumulation of fat in the liver by adding an extra amount of fat or of fatty acids to the feed. Nevertheless, the experiment conducted by Leveille et al. (1975) showed that the de novo - synthesis of fatty acids may be reduced by such a measure. Thus, the reducing effect of wheat-soy diets on FLS or FLHS in the experiments using dietary fat could be from the oil supplying the energy rather than wheat per se. The results obtained in these experiments clearly confirm this concept. Corn oil routinely decreased fat content of livers from hens fed wheat-soy diets. Either corn starch or wheat starch used in wheat-soy diets produced the same intensity of FLHS as did corn-soy diet. In these experiments the percentage of fat in the liver of the birds force-fed a wheat-soy corn oil diet was lower than in those force-fed a corn-soy or wheat-soy starch diets.

No significant difference was obtained between corn starch and wheat starch used in the wheat-soy diets concerning their effect on fatty liver or FLHS. The birds force-fed corn-soy diet had almost the same intensity of FLS or FLHS as those force-fed wheat-soy-starch diets, suggesting that there may be nothing in the wheat to prevent FLS or FLHS in laying hens. Therefore, the FLS or FLHS reduction effect from wheat-soy-corn oil diet must be due to the addition of the corn oil and not due to the wheat or its starch in the diet. This conclusion is confirmed by the

ad libitum feeding experiment in which corn oil, corn starch, and wheat starch were used to make wheat-soy diets isocaloric with the corn-soy diet.

The antilipogenic effect of dietary fat is related to the long chain acyl-CoA derivatives, produced partly from dietary lipids, which behave as inhibitors of fatty enzymes (Pearce, 1968 and 1971a; Balnave and Pearce, 1969; Goodridge, 1972 and 1973; Leveille, 1975; and Weiss et al., 1967). Based on their own research and on the work by Goodridge (1972 and 1973), Leveille et al. (1975) suggested the following regulation of the fatty acid synthesis by fatty acids. The citrate-cleavage reaction, which yields acetyl-CoA, and the activation of fatty acids from feed fat or storage fat requires CoA. Because of this competition for CoA, a quick activation of free fatty acids, derived from feed fat or the fat deposits, may reduce the availability of CoA for the citrate-cleavage reaction and therefore for fatty acid synthesis, resulting in a reduced fatty acid synthesis. Besides, long chain fatty acid-CoA molecules may also inhibit the transport of citrate from the mitochondria to the cell plasma and the activity of acetyl-CoA-carboxylase directly.

Liver lipids are derived from two main sources, de novo synthesis, and feed fat. Obvious causes of an accumulation of fat in the liver are (a) an intensified de novo synthesis (Butler, 1975 and 1976), (b) a defect in the transport of fat from the liver cell plasma to the blood stream (Butler, 1975 and 1976), and (c) decrease in energy utilized in relation to energy intake.

According to Butler (1976), the change in fatty acid composition and the presence of hyperlipidaemia in FLS indicate that the excess of liver fat is mainly derived from a too intensive de novo synthesis.

In the blood, 65 to 75 percent of the lipids may be present as low-density lipoproteins (O'Hea and Leveille, 1969, and Leveille et al., 1975), a form for the transport of lipid. So, a defect in this transport might be a consequence of a decreased formation or release of low-density lipoproteins (Butler, 1976). It was supposed that this might result from a choline deficiency (Lombardi et al., 1968 and Butler, 1975 and 1976). Choline is a component of lecithin (phosphatidyl-choline), a main constituent in the lipid fraction of lipoproteins (Wright, 1970) and therefore important for the permeability of fat through cell membranes (Greuel and Hartfiel, 1968). Lombardi et al. (1968), did observe an inhibition of the release of triglycerides from the liver cells to the blood plasma in choline-deficient rats. The mixture of choline, vitamins B_{12}, and vitamin E without inositol did not reduce lipid and hemorrhagic score of livers in force-fed hens (Wolford and Polin 1975), substantiating an earlier study by Wolford and Murphy (1972).

The results of these experiments and the data from previous research (Annison, 1971, and Butler, 1975 and 1976) lead to the conclusion that the fat accumulation in the liver and FLHS are probably caused by a high lipogenesis in the liver which might be a consequence of (a) an abundant supply of carbohydrates, (b) a deficiency of essential fatty acids, or (c) an increased estrogen level in the blood.

Glycolysis and the citric acid cycle provide the essential materials for fatty acid synthesis, pyruvate as a substrate, NADPH, as a coenzyme,

and citrate as a stimulating factor (Goodridge, 1972 and 1973). These factors mainly determine the extent of fatty acid synthesis. So fatty acid synthesis is highly dependent on the glycolysis activity and therefore on the supply of carbohydrates through the feed. In practical rations for laying hens about 70-80% of energy is supplied as carbohydrate, 2-4% from fat and the rest from protein. Thus, carbohydrates supplied mostly as grains (corn, sorghum, wheat, barley, etc.) would appear to have a major role in inducing FLS and FLHS in laying hens.

Hartfiel and Tuller (1973) observed a drop in liver fat content, when including 3 percent oleic acid rich fat (68.1% oleic acid) or 4 percent oleic acid in the feed. No explanation for this relatively strong effect of oleic acid has been suggested except for general long chain fatty acids antilipogenesis activity. The linoleic acid content of the feed seems to have a great influence on the liver fat content as well. In two experiments by Hartfiel et al. (1972 and 1973), the liver fat content was significantly reduced when feed was supplied with linoleic acid-rich vegetable oils, 2.5 percent soya oil, and 3 to 6 percent sunflowerseed oil. Favorable results were also obtained by Balnave and Pearce (1969) when including 2% corn oil in the feed. "It is tempting to ascribe the favourable effect of linoleic acid to its conversion into arachidonic acid, an essential element of the cell membranes" (Annison, 1971 and Dorp, 1975). This might benefit the permeability of the cell membrane and therefore the transport of lipids.

The facts, mentioned above, lead to the hypothesis that the beneficial effect of corn oil on FLS and FLHS might be due to (a) its lipogenesis inhibition, and (b) its oleic and linoleic acid content.

Therefore, more research is required to explain the effect of dietary fat and linoleic or oleic acids on FLS and FLHS in laying hens.

Data from experiment 3 indicate that sucrose used in wheat-soy diet reduced FLS and FLHS in the force-fed hens. No additional information has been reported about the effect of sucrose on FLS or FLHS. Thus, sucrose could be used as a source of energy in future studies to understand if it has any favorable effect on FLHS.

Influence of Dietary Selenium on Fatty Liver and FLHS

In the force-feeding experiments, FLHS occurred despite 0.16 and 0.24 mg of selenium per kg of corn-soy and wheat-soy diets respectively (0.1 mg/kg from the mineral mix). Karpov (1967) indicated that supplementation of a practical ration with selenium (0.9 mg per kg) reduced the incidence of lipid dystrophy of the liver in laying hens. Jensen et al. (1974b) reported that adding 1 ppm selenium to corn-soybean meal rations, significantly reduced total fat accumulation per liver. These results are contradictory to the data reported from the same laboratory by Maurice et al. (1979), in which selenium had no significant effect on liver fat content. Maurice et al. (1979) indicated that selenium at 0.3 mg/kg diet, mostly from fish meal in a corn-fish meal diet, possesses an anti-hepatohemorrhagic property in caged hens. However, these experiments and those of Polin and Wolford (1977) did not indicate that practical dietary levels of selenium in a hen's diet prevented FLHS. In the latter situation, the evaluation was performed in force-feeding experiments. Also, selenium supplemented (0.24 mg/kg) to a corn-soy diet did not prevent the induction of FLHS in force-fed immature female and male White

Leghorn chickens which were injected with 17-β-estradiol propionate (Polin and Wolford, 1977).

Since FLHS was not of sufficient intensity in the experimental birds feeding ad libitum, it was not possible to evaluate any preventive effect of selenium on FLHS. However, FLHS was induced at sufficient intensity in force-fed chickens to allow such an evaluation on selenium's presumed anti-hemorrhagic effect. No such effect from selenium at level of 0.24 mg per kg of diet was detected.

The force-feeding technique has indicated (1) dietary ingredients contain an anti-hemorrhagic factor (Polin and Wolford, 1976), that (2) data on ad libitum feeding with supplemented inositol, vitamin B_{12}, vitamin E, are not factors preventing FLHS induction (Wolford and Polin, 1975), and (3) that estrogen (Polin and Wolford, 1977) is a factor besides energy for inducing FLHS. Therefore, the force-feeding technique appears to be a valid approach to assess factors inducing or alleviating FLHS,

Methods of Control

Since the first description of the FLS and FLHS, curative and/or preventive measures have been searched for in the field of nutrition. Depending on the hypothesis on the physiological causes of the syndrome, various aspects of nutrition have been regarded, a survey of which will follow here.

1. Restriction of Energy Intake

Energy consumed beyond the requirement for maintenance and egg production by mature hens (36 weeks of age or older) converted to fat.

It is therefore obvious that attempts have been made to counteract the undesirable, excessive fat accumulation in the liver by restricting the energy intake. The most direct way to attain this is feed restriction. In a trial by Wolford and Polin (1974) a rather severe feed restriction, 80% of the intake of the control group during six weeks, resulted in a drop in body weight, weight of the abdominal fat pad, liver weight and mean liver fat content (28.4% vs 37.9% in dry matter). Though feed restriction may be an effective curative measure when a direct problem with FLHS exists, it may be less suited as a preventive measure because of the difficulties encountered in applying the right level of restriction in practical situations. In the years 1960-1965 the commercial feed industry in the U.S.A. treated FLS by supplying diets with a reduced energy level and an increased protein percentage (Wolford, 1971). By reducing the energy level it is possible to depress the energy intake and prevent energetic overconsumption. It was found that a reduction in the M.E. content of the feed from 2900 to 2400 kcal M.E./kg had a favorable effect on the liver fat content (Barton et al., 1967; Duke et al., 1968 and Wolford and Murphy, 1972). According to Wolford and Polin (1974) energy restriction is the most effective method to reduce liver fat content dramatically.

2. Fat Content of the Diet

The liver lipogenesis, FLS and FLHS can be declined when an increasing percentage of feed energy is derived from fat. However, more studies are required to measure the effect of fat and essential fatty acids on FLS and FLHS.

VI. SUMMARY AND CONCLUSION

In this study five experiments were conducted for further investigations about FLHS in adult female Single Comb White Leghorn (SCWL). The relation of plasma estradiol and progesterone levels with FLHS was determined in experiment I to III. The results obtained from these force-feeding experiments revealed that the plasma estradiol levels were positively correlated to the score of FLHS. The correlation between values for plasma estradiol and hepatic fat, the correlation for hepatic fat levels and FLHS score, and the correlation of plasma estradiol vs FLHS score obtained from experiments I to III, implicate higher plasma estradiol, and larger fat accumulation for FLHS to occur. These results substantiated that estradiol is associated with the cause of liver hemorrhages, in agreement with conclusions by Polin and Wolford (1977), Pearson and Butler (1978b), and Maurice et al. (1979). No significant correlation was obtained between plasma progesterone level vs FLHS.

The effect of carbohydrate source and corn oil on FLHS was studied in experiments II to V.

The energy content of the wheat-soybean diets in these experiments (experiments II to IV) has been balanced with the corn-soybean diet using either corn oil, corn starch, sucrose, or wheat starch. The results revealed that either corn starch or wheat starch used in wheat-soybean diets produced almost the same intensity of FLHS as the corn-soybean diet. The liver fat percentage and the score of FLHS from the

birds force-fed wheat-soybean diets supplemented with corn oil or sucrose were significantly lower than other force-fed groups. The data indicate that a wheat-soybean diet has a beneficial effect on prevention of FLHS only when it is supplemented with corn oil or sucrose.

The favorable effect by sucrose on FLS or FLHS will need additional study for confirmation. The decreasing effect of corn oil on FLHS may be due to its antilipogenic activity, which has been reported by many investigators (Pearce, 1968 and 1971a; Balnave and Pearce, 1969; Goodridge, 1972 and 1973; Leveille et al., 1975; and Weiss et al., 1967). Therefore, the effect of wheat-soybean-corn oil diet to reduce FLS or FLHS is due to the addition of corn oil and not due to the wheat per se. This conclusion was confirmed by the ad libitum feeding experiment (experiment #5) in which wheat-soy diets were supplemented with either corn oil, corn starch, or wheat starch.

Selenium at a level of 0.24 mg/kg of diet did not reduce FLHS in laying hens (SCWL) by force-feeding (Polin and Wolford, 1977). Maurice et al. (1979), reported that selenium possesses an anti-hepatohemorrhagic property in cage hens. In the experiment conducted by Polin and Wolford (1977) selenium supplementation (0.24 mg/kg) to a corn-soy diet did not prevent FLHS in force-fed immature female and male chickens receiving estrogen. The data obtained in this study from an ad libitum experiment revealed that dietary selenium at a level of 0.45 mg/kg, which is about four times the level of requirement according to NRC (1977), did not have an effect on hepatic lipid concentration. The scores obtained for FLHS in the ad libitum experiment (experiment V) were not high enough to study

the effect of selenium on FLHS. However, in experiments using the force-feeding technique (experiments I to IV) selenium did not act as an anti-hepatohemorrhagic factor.

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