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ADIPOCYTE HYPERPLASIA AND FAT DEPOSITION IN HIGH-WEIGHT AND LOW-WEIGHT CHICKENS

by

Walter Wade Robey

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APPROVED:

H. P. Van Krey, Chairman

J. A. Cherry

J. H. Wolford

P. B. Siegel

G. E. Bunce

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Blacksburg, Virginia

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(ABSTRACT)

Four experiments were conducted to compare adipocyte dynamics and body composition among adult chickens from lines differing in growth rate and feed consumption. In Experiment I, forced caloric overconsumption increased weight gain and fat deposition, with concomitant increases in the total adipocyte DNA content of the assayed fat depots. Injections of DNA-specific [methyl^{3}H] thymidine resulted in higher radioactivity levels in the fat depots of forced versus *ad libitum* or restricted-fed birds. Evidently, stimulation of fat deposition in adult chickens can be associated with adipocyte hyperplasia.

A second experiment was conducted to histologically examine the effects of forced caloric overconsumption on adipocyte size, number, and distribution in the retroperitoneal fat depot of adult weight-line birds. Similarly to Experiment I, force feeding increased fat deposition, resulting in increases in the retroperitoneal fat depot total adipocyte DNA content. The lipid/adipocyte DNA ratio also decreased during rapid depot expansion. This resulted in an increase in the small adipocyte population of the force fed birds when adipocyte diameter was increasing. Subsequently, when the constraints of force feeding were removed, adipose

depot reduction occurred, with the mobilization of lipid from small as well as large adipocytes.

In a third experiment, the effects of estrogen administration and partial lipectomy were examined. In addition to the weight-line birds, a commercial broiler breeder stock was utilized. Estrogen administration depressed body weight gain and abdominal fat deposition when compared to placebo implanted birds. Partial lipectomy depressed body weight, fat deposition, and abdominal fat total adipocyte DNA content. Lipectomy modified several components of carcass composition without altering feed intake. No indication of a compensatory hyperplastic response to lipectomy was indicated.

A final experiment was conducted examining the effects of forced body weight fluctuations on body composition of male and females from the weight-selected lines. Although numerous first-order interactions were detected, force-feeding resulted in transient modifications in carcass composition and fat deposition which were more apparent in the high than low weight line, in females than males. After release to *ad libitum* feeding, the differences resulting from force feeding were reduced.

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This dissertation is dedicated to my wife who has made this work possible, and my newborn child which has given it purpose.

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INTRODUCTION

Recently, there has been growing interest in gaining a better understanding of lipogenesis in animals and man, and how it may be modified by diet or exercise. In most animals obesity is associated with many negative attributes. Excessive fat deposition is also of considerable economic importance in poultry meat production. Large quantities of fat in the abdominal and visceral regions is considered to be a waste of dietary energy, and adversely affects many facets of commercial production. Health conscious consumers are also demanding the availability of lean meats to avoid the problems which are associated with the consumption of a high fat diet.

Previously, adipose tissue was considered to be metabolically inert, a passive fat storage depot that provided insulation against heat loss, and functioned as a structural buttress for certain regions of the body. More recently, adipose tissue has become recognized as a diffuse organ of primary metabolic importance.

Because most animals feed intermittently, there must be a provision for the temporary storage of excess fuel. Lipid is the most favorable substance for this purpose because it weighs less and occupies less volume per calorie of stored chemical energy than either carbohydrate or protein. Although many tissues contain small amounts of carbohydrate and fat, adipose depots serve as the body's most capacious reservoir of energy. Approximately 15-20% of the total body weight of an average man is fat, representing approximately a 40-day reserve of energy. In obese

individuals this may increase to the equivalent of a year or more of normal metabolism. By ammassing lipid in periods of excess dietary intake and releasing fatty acids in periods of fasting, adipose depots play an important role in maintaining a stable supply of body fuel. Far from being inert, the cells of this tissue in some species actively synthesize fat from carbohydrate and are highly responsive to hormonal and nervous stimulation.

As a result of the growing interest in health and fitness which has developed in the past few years, scientific research into the mechanisms which regulate adiposity have been intensely investigated. These efforts have led to a more complete understanding of how the body regulates its adipose stores, and revealed many previously held misconceptions. However, complete understanding of cellular obesity has been hindered due to the complexity of its etiology. Many factors including diet, exercise, pregnancy, basal metabolic rate, and age related changes in lean body mass have been implicated.

The objective of this dissertation was to determine whether adipocyte hyperplasia can be stimulated in adult chickens. Secondly, to examine the effects of caloric overconsumption on adipocyte size, number, and distribution in the abdominal fat depot of adult chickens. Lastly, to investigate the effects of forced body weight fluctuations on the body composition of adult male and female chickens.

PREVIOUS WORK

Histogenesis of uniocular adipose tissue. Two types of adipose tissue have been identified, characterized by the morphology of their cells, localization, and color.

Yellow, or unilocular adipose tissue, is comprised of cells which, when mature, contain a single large central droplet of cytoplasmic fat. Brown, or multilocular, adipose tissue is composed of cells which contain many small lipid droplets and mitochondria. Yellow, or unilocular adipose cells, develop from mesenchymally-derived lipoblasts. Adipocytes are structurally similar to fibroblasts, but have the ability to accumulate fat in their cytoplasm. Experiments by Goodridge (1968) and O'Hea and Leveille (1968) demonstrated that adipose tissue of birds, in contrast to that of most mammals with the exception of man, displays only minimal *de novo* fatty acid synthesis. The lipid stored in avian adipose tissue is, therefore, of dietary origin or originates from hepatic lipogenesis. Enlargement of adipose tissue can theoretically be controlled through hepatic lipogenesis, lipoprotein transport, or the release of fatty acids from portomicrons and other lipoproteins.

Enlargement of adipose depots can result from either increases in the number of adipocytes (hyperplasia), enlargement of existing adipocytes (hypertrophy), or both. In the early period of an animal's development, hyperplasia rather than hypertrophy is the predominant factor affecting the size of fat depots. This pattern of development

changes as an animal ages until adipocyte hypertrophy becomes the principal factor influencing fluctuations in body fat in the sexually mature animal (Hirsch and Han, 1969; Hirsch and Knittle, 1970; Johnson *et al.*, 1971; Greenwood and Hirsch, 1974). This tendency has resulted in the concept that adipocyte number is fixed in sexually mature individuals.

Adipose tissue cellularity in mammalian species. Although adipose tissue cellularity has been intensively investigated, the concept that adipocyte numbers remain fixed in adult animals is still controversial. Until recently, it has been generally accepted that adipocyte hyperplasia is the major factor determining fat accretion during early stages of growth, but as sexual maturity is approached, adipocyte hypertrophy becomes increasingly more pronounced until, at some point, changes in body fat are due solely to changes in adipocyte size. Several experimental approaches have been used to verify this hypothesis. Hubbard and Mathew (1971), for example, reported that chronic underfeeding of young rats delayed but did not prevent the attainment of normal epidydimal fat cell populations. Similar observations were made for porcine adipose tissue (Hood and Allen, 1973). These experiments have been interpreted as evidence for a genetically predetermined adipocyte number which is independent of nutrient intake and other environmental factors. Moreover, there is evidence that once attained, the number of constituent cells remains constant. Several experiments, for instance, indicated that adult rats do not lose adipocytes when starved nor acquire new adipocytes during a period of weight gain induced by hypothalamic damage

(Hirsch and Han, 1969; Johnson *et al.*, 1971; Johnson and Hirsch, 1972). Sims *et al.* (1968) also reported that increased body fat induced experimentally in adult human males was directly associated with increases in the size of existing adipocytes; no indication of any increase in cell number was observed. These and other reports led James (1976) to formulate the adipose cell hypothesis which includes the following postulates:

1) The adipocyte number of normal or obese adults is fixed.

2) Adult-onset obesity is associated only with the enlargement of existing fat cells (hypertrophic obesity).

3) Pre-puberal obesity is associated with an increase in cell number (hyperplastic obesity).

4) Adipose tissue hypercellularity predisposes to obesity since each adipocyte tends to accumulate a "normal" amount of fat.

5) High energy dietary intakes stimulates fat cell hyperplasia in infants.

6) Besides having a different morbidity and mortality, hyperplastic obesity is more resistant to weight-reduction therapy than hypertropic obesity.

Recent reports have indicated that much of the early experimental research of adipose cellularity in growing and mature animals may have been too hastily accepted. Many of the six postulates of the adipose cell hypothesis have been challenged because of basic experimental deficiencies.

The epididymal fat pad has been widely utilized. However, Ashwell *et al.* (1975) and Faust *et al.* (1977) reported that comparison of different adipose depots reveals that the epididymal fat pad does not have as great a proliferative capacity as do other sites. As a result, conclusions based on experiments using the epididymal fat pad exclusively may be suspect since the results may not be representative of other depot sites, or adipose tissue in other species.

In many cases, adipose tissue samples too small to provide an accurate determination of adipose tissue cellularity are analyzed. Needle biopsies are commonly used to examine approximately 20 mg of tissue, which may then be used to evaluate up to 100 kg of body fat distributed throughout the body. Also, many studies on adipose size distributions report only mean cell weights or volume. Eisen *et al.* (1978) reported that such means are only valid when the distribution is Gaussian. If adipocyte distributions are skewed or multiphasic, a comparison of mean values can be very misleading.

In contrast to the experiments previously discussed, evidence that adipocyte hyperplasia occurs in adult animals has also been published. Lemonnier (1972) reported that mature rats and mice fed high-fat diets exhibited increases in the number of adipocytes in certain fat depots. Faust *et al.* (1978) reported a series of experiments involving three strains of adult rats fed chow, sucrose, or high-fat (55%) diets. The high-fat diet caused an increase in the number of fat cells, which was noticeable in the retroperitoneal fat pad. These results indicate that some adult adipose tissue depots are capable of hyperplasia, or the

recruitment of new adipocytes, when the animal is faced with a challenge to store an increased amount of fat. Faust speculated that some portion of the adipocyte population had to reach a critical size for hyperplasia to be reinitiated (1.2-1.6 ug lipid/cell).

Therriault and Mellin (1981) reported that exposing adult rats to cold temperatures induced the formation of new adipocytes. Several other studies with rats are also inconsistent with the concept that postmaturational changes in adipose tissue mass depend solely upon changes in cell size (Stiles *et al.*, 1976; Faust *et al.*, 1978; Bertrand *et al.*, 1978, 1980, 1984; Klyde and Hirsch, 1979a,b).

Greenwood and Hirsch (1974) suggested that the rate at which performed adipocytes fill with lipid is determined by the amount of lipid presented to the tissue and may provide a signal to regulate increased cell proliferation in the rat. It has also been suggested that species may differ in the manner in which fat accretion occurs. DiGirolamo and Mendlinger (1971) reported that adult rats and hamsters enlarged adipose depots solely through the lipid filling of existing cells, while the guinea pig was capable of periodic adipocyte hyperplasia. They reported that the capacity of the fat cells from the adult guinea pig to increase in volume is rather limited when compared to the rat or hamster. However, the guinea pig has a marked capacity to increase the number of adipose cells from 6 weeks to 1 year of age, while the rat and hamster showed limited capacity for hyperplastic growth after reaching maturity. In contrast, Faust *et al.* (1978) argued diet-induced increases in adipocyte number in the adult rat is a general phenomenon independent of strain,

sex, depot, or diet. They suggested that diet-induced increases in cell number persist during subsequent weight loss and, therefore, represent new cell formation rather than lipid filling of differentiated cells normally too small for detection. Moreover, they hypothesized that there is an orderly morphologic sequence of adipose depot enlargement so that initially only mean adipocyte size increases, but after a certain size is reached further fat deposition is due to increases in adipocyte number.

It has also been proposed that adipocyte multiplication may occur during pregnancy or lactation. Billewicz and Thompson (1970) reported that fat accumulation during pregnancy is more difficult to lose because fat gain in the pregnant female results from not only increases in cell size, but also increases in cell number. Bershtein and Aleksandrov (1977) reported that female rats increased adipocyte cell numbers if prevented from lactating. Roncari and Van (1978a) reported that the levels of 17B estradiol are elevated during puberty and pregnancy; this hormone can stimulate adipocyte multiplication during *in vitro* culture.

An alternate means of studying adipose cellularity in the animal is by the process of lipectomy. In this procedure, part or all of the adipose depot is removed by suction. Lipectomy, however, has been shown to stimulate a compensatory hyperplastic response in some situations. Faust *et al*. (1977) reported that when 48% of the subcutaneous inguinal fat pad was excised from young rats, complete adipocyte regeneration occurred in rats fed a high-fat diet for a period of 7 months. Rats fed a low-fat diet demonstrated partial regeneration. This study, as well as others, caused Faust to speculate that the regeneration of adipose

depots after lipectomy is dependent upon depot site as well as diet. The occurrance of regeneration is regarded as proof for the existence of regulated cellular proliferation. Lipectomies are also used as a means of studying the proliferative capacity of adipose depots without inducing obesity.

Adipose tissue cellularity in avian species. In comparison to studies with mammals, adipose cellularity studies with domestic fowl are limited. Using adipose tissue DNA concentrations as a measurement of cell proliferation, Pfaff and Austic (1976) reported that hyperplastic growth of adipose tissue in White Leghorn chickens fed *ad libitum* ceased by about 12 weeks of age. Hood (1982) conducted similar experiments with meat-type chickens. The number of adipose cells in the retroperitoneal fat depot increased rapidly up to about 14 weeks of age, after which the number (270 x 10^6 cells/depot) tended to remain constant as the birds aged. This estimate of adipocyte cell number in the retroperitoneal depot was similar to the results of Ballam and March (1979).

Several investigators have examined adipose tissue cellularity in response to nutrition. White Leghorn pullets fed a low energy diet for the first 20 weeks of life had smaller adipocytes than *ad libitum*-fed controls, but no differences in cell number due to feeding treatment were observed (Pfaff and Austic, 1976). They concluded that adipocyte number was independent of early-life nutrition. March *et al.* (1982) postulated that cell size was also, within certain limits, independent of diet. In their study, adipocyte diameter increased with age in meat-type stocks although feed intake was restricted. The most frequent size observed was

70 μm at 27 weeks of age and 119 μm at 66 weeks of age. Moreover, a bimodal distribution of adipocytes in the retroperitoneal fat depot was observed. As adipocyte diameter increased, the percentage of cells in the primary model group decreased which was interpreted to mean that a maximum size limitation for adipocyte diameter exists beyond which further absorption of lipid into the cell is impeded. With feed restriction, loss of the small diameter adipocyte population eliminated the biomodality of the adipocyte distribution, suggesting that lipid is mobilized from small as well as large adipocytes.

In addition to nutrition differences, several experiments indicate that genetic differences in adipose tissue cellularity occur. March had Hansen (1977) reported that White Leghorn chickens had smaller adipocytes than broiler-type chickens. Simon and Leclercq (1982) reported that approximately 90% of the difference in abdominal fat weight between fat and lean lines of chickens was explained by differences in adipocyte volume. Dwarf chickens have larger fat cells than their normal siblings (Swartworth, 1981). Differences in adiposity between two commercial broiler stocks, however, were primarily due to differences in adipocyte number (Cherry *et al.*, 1984).

Although Ballam and March (1979) suggested that the retroperitoneal fat depot may undergo periodic hyperplasia, there is little information indicating that postmaturational changes in adipocyte number occur in the domestic chicken (Pfaff and Austic, 1976; March and Hansen, 1977; Hood, 1982; March *et al.*, 1982). Evidence that Japanese quail are capable of adipocyte hyperplasia has been published, but it was speculated that

premigratory fattening may have been involved in the response (Oruwari *et al.*, 1986). Recent data provided by March *et al.* (1984), however, may be suggestive of adipocyte multiplication in the domestic chicken. Using histological techniques, they consistently observed a bimodal distribution of fat cells which appeared to represent two distinct populations of adipocytes. The population composed of the smaller adipocytes continued to increase to 22 weeks of age when the experiment was terminated. With their methodology, however, it was impossible to determine if the small adipocyte population resulted from cellular multiplication or from the lipid filling of existing preadipocytes.

EXPERIMENT I

HYPLASTIC RESPONSE OF ADIPOSE TISSUE TO CALORIC OVERCONSUMPTION IN SEXUALLY MATURE CHICKENS

INTRODUCTION

Excessive body weight and obesity have negative health implications in both humans and animals. In addition, adiposity that exceeds normal requirements for thermoregulation and energy storage can adversely affect many aspects of commercial poultry production, including hatching egg production, processing waste management, by-product composition, and, especially, consumer acceptance (Heath *et al.*, 1980).

The adipose tissue of chickens, in contrast to most mammals with the exception of man, displays only minimal de novo fatty acid synthesis from acetate and glucose. Most lipid stored in avian adipose tissue is, therefore, either of dietary origin or originates from hepatic lipogenesis. Numerous investigators have reported that caloric overconsumption by chickens results in fat deposition, principally in the abdominal and visceral regions (Essary *et al.*, 1960; Kubena *et al.*, 1974; Griffiths *et al.*, 1977a), with the comparative size of individual adipose depots being influenced by both genetic (Pym and Solvyns, 1979) and nutritional factors (Farrell 1974; Griffiths *et al.*, 1977a; 1977b).

Enlargement of adipose depots can result from either hypertrophy, hyperplasia, or both of these phenomena. Although adipose tissue cellularity has been intensively investigated, the concept that adipocyte numbers are static in adult animals remains equivocal. Until recently, it has generally been accepted that adipocyte hyperplasia is the primary means of fat accretion during the early stages of growth, but as sexual maturity is approached, adipocyte hypertrophy becomes increasingly

pronounced until, at some point, changes in body fat are due solely to changes in adipocyte size. Support for this hypothesis has been obtained by experimentation with adipose tissue from several species including man (Sims *et al.*, 1968), rats (Hubbard and Matthew, 1971), bovine (Hood and Allen, 1973), and chickens (Pfaff and Austic, 1976).

In contrast to the experiments previously cited, evidence that adipocyte hyperplasia can occur in adult animals also exists. Therriault and Mellin (1981) reported that exposing adult rats to cold temperatures induced the formation of new adipocytes. Lemmonier (1972) found that mature rats and mice fed high fat diets experienced adipocyte hyperplasia in certain, but not all, fat depots. Greenwood and Hirsh (1974) suggested that in rats, the rate at which preformed adipocytes fill with lipid is determined by the amount of lipid presented to the tissue, and that the latter acts as a signal to regulate increased cellular proliferation. Data from several other studies also question the concept that post-maturational changes in adipose tissue mass are solely the result of changes in cell size (Stiles *et al.*, 1976; Faust *et al.*, 1978; Bertrand *et al.*, 1978; 1980; Klyde and Hirsch, 1979a; 1979b). Faust *et al.* (1978) argued that a diet-induced increase in adipocyte number in the adult rat is a general phenomenon independent of strain, sex, or adipose depot.

Information regarding post-maturational changes in adipocyte number in the chicken is limited. Oruwari *et al.* (1986) reported that the adipose tissue of adult Japanese quail is capable of adipocyte hyperplasia, but speculated that premigratory fattening may have been involved in the response. The objective of the study reported here was

to determine if adipocyte hyperplasia can be induced in adult domestic fowl, a non-migratory species, by caloric overconsumption, or if enlargement of the adipose depots is restricted simply to hypertrophy of existing cells. The response was tested in lines of chickens divergently selected for growth potential.

MATERIALS AND METHODS

Experiment 1. Adult female chickens, 32 weeks of age, from the high weight (HW) and low weight (LW) lines developed through bidirectional selection for juvenile body weight (Dunnington and Siegel, 1985), were chosen at random and placed in individual wire cages. Twelve hens of each line from the S_{28} generation were utilized, six fed *ad libitum* (AL) with the remaining six force-fed (FF). The force-fed chickens received a blend of 50% feed and 50% water. To force feed, polyethylene tubing (25 cm) attached to a pneumatic gun was directed via the esophagus to the level of the crop, and a quantity of the blend approximating crop capacity was administered twice daily. A simple corn-soy ration containing 3445 kcal ME per kg, and 10% protein was fed to all birds (Experiments 1 and 2). The diet was formulated to contain a high calorie:protein ratio to stimulate lipogenesis (Table 1).

On day 15 of the experiment, each bird received an intramuscular injection of tritiated [methyl-^{3}H]thymidine (.05 uCi/g body weight) into the pectoral muscle. Body weights were recorded on days 1, 10, 15, and 20 of the experiment.

Twenty days after initiating the feeding treatments, the birds were sacrificed and the retroperitoneal (abdominal) fat depot was removed and weighed. Adipose tissue (2-3g) samples were excised from each of the following four locations: the dorsal and the ventral surfaces of the retroperitoneal fat depot immediately posterior to the gizzard, the lateral margin of the retroperitoneal fat depot, and the medial right

sartorial fat depot. All samples were maintained in cold physiological saline for subsequent analyses.

Adipose tissue from each bird was used for DNA isolation and determination of radiotracer uptake. DNA and radiotracer measurements were performed in duplicate. Adipocytes were isolated using the procedure of Rodbell (1964), and the DNA content of adipocytes was measured (Beckman Model DU-6 Spectrophotometer) as described by Curtis-Prior *et al.* (1975). Tissue samples used for radiotracer measurements were treated identically to those for DNA analysis up to, but not including, hydrazone formation. Instead, the samples were dried in scintillation vials and scintillation cocktail (Beckman Ready-Solv EP) was added for liquid scintillation counting (Beckman Model LS 7500). Radioactivity measurements were corrected for background and counting efficiency determined using a reference standard (Amershan Searle H-3 Standard).

Experiment 2. Seventeen HW line and 17 LW line males (S_{28} generation), 44 weeks of age, were selected at random and placed in individual wire cages. Five males from each line were fed *ad libitum*, six were restricted to approximately 50% of *ad libitum* intake, and the remaining six were force-fed. Feed allotments for the restricted-fed birds were based on the average feed consumption of the *ad libitum*-fed birds of the same line for the preceding 3-day period.

On days 1 and 10 of the experiment, each bird received an intramuscular injection of tritiated [methyl-^{3}H]thymidine (.025 uCi/g body weight) into the pectoral muscle. Body weights were recorded on days 1, 5, 10, and 20 of the experiment.

Twenty days after initiating the feeding treatments, the birds were sacrificed and adipose tissue (2-3g) samples were excised from the ventral surface of the retroperitoneal (abdominal) fat depot. Adipose tissue samples from each bird were used for DNA isolation and determination of radiotracer uptake.

Statistical Analyses. Comparisons within traits were made by analysis of variance using the general linear models procedure (Ray *et al.*, 1982) as a 2 x 2 factorial arrangement and a 2 x 3 factorial arrangement in Experiments 1 and 2, respectively. All percentage data were analyzed after $\sqrt{}$arcsin transformation. Significance implies ($P \leq .05$) unless otherwise indicated. Line and treatment were considered as fixed effects. The statistical model was:

$$Y_{ijk} = u + L_i + T_j + (LT)_{ij} + e_{ijk}$$

where i = 1..n lines, j = 1..n feeding regimes, and k = 1..n individuals.

RESULTS

Experiment 1. Body Weight, Cumulative Percent Gain, Abdominal Fat Weight, and Right Sartorial Fat Weight. The HW line birds were heavier and had greater amounts of abdominal fat and larger right sartorial fat depots than LW line birds (Table 2). Nevertheless, there were no line differences in cumulative percent body weight gain (Table 2) at any time during the experiment, indicating that the HW and LW line birds responded similarly per unit body weight when force-fed. When analyzed as a percentage of body weight, abdominal fat pads from the HW line birds were greater than those from the LW line; line differences were not significant for the right sartorial fat depot when analyzed on a body weight basis.

Force-fed birds were heavier than the *ad-libitum* fed birds by day 15, and this relationship continued to the end of the experiment (Table 2). The cumulative percent body weight gain was also greater in force-fed than in the *ad libitum*-fed birds. When expressed as a percentage of body weight, abdominal and right sartorial fat from the force-fed birds was greater than that of the *ad libitum*-fed birds. The line-by-feeding regimen interactions for body weight, cumulative percent gain, abdominal fat weight, and sartorial fat weight were not significant, demonstrating that the effects of force-feeding were similar in both lines.

Total DNA Content and Radiotracer Uptake. Sampling sites within the abdominal fat depot were not different for either DNA content or radiotracer uptake; therefore, sites were pooled and data were analyzed

by fat depot. Total DNA content and total radiotracer uptake were greater in the abdominal fat depot of the HW line than those of the LW line chickens (Table 3). The right sartorial fat depot of HW line birds demonstrated greater total DNA content than LW line birds. Force-fed birds showed a greater total DNA content and radiotracer uptake in the abdominal and right sartorial fat depots than did the *ad libitum*-fed chickens. No differences in specific activity (DPM/ug DNA) were detected by weight line or feeding regimen in the abdominal or right sartorial fat depots. However, due to the presence of significant radioactivity within the fat depots of birds from both feeding regimens, it can be surmized that hyperplasia played a role in the fat depot enlargement of both *ad libitum* and force-fed birds. Line-by-feeding regimen interactions were not significant.

Experiment 2. Feed Intake, Body Weight, Cumulative Percent Body Weight Change and Abdominal Fat Depot Weight. A significant line-by-feeding regimen interaction was detected for average daily feed intake. Average daily feed intakes for the LW restricted, *ad libitum*, and force fed birds, and the HW restricted, *ad libitum*, and force fed birds were 16.25, 49.14, 94.27, 24.00, 120.82, and 191.41 grams/day respectively. Feeding treatment resulted in differences in cumulative percent body weight change by day 5, and this difference continued throughout the 20 day experimental period (Figure 1). At the conclusion of the experiment, the restricted-fed birds had lost approximately 12% of their original body weight, the *ad libitum*-fed birds had gained

approximately 6%, and the force-fed birds had gained approximately 17% above their original body weight.

A significant line-by-feeding regimen interaction for body weight was detected (Figure 2), the result of differences in response by weight lines in the *ad libitum* feed groups. *Ad libitum*-fed LW line birds exhibited only slight increases in body weight when compared to restricted-fed birds of the same line. However, when HW line birds were allowed to consume feed *ad libitum*, their body weight gain resembled that of force-fed birds of the same line. This phenomenon resulted in a significant line-by-feeding regimen interaction for body weight (Figure 3). Similarly, the line-by-feeding regimen interaction was significant for abdominal fat weight (Figure 3). Abdominal fat depot weights of *ad libitum* fed LW line birds was only slightly above that of the restricted-fed birds of the same line. A significant response due to feeding regimen was detected for abdominal fat weight as a percentage of body weight. *Ad libitum* feeding resulted in a significant increase in abdominal fat per unit body weight over restricted birds (.82 vs .15% respectively). This response was potentiated when birds were force-fed (1.87%).

Total DNA Content and Radiotracer Uptake. Line-by-feeding regimen interactions for total DNA content and for total radiotracer uptake were significant (Figures 4 and 5, respectively). Again, limited fat deposition, the result of reduced feed intake by some of the *ad libitum* fed LW line birds, created the interaction. Feeding regimen also resulted in significant differences in specific activity (DPM/ug DNA) in the

abdominal fat depot. *Ad libitum* feeding increased specific activity over restricted-fed birds (3.5 vs 1.3 DPM/ug DNA respectively). This response was potentiated when birds were force-fed (8.7 DPM/ug DNA).

DISCUSSION

Force-feeding a high calorie:protein ratio diet increased body weight and cumulative percent body weight gain over the *ad libitum*-fed birds of both the HW and LW lines (Experiment 1). These results are consistent with those observed with rats where caloric overconsumption, as a result of feeding a high fat or high carbohydrate diet, increased both body fat, and body weight gain (Faust *et al.*, 1978).

The significant line-by-feeding regimen interactions noted in Experiment 2 were possibly the result of an apparent dietary self-restriction of some of the *ad libitum*-fed LW line birds, for over the course of the experiment, LW *ad libitum*-fed birds showed only a 200% increase in consumption over LW restricted birds, compared to a 400% increase seen between feeding regimes in the HW line. Although LW force-fed chickens exhibited greater body weight gain, abdominal fat deposition, DNA content, and radiotracer uptake than the restricted-fed birds of the same line, the degree of response was less than that seen when comparing similar treatments in the HW line. Calabotta *et al.* (1985) reported earlier that LW line birds exhibited a relatively greater lipogenic and lipolytic competence when compared to HW line birds. Furthermore, when LW line birds were force-fed, they exhibited large increases in both DNA content and radiotracer uptake by the abdominal and right sartorial fat depots, indicating that when the constraints of dietary self-restriction were removed, adipose tissue from LW line birds was capable of hypercellularity. Thus, it is conceivable that feed intake

along with lipid turnover are major reasons why net fat deposition differs among weight-selected populations of chickens.

In both Experiments 1 and 2, force-feeding increased the total DNA content of the adipose tissue. The elevated levels of DNA noted in the two experiments paralleled an increase in radiotracer uptake by the fat depots. The increased uptake of DNA-specific thymidine reflected adipose hyperplastic activity. Thus, adipose tissue hyperplasia was observed in adult chickens, as in Japanese quail (Oruwari *et al.*, 1986), and rats (Faust *et al.* 1978; Lemmonier, 1972). Apparently, adipocyte hyperplasia in Aves is not soley a premigratory response.

Both the abdominal and right sartorial fat depot responded to caloric overconsumption by initiating periods of hyperplastic activity and depot enlargement. A lack of differences in abdominal and sartorial fat depot specific activity (Experiment 1) indicates that these depots are dynamically similar. Since restricted-fed birds of both lines demonstrated little radiotracer uptake, it can be surmised that adipocyte hyperplasia did not occur when feed intake was limited. The methodology employed in this experiment precluded determining if adipocyte number decreased in the restricted-fed birds from either line.

Adipocyte hyperplasia in the adult chicken can have negative implications, for animals which become hypercellular are likely candidates for obesity. Egg or meat producing birds which are allowed to consume calories in excess of the level necessary for normal function, whether pre- or post-puberal, incur the risk of permanent hypercellularity and potential obesity. There is evidence that once

attained, the number of constituent cells never decreases and they tend to accumulate lipid at a normal rate. Hirsch and Han (1969) found that even when starved, adult rats did not lose adipocytes. Strict regulation of caloric intake and body weight gains are, therefore, prudent during both growth and production. The negative implications of obesity on egg production and sexual function are well documented in the fowl (Siegel and Dunnington, 1986).

Allen (1976) proposed that a relatively fixed percentage of adipocytes must reach a critical size (135-150 μm diameter or about 1.2-1.6 ug lipid/cell) before hyperplasia can be initiated in meat animals. Faust *et al.* (1978) reported that a high-fat diet resulted in a significant increase in adipocyte size, and that after a certain size is attained, continued depot enlargement is due to increase adipocyte number. This thesis is consistent with the data in this study in that force-fed chickens demonstrated higher radiotracer uptake than *ad libitum*-fed birds of the same line. Secondly, LW line restricted-fed chickens (Experiment 2), which exhibited limited lipid deposition, had less total DNA content in the abdominal fat pad, and had no significant radiotracer levels above background. However, additional experimentation is necessary to determine if adipocytes of the adult chicken have a critical size or lipid volume limitation which must be approached or exceeded before hyperplasia is initiated. Such information could provide further insight into the factors which regulate the dynamic state of avian adipose tissue.

SUMMARY

Two experiments were conducted to determine the hyperplastic potential of adipose tissue from sexually mature chickens in response to caloric overconsumption. In the first experiment, birds were either force-fed or allowed to consume feed *ad libitum*, while in the second experiment, a feed-restriction regimen was also implemented. In both experiments, the feeding treatments were applied for 19 consecutive days, and a diet high in energy and low in protein was fed to all birds to promote lipogenesis. To monitor adipocyte dynamics, birds in Experiment 1 received an intramuscular injection of [methyl-^{3}H]thymidine on day 15, while in Experiment 2 birds were injected both on days one and ten.

In both Experiments 1 and 2, body weight gain and percentage weight gain were greater in the force-fed birds. Furthermore, the retroperitoneal (abdominal), and right sartorial fat depots increased in weight as a result of force-feeding, and there were concomitant increases in total DNA content and total radiotracer uptake by these tissues. The results indicate that expansion of adipose tissue in sexually mature chickens is not restricted solely to cellular hypertrophy, but periodic hyperplastic activity also occurs in response to caloric overconsumption.

Table 1. Composition of the experimental diet

Ingredient	Percent
Ground yellow corn	86.41
Dehulled soybean meal (48.5% protein)	4.82
Defluorinated phosphate	.99
Ground limestone	1.99
Stabilized feed-grade fat	4.99
Sodium chloride	.15
DL-Methionine	.10
Vitamin premix[1]	.50
Mineral mix[2]	.05
<u>Calculated analysis</u>	
Energy, kcal ME[3]/kg	3445
Protein, %	10
Calorie:Protein	344.5

[1]Vitamin premix supplied per kilogram of diet: 4400 IU vitamin A, 1100 IU vitamin D_3, 4.4 IU vitamin E, 7.0 mg menedione sodium bisulfite, 4.4 mg riboflavin, 11.0 mg d-calcium pantothenate, 33 mg niacin, 250 mg choline chloride, 6.6 mg vitamin B_{12}, .6 mg folic acid, and 125 mg ethoxyquin.

[2]Mineral mix supplied per kilogram of diet: 60 mg manganese, 60 mg zinc, 20 mg iron, 2.5 mg copper, 1 mg iodine, and .22 mg cobalt.

[3]Metabolizable energy.

Table 2. Least-squares means of average daily feed intake, body weight, cumulative percent gain, abdominal fat weight, and right sartorial fat weight summarized by high (HW) or low weight (LW) line and feeding regimen[1] (Experiment 1).

	Line				Feeding Regimen			
	LW	HW	$T_{S.E.}$[2]	Significance[3]	Ad-Libitum	Force-fed	$T_{S.E.}$	Significance
Intake								
feed (grams)	76.35	134.99	29.08	***	68.85	142.49	28.94	***
protein (grams)	7.66	13.54	2.92	***	6.91	14.29	2.90	***
energy (kcal)	263.03	465.06	100.19	***	237.19	490.90	99.69	***
Body weight, kg								
Day (1)	1.44	3.39	.11	***	2.41	2.42	.11	NS
(10)	1.50	3.58	.11	***	2.43	2.65	.11	NS
(15)	1.59	3.75	.11	***	2.45	2.90	.11	**
(20)	1.63	3.79	.10	***	2.46	2.96	.10	**
Cumulative gain, %								
Days (1-10)	4.1	5.7	1.1	NS	.4	9.3	1.1	***
(1-15)	10.6	11.0	1.5	NS	1.2	20.3	1.5	***
(1-20)	12.7	12.3	1.4	NS	1.7	23.4	1.4	***
Abdominal fat								
grams	69.4	234.0	16.9	***	126.7	176.7	17.1	.056
% body wt.	4.0	6.5	.5	**	4.4	5.8	.5	*
Sartorial fat								
grams	4.30	9.29	1.00	**	5.37	8.23	1.0	NS
% body wt.	.25	.24	.03	NS	.20	.29	.03	*

[1]Line-by-feeding regimen interactions were not significant.
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3] * ($P \leq .05$)
** ($P \leq .01$)
*** ($P \leq .001$)

Table 3. Least-squares means of total DNA, total radiotracer uptake and specific activity by the abdominal and right sartorial fat depots summarized by high (HW) or low weight (LW) line and feeding regimen[1] (Experiment 1).

	Line				Feeding Regimen			
	LW	HW	$T_{S.E.}$[2]	Signif-icance[3]	Ad-libitum	Force-fed	$T_{S.E.}$	Signif-icance
Abdominal fat								
DNA (ug) x 10^4	1.49	5.05	.55	***	2.35	4.18	.56	*
DPM x 10^5	4.19	13.03	1.30	***	5.66	11.58	.13	**
SA[4] (DPM/ug DNA)	26.29	29.74	4.32	NS	28.19	27.84	4.34	NS
Sartorial fat								
DNA (ug) x 10^4	.09	.21	.04	*	.10	.21	.04	*
DPM x 10^5	.03	.45	.08	NS	.22	.55	.08	**
SA[4] (DPM/ug DNA)	29.82	27.49	5.19	NS	27.06	30.25	5.22	NS

[1]Line-by-feeding regimen interactions were not significant.
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3] * ($P \leq .05$)
** ($P \leq .01$)
*** ($P \leq .001$)
[4]Specific activity

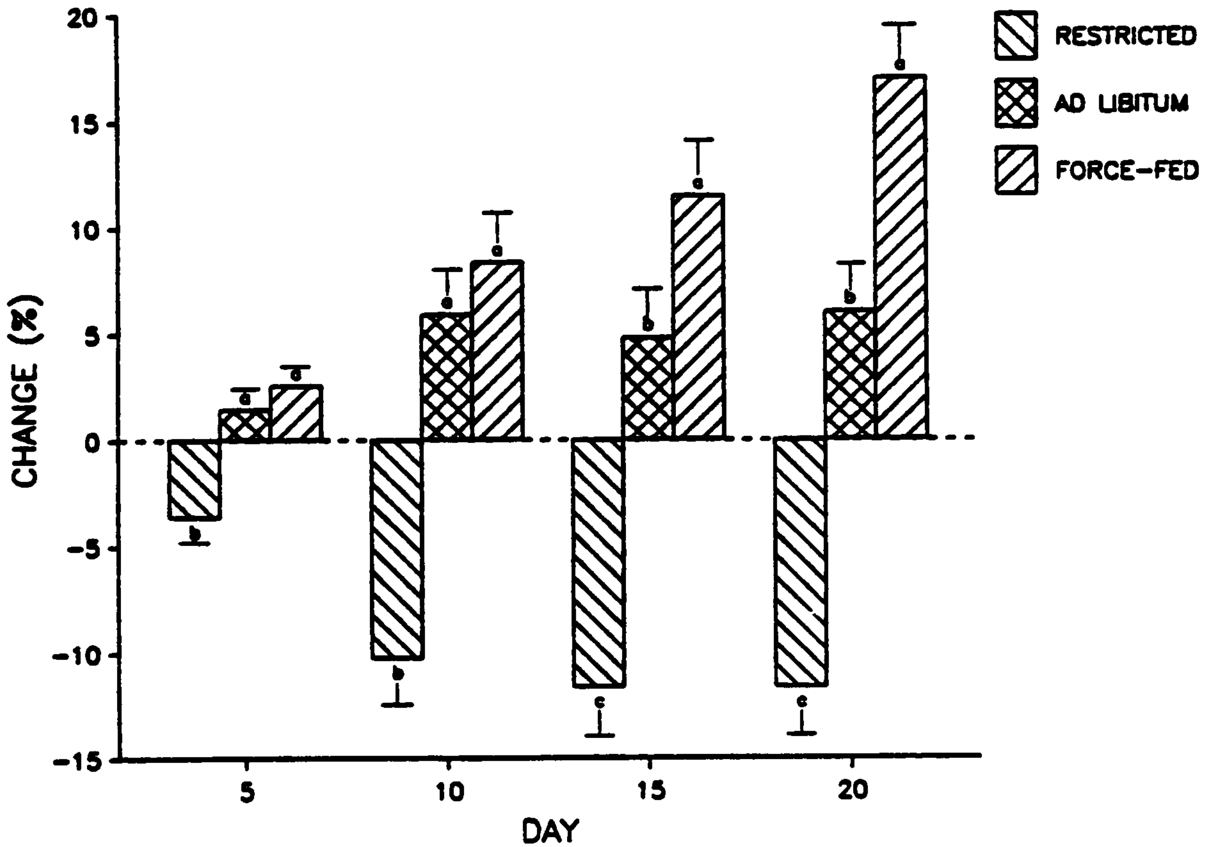

Figure 1. Least-squares means (± standard error) of cumulative percent body weight change during the experimental period (Experiment II). Means within a time period with different letter notations are significantly different (P≤.05).

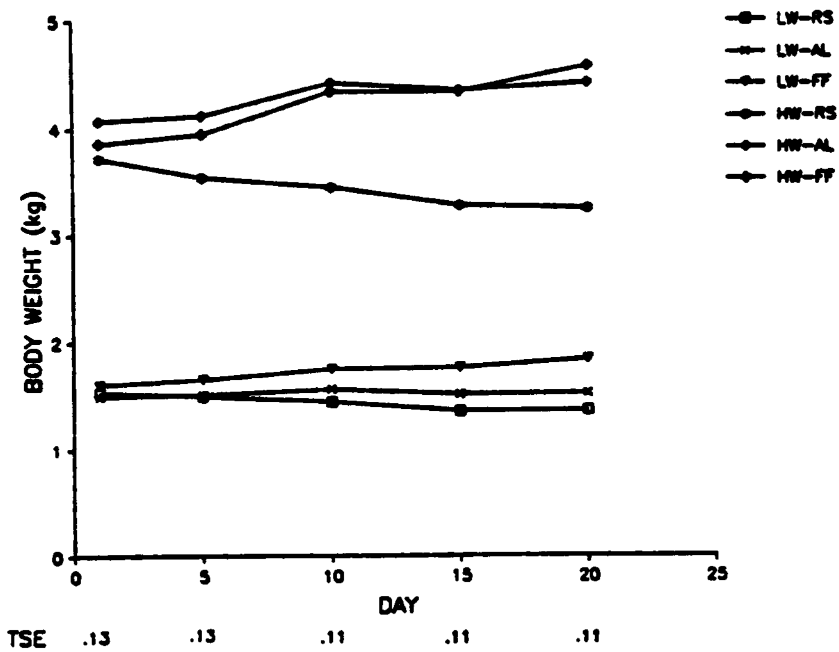

Figure 2. Least-squares means of body weight during the experimental period (Experiment II) ($T_{S.E.}$ - Standard error of the treatment means).

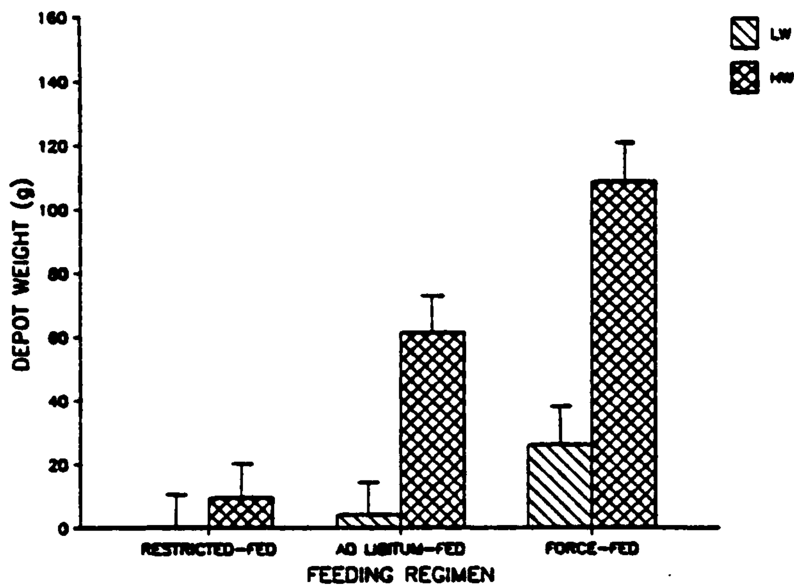

Figure 3. Least-squares means (± standard error) of abdominal fat depot weight, for each line-by-feeding regimen subclass (Experiment II).

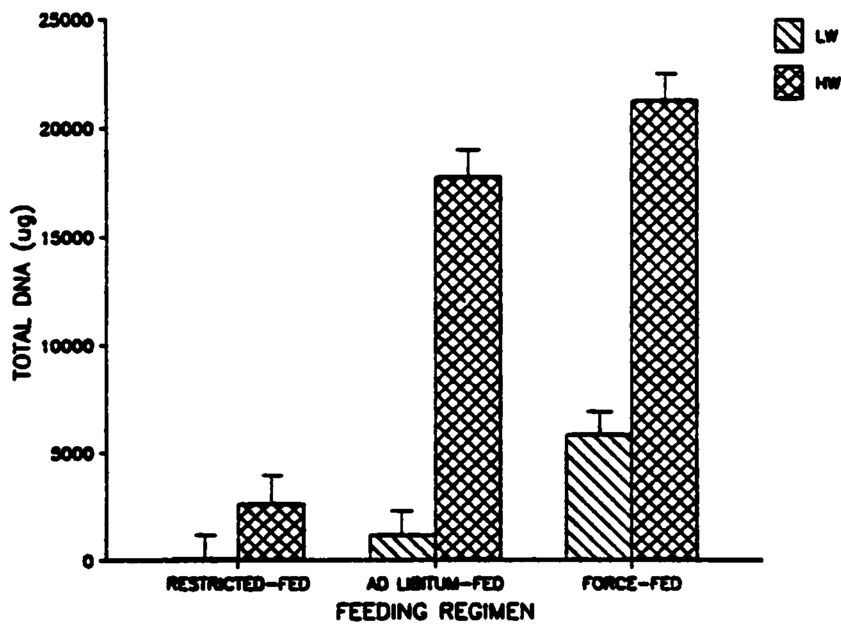

Figure 4. Least squares means (± standard error) of total DNA content in the abdominal fat depot for each line-by-feeding regimen subclass (Experiment II).

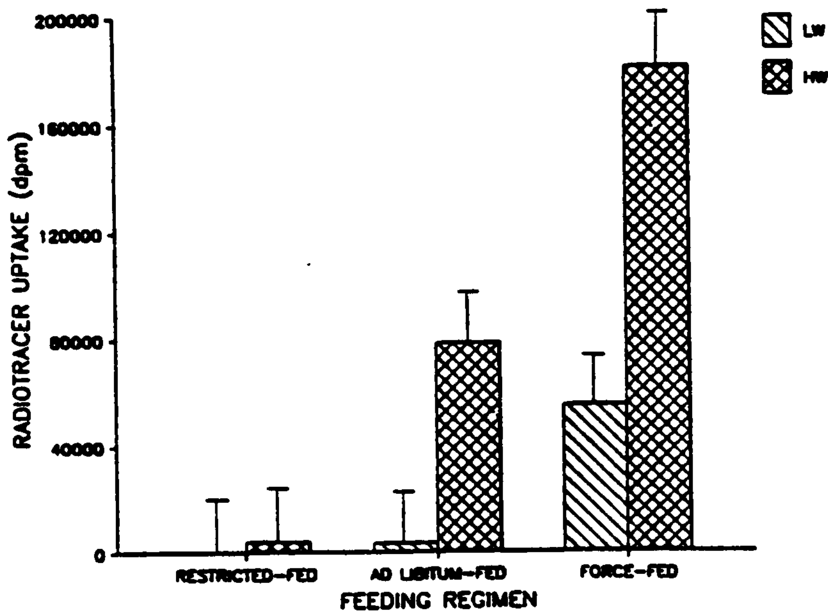

Figure 5. Least-squares means (± standard error) of total radiotracer uptake by the abdominal fat depot for each line-by-feeding regimen subclass (Experiment II).

EXPERIMENT II

INFLUENCE OF CALORIC OVERCONSUMPTION ON ADIPOCYTE SIZE NUMBER, AND DISTRIBUTION IN THE RETROPERITONEAL FAT DEPOT OF SEXUALLY MATURE HIGH- AND LOW-WEIGHT CHICKENS

INTRODUCTION

The premise that adipocyte hyperplasia is confined solely to pre-puberal increases in adipose tissue mass has been questioned. Previously it was hypothesized that the attainment of final adipocyte number was accomplished prior to the age of sexual maturation (Anderson and Kauffman, 1973; Hood and Allen, 1973: Pfaff and Austic, 1976). However, recently evidence has arisen which questions the concept that post-maturational increases in adipose tissue are the sole result of cellular hypertrophy. In the adult rat, adipose cell number has been shown to increase in response to dietary manipulation (Lemonier, 1972), exposure to cold (Therriault and Mellin, 1971), and partial lipectomy (Faust *et al.*, 1977). DiGirolamo and Medlinger (1971) reported that the adult guinea pig is also capable of periodic hyperplasia.

Evidence of post-maturational hyperplastic episodes in avian adipose tissue also exists (Oruwari *et al.*, 1986), although it was hypothesized as a possible pre-migratory response. Results of March *et al.* (1984) may also be indicative of post-puberal adipocyte hyperplasia; however, the methodology employed precluded separation of new adipocytes from those resulting from simple lipid filling of existing pre-adipocytes.

Results from Experiment I of this dissertation indicated that adipocyte hyperplasia can occur in the adult chicken in response to self-induced or forced caloric overconsumption. The objective of the present study was to examine the effects of caloric overconsumption on

adipocyte number and distribution in the retroperitoneal (abdominal) fat depot of sexually mature high- and low-weight chickens.

MATERIALS AND METHODS

Adult male chickens, 40 weeks of age, from the high- (HW) and low-weight (LW) lines developed through bidirectional selection for juvenile body weight (Dunnington and Siegel, 1985) were chosen at random and placed in individual wire cages. Forty males of each line from the S_{29} generation were utilized. One half of the birds in each line were fed *ad libitum* (AL), with the remaining being force-fed (FF). The force-fed chickens received a blend of 50% feed and 50% water. To force-feed, polyethylene tubing (25 cm) attached to a pneumatic gun was directed via the esophagus to the level of the crop, and a quantity of the blend approximating crop capacity was administered twice daily. A simple corn-soy ration containing 3445 kcal ME per kg, and 10% protein was fed to all birds. The diet was formulated to contain a high level of carbohydrates to stimulate lipogenesis (Table 1).

Six, 12, and 18 days after treatment application, five birds from each line-by-feeding regimen combination were randomly selected and sacrificed. Duplicate adipose tissue samples (4-6 grams) were removed from the ventral surface of the retroperitoneal (abdominal) fat depot for the determination of adipocyte DNA content (Curtis-Prior *et al.*, 1975), adipocyte size and distribution (March *et al.*, 1982), and depot lipid content (Folch *et al.*, 1957). Adipocyte cell suspensions were prepared as outlined by Rodbell (1964), and processed using a Zeiss MOP image analyzer as described by March *et al.* (1982). Total adipocyte number was

calculated from the average cell diameter (Goldrick, 1967) and the weight of the extractable lipid contained in the adipocytes.

After day eighteen, the remaining force-fed birds were released to *ad libitum* feeding until their body weights stablized. At this latter point, the birds were sacrificed and treated as previously described.

Body weights were recorded on days 1, 6, 12, 18, and thereafter at three-day intervals. Prior to sacrifice, venous blood was collected from each bird (post 14-hr fast) for the determination of plasma lipids (Zollner and Kirsch, 1962), total cholesterol (Allain *et al.*, 1974), glucose (Raabo and Terkildsen, 1960) and protein (Lowry *et al.*, 1951).

Statistical Analysis: Comparisons within traits were made by analysis of variance using the general linear models procedure (Ray *et al.*, 1982) as a 2 x 2 factorial arrangement. Line and feeding treatment were considered as fixed effects. All percentage data were analyzed after $\sqrt{}$arcsin transformation. Significance implies ($P \leq .05$) unless otherwise noted). The statistical model was:

$$Y_{ijk} = u + L_i + T_j + (LT)_{ij} + e_{ijk}$$

where i = 1,2 lines, j = 1,2 feeding regimes and k = 1,2...n individuals.

RESULTS

Feed Intake: A line-by-feeding regimen interaction for feed intake was detected at each measuring period through day 21 (Figure 1). Force-fed birds from both lines showed similar patterns of consumption, with the interaction the apparent result of differences by weight line within the *ad libitum* regimen. When released from force-feeding, both LW, and HW birds demonstrated immediate decreases in intake, which then recovered to a level similar to their *ad libitum*-fed counterparts. From day 24 through the conclusion of the experiment, HW birds consumed more feed than LW birds at all but two measuring periods.

Body Weight: As anticipated, HW birds were heavier than LW birds throughout the experimental period (Table 2). Feeding regimen did not alter body weight until day 18, at which point force-fed birds were heavier than *ad libitum*-fed birds. This relationship continued through day 36, after which no differences in body weight by feeding regimen were detected.

Plasma Glucose, Total Lipid, and Protein: Other than at the termination of the experiment, when LW birds had higher levels of plasma glucose than HW birds (Table 4), no differences in plasma glucose were detected by weight-line or feeding regimen. The HW birds had higher plasma lipid levels than LW birds when measured on day 6, and at the termination of the experiment. Force-fed birds had higher plasma total lipid levels than *ad libitum*-fed birds on day 12, and at the termination

of the experiment. No differences in plasma protein were detected by weight-line or feeding regimen.

Abdominal Fat: At all but the initial measuring period, HW birds had larger abdominal fat depots than LW birds (Table 4). Force-fed birds had more abdominal fat than *ad libitum*-fed birds on days 6, 12, and 18, demonstrating the bilateral effects of force-feeding on abdominal fat deposition.

Percent lipid content of the abdominal fat depot was similar for both lines (Table 4). However, force-feeding increased the percent lipid content of the abdominal fat depot when measured on days 12 and 18.

At each measuring period, HW birds had more total lipid in the abdominal fat depot than LW line birds (Table 4). Force-fed birds had more total lipid than *ad libitum*-fed birds on days 12 and 18, with differences no longer apparent after release to a period of *ad libitum* feeding (day 57).

Force-feeding increased the total adipocyte DNA content in the abdominal fat depot on day 12. On day 18, a line-by-feeding regimen interaction for total adipocyte DNA was detected (Figure 2), however, the incremental effects of forced caloric over-consumption are clearly demonstrated across weight line.

No differences in the lipid/adipocyte DNA ratio within the abdominal fat depot were detected by weight-line (Table 4). However, force-fed birds demonstrated a sharply decreasing ratio which was lower than *ad libitum*-fed birds on day 18. Because the fat content of this depot was undergoing a period of rapid expansion, a decreasing ratio is

indicative of hyperplastic development. On day 57, the differences were no longer apparent.

Adipocyte Distributions: Adipocyte distributions are depicted in Figures 3-6. *Ad libitum*-fed birds from both lines had similar patterns of change, demonstrating a gradual shift toward bimodality. A similar relationship was apparent for force-fed birds; however, the shift was much more pronounced at day 18. It is important to note the marked increase in the small adipocyte population of the force-fed birds on day 12. This population shift, which was maintained through day 18, was lost when depot reduction occurred after the birds were released to *ad libitum* feeding. The loss of the small adipocyte population was not as apparent within the LW line, which retained a large percentage of adipocytes with diameters greater than 130 um.

Adipocyte Diameter and Number: No differences in average adipocyte diameter by weight-line or feeding regimen were detected during the experiment. However, the adipocyte distribution curves shown in Figures 3-6 clearly indicate that diameter is increasing, leading to a translocation towards bimodality in the fat depots of force-fed birds. Because the proportion of the population comprised of small adipocytes was also increasing, this shift was masked, and as a result, no differences in average adipocyte diameter were detected. This relationship is also apparent to a lesser extent in the *ad libitum*-fed population.

Adipocyte diameter of the force-fed birds increased at day 57, even though the abdominal fat depot for these birds was decreasing. By

examining Figures 4 and 6, it is apparent that the increase in adipocyte diameter was due to the loss of the small adipocyte population. This resulted in an upward shift in overall diameter size even though the diameter of the large adipocytes was in a state of decline. Differences in adipocyte number in the abdominal fat depot were not detected by feeding regimen. However, the relationship seen in the adipocyte distribution curves was apparent. Cell number in the force-fed birds increased at day 12, was maintained through day 18, and decreased after release to *ad libitum* feeding.

DISCUSSION

Force-feeding increased body weight, deposition of abdominal fat, and adipocyte DNA content within the abdominal fat depot of both LW and HW birds. These effects were transient, however, and disappeared after a 33-day period of *ad libitum* feeding.

In agreement with Lepkovsky and Furuta (1971), force-fed birds from both lines essentially eliminated their *ad libitum* consumption when initially released from the constraints of force-feeding. However, they increased their voluntary intake to a level comparable to the continuously *ad libitum*-fed birds after only three days. As this experiment was conducted in the early winter months, when ambient temperature was beginning to decline. Therefore, it is possible that the rapid resumption of dietary intake was the result of an attempt to prevent a loss of insulating body fat, or meet a critical need to maintain heat production. In spite of this phenomenon, however, these birds were unable to prevent the loss of abdominal fat and adiposity previously described. Even though feed intake of the force-fed birds returned to normal levels after release, their injestive behavior fluctuated irregularly. This may indicate a transient inability to closely regulate their dietary intake.

March and Hansen (1977) reported that White Leghorn chickens had smaller, and fewer adipocytes in the abdominal fat depot than broiler-type chickens. Although it would be incorrect to equate the LW and HW birds used in this experiment with commercial strains, it is interesting to note that other than at the initial measuring period, no differences in average

adipocyte diameter were detected by weight line. Evidently, in regard to these lines, selection for 8-week body weight has not altered adipocyte diameter.

Even though differences in total adipocyte number were not detected by feeding regimen, our results indicate that adipocyte number increased in the abdominal fat depots of force-fed birds. This premise is supported by the increase seen in total adipocyte DNA content, the decrease in the lipid/adipocyte DNA ratio during a period of rapid depot expansion, and an increase in the small adipocyte population during force-feeding (Figures 4, 6).

My results revealed a greater number of adipocytes within the abdominal fat depot than previous studies (Hood, 1982; Cherry *et al.*, 1984). However, most of the early reports involved the use of the Coulter electronic counter, which is unable to record cells smaller than 20-25 um. Our procedure allowed for the histologic examination of the tissue, and, thus, the ability to measure smaller cells. This refinement of technique resulted in a decrease in average adipocyte diameter, and a resultant increase in apparent total cell number.

In agreement with March *et al.* (1982), when the force-fed birds were released to *ad libitum* feeding, and abdominal fat mass decreased, lipid was mobilized from small as well as large adipocytes, leading to a reduction in the small adipocyte population. Contrary to Haugebak *et al.* (1974), my results indicate a decrease in total adipocyte number during this period. However, it is probable that this was the result of an inability to recover, or measure extremely small adipocytes containing

limited lipid volume. This may also have contributed to our inability to show differences in adipocyte size and number between the LW and HW lines. Unexplainably, the changes associated with depot reduction were not as clearly defined within the LW population. Although LW birds showed a slight reduction in the small adipocyte population when released from force-feeding, an increase in the percentage of adipocytes with diameters greater than 130 um was also manifested. This phenomenon may be related to the need of these birds to meet critical thermogenic requirements, or a decreased ability to regulate adipocyte size in the abdominal fat depot.

Additional experimentation is necessary before a more complete understanding of adipocyte regulation in the adult animal is attained. The effects of repeated forced fluctuations in adult body weight are not clearly understood. In some instances, partial lipectomy has also been demonstrated as a means of inducing adipocyte multiplication (Faust *et al*, 1977). It is clear, however, that the use of immature individuals to predict adipocyte dynamics in adults may be misleading.

SUMMARY

An experiment was conducted to determine the effects of caloric overconsumption on adipocyte size, number, and distribution in the abdominal fat depot of adult high- and low-weight chickens. Birds were either force-fed or allowed to consume feed *ad libitum*. Adipocyte dynamics were histogically monitored using a Zeiss MOP Image Analyzer.

Force-feeding resulted in a transient increase in body weight, abdominal fat deposition, abdominal fat total lipid and DNA content, and a decrease in the lipid/adipocyte DNA ratio. These effects were no longer apparent after a period of release to *ad libitum* feeding.

Although no differences in total adipocyte number of the abdominal fat depot were detected by feeding regimen, adipocyte number increased in force fed birds as the lipid/adipocyte DNA content decreased. These phenomena occurred as the small adipocyte population was growing. Evidently, adipocyte number can increase as a result of rapid depot expansion.

Table 1. Composition of the experimental diet

Ingredient	Percent
Ground yellow corn	86.41
Dehulled soybean meal (48.5% protein)	4.82
Defluorinated phosphate	.99
Ground limestone	1.99
Stabilized feed-grade fat	4.99
Sodium chloride	.15
DL-Methionine	.10
Vitamin premix[1]	.50
Mineral mix[2]	.05
<u>Calculated analysis</u>	
Energy, kcal ME[3]/kg	3445
Protein, %	10
Calorie:Protein	344.5

[1]Vitamin premix supplied per kilogram of diet: 4400 IU vitamin A, 1100 IU vitamin D_3, 4.4 IU vitamin E, 7.0 mg menadione sodium bisulfite, 4.4 mg riboflavin, 11.0 mg d-calcium pantothenate, 33 mg niacin, 250 mg choline chloride, 6.6 mg vitamin B_{12}, .6 mg folic acid, and 125 mg ethoxyquin.

[2]Mineral mix supplied per kilogram of diet: 60 mg manganese, 60 mg zinc, 20 mg iron, 2.5 mg copper, 1 mg iodine, and .22 mg cobalt.

[3]Metabolizable energy.

Table 2. Least-squares means of body weight summarized by weight line and feeding regimen[1]

	Weight-Line				Feeding Regimen			
Day	LW	HW	$T_{S.E.}$[2]	Sig[3]	AL	FF	$T_{S.E.}$	Sig
1	2.19	4.32	.06	***	3.26	3.25	.06	NS
6	2.23	4.47	.07	***	3.28	3.42	.07	NS
12	2.29	4.66	.09	***	3.41	3.55	.09	NS
18	2.33	4.93	.09	***	3.49	3.77	.09	*
21	2.29	4.94	.09	***	3.44	3.78	.09	*
24	2.30	4.95	.09	***	3.46	3.78	.09	*
27	2.29	4.99	.10	***	3.47	3.82	.10	*
30	2.32	5.02	.10	***	3.50	3.83	.10	*
33	2.32	5.05	.10	***	3.52	3.85	.10	*
36	2.31	5.02	.10	***	3.52	3.82	.10	*
39	2.32	5.01	.10	***	3.53	3.79	.10	NS
42	2.33	5.01	.10	***	3.53	3.80	.10	NS
45	2.34	5.06	.10	***	3.56	3.84	.10	NS
48	2.33	5.07	.10	***	3.55	3.85	.10	NS
51	2.32	5.07	.10	***	3.57	3.82	.10	NS
54	2.32	5.06	.10	***	3.56	3.82	.10	NS
57	2.34	5.05	.10	***	3.57	3.82	.10	NS

[1]First order interactions were not significant.
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3]Significance: NS - Not Significant, * ($P \leq .05$), ** ($P \leq .01$), *** ($P \leq .001$).

Table 3. Least-squares means of plasma glucose, lipid, and protein summarized by weight line and feeding regimen[1]

		Weight-Line				Feeding Regimen			
Plasma	Day	LW	HW	$T_{S.E.}$[2]	Sig[3]	AL	FF	$T_{S.E.}$	Sig
Glucose (mg/DL)	1	217.91	203.33	8.33	NS	205.63	215.62	8.33	NS
	6	186.47	177.29	4.97	NS	177.64	186.11	4.87	NS
	12	128.73	125.67	2.29	NS	129.88	124.53	2.28	NS
	18	124.33	122.79	2.19	NS	125.35	121.77	2.21	NS
	57	135.21	122.00	3.97	*	130.41	126.81	3.97	NS
Lipid (mg/DL)	1	370.53	448.55	38.77	NS	383.79	435.29	38.51	NS
	6	343.77	462.17	24.15	**	370.92	435.03	23.99	NS
	12	493.83	531.77	27.01	NS	421.28	604.32	26.89	***
	18	679.20	719.03	30.54	NS	681.42	716.81	31.19	NS
	57	463.49	583.92	48.03	*	599.78	447.64	48.03	*
Protein (g/DL)	1	2.58	2.69	.09	NS	2.62	2.65	.09	NS
	6	2.31	2.49	.08	NS	2.48	2.32	.08	NS
	12	2.25	2.08	.07	NS	2.21	2.13	.07	NS
	18	2.10	2.19	.08	NS	2.21	2.09	.09	NS
	57	1.95	2.06	.06	NS	2.05	1.96	.06	NS

[1]First order interactions were not significant.
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3]Significance: NS - Not Significant, * ($P \leq .05$), ** ($P \leq .01$), *** ($P \leq .001$).

Table 4. Least-squares means of abdominal fat weight, lipid content and total adipocyte DNA content summarized by weight line and feeding regimen

		Weight-Line				Feeding Regimen			
Abdominal Fat	Day	LW	HW	$T_{S.E.}$[2]	Sig[3]	AL	FF	$T_{S.E.}$	Sig
Weight (g)	6	38.73	58.07	6.79	NS	36.41	60.39	6.79	*
	12	75.54	128.06	7.96	***	54.38	149.22	8.27	***
	18	93.20	154.90	10.67	***	51.18	196.93	11.09	***
	57	61.24	130.46	20.08	*	83.52	108.18	20.08	NS
Lipid content (%)	6	81.79	89.28	3.59	NS	84.82	86.25	3.59	NS
	12	83.70	87.12	1.12	NS	77.83	92.99	1.12	***
	18	89.99	90.88	1.08	NS	84.29	96.59	1.08	***
	57	85.03	84.29	1.97	NS	84.52	83.89	1.97	NS
Lipid content (g)	6	32.49	51.84	5.84	**	38.00	53.56	5.84	NS
	12	67.07	115.47	6.16	***	43.48	139.06	6.16	***
	18	87.76	144.31	10.37	**	43.41	188.66	10.77	***
	57	53.11	111.53	17.78	*	72.83	91.82	17.78	NS
Total adipocyte DNA content (ug x 10^3)[1]	6	1.64	2.44	.46	NS	1.48	2.57	.46	NS
	12	5.93	11.52	1.92	NS	4.10	13.35	1.92	**
	18								
	57	1.78	4.00	.84	NS	2.39	3.39	.84	NS
Lipid/DNA ratio (mg/ug)	6	30.88	22.21	4.95	NS	26.84	26.25	4.95	NS
	12	17.08	12.32	2.88	NS	17.06	12.34	2.88	NS
	18	26.76	30.79	9.42	NS	51.27	6.28	9.42	**
	57	69.81	31.29	27.76	NS	39.32	61.78	27.76	NS

[1]Significant line-by-treatment interaction for total adipocyte DNA content on day 18 (see Figure 2).
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3]Significance: NS - Not Significant, * ($P \leq .05$), ** ($P \leq .01$), *** ($P \leq .001$).

Table 5. Least-squares means of adipocyte diameter and number in the abdominal fat depot[1]

		Weight-Line				Feeding Regimen			
	Day	LW	HW	$T_{S.E.}$[2]	Sig[3]	AL	FF	$T_{S.E.}$	Sig
Adipocyte diameter (um)	6	35.31	26.33	3.90	NS	30.25	31.38	3.90	NS
	12	47.33	50.45	6.89	NS	51.39	46.38	6.89	NS
	18	42.38	39.84	4.12	NS	38.08	44.15	4.12	NS
	57	68.84	52.34	11.48	NS	62.84	58.34	11.48	NS
Adipocyte number (x 10^8)	6	7.11	18.95	2.71	**	13.63	12.43	2.71	NS
	12	10.11	19.54	8.49	NS	9.51	20.13	8.21	NS
	18	8.77	22.62	6.39	NS	13.29	18.12	6.62	NS
	57	.89	7.74	2.51	NS	3.31	5.32	2.51	NS

[1]First order interactions were not significant.
[2]$T_{S.E.}$ - Standard error of the treatment means.
[3]Signficance: NS - Not Significant, ($P \leq .05$), ($P \leq .01$), ($P \leq .001$).

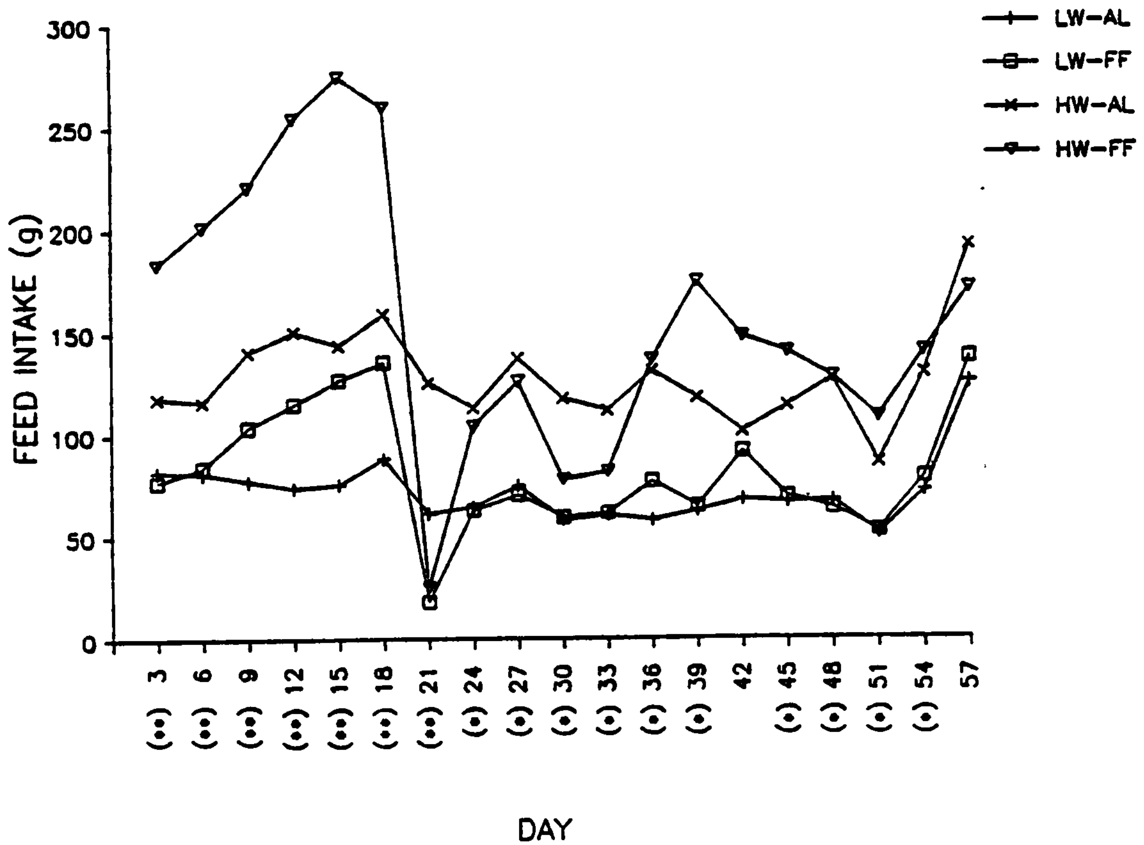

Figure 1. Least-squares interaction means of food intake during the 57-day experimental period (** - line-by-feeding regimen interaction, * - significant line effect).

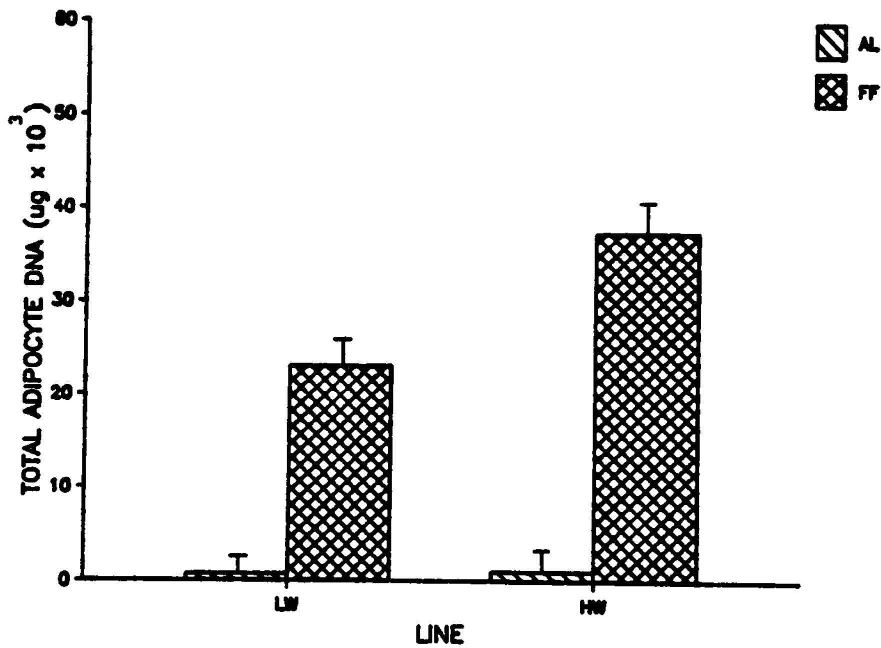

Figure 2. Least-squares interaction means (± standard error) of total DNA content in the abdominal fat depot determined on day 18.

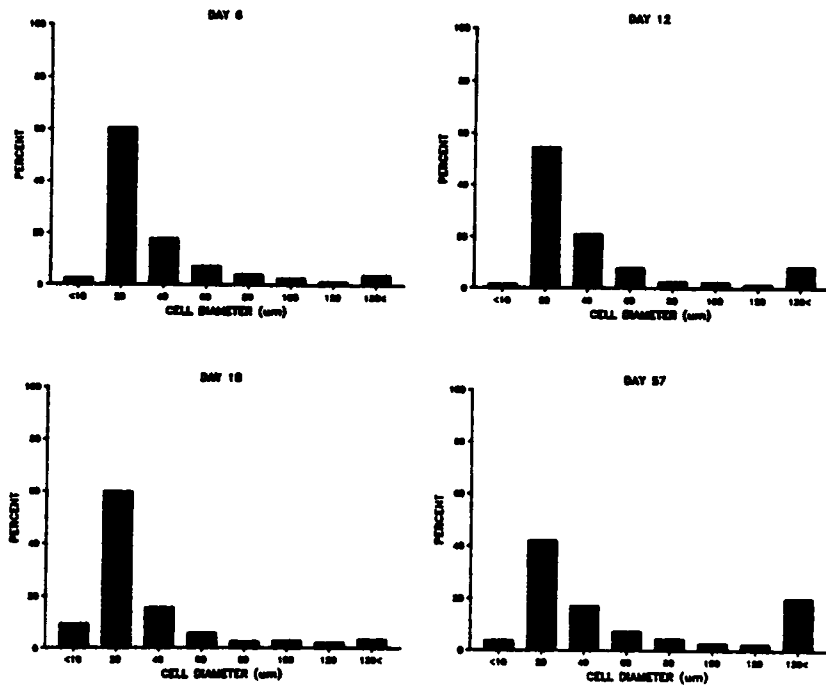

Figure 3. Adipocyte frequency distributions from the abdominal fat depot of LW *ad libitum*-fed birds.

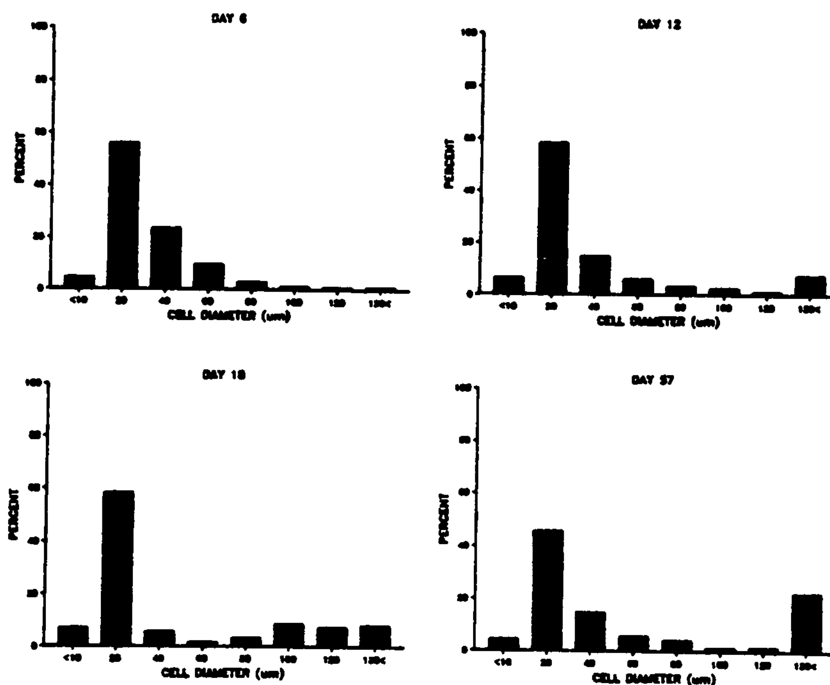

Figure 4. Adipocyte frequency distributions from the abdominal fat depot of LW force-fed birds.

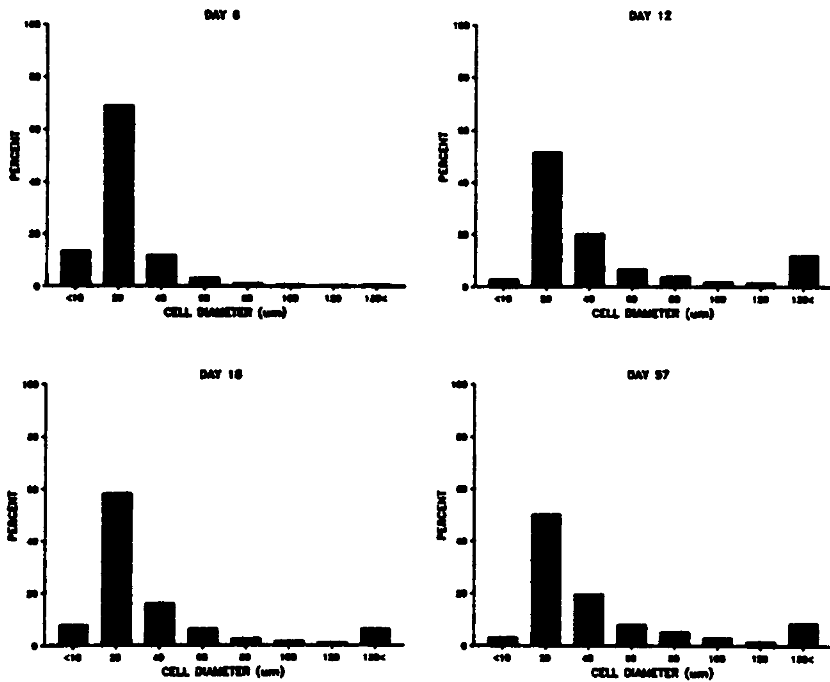

Figure 5. Adipocyte distributions from the abdominal fat depot of HW *ad libitum*-fed birds.

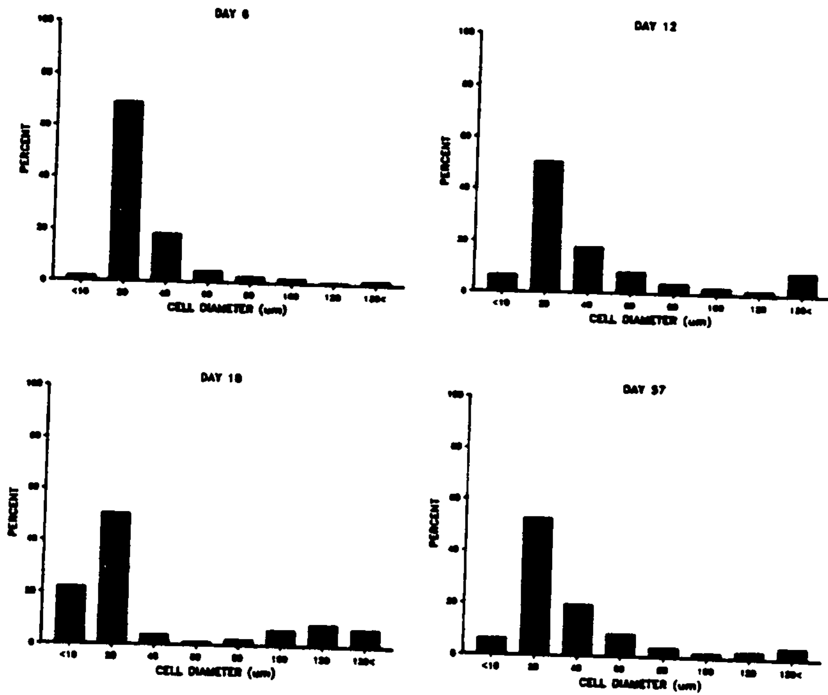

Figure 6. Adipocyte distributions from the abdominal fat depot of HW force-fed birds.

EXPERIMENT III

REGENERATIVE CAPACITY OF AVIAN ADIPOSE TISSUE IN RESPONSE TO PARTIAL LIPECTOMY AND ESTROGEN ADMINISTRATION

INTRODUCTION

The potential for using the domestic chicken as a model for human obesity studies has long been recognized. This is because lipogenesis in the fowl, like man, occurs primarily in the liver (Goodridge, 1968; O'Hea and Leveille, 1968). Nevertheless, avian adipose tissue does differ from most mammalian adipose tissue in that it appears to be principally a lipid reservoir rather than an active lipogenic tissue.

Recently, interest has focused on adipose tissue and its diverse roles as a dynamic body constituent. Lipectomy has been proposed as a means by which the cellularity and regenerative capacity of adipose tissue may be studied without inducing obesity. Faust *et al.* (1976) reported that 40% lipectomy of the epididymal fat depot of young genetically obese Zucker rats did not stimulate tissue regeneration; however, when 48% of the subcutaneous inguinal fat pad was lipectomized (Faust *et al.*, 1977), complete regeneration occurred. Regeneration was found to be diet dependent since rats fed high-fat diets showed complete tissue regeneration, while those fed low-fat diets showed only partial regeneration. Thus, adipose depot site and dietary components can influence tissue regeneration, and determine whether regeneration results from a hypertrophic or hyperplastic episode.

It has been reported that the carcass composition of broiler chickens can be influenced by diverse factors, including environmental temperature, age, sex, type of housing, and diet (Kubena *et al.*, 1972; Deaton *et al.*, 1974). However, little information is available regarding

the regenerative capability of avian adipose tissue after lipectomy, and the effect of lipectomy on whole body composition. With human subjects, the most common recipients of liposuction surgery, dietary modifications are generally necessary to insure permanent post-operative alterations of body fat. Thus, the objective of the study reported here was to determine the effects of estrogen administration and partial lipectomy of the retroperitoneal (abdominal) fat depot in birds fed a diet formulated to promote lipogenesis.

MATERIALS AND METHODS

Adult male chickens from the high weight (HW) and low weight (LW) lines (S_{29} generation), developed through bidirectional selection for juvenile body weight (Dunnington and Siegel, 1985), and commercial broiler (CB) breeder males were chosen at random and placed in individual wire cages. The weight line males and the CB were 60 and 56 weeks of age, respectively. Twenty-four birds from each line were utilized. Daily *ad libitum* feed intake was measured for 14 days after which 12 males from each line were randomly chosen for partial lipectomy; the remaining 12 birds from each line were sham-operated. Six males from each surgery-by-line subclass were also designated for implantation of silastic capsules containing 17-B estradiol, while the remaining six received placebo implants.

For lipectomies, birds were anesthetized with sodium pentabarbitol (25mg/kg), and a transverse incision (5 cm) was made through the mid-ventral abdominal wall exposing the retroperitoneal (abdominal) fat depot. Based on previous necropsies, approximately 10-15% of the abdominal adipose tissue was excised from the ventral surface of the depot, posterior to the gizzard. Identical surgical procedures were utilized for the sham-operated birds with the exception that no tissue was removed. Prior to closure of the abdominal cavity, two, four, or six silastic capsules (2cm x 2.4mm ID) were inserted into the abdominal cavity of the LW, HW, and CB, respectively. Incisions were then sutured and the birds were returned to their individual cages.

Ad libitum food intake was monitored for an 8-week period post-lipectomy. The diet fed to all birds (Table 1) was a high calorie-low protein feed formulated to promote lipogensis. Body weights were recorded at one week intervals. At two week intervals blood samples were collected for the determination of plasma lipids (Zollner and Kirsch, 1962). Blood plasma at the termination of the experiment was also analyzed for total cholesterol (Allain *et al.*, 1974).

Eight weeks post-lipectomy, the experiment was terminated and the birds were sacrificed. The abdominal, right sartorial, and left sartorial fat depots were removed and weighed. Adipose tissue samples from the abdominal fat depot were excised for duplicate determinations of lipid (Folch *et al.*, 1957), and DNA content of isolated adipocytes (Curtis-Prior *et al.*, 1975). The remaining tissue was returned to the abdominal cavity, the gastrointestinal tract cleaned of residual feed and frozen for subsequent body composition analysis. The carcasses were handled as described by Nir *et al.* (1974), except carcass protein was determined by micro-kjeldahl, and carcass lipid was determined as described by Folch *et al.* (1957).

Statistical Analysis. Comparisons within traits were made by analysis of variance using the general linear models procedure (Ray *et al.*, 1982) as a factorial arrangement. Line, surgical treatment, and form of implant were considered to be fixed effects. All percentage data were analyzed after $\sqrt{}$arcsin transformation. Significance imples ($P \leq .05$) unless otherwise indicated.

The statistical model was:

$$Y_{ijkl} = u + L_i + S_j + I_k + (LS)_{ij} + (LI)_{ik} + (SI)_{jk} + (LSI)_{ijk} + e_{ijkl}$$

where $i = 1,2,3$ populations, $j = 1,2$ methods of surgery, and $k = 1,2$ forms of implant, and $l = 1,2...n$ individuals.

RESULTS

Body Weight and Feed Intake. The body weights of each of the three bird-types were significantly different from each other throughout the experiment (Table 2). The CB stock was heaviest, the LW line lightest, with HW birds intermediate and different from both. Estrogen implantation depressed body weight gain by 7 days post-lipectomy, and as a result, the body weight of the placebo-implanted group was greater through the conclusion of the experiment. The birds which had undergone partial lipectomy were lighter from days 21 through 56, after which no differences in body weight were detected.

A significant effect on food intake by bird-type was found throughout the experimental period (data not shown). The CB males had the greatest daily food intake, the HW birds were intermediary, and the LW birds had the lowest average daily intake. Estrogen implantation depressed *ad libitum* feed intake at days 42, 56, and 70 of the experimental period (Figure 1). Lipectomy did not alter feed intake or feed efficiency at any point during the experiment (data not shown).

Abdominal Fat Weight, DNA and Lipid Content, Left and Right Sartorial Fat Weights. Consistent with the relationship seen in body weight, each of the three bird-types differed in total abdominal fat, and total abdominal fat DNA content at the termination of the experiment (Table 3). However, when abdominal fat was analyzed as a percentage of body weight, no differences by bird-type existed. The LW and HW line birds had less adipose tissue in the right and left sartorial fat depots

than the CB stock, both on an absolute basis and as a percentage of body weight. The birds which received estrogen implants had less abdominal fat than their placebo-implanted counterparts. This was also true when abdominal fat was analyzed as a percentage of body weight.

Lipectomized birds had less abdominal fat than sham-operated birds when expressed on an absolute basis and when expressed as a percentage of body weight. Total adipocyte DNA content of the abdominal fat depot was depressed in birds which were partially lipectomized. No differences in the lipid content (%) of the abdominal fat depot was seen by bird-type, form of implant, or method of surgery. The left sartorial fat depot weight was less in lipectomized birds than in sham-operated birds, and this relationship was also apparent when tissue from the left and right sartorial fat depot were analyzed as a percentage of body weight.

Plasma Total Lipid and Cholesterol Content. Differences among genetic stocks in plasma total cholesterol (Table 3) were detected at the conclusion of the experiment. The HW line birds had lower total cholesterol levels than either the LW or CB birds. Form of implant or method of surgery had no effect on plasma total cholesterol.

Differences among bird-type for plasma total lipid content were found at day 14, when the CB birds had the greatest blood lipid level (519.49 ± 15.57 mg/DL); no differences in blood lipid level existed among the LW and HW line birds (450.80 ± 15.83 and 466.02 ± 17.41 mg/DL, respectively). Differences in blood lipid level were not detected at any other period during the experiment (data not shown).

Body Composition. Differences in carcass lipid:protein ratio were detected by bird-type (Table 4). Birds from the LW line had significantly more total carcass protein (%) and less total carcass lipid (%) than either the HW or CB birds. Estrogen decreased abdominal fat, but did not affect whole body composition. Lipectomy decreased total carcass dry matter (%), protein (%), and lipid content (%), while increasing total carcass water content (%). Carcass lipid:protein ratios of the lipectomized and sham-operated birds were not different. No differences in total carcass ash were detected by bird-type, form of implant, or method of surgery.

DISCUSSION

Implantation of capsules containing 17-B estradiol had marked effects on the feed intake and body weight of adult chickens. Cumulative feed intake of the estrogen-treated birds was depressed from day 21, through the conclusion of the experiment (Figure 1). These resulted are in contrast with those of Bird (1946) who reported that estrogen administration increased the feed intake of male Barred Plymouth Rock chickens. However, Gentry and Wade (1976), Wurtman and Baum (1980), and Gavin *et al.* (1984) reported that estrogen depressed the feeding behavior of adult male and female rats. Donohoe and Stevens (1982) speculated that estrogens may depress feed intake by acting directly on the neurons of the ventromedial hypothalmus. The depression in food intake in this experiment resulted in the body weight of the estrogen-treated birds being lower than their placebo-implanted counterparts throughout the post-surgery period.

Estrogen implantation depressed abdominal fat deposition, both on an absolute basis and as a percentage of body weight. These results are in contrast to those of Lorenz (1945b) who reported that estrogen implantation increased abdominal fat deposition when fed to growing Leghorn males. Bird (1946) reported that estrogen reduces the basal metabolic rate allowing for the greater availability of carbohydrates for fat synthesis. Results of research with other species has been somewhat inconclusive. Gavin *et al.* (1984), reported no effects of estrogen on the size or cellularity of the retroperitoneal fat depot of adult rats;

however, they did report an estrogen-induced non-significant trend towards depot weight depression.

No differences in body composition as a result of estrogen supplementation were found in our study. These results differ from those of Gray and Wade (1981) who reported that total carcass lipid is lowered during estrogen administration, but agree with those of Gavin *et al.* (1983) who noted that estrogen administration had no effect on the percent carcass lipid, protein or water content of adult rats. Earlier experimentation with the domestic chicken yielded contrasting results. Thayer *et al.* (1945), reported that synthetic estrogen (Dianisylhexene) increased the market quality of male birds, chiefly through increases in subcutaneous fat deposition. It is possible that estrogen application in our experiment, although at high enough levels to alter feed intake and body weight, was not available at sufficient circulating levels to significantly modify body composition.

The LW birds had a higher percent carcass protein and a lower percent carcass lipid than the HW or CB birds, an apparent result of the differential selection pressure for juvenile body weight. These results are in contrast with those of Pym and Solvyns (1979) which indicated that selection for body weight gain in chickens had no effect on body composition.

Partial lipectomy affected body composition and adipose tissue mass without modifying feed intake or efficiency. These results are consistent with those of Gavin *et al.* (1984) who reported that partial lipectomy of adult rats, fed a high carbohydrate diet, did not influence feed intake.

The latter authors speculated that the lack of a significant effect of lipectomy on food intake may have been the result of the relatively small amount of tissue excised (10-15% of total body lipid). If this premise is correct, it could explain why lipectomy did not affect food intake in our experiment.

Lipectomy depressed body weight gain for six weeks post-surgery, after which no differences in body weight were detected. Similar results were noted by Gavin *et al.* (1984) who reported that partial lipectomy depressed body weight in adult rats when compared to their sham-operated controls. The lack of a difference in body weight at the conclusion of our experiment was the result of an apparent decrease in the rate of body weight gain by the sham-operated controls. This allowed the lipectomized birds to diminish the difference in body weight which had developed.

Partial lipectomy of the retroperitoneal fat depot did not stimulate adipose tissue proliferation in either the depot resected or in the sartorial fat depots. Moreover, although only a few grams of adipose tissue were excised, the retroperitoneal fat depots of the lipectomized birds were significantly smaller than their sham-operated counterparts. Because the total adipocyte DNA content of the retroperitoneal fat depot was also depressed, it can be surmised that partial lipectomy does not stimulate adipocyte regeneration in the adult chicken.

Since partial lipectomy of the retroperitoneal fat depot resulted in the depression of discrete as well as general adipose stores, it appears that the surgical removal of body fat may initiate the

mobilization of energy reserves in an attempt to compensate for alterations of total adipose tissue mass. Results published by Faust *et al.* (1976, 1977) suggest that there is a minimum amount of adipose tissue which must be removed to initiate a compensatory hyperplastic episode. However, the phenomenon appeared to be modulated by depot site, and post-operative caloric intake. The results of this study suggest that a minimal lipectomy of the retroperitoneal fat depot may induce decreases in adipose tissue mass, and depress total carcass lipid without prompting a compensatory hyperplastic response.

A greater percentage of the retroperitoneal fat depot was not removed in an attempt to limit the degree of surgical trauma and to prevent extensive vascular disruption of the resected fat depot. The concomitant weight depression seen in the sartorial fat depots of lipectomized birds indicated that the effects of lipectomy were not due simply to a localized disruption of the retroperitoneal depot vasculature. Furthermore, the partial lipectomy modified several components of whole carcass composition without altering feed intake. Within the dry matter component, lipectomy decreased carcass protein and lipid, while the ash content remained unchanged. It is difficult to explain how the small amount of adipose tissue excised could precipitate differences in carcass protein and lipid. Perhaps lipectomy influences the ability of the adult bird to partition their dietary intake.

Additional experimentation is necessary to understand the varied effects of lipectomy on carcass composition. Although partial lipectomy did not stimulate adipose tissue proliferation in our experiment, it is

possible that increasing the quantity of tissue excised or increasing the caloric intake of the lipectomized birds would induce cellular regeneration.

Nevertheless, current results suggest that partial lipectomy may be an effective means of modifying carcass composition, without resulting in a permanent or long-term depression of body weight. Since man and domestic fowl exhibit many similarities in adipose tissue dynamics, the effects of lipectomy on body composition of both humans and excessively obese broiler breeder stock would seem worthy of investigation.

SUMMARY

The regenerative capability of adipose tissue in response to partial lipectomy and estrogen administration was determined in sexually mature male chickens. Daily *ad libitum* food intake was monitored for 14 days after which approximately 10-15% of the retroperitoneal (abdominal) fat depot was surgically lipectomized from one half of the birds; the remaining birds underwent a sham operation. In addition, half of the birds in each surgery class were implanted with silastic capsules containing 17-B estradiol, and half the birds received a placebo implant. Body weights and *ad libitum* feed intakes were measured for an 8-week period post-lipectomy.

Estrogen implantation depressed the rate of body weight gain, abdominal fat, and food intake when compared to placebo-implanted birds. Lipectomy depressed body weight from 2-7 weeks post-surgery, after which no differences in body weight were detected. Lipectomy also depressed abdominal and sartorial fat deposition, abdominal fat total DNA content, carcass dry matter, carcass protein, and carcass lipid; carcass water content increased after lipectomy. Feed intake of lipectomized birds was not effected.

These results indicate that partial lipectomy of the abdominal fat depot of sexually mature male chickens does not stimulate adipose tissue proliferation. Lipectomy can also modify several components of carcass composition without altering feeding behavior.

Table 1. Composition of the experimental diet

Ingredient	Percent
Ground yellow corn	86.41
Dehulled soybean meal (48.5% protein)	4.82
Defluorinated phosphate	.99
Ground limestone	1.99
Stabilized feed-grade fat	4.99
Sodium chloride	.15
DL-Methionine	.10
Vitamin premix[1]	.50
Mineral mix[2]	.05
Calculated analysis	
Energy, kcal ME[3]/kg	3445
Protein, %	10
Calorie:Protein	344.5

[1]Vitamin premix supplied per kilogram of diet: 4400 IU vitamin A, 1100 IU vitamin D_3, 4.4 IU vitamin E, 7.0 mg menadione sodium bisulfite, 4.4 mg riboflavin, 11.0 mg d-calcium pantothenate, 33 mg niacin, 250 mg choline chloride, 6.6 mg vitamin B_{12}, .6 mg folic acid, and 125 mg ethoxyquin.

[2]Mineral mix supplied per kilogram of diet: 60 mg manganese, 60 mg zinc, 20 mg iron, 2.5 mg copper, 1 mg iodine, and .22 mg cobalt.

[3]Metabolizable energy.

Table 2. Least-squares means of body weight (kg) summarized by bird-type[1], form of implant[2], and surgery-type[3]

	Bird-type					Form of Implant				Surgery-type			
Day	LW	HW	CB	$T_{S.E.}$[4]	Sig[5]	E	P	$T_{S.E.}$	Sig	L	S	$T_{S.E.}$	Sig
1	2.04^{a}	4.06^{b}	6.01^{c}	.08	**	4.03	4.09	.07	NS	4.07	4.06	.07	NS
7	2.09^{a}	4.14^{b}	6.13^{c}	.09	**	4.08	4.16	.08	NS	4.09	4.14	.08	NS
14	2.05^{a}	4.15^{b}	6.13^{c}	.08	**	4.03	4.18	.07	NS	4.09	4.12	.07	NS
Surgery and Implantation													
21	2.01^{a}	4.11^{b}	6.38^{c}	.09	**	4.04	4.29	.08	*	4.04	4.29	.08	*
28	2.04^{a}	4.26^{b}	6.52^{c}	.11	**	4.09	4.45	.09	**	4.14	4.44	.09	*
35	2.02^{a}	4.29^{b}	6.48^{c}	.13	**	4.12	4.45	.10	*	4.12	4.45	.10	*
42	2.05^{a}	4.36^{b}	6.54^{c}	.13	**	4.19	4.54	.10	*	4.19	4.53	.10	*
49	2.09^{a}	4.37^{b}	6.66^{c}	.13	**	4.18	4.57	.10	**	4.23	4.53	.10	*
56	2.12^{a}	4.43^{b}	6.67^{c}	.14	**	4.22	4.59	.11	*	4.25	4.57	.11	*
63	2.12^{a}	4.40^{b}	6.75^{c}	.13	**	4.20	4.65	.11	**	4.29	4.55	.11	NS
70	2.14^{a}	4.44^{b}	6.66^{c}	.14	**	4.14	4.68	.12	**	4.26	4.56	.12	NS

[1]Means within a row within a grouping with different superscripts are significantly different.
[2]E - estrogen, P - placebo
[3]L - lipectomized, S - sham-operated
[4]$T_{S.E.}$ - Standard error of the treatment means.
[5]Significance: NS - not significant, *($P \leq .05$), **($P \leq .01$).

Table 3. Least-squares means of fat depot weights, total DNA content, and plasma total cholesterol summarized by bird-type[1], form of implant[2], and surgery-type[3]

	Bird-type					Form of Implant				Surgery-type			
Fat Depot	LW	HW	CB	$T_{S.E.}$[4]	Sig[5]	E	P	$T_{S.E.}$	Sig	L	S	$T_{S.E.}$	Sig
Abdominal													
grams	38.98^{a}	100.57^{b}	153.50^{c}	16.27	**	71.99	123.37	13.23	**	74.69	120.68	13.23	*
% body wt	1.73^{a}	2.24^{a}	2.17^{a}	.34	NS	1.60	2.49	.28	*	1.47	2.62	.28	**
% lipid	59.73^{a}	61.44^{a}	62.01^{a}	2.34	NS	61.28	60.84	1.92	NS	59.51	62.61	1.92	NS
DNA(ug)x10^{4}	.96^{a}	2.05^{b}	5.58^{c}	.54	**	2.16	2.75	.44	NS	1.74	3.12	.44	*
Right Sartorial													
grams	1.41^{a}	2.05^{a}	5.58^{b}	.57	**	2.53	3.41	.22	NS	2.41	2.63	.22	NS
% body wt	.06^{a}	.05^{a}	.07^{b}	.01	**	.06	.07	.01	NS	.05	.08	.01	**
Left Sartorial													
grams	1.49^{a}	2.33^{a}	5.05^{b}	.59	**	2.46	2.45	.49	NS	2.08	3.83	.49	*
% body wt	.06^{a}	.05^{a}	.07^{b}	.01	**	.06	.07	.01	NS	.05	.08	.01	**
Plasma Total Cholesterol													
(mg/DL)	129.72^{a}	100.92^{b}	130.96^{a}	8.73	*	116.21	123.66	7.15	NS	123.52	116.35	7.11	NS

[1]Means within a row within a grouping with different superscripts are significantly different.
[2]E - estrogen, P - placebo
[3]L - lipectomized, S - sham-operated
[4]$T_{S.E.}$ - Standard error of the treatment means.
[5]Significance: NS - not significant, *($P \leq .05$), **($P \leq .01$).

Table 4. Least-squares means of carcass composition (%) summarized by bird-type[1], form of implant[2], and surgery-type[3]

Carcass Component (%)	Bird-type					Form of Implant				Surgery-type			
	LW	HW	CB	$T_{S.E.}$[4]	Sig[5]	E	P	$T_{S.E.}$	Sig	L	S	$T_{S.E.}$	Sig
Water Content	59.51[a]	59.13[a]	60.92[a]	1.06	NS	60.37	59.33	.87	NS	61.68	58.03	.87	**
Dry Matter	40.49[a]	40.88[a]	39.07[a]	1.05	NS	39.63	40.67	.86	NS	38.32	41.97	.86	**
Ash	6.76[a]	6.12[a]	4.70[a]	.72	NS	5.71	6.01	.59	NS	5.94	5.78	.59	NS
Protein	31.14[a]	27.75[b]	28.21[b]	.76	**	28.36	29.70	.63	NS	27.74	30.32	.63	**
Lipid	4.63[a]	6.75[b]	6.43[b]	.57	*	5.63	6.25	.48	NS	5.24	6.64	.48	**
Lipid:Protein Ratio	.14[a]	.25[b]	.23[b]	.02	**	.19	.22	.02	NS	.19	.22	.02	NS

[1]Means within a row within a grouping with different superscripts are significantly different.
[2]E - estrogen, P - placebo
[3]L - lipectomized, S - sham-operated
[4]$T_{S.E.}$ - Standard error of the treatment means.
[5]Significance: NS - not significant, *($P \leq .05$), **($P \leq .01$).

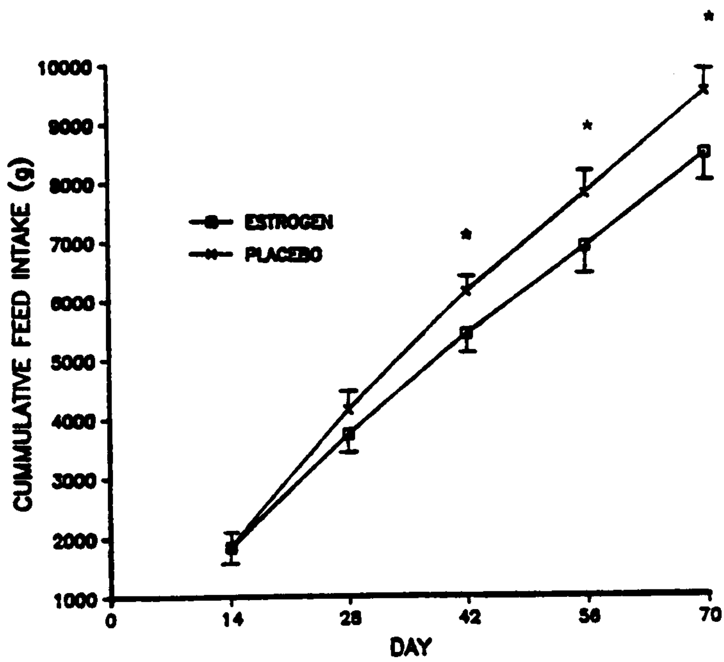

Figure 1. Least-squares means (± standard error) of cumulative feed intake summarized by form of implant (* - P≤.05).

EXPERIMENT IV

THE EFFECT OF CALORIC OVERCONSUMPTION ON FAT DEPOSITION AND WHOLE BODY COMPOSITION OF SEXUALLY MATURE HIGH- AND LOW-WEIGHT CHICKENS

INTRODUCTION

Commercial poultry breeders have long recognized the necessity of feed restriction in adult birds, since excess dietary energy is readily digested (Nir *et al.*, 1974; Washburn *et al.*, 1975a), and a highly significant positive correlation exists between dietary calorie:protein ratio and fat content of the whole carcass (Donnaldson *et al.*, 1956). Pym and Farrell (1977) reported that the energy cost of fatty tissue deposition is much greater than that of lean tissue. Obesity may also have important ramifications on reproductive fitness. Siegel and Dunnington (1985) recently documented some of the negative aspects of obesity on egg production and sexual function in broiler breeders.

Interest in caloric overconsumption and its influence on animal health and welfare have stimulated research into the condition of obesity. Several methods of caloric overconsumption have been utilized (force-feeding, cafeteria-feeding) to demonstrate the problems which may arise when adult animals injest calories in excess of their normal daily requirements. Levin (1944) reported that 67% of the increase in body weight seen in force-fed rats can be attributed to the deposition of carcass fat. Dietary modifications of carcass quality can also be modulated by altering the method by which calories are injested. Cohn *et al.* (1955, 1957) reported that feeding adult rats by stomach tube increased body fat when compared to rats fed *ad libitum*, even though rats from both feeding regimens demonstrated similar body weight gains and feed intakes.

Other factors, including genetic selection, may influence the carcass composition of domestic fowl. Proudman *et al.* (1970) reported that with selection pressure for increased body weight, there is an associated increase in carcass fat. The objective of the study reported here was to determine the effects of caloric overconsumption on carcass composition among adult male and female chickens divergently selected for growth potential, and to determine the effects of forced body weight gain on body weight "set point".

MATERIALS AND METHODS

Adult male and female chickens, 44 weeks of age, from the high weight (HW) and low weight (LW) lines developed through bidirectional selection for juvenile body weight (Dunnington and Siegel, 1985) were chosen at random and placed in individual wire cages. Twenty-six male and twenty-six female birds of each line from the S_{29} generation were utilized. Thirteen birds of each sex were fed *ad libitum* (AL) and the remaining thirteen were force-fed (FF). The force-fed chickens received a blend of 50% feed and 50% water. To force-feed, a polyethylene tube (25 cm) attached to a pneumatic gun was directed via the esophagus to the level of the crop, and a quantity of the blend approximating crop capacity was administered twice daily. A simple corn-soy ration containing 3445 Kcal ME per kg, and 10% protein was fed to all birds. The diet was formulated to contain a high level of carbohydrates to stimulate lipogenesis (Table 1).

After 14 days of force-feeding, half of the birds in each line-by-sex-by-feeding regimen subclass were sacrificed. Immediately after sacrifice the birds were laparotomized, the liver and retroperitioneal fat depot were excised, weighed, and duplicate samples (2-4 grams) of each of these tissues were processed for lipid content determinations (Folch *et al.*, 1957). The remaining tissue was returned to the carcass and frozen for subsequent body composition analyses. The carcasses were handled as described by Nir *et al.* (1974), with the exceptions that carcass protein was determined by micro-kjeldahl, carcass

lipid was determined by Folch *et al.* (1957), and residual feed was removed from the gastrointestinal tract prior to analysis.

The remaining birds which had been force-fed, were placed on *ad libitum* feeding until their body weights stabilized. Body weight was recorded at six-day intervals until the termination of the experiment. At this latter point (day 66), the birds were sacrificed and subjected to the measurements previously described.

On days one, 14, and at the termination of the experiment, all birds were fasted for a period of 14 hours, and then bled. Venous blood was collected from the brachial vein for plasma lipid (Zollner and Kirsh, 1962) and cholesterol (Allain *et al.*, 1974) determinations.

Statistical Analysis. Comparisons within traits were made by the analysis of variance using the general linear models procedure (Ray *et al.*, 1982) as a 2 x 2 x 2 factorial arrangement. All percentage data were analyzed after $\sqrt{}$arcsin transformation. Line, treatment, and sex were considered as fixed effects. The statistical model was:

$$Y_{ijkl} = u + L_i + T_j + S_k + (LT)_{ij} + (LS)_{ik} + (TS)_{jk} + (LTS)_{ijk} + e_{ijkl}$$

where i = 1,2 lines, j = 1,2 feeding regimens, k = 1,2 sexes, and l = 1,2...n individuals.

RESULTS AND DISCUSSION

Body Weight. A highly significant sex-by-feeding regimen interaction for body weight was detected at each measuring period throughout the experiment (Figure 1). The body weights of the force-fed male and female chickens demonstrated similar patterns of change; however, this relationship was not apparent between *ad libitum*-fed birds from the two sexes. When male chickens were placed on *ad libitum* feeding, they demonstrated an immediate marked increase in body weight which continued through the duration of the experiment. Although the final body weights of the *ad libitum*-fed female chickens was slightly greater than their starting weight, they did not demonstrate the rapid sustained increase which was apparent in male birds from the same feeding regimen. Force-fed male and female birds showed sharp increases in body weight. However, in agreement with Lepkovsky and Furuta (1971), these birds reduced their body weight to near original levels when released.

Abdominal Fat, Liver, Body Composition, and Blood Plasma Measurements. Significant main effects are listed in Table 2. Numerous first-order interactions were demonstrated because of the wide divergence in response shown between line, sex, and feeding regimen. In order to present the data in a clear manner, and avoid a laborious description of each interaction, the main effects will be presented in Table 2, first-order interactions in Tables 3-6, and their overall significance discussed.

Force-feeding increased abdominal fat deposition both on an absolute basis, and as a percentage of body weight (Table 2). These effects were transient, however, and were not apparent at the termination of the experiment after the birds had been released to *ad libitum* feeding (day 66). These results are in agreement with those of Nir *et al*. (1977) who reported that force-feeding significantly increased abdominal fat deposition in the Japanese Quail.

The main effect interactions for abdominal fat (Table 3), suggest that selection pressure for 8-week body weight may not be exerting a uniform influence on abdominal fat deposition across sex. Female chickens showed line differences in abdominal fat both on an absolute basis and as a percentage of body weight; male chickens did not. These results are in contrast to those of Hood and Pym (1982) who reported no line differences by sex in abdominal fat, either on an absolute or a percentage of body weight basis, between a line selected for increased body weight gain and a random-bred control. Gyles *et al* (1982) reported that spent sires carry little abdominal fat, but, as dams age, the percentage of abdominal fat increases.

The main effect interactions for the various liver measurements at day 14 (Table 4), were primarily the result of sex differences across line and feeding regimen. The liver of female chickens was more responsive to force-feeding than its male counterpart. This relationship was not true for *ad libitum*-fed birds, and may be reflective of the body weight relationship demonstrated in Figure 1. However, these results agree with those of Leclerq *et al*. (1974) who concluded that overfeeding chickens

could saturate VLDL synthesis and transport, and thus lead to liver triglyceride synthesis. After release from force-feeding, the differences in liver weight by sex and feeding regimen were no longer apparent. At day 66, HW birds had larger livers than LW birds, with no differences in the percent lipid content of this tissue.

Force-feeding increased carcass day matter at day 14 (Table 2). The main effect interaction for carcass dry matter (Table 5) was the result of an inverse relationship between male and female birds across weight-line.

At day 14 and 66, male birds had more carcass protein (%) than female birds; HW birds had more carcass protein (%) than LW birds (Table 2). In agreement with Cohn and Joseph (1962) force-feeding increased carcass lipid, with differences no longer apparent after release to *ad libitum* feeding. A line-by-sex interaction for percent carcass lipid was also detected. The HW female birds had a higher percent carcass lipid than LW female birds, no differences by line in the male population were found. At day 66, female birds had more carcass lipid than male birds.

No sex differences in plasma total cholesterol were detected at day 14. However, LW birds had more plasma cholesterol than HW birds, and force-fed birds had higher levels than *ad libitum*-fed birds. By day 66, no differences in total cholesterol were detected by sex, weight-line, or feeding regimen.

Numerous first-order interactions were detected for plasma total lipid content during the experiment, the result of differences by sex,

and weight line (Table 6). Generally, force-feeding increased plasma lipid levels in females and in the LW population.

The first-order interactions which were demonstrated in this experiment were somewhat expected, since the weight-line birds display wide divergence in body weight and *ad libitum* feed intake. These results also indicate that although male birds from both lines have a greater propensity for self-induced overconsumption, the direct effects on abdominal fat, liver, and the various components of body composition are less dramatic than those seen in female birds across weight line. However, changes in abdominal fat mass, liver, and whole body composition induced by forced caloric overconsumption were readily reversible. It would have been interesting to have imposed a second period of force-feeding to investigate the "yo-yo" dietary syndrome which is seen in human populations. When adult men and women are subjected to several periods of weight gain and loss, they become very food efficient. The second time they are forced to lose weight, their rate of weight loss is decreased even when maintained on the same caloric intake. Repeated fluctuations in body weight may decrease or eliminate the ability of adult birds to return their body weight to original levels, resulting in permanent modifications of whole body adiposity.

SUMMARY

An experiment was conducted to examine the effects of forced body weight fluctuations on the body composition of adult male and female high- and low-weight chickens. Birds were either force-fed or allowed to consume feed *ad libitum*.

Force-feeding resulted in transient increases in body weight, abdominal fat deposition, and liver weight, with differences no longer apparent after release to a period of *ad libitum* feeding. Force-feeding also modified body composition, increasing carcass dry matter and lipid content (%). At the termination of the experiment, most of the effects of force-feeding were no longer apparent; however, differences by sex and weight line persisted.

Numerous first order interactions were detected during the experiment, the result of wide differences in response by sex and weight line. Generally, adult female chickens were more responsive to force feeding than male chickens; however, the effects were not always consistent across weight line. Evidently, body weight fluctuations can have varied effects dependent upon sex and original body weight status.

Table 1. Composition of the experimental diet

Ingredient	Percent
Ground yellow corn	86.41
Dehulled soybean meal (48.5% protein)	4.82
Defluorinated phosphate	.99
Ground limestone	1.99
Stabilized feed-grade fat	4.99
Sodium chloride	.15
DL-Methionine	.10
Vitamin premix[1]	.50
Mineral mix[2]	.05
<u>Calculated analysis</u>	
Energy, kcal ME[3]/kg	3445
Protein, %	10
Calorie:Protein	344.5

[1]Vitamin premix supplied per kilogram of diet: 4400 IU vitamin A, 1100 IU vitamin D_3, 4.4 IU vitamin E, 7.0 mg menadione sodium bisulfite, 4.4 mg riboflavin, 11.0 mg d-calcium pantothenate, 33 mg niacin, 250 mg choline chloride, 6.6 mg vitamin B_{12}, .6 mg folic acid, and 125 mg ethoxyquin.

[2]Mineral mix supplied per kilogram of diet: 60 mg manganese, 60 mg zinc, 20 mg iron, 2.5 mg copper, 1 mg iodine, and .22 mg cobalt.

[3]Metabolizable energy.

Table 2. Least-squares means of abdominal fat, liver measurements, carcass composition, and blood parameters summarized by bird-sex, weight-line, and feeding regime

	Day	I[1]	Female	Male	$T_{S.E.}$	Sig[2]	LW	HW	$T_{S.E.}$	Sig	AL	FF	$T_{S.E.}$	Sig
Abdominal Fat														
grams	14	2									82.26	130.56	9.27	***
	66	2									111.30	103.43	10.32	NS
% body wt	14	2									3.05	4.48	.28	***
	66		6.25	2.07	.30	***	3.74	4.58	.30	NS	4.37	3.95	.30	NS
% lipid	14	1	89.66	88.61	.28	NS								
	66		89.71	86.59	.91	*	86.86	89.42	.91	NS	88.36	87.93	.91	NS
Liver														
grams	14	2,3												
	66		33.41	33.49	1.39	NS	26.89	39.90	1.39	***	33.83	32.96	1.39	NS
% body wt	14	2,3												
	66	2,3												
% lipid	14	3					9.49	10.83	1.04	NS				
	66		8.54	5.97	.06	***	7.62	6.89	.06	NS	7.39	7.12	.06	NS
Carcass Composition														
Dry Matter	14	2									42.66	47.38	.67	***
	66	1	50.19	42.81	.80									
Protein	14		25.57	28.84	.73	**	29.71	24.71	.73	***	27.08	27.33	.73	NS
	66		27.92	30.97	.65	**	30.44	28.46	.65	*	28.69	30.20	.65	NS
Lipid	14	2									10.75	15.18	.85	***
	66		16.43	7.06	.87	***	9.37	14.12	.87	***	12.02	11.47	.87	NS
Ash	14		2.76	3.11	.21	NS	3.06	2.81	.21	NS	3.21	2.66	.21	NS
	66		3.06	3.24	.29	NS	3.06	3.24	.29	NS	3.04	3.25	.29	NS
Blood Measurements														
Plasma lipid (ug/DL)														
	1	2									649.84	640.81	54.80	NS
	14	1,2,3												
	66	1,2												
Plasma total cholesterol (ug/DL)														
	1	2,3												
	14		184.23	192.74	8.64	NS	203.93	173.04	8.64	**	175.45	201.52	8.64	*
	66	1	140.33	102.72	9.98	NS	132.53	110.52	9.91	NS	111.77	131.28	10.01	NS

[1]First order interactions: 1-Line-by-treatment, 2-Line-by-sex, 3-Treatment-by-sex.
[2]Significance: * (P≤.05), ** (P≤.01), *** (P≤.001).

Table 3. Least-squares means of main effect interactions of abdominal fat measurements (Day 1 and 66)[1]

	Day	LW		HW	$T_{S.E.}$	AL		FF	$T_{S.E.}$
Abdominal fat (g)	14								
Females		74.98	← *** →	234.29					
		↑ * ↓		↑ *** ↓					
Males		41.01	← NS →	75.37	13.17				
Abdominal fat (g)	66								
Females		82.35	← *** →	202.12					
		↑ ** ↓		↑ ** ↓					
Males		44.76	← ** →	100.24	14.96				
Abdominal fat (% bwt)	14								
Females		4.77	← * →	6.79					
		↑ *** ↓		↑ *** ↓					
Males		1.79	← NS →	1.71	.40				
Abdominal fat (% lipid)	14								
Low-weight						83.31	← ** →	90.07	
						↑ NS ↓		↑ NS ↓	
High-weight						93.31	← NS →	90.02	1.78

[1]* - ($P \le .05$), ** - ($P \le .01$), *** - ($P \le .001$).

Table 4. Least-squares means of main effect interactions of liver measurements (Day 1 and 66)[1]

	Day	LW		HW	$T_{S.E.}$	AL		FF	$T_{S.E.}$
Liver weight (g)	14								
Females		42.41	← * →	65.88		34.64	← *** →	73.25	
		↑ NS ↓		↑ NS ↓		↑ * ↓		↑ NS ↓	
Males		36.51	← *** →	75.38	3.42	48.29	← NS →	63.60	3.46
Liver weight (% bwt)	14								
Females		2.85	← * →	1.89		1.79	← *** →	2.95	
		↑ *** ↓		↑ NS ↓		↑ NS ↓		↑ *** ↓	
Males		1.66	← NS →	1.76	.13	1.55	← ** →	1.86	1.33
Liver weight (% bwt)	66								
Females		1.94	← *** →	1.27		1.75	← NS →	1.46	
		↑ *** ↓		↑ * ↓		↑ *** ↓		↑ * ↓	
Males		1.12	← NS →	1.02	.08	1.01	← NS →	1.13	.08
Liver weight (% lipid)	14								
Females						6.20	← *** →	21.15	
						↑ ** ↓		↑ *** ↓	
Males						5.09	← *** →	8.19	1.49

[1]* - ($P \leq .05$), ** - ($P \leq .01$), *** - ($P \leq .001$).

Table 5. Least-squares means of main effect interactions of carcass compositions (Day 1 and 66)[1]

	Day	LW		HW	$T_{S.E.}$	AL		FF	$T_{S.E.}$
<u>Carcass dry matter</u>	14								
Females		48.75	← NS →	52.28					
		↕ ***		↕ ***					
Males		40.18	← NS →	38.86	.95				
<u>Carcass lipid (%)</u>	14								
Females		13.35	← *** →	25.75					
		↕ **		↕ **					
Males		5.49	← NS →	7.47	1.20				
<u>Carcass dry matter (%)</u>	66								
Low-weight						43.95	← NS →	46.72	
						↕ NS		↕ NS	
High-weight						48.89	← NS →	46.45	1.08

[1]* - ($P \leq .05$), ** - ($P \leq .01$), *** - ($P \leq .001$).

Table 6. Least-squares means of main effect interactions for plasma total lipid, and cholestrol content (Day 1, 14, and 66)[1]

Day	LW		HW	TS.E.	AL		FF	TS.E.
Lipid (mg/DL): 1								
Females	1032.77	← ** →	604.34					
	↕ ***		↕ NS					
Males	462.29	← NS →	481.91	76.69				
Lipid (mg/DL): 14								
Females	1073.24	← ** →	620.63		599.93	← * →	1094.04	
	↕ ***		↕ NS		↕ NS		↕ ***	
Males	381.39	← * →	514.86	69.04	457.68	← NS →	438.58	68.94
LW					511.66	← * →	943.07	
					↕ NS		↕ NS	
HW					545.94	← NS →	589.55	69.15
Lipid (mg/DL): 66								
Females	1826.96	← ** →	1033.09					
	↕ ***		↕ NS					
Males	527.21	← NS →	683.76	119.47				
LW					1068.87	← * →	1285.29	
					↕ NS		↕ NS	
HW					1028.80	← NS →	688.05	119.64
Cholesterol (mg/DL)								
Females	151.34	← ** →	113.32		145.59	← NS →	119.06	
	↕ ***		↕ NS		↕ **		↕ NS	
Males	101.27	← NS →	113.27	8.02	103.75	← NS →	110.79	8.01

[1]* - (P ≤ .05), ** - (P ≤ .01), *** - (P ≤ .001).

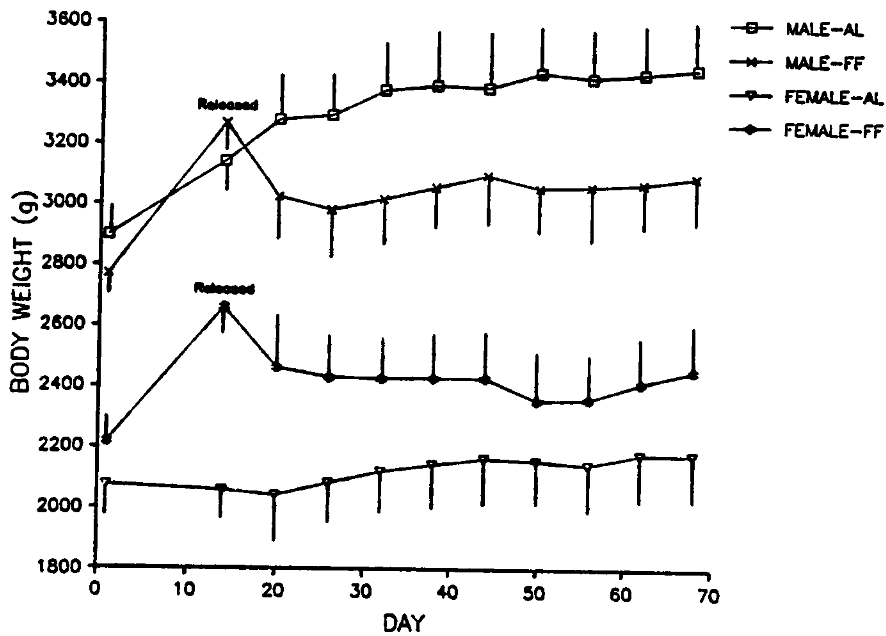

Figure 1. Least-squares means of (± standard error) body weight summarized by each sex-by-feeding regimen subclass.

SUMMARY AND CONCLUSIONS

Unregulated body weight and adiposity can have detrimental effects on animal management (Siegel and Dunnington, 1985). In recent years, the genetic potential of domestic poultry has improved, and in concert with better feeding practices, has increased the likelihood of unwanted obesity. Tight regulation of dietary intake has been the chief tool of animal caretakers to effectively control body weight, particularly with meat-type breeders.

Previously it was hypothesized that adipocyte hypercellularity in adult animals, is the sole result of pre-puberal adipocyte hyperplasia (Anderson and Kauffman, 1973; Pfaff and Austic, 1976). However, recent reports with numerous species have questioned this concept (Lemmonier, 1972; Therriault and Mellin, 1981; Oruwari *et al.*, 1986). The experiments reported in the dissertation examined the effects of caloric overconsumption (forced, voluntary) on adipocyte dynamics, and tested the regenerative capacity of adult avian adipose tissue.

The results of Experiment I indicated that adipocyte hyperplasia plays a role in adipose depot enlargement in adult chickens. This phenomenon did not occur when dietary intake was restricted. Whether hyperplasia was initiated by elevated blood lipid levels (Greenwood and Hirsch, 1974) or a critical adipocyte size limitation (Faust *et al.*, 1978), remains equivocal.

Although previous reports with the domestic chicken have indicated that adipocyte hyperplasia ceases at some point prior to sexual

maturation, Oruwari *et al.* (1986) reported that adult Japanese quail could initate periods of adipocyte multiplication. Hypercellularity in adult animals usually leads to adipocyte expansion and obesity.

In agreement with Lepkovsky and Furuta (1971), the effects of force feeding in our experiments were transient and could be reversed after a period of *ad libitum* feeding. Adipocyte distribution plots in Experiment II indicated that the small adipocyte population increased during depot expansion, and, in agreement with March *et al.* (1986) disappeared when lipid was mobilized.

Recently, growing public awareness of health and fitness has increased the popularity of partial lipectomy as a tool for fat reduction. However, the possibility that adipocyte regeneration may occur (Faust *et al.*, 1978) has raised questions as to the permanency of this treatment. Questions also persist as to the regenerative potential of adipose tissue based upon depot location. Results from Experiment III indicate that mimimal (10-15%) lipectomy can modify adipose tissue mass without inducing a compensatory response. However, it is possible that a minimal amount of tissue must be removed to stimulate depot compensation. Additional experimentation is necessary before the long-term effectiveness of this procedure is known.

Results from Experiment I on the role of adipocyte hyperplasia in adult chickens conflict with previous studies. There are numerous reasons for this outcome. Typically, the coulter counter has been utilized (Hirsch and Gallian, 1968) which precludes the identification of adipocytes with diameters less than 20-25 um. This procedure can result

in an inability to measure a percentage of the total adipocyte population. Secondly, in rodent studies, the epidydimal fat depot is normally utilized, which has been shown to have limited proliferative capability in comparison to other depot sites (Ashwell *et al.*, 1975). Lastly, small adipose samples are utilized to estimate whole body adiposity, and thus can result in the underestimation of whole body fat mass.

Further experimentation is necessary to elucidate the various factors which may stimulate adipocyte multiplication in adult birds. Numverous factors, including environmental, nutritional, and genetic may be interrelated in the timing and magnitude of the response.

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APPENDIX I

BLOOD ASSAY PROCEDURES

COLORIMETRIC DETERMINATION OF PLASMA GLUCOSE

Reference: (Raabo and Terkildsen, 1960)

Reagents:

1) PGO enzymes (Sigma). 1 capsule in 100 ml distilled water (gentle shaking)

2) Dianisidine dihydrochloride color reagent (Sigma). Reconstitute vial with 20 ml distilled water

3) Combined enzyme-color reagent solution. To 100 ml PGO enzymes, add 1.6 ml of color reagent (dianisidine dihydrochloride)

4) Glucose standard solution (Sigma).

Procedure:

1) Label tubes as follows: BLANKS, STANDARDS, SAMPLE 1, etc.

2) To BLANK add .02 ml distilled water

To STANDARD add .02 ml standard

To each SAMPLE add .02 ml unknown sample

To BLANK, STANDARD and each SAMPLE, add an additional .5 ml distilled water.

3) To each tube add 5.0 ml combined enzyme-color reagent solution and mix each tube thoroughly.

4) Incubate all tubes at room temperature for 45 minutes. NOTE: Avoid exposure to bright lights during incubation.

5) Read absorbance (Abs) at 450 nm, using BLANK as reference set at zero.

6) Calculate SAMPLE concentration as follows: Plasma glucose (mg/100 ml) = (Abs sample/Abs standard) x 100

COLORIMETRIC DETERMINATION OF TOTAL LIPIDS IN BLOOD SERUM
Reference: (Zollner and Kirsh, 1962)

Reagents:

1) Total lipid reagent (phospho-vanillin)

2) Concentrated sulfuric acid

3) Total lipid standard

Procedure:

1) Pipet .05 ml water into a 10-15 ml glass test tube.

2) Pipet .05 ml serum or standard into similar test tubes (duplicate).

3) To each tube add 3 ml concentrated sulfuric acid. Vortex repeatedly.

4) Place all tubes into a boiling waterbath for 10 minutes.

5) Remove and cool to room temperature.

6) Pipet .2 ml aliquots of sulfuric acid digests into new tubes.

7) Add 3 ml total lipid reagent into each tube. Vortex repeatedly.

8) Place tubes in the dark at room temperature for 45 minutes.

9) Transfer to cuvettes and tap to remove bubbles.

10) Read absorbance (Abs) at 520 nm against blank set for zero absorbance.

Calibration curve:

Tube	750 mg/dl std (ml)	Absolute ethanol (ml)	Value mg/dl
1	1	2	250
2	2	1	500

Results: (Abs unknown/Abs std) x value std. = mg/dl total unknown

LOWRY PROTEIN DETERMINATION
Reference: (Lowry et al., 1951)

Reagents:

1) Bovine serum albumin

2) Sodium hydroxide, in pellet form, A.R.

3) Sodium carbonate, Na_2CO_3. A.R.

4) Sodium potassium tartrate, $NaKC_4H_4O_6$-$4H_2O$, A.R.

5) Copper sulphate, $CuSO_4$-$5H_2O$, A.R.

6) Folin-Ciocalteu reagent (Mereck)

7) Trichloracetic acid, CCL_3COOH, A.R.

Preparation of Solutions:

1) Albumin solution: Dissolve 25 mg bovine serum albumin in doubly distilled water and make up to 25 ml with water in a graduated flask.

2) Sodium hydroxide solution (0.1 N): Dissolve 4 grams NaOH pellets in water and make up to 1000 ml.

3) Sodium carbonate/Na,K tartrate solution: Dissolve 2 grams anhydrous Na_2CO_3 and 20 mg. $NaKC_4H_4O_6$-$4H_2O$ in NaOH (2) and make up to 100 ml.

4) Copper sulphate solution: Dissolve 50 mg $CuSO_4$-$5H_2O$ in water and make up to 10 ml.

5) Mixture A: Add 1 ml solution (4) to 50 ml solution (3) with stirring.

6) Mixture B: Mix 5 parts of Folin-Ciocalteu reagent (Merck) with 9 parts water.

7) Trichloroacetic acid (3 M): Dissolve 49 grams trichloracetic acid in water and make up to 100 ml.

Preparation of Sample:

Use aqueous solution containing 50-200 ug. protein/ml (sample solution) without dilution. Where the protein concentration is higher, use a correspondingly lower volume. a) Protein solutions in 50% glycerol or in ammonium sulfate solution: Mix a portion corresponding to 50-200

ug. protein with 1 ml 3 M trichloroacetic acid (7) (heat in a hot water bath if necessary). Centrifuge for 10 min. at about 3000 rpm., pour off supernatant, and dissolve precipitate in 1 ml. 0.01 N sodium hydroxide solution (dilute solution (2) 1+9 with water)(=sample solution).

b) Protein not precipitated by trichloroacetic acid, in ammonium sulphate solution: For the determination use 0.1 ml. of the protein solution containing 0.5-2 mg. protein/ml. Adjust volume to 1.0 ml. with water. Add ammonium sulphate to the standards in a quantity equal to that present in 0.1 ml. of the sample solution.

ASSAY SYSTEM:

Wavelength: Hg 578 nm; light path: 1 cm; final volume: 6.5 ml; room temperature.

Pipette into test tubes	Blank (ml)	Standard (ml)				Sample (ml)
Water	1.0	0.975	0.95	0.90	0.85	----
Serum albumin (1)	---	0.025	0.05	0.10	0.15	----
Sample	---	-----	----	----	----	1.00
Mixture A (5)	5.0	5.00	5.00	5.00	5.00	5.00
Mix and incubate for exactly 10 min. at room temperature.						
Mixture B (6)	0.5	0.50	0.50	0.50	0.50	0.50

Mix and allow to stand for 30 min. at room temperature; then measure extinctions of the sample and of the standard against that of the blank.

Calculations:

Plot the extinctions obtained for the standards against the amount in ug. of protein used and draw the calibration curve. Read off the protein value for the sample from the calibration curve.

COLORIMETRIC DETERMINATION OF PLASMA TOTAL CHOLESTEROL
(Allain et al., 1974)

MICRO METHOD (1 ml Reaction Volume)

Reagents

1) Cholesterol aqueous standard, concentration 200 (Sigma)

2) Cholesterol reagent (Sigma)
   Reconstitute with distilled water to form cholesterol assay solution.

Procedure Outline

1) To cuvet labeled BLANK, add .01 ml distilled water.
   To cuvet labeled STANDARD, add .01 ml cholesterol aqueous standard.
   To cuvet labeled TEST 1, TEST 2, etc., add .01 ml plasma which has been gently vortexed. (Run all blanks, standards and tests in duplicate.)
2) To each cuvet, add 1.0 ml cholesterol assay solution. Cover cuvets with Parafilm and invert several times to mix.
3) Incubate cuvets at 37 degrees Celcius for ten minutes.
4) Read and record absorbance of STANDARD and TEST vs. BLANK as reference at 500 nm. Complete test readings within 30 minutes.

Results

Calculate plasma total cholesterol as follows:

(Abs. TEST/ Abs. STANDARD) x 200 = Plasma total cholesterol (mg/dl)

APPENDIX II

TISSUE ASSAY PROCEDURES

ISOLATION OF A HOMOGENEOUS PREPARATION OF FAT CELLS

Reference: Martin Rodbell, 1964.

Materials and Reagents:

1) Bovine serum albumin

2) Collagenase (prepared from C. histolyticum)

3) Plastic or siliconize glass incubation vessels

4) Metabolic shaker

5) Krebs ringer bicarbonate buffer (1/2 the prescribed CaC13)

6) Centrifuge and appropriate tubes

Procedure:

1) Immediately after sacrifice, remove approximately 3 grams of fresh adipose tissue from the ventral surface of the retroperitoneal fat depot, just below the gizzard, and place in cold physiological saline. (see preparation instructions at the end of the assay).

2) Next, place up to 1 gram of tissue in the incubation vials containing 3 ml of albumin-bicarbonate buffer, and 10 mg of collagenase.

3) Incube the tissue for approximately 1-2 hours, after which time any remaining fragments of undigested tissue should be removed with forceps.

4) Centrifuge the adipocyte cell suspensions for 1 minute at 400 x g, and remove the stromal-vascular cells (sedimented), by aspiration.

5) Resuspend the adipocytes in 10 ml of warm (37 C) albumin buffer without collagenase.

Note: Repeat steps 4-5 a minimum of three times to insure adequate removal of contaminating stromal-vascular cells.

Transfer of isolated cell suspensions:

1) Swirl the cell suspension to ensure delivery of uniform suspensions of cells and immediately draw up desired vol. into a piece of 18 cm plastic tubing attached to a 2-ml calibrated syringe (lubricate to insure a tight fit).

2) Dispense desired volume into assay tube and proceed with selected assay.

Preparation of physiological saline: (Chicken - .87%)

1) To one liter of distilled water add 8.7 grams Nacl. Preparation of albumin-bicarbonate buffer:

Ca++ free Krebs Ringer

Salt	g/500 ml water (5x conc.)
I NaCl	22.50
II KCl	28.71
III KH_2PO_4	52.39
IV $MgSO_4$-$7H_2O$	94.89
V $NaHCo_3$	32.34

A COLORIMETRIC METHOD FOR THE DETERMINATION OF DEOXYRIBONUCLEIC ACID IN ADIPOSE TISSUE

References: Curtis-Prior et al. (1975)

Reagents:

1) De-ionized, glass-distilled water

2) Diethyl ether-ethanol (3+1)

3) Ethanol-diethyl ether (3+1)

4) 10% Trichloroacetic acid (10 g TCA dissolved in 100 ml of the diethyl ether-ethanol solutions)

5) 5% Trichloroacetic acid (aqueous extraction mixture) (dissolve 5 g TCA in 100 ml of water)

6) p-Nitrophenylhydrazine color reagent (20 mg in 2 ml ethanol with gently warming)

7) N-Butyl acetate

8) DNA standard solutions (Prepare stock DNA solution by adding 50 ml of a 5% m/V aqueous solution of TCA to 25 mg of salmon-sperm DNA in a calibrated flask fitted with an air condenser. Heat on a boiling water bath for 60 min. Allow to cool to room temperature and add water to replace the small amount lost by evaporation. Mix well and store at 4 C.

9) Prepare desired standard solution concentrations.

Procedure:

1) Dispense 1-ml portions of a suspension of isolated fat cells (lipid content 20 to 80 mg) into polypropylene test tubes, add 4 ml of cold ethanol-diethyl ether (3+1) containing 10% m/V TCA. Homogenize for 1 min, and transfer to a 15-ml polypropylene centrifuge tube using a further 6 ml of the TCA solution to wash the Polytron.

2) Pool the first homogenate and the TCA rinsings, and centrifuge for 10 min at 4000 *g* in chilled centrifuge buckets and discard the supernatant fluid. Next Re-suspend the precipitate in 5 ml of cold ethanol-diethyl ether (3+1) without TCA. Mix well, centrifuge as before and discard the supernatant fluid.

3) Add 3 ml of the 5% m/V aqueous solution of TCA, and several anti-bumping granules to the protein pellet. Fragment the pellet with a

glass rod, and incubate at 90 C in a metabolic shaker for 20 min (disperse the protein frequently through agitation with the glass stirring rod).

4) Allow the samples to cool, then centrifuge for 20 min and retain the maximum possible volume of supernatant.

5) Transfer 2 ml of the supernatant,or 2 ml of the DNA standard solution, or 2 ml of aqueous 5% m/V TCA as as a blank into glass test tubes.

6) Add 40 ul of freshly prepared color reagent to each sample and mix. Place the samples in a boiling water bath for 20 min and allow to reflux.

7) After the samples have cooled to room temperature, transfer the whole of each sample to a 15-ml centrifuge tube and add 7-ml of n-butyl acetate. Sake the samples vigorously for 2 min. Centrifuge briefly to separate the phases, and aspirate and discard the upper phase. (repeat twice more).

8) Pipette 1 ml of the washed lower phase into a 1 ml glass centrifuge tube and aspirate any visible n-butyl acetate, plus the uppermost layer of the aqueous solution.

9) Transfer 500 ul of the remaining solution into a glass microcuvette of 2-cm light path, and add 200 ul of fresh aqueous 4 N sodium hydroxide solution. Stopper the cuvette and mix by repeated inversion.

10) Read the absorbance of the solution at 560 nm, exactly 3 min after the addition of the alkali.

Calibration equation:

- DNA conc. in ug/ml (x)

- Absorbance at 560 nm (y)

- constant (0.02533)

$y = 0.02533\,(x)$

CARCASS EVALUATION

Procedures:

1) Weigh carcass.

2) Place carcass in large can. Autoclave 3 hours at 121 degrees Celcius.

3) Homogenize. If large, use meat grinder, and blender.

4) Determine:

DM - dry matter, reciprocal - moisture ASH - muffle furnace FAT - gravimetrically - chlor-meth PROTEIN micro-kjedhal.

PROCEDURE FOR THE DETERMINATION OF DRY MATTER IN TISSUE (A.O.A.C., 1975)

Procedure Outline

1) Label dry crucibles and weigh to four decimal places.

2) Place 2-4 grams of sample in crucible. Reweigh on Mettler balance, again to four decimal places.

3) Place samples in oven overnight at 105 degrees Celcius.

4) Remove from oven with tongs and cool in desiccator.

5) Reweigh crucibles with sample and record new weight.

6) Proceed to ash determination.

Calculations:

wt. of crucible with wet sample - wt. of crucible = weight of wet sample.

wt. of crucible with dry sample - wt. of crucible = weight of dry sample.

(wt. of dry sample/wt. of wet sample) x 100 = % Dry matter.

PROCEDURE FOR THE DETERMINATION OF ASH IN TISSUE (A.O.A.C., 1975)

Procedure Outline:

1) Proceed with same crucibles and samples used to determine dry matter.

2) Use same dry weight at end of dry matter determination for beginning weight of sample.

3) Place samples in cool muffle furnace.

4) Turn furnace on and allow to heat to 600 degrees Celcius.

5) Ash for approximately six hours.

6) Remove samples from furnace and place in desiccator. Allow to cool and weigh on Mettler balance to four decimal places).

Calculations:

wt. of crucible with sample - wt. of crucible = weight of sample.

wt. of crucible with ash - wt. of crucible = weight of ash

(wt. of ash/wt. of sample) x 100 = % Ash.

CHLOROFORM-METHANOL PROCEDURE FOR THE
EXTRACTION OF LIPID FROM TISSUE
References: (Folch et al., 1957)

1) 2:1 solution of chloroform:methanol

Procedure:

1) Label and tare large glass tube. Next add 4-5 grams of tissue reweigh and record weight.

2) Add chloroform-methanol solvent at a ratio of 10:1 solvent:sample on a volume-to-wet weight-of tissue basis.

3) Homogenize tissue for 15 seconds.

4) Cap tube and allow to set at room temperature for 24 hours.

5) Filter solution into labeled preweighed beakers rinsing tube and filter paper with a wash bottle filled with chloroform.

6) Allow samples to dry and set aside filter paper with sediment for protein determination. Weigh beaker plus extract.

Calculations:

(Weight of beaker + extract) - beaker weight = weight of extract

(wt. of extract/wt. of sample) x 100 = % Fat (on wet weight basis)

APPENDIX III

MEANS SQUARES TABLES

Appendix Table 1. Means squares and degrees of freedom for average daily feed intake, protein intake, and energy consumption of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 1a.

Source	df	Feed intake (g)	Protein intake (g)	Energy intake (g)
		($x10^3$)		($x10^3$)
Line	1	16.84***	169.46***	199.91***
Trt	1	26.56***	267.23***	315.26***
Line x Trt	1	.37	3.69	4.36
Error	16	.17	1.70	2.01

***$P \leq .001$.

Appendix Table 2. Means squares and degrees of freedom for body weight of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 1a.

Source	df	Weight 1 (g)	Weight 2 (g)	Weight 3 (g)	Weight 4 (g)
Line	1	18.59***	21.09***	22.68***	23.03***
Trt	1	.0003	.26	1.02**	1.24**
Line x Trt	1	.06	.0001	.01	.01
Error	16	.11	.12	.12	.10

**P≤.01, ***P≤.001.

Appendix Table 3. Means squares and degrees of freedom for cumulative percent body weight gain of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 1a.

Source	df	Gain 1 (%)	Gain 2 (%)	Gain 3 (%)
Line	1	30.98	57.71	.49
Trt	1	392.70***	1505.18***	2308.98***
Line x Trt	1	.01	59.58	42.48
Error	16	12.77	16.35	19.33

Percentage data were transformed to arc sine square roots prior to analysis.

***$P \leq .001$.

Appendix Table 4. Means squares and degrees of freedom for abdominal and right sartorial fat of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 1a.

Source	df	Abdominal fat weight (g)	% of Body weight	Sartorial fat weight (g)	% of Body weight
		($x10^3$)			
Line	1	132.77***	44.31**	122.04**	.004
Trt	1	12.25	23.71*	40.02	1.27*
Line x Trt	1	.01	9.84	4.34	1.13
Error	16	2.89	5.18	10.01	.26

Percentage data were transformed to arc sine square roots prior to analysis.

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 5. Means squares and degrees of freedom for abdominal and right sartorial fat total DNA and radiotracer uptake of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 1a.

Source	df	Abdominal fat		Sartorial fat	
		Total DNA (μg)	Total DPM	Total DNA (μg)	Total DPM
		($x10^7$)	($x10^{10}$)	($x10^4$)	($x10^6$)
Line	1	618.16***	382.30***	678.05*	680.02
Trt	1	165.12*	171.49**	568.42*	5370.71**
Line x Trt	1	21.37	22.54	25.43	47.24
Error	16	30.74	173.21	122.55	692.58

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 6. Means squares and degrees of freedom for average daily feed intake, protein intake and energy consumption of High- (HW) and Low-weight (LW) birds in response to restricted, *ad libitum*, or force feeding, Experiment 1b.

Source	df	Feed intake (g)	Protein intake (g)	Energy intake (kcal)
		($x10^3$)	($x10^1$)	($x10^4$)
Line	1	28.70***	28.84***	34.02***
Trt	2	41.97***	42.23***	42.82***
Line x Trt	2	6.07***	6.10***	7.19***
Error	26	.13	.13	.16

***$P \leq .001$.

Appendix Table 7. Means squares and degrees of freedom for body weight of High- (HW) and Low-weight (LW) birds in response to restricted, *ad libitum*, or force feeding, Experiment 1b.

Source	df	Weight 1 (g)	Weight 2 (g)	Weight 3 (g)	Weight 4 (g)	Weight 5 (g)
Line	1	43.62***	42.78***	49.06***	47.75***	49.97***
Trt	2	.07	.31*	1.19**	1.18***	2.49***
Line x Trt	2	.12	.22	.49**	.59***	.81***
Error	26	.09	.09	.06	.07	.07

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 8. Means squares and degrees of freedom for cumulative percent body weight gain of High- (HW) and Low-weight (LW) birds in response to restricted, *ad libitum*, or force feeding, Experiment 1b.

Source	df	Gain 1 (%)	Gain 2 (%)	Gain 3 (%)	Gain 4 (%)
Line	1	23.81	37.89	61.81*	67.98
Trt	2	1132.33***	3531.98***	4555.45***	5560.41***
Line x Trt	2	11.79	42.69	46.45	75.84
Error	16	38.91	74.14	84.94	85.64

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, ***$P \leq .001$.

Appendix Table 9. Means squares and degrees of freedom for abdominal fat, total adipocyte DNA content, and total radiotracer uptake of High- (HW) and Low-weight (LW) birds in response to restricted, *ad libitum*, or force feeding, Experiment 1b.

Source	df	Abdominal fat			
		Weight (g)	% of body weight	Total DNA (μg)	Total DPM
		($x10^3$)		($x10^7$)	($x10^9$)
Line	1	19.69***	4.81***	105.78***	37.29***
Trt	2	10.76***	8.19***	42.14***	38.31***
Line x Trt	2	3.81**	.57	16.27*	10.31*
Error	26	.75	.37	3.45	2.32

Percentage data were transformed to arc sine square roots prior to analysis.

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 10. Means squares and degrees of freedom for body weight (g) of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Body weight (day 1)	df	Body weight (day 18)	df	Body weight (day 57)
		($x10^4$)		($x10^5$)		($x10^5$)
Line	1	8328.35***	1	561.29****	1	299.48***
Trt	1	.17	1	6.47*	1	2.35
Line x Trt	1	3.99	1	.40	1	.18
Error	70	14.76	30	1.26	13	.85

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 11. Means squares and degrees of freedom for abdominal fat of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
		($x10^2$)		($x10^3$)		($x10^3$)		($x10^3$)
Line	1	16.61	1	11.62***	1	15.23***	1	19.49*
Trt	1	25.58*	1	37.88***	1	84.98***	1	2.47
Line x Trt	1	2.74	1	.002	1	3.46	1	2.17
Error	14	4.16	13	.46	13	.82	13	3.43

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, ***$P \leq .001$.

Appendix Table 12. Means squares and degrees of freedom for abdominal fat lipid content of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
		$(x10^2)$		$(x10^3)$		$(x10^3)$		$(x10^3)$
Line	1	27.32**	1	9.86***	1	12.79***	1	13.88*
Trt	1	9.37	1	38.47***	1	84.39***	1	1.47
Line x Trt	1	4.76	1	.04	1	3.17	1	2.88
Error	12	2.73	13	.33	13	.77	13	2.69

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 13. Means squares and degrees of freedom for abdominal fat total adipocyte DNA content of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
		($x10^5$)		($x10^7$)		($x10^7$)		($x10^5$)
Line	1	30.58	1	13.72	1	24.29***	1	201.18
Trt	1	52.82	1	37.68**	1	335.05***	1	40.14
Line x Trt	1	13.09	1	.19	1	17.05***	1	27.26
Error	14	19.10	14	3.31	13	.42	13	60.23

**P≤.01, ***P≤.001.

Appendix Table 14. Means squares and degrees of freedom for abdominal fat lipid/adipocyte DNA ratio of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
								($x10^2$)
Line	1	290.99	1	95.43	1	64.83	1	60.33
Trt	1	1.33	1	93.82	1	8097.08**	1	20.52
Line x Trt	1	17.41	1	9.15	1	72.84	1	29.61
Error	12	192.54	13	70.30	13	639.32	13	65.51

**$P \leq .01$.

Appendix Table 15. Means squares and degrees of freedom for average adipocyte diameter of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
Line	1	358.38	1	40.86	1	20.61	1	933.78
Trt	1	5.64	1	105.53	1	117.69	1	69.38
Line x Trt	1	167.57	1	163.71	1	35.95	1	1687.59
Error	14	136.99	13	403.87	11	122.25	10	924.11

Appendix Table 16. Means squares and degrees of freedom for adipocyte cell number in the abdominal fat depot of High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 2.

Source	df	Day 6	df	Day 12	df	Day 18	df	Day 57
		($x10^{30}$)		($x10^{29}$)		($x10^{29}$)		($x10^{29}$)
Line	1	54.29**	1	37.37	1	61.39	1	16.08
Trt	1	56.15	1	47.39	1	743.35	1	138.65
Line x Trt	1	13.52	1	30.46	1	2274.43	1	177.16
Error	12	6.61	13	52.06	11	29.39	10	4.39

**$P \leq .01$.

Appendix Table 17. Means squares and degrees of freedom for body weight of High- (HW), Low-weight (LW), and commercial broiler breeders (CB) in response to lipectomy and estrogen supplementation, Experiment 3.

Source	df	Day 1	df	Day 21	df	Day 70
Line (L)	2	93.11***	2	80.45***	2	71.51***
Implant (I)	1	.03	1	.72*	1	2.96**
Surgery (S)	1	.01	1	.85*	1	.94
L x I	2	.21	2	.09	2	.76
L x S	2	.05	2	.11	2	.18
I x S	1	.34	1	.08	1	.07
L x I x S	2	.26	2	.31	2	.06
Error	60	.17	37	.15	31	.31

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 18. Means squares and degrees of freedom for abdominal fat of High- (HW), Low-weight (LW), and commercial broiler breeders (CB) in response to lipectomy and estrogen supplementation, Experiment 3.

Source	df	Weight (g)	% of Body Weight	Lipid (%)	Total DNA (μg)
		($x10^4$)			($x10^9$)
Line (L)	2	4.99***	11.23	19.44	2.67**
Implant (I)	1	3.04**	56.17**	1.01	.49
Surgery (S)	1	2.27*	94.48***	98.46	1.77*
L x I	2	.93	4.17	93.65	.19
L x S	2	1.15	7.16	60.15	.57
I x S	1	.65	9.94	.07	.19
L x I x S	2	.71	24.26	6.35	1.04
Error	31	.36	6.15	83.85	.38

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

Appendix Table 19. Means squares and degrees of freedom for right and left sartorial fat of High- (HW), Low-weight (LW), and commercial broiler breeders (CB) in response to lipectomy and estrogen supplementation, Experiment 3.

Source	df	Rt Sartorial Fat Weight (g)	% of Body Weight	Lft Sartorial Fat Weight (g)	% of Body Weight
Line (L)	2	71.99***	164.65***	50.50***	113.04***
Implant (I)	1	11.36	38.33*	11.48	44.61*
Surgery (S)	1	14.55	56.87**	30.44*	108.77**
L x I	2	2.34	2.34	1.79	2.49
L x S	2	4.79	8.99	7.56	14.01
I x S	1	13.13	29.46	4.45	12.26
L x I x S	2	12.40	23.96	10.45	25.97
Error	31	4.19	7.69	4.73	9.96

Percentage data were transformed to arc sine square roots prior to analysis.

$*P \leq .05$, $**P \leq .01$, $***P \leq .001$.

Appendix Table 20. Means squares and degrees of freedom for carcass composition of High- (HW), Low-weight (LW), and commercial broiler breeders (CB) in response to lipectomy and estrogen supplementation, Experiment 3.

Source	df[a]	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Lipid/ Protein
Line (L)	2	4.09	20.24**	32.66**	23.27	.04**
Implant (I)	1	4.57	8.16	10.09	2.26	.002
Surgery (S)	1	48.31**	30.24**	39.18*	1.37	.008
L x I	2	17.73	4.54	6.66	2.22	.004
L x S	2	4.33	3.74	2.83	26.57	.002
I x S	1	2.02	5.97	7.56	8.14	.003
L x I x S	2	1.56	3.08	16.08	4.78	.02
Error	31	5.73	3.30	6.52	14.18	.01

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, **$P \leq .01$.

Appendix Table 21. Means squares and degrees of freedom for abdominal fat characteristics of male and female High- (HW) and Low-weight (LW) birds (Day 14), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Weight (g)	% of Body weight	Lipid (%)
		($x10^3$)		
Line (L)	1	111.09***	20.56*	4.99
Trt (T)	1	28.01***	87.37***	216.77*
Sex (S)	1	108.53***	452.62***	5.29
L x T	1	0.0	14.15	211.63*
L x S	1	46.67***	26.03*	.10
T x S	1	.06	3.77	60.68
L x T x S	1	2.71	14.92	22.94
Error	40	2.04	4.16	33.98

Percentage data were transformed to arc sine square roots prior to analysis.

*P≤.05, ***P≤.001.

Appendix Table 22. Means squares and degrees of freedom for liver characteristics of male and female High- (HW) and Low-weight (LW) birds (Day 66), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Weight (g)	% of Body weight	Lipid (%)
		($x10^3$)		
Line (L)	1	85.63***	15.38	53.73
Trt (T)	1	.82	4.81	2.48
Sex (S)	1	53.18***	399.04***	74.17
L x T	1	1.94	.23	18.03
L x S	1	12.30*	4.98	46.85
T x S	1	.55	1.15	.09
L x T x S	1	3.75	2.43	10.79
Error	37	2.47	4.82	15.41

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, ***$P \leq .001$.

Appendix Table 23. Means squares and degrees of freedom for abdominal fat characteristics of male and female High- (HW) and Low-weight (LW) birds (Day 14), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Weight (g)	% of Body weight	Lipid (%)
		($x10^3$)		
Line (L)	1	113.58***	6.98**	10.29
Trt (T)	1	86.32***	23.79***	742.45***
Sex (S)	1	.48	17.54***	410.51***
L x T	1	2.75	.24	12.96
L x S	1	7.14	11.21***	.001
T x S	1	15.68	5.87**	240.42***
L x T x S	1	.45	.29	.32
Error	40	.14	.66	15.33

Percentage data were transformed to arc sine square roots prior to analysis.

**$P \leq .01$, ***$P \leq .001$.

Appendix Table 24. Means squares and degrees of freedom for liver measurements of male and female High- (HW) and Low-weight (LW) birds (Day 66), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Weight (g)	% of Body weight	Lipid (%)
Line (L)	1	1857.54***	8.74***	6.21
Trt (T)	1	8.55***	.27	1.87
Sex (S)	1	.36	17.82***	81.97***
L x T	1	34.37	.11	8.54
L x S	1	147.79	3.99**	1.49
T x S	1	2.79	2.29*	7.36
L x T x S	1	.73	.004	5.44
Error	37	44.73	.45	3.78

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

Appendix Table 25. Means squares and degrees of freedom for carcass composition of male and female High- (HW) and Low-weight (LW) birds (Day 14), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)
				($x10^2$)	
Line (L)	1	5.03	129.49***	4.03***	1.25
Trt (T)	1	91.52***	.39	2.17***	8.19
Sex (S)	1	462.15***	56.35**	14.39***	5.02
L x T	1	4.27	8.72	.41	3.76
L x S	1	24.74**	20.75	1.58**	1.31
T x S	1	.03	6.34	.08	.19
L x T x S	1	9.33	.75	.12	1.27
Error	40	3.52	6.03	.13	3.37

Percentage data were transformed to arc sine square roots prior to analysis.

$^{**}P \leq .01$, $^{***}P \leq .001$.

Appendix Table 26. Means squares and degrees of freedom for carcass composition of male and female High- (HW) and Low-weight (LW) birds (Day 66), in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)
Line (L)	1	21.92*	16.72*	185.98***	1.52
Trt (T)	1	.002	9.69	.77	2.71
Sex (S)	1	197.18***	40.06**	778.57***	.11
L x T	1	24.49*	.05	27.74	7.76
L x S	1	7.41	.37	19.09	10.87
T x S	1	6.01	1.94	31.37	1.35
L x T x S	1	4.34	2.12	42.62	.76
Error	36	4.17	3.72	11.90	5.14

Percentage data were transformed to arc sine square roots prior to analysis.

*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

Appendix Table 27. Means squares and degrees of freedom for blood plasma total lipid of male and female High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Day 1	df	Day 14	df	Day 66
		($x10^5$)		($x10^5$)		($x10^5$)
Line (L)	1	10.83**	1	5.51*	1	10.53**
Trt (T)	1	.02	1	13.46***	1	.49
Sex (S)	1	30.69***	1	32.89***	1	73.79***
L x T	1	.27	1	7.82**	1	9.22**
L x S	1	11.81**	1	19.23***	1	22.73***
T x S	1	.11	1	13.54***	1	1.33
L x T x S	1	1.23	1	5.61*	1	9.49**
Error	99	1.59	83	1.06	36	1.29

*P≤.05, **P≤.01, ***P≤.001.

Appendix Table 28. Means squares and degrees of freedom for blood plasma total cholesterol of male and female High- (HW) and Low-weight (LW) birds in response to *ad libitum* or force feeding, Experiment 4.

Source	df	Day 1	df	Day 14	df	Day 66
		($x10^3$)		($x10^3$)		($x10^3$)
Line (L)	1	4.52	1	1.65	1	4.92
Trt (T)	1	2.46	1	15.81*	1	3.82
Sex (S)	1	17.34***	1	21.75*	1	15.42
L x T	1	5.83	1	1.28	1	.97
L x S	1	19.21***	1	3.68	1	19.78
T x S	1	7.10*	1	.28	1	.89
L x T x S	1	3.58	1	.36	1	4.59
Error	102	1.48	86	3.58	37	5.34

*$P \leq .05$, ***$P \leq .001$.

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