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## ABSTRACT

### FATTY LIVER STUDIES IN LAYING HENS

by Thomas Lionel Barton

A series of seven experiments was conducted relative to the production and/or prevention of fatty livers. A specific high-energy (3,000 Metabolizable Energy Cal/Kg), commercially available diet was used to induce fatty livers in a commercial strain of Single Comb White Leghorn hens. Experiment I was conducted in floor pens and the remainder of the tests were conducted in individual-bird cages.

A scoring system from one to four was devised to evaluate the livers when birds were sacrificed. A score of one was a mahogany-colored "normal" liver; two was a mild degree of fat infiltration producing a slightly yellow color; three was a very fatty appearing liver, very yellow in color, but with no hemorrhages. If the livers had hemorrhages and/or scar tissue present, a score of four was given. Birds were sacrificed at different intervals in the various experiments, but all birds were evaluated in each experiment.

Fatty liver incidence was much higher in the caged birds than in floor birds fed the same ration and under similar environmental conditions. Methionine supplementation of the basal high-energy diet was not effective in reducing the incidence of fatty livers in caged birds. A specific low-energy, high-fiber diet was very effective in preventing

fatty livers from developing. Most non-laying birds had normal appearing livers, indicating that hormonal balance is involved in this condition.

Varying protein and energy levels were fed in one experiment and higher liver scores were obtained as the energy content of the diet was increased. Higher protein (18%) had no effect on the incidence of fatty livers.

In an experiment concerning methods of correcting fatty livers once they had been induced, the low-energy, high-fiber diet was again very effective in correcting fatty livers. Fifteen percent of wheat bran and three percent of fish meal were also effective in this respect, but required a longer period of time than the low-energy diet. Choline, vitamin B<sub>12</sub> and vitamin E showed very erratic results and a combination of aureomycin and furazolidone was ineffective in correcting fatty livers.

Most of the mortality from fatty liver in these experiments occurred during periods of hot weather. One experiment in which birds were artificially heated to 32° C for 12 weeks showed that mortality was higher in a high temperature environment than in a cool environment (10 - 15.5° C). However, fatty liver incidence of living birds was just as high in the cool environment when fed a fatty liver producing diet.

In a preliminary fractionation experiment, a water extract of wheat bran and its residue corrected the fatty livers to some extent. Numbers of birds were not large in this experiment, but the results suggest possible factor(s) in wheat bran that correct fatty livers.

Thomas Lionel Barton

That the fatty liver syndrome is a nutritional problem is suggested by the fact that fatty livers were induced and/or corrected by diet manipulation. Hormonal balance is also a factor in this condition since most non-layers did not have fatty livers.

FATTY LIVER STUDIES IN LAYING HENS

By

Thomas Lionel Barton

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## INTRODUCTION

Many changes have occurred in the laying hen industry during the past 15 years. Feed efficiency has been improved by the addition of fat to the feed and substitution of corn for the more fibrous ingredients. Due to the competitive nature of the poultry industry, increasing the bird density has been one method of lowering production costs. A much higher percentage of laying hens are now kept in cages because of economics. One problem that has developed in the midst of this progress is a condition called "fatty liver syndrome".

In September, 1964, a conference concerning this problem was held at Michigan State University with various feed industry representatives and members of the Department of Poultry Science participating. It was the opinion of this group that the fatty liver syndrome is a complex problem. It is quite general throughout the United States and Canada and may cause two percent mortality a month. Some of the industry representatives had tried to produce the syndrome experimentally by using different levels of energy or different types of fat. These attempts were unsuccessful. It was the opinion of this group that fatty livers had to be produced experimentally so that a fundamental approach could be made.

This study was initiated as a result of that conference. The objectives of this study were: (1) to try to create the fatty liver syndrome as it occurs under field conditions; (2) to determine whether

the condition is more prevalent in cage or floor pen operations and;  
(3) to study ways of correcting the fatty liver condition once it had occurred.

## REVIEW OF LITERATURE

The pathogenesis of fatty liver is complex. Popper and Shaffner (1957) stated that excess fat deposition in the liver results from a disturbance of one or more pathways of the movement of fat to and from the liver. These are: (1) deposition of dietary fat; (2) mobilization of fat from fat depots; (3) transformation of dietary carbohydrate and protein into fat; (4) lipogenesis from the metabolic pool; (5) oxidation of fat in the liver; and (6) release of fat to the depots.

Hershey (1930) discovered that egg-yolk lecithin could replace raw pancreases in the diet of the depancreatized dog and restore liver fat levels to normal. Best et al. (1932) reported that varying amounts of crude or purified lecithin prepared from egg yolk or beef liver also prevented an increase in liver fat of normal rats fed saturated fat in the ration. The active ingredient in lecithin was reported to be choline and this was further substantiated by the same group later that year (Best and Huntsman, 1932a).

In a report by Gavin and McHenry (1941), fatty liver produced in rats by feeding beef liver fraction was only slightly affected by giving choline, but was completely prevented by the simultaneous administration of lipocaic. Inositol prevented the development of the fatty liver and the accumulation of cholesterol in the liver. They suggested that the action of inositol resembles that of lipocaic.

Similarly, Abels et al. (1943) reported reduced liver fat of human patients with carcinoma of the gastro-intestinal tract who each

received eight grams of lipocaic during the night prior to laparotomy. The lipotropic properties of the lipocaic could be accounted for by its content of inositol alone.

Amdur et al. (1946), in studies on the effect of manganese and choline on bone formation in the rat, found that manganese as well as choline prevented the deposition of excess fat in the liver. At a given level of choline, more fat was observed to be present in the livers of manganese-deficient rats than in the livers of rats receiving adequate manganese. The lipotropic action of manganese was much greater when the choline content of the diet was low, thus indicating an interaction between manganese and choline.

Singal et al. (1949), while studying the nicotinic acid-tryptophan relationship in rats fed a nine percent casein diet containing five percent fat and 0.2 percent cystine and choline, observed yellowish livers at autopsy. Upon analysis, the liver lipids were 16 percent. Supplementary nicotinic acid, tryptophan, valine, histidine, phenylalanine or serine were without effect. The addition of threonine reduced the liver lipids to 5.1 percent.

Harper et al. (1954) failed to obtain greater deposition of fat in the liver of rats by increasing the amount of fat in the diet. This indicated that dietary fat per se did not accumulate to any great extent in the livers of the animals used in this study. Since the amount of fat which was deposited in the liver was decreased slightly as the amount of dietary fat was increased, it appeared that the liver fat arose from the conversion of carbohydrate or protein to fat. Additionally, evidence supporting this conclusion was obtained from the

observation that high fat deposition occurred in the livers of rats receiving fat-free diets.

Yoshida and Harper (1960), using C<sup>14</sup> labeled acetate and palmitate, reported that stimulation of fat synthesis accompanied the fat accumulation in the livers and carcasses of rats fed a low protein diet deficient in threonine. The effects of choline deficiency were less clear-cut. They included increased fat synthesis in the liver, but not in the carcass, and some evidence of impaired transport of fat from the liver. In a later report, Yoshida et al. (1961) found that a moderate restriction of caloric intake (70% of controls) prevented the development of fatty livers in rats fed a threonine-deficient diet, but an equivalent restriction of caloric intake did not prevent the accumulation of liver fat in rats fed a choline-deficient diet.

Similarly, Donovan and Balloun (1955) reported that chicks consuming a high-fat (10%) diet accumulated no more fat in their livers than those consuming a low-fat (1%) diet. They concluded that dietary fat per se was not deposited in the chick liver to any great extent.

Shils and Stewart (1954a) observed that rats subsisting on a diet containing 76 percent corn and three percent or less casein developed a fatty liver characterized by initial and preponderant accumulation of lipid in the portal areas. Rats of the same strain on a diet in which the only protein was casein developed a centrolobular type of fatty liver. Methionine, choline and vitamin B<sub>12</sub> were able to decrease the liver lipid. However, methionine was rarely capable of preventing the deposition of some excess fat, while under certain conditions, the lipotropic action of vitamin B<sub>12</sub> was unpredictable. Later, Shils et al. (1954) reported that a portal type of fatty liver was rapidly and



consistently produced in stock weanling rats by feeding diets in which the protein was exclusively of plant origin (corn, rice, wheat or cassava). Within one week on a corn meal diet excess fat appeared in the portal region of the liver lobule and increased in amount over the 12-week period of study.

Shils and Stewart (1954b) found that DL-tryptophan (0.5%) and L-lysine (1%), alone and together, greatly reduced or prevented the accumulation of hepatic fat usually seen on the corn meal diet. Threonine had little or no effect. Additionally, Vennart et al. (1958) reported that the type of fatty liver produced by corn diets was reversed by the addition of lysine and tryptophan. In no instance was there any demonstrable permanent damage. They suggested a possible relationship between protein and lipid metabolism that has not yet been elucidated.

Gyorgy et al. (1951) reported that a delay in the production of hepatic necrosis in rats fed a low choline diet was obtained from aureomycin and, to a lesser extent, from terramycin and streptomycin. Chloromycetin, polymixin and penicillin were not protective. The beneficial effect of aureomycin was not limited to the delay of hepatic necrosis but manifested itself also in the prevention of hepatic cirrhosis in rats fed a low-protein (casein)-high-fat diet. They suggested an antimicrobial effect on the intestinal flora.

Baxter and Campbell (1952) found that the renal lesions and mortality of rats caused by a purified diet deficient in choline were largely prevented by supplementing the diet with rather high levels of crystalline aureomycin. Fatty changes in the liver also appeared to be reduced to some extent. The levels of choline in liver and kidney

tissue and in feces of deficient animals appeared to be increased slightly as a result of aureomycin administration.

Di Luzio and Zilversmit (1956) reported that the triglyceride concentration in livers of dogs maintained for three weeks on a high-fat, low-protein, choline-deficient diet was lowered by daily injections of heparin and by supplementation of the diet with choline. Oral administration of aureomycin (0.5 grams twice daily) increased liver triglyceride concentrations. One gram of aureomycin per day fed to dogs on normal rations was without any visible or chemical effect on liver lipids.

Rutenburg et al. (1957) also studied the role of intestinal bacteria in the development of dietary cirrhosis in rats. They found that absorbable broad spectrum antibiotics added to the daily diet did not prevent the development of fatty infiltration, but they delayed the development of cirrhosis for about 100 days more. Non-absorbable antibiotics added to the daily diet prevented the development of cirrhosis in most rats for as long as 750 days but did not prevent fatty infiltration. Since non-absorbable antibiotics were superior to absorbable antibiotics, the authors excluded systemic activity of the antibiotic as having anything to do with the protective effect. They asserted that cirrhosis in rats on a choline-deficient diet is caused by intestinal bacteria and not by the choline deficiency.

Leevy (1962) reported on observations in 270 human patients with fatty liver demonstrated by percutaneous liver biopsy in a large municipal hospital. Seventy-seven percent of the patients were alcoholic. Similar degrees of hepatic steatosis were encountered in non-alcoholic patients with a variety of diseases, diabetes and heart failure being

most frequent in this series. Dietary abnormalities were present in each of the patients with fatty liver and appeared to play a central role in its evolution. All dietary regimes tested including a general hospital diet, a high-fat diet, or a low-protein, low-choline diet were associated with elimination of all liver fat in the malnourished alcoholic in 4 to 6 weeks.

Couch (1956) first reported the fatty liver syndrome in laying hens. He stated that the syndrome was first called to his attention by veterinarians at the Poultry Diagnostic Laboratory of the School of Veterinary Medicine, Texas A & M University who stated that the syndrome had first been observed in late 1954.

The symptoms were described as follows: "The hens usually increase in weight from 25 to 30 percent, and the rate of egg production decreases approximately one-third. The birds appear to be healthy and in good condition. A post-mortem examination reveals an excess of abdominal fat, fatty livers, capillary hemorrhages in the liver and hematoma." The author stated that the syndrome may occur as a result of increased strain brought on by such developments as improved breeding and higher egg production, increased energy content of feeds and the caged layer system of management. For prevention, it was recommended that laying mash formulas of the high-energy type have a minimum of 500 grams of added choline, 12 milligrams of vitamin B<sub>12</sub> and 5,000 to 10,000 units of added vitamin E per ton and that the ration contain at least 17 percent protein.

Shortly thereafter, Fisher and Weiss (1956) in a preliminary report, stated that a similar, if not identical, condition to the fatty liver disease was first induced in their laboratory during the fall of

1955. Animal fat was the variable in their studies with the diets well fortified with choline, vitamin B<sub>12</sub>, methionine and higher than normal protein levels. Young pullets fed a ten percent animal fat diet for four months after the start of egg production showed a significant increase in the lipid and cholesterol content of the blood with fatty encapsulation and infiltration of the kidneys.

Fatty livers, while observed to a larger extent in fat-fed birds, also occurred with remarkable frequency in well-laying pullets on fat-free diets. No heavy fat deposits were noticeable in these young birds. On the other hand, quite heavy visceral fat deposits were observed after only eight weeks on a five percent animal fat ration when the birds used were over one year old and thus at the end of their first laying season. In view of the high levels of choline, protein and vitamin B<sub>12</sub> in the animal-fat containing diets, they concluded that these nutrients were not helpful in alleviating or preventing this condition.

A few months later in a more extensive report, Weiss and Fisher (1957) reported that, depending on the age of birds and the duration of feeding trials, 5 to 10 percent of added fat to laying rations resulted in the development of an apparent derangement in lipid metabolism characterized by one or more of the following symptoms: elevated plasma cholesterol and total plasma lipid; excess deposits of body fat; friable and fatty livers; fatty deposits in and around the kidneys; and greater severity of aortic atherosclerosis.

The incidence of abnormal appearing livers and kidneys, as determined by gross observation at autopsy, was approximately twice as high in the fat supplemented birds as in control birds. Abdominal fat

scores indicated a significant deposition of excess body fat in the fat supplemented birds. Histological examination of livers and kidneys from some of the treated and control birds revealed evidence of fatty metamorphosis in both organs. However, the incidence and severity seemed about the same in the liver of the two groups and only slightly greater in the kidneys of the treated birds.

Sutton et al. (1957) found that mature White Leghorn hens housed in individual cages and fed normal low-fat diets exhibited a tendency to increase liver fat deposition. The addition of one percent cholesterol to this diet increased their blood cholesterol level materially and also the total liver fat. Choline chloride (1%) fed with cholesterol exerted a lipotropic action and was effective in reducing both the blood cholesterol level and the total liver fat. Methionine (1%) reduced blood cholesterol levels but did not reduce total liver fat.

Donaldson and Millar (1958) reported on the use of 3 percent added animal fat in a laying ration containing 19 percent crude protein. Fat addition over a 350-day test period resulted in significantly poorer hen-housed egg production and feed conversion but greater energy consumption, body weight gains and mortality during the laying year. The entire increase in mortality was associated with obesity and/or fatty degeneration and hemorrhage in the liver and kidneys.

Turk et al. (1958) fed diets ranging from 690 to 1210 Calories of productive energy per pound during the growing and laying periods. Energy level did not affect egg production. Hens fed more energy deposited large amounts of fat in the viscera and liver but this could be removed by a cool environment and a low-energy ration.

McDaniel et al. (1957) studied the effect of dietary caloric density in relation to protein level on the plasma lipids and liver fat of caged layers. Liver fat values, expressed on a dry weight basis, were found to be higher in caged birds as compared to birds in floor pens. On a given diet, non-laying birds showed more liver fat accumulation than did birds in production. Among birds maintained on litter, this was not the case. The non-layers on litter exhibited the same or lower liver fat when compared to the layers. Liver fat was increased slightly with an increase in energy content of the diet and decreased slightly with an increase in dietary protein at a given calorie level.

Later, McDaniel et al. (1959) reported on the effect of different dietary protein and calorie levels in the presence of dietary fat on performance and on the occurrence of the fatty liver syndrome, obesity and atherosclerotic conditions in caged layers. Caloric consumption per hen per day differed appreciably between the low and higher energy levels. Liver fat values were found to be similar except in a 15 percent protein, 935 Calorie productive energy/lb ration that contained no added fat. Liver fat values were about double in this treatment. All diets tested, except a 15 percent protein - 750 Calorie diet, were scored as producing a high incidence of fatty livers. The presence of added fat in the diet, either from an animal or vegetable source, did not appear to influence the fatty liver condition.

Excessive deposition of abdominal fat was observed in approximately the same degree and incidence as the fatty liver condition. Abdominal fat appeared to be closely related to the increase in body weight. Fatty encapsulation of the kidneys was observed in all hens with normal to excessive amounts of abdominal fat. This condition was

considered to be normal in that the kidney itself showed no fat infiltration, as evidenced by ether extraction and histological examination. A highly significant correlation was obtained between egg production and percent liver fat.

Treat et al. (1960) also reported that liver fat and liver weight of caged layers were higher in treatments with no added fat than in any of the groups receiving added fat.

Donaldson and Gordon (1960) added three percent animal fat to laying hen diets in a floor pen experiment and reported that all fat-fed groups gained more body weight than the controls, but the gains were appreciably greater in heavy as compared to light breeds. Added fat resulted in higher mortality with heavy breeds but not with light breeds.

March and Biely (1962) studied mortality in White Leghorn pullets subjected to three dietary regimens under which the birds received 2.5, 7.5 or 12.5 percent of fat from the time of hatching until they were three years old. Mortality from liver derangement was approximately doubled when either 7.5 or 12.5 percent fat was fed but no other untoward effects on mortality rate were noted. In a later study, March and Biely (1963) introduced supplementary fat into the diet of mature birds which had been fed a ration without added fat. There was no effect on mortality attributable to the level of fat.

Ringer and Sheppard (1963) reported the occurrence of fatty liver syndrome in a Michigan caged layer operation. Postmortem examination revealed excessive fat lining all the visceral organs, an enlarged and very friable liver of a light yellowish-brown color indicative of fat infiltration, a massive hemorrhage from the liver and multiple antemortem

hematomas of the liver. Egg production, based on a percentage of birds surviving did not drop below that for birds of an equal age. The addition of choline chloride to the diet did not alleviate the condition.

Couch (1964) reported on attempts by veterinarians to transmit the fatty liver condition to normal laying hens through the feeding of fecal material, transplantation of respiratory discharge and through the injection of blood from affected birds into normal ones. All such attempts were without effect. It was recommended at this time that treatment of fatty liver include 12 milligrams of vitamin B<sub>12</sub>, 1,000 grams of choline chloride and 10,000 units of vitamin E per ton added on top of the current micronutrient fortification for four weeks along with furazolidone at 200 grams per ton for two weeks and at 50 grams per ton for two weeks.

Summers et al. (1966) recommended that if a flock becomes affected with fatty liver syndrome, 2 ounces of copper sulfate, 500 grams of choline, 3 milligrams of vitamin B<sub>12</sub> and 5,000 I.U. of vitamin E should be added per ton of laying diet. Increasing the level of dietary protein by one to two percent was also reported to help alleviate the condition.

Quisenberry (1966) added iodinated casein (Protamone) at 100 and 200 grams per ton to diets containing either 937 or 1,051 Calories of Productive Energy per pound. One hundred grams of Protamone decidedly reduced the visual fat scores of the livers taken from birds on autopsy at the end of the experimental period of eleven months. In spite of the lower liver fat score the mortality of the Protamone-fed birds was considerably increased. Liver scores from birds fed 200 grams Protamone per ton were higher than those of birds fed 100 grams per ton but less



than livers of birds from the unsupplemented diets. The reason for this was not clear. It was stated that solution of the fatty liver problem has been delayed because of the inability to create the condition at will.

Sunde (1966) fed pullets in floor pens on eight different energy levels ranging from 848 to 1190 Productive Energy Calories per pound and, after five months of production, five birds from each pen were killed. The livers were scored for fatty livers by visual means and later analyzed for fat content. As the energy level increased, the liver fat increased although a plateau seemed to occur during the middle range of energy levels. Liver scores determined visually also seemed to increase. Visual scores were not as dependable as would be desired due to the range in fat content for each visual score. By determining moisture content of the livers, percent solids was a better guide to the liver fat content than a visual score. However, the visual fat score was obtained much faster.

The hens that remained in each experimental group after the first phase of the experiment was concluded were placed on a low-energy diet in a cool environment. Several weeks later additional hens were killed. At this time, all average liver weights had decreased and liver fat had also dropped to much lower levels. Data were presented which indicated that laying hens usually have higher fat in their livers than non-layers. The author reported inability to reproduce the fatty liver condition in the laboratory as it occurs in field cases.

Thornton and McPherron (1962), in an effort to alleviate excessive body and liver fat often observed in chickens maintained for long periods of time in cages, added twenty percent brewers dried grains to

a normal ration at the expense of corn, milo and soybean meal. The gain in body weight and liver fat accumulation during the last fifty weeks was less in the experimental birds to a highly significant degree. The experimental birds also grew faster, matured earlier and produced a greater number of eggs. The results suggested that brewers grains may either contain an unknown factor or that the biological availability of recognized nutrients is greater.

Heald (1963) reported that the metabolism of carbohydrate by slices of liver from the domestic fowl is quantitatively different from that of mammalian liver in several ways. Slices of liver from the domestic fowl metabolized fructose with an increased oxygen uptake, in contrast with mammalian liver where fructose metabolism does not increase the oxygen uptake. Determination of the respiratory quotient, R.Q., showed that slices of fowl liver from fed birds metabolized fructose with an R.Q. of 1.10, and occurred in both laying and non-laying pullets. The increased R.Q. was not found with slices of liver from birds starved for 18 - 24 hours.

Heald and Rookledge (1964) found that estrogen treatment increased the levels of plasma-free fatty acids simultaneously with those of the total lipids and lipophosphoprotein in the immature fowl. Testosterone and thyroxine decreased the levels of plasma lipids and of lipophosphoprotein in the laying fowl, but had no effect upon the levels of free fatty acids in either the immature or laying fowl. Gonadotrophins were without effect upon the plasma free fatty acids in the immature pullet but increased the levels in laying or molting birds. They considered the increases in plasma free fatty acids found when

the bird comes into lay to be an indirect result of ovarian stimulation by pituitary hormones.

Hawkins and Heald (1966) reported that slices of liver from the domestic fowl, when incubated in media containing  $C^{14}$  palmitic acid, incorporated the isotope into the neutral fat fraction, principally triglycerides. Both on a dry weight basis and a DNA basis, slices of liver from the laying hen incorporated more palmitate into the neutral lipid fraction than did slices from immature birds. Treatment of immature birds with estrogen enhanced the ability of the liver to incorporate palmitate into the neutral lipid fraction. Based on their data, it was calculated that the liver of the laying hen synthesizes 15 to 25 times as much lipid per unit time as does that of immature birds when compared on a cellular basis.

Interestingly, in a report by Leach et al. (1966) on the choline requirements of chicks and hens, it would appear that the hen is able to synthesize substantial amounts of choline providing there are sufficient methyl groups present in the diet. Most responses to dietary choline were achieved with marginal levels of methionine. The limiting factor in the hen's ability to synthesize choline appears to be in the formation of methyl groups. Thus, the hen differs from the young chick, which cannot synthesize choline even in the presence of adequate methyl groups.

Therefore, the objectives of this study were: (1) to try to create the fatty liver syndrome as it occurs under field conditions; (2) to determine whether the condition is more prevalent in cage or floor pen operations; and (3) to study ways of correcting the fatty liver condition once it had occurred.

## EXPERIMENT I

### Procedure:

This experiment was conducted with birds in floor pens. All subsequent experiments were conducted in cages. The birds used, as in all experiments in this thesis, were a commercial strain of Single Comb White Leghorns. In this experiment the birds were raised to 21 weeks of age using standard procedures of the Michigan State University Poultry Science Research and Teaching Center. At this time, the birds were transferred to the laying house and randomly distributed into the pens, eliminating the large and small extremes in size and any obvious culls. Wing badges were applied for identification purposes and "Specs" to prevent cannibalism.

Twenty-four pens of 25 birds each were fed the experimental diets shown in Table 1. Each diet was fed to six pens. Diet 1 is a commercially available, all-mash laying formula that has experienced some problem with the fatty liver syndrome under field conditions. All birds were fed this diet from 21 to 26 weeks of age when the experiment started. This formula is available in a concentrate and can be mixed by using 75 percent ground corn, 20 percent concentrate and 5 percent ground limestone. This diet will be referred to in this and following experiments as the basal diet.

When the experiment started, six pens of birds were changed to diet 4 in which 12 milligrams of vitamin B<sub>12</sub>, 5,000 I.U. of vitamin E

Table 1. Composition (%) of experimental diets used in Experiment I

| Ingredient                      | Diet 1 | Diet 2 | Diet 3 | Diet 4   |
|---------------------------------|--------|--------|--------|----------|
| Ground yellow corn              | 75.00  | 71.00  | 67.50  | 75.00    |
| Soybean meal (50%)              | 11.90  | 15.90  | 16.36  | 11.90    |
| Alfalfa meal (17%)              | 0.90   | 0.90   | 1.24   | 0.90     |
| Meat and bone meal (50%)        | 2.20   | 2.20   | 3.02   | 2.20     |
| Fish meal (60%)                 | 0.40   | 0.40   | 0.55   | 0.40     |
| Dried fish solubles             | 0.10   | 0.10   | 0.14   | 0.10     |
| Feather meal                    | 0.20   | 0.20   | 0.27   | 0.20     |
| Blood meal                      | 1.00   | 1.00   | 1.38   | 1.00     |
| Dicalcium phosphate             | 1.40   | 1.40   | 1.92   | 1.40     |
| Ground limestone                | 5.85   | 5.85   | 6.17   | 5.85     |
| Salt                            | 0.35   | 0.35   | 0.48   | 0.35     |
| Fat (animal and vegetable)      | 0.50   | 0.50   | 0.69   | 0.50     |
| Vitamin-trace mineral premix    | 0.20*  | 0.20*  | 0.28   | 0.20*    |
| Methionine hydroxy analogue, Ca | --     | --     | 0.05   | --       |
| Vitamin B <sub>12</sub>         | --     | --     | --     | 600 mcg. |
| Vitamin E                       | --     | --     | --     | 250 I.U. |
| Choline                         | --     | --     | --     | 50 gms.  |
| Total                           | 100.00 | 100.00 | 100.00 | 100.00   |
| % Protein (calc.)               | 15.20  | 16.84  | 17.70  | 15.20    |
| Metab. energy (Cal/kg)          | 3004   | 2986   | 2905   | 3004     |

\* Supplied the following per kg. of diet: Vitamin A, 4400 IU; Vitamin B<sub>12</sub>, 3.52 mcg; Vitamin D<sub>3</sub>, 1198.5 ICU; Riboflavin, 2.64 mg; Niacin, 17.0 mg; Calcium pantothenate, 4.8 mg; Choline, 172 mg; BHT, 11.35 mg; Methionine, 74.9 mg; Vitamin K, .84 mg; Manganese, 80 mg; Iodine, 0.8 mg; Iron, 16 mg; Copper, 4 mg; Zinc, 40 mg; Cobalt, 4 mg.

and 1,000 grams of choline chloride were added per ton of basal diet. Eighteen pens of birds remained on the basal diet to allow further changes as the experiment progressed. After 12 weeks, six pens of birds were changed from diet 1 to diet 2. In diet 2, 4 percent soybean meal (50% protein) was substituted for corn. After 20 weeks, six pens of birds were changed from diet 1 to diet 3. Diet 3 was formulated by increasing the percentage of concentrate to 27.5 percent of the ration and adding .05 percent methionine hydroxy analogue.

Data were recorded for each pen by 28-day periods and included hen-day production, feed consumption, and body weight. These data are shown in Table 2 by periods at which ration changes were made. Data for each 28-day period are shown in Appendix Table 1. One bird was sacrificed from each pen after 20 and 28 weeks on test for liver fat determination and observation. These data are shown in Table 3.

Due to the large numbers of birds involved in this and subsequent experiments and the time required to perform liver fat analyses, a scoring system was devised to evaluate the livers when birds were sacrificed. The scores were from one to four with a score of one being a mahogany-colored "normal" liver. A score of two was indicative of a mild degree of fat infiltration being slightly yellow in color. A score of three was an indication of a very fatty appearing liver, very yellow in color but with no hemorrhages. If the livers had hemorrhages and/or scar tissue present, then a score of 4 was used. After ten 28-day periods, all remaining birds were sacrificed and the livers visually scored for incidence and degree of fatty livers. Liver score values are presented in Table 4.

Table 2. Summary of data collected in Experiment I\*

| Treatment*                               | 4-week periods |       |        |         |
|------------------------------------------|----------------|-------|--------|---------|
|                                          | 0 - 3          | 4 - 5 | 6 - 10 | Overall |
| Average egg production (Percent hen-day) |                |       |        |         |
| 1                                        | 73.4           | 72.5  | 67.6   | 70.5    |
| 2                                        | 74.0           | 72.0  | 68.7   | 71.2    |
| 3                                        | 71.1           | 69.4  | 73.1   | 71.6    |
| 4                                        | 74.0           | 70.9  | 68.2   | 70.7    |
| Average feed consumption (gms/bird/day)  |                |       |        |         |
| 1                                        | 105            | 113   | 103    | 105     |
| 2                                        | 108            | 114   | 105    | 108     |
| 3                                        | 105            | 113   | 107    | 105     |
| 4                                        | 106            | 111   | 103    | 106     |
| Average body weight (grams)              |                |       |        |         |
|                                          | <u>Initial</u> |       |        |         |
| 1                                        | 1730           | 1675  | 1734   | 1816    |
| 2                                        | 1703           | 1643  | 1730   | 1798    |
| 3                                        | 1707           | 1639  | 1716   | 1857    |
| 4                                        | 1712           | 1634  | 1716   | 1789    |

\* Each diet is an average of 6 pens of 25 birds each.

Table 3. Fat content of livers from birds sacrificed after 5 and 7 28-day periods

| Treatment | Percent fat (wet weight) after |                  |
|-----------|--------------------------------|------------------|
|           | 5 28-day periods               | 7 28-day periods |
| 1         | 15.5 $\pm$ 2.8*                | 19.2 $\pm$ 5.0   |
| 2         | 11.6 $\pm$ 1.8                 | 11.8 $\pm$ 1.7   |
| 3         | 11.1 $\pm$ 3.0                 | 11.4 $\pm$ 1.7   |
| 4         | 10.9 $\pm$ 1.2                 | 14.4 $\pm$ 2.8   |

\* Mean  $\pm$  standard error. Each mean is an average of 6 birds.



Table 4. Liver score values obtained in Experiment I

| Treatment | No.<br>birds | Liver score expressed as<br>% of treatment |       |       |       | Average<br>liver<br>score |
|-----------|--------------|--------------------------------------------|-------|-------|-------|---------------------------|
|           |              | No. 1                                      | No. 2 | No. 3 | No. 4 |                           |
| 1         | 115          | 50                                         | 35    | 15    | 0     | 1.66                      |
| 2         | 106          | 73                                         | 24    | 3     | 0     | 1.31                      |
| 3         | 104          | 83                                         | 9     | 8     | 1     | 1.27                      |
| 4         | 114          | 79                                         | 17    | 3     | 1     | 1.28                      |

Pen average hen-day egg production, feed consumption and body weights were subjected to analysis of variance (Snedecor, 1956) and Duncan's multiple range test (Duncan, 1955) was used where applicable.

#### Results and Discussion:

The basal diet used in this experiment was a high energy diet with most of the energy supplied from carbohydrate sources. With this energy level, the 15.2 percent protein content was considered marginal. At the end of four weeks, birds on all treatments had lost weight (Appendix Table 1). The weight loss continued through the third 4-week period of production. During the second 4-week period, a drop in egg production was noted. During the early stages of lay, body weight usually increases rapidly for 6 to 8 weeks and then increases gradually until mature body weight is reached. Likewise, egg production should rapidly increase until peak production is attained at approximately 32 - 34 weeks of age.

The weight loss and subsequent drop in egg production observed in the early weeks of this experiment could have been due to inadequate protein intake. Therefore, the protein content was increased in diet 2 after 12 weeks and in diet 3 after 20 weeks of the experiment.

There were no significant differences between treatments for egg production, feed consumption or body weights. The response in egg production when birds were switched to diet 3 after 20 weeks on test (Table 2) approached but was not significant at the five percent level.

Two birds on each of diets 1, 2 and 4 were diagnosed as having died from a fatty liver during the eighth and ninth periods. This was during July and August, 1965. Couch (1956) reported increased mortality

in warmer weather and the mortality due to fatty liver in this experiment would tend to confirm this. However, when all remaining birds were sacrificed after forty weeks on test, there was not much evidence of fatty liver occurrence. As shown in Table 4, only two birds had hemorrhages present in their livers. Most of the livers were considered normal.

Since Couch (1956) reported that birds with fatty liver are usually 25 to 30 percent heavier than normal birds, final body weights obtained in this experiment were plotted by liver scores. These data are presented in Table 5. As body weight increased, the birds tended to have higher liver scores. However, the birds in this experiment were smaller, on average, than would normally be expected of birds of this strain. Likewise, the birds from diet 3 were larger than the other treatments and yet had a lower average liver score.

Table 5. Average body weights by liver scores

| Treatment | No.<br>birds | No. 1 | No. 2 | No. 3 | No. 4 |
|-----------|--------------|-------|-------|-------|-------|
| 1         | 115          | 1739  | 1866  | 1948  | --    |
| 2         | 106          | 1775  | 1884  | 2102  | --    |
| 3         | 104          | 1798  | 1784  | 2229  | 2225  |
| 4         | 114          | 1757  | 1898  | 2102  | 2088  |

## EXPERIMENT II

### Procedure:

The purpose in starting this experiment nine weeks later than Experiment I was to take advantage of any early observations that developed as Experiment I progressed. Since the birds used in Experiment I experienced a weight loss and drop in egg production, it seemed desirable to add methionine to one diet (Diet 6). Supplemental vitamin E was added since Couch (1956) reported that it was beneficial in preventing fatty livers. Ten percent oats were substituted for corn as a method of lowering the energy content of the ration.

Experiment II was conducted in cages with birds of the same population as those in Experiment I. The birds were placed in individual 8 x 16-inch cages at 21 weeks of age and fed the basal diet designated as diet 1 in Experiment I until the experiment started when the birds were 35 weeks of age. Egg production (hen-day), feed consumption and body weight were measured by 28-day periods during the 28-week experiment. Four groups of 8 birds were fed each experimental diet and the experimental outline is shown in Table 6. The composition of diet 7 is shown in Table 7.

One bird from each treatment was sacrificed after 3 and 5 28-day periods on test for liver fat analysis and observation for fatty livers. This corresponds to the fifth and seventh period in Experiment I. At the end of the test all remaining birds were sacrificed and livers were scored for fatty liver incidence according to the system described

Table 6. Experimental outline for Experiment II

| Diet | Supplement                                |
|------|-------------------------------------------|
| 5    | Basal diet as in Experiment I             |
| 6    | Diet 5 + 0.1% methionine hydroxy analogue |
| 7    | See Table 7                               |
| 8    | Diet 7 + 10,000 I.U. vitamin E/ton        |
| 9    | Diet 7 with 10% oats replacing corn       |

Table 7. Composition of diet 7 used in Experiment II

| Ingredient                      | Percent of ration |
|---------------------------------|-------------------|
| Ground yellow corn              | 66.85             |
| Soybean meal (50%)              | 17.50             |
| Fish meal (57%)                 | 3.00              |
| Alfalfa meal (17%)              | 2.00              |
| Animal fat                      | 1.50              |
| Ground limestone                | 7.00              |
| Dicalcium phosphate             | 1.50              |
| Salt                            | 0.35              |
| Methionine hydroxy analogue, Ca | 0.05              |
| Vitamin-trace mineral premix    | 0.25*             |
| Total                           | 100.00            |
| Percent protein (calc.)         | 16.8              |
| Metabolizable energy (Cal/kg)   | 2944              |

\* Supplied the following per kg of feed; Vitamin A, 6,600 I.U.; Vitamin D<sub>3</sub>, 1,650 ICU; Vitamin E, 8.25 IU; Riboflavin, 4.4 mg; Niacin, 22 mg; d-Pantothenic acid, 7.04 mg; Choline chloride, 220 mg; Vitamin B<sub>12</sub>, 11 mcg; Folic acid, 0.275 mg; Menadione sodium bisulfite complex, 2.2 mg; BHT, 124.7 mg; Manganese, 60 mg; Zinc, 27.5 mg; Iodine, 1.2 mg; Iron, 20 mg; Copper, 2 mg; Cobalt, 0.2 mg.

in Experiment I. Average egg production, feed consumption and final body weight data are shown in Table 8. Data for these measurements are presented by 28-day periods in Appendix Table 2. Liver fat analyses for birds sacrificed after 3 and 5 28-day periods are shown in Table 9. Fat content of livers from birds fed a low-energy, high-fiber diet from an unrelated experiment are also presented in this table. Average liver scores and the percentage of each treatment having the various scores are presented in Table 10.

#### Results and Discussion:

The birds used in this experiment became considerably larger than those in Experiment I. These birds apparently did not experience a weight loss during the early stages of production and thus were considerably larger at 35 weeks of age than the birds from Experiment I (See Appendix Table 2).

There were no statistical differences between treatments for egg production, feed consumption or final body weight when the entire experimental period was considered. Egg production was higher at the start but lower at the end of this experiment than in Experiment I. Egg production was lower for the entire experiment than in Experiment I, but it should be pointed out that peak production had occurred before this experiment started and thus is not included.

Birds sacrificed at the end of the third and fifth periods (equivalent to the 5th and 7th periods in Experiment I) had higher liver fat content than did the birds in Experiment I (See Table 9). Liver fat content was higher after five periods than at the earlier interval. Four birds that died during the fifth period were diagnosed as having



Table 8. Average egg production, feed consumption and final body weight obtained in Experiment II\*

| Diet | Egg<br>production<br>(% Hen-day) | Feed<br>consumption<br>(gms/bird/day) | Final body<br>weight (gms) |
|------|----------------------------------|---------------------------------------|----------------------------|
| 5    | 65.8                             | 109                                   | 2007                       |
| 6    | 66.6                             | 113                                   | 2106                       |
| 7    | 69.9                             | 109                                   | 2116                       |
| 8    | 68.9                             | 108                                   | 2070                       |
| 9    | 69.6                             | 109                                   | 2052                       |

\* Each diet is an average of four groups of eight birds.

Table 9. Fat content of livers from birds sacrificed after 3 and 5 periods in Experiment II

| Diet                   | Percent fat (wet weight) |           |
|------------------------|--------------------------|-----------|
|                        | 3 periods                | 5 periods |
| 5                      | 21.9                     | 22.6      |
| 6                      | 26.8                     | 42.1      |
| 7                      | 13.3                     | 19.4      |
| 8                      | 30.7                     | 39.1      |
| 9                      | 24.8                     | 30.1      |
| Low-energy, high-fiber | 5.8                      | 9.8       |
| " " " "                | 7.2                      | 6.9       |
| " " " "                |                          | 17.5      |
| " " " "                |                          | 7.8       |

Table 10. Liver scores obtained in Experiment II

| Treatment | Average<br>liver score | Liver score<br>expressed as percent of treatment |       |       |       |
|-----------|------------------------|--------------------------------------------------|-------|-------|-------|
|           |                        | No. 1                                            | No. 2 | No. 3 | No. 4 |
| 5         | 2.79                   | 13                                               | 25    | 33    | 29    |
| 6         | 2.46                   | 21                                               | 29    | 32    | 18    |
| 7         | 1.88                   | 40                                               | 40    | 12    | 8     |
| 8         | 2.10                   | 34                                               | 35    | 17    | 14    |
| 9         | 2.04                   | 35                                               | 35    | 23    | 7     |

died from a fatty liver (2 birds were from diet 5 and one each from diets 8 and 9). This was during June and the early part of July which was slightly earlier than the fatty liver mortality experienced in Experiment I. The mortality during this period and the fat contents of sacrificed birds indicate that fatty liver was present during this time and of greater severity than in Experiment I.

Liver fat levels of some birds fed a low-energy, high-fiber diet in an unrelated experiment were much lower than those of birds sacrificed in this experiment. This observation was the basis of Experiment III and will be discussed later.

When all remaining birds were sacrificed at the end of the experiment, liver fat scores were much higher than those obtained in Experiment I (See Table 10). Twenty-nine percent of the birds fed the basal diet had hemorrhages present. Supplementation of the basal diet with methionine had little effect, but the average liver score of birds fed a higher quality, higher protein diet (Diet 7) were considerably less than those from the basal diet. There was no effect from supplemental vitamin E or the substitution of ten percent oats for corn.

Average body weights were again plotted for each treatment by liver scores and are presented in Table 11. As body weight increased, the average liver score also increased. However, this was only true within treatments since the birds fed diet 7 were larger at the end of the test but had lower liver scores.

The results of this experiment indicate that fatty liver is more prevalent in cages. Consequently, all subsequent experiments were conducted in cages.

Table 11. Average body weight by liver scores

| Treatment | No. 1 | No. 2 | No. 3 | No. 4 |
|-----------|-------|-------|-------|-------|
| 5         | 1740  | 2012  | 1901  | 2140  |
| 6         | 1869  | 1969  | 2260  | 2360  |
| 7         | 1875  | 2293  | 2194  | 2315  |
| 8         | 1870  | 2179  | 2252  | 2247  |
| 9         | 1851  | 2017  | 2285  | 2360  |

### EXPERIMENT III

#### Procedure:

As mentioned previously, it was observed that birds from an unrelated experiment fed a low-energy, high-fiber ration did not develop fatty livers. Experiment III was conducted, therefore, to compare this low-energy, high-fiber ration with the basal diet used in Experiments I and II. Twenty-four groups of four birds each that had been fed the low-energy ration for approximately six months of production were randomly distributed into 12 x 18-inch cages. Twelve groups of four birds each were fed the basal diet and a like number of birds remained on the low-energy, high-fiber diet. In this experiment, the basal diet was designated as diet 1 and the low-energy, high-fiber diet was designated as diet 2. Diet 2 was quite different in composition from the high-energy basal but for purposes of discussion in this study, will be referred to as the low-energy, high-fiber diet.

The objective of this experiment was to determine if fatty livers could be created in a relatively short time during the summer months. The experiment started June 30, 1965 and was conducted for a period of 14 weeks. Feed consumption, body weight and hen-day egg production were measured by two-week periods. At the end of 14 weeks all birds were sacrificed and livers were scored for fatty livers as described in Experiment I. The composition of the low-energy, high-fiber diet is presented in Table 12. Egg production, feed consumption and body

Table 12. Composition (%) of low-energy diet used in Experiment III

| Ingredient                    | Percent |
|-------------------------------|---------|
| Ground yellow corn            | 34.5    |
| Ground oats                   | 20.0    |
| Wheat bran                    | 15.0    |
| Wheat middlings               | 10.0    |
| Alfalfa meal (17%)            | 3.0     |
| Dried skim milk               | 2.0     |
| Fish meal (60%)               | 2.5     |
| Meat and bone scraps          | 3.0     |
| Soybean meal (44%)            | 2.5     |
| Oyster shell flour            | 5.0     |
| Steamed bone meal             | 1.5     |
| Salt                          | 0.6     |
| Fish oil (2000 A; 400 D)      | 0.4     |
| Total                         | 100.0   |
| Percent protein (calc.)       | 14.8    |
| Metabolizable energy (Cal/kg) | 2359    |

weight data are presented in Table 13 and liver score results are presented in Table 14.

#### Results and Discussion:

The low-energy diet used in this experiment was formulated in the mid 1930's and the only supplemental vitamins added were vitamins A and D from the fish oil. It also contained relatively large amounts of oats, wheat bran and wheat middlings, which are of lower energy content than corn.

When birds were switched from this diet to the high-energy basal diet used in Experiment I, they did not readily adjust to the higher energy content by lowering feed intake (Table 13). During the third two-week period, birds fed the high-energy basal diet consumed less feed than birds remaining on the low-energy diet and this trend was maintained for the remainder of the experiment. Feed intake differences over the entire experimental period approached but were not significantly different ( $P < 0.05$ ). Thus, birds fed the high-energy basal diet consumed approximately 55 M.E. Calories more per day than birds fed the low-energy diet. Body weight was a reflection of energy intake and thus birds fed the basal diet were heavier at the end of the test than birds receiving the low-energy diet, but this difference was not statistically significant ( $P < 0.05$ ). No significant differences were observed between treatments for egg production, although birds switched to the high-energy basal diet laid at a lower rate.

When all birds were sacrificed at the end of the test, there was a wide difference in the average liver scores of the two treatments. Six of the livers from the birds fed the high-energy basal diet had



Table 13. Summary of results for Experiment III

| Diet                       | Two-week periods                 |      |      |      |      |      |      | Overall |
|----------------------------|----------------------------------|------|------|------|------|------|------|---------|
|                            | 1                                | 2    | 3    | 4    | 5    | 6    | 7    |         |
|                            | Percent egg production (hen-day) |      |      |      |      |      |      |         |
| 1 (high-energy, basal)     | 74.8                             | 70.1 | 66.7 | 67.9 | 68.9 | 66.7 | 57.1 | 67.6    |
| 2 (low-energy, high-fiber) | 76.3                             | 77.5 | 72.3 | 71.4 | 69.1 | 72.8 | 61.6 | 71.5    |
|                            | Feed intake (gms/bird/day)       |      |      |      |      |      |      |         |
| 1 (high-energy, basal)     | 125                              | 114  | 106  | 103  | 114  | 114  | 99   | 110.7   |
| 2 (low-energy, high-fiber) | 123                              | 112  | 119  | 112  | 123  | 122  | 115  | 118.0   |
|                            | Body weight (gms)                |      |      |      |      |      |      |         |
| Pre-experimental           |                                  |      |      |      |      |      |      |         |
| 1                          | 1834                             | 1889 | 1880 | 1847 | 1852 | 1882 | 1877 | 1864    |
| 2                          | 1793                             | 1761 | 1761 | 1735 | 1739 | 1763 | 1758 | 1765    |

Table 14. Liver scores, Experiment III

| Diet                       | Average<br>liver score | Liver score expressed as<br>percent of treatment |       |       |       |
|----------------------------|------------------------|--------------------------------------------------|-------|-------|-------|
|                            |                        | No. 1                                            | No. 2 | No. 3 | No. 4 |
| 1 (Basal, High-energy)     | 2.44                   | 11                                               | 47    | 29    | 13    |
| 2 (Low-energy, high-fiber) | 1.77                   | 85                                               | 13    | 2     | 0     |

hemorrhages and/or scar tissue present and only five of the birds on this treatment had mahogany-colored livers. On the other hand, 85 percent of the birds fed the low-energy diet had livers that were scored one and only one bird was scored a number three liver. The average liver score of birds fed the basal diet was lower in this experiment than in Experiment II, but probably was a result of this shorter test period.

Although the two diets fed in this experiment were quite different in composition, one of the major differences was energy content and resulting energy intake. The results of this experiment indicated that fatty liver could be developed in as short as 14 weeks during the summer months.

## EXPERIMENT IV

### Procedure:

Since the birds in Experiment III did not readily adjust feed intake downward when switched to a high-energy diet, Experiment IV was designed to test the effect of varying energy and protein levels on the incidence of fatty livers. Sixteen birds of the same strain used in previous experiments were fed individually in 8 x 16-inch cages each of the six experimental diets shown in Table 15. Wheat bran and wheat middlings were used to achieve the low-energy levels and these ingredients were removed and animal fat was added in the higher energy diets. Corn and soybean meal were altered to keep the protein constant at either 15 or 18 percent. Egg production, feed consumption and body weight were determined individually by two-week periods during the course of the 24-week experiment. The birds in this experiment were purchased at 20 weeks of age as started pullets and were fed a standard laying ration until they were 34 weeks of age when the experiment started.

After 12 weeks on the test diets, four birds were sacrificed from each treatment for liver scoring. However, if a bird had died from a given treatment, only three birds were sacrificed so as to leave 12 birds remaining on each treatment. A similar number of birds were sacrificed from each treatment after 16, 20 and 24 weeks of the test. The results for egg production, feed consumption and body weight

Table 15. Composition (%) of experimental diets used in Experiment IV

| Ingredient          | Diets  |        |        |        |        |        |
|---------------------|--------|--------|--------|--------|--------|--------|
|                     | 1      | 2      | 3      | 4      | 5      | 6      |
| Ground yellow corn  | 50.58  | 62.36  | 72.92  | 58.41  | 63.16  | 57.68  |
| Soybean meal (50%)  | 7.82   | 10.04  | 12.48  | 17.99  | 20.24  | 21.22  |
| Alfalfa meal (20%)  | 2.00   | 2.00   | 2.00   | 2.00   | 2.00   | 2.00   |
| Wheat bran          | 14.00  | 7.00   | --     | 5.00   | --     | --     |
| Wheat middlings     | 14.00  | 7.00   | --     | 5.00   | --     | --     |
| Fish meal (60%)     | 3.00   | 3.00   | 3.00   | 3.00   | 3.00   | 3.00   |
| Ground limestone    | 6.50   | 6.50   | 6.50   | 6.50   | 6.50   | 6.50   |
| Dicalcium phosphate | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   |
| Salt                | 0.35   | 0.35   | 0.35   | 0.35   | 0.35   | 0.35   |
| Animal fat          | --     | --     | 1.00   | --     | 3.00   | 7.50   |
| Vitamin premix*     | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   |
| Total               | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| % protein (calc.)   | 15.00  | 15.00  | 15.00  | 18.00  | 18.00  | 18.00  |
| M.E. Cal/kg         | 2472   | 2716   | 2991   | 2715   | 2984   | 3127   |

\* Supplied the following per kilogram of feed: Vitamin A, 6600 I.U.; Vitamin D<sub>3</sub>, 1650 ICU; Vitamin E, 8.25 IU; Riboflavin, 4.4 mg; Niacin, 22 mg; d-Pantothenic acid, 7.04 mg; Choline chloride, 220 mg; Vitamin B<sub>12</sub>, 11 mcg; Folic acid, 0.275 mg; Menadione sodium bisulfite complex, 2.2 mg; BHT, 124.7 mg; Manganese, 60 mg; Zinc, 27.5 mg; Iodine, 1.2 mg; Iron, 20 mg; Copper, 2 mg; Cobalt, 0.2 mg.

corresponding to the intervals at which birds were sacrificed are shown in Tables 16, 17 and 18, respectively. The results for these measurements during the first 12 weeks of the experiment are presented by two-week periods in Appendix Table 3. Average liver scores for the various intervals are presented in Table 19.

#### Results and Discussion:

Feed intake was essentially the same for all treatments during the first two weeks of the experiment (See Appendix Table 3). Thus, the birds fed the lower energy diets lost weight during this period while birds fed the higher energy diets gained weight. During the second two-week period, birds on all treatments adjusted their intake to the energy content of the diet and thus birds on all treatments gained weight. However, complete adjustment to the energy level of the diet did not occur, since birds fed higher energy diets consumed more calories (See Appendix Table 3). A portion of this difference in energy intake could be due to the fact that birds fed higher energy diets did not lose weight during the initial two-week period. Thus, birds fed higher energy diets were larger and would require more calories for maintenance.

Protein intake was decreased as the energy level of the diet increased since energy level was the primary factor governing feed intake. Possibly the increased intake on the low-protein diets was an attempt by the birds to consume adequate protein.

Feed intake was significantly different ( $P < 0.01$ ) between treatments as shown in Table 17. There was a step-wise decrease in feed intake as the energy content of the diet was increased. Feed

Table 16. Egg production (% hen-day) obtained in Experiment IV

| Diet | 2-week periods |       |        |         |
|------|----------------|-------|--------|---------|
|      | 0 - 6          | 7 - 8 | 9 - 10 | 11 - 12 |
| 1    | 80.4           | 75.6  | 73.7   | 72.3    |
| 2    | 81.0           | 80.6  | 78.1   | 65.2    |
| 3    | 83.8           | 76.2  | 65.6   | 76.8    |
| 4    | 77.6           | 72.0  | 59.4   | 73.2    |
| 5    | 80.1           | 77.6  | 70.1   | 77.4    |
| 6    | 79.2           | 74.0  | 73.2   | 71.4    |

Table 17. Average feed intake (gms/bird/day) for Experiment IV\*

| Diet | 2-week periods |         |          |           |
|------|----------------|---------|----------|-----------|
|      | 0 - 6          | 7 - 8   | 9 - 10   | 11 - 12   |
| 1    | 111 Aa         | 115 Aa  | 110 Aa   | 103 ABab  |
| 2    | 107 ABab       | 107 ABb | 97 ABbc  | 99 ABCabc |
| 3    | 100 BCbcd      | 102Bb   | 94 Bbc   | 90 BCcd   |
| 4    | 105 ABabc      | 107 ABb | 103 ABab | 108 Aa    |
| 5    | 100 BCcd       | 102 Bb  | 98 ABbc  | 95 ABCbcd |
| 6    | 95 Cd          | 93 Cc   | 90 Bc    | 88 Cd     |

\* For any given time interval, means having different letters are significantly different according to Duncan's Multiple Range Test. Capital letters denote  $P < 0.01$ ; small letters denote  $P < 0.05$ .



Table 18. Average body weight (gms) obtained in Experiment IV\*

| Diet | 2-week periods |          |         |         |
|------|----------------|----------|---------|---------|
|      | 0 - 6          | 7 - 8    | 9 - 10  | 11 - 12 |
| 1    | 1719 Aa        | 1778 a   | 1756 ab | 1806 a  |
| 2    | 1758 Aab       | 1782 ab  | 1706 b  | 1715 a  |
| 3    | 1782 ABabc     | 1816 abc | 1800 ab | 1686 a  |
| 4    | 1755 ABab      | 1825 abc | 1841 ab | 1855 a  |
| 5    | 1825 ABbc      | 1885 bc  | 1906 a  | 1867 a  |
| 6    | 1862 Bc        | 1906 c   | 1895 a  | 1845 a  |

\* For any given time interval, means having different letters are significantly different. Capital letters denote  $P < 0.01$ ; small letters denote  $P < 0.05$ .

Table 19. Liver scores, Experiment IV

| Diet | Average liver scores |         |         |         | Overall average |
|------|----------------------|---------|---------|---------|-----------------|
|      | 12-week              | 16-week | 20-week | 24-week |                 |
| 1    | 2.25                 | 2.38    | 2.00    | 2.87    | 2.38            |
| 2    | 2.75                 | 2.63    | 2.25    | 2.25    | 2.47            |
| 3    | 2.83                 | 3.00    | 2.75    | 3.00    | 2.90            |
| 4    | 2.33                 | 2.50    | 2.67    | 2.12    | 2.41            |
| 5    | 3.00                 | 3.00    | 2.83    | 2.67    | 2.88            |
| 6    | 2.90                 | 3.00    | 2.50    | 2.38    | 2.70            |

intake was more variable between treatments during the latter part of the test. This was because fewer birds were in each treatment during this time and thus the values were less repeatable.

Body weights shown in Table 18 were significantly different ( $P < 0.01$ ) after 12 weeks on test. These weights increased as the energy content of the diet was increased. After 16 and 20 weeks on test, body weights between treatments were not as great, but were significantly different ( $P < 0.05$ ). Body weights at the end of the test were not significantly different since only four birds remained on each treatment.

Liver scores presented in Table 19 indicate that there was a trend toward higher liver scores as the energy content of the diet was increased. However, there was a considerable degree of fatty livers in all treatments, indicating that other factors are involved besides energy. None of the birds that died during this test were diagnosed as having died from a fatty liver. Four birds (one each from diets 1 and 3; two from diet 6) were scored as having hemorrhages and/or scar tissue present.

The results of this experiment indicate that energy level of the diet is a factor in the incidence of fatty livers, but other factors are also involved since some fatty livers occurred in all treatments.

## EXPERIMENT V

### Procedure:

At this point in the study, it seemed desirable to test various possibilities of correcting fatty livers once they had developed. Approximately 180 birds from the same population as those used in Experiment IV were placed on the basal diet described in Experiment I at 38 weeks of age. Forty similar birds were fed the low-energy, high-fiber diet used in Experiment III. The birds were fed these diets until they were 57 weeks of age. During the week prior to the start of the experiment all non-layers and extremes in size were eliminated. Eight birds fed the basal diet and six birds fed the low-energy, high-fiber diet were sacrificed to determine the incidence of fatty livers.

The experiment started June 30, 1966 and was conducted for a period of nine weeks. Six groups of four birds each were fed diets 1 - 6 and three groups of four birds each were fed diets 7 and 8. The experimental outline of these diets is shown in Table 20. Egg production, feed consumption and body weight changes were measured by groups of four birds and are presented in Tables 21, 22 and 23, respectively. Two groups of four birds were sacrificed from each of the first six treatments after 3, 5 and 9 weeks on test and scored for fatty livers as described previously. One group of four birds was sacrificed from treatments 7 and 8 at these intervals. The average liver scores are presented in Table 24.

Table 20. Experimental outline for Experiment V

| Treatment | Previous treatment                          | Treatment during Experimental period                                                                                                          |
|-----------|---------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| 1         | Basal diet, Exp. I.                         | No change                                                                                                                                     |
| 2         | " " " "                                     | Switch to low-energy, high-fiber diet used in Experiment III                                                                                  |
| 3         | " " " "                                     | Basal diet with 15% wheat bran and 3% fish meal replacing corn                                                                                |
| 4         | " " " "                                     | Basal diet plus 1,000 gms choline chloride, 12 mg Vitamin B <sub>12</sub> and 5,000 I.U. vitamin E/ton                                        |
| 5         | " " " "                                     | Diet 4 + 100 gms aureomycin and 150 gms furazolidone/ton                                                                                      |
| 6         | " " " "                                     | Basal diet + the following per Kg of feed: 4.4 mg riboflavin, 8.8 mg pantothenic acid, 22 mg niacin, 0.55 mg folic acid and 3.3 mg pyridoxine |
| 7         | Low-energy, high-fiber diet, Experiment III | No change                                                                                                                                     |
| 8         | " " "                                       | Switch to basal diet                                                                                                                          |

Table 21. Percent hen-day egg production in Experiment V

| Treatment | Pre-exp. * | Weeks |       |       |
|-----------|------------|-------|-------|-------|
|           |            | 0 - 3 | 4 - 5 | 6 - 9 |
| 1         | 67.7       | 63.4  | 57.6  | 54.6  |
| 2         | 69.5       | 61.3  | 57.6  | 61.3  |
| 3         | 69.8       | 62.9  | 56.7  | 48.1  |
| 4         | 66.7       | 59.7  | 60.5  | 57.1  |
| 5         | 71.6       | 66.9  | 65.7  | 60.0  |
| 6         | 70.6       | 65.9  | 61.6  | 68.8  |
| 7         | 64.6       | 65.1  | 60.7  | 44.2  |
| 8         | 60.4       | 60.7  | 50.9  | 40.0  |

\* Two weeks.

Table 22. Feed intake (gms/bird/day) in Experiment V

| Treatment | Weeks |       |       |
|-----------|-------|-------|-------|
|           | 0 - 3 | 4 - 5 | 6 - 9 |
| 1         | 91.7  | 91.7  | 98.5  |
| 2         | 83.7  | 94.9  | 112.1 |
| 3         | 89.4  | 90.8  | 97.2  |
| 4         | 88.3  | 96.9  | 101.3 |
| 5         | 94.9  | 96.9  | 96.5  |
| 6         | 90.5  | 93.2  | 103.6 |
| 7         | 93.3  | 100.5 | 90.8  |
| 8         | 91.7  | 88.9  | 101.3 |

Table 23. Body weight change (gms) in Experiment V\*

| Treatment | Initial weight | Weeks |        |       |
|-----------|----------------|-------|--------|-------|
|           |                | 0 - 3 | 4 - 5  | 6 - 9 |
| 1         | 1789           | - 26  | - 6 a  | - 18  |
| 2         | 1774           | - 82  | + 11 a | + 40  |
| 3         | 1800           | - 18  | - 31 a | + 10  |
| 4         | 1791           | 0     | - 11 a | + 10  |
| 5         | 1800           | - 2   | - 5 a  | - 6   |
| 6         | 1783           | - 33  | + 59 b | - 32  |
| 7         | 1630           | - 36  | - 30 a | + 21  |
| 8         | 1654           | - 2   | - 4 a  | + 29  |

\* Means having different letters are significantly different ( $P < 0.05$ ).



Table 24. Average liver scores obtained in Experiment V

| Treatment | Pre-exp. | 3 weeks | 5 weeks | 9 weeks |
|-----------|----------|---------|---------|---------|
| 1         | 3.13     | 3.13    | 3.14    | 2.94    |
| 2         |          | 1.94    | 1.31    | 1.37    |
| 3         |          | 3.00    | 1.71    | 1.64    |
| 4         |          | 2.63    | 2.50    | 2.06    |
| 5         |          | 2.88    | 2.29    | 3.00    |
| 6         |          | 3.06    | 2.94    | 3.19    |
| 7         | 1.67     | 1.50    | 1.25    | 1.13    |
| 8         |          | 2.37    | 2.50    | 2.88    |

### Results and Discussion:

Two birds from the basal diet were diagnosed as having died from fatty livers during the month preceding the start of the experiment. Three birds died as a result of fatty livers during the first two weeks of the experiment (one each from diets 1, 4 and 5). No other birds died as a result of fatty liver during the nine-week experimental period.

Body weight change was used in this experiment to reflect the status of remaining birds, since one-third of the birds fed each diet were sacrificed at each time interval. During the first three weeks of the experiment, birds on all treatments except treatment 4 lost weight (See Table 23). Birds switched to the low-energy, high-fiber diet lost the most weight and this was probably a result of failing to adjust to the energy content of the diet during this period.

The weather was quite hot (over 90° on several days) during this period and feed intake of birds on all treatments was lowered, which accounts for the weight loss during this period. During the fourth and fifth weeks of the test birds fed treatment 6 gained significantly more weight ( $P < 0.05$ ) than birds fed any other diet. The large fluctuation in body weight of this group is not readily explainable. No other significant differences between treatments for body weight change were noted in this experiment.

The average liver scores presented in Table 24 indicate wide differences between treatments. Birds sacrificed before the experiment started indicated that the basal diet once again caused fatty livers while other birds did not develop fatty livers when fed the low-energy,

high-fiber diet. Birds switched from the basal diet to the low-energy diet (treatment 2) showed complete correction of fatty livers after five weeks on test and these were largely corrected after three weeks. Wheat bran and fish meal supplementation of the basal diet (Treatment 3) had no effect after three weeks on test, but had corrected the fatty livers after 5 and 9 weeks.

Supplemental choline, vitamin B<sub>12</sub> and vitamin E (treatment 4) showed very erratic results. In some birds, there were no fatty livers while other birds had very fatty livers with hemorrhages present. The same was true for treatment 5 in which aureomycin and furazolidone were added along with these vitamins. It is interesting to note that Quisenberry et al. (1967) also obtained no benefit from either low or high levels of supplemental choline, B<sub>12</sub> or E as far as mortality due to fatty liver was concerned.

Birds fed the low-energy, high-fiber diet during both the pre-experimental periods and the experimental period had the lowest liver scores. The average liver score for treatment 8, in which birds were switched from the low-energy diet to the basal diet, increased at each time interval.

The results of this experiment indicate that fatty livers can be created in laying hens by switching them to the basal diet described previously. Fatty livers can also be corrected by placing the birds on the low-energy, high-fiber diet described in Experiment III. Wheat bran and fish meal substitution for corn will also correct fatty liver, but require longer than the low-energy diet to do so. Multiple B-vitamin supplementation (Treatment 6) had no effect and the recommendations of Couch (1956) were erratic if not ineffective.

## EXPERIMENT VI

### Procedure:

In previous experiments, mortality from fatty liver had occurred during periods of hot weather. This experiment was designed to test the effect of high temperatures on the incidence and severity of fatty livers.

The birds used in this experiment were of the same population as those used in Experiments IV and V and had previously been used in an unrelated floor pen experiment. At the end of July, 1966, approximately 400 of these birds were force-molted by withdrawal of feed and water in total darkness for 48 hours. Then, only water and eight hours of light were provided for three additional days. After the fifth day, feed and water were supplied ad libitum but light was restricted to eight hours per day for seven additional weeks at which time the birds were placed in individual 8 x 16-inch cages. The feed used after the molt was initiated was the basal diet described in Experiment I.

These force-molted birds were used since field cases of fatty liver syndrome occurs more frequently during the latter stages of lay. The forced-molt was accomplished to eliminate any previous dietary effects and to increase the level of production of this group of birds. The fact that birds used in this experiment were force-molted was not considered relevant to the experimental results obtained.

When the experiment started, three groups of eight birds were fed each of the diets described in Table 25 in an environment of 10 to

Table 25. Experimental outline for Experiment VI

| Diet | Supplement                                                                                |
|------|-------------------------------------------------------------------------------------------|
| 1    | Basal diet, Experiment I                                                                  |
| 2    | Diet 1 with 15% wheat bran substituted for corn                                           |
| 3    | Diet 1 with 1.5% dicalcium phosphate substituted for<br>1% ground limestone and 0.5% corn |
| 4    | Low-energy diet, Experiment III                                                           |
| 5    | Diet 4 + 12% animal fat (See Table 26)                                                    |

Table 26. Composition (%) of diet 5 used in Experiment VI

| Ingredient                    | Percent |
|-------------------------------|---------|
| Ground yellow corn            | 19.5    |
| Ground oats                   | 20.0    |
| Wheat bran                    | 15.0    |
| Wheat middlings               | 10.0    |
| Alfalfa meal (17%)            | 3.0     |
| Dried skim milk               | 2.0     |
| Fish meal (60%)               | 2.5     |
| Meat and bone scraps          | 3.0     |
| Soybean meal (44%)            | 5.5     |
| Oyster shell flour            | 5.0     |
| Steamed bone meal             | 1.5     |
| Salt                          | 0.6     |
| Fish oil (2000 A; 400 D)      | 0.4     |
| Animal fat                    | 12.0    |
|                               | <hr/>   |
| Total                         | 100.00  |
| Percent protein (calc.)       | 14.8    |
| Metabolizable energy (Cal/kg) | 2789    |

---

15.5° C (50 to 60° F). A like number of birds were fed the same diets in an environment artificially heated to 32° C (90° F). Both of the rooms were equipped with thermostatically controlled exhaust fans. Egg production, feed consumption and body weight change were measured at weekly intervals for one month, two-week intervals for the second month, and for the final month during the 12-week study. Sufficient extra birds were fed the basal diet to sacrifice six birds in each environment after 1, 2 and 3 weeks on test. Eight birds were sacrificed from each of the treatments in both environments after 4, 8 and 12 weeks on test. The data for egg production, feed consumption and body weight change are presented in Tables 27, 28 and 29, respectively. The average liver scores are presented in Table 30. This experiment started December 6, 1966, and ended February 28, 1967.

#### Results and Discussion:

Two birds fed diet 1 died of a fatty liver in the unheated environment and eight birds died of a fatty liver in the heated environment (one each from diets 2 and 3; two from diet 5; four from diet 1) during the experimental period. This indicates that laying hens are less able to withstand fatty livers during periods of high environmental temperatures.

The effect of heat on body weight change (Table 29) was highly significant ( $P < 0.0005$ ) after one week. Birds fed all treatments in the heated environment lost weight during the first week. There was no significant interaction between diet and temperature environment for any of the traits measured during any part of the test. Thus, the two environments were pooled to determine differences between treatments.

Table 27. Egg production (percent hen-day) for Experiment VI

| Diet       | 2-wk Pre-exp | Weeks |      |      |      |       |       |        |  |  |  |
|------------|--------------|-------|------|------|------|-------|-------|--------|--|--|--|
|            |              | 1     | 2    | 3    | 4    | 5 & 6 | 7 & 8 | 9 - 12 |  |  |  |
| 1 Unheated | 64.6         | 58.3  | 62.8 | 62.7 | 62.7 | 51.9  | 54.6  | 47.8   |  |  |  |
| 1 Heated   | 71.0         | 69.6  | 59.5 | 54.2 | 50.3 | 53.3  | 35.1  | 27.5   |  |  |  |
| 2 Unheated | 71.7         | 67.3  | 62.5 | 64.9 | 65.5 | 62.0  | 61.3  | 69.6   |  |  |  |
| 2 Heated   | 67.5         | 64.9  | 67.2 | 59.0 | 62.7 | 51.4  | 45.0  | 22.8   |  |  |  |
| 3 Unheated | 71.4         | 65.5  | 67.9 | 68.4 | 63.1 | 64.4  | 59.6  | 45.1   |  |  |  |
| 3 Heated   | 65.9         | 63.1  | 63.1 | 51.8 | 47.6 | 44.7  | 32.7  | 33.3   |  |  |  |
| 4 Unheated | 67.5         | 63.7  | 62.5 | 62.5 | 72.6 | 64.4  | 66.3  | 55.4   |  |  |  |
| 4 Heated   | 74.3         | 67.3  | 61.3 | 65.5 | 55.4 | 54.8  | 64.2  | 49.0   |  |  |  |
| 5 Unheated | 72.9         | 65.5  | 61.3 | 60.1 | 59.5 | 61.8  | 65.3  | 59.2   |  |  |  |
| 5 Heated   | 72.2         | 61.7  | 56.5 | 55.9 | 47.8 | 46.7  | 45.7  | 41.1   |  |  |  |



Table 28. Feed intake (gms/bird/day) for Experiment VI\*

| Diet       | Weeks            |     |     |                    |                     |                     |        |  |    |
|------------|------------------|-----|-----|--------------------|---------------------|---------------------|--------|--|----|
|            | 1                | 2   | 3   | 4                  | 5 & 6               | 7 & 8               | 9 - 12 |  |    |
| 1 Unheated | 102              | 104 | 103 | 97 <sup>bc</sup>   | 92 <sup>Bb</sup>    | 90 <sup>Bbc</sup>   | 91     |  |    |
| 1 Heated   | 87 <sup>a</sup>  | 92  | 87  | 87                 | 79                  | 69                  | 66     |  |    |
| 2 Unheated | 99               | 111 | 113 | 116 <sup>a</sup>   | 114 <sup>Aa</sup>   | 109 <sup>Aa</sup>   | 112    |  |    |
| 2 Heated   | 79 <sup>ab</sup> | 95  | 94  | 96                 | 92                  | 83                  | 78     |  |    |
| 3 Unheated | 103              | 110 | 121 | 107 <sup>abc</sup> | 104 <sup>ABab</sup> | 101 <sup>ABab</sup> | 99     |  |    |
| 3 Heated   | 81 <sup>a</sup>  | 90  | 84  | 83                 | 80                  | 71                  | 68     |  |    |
| 4 Unheated | 88 <sup>b</sup>  | 104 | 99  | 108 <sup>ab</sup>  | 104 <sup>ABa</sup>  | 103 <sup>Aa</sup>   | 105    |  | 62 |
| 4 Heated   | 72 <sup>b</sup>  | 89  | 96  | 100                | 93                  | 89                  | 92     |  |    |
| 5 Unheated | 88 <sup>b</sup>  | 99  | 98  | 97 <sup>c</sup>    | 96 <sup>Bb</sup>    | 95 <sup>Bc</sup>    | 93     |  |    |
| 5 Heated   | 73 <sup>b</sup>  | 89  | 85  | 80                 | 68                  | 63                  | 75     |  |    |

\*For any given time interval, diets having different letters are significantly different.  
Capital letters denote  $P < 0.01$ ; small letters denote  $P < 0.05$ .

Table 29. Body weight changes (gms) in Experiment VI\*

| Diet       | Weeks              |      |      |      |       |       |        |
|------------|--------------------|------|------|------|-------|-------|--------|
|            | 1                  | 2    | 3    | 4    | 5 & 6 | 7 & 8 | 9 - 12 |
| 1 Unheated | + 12               | + 21 | + 23 | - 29 | + 2   | - 10  | - 51   |
| 1 Heated   | - 71 <sup>a</sup>  | - 6  | - 9  | + 6  | - 35  | 0     | + 9    |
| 2 Unheated | - 14 <sup>ab</sup> | + 17 | + 18 | - 1  | + 18  | + 21  | - 22   |
| 2 Heated   | - 95               | + 15 | - 21 | - 3  | - 10  | + 11  | - 40   |
| 3 Unheated | 0 <sup>ab</sup>    | + 13 | + 19 | + 4  | - 8   | - 22  | - 22   |
| 3 Heated   | - 84               | + 18 | - 10 | + 13 | - 3   | + 39  | - 50   |
| 4 Unheated | - 45 <sup>b</sup>  | + 8  | - 5  | - 2  | + 2   | - 1   | - 1    |
| 4 Heated   | - 102              | - 50 | - 16 | + 5  | + 12  | - 15  | + 53   |
| 5 Unheated | - 27 <sup>a</sup>  | + 30 | + 22 | + 14 | + 16  | + 14  | - 21   |
| 5 Heated   | - 45 <sup>a</sup>  | + 14 | + 3  | - 4  | + 7   | - 55  | + 14   |

\* Diets with different letters are significantly different ( $P < 0.05$ ).

Table 30. Average liver scores for Experiment VI

| Diet |          | Weeks |      |      |      |      |      |
|------|----------|-------|------|------|------|------|------|
|      |          | 1     | 2    | 3    | 4    | 8    | 12   |
| 1    | Unheated | 2.93  | 2.75 | 3.08 | 2.50 | 2.88 | 2.28 |
| 1    | Heated   | 2.67  | 3.33 | 3.25 | 2.94 | 2.93 | 2.70 |
| 2    | Unheated |       |      |      | 2.06 | 2.63 | 2.06 |
| 2    | Heated   |       |      |      | 1.86 | 2.00 | 2.12 |
| 3    | Unheated |       |      |      | 2.81 | 3.00 | 2.93 |
| 3    | Heated   |       |      |      | 2.94 | 2.75 | 3.83 |
| 4    | Unheated |       |      |      | 1.75 | 1.81 | 1.31 |
| 4    | Heated   |       |      |      | 1.63 | 1.31 | 1.71 |
| 5    | Unheated |       |      |      | 1.38 | 1.38 | 1.36 |
| 5    | Heated   |       |      |      | 1.75 | 1.50 | 1.31 |

Birds fed the low-energy diet (diet 4) lost significantly more weight ( $P < 0.05$ ) than birds remaining on the basal diet during the first week. This weight loss was due to inadequate feed intake since fat supplementation (diet 5) of the low-energy diet prevented a portion of the weight loss.

Feed intake was significantly ( $P < 0.0005$ ) depressed by the high temperature environment during the first week of the test (Table 28). This accounts for the body weight loss in the heated environment during this period. Birds fed diets 4 and 5 consumed significantly less feed during the first week. Apparently density of the ration governed feed intake during this period since birds fed diets 4 and 5 consumed the same amount of feed and their energy content is quite different.

During the second week of the test, birds on all treatments adjusted their feed intake toward the energy content of the diet. There was no significant diet effect during this period. However, the high temperature continued to significantly depress ( $P < 0.0005$ ) feed intake. The increase in feed intake among birds fed the lower energy diets in the unheated environment and among birds of all treatments in the high temperature environment resulted in a gain in body weight for most treatments. However, birds fed the low-energy diet (diet 4) in the heated environment continued to lose weight. Since birds fed most of the treatments gained weight during the second week of the test, the effect of high temperature was less depressing ( $P < 0.05$ ) on body weight change.

A significant drop ( $P < 0.05$ ) in egg production was observed during the third week of the test due to the high temperature environment. Apparently, by drawing on body stores and increasing feed intake,

the birds were able to withstand the heat stress during the first two weeks of the test. As the experiment progressed, the effect of heat became more pronounced as far as egg production was concerned.

As the experiment progressed, birds fed the lower energy diets increased their feed intake to more nearly reflect the energy content of the diet. By the fourth week of the test, the feed intake of birds fed diet 2 was significantly greater ( $P < 0.05$ ) than those from diet 1. Likewise, birds fed diet 4 consumed significantly more feed than those from diet 5 during this period. These differences were further magnified during the fifth and sixth weeks of the test.

Average liver scores of birds fed diet 1 that were sacrificed after 1, 2 and 3 weeks on test indicated that fatty liver was present in both temperature environments (see Table 30). Apparently, since more birds died due to fatty liver in the high temperature environment, laying hens with fatty livers are less able to withstand high temperature stress. When birds from all treatments were sacrificed after four weeks on test, the low-energy diet (diet 4) had once again corrected the fatty livers. Interestingly, this low-energy diet supplemented with 12 percent animal fat (diet 5) had also corrected the fatty livers. This further substantiated the results of Experiment IV that energy is not the primary cause of fatty livers.

Wheat bran also corrected the fatty livers to an extent, but less so than the wheat bran and fish meal supplementation used in Experiment V. In this experiment, the wheat bran was more effective in the heated environment.

One of the differences, in addition to energy content, between the basal diet and the low-energy diet was the phosphorus content. In

diet 3 of this experiment, the phosphorus content was increased to equal that of the low-energy diet. This increase in phosphorus content had no effect in reducing the incidence of fatty livers.

This experiment indicates that there is/are some factor(s) in the low-energy diet that corrects fatty livers. Wheat bran is less effective, but the low-energy diet also contains ten percent wheat middlings. Since wheat bran is low in energy and bulky, a reduction in energy intake results when it is substituted for corn in a ration. The reduction of energy intake when wheat bran is used makes it difficult to assess the value of this ingredient in correcting fatty livers. Experiment VII was a preliminary attempt in this respect.

## EXPERIMENT VII

### Procedure:

Three groups of six birds that had been fed the basal diet in the unheated environment of Experiment VI were available for this preliminary study. A water extract of wheat bran was prepared as follows: 4 kilograms of wheat bran was soaked with constant stirring in 40 liters of distilled water for 7 hours. This material was allowed to set for 30 minutes and then most of the filtrate was drawn off by siphon. The remaining material was filtered through 8 layers of cheesecloth and this filtrate added to that previously siphoned. The residue was air-dried in thin layers with a circulating fan for 36 hours. The filtrate was dried in shallow trays onto corn in a hot-air oven at 50° C for 16 hours. Both products were reground after drying. This procedure was repeated to obtain enough material for this experiment.

Approximately 65 percent of the wheat bran remained in the residue after extraction. This does not take into account any losses when transferring the product, but these losses were considered small. The residue was included in the ration at ten percent of the ration based on this yield. This extraction was considered incomplete and the extract was added to the basal diet at twice the level of wheat bran. (The water extract of 300 grams of wheat bran was added per kilogram of feed.)

These materials were fed for 28 days and the results are presented in Table 31.

Table 31. Data collected in Experiment VII

| Diet | Supplement                 | Feed intake<br>(gms/bird/day) | Percent<br>egg prod. | Body wt. (gms) |        | Average<br>liver<br>score |
|------|----------------------------|-------------------------------|----------------------|----------------|--------|---------------------------|
|      |                            |                               |                      | Initial        | Change |                           |
| 1    | Basal diet, Experiment I   | 114                           | 56.6                 | 2154           | + 2    | 3.20                      |
| 2    | Basal + wheat bran extract | 89                            | 67.2                 | 1669           | + 64   | 1.92                      |
| 3    | Basal + wheat bran residue | 115                           | 65.5                 | 1993           | - 54   | 1.75                      |



Results and Discussion:

The average liver scores were reduced by both the extract and residue of wheat bran. It would appear that there is/are some factor(s) in wheat bran responsible for the correction of the fatty liver. However, it should be pointed out that the birds fed the wheat bran extract were much smaller than the other two groups. Unfortunately, this was not observed at the start of the test. To help substantiate this effect of the wheat bran extract, the liver scores of all birds fed the basal diet in Experiment VI that weighed between 1550 and 1750 grams were averaged. There were 17 such birds in Experiment VI and their average body weight and liver scores were 1630 and 2.88, respectively. This would indicate that birds of this size had fatty liver in Experiment VI.

Due to the small number of birds in this experiment (6 per treatment), this experiment does not definitely prove that wheat bran contains factor(s) that correct fatty liver. However, the results are suggestive of the value of further work in this area.

## GENERAL DISCUSSION

The basal diet used in these experiments was of the high-energy, commercial-type and most of this energy (75% corn and 0.5% fat) was supplied by carbohydrate sources. This level of energy was accomplished by maximum utilization of corn and minimum use of fibrous ingredients in the diet. Methionine was considered a borderline deficiency in this diet but methionine supplementation in Experiment II did not correct the fatty liver.

The results of Experiment I and II demonstrated that fatty liver is much more prevalent in cages than in floor birds. The difference in opportunity for exercise between cage and floor birds might allow energy used by floor birds for this purpose to be unused and reflected as fat in cage birds. Another possibility could be that floor birds obtain some factor(s) from the litter that is beneficial in preventing fatty livers.

After sacrificing the birds used in these experiments, it would appear that fatty liver mortality occurs as a result of rupture of the liver capsule causing massive bleeding into the abdominal cavity. However, some birds survived these liver ruptures as evidenced by blood in the abdominal cavity as well as abdominal wall discoloration of some birds in these studies. Apparently, if the liver capsule did not rupture, most birds survive these hemorrhages. Interestingly, most non-layers did not have fatty livers. This indicates that hormonal balance is involved in this condition.

The results of Experiment III indicated that development of fatty livers could be prevented by use of a specific low-energy, high-fiber diet. This diet was much lower in energy content than the basal diet used to create fatty livers. Hill et al. (1956) found that increasing the energy level of the diet by the use of fat reduced the feed requirement by a rate of two percent for each one percent of added fat. Body weight was maintained at a higher level by high-energy diets.

Birds fed the basal diet in Experiment III gained more weight than those fed the low-energy, high-fiber diet and also developed fatty livers. Yet, in Experiment IV where varying energy and protein levels were fed, birds from all diets had some fatty livers, although the incidence was higher in the higher-energy diets. This indicated that energy level of the diet has some effect on the incidence of fatty livers, but other factors are also involved.

Experiment V demonstrated that choline does not correct fatty livers or its effect is very erratic. This is in agreement with reports by Ringer and Sheppard (1963), Fisher and Weiss (1956), and Quisenberry (1967). The failure of aureomycin and furazolidone to affect fatty livers discounts the theory of low-level infection. Michigan State University Poultry Pathologists have not detected any disease organisms related to the fatty liver condition. This is in agreement with the report of Texas veterinarians in which unsuccessful attempts were made to transmit the condition.

In Experiment V, wheat bran and fish meal were demonstrated to correct the fatty liver condition. This effect was probably due to the wheat bran as evidenced by Experiment VI. Wheat bran is a low-energy

material as compared to corn and is a good source of B-vitamins and trace minerals. However, the high-energy basal diet was considered well-fortified with vitamins and a multiple B-vitamin supplementation had no effect on fatty livers in Experiment V.

It could be postulated that the higher calcium level in present-day laying rations increases the requirement for certain trace minerals. This possibility was not investigated in this study. However, the low-energy diet used in Experiment III contains about the same level of calcium as the basal diet.

In most of these experiments, mortality due to fatty liver was noted during periods of hot weather. Experiment VI demonstrated that more fatty liver mortality does occur during hot weather, but the actual incidence of fatty livers in living birds was just as high in a cool environment (10 - 15.5° C). Mueller (1961) reported that continuous exposure to temperature of 32° C reduced egg production 28 percent, and feed intake 32 percent. In a later report, Mueller (1967) found that supplemental methionine had no effect on these traits at 32° C.

Experiment VI demonstrated the effects of high environmental temperatures (32° C) on egg production and feed intake. Yet, this depressed feed intake and lower egg production seemed to be unrelated to the occurrence of fatty livers. When birds were switched to the low-energy diet described in Experiment III, a weight loss occurred. This weight loss was largely prevented by the addition of 12 percent fat to this diet in Experiment VI. Even with 12 percent added fat, this diet still corrected fatty livers. This suggests that dietary fat is not the source of liver fat accumulation and agrees with the work of Harper et al. (1954) with rats.

The results of Experiment VII suggest that there is some water soluble factor(s) in wheat bran that helps to correct fatty livers. If so, the properties of this material(s) may be similar to that reported by Thornton and McPherron (1962) in brewers' dried grains.

It should be mentioned that fatty liver studies with laying hens are difficult and time-consuming. It takes several weeks or even months to develop the condition and, since no tests are available, some birds must be sacrificed to determine the incidence of fatty livers. Corrective treatments employed should not be so drastic as to stop egg production, since most non-layers do not have fatty livers. As mentioned earlier, this indicates that hormonal balance is involved in this condition also.

## SUMMARY

In this study, a commercially available, high-energy diet that had experienced some fatty liver problems in field conditions was used.

Fatty liver was much more prevalent in birds in cages than those in floor pens. Methionine failed to correct the condition, but a specific diet low in energy, and high in fiber was very effective in preventing or correcting the fatty liver syndrome. In one experiment, energy and protein levels were varied. The incidence of fatty liver was increased as the energy content of the diet was increased. However, some fatty livers occurred among all treatments in this experiment. A higher protein level in the diet was without apparent effect.

In another experiment, fatty livers were corrected by either switching birds to a low-energy, high-fiber diet or by substituting 15 percent wheat bran and 3 percent fish meal for corn. Supplemental choline, vitamin B<sub>12</sub> and vitamin E gave very erratic results. In some birds fed these vitamins, there were no fatty livers while other birds had very fatty livers with hemorrhages present. There was no effect in this experiment from supplemental aureomycin or furazolidone or from a multiple B-vitamin supplement.

An experiment in which some birds were held in an artificially heated environment (32° C) during a 12-week experiment indicated that mortality from fatty livers was increased during the high temperatures. However, similar birds in a cool environment had the same degree of

fatty livers but mortality was lower. The low-energy diet effectively corrected fatty livers in this experiment and wheat bran had a similar but less effective action.

A water extract of wheat bran was effective in connecting fatty livers in a small preliminary test, but the residue was more effective. This suggests that some factor(s) in wheat bran was partially responsible for correction of fatty livers.

That the fatty liver syndrome is a nutritional problem is suggested by the fact that fatty livers were induced and/or corrected by diet manipulation. Non-laying birds in these experiments generally had normal appearing livers, implicating hormonal balance as a factor in this condition.

The results of this study help to explain the failure of some research workers to create the fatty liver syndrome. Most previous attempts had utilized high-fat diets, while a high-energy, high-carbohydrate diet was used in this study.

In this study, fatty livers were produced in birds in cages using a high-energy diet in which the energy was supplied primarily from carbohydrate sources. Birds in floor pens had a much lower incidence of fatty livers when fed the same diet. A specific low-energy, high-fiber diet was very effective in preventing fatty livers from developing and corrected the fatty livers once they had been produced. Fifteen percent wheat bran was also effective in this respect, but required a longer period of time for correction of fatty livers than the low-energy, high-fiber diet.

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Appendix Table 1. Summary of data collected in Experiment I by 28-day periods

| Treatment* | 4-week periods                     |      |      |      |      |      |      |      |      |      |
|------------|------------------------------------|------|------|------|------|------|------|------|------|------|
|            | 1                                  | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
|            | Average egg production (% hen-day) |      |      |      |      |      |      |      |      |      |
| 1          | 75.7                               | 72.3 | 72.2 | 72.3 | 72.7 | 71.9 | 66.1 | 67.7 | 67.5 | 65.1 |
| 2          | 72.5                               | 76.5 | 73.1 | 73.0 | 71.1 | 73.8 | 72.3 | 69.9 | 66.2 | 61.8 |
| 3          | 74.7                               | 71.4 | 67.3 | 67.5 | 71.2 | 76.0 | 74.0 | 71.6 | 71.9 | 70.8 |
| 4          | 76.0                               | 74.9 | 71.0 | 70.8 | 71.1 | 69.8 | 70.6 | 68.6 | 67.3 | 64.7 |
|            | Feed consumed (grams/bird/day)     |      |      |      |      |      |      |      |      |      |
| 1          | 98                                 | 105  | 113  | 114  | 112  | 107  | 104  | 102  | 98   | 101  |
| 2          | 96                                 | 113  | 115  | 114  | 113  | 115  | 109  | 104  | 98   | 101  |
| 3          | 101                                | 105  | 110  | 113  | 112  | 111  | 109  | 107  | 100  | 107  |
| 4          | 101                                | 108  | 110  | 111  | 111  | 106  | 104  | 103  | 98   | 103  |
|            | Average body weight (gms.)         |      |      |      |      |      |      |      |      |      |
| 1          | 1693                               | 1657 | 1675 | 1707 | 1734 | 1748 | 1771 | 1784 | 1748 | 1816 |
| 2          | 1639                               | 1634 | 1643 | 1703 | 1730 | 1734 | 1757 | 1766 | 1734 | 1798 |
| 3          | 1684                               | 1643 | 1639 | 1675 | 1716 | 1743 | 1766 | 1798 | 1784 | 1857 |
| 4          | 1675                               | 1630 | 1634 | 1689 | 1716 | 1725 | 1748 | 1739 | 1734 | 1789 |

\* Each diet is an average of 6 pens of 25 birds each.

Appendix Table 2. Summary of data collected in Experiment II by 28-day periods\*

| Diet                             | 28-day periods |      |      |      |      |      |      |
|----------------------------------|----------------|------|------|------|------|------|------|
|                                  | 1              | 2    | 3    | 4    | 5    | 6    | 7    |
| Percent egg production (hen day) |                |      |      |      |      |      |      |
| 5                                | 77.5           | 75.8 | 73.6 | 68.6 | 62.0 | 56.4 | 42.9 |
| 6                                | 74.7           | 72.8 | 69.8 | 64.1 | 64.0 | 64.1 | 53.6 |
| 7                                | 76.6           | 77.6 | 72.2 | 70.2 | 64.5 | 63.7 | 60.4 |
| 8                                | 75.5           | 71.2 | 69.3 | 69.7 | 73.0 | 66.3 | 59.0 |
| 9                                | 78.2           | 75.6 | 70.7 | 71.3 | 69.3 | 63.8 | 55.3 |
| Feed consumption (gms/bird/day)  |                |      |      |      |      |      |      |
| 5                                | 114            | 119  | 115  | 107  | 110  | 103  | 93   |
| 6                                | 112            | 119  | 120  | 107  | 119  | 112  | 100  |
| 7                                | 113            | 115  | 114  | 110  | 108  | 105  | 100  |
| 8                                | 113            | 113  | 117  | 108  | 114  | 99   | 92   |
| 9                                | 109            | 115  | 112  | 110  | 113  | 103  | 93   |
| Average body weight (gms)        |                |      |      |      |      |      |      |
| Init.<br>wt.                     |                |      |      |      |      |      |      |
| 5 1966                           | 1943           | 1979 | 1998 | 1998 | 2016 | 1998 | 2007 |
| 6 1938                           | 1947           | 1993 | 2043 | 2070 | 2116 | 2070 | 2106 |
| 7 1907                           | 1902           | 1934 | 1957 | 1988 | 2075 | 2057 | 2116 |
| 8 1893                           | 1934           | 1970 | 2025 | 2034 | 2061 | 2011 | 2070 |
| 9 1879                           | 1875           | 1938 | 1979 | 1966 | 2047 | 2011 | 2052 |

\* Each diet is an average of four groups of eight birds.

Appendix Table 3. Summary of data collected during the first 12 weeks in Experiment IV by 2-week periods

| Diet                            | Pre-exp. | Periods |      |      |      |      |      |
|---------------------------------|----------|---------|------|------|------|------|------|
|                                 |          | 1       | 2    | 3    | 4    | 5    | 6    |
| Percent egg production          |          |         |      |      |      |      |      |
| 1                               | 84.8     | 80.6    | 81.7 | 81.2 | 80.8 | 79.5 | 79.5 |
| 2                               | 86.4     | 82.6    | 84.8 | 81.7 | 82.1 | 76.3 | 78.1 |
| 3                               | 87.1     | 87.5    | 85.7 | 84.4 | 84.4 | 80.8 | 80.0 |
| 4                               | 87.1     | 80.4    | 80.8 | 77.6 | 75.7 | 79.1 | 77.1 |
| 5                               | 85.0     | 82.1    | 83.0 | 79.5 | 78.1 | 77.4 | 80.6 |
| 6                               | 84.4     | 81.3    | 81.7 | 78.5 | 77.2 | 79.5 | 76.8 |
| Body weight (gms)               |          |         |      |      |      |      |      |
| 1                               | 1733     | 1686    | 1710 | 1719 | 1731 | 1707 | 1719 |
| 2                               | 1738     | 1709    | 1724 | 1721 | 1756 | 1719 | 1741 |
| 3                               | 1745     | 1735    | 1768 | 1774 | 1795 | 1764 | 1782 |
| 4                               | 1740     | 1716    | 1735 | 1748 | 1774 | 1730 | 1755 |
| 5                               | 1740     | 1774    | 1779 | 1795 | 1842 | 1796 | 1825 |
| 6                               | 1745     | 1807    | 1838 | 1857 | 1901 | 1854 | 1862 |
| Gms. feed/bird/day              |          |         |      |      |      |      |      |
| 1                               |          | 99      | 116  | 114  | 115  | 105  | 115  |
| 2                               |          | 100     | 108  | 108  | 109  | 104  | 112  |
| 3                               |          | 98      | 102  | 99   | 104  | 94   | 103  |
| 4                               |          | 98      | 106  | 106  | 110  | 101  | 109  |
| 5                               |          | 102     | 104  | 97   | 98   | 95   | 104  |
| 6                               |          | 101     | 100  | 94   | 95   | 86   | 94   |
| Metabolizable Calories/bird/day |          |         |      |      |      |      |      |
| 1                               |          | 245     | 288  | 282  | 284  | 259  | 284  |
| 2                               |          | 271     | 293  | 292  | 296  | 282  | 304  |
| 3                               |          | 292     | 306  | 298  | 311  | 282  | 309  |
| 4                               |          | 266     | 288  | 288  | 298  | 274  | 296  |
| 5                               |          | 303     | 309  | 291  | 292  | 282  | 310  |
| 6                               |          | 315     | 314  | 293  | 297  | 269  | 293  |
| Gms. protein/bird/day           |          |         |      |      |      |      |      |
| 1                               |          | 14.9    | 17.5 | 17.1 | 17.2 | 15.7 | 17.2 |
| 2                               |          | 15.0    | 16.2 | 16.1 | 17.3 | 15.5 | 16.8 |
| 3                               |          | 14.6    | 15.3 | 14.9 | 15.6 | 14.1 | 15.5 |
| 4                               |          | 17.7    | 19.1 | 19.1 | 19.8 | 18.2 | 19.7 |
| 5                               |          | 18.3    | 18.7 | 17.5 | 17.6 | 17.0 | 18.7 |
| 6                               |          | 18.2    | 18.1 | 16.9 | 17.1 | 15.5 | 16.9 |

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