THE EFFECTS OF DIETARY RESTRICTION DURING THE GROWTH PERIOD ON RATE OF GROWTH, MATURE BODY WEIGHT, TISSUE PROPORTIONS, AND ADIPOSE TISSUE CELLULARITY OF BROILER-TYPE CHICKENS

by

GORDON C. BALLAM

B. Sc., University of Victoria, 1976

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Department of Poultry Science

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December 1978

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Poultry Science

The University of British Columbia
2075 Wesbrook Place
Vancouver, B.C.
V6T 1W5

Date January 1979

ABSTRACT

Male and female broiler-type chicks were subjected to different periods of dietary restriction between the ages of 0 and 14 weeks of age. Feed was restricted during this period of time by limiting feed consumption to 30 minutes of feeding per day. Growth rate of the birds and mature body weights were measured. The proportion of organs and tissues, and adipocyte diameter and number in the retroperitoneal and M. sartorius depots were determined in mature female birds subjected to the different periods of dietary restriction. The following summarizes the findings:

1. Male and female birds subjected to different periods of dietary restriction from 0-14 weeks of age had similar body weights at the end of any given period of feed restriction. However, following ad libitum feeding, the male birds previously subjected to different periods of dietary restrictions, grew at a greater rate and obtained a greater final body weight than did the females.
2. Dietary restriction increased mortality in both male and female birds. There was, however, no sex difference in mortality in response to the early dietary restriction. Cropbound birds and birds with leg weakness accounted for most of the mortality.

3. Dietary restriction from 0-12 and from 0-14 weeks of age caused significant decreases in the mature body weights of female birds. The lighter weights appeared to be due to a reduction in the growth of all tissues since the proportional weights of the M. pectoralis major, liver, tibiotarsus, retroperitoneal and M. sartorius adipose depots were similar in the restricted and the control birds. The tibiotarsus and the M. sartorius adipose depot were the tissues most sensitive to the dietary restriction. Since the weight of the retroperitoneal adipose depot was not significantly affected by dietary restriction, there may be differences in the responses of the retroperitoneal depot and the M. sartorius depot, to early dietary restriction.

4. Determining the average retroperitoneal adipocyte diameter at 17-19 weeks and 40-43 weeks of age, revealed that adipocyte enlargement in the retroperitoneal depot occurred in all treatments between the two ages.

5. Adipocytes from the retroperitoneal depot were significantly larger than adipocytes from the M. sartorius depot regardless of dietary treatment.

6. Dietary restriction reduced the average adipocyte diameter in the retroperitoneal and the M. sartorius depots of birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age; and the effect on cell size was still apparent at 40-43 weeks of age.

7. Adipocyte cellularity in the M. sartorius depot was similar for all treatments studied, indicating that the number of adipocytes in this depot was unaffected by early dietary restriction. In the retroperitoneal depot, however, birds restricted from 0-12 and from 0-14 weeks of age had significantly more adipocytes than did the control birds. Whether this increase in observable adipocytes reflected an increase in adipocyte cellularity or an increase in the lipid-filling of pre-adipocytes is not clear from this study. The difference in response to early dietary restriction exhibited by the retroperitoneal and the M. sartorius depots may reflect a greater propensity of adipocytes in the retroperitoneal depot to multiply.

TABLE OF CONTENTS Page

LIST OF TABLES

LIST OF FIGURES

LIST OF TABLES IN APPENDIX

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
Å	angstrom
Avg	average
BW	body weight
cAMP	cyclic adenosine 3' - 5' monophosphate
DNA	deoxyribonucleic acid
g	grams
I.U.	international unit
I.C.U.	international chick unit
mcg	microgram
LH	luteinizing hormone
µm	micron
mg	milligram
NADH	nicotinamide adenosine dinucleotide (reduced form)
NADPH	nicotinamide adenosine dinucleotide phosphate (reduced form)
pl	picoliters
TSH	thyrotropic hormone

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor B.E. March of the Department of Poultry Science for her contribution to my academic development. Her inspiration and encouragement are deeply appreciated.

I would also like to extend a full measure of appreciation to the other members of my Thesis Committee:

Dr. D.B. Bragg	Chairman, Department of Poultry Science
Dr. R.C. Fitzsimmons	Department of Poultry Science
Dr. R. Reeves	Department of Zoology

Special consideration must be given Dr. J. Biely (Research Professor, Department of Poultry Science) for his encouragement in my academic achievements and for his helpful advice.

I would also like to thank Dr. Bragg and Dr. Fitzsimmons for their interest in my progress and for the stimulatory environment which they help to provide.

Lastly, it is my pleasure to include a note of appreciation to the technical staff and farm operators for service so often and congenially rendered.

INTRODUCTION

The adipose tissue can no longer be termed, as it was by Wells in the early 40's, "the neglected subject", (Wells, 1940). Research in the last 35 years has evolved the concept of adipose tissue, from, "an inert site of fat storage" to the view that adipose tissue is an important hormone-regulated metabolically functioning organ.

Two types of differentiated adipose tissue occur in many species of vertebrates: white fat, which comprises the bulk of all adipose tissue, and brown fat, which occurs in restricted locations such as the interscapular region and is especially prominent in rodents and hibernating animals. Brown fat functions as a source of heat production in animals, especially during arousal from hibernation. White fat, the only type present in the avian species, serves primarily as an energy source, as insulatory material and as a protective cushion.

Interest in adipose tissue development in the past 10 years has focused on the involvement of white adipose tissue in the problem of obesity. Obesity results from the presence in the body of excessive amounts of adipose tissue. The enlargement of adipose tissue may result from an increase in either adipocyte size, (hypertrophy) or cell number, (hyperplasia). Cellular development of adipose tissue has been investigated in man, rats, meat animals and poultry. In all species, adipose tissue develops by hyperplastic growth until one or more specific periods in the development of the animal after which further increases in adiposity result primarily from adipocyte hypertrophy.

It is important to know whether adipose tissue contains, after some physiological age is attained, a fixed number of adipocytes, because if so, it may be possible to control cell multiplication by diet and thus control the extent to which obesity can subsequently occur.

The problem of obesity is not restricted to the mammalian species. The commercial poultry breeders have long been aware of the problem of excessive fat deposition in breeding flocks of broiler-type birds. The following investigation was undertaken to determine the effects of early dietary restriction on the adipocyte size and cellularity of adipose depots in broiler-type chickens.

LITERATURE REVIEW

Historical Perspective

Adipose tissue was originally thought of as ordinary connective tissue in which fat had been deposited (Flemming, 1871). The concept that connective tissue acted as a storage organ for fat challenged the earlier views of Toldt (1870). Toldt had originally stated the adipose tissue was a specific organ, entirely distinct from the connective tissue in which it frequently occurred. However, since Toldt could not show how the vascularized embryonic fat lobules developed, and he had no evidence for the specificity of the fat cell, the controversy seemed to rest in favour of Flemming (Hammar, 1895; Bell, 1909; Foot, 1912).

Reflection on Flemming's views, indicates the impossibility of this concept, for it is known that fat does not become generally and diffusely deposited in connective tissue throughout the human body, even in obesity. The hands, the feet, the ears, and the nose, are seen to undergo much less thickening in obesity than the abdominal wall, thighs, buttocks and shoulders.

The individuality of adipose tissue was clearly demonstrated by Strandberg (1915). Skin from the anterior abdominal wall was grafted to the dorsum of the hand for treatment of a burn in a 12 year old girl. Later in life this transplanted skin deposited fat to produce a grotesque boxing glove effect.

Histological and embryological studies in the 1920's supported Toldt's concept of the individuality of adipose tissue (Rasmussen, 1923; Inglis, 1927).

Final resolution of the views held by Toldt and Flemming was achieved when it was demonstrated that adipocytes develop from non-differentiated mesenchymal cells (Maximow, 1927).

The key to the understanding of the development of adipose tissue was found to be in the histogenesis of this primitive organ. Studies from the laboratory of Frederick Wasserman from 1926-1931 gave rise to the hypothesis that primitive adipose cells belonged to the reticuloendothelial system (Wasserman, 1965). The concept that adipose tissue is part of the reticuloendothelial system is in harmony with the gland-like histological structure of the adipose tissue before fat accumulation, and establishes the morphological basis for the complex metabolic activities of the fat cell. Portis (1924) demonstrated that the cells of the omentum were able to form antibodies and that this ability was correlated with the occurrence, in this adipose organ, of aggregates of reticuloendothelial cells. Histologically, this cell closely resembled primitive adipose cells, prior to fat storage. According to Wasserman (1926), the blood-forming function of adipose tissue could be re-established in depleted adipose tissue under appropriate conditions. An additional property of the reticuloendothelial system is that its cells are capable of ingesting vital dyes. Dogliotti (1928) first showed that both brown and white fat cells stored vital dyes, and this was especially well seen in cells depleted of lipid

(McCullough, 1944). Bremmer (1938) demonstrated that even the thin cytoplasmic ring of mature adipocytes exhibited the property of taking up colloidal particles of vital dyes. Investigations by Hausberger, (1938) further supported the concept that adipose tissue was distinct from connective tissue and that adipocytes arose from special mesenchymal cells. Hausberger (1938) transplanted embryonic tissue, from a site that developed adipose tissue, into normal adult rats and found the transplanted tissue formed normal adipose tissue. Upon similar transplantation of embryonic connective tissue, no adipose tissue was formed. The transplanted embryonic adipose tissue was lipid-free and was morphologically undistinguishable from embryonic connective tissue. The tissue was recognized by its specific location and development. Subsequent investigations on the histogenesis of adipose tissue have supported Wasserman's reticuloendothelial theory, on the origin of fat cells (Simon, 1965; Wasserman, 1965).

During the period of time investigations were being conducted to determine the origin of adipocytes, other researchers were determining the physiological role of adipose tissue in the body. The discoveries that pituitary extracts caused an acute mobilization of adipose lipid (Best and Campbell, 1936), and that there was a rapid turnover of adipose lipid in the tissue (Schoenheimer and Rittenberg, 1937), indicated a more dynamic role for the adipose tissue in body metabolism. In a review article by Wertheimer and Shapiro (1948), it was concluded that adipose tissue was a tissue with a special structure and a special type of cell. At this time, it was evident that the adipose depots were supplied by a comparatively dense capillary network (Gersh and

Still, 1945), and were innervated by sympathetic nerve fibers (Beznak and Harris, 1937). It was also known that the deposition and mobilization of fat in the adipose tissue was an active process, involving accumulation of glycogen (Tuerkischer and Wertheimer, 1942); and that synthesis of new fatty acid from carbohydrate, as well as the transformation of one fatty acid into another, proceeded continuously in this tissue (Schoenheimer and Rittenberg, 1937).

The discovery in 1956 of circulating free fatty acids, the recognition that adipose tissue was the main source of fatty acids in the plasma and the demonstration that the levels of fatty acids in the plasma fluctuated in response to changes in carbohydrate intake,led to the conclusion that the adipose tissue was participating on a minute to minute basis in the accumulation and liberation of this metabolic fuel (Gordon and Cherkes, 1965; Dole, 1956; Laurell, 1956). It was recognized that since adipose tissue was the major site of synthesis, oxidation, storage and release of fatty acids, it was a major site for the metabolic interrelationships between carbohydrates and lipids (Wertheimer and Shafrir, 1960; Renold et al., 1960).

The late 1950's and early 1960's represented an explosive period in the field of adipose tissue research. A tremendous number of papers were published elucidating metabolic pathways and their hormonal control in adipose tissue. By 1960 it was clearly established that the metabolism of the rat adipose tissue was altered by nearly every known hormone (reviews by Ball and Jungas, 1964; and Renold, et al., 1965).

The observation that adipose tissue responded to such an array of hormones placed in question the previous concept of target tissue selectivity for hormones, and of course, the specificity of hormone action.

It was not until a method was developed by Rodbell (1964a), for isolating adipocytes from the stromal-vascular fraction of the adipose tissue, making adipocytes more sensitive to the actions of hormones, that a clearer understanding of the hormonal control of adipose tissue was achieved. By 1965 it was clear that hormones could be separated into different classes depending upon their intracellular mode of action. The extraordinary investigations of Sutherland, Butcher, Robinson, and their colleagues, showed that glucagon, ACTH, LH, TSH and catecholamines altered the metabolism of fat, glycogen and protein by stimulating the production of cyclic AMP in isolated fat cells, (Robinson, Butcher and Sutherland, 1967). Subsequent reports by Butcher (1966) demonstrated that in isolated adipocytes, insulin diminished the production of cyclic AMP formed in response to the lipolytic hormones. Recent investigations have shown one effect of insulin on the isolated adipocyte to be elicited through inhibition of adenylate cyclase activity (Rodbell, 1970).

During the period of time when investigations were being conducted to determine the role of hormones on adipose tissue, it became apparent that there was considerable interspecies variation in the physiological response of adipocytes to hormones. Examination of adipose tissue from a number of species demonstrated that mouse adipose

tissue was identical in metabolic organization and hormonal responsiveness to that of the lean rat but that the adipose tissues of other species studied (hamster, guinea pig and pigeon), differed from that of the lean rat or mouse in one or more of the following respects: the form in which stored triglyceride fatty acids were mobilized; the nature of the principal extracellular carbohydrate which was metabolized by the fat cell; the capacity to convert glucose to glyceride fatty acids; the responsiveness to glucose-transport effect of insulin; the responsiveness to the antilipolytic action of insulin; the responsiveness to the various naturally occurring lipolytic agents (Rudman and Di Girolamo, 1967). In this regard, the adipose tissue of the avian species was shown to have negligible lipogenic activity; to be unresponsive to insulin; and to be highly responsive to the lipolytic action of glucagon (Goodridge, 1964; Leveille et al., 1968; Hazelwood, 1971).

In the 1970's research on adipose tissue has focused on its involvement in obesity. Adipose tissue development in animals and humans, has been shown to result from adipocyte hyperplasia and hypertrophy until sexual maturity, after which time adipose enlargement continues solely by adipocyte hypertrophy (Hirsch and Han, 1969; Hirsch, 1972).

The adipose tissue consists of two basic cell types, the adipocyte and the stromal vascular cells (capillary, endothelial, mast, macrophage, and epithelial cells). The adipose organ is highly vascularized so that few fat cells escape close contact with at least

one capillary (Wertheimer and Shapiro, 1948), and is innervated by a network of nerves (Gersh and Still, 1948). Investigations by Rodbell (1964b) have shown that the stromal-vascular fraction of adipose tissue contains 35% of the DNA and 50% of the protein in adipose tissue.

The first adipose cells appear in the "prestructural" territories, the mesenchymatous lobules (Hausberger, 1938). The beginning of adipogenesis occurs simultaneously with the penetration of capillary buds into the lobule. The primitive fat cells or preadipocytes differentiate from reticular cells, not from pre-existent fibroblasts (Wasserman, 1965; Poznanski, et al., 1973). The cytological change in a cell as it proceeds through the stages of white fat cell differentiation was concisely reviewed by Napolitano (1965). "There is a change in shape of the cell, from the spindle-shaped fibroblast-like structure to the nearly spherical form of a mature adipose cell. There is an ever-increasing accumulation of lipid, first as small inclusion concentrated toward one pole of the cell and finally as large lipid masses in the central region of the cell. As the preadipocyte accumulates more and more lipid, the droplets fuse and surround the nucleus which remains central. Initially all mitochondria are of spherical shape, but as differentiation progresses more filamentous forms are observed. The endoplasmic reticulum is abundant and highly organized in the earliest stages but by the time differentiation is complete, its organized form has disappeared and it now occurs only as short discrete sections of granulated membrane lying scattered in the cytoplasm. The Golgi apparatus is never very prominent in the developing

adipose cell. Glycogen is first observed at midstage in differentiation, appearing as an aggregation of particles approximately 200-300 Å in diameter in the immediate vicinity of lipid droplets. Towards the end of differentiation the nucleus becomes flattened at one side of the cell, and the large accumulations of lipid appear to coalesce in the center. Ultimately the cell displays the characteristic mature signet-ring shape associated with mature adipose tissue."

The mature lipid laden adipocyte is unable to divide (Cameron and Seneviratne, 1947; Gross, 1966). Therefore expansion of the adipose tissue during the early development of the tissue, proceeds by adipocyte enlargement and/or adipocyte multiplication or lipid-filling of preadipocytes (Hirsch, 1972).

Hollenberg and Vost (1968) reported that when adipose tissue was removed from rats in their postweaning, prepubertal growth period 0-2 days after an injection with (^{3}H)-thymidine, less than 1% of the radioactivity was present in the adipocyte fraction. However, during the following 2-5 days, the specific activity of the adipocyte fraction rose. A similar effect, in in vitro cultured adipose tissue from rats at weaning was also demonstrated; there was a slight rise in DNA specific activity of the adipocyte fraction after 3 days (Frohlich et al., 1972). These studies (Hollenberg and Vost, 1968; and Frohlich et al., 1972) were interpreted to mean that within the stromal elements of the adipose tissue, there are primordial adipose cells that give rise to new adipocytes during this period of prepubertal growth.

In the adult, however, it is uncertain whether or not preadipocyte multiplication occurs. Although some forms of obesity in man and animals are characterized by adipocyte hyperplasia (Hirsch et al., 1966; Salans et al., 1968), it is generally agreed that in the "normal adipose tissue" multiplication of preadipocytes is restricted to the period of prepubertal growth, and that once sexual maturity is reached, adipose tissue expansion proceeds solely by adipocyte hypertrophy or by lipid filling of preadipocytes (Greenwood and Hirsch, 1974).

Adipose Tissue Cellularity

Mature adipocytes are often considered to be "non-mitotic" cells and therefore may have a fixed number in the adult animal, even though each cell remains capable of great change in size (Cameron and Seneviratne, 1947; Simon, 1965; Gross, 1966). A study of the possibility of fixed adipocyte number in adult organisms is of general biological interest as well as clinical relevance, since it has been shown that some obese individuals have a great increase in adipocyte number, (Hirsch et al., 1966) and also, that adipocyte size and numbers are important parameters of adipose tissue metabolism (Salans et al., 1968). Furthermore, it is important to know whether adipose depots contain, after some physiological age is attained, a fixed number of adipocytes; because if so, it may be possible to control adipocyte multiplication by early dietary manipulation and thus control the extent to which obesity can subsequently occur.

The size achieved by a particular adipose depot depends upon the number of adipocytes, the size of the adipocytes, and the extent of stromal-vascular tissue (Zing et al., 1961, 1962; Enesco and Leblond, 1962); and is thus influenced by factors affecting either adipocyte division and/or adipocyte enlargement.

Investigators have been puzzled as to whether, in the adult animal, adipocyte hyperplasia contributes to an increase in the amount of adipose tissue. The early studies investigating this problem gave equivocal results. Reh, in his histological studies of human adipose tissue, concluded that nutritional effects induce great changes in cell size, thus emphasizing cell enlargement or hypertrophy as a mechanism for changing the size of the adult adipose organ (Reh, 1953). Enesco and Leblond (1962), Peckman, Entenman and Carrol (1962), as well as Zing, Angel and Steinberg (1961, 1962), concluded that changes in both cell size and cell number could occur in adult rats. These investigators used either total fat-free dry weights of tissue or total DNA content of the tissue as a measure of cellularity. In the light of recent investigations, however, their findings must be re-evaluated. Modern methods for determining adipose tissue cellularity have indicated that a large proportion of the adipose tissue DNA is present in the supportive tissue matrix (stromal-vascular cells) but not in the adipocytes (Rodbell, 1964b; Hirsch and Han, 1969).

A method for isolating adipocytes from the stromal-vascular tissue of the adipose depot was developed by Rodbell (1964a), and for the first time permitted a direct determination of adipocyte cellularity.

Two of the major techniques for measuring adipocyte cellularity, developed following the isolation of adipocytes by Rodbell, were the microscopic technique (Bray, 1969) and the osmium fixation technique of Hirsch and Gallian (1968). These techniques provided excellent methods for determining adipocyte cellularity, although the former has had subsequent modification (Di Girolamo et al., 1971; Lavau et al., 1977).

Goldrick (1967) suggested, from histological studies, that adult rat adipose tissue grew by cellular enlargement. This was immediately confirmed by Hirsch and Han (1969), who, using the osmium fixation technique of Hirsch and Gallian (1968), demonstrated that the rat epididymal adipose depot grew by an increase in adipocyte size and number until 15 weeks of age when the increase in adipocyte number ceased and further change in depot size occurred by adipocyte enlargement. This was supported by the experiments of Johnson et al., (1971), who demonstrated that the epididymal and retroperitoneal adipose depots of rats grew by adipocyte hyperplasia and hypertrophy until the 14th week of age, when the number of adipocytes became fixed and further increases in depot size occurred by adipocyte hypertrophy.

Similar studies on the hyperplastic and hypertrophic nature of adipose tissue have been undertaken in man (Sims et al., 1968; Hirsch and Knittle, 1970; Salans et al., 1971; Björntrop and Sjöström, 1971) rats, (Hubbard and Matthew, 1971; Hirsch and Gallian, 1968; Johnson et al., 1971; Lau et al., 1976) mice (Johnson and Hirsch, 1972) cattle, (Hood and Allen, 1973, Allen, 1976) swine, (Anderson and Kauffman, 1973;

Hood and Allen, 1977) chickens, (Pfaff and Austic, 1976) and ducks, (Evans, 1972b). From the results of the above studies it was concluded that during the postnatal, prepubertal growth period adipose tissue develops by both hyperplasia and hypertrophy. When sexual maturity is reached, a maximum number of adipocytes is attained and further growth is a result of adipocyte enlargement rather than adipocyte proliferation. In addition, studies have shown that in obese animals, including man, reduction of the adipose depot size does not reduce cell number (Hirsch and Han, 1969; Hirsch and Knittle, 1970); lending further support to the hypothesis of a constant adipocyte number in the adult animal.

Investigations of adipose tissue cellularity in meat animals, have shown that there is variability in the size and number of adipocytes among the adipose depots of these animals (Allen, 1976; Hood and Allen, 1977). In the porcine animal, subcutaneous, interscapular and thigh adipocytes had the smallest mean diameter and the perirenal adipocytes had the largest mean diameter, (Anderson, et al., 1972). On the other hand Hood, and Allen (1977), demonstrated that the adipocyte diameter of the middle back fat subcutaneous fat layer, was similar to the adipocyte diameter of the perirenal adipose tissue. It appears that the perirenal adipocytes of the ovine (Haugebak et al., 1974) and bovine (Hood and Allen, 1973) are also larger than the subcutaneous adipocytes. Similar differences in adipocyte sizes between adipose depots have been observed in rats and man (Bjurulf, 1959; Lemmonier, 1972; Johnson & Hirsch, 1972; Salans et al., 1971; Brook, 1971).

There is considerable variability among adipose depots of the same species in the time at which hyperplasia in the depots ceases and development continues by adipocyte hypertrophy (Hood and Allen, 1973; Anderson and Kauffman, 1973). In contrast to the interdepot differences in adipose tissue hyperplasia, it appears that in the bovine (Hood and Allen, 1973) and porcine (Anderson and Kauffman, 1973; Hood and Allen, 1977) species adipocyte hyperplasia is a relatively more important factor in the accumulation of lipid in adipose tissue. In this respect, in very obese animals, adipocyte hyperplasia may not be complete in the subcutaneous depot of the bovine animal by 14 months of age (Hood and Allen, 1973) or in the total carcass adipose tissue of the porcine animal by 5 months of age (Anderson and Kauffman, 1973).

The results of studies with meat animals, when coupled with the similar observations in obese and nonobese individuals of the same species, (Bjurlf, 1959; Bray, 1969; Hausberger, 1965; Herberg et al., 1974; Hirsch and Knittle, 1970; Hirsch et al., 1966; Johnson et al., 1971) that substantial variations exist in the stage of development beyond which no increase in adipocyte number occurs, indicate that the number of adipocytes in the mature adult may not be fixed. In order to interpret the observations in obese individuals, obesity has been classified according to type: either hyperplastic obesity usually occurring in early life when adipocyte hyperplasia is active (Hirsch and Knittle, 1970; Salans et al., 1971); or hypertrophic obesity which occurs in the sexually mature adult after adipocyte hyperplasia has ceased (Sims et al., 1968; Hirsch and Han, 1969; Björntorp and Sjöström, 1971). Hyperplastic obesity involves adipocyte

hypertrophy -- but hypertrophic obesity never involves adipocyte hyperplasia. Hyperplastic obesity also includes those forms of genetic obesity in which there is both adipocyte hyperplasia and hypertrophy (Bray, 1969; Hausberger, 1965; Hirsch et al., 1966; Lemmonier, 1972; Johnson and Hirsch, 1972; Johnson et al., 1971; Salans et al., 1968; Walkley et al., 1978).

Conclusions regarding adipose cellularity in man and animals must be drawn with caution. Although most of the experimental evidence in man and rats (Greenwood and Hirsch, 1974) and in meat animals (Allen, 1976), supports the contention of a fixed number of adipocytes in the sexually mature animal -- variability in the degree of hyperplasia and hypertrophy observed in adipose depots of obese animals, complicates the interpretation of data on the control of adipose development in the adult animal. Furthermore it is not entirely clear, whether the increase in mass of adipose tissue in the adult animal is due to multiplication of precursor adipocytes or to the filling of pre-adipocytes (Hirsch, 1972). Greenwood and Hirsch (1974), maintain that the multiplication of preadipocytes is restricted to the period of growth prior to sexual maturity and that once sexual maturity is attained, an increase in the observed adipose cellularity is derived from lipid filling of the existent preadipocytes. This concept was based on the early in vitro experiments of Hollenberg, and Vost (1968) and the organ culture experiments of Frolich, et al. (1972), who found an increase in the specific activity of the adipocyte fraction at various times after exposure to (^{3}H)-thymidine. These studies have been interpreted to mean that within the stromal-vascular elements of the adipose tissue, there are primordial adipose cells that give rise to new adipocytes during the period of prepubertal growth. Subsequent experiments by

Poznanski et al. (1973) have also confirmed these findings. Poznanski (1973); also demonstrated in vitro, however, that the stromal cells of the adult human adipose tissue are differentiated precursors (pre-adipocytes) with a potential for multiplication and for evolving into mature adipocytes. Similarly the studies by Hollenberg et al. (1970), have shown that adipocyte DNA in adult animals incorporates some tritiated thymidine, indicating some turnover of preadipocytes.

The controversy as to whether or not preadipocytes continue to divide in the adult animal is unresolved. The small turnover of (^{3}H)-thymidine in the studies of both Poznanski (1973) and Hollenberg et al. (1970), has been taken to indicate that the contribution of preadipocyte multiplication to adipose tissue development in the adult is small; and that the predominant factors determining adipose tissue expansion in the sexually mature animal are the enlargement of adipocytes and the lipid filling of preadipocytes already present within the stromal fraction of the adipose tissue (Greenwood and Hirsch, 1974). Further clarification of the role of preadipocytes in the development of adipose tissue should arise from the new procedure developed by Björntorp and associates for isolating preadipocytes from the fat depots of rats (Björntorp et al., 1978).

Avian Adipose Tissue

Until recently the metabolism of the avian adipose tissue has received little attention compared to the metabolism of the mammalian adipose tissue. The avian species differs considerably from most mammalian species in many aspects of carbohydrate and lipid metabolism. Also

several differences with regard to the influence of hormonal factors on carbohydrate and lipid metabolism have been demonstrated.

The adipose tissue of the rat and mouse has been most extensively investigated and seems to be the most important site for lipogenesis in these animals, with the liver playing a less important role (Feller, 1954; Hausberger et al., 1954; Jansen, 1966). In fact, in vivo studies have demonstrated that over 50 percent of total fatty acid synthesis in the mouse and rat occurs in adipose tissue (Favarger, 1965; Leveille, 1967; Romsos and Leveille, 1974). Adipose tissue is responsible for virtually all fatty acids synthesized in the pig (O'Hea and Leveille, 1969b). Adipose tissue of rats and mice is also markedly insulin sensitive (Winegrad and Renold, 1958; Randle, 1963). The major effect of insulin in this tissue is related to its facilitation of glucose uptake (Renold and Cahill, 1965). The many reports of adipose tissue metabolism have led to the generally accepted concept that, in mammals, this tissue is a major target organ for insulin and that it is largely responsible for the synthesis as well as storage and release of fatty acids (Wertheimer and Shafrir, 1960; Dole, 1965).

A major difference between avian and mammalian adipose tissue is that the avian species contain only white adipose tissue; brown adipose tissue is absent at all ages (Freeman, 1967; Johnston, 1971). The circulating level of glucose is approximately twice that of a number of mammals (Bell and Sturkie, 1965), yet the chicken is resistant to acute hypoglycemia (Houpt, 1958) and tolerant of low-carbohydrate, high-fat diets (Hazelwood, 1965; Brambolia and Hill, 1966).

In vitro, chick adipose tissue synthesizes only minute quantities of fatty acids but has substantial ability to convert precursors such as glucose and pyruvate to glyceride-glycerol; whereas liver tissue is extremely active in de novo fatty acid synthesis (O'Hea and Leveille, 1968; Leveille, 1969; Goodridge, 1968a). Although chick adipose tissue has a low capacity for fatty acid synthesis, it does have the ability to esterify fatty acids to triglycerides (Leveille et al., 1975). Studies in vivo have demonstrated that chick liver accounts for at least 70 percent of de novo fatty acid synthesis (Leveille et al., 1968), suggesting that the function of adipose tissue in the chick is mainly one of storage and release of lipids rather than synthesis. In vivo studies by O'Hea and Leveille (1969a), confirmed these findings, demonstrating that between 90 and 95 percent of de novo fatty acid synthesis in the chick took place in the liver; and that the newly formed triglycerides in the plasma occurred as low density lipoproteins which were the main transport form of lipid from the liver to the adipose tissue (O'Hea and Leveille, 1969a; Leveille et al., 1975). It has also been shown that the liver is the major site of lipogenesis in the pigeon (Goodridge and Ball, 1966, 1967) and in the duck, (Evans, 1972a).

Korn and Quigley (1957) were the first to report that chick adipose tissue possessed an active lipoprotein lipase system. Subsequent investigations by Husbands (1972) and Benson and Bensadoun (1977), have also revealed an active lipoprotein lipase system in chick adipose tissue; and a similar observation has been reported for the pigeon adipose tissue (Goodridge and Ball, 1967) and for the duck adipose tissue

(Evans, 1972a). The necessity for an active lipoprotein lipase is apparent if it is assumed that fatty acids and not triglycerides enter the adipose tissue cells.

Reduced NADP is essential for the reductive stages of fatty acid biosynthesis, and in mammals the pentose phosphate pathway is an important source of the cytoplasmic reducing equivalents. Studies of the metabolism of specifically labelled radioactive glucose to carbon dioxide (Duncan and Common, 1967) by liver slices showed that glucolysis is the major pathway of glucose dissimilation in both the immature and mature domestic fowl, and that the pentose phosphate pathway is not an important source of NADPH for fatty acid synthesis in the chick. Goodridge (1968c, d) has supported these findings. Pearce (1972a) investigated the specific activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in liver extracts from laying hens and cockerels and found no significant sex differences. The lack of difference is further evidence that this pathway is not involved in hepatic lipogenesis in the fowl. Similar data have been reported for the pigeon (Goodridge and Ball, 1966, 1967). In addition NADP-dependent isocitrate dehydrogenase, another potential source of cytoplasmic reducing equivalents, does not appear to be important in this regard for the support of lipogenesis in chick liver (Goodridge, 1968c).

Malic enzyme activity however, is much higher in chick livers than either the pentose phosphate pathway or the NADP-dependent isocitrate dehydrogenase enzyme system, which implies that it may serve an important function in the production of reducing equivalents for fatty acid

synthesis in this organ (O'Hea and Leville, 1968; Goodridge, 1968a, b). Goodridge and Ball (1964) arrived at a similar conclusion in their studies with the pigeon.

The activities of the lipogenic enzymes ATP-citrate lipase and the "malic" enzyme have been shown to be closely correlated with the rate of lipogenesis in the chicken liver (Goodridge, 1968c, d; Yeh et al., 1970; Pearce, 1971a) and the activities of these enzymes have been shown to be greater in the liver than the adipose tissue (Goodridge, 1968c; O'Hea and Leveille, 1968).

Dietary lipid has been reported by Yeh and Leveille (1969), Yeh et al. (1970), Mason and Donaldson (1972), Pearce (1968, 1971a) and Cunningham and Morrison (1977) to significantly reduce hepatic lipogenesis and malic enzymes. Cunningham and Morrison (1977), however, have shown that the induced depressions in lipogenic enzyme activities (citrate-cleavage and malic enzymes) will not lower total carcass fat content since the dietary lipid seems to be deposited in the adipose tissue in place of de novo synthesized lipid.

Pearce (1972b) reported a high level of activity of both citrate-cleavage and malic enzymes in replacement pullets from 4 to 7 weeks of age; but between 7 to 10 weeks of age the activities of both enzymes decreased and remained unchanged from 10 to 22 weeks of age. Raheja et al. (1971) obtained similar results for hepatic malic enzyme activity in the cockerel. The specific activities of the lipogenic enzymes, citrate-cleavage, and malic in the liver of laying hens have been reported to be greater than in pullets immediately prior to the onset of sexual

maturity (Pearce, 1971a, 1972b) and similar to the activity of the young pullet between 4 and 7 weeks of age (Pearce, 1972b). In the mature cockerel, where there is no demand for egg production and thus high lipogenic activity, the lipogenic enzyme activities remain low (Pearce, 1971a).

The endocrine control of glucose metabolism in the chicken is known to differ in many respects from other species (Hazelwood, 1971; Pearce, 1971b). Insulin is hypoglycemic in the chicken but, in contrast to its effect in most species, insulin elevates circulating free fatty acid levels (Goodridge, 1964; Lepkovsky et al., 1967; Langslow et al., 1970). Glucagon is lipolytic in the chicken and may be one of the most important endocrine secretions controlling avian metabolism (Hazelwood, 1971). Chicken adipocytes are extremely sensitive to the lipolytic action of glucagon (Goodridge, 1968b; Langslow, 1972). The adipocytes of chicks are insensitive to the lipolytic action of adrenaline, noradrenaline and ACTH, hormones which potentiate lipolysis in rat adipose tissue; insulin has neither lipolytic nor anti-lipolytic actions in chick adipocytes (Goodridge, 1968b; Langslow, 1971).

Despite the recent interest devoted to lipid biosynthesis in the chick, there has been little research performed on the structure and functional development of adipose tissue within the avian embryo or growing chick. Langslow and Lewis (1972), showed that the amount of subcutaneous adipose tissue in the thoracic and thigh muscles of Rhode Island Red chicks increased during embryonic life until the first day post-embryonic life, at which time it began to decline. From the

twelfth day of incubation, the subcutaneous adipocytes rapidly filled with triglyceride which serves as an important source of energy during the first 3 weeks of life (Langslow and Lewis, 1972). March and Hansen (1977), found in broiler-type chicks, that although the amount of lipid present in the lateral thoracic depot declined after hatching, the decline was quickly reversed, and by 29 days of age the amount of lipid present in this depot was more than double that at one day of age.

A species difference in adipocyte size and number was found between broiler-type chicks and White Leghorns, with the latter having a smaller adipocyte number and size in the retroperitoneal adipose depot, (March and Hansen, 1977). A difference in the growth rate between adipose depots was found, with the retroperitoneal depot growing at a faster rate than the sartorial depot, which in turn incorporated more lipid than the lateral thoracic depot. Furthermore a difference in the size of adipocytes in young chicks was found between the lateral thoracic and the retroperitoneal adipose depots -- with the latter having smaller adipocytes. However the faster rate of hyperplasia observed in the adipocytes of the retroperitoneal adipose depot could account for the slower rate of adipocyte enlargement seen in this depot (March and Hansen, 1977). Adipocyte hyperplasia was found to continue in both depots up to 6 weeks of age. Investigations by Pfaff and Austic (1977) showed that adipocyte hyperplasia in the retroperitoneal adipose depot of White Leghorn pullets continued until 12 to 16 weeks of age, at which time adipocyte hyperplasia ceased, and the adipose development continued by adipocyte hypertrophy.

Although hypertrophic growth occurred throughout development of the retroperitoneal adipose depot, it was most pronounced after 7 weeks of age. The relatively lower adipocyte hypertrophy observed before 7 weeks of age (Pfaff and Austic, 1977), may explain the low rate of adipose tissue growth seen in previous experiments (Langslow and Lewis, 1972; March and Hansen, 1977). Between 7 and 24 weeks of age, large amounts of lipid were deposited in the retroperitoneal adipose depot, producing a 27-fold increase in lipid content of the depot (Pfaff and Austic, 1977). Pearce (1972b) showed a continued decrease in the activity of lipogenic enzymes of the liver during the prelaying growth period. It seems likely, therefore, that decreased mobilization of lipid stores from adipose tissue may be an important factor in the development of adipose tissues in growing chicks.

The Influence of Nutrition on the Cellularity of Adipose Tissue

Cellular development of adipose tissue has been studied in man (Hirsch and Knittle, 1970), swine (Hood and Allen, 1977), cattle (Hood and Allen, 1973), rats (Hirsch and Han, 1969), mice (Greenwood et al., 1970) and pullets (Pfaff and Austic, 1976). In all species, adipose tissue develops by hyperplastic growth until one or more specific periods in the animals development is reached, after which further increases in adiposity result primarily from cellular hypertrophy. Thus, in the course of the growth and development, the final dimension achieved by this or any organ in the body will be modified by factors that exert their effect on cell division and/or cell enlargement. It has been demonstrated in other

organ systems that the degree to which either of these mechanisms is modulated by nutritional factors depend in part on the age of the animal. The earlier in life that they exert their influence, the greater the likelihood that permanent alterations in body size and organ size will occur (Dickerson and McCance, 1960; Pratt and McCance, 1961; McCance and Widdowson, 1962; Winick and Noble, 1965; Lister et al., 1966).

Clark and Clark (1940) were the first to show the effects of nutrition on the development of adipose tissue in the rabbit. Pace and Rathbun (1945) pointed out that fat is the only component of the whole body which may be expected to fluctuate widely in a "normal population". Montemurro and Stevenson (1957) concluded that the only major change in the gross body composition, even as a result of hypothalamic hyperphagia and obesity in the rat, was excessive fat. Diets different in fat content were shown to produce differences in body weight; the composition of which could be accounted for by the differences in fat content (Peckman et al., 1962).

Knittle and Hirsch (1968) were the first to show an effect of early nutrition on the ultimate size of specific adipose bodies. Rats nutritionally deprived during the first 21 days of life prior to weaning showed a permanent reduction in the size and number of adipocytes developing in the epididymal adipose depots. Subsequent experiments have shown that exercise in addition to food restriction early in life (Oscai et al., 1972), and protein restriction imposed immediately after weaning (Lau et al., 1976) may cause a permanent reduction in the number

of adipocytes in the epididymal adipose depots or parametrial and retroperitoneal adipose depots respectively.

These studies imply that a permanent reduction in the size of adipose depots can result only from a decrease in the adipose cell number; and that this can only be achieved during the hyperplastic period of adipose development early in life. The inability of starvation (Hirsch and Han, 1969; Rakow, 1971), exercise (Palmer and Tipton, 1973; Oscai et al., 1972), and cold exposure (Therricault and Mellin, 1971); to reduce adipocyte number permanently in sexually mature rats leads to the conclusion that a permanent reduction in the size of the adipose depots can result only from a decrease in adipocyte cell numbers (Hirsch and Knittle, 1970). Similarly, the inability of hypothalamic obesity or overfeeding in rats (Hirsch and Han, 1969; Johnson et al., 1971) or humans (Sims et al., 1968; Hirsch and Knittle, 1970; Salans et al., 1971), to alter adipose cellularity supports the concept of a constant number of adipocytes in the adult. Furthermore, investigations have shown that in obese man or animals, reduction in depot size does not reduce adipocyte number (Hirsch and Han, 1969; Hirsch and Knittle, 1970).

Lemonnier (1972), who reported an increase in adult adipocyte number in rats and mice when fed high fat diets, found that the greatest effect on cellularity occurred when the nutritional manipulation was started during the period of intensive adipocyte formation before weaning. Subsequent investigations by Herberg et al. (1974) in mice, and Ruzyllo and Szostak (1978) in rats, have also found an increase in the number of adipocytes following early dietary manipulation. These studies can

certainly be interpreted to mean that in "normal" adipose tissue, a predetermined adipocyte number exists in the depot, that when reached permits no further adipocyte addition but only cell size fluctuation.

The investigations of Lemonnier (1972) demonstrated that different adipose depots within mice responded differently to high fat diets. Increases in adipose depot size following high fat diets during weaning, were accounted for in the perirenal depot by hyperplasia; in the epididymal and subcutaneous depots by hypertrophy and in the parametrial depot by hyperplasia and hypertrophy. Results from Walkley et al. (1978) have also found site-specific responses of the adipose depots to overnutrition. It must be remembered, however, the observed increase in adipocyte number, induced genetically or by diet, does not exclude the possibility of lipid filling of preadipocytes laid down early in life.

Johnson and associates (1973) demonstrated that the degree of obesity established in mice was due to an interaction between genotype and early nutrition. While early overfeeding significantly increased adipocyte number in both obese and nonobese rats, early underfeeding reduced adipocyte number only in the nonobese. Therefore, early nutrition had only limited effects in the genetically obese rats -- increasing adipocyte number by preweaning overnutrition and by decreasing adipocyte size but not adipocyte number in preweaning undernutrition. Investigations in meat animals have also shown the importance of genetic background in determining adipose tissue cellularity. Hood and Allen (1977) have shown that the adipose depots of pigs of the same litter have similar

adipocyte numbers, but which may be different from the adipose numbers in the adipose depots of pigs from the same breed but from different litters.

There have been several investigations regarding the effect of early dietary manipulation on adipose tissue cellularity in adult meat animals. Early dietary restriction in lambs failed to reduce adipocyte numbers in the adult (Haugebak et al., 1974). Similarly Lee et al. (1973a, b) demonstrated that early dietary restriction in pigs was unable to reduce cell numbers despite a 2-4 fold reduction in the subcutaneous adipose depot. The inability of dietary manipulations early in life to limit adipocyte cellularity in meat animals may be due to the ability of certain adipose depots within the animal to achieve their maximum number of adipocytes early in life (Allen, 1976).

Some investigations have focused on the role of maternal nutrition during lactation in determining adipose tissue cellularity and metabolism in the young (Knittle, 1972). Recovery from undernutrition has been found to be less likely if the insult occurs early in life, for example, during gestation or the neonatal period (McCance and Widdowson, 1962; Knittle and Hirsch, 1968; Knittle, 1972). Investigations in which there have been manipulations of the diets or feeding habits of mice and rats (Lemonnier, 1972; Herberg et al., 1974), during gestation or suckling have produced increased in adipocyte cellularity in the adult animal. These investigators, however, have concluded that differences in cellularity in the adult animals cannot be attributed exclusively to early nutrition, but must also be attributed to later feeding patterns and behavior.

In addition, Schemmel and associates (1973) have observed that, depending upon the dietary manipulation early in life, the nature of the diet after weaning has a profound effect on the ultimate obesity, which may completely overshadow the effect of dietary manipulation during the first 3 weeks of age.

The Influence of Nutrition on the Development of Adipose Tissue in the Avian Species

The importance of considering carcass composition of chickens reared for meat purposes has long been appreciated (Pfeiffer, 1887; Köhler, 1900; Lee, 1911; Jull and Maw, 1923; Hepburn and Holder, 1922; and Harshaw, 1936, 1938). Although much work has been done in developing rations to finish broiler chickens (Donaldson et al., 1955, 1956; Biely and March, 1957; Summers et al., 1965), ducks (Scott et al., 1959; Evans, 1972b) and turkeys (Donaldson et al., 1958; Bixler et al., 1968) much less information is available on the influence of diet on the carcass composition of replacement pullets at sexual maturity.

The degree of fatness on pullets is affected by both non-nutritional and nutritional factors. Age and sex seem to be the most important non-nutritional factors, the percentage of fat in the carcass increasing with age (Combs, 1968; Kubena et al., 1972) while females contain more fat than males (Summers et al., 1965; Edwards et al., 1973; Farrell, 1974; Kubena et al., 1974).

Early experiments considered the effects of nutrition on the carcass composition of pullets between 0 and 20 weeks of age (reviews by Lee et al., 1971; and Balnave, 1973). Various nutrient restriction methods have been tested including daily restriction of eating and/or feed quantity; skid-a-day programs; restriction of energy, water or protein intake; low essential amino acid(s) formulas (e.g. low lysine formula) and various combinations of light restriction to complement nutrient restriction.

The first reported study on the effects of dietary restriction on subsequent growth performance was by Temperton and Dudley (1941). Since then numerous studies have been performed limiting daily feeding time (Heuser et al., 1945; Novikoff and Biely, 1945; Luther et al., 1976) or using "skip-a-day" feeding (Yates and Schaible, 1963; Pepper et al., 1966). However, these experiments have not met with success because the birds soon learn to eat quickly and satisfy their requirements in the limited time available (Lepkovsky et al., 1960).

Early experiments using quantitative feed restriction (Schneider et al., 1955; Fuller and Dunahoo, 1962; Deaton and Quisenberry, 1963; Strain et al., 1965) were shown to lower total feed consumption, increase feed conversion, delay sexual maturity, and increase egg production. However, no information was found on the effects of quantitative restriction on body composition. More recently, studies by Watson (1975), Gous, and Strielau (1976) and Luther et al. (1976) have shown that quantitative feed restriction reduces body weight, either increases or decreases egg production depending upon the severity of the dietary

restriction; and in some instances decreases carcass fat. Other reports (Kari et al., 1977) demonstrate no difference in egg production or fat content with feed restriction. The different responses in egg production and carcass fat composition to dietary restriction observed in these studies arise from differences in the severity of the restriction and from differences in the stage of growth at which the dietary restriction was implemented (either growing or laying period).

The effects of nutritional factors on the body composition of pullets were first described by Fraps (1943) who was able to produce carcasses with widely varying amounts of body fat by adjusting dietary constituents. Subsequent investigations on qualitative feed restriction using low energy diets (Berg and Bearse, 1958; Berg, 1959; Summers et al., 1967; Bolton et al., 1970; or low protein diets or amino acid imbalances (Berg and Bearse, 1958; Berg, 1959; Harms and Waldrup, 1962; Singsen et al., 1965; Lillie and Deaton, 1966, 1967; Lipstein et al., 1975; Balnave, 1976) have also been shown to reduce body weight gain, feed consumption, delay onset of sexual maturity, increase egg production and in some studies decrease fat content of the bird.

Other researchers have investigated the effects of calorie to protein ratio in subsequent performance and body composition (Hill and Dansky, 1954; Donaldson et al., 1956; Combs et al., 1964; Kubena et al., 1972; Farrell, 1974; Bartov et al., 1974, 1976; Griffiths et al., 1977). These studies demonstrated that as the dietary C:P ratio was widened, energy intake and carcass fat deposition increased while water content decreased.

Kubena et al. (1974) showed that as the rearing temperature increased, the percentage of retroperitoneal fat increased in broilers. Deaton and associates (1974) showed that broilers reared on litter-floor pens had less fat than those reared in cages at 9 weeks of age.

Despite the numerous reports describing the effects of dietary or rearing manipulations on subsequent carcass composition, few such reports appear in the literature relating to broiler-type replacement pullets. The importance of investigating nutritional factors affecting the body composition in broiler breeders results from the broiler's rapid growth and propensity to become obese in adulthood. The excessive fat deposition occurs during the growth period and well into adulthood, resulting in obesity and uneconomical feed:egg ratios. It is uncertain whether or not the excessive fat deposition decreases egg production (Bolton, et al., 1970) or increases mortality and thereby indirectly reducing egg production (Chaney and Fuller, 1975).

Brobeck (1946) was the first to show that obesity observed in rats with hypothalamic lesions was the consequence of overeating. White Leghorn cockerels in response to hypothalamic ventromedial lesions were shown to acquire a new "set-point" which required larger amounts of fat to be deposited in the adipose depots (Lepkovsky and Yasuda, 1966). The lesions in the hypothalamus were postulated either to act centrally, evoking urges to eat (appetite), or to act indirectly, and upset homeostasis in the adipose tissue, and thereby elicit the accumulation of abnormally large amounts of fat in the depots. Lepkovsky (1973) supports the latter

hypothesis, and maintains that the hypothalamus regulates the amount of fat deposition by giving the adipose tissue in the avian species a "set-point control" to regulate its own homeostasis. The amount of fat in the adipose depot is regulated by lipolysis and lipogenesis. When the amount of fat in the adipose tissue increases above the "set-point" level, lipolysis is accelerated, the rate of absorption of food is decreased and food intake decreases. Conversely, when the amount of fat in the adipose tissue is below the "set-point", lipogenesis and deposition of fat in the adipose tissues increases, absorption of food is accelerated and food intake increases (Lepkovsky, 1973). Lesions in the ventromedial area of the hypothalamus of cockerels accordingly change the "set-point" of the control system of the adipose tissues which then require more fat (Lepkovsky and Yasuda, 1966). On the other hand, investigations by Lepkovsky and Furuta (1971) showed that excessive fat deposition resulting from force-feeding excessive amounts of food, without changing the "set-point" in the adipose tissue, results in the deposition of fat above the level required by the "set-point" in the adipose tissue. When the birds were fed ad libitum, they stopped eating until the amount of fat in the adipose tissues decreased toward the level established by the "set-point", at which time the birds started to eat and ate normally until the amount of fat in the depots returned to normal (Lepkovsky and Furuta, 1971).

Similar results have been reported for normal rats made obese by force feeding (Cohn and Joseph, 1962) and by electrical stimulation of the lateral hypothalamus (Steinbaum and Miller, 1965).

Relatively few studies have been undertaken to determine the effects of nutritional manipulation on the development of adipose tissue in chickens. March and Hansen (1977) demonstrated that restricting nutrient intake by dietary dilution from hatch until 6 weeks of age, decreased growth rate and lipid accumulation in the retroperitoneal adipose depot of both broiler-type and White Leghorn chicks (although adipocyte multiplication continued). When feed was withheld from newly hatched chicks, a loss of lipid from the retroperitoneal adipose depot did not occur until the subcutaneous adipose depots had undergone considerable depletion (March and Hansen, 1977). Upon refeeding, multiplication of adipocytes occurred in 20 hours, at a reduced rate compared to the controls, but rapidly attained a normal rate. On the other hand, when fasting was imposed on 10-day old chicks, refeeding did not immediately stimulate adipocyte multiplication although the lipid lost from the cells was rapidly repleted (March and Hansen, 1977).

Pfaff and Austic (1976) showed that, although feeding low energy or high protein diets to White Leghorn chickens, limited adipose tissue accumulation and delayed the time at which hyperplastic growth ceased, it did not alter the cellularity of the retroperitoneal adipose depot at maturity. Feeding diets low in energy or high in protein from hatch to 9.5 weeks of age, or from hatch to 22 weeks of age, tended to lower the level of fat accumulation in the retroperitoneal adipose depots by 22 weeks of age. In both cases, the adipose depot cellularity was similar among groups; the reduced adipose depot stature resulted from a reduced adipocyte size (Pfaff and Austic, 1976). It is not known, hoever, whether these effects of early diet would persist indefinitely.

At variance with the finding of Pfaff and Austic (1976) that adipose depot growth could be reduced by the feeding of either low energy or high protein diet. Cunningham and Morrison (1976) reported that the energy level of the diet had no effect at sexual maturity on retroperitoneal adipose depots weights, liver water, fat, protein, total body water or ash, of White Leghorn pullets. Survey of the literature did not reveal further reports on the development of adipose tissue in the avian species.

EXPERIMENTAL

I. Experimental Treatments

Two-hundred and fifty-six day-old broiler-type birds (Hubbard), of mixed sexes were used. The diet, (Table 1), was calculated to contain 15.2% of protein and 2861 kcal of metabolizable energy per kg. The diet was fed throughout the entire experiment. Oyster-shell was provided ad libitum after the onset of lay.

A. Management of birds

Birds were given free access to water immediately after hatch; however feed was withheld for 2-3 days (48-72 hrs), depending upon the treatment. The birds were then randomly distributed into the following treatments. Each treatment was imposed on 28 birds distributed into two replicate lots of 14 birds each.

(1) Treatment 1, the control lot was fed 48 hours after hatch, and fed ad libitum throughout the experimental period.

(2) Treatments 2-10, the remaining chicks were given free access to feed at 72 hours (day 4) and fed for 24 hours. Following 24 hours feeding, the feed was withdrawn for an additional 24 hours (water ad libitum), and the birds were refed on

the 6th day. On day 7 and thereafter, the birds were restricted to 30 minutes feeding/day.

Treatment 2, subjected to dietary restriction for an additional week; ad libitum feeding from 2 weeks of age.

Treatment 3, subjected to dietary restriction for an additional 2 weeks; ad libitum feeding from 3 weeks of age.

Treatment 4, subjected to dietary restriction for an additional 3 weeks; ad libitum feeding from 4 weeks of age.

Treatment 5, subjected to dietary restriction for an additional 4 weeks; ad libitum feeding from 5 weeks of age.

Treatment 6, subjected to dietary restriction for an additional 5 weeks; ad libitum feeding from 6 weeks of age.

Treatment 7, subjected to dietary restriction for an additional 7 weeks; ad libitum feeding from 8 weeks of age.

Treatment 8, subjected to dietary restriction for an additional 9 weeks; ad libitum feeding from 10 weeks of age.

Treatment 9, subjected to dietary restriction for an additional 11 weeks; ad libitum feeding from 12 weeks of age.

Treatment 10, subjected to dietary restriction for an additional 13 weeks; ad libitum feeding from 14 weeks of age.

The birds were reared in battery brooders. Water was given ad libitum. The birds were transferred to large battery cages following 2 weeks ad libitum feeding after termination of the imposed feed restriction. The birds were housed 4-5 birds per cage. Males and females were segregated at 15 weeks of age, except for those birds on treatments 9 and 10 whose sexual development had been retarded. Treatments 9 and 10 were sexed at 17 weeks of age, and as with the previous treatments, the birds were held in their respective cages for the duration of the experiment.

The birds placed on dietary restriction were weighed weekly, before the daily feeding period, until 14 weeks of age and then bi-weekly without the removal of feed. The control birds were weighed weekly without the removal of feed until 14 weeks of age, and bi-weekly thereafter.

The age at which birds on each treatment commenced laying was noted. Egg production was recorded from 24 weeks of age until the end of the experimental period. Egg weights for treatments 1, 2, 9 and 10 were measured for 3 weeks between 36 and 38 weeks of age.

Mortality was recorded for each treatment. Severely cropbound birds, whose growth rate was impaired, were killed and included in the figures of mortality. Similarly, birds with weak legs, whose growth rate was impaired, were killed and included in the figures for mortality.

II. Experimental Materials and Methods

A. Determination of the average adipocyte diameter at 17-19 weeks of age

Between 17 and 19 weeks of age four pullets from treatment 1, (control), and five pullets from treatments 9 and 10 respectively, were selected at random for biopsy of adipose tissue. On each day of adipose biopsy, three birds were examined, one bird randomly selected from each treatment.

The birds were anesthetized by intravenous injection of sodium pentobarbital at a dose of 30 mg per kilogram of body weight (Clarkson et al., 1957). Adipose tissue weighing 400-600 mg was removed from the retroperitoneal depot of each bird. The adipose tissue was removed from the midline of the retroperitoneal depot, 2-5 cm distal to the point of adhesion with the musculature of the abdominal wall. The small incision in the abdominal wall was sutured, and after the birds had regained consciousness, the birds were returned to the cages from which they had been taken. The entire procedure lasted approximately one hour per bird.

Two samples of the adipose tissue from each bird, weighing 200-300 mg each were incubated in 3 ml of Krebs-Ringer bicarbonate medium ($CaCl_2$ concentration 1.22%) containing, per ml: 3 µmoles glucose, 40 mg bovine serum albumin (Sigma Chem. Co., Fraction V), and collagenase from Clostridium histolyticum (Sigma Chem. Co.) at a concentration of 10 mg/g

adipose tissue (DiGirolamo et al., 1971). The medium was gassed for 5 minutes with 95% O_2-5% CO_2. The pH of the medium was adjusted to 7.4. The incubation was carried out in a shaker bath at 37°C for 1½ hours, at 20-30 strokes/minute. The glassware was siliconized with Dri-cote (Fisher Scientific Co.).

The isolation, washing (in medium lacking collagenase), collection and dispersing of adipocytes were done following the procedure described by Rodbell (1964a), but without centrifugation, Martinsson (1968).

After shaking, the liberation of cells was manifested by an increase in the turbidity of the medium. Fragments of tissue still remaining after the treatment were removed with forceps. The incubation tubes were allowed to stand and the adipocytes rose to the surface; the underlying solution was removed by aspiration and replaced by fresh medium (not containing collagenase). This was repeated three times. The fat liberated by the procedure floated to the surface and was partially removed. Complete removal was not required since the fat layers appeared to be intact during rotation of the vessel for removal of adipocytes.

The final cell suspension was immediately drawn up into 18 cm of plastic tubing (polyethylene) I.D. 1.14 mm, attached to a 2 ml calibrated syringe and then discharged into vials. The volume of the tubing was sufficient to contain 1.0 ml of cell suspension. The 1 ml aliquot of isolated fat cell suspension was added to 4 ml of warm medium in a vial containing 1 mg of methylene blue. After staining for 2-5 minutes, successive 0.2-0.4 ml aliquots of the stirred suspension of stained cells

were placed on siliconized glass slides and examined microscopically. The insertion of a micrometer disc in a focussing eyepiece produced a projected caliper scale. At a magnification of 160X, the micrometer scale was calculated to have a constant interval of 6 μm between the smallest calibrations. The free adipocytes floating on the surface of the medium, were recognized by their spherical shape, stained cytoplasm and stained nucleus; all features readily distinguished the adipocytes from occasional droplets of floating lipid.

Six slides of each fat cell suspension were prepared and 300-400 adipocytes were measured by bringing the cells into the caliper field with systematic motion of the stage control knobs. The cells were aligned on the caliper scale, the equitorial plane of the cell was brought into focus, and the adipocyte diameter was determined. In this fashion, cell diameters between 24 and 150 μm were recorded in classes with midpoints of successive 6 μm multiples to provide a frequency distribution of diameters in 21 categories of size. Cells less than 24 μm were not considered in the frequency distribution because of the difficulty in distinguishing them from small lipid droplets.

The mean adipocyte diameter and standard deviation were determined for each frequency distribution of 300-400 adipocytes using the appropriate formulas (Zar, 1974). Each mean and standard deviation of 300-400 cells, represented measurements of one tissue fragment or cell suspension. The average adipocyte diameter for an adipose depot was taken as the mean of the two mean adipocyte diameters of the duplicate samples of adipose

tissue removed upon biopsy. The slides were coded so that the experimental treatment of the birds from which the cells were taken was unknown at the time the cells were measured.

Many of the preparations of adipocytes were found to contain clumps of 10 to 100 small adipocytes less than 30 μm in diameter. The number of these small clumped cells were recorded in two size categories, viz. 6-12 μm and 18-30 μm, whenever they were seen.

B. Removal of organs and tissues at 40-43 weeks of age for determination of tissue proportions and for determination of adipocyte diameter and cellularity

At the conclusion of the experiment (40-43 weeks of age), birds from treatments 1, 2, 9 and 10 were killed for the study of tissue proportions, adipocyte diameter and adipocyte cellularity. On any one day of examination, 3 birds were randomly selected (one from each treatment), weighed, and killed by cervical dislocation. The adipose tissue was immediately removed for determination of adipocyte diameter. Two adipose depots with well defined boundaries were selected for examination. The adipose depot lying the length of the Muscularis sartorius (M. sartorius depot) was removed and weighed. Immediately after removal, samples of tissue weighing 200-300 mg were cut from the distal portion of the depot, rinsed with warm isotonic saline (37°C) and blotted dry (Hirsch and Gallian, 1968), and weighed. Three samples were frozen at -20°C for subsequent lipid extraction, and two were placed in warm medium (37°C) containing collagenase for isolation of adipocytes as described above.

The retroperitoneal adipose depot was similarly removed and weighed. The retroperitoneal adipose tissue that was removed and immediately weighed was the fat that surrounds the gizzard and lay between the abdominal muscles and the intestines. The layer of adipose tissue extended within the ischium and surrounded the bursa of Fabricius and cloaca where it was attached to the abdominal muscles in the area of the bursa of Fabricius and the cloaca (Kubena et al., 1974).

After the retroperitoneal adipose depot was removed and weighed, samples of tissue weighing 200-300 mg were removed from the area to the right of the mid-line near the attachment of the adipose depot to the gizzard. Samples of adipose tissue from this area were preferred for morphological studies because this region was the least damaged upon removal of the abdominal depot. Samples of adipose tissue taken from other areas had considerable cell breakage. The samples of tissue were rinsed with warm isotonic saline, and blotted dry, and weighed. Three samples were frozen at -20^{o}C for subsequent lipid extraction. Two samples were placed in warm medium containing collagenase (37^{o}C), for isolation of adipocytes as described above.

Following the removal of the adipose tissue, specific tissues and organs were removed for examination. The left pectoral muscle (Muscularis pectoralis major), the liver, ovary and oviduct were removed and immediately weighed. The left tibiotarsus (Tibia), was also removed, scraped clean, placed in an oven for 48 hours to dry and then weighed. In addition, the length of the tibiotarsus was measured. Tissue and organ weights, including the weights of the adipose depots, were recorded and expressed as percentages of the body weight.

C. Determination of the average adipocyte diameter and cellularity at 40-43 weeks of age

An estimate of the number of adipocytes in an adipose depot can be obtained by dividing the total lipid content of the adipose depot by the average lipid content of the adipocytes. The lipid content of the average adipocyte was derived by multiplying the mean adipocyte volume by the density of the lipid. The mean adipocyte volume of an adipose depot was derived from an estimate of the mean adipocyte diameter using Goldrick's formula (Goldrick, 1967). This method is based on two assumptions: that all or most of the adipose tissue lipid is intracellular (Rodbell, 1964); and that the isolated adipocytes assume spherical configuration so that determination of the transverse diameter of the cell essentially represents the diameter of the lipid droplet of the cell (Reh, 1953; Di Girolamo et al., 1971; and Martinsson, 1968).

The two important operations required for determination of adipocyte cellularity are:

1) the determination of the mean cell volume, and
2) the determination of the lipid content of the adipose depot.

1. Determination of the average adipocyte diameter and the mean adipocyte volume of the adipose depots

Adipose tissue samples were excised from both the retroperitoneal and the M. sartorius depots. Two samples from each depot were removed and incubated in medium containing collagenase, as previously described. The isolation and dispersing of the adipocytes were performed in a fashion similar to that mentioned earlier, except the adipocytes were not stained and the average adipocyte diameter was determined using photomicroscopy.

From each adipose tissue sample the isolated adipocytes were dispersed on six siliconized slides and 3 or 4 photomicrographs were taken per slide. Photographs were taken using yellow light and a total magnification in the film plane between 65 and 75X. Under these conditions, the depth of the field was large compared with the mean cell radius of the adipocyte populations, so that all cells, regardless of their size, appeared clearly defined, indicating that their equitorial planes were in focus. A micrometer scale was photographed after every 12th picture to determine the correct magnification.

Oval and circular areas in the photographs, were shown to represent membranes of intact fat cells floating on the surface. The adipocytes were translucent and could be easily distinguished from the more transparent lipid droplets which were occasionally present. A membrane in clear definition was taken to indicate that the plane of focus coincided with the maximal cell diameter, and only these cells exhibiting this characteristic were evaluated. Measurement of cells was confined to the central regions of each photograph (all cells being measured), and cells bordering on the perimeter of the photograph ignored. This

was done to avoid any distortion of cell size due to the developing and printing procedures.

Adipocyte diameters between 24 and 150 μm were recorded in classes with midpoints of successive 7 μm multiples to provide a frequency distribution in 18 categories of size. Cells less than 24 μm were not included in the frequency distribution because of the difficulty of distinguishing them from small lipid droplets. The slides were coded so that the experimental treatment of the birds from which the cells were taken was unknown at the time the cells were measured.

Adipocyte diameters were measured by hand and the mean adipocyte diameter and standard deviation for each cell suspension was determined using the appropriate formulas (Zar, 1974). The mean adipocyte diameter was determined from the diameters of 300-400 cells.

The mean adipocyte volume was then calculated from the mean adipocyte diameter and standard deviation using Goldrick's formula, (Goldrick, 1967); and the mean adipocyte weight was determined by using the density of triolein (0.915). Adipose cell volume was calculated on the assumption that the adipocytes were spherical from the mean diameter ($\bar{x}$) and the variance of the diameter (σ^2) using the formula, $(\pi/6)(3\sigma^2+\bar{x}^2)\bar{x}$. [1] Adipocyte volume was expressed as picoliters (pl) per adipocyte.

---

1 The diameter D, is a normally distributed variable, but its cube is skewed. Hence, the arithmetic mean of (D^3) cannot be used in the calculation of cell volume. The expected volume (E) of $(D^3) = E(D^3)$, where $\bar{x}$ is the normally distributed variable and $\bar{x}$, σ, and σ^2 are, respectively, the mean, standard deviation and variance.

$$(E(D^3) = \int_{-\infty}^{+\infty} (\bar{x}^3) \left(\frac{e}{\sigma \sqrt{2\pi}} \quad \frac{-\frac{1}{2}(x-\bar{x})^2}{\sigma 2}\right) dx$$

This resolves to $E(D^3)=(3\sigma^2+\bar{x}^2)\bar{x}$. Thus E(cell volume) = $(\pi/6)(3\sigma^2+\bar{x}^2)\bar{x}$. (cited from Goldrick, 1967).

The average cell volume for each depot was determined as the mean of the mean cell volume of the two tissue fragments (duplicates) per adipose depot.

The results of this method of determining cell volume for the purpose of determining cellularity are, according to Lavau et al. (1977), in excellent agreement with the more elaborate osmium fixation method of Hirsch and Gallian (1968).

2. Determination of the lipid content of the adipose tissue

Three aliquots of adipose tissue weighing 200-300 mg from each adipose depot were placed in chloroform-methanol (2:1) and the total lipid was extracted according to the procedure described by Folch et al., 1957. This procedure consisted of two successive steps. In the first step the tissues were homogenized in a Potter-Elvehjem type homogenizer with a 2:1 chloroform-methanol mixture (w/v) to a final dilution 20-fold the volume of the tissue sample. The homogenate was then filtered through fat-free paper into a glass-stoppered vessel. The second step consisted of mixing the extract with 20% of its volume of a specified salt solution (Folch et al., 1957), and allowing the mixture to separate into two phases. The lower phase contains all the tissue lipids. As much of the upper phase as possible was removed by aspiration and complete removal was accomplished by rinsing the interphase three times with small amounts of pure solvents upper phase according to Folch et al. (1957). Finally, the lower phase

and the remaining rinsing fluid were made into a simple phase by the addition of methanol and the resulting solution was evaporated in a water bath.

The lipid content of the tissue was determined on triplicate aliquots of extract by microgravimetric determination after complete evaporation of the solvent. The ratio of lipid to wet weight of the tissue was expressed as a percentage.

The defatted dry residue (DDR) of adipose tissue was determined by microgravimetric determination of the tissue fragment, removed from the first extract, placed in 10 ml of fresh solvent for an additional 24 hours, removed from the solvent and dried overnight in an oven. The ratio of DDR to wet weight of adipose depot was expressed as a percentage.

3. Determination of the adipocyte cellularity of the adipose depots

The estimate of the number of adipocytes in a sample of adipose tissue was obtained by dividing the total lipid content of the tissue by the average lipid content of the adipocytes. The lipid content of the average adipocyte was derived by the mean cell volume X the density of the lipid (taken as the density of triolein, 0.915). The operations involved are illustrated in Figure 1.

Figure 1. Summary of procedures involved in the determination of adipocyte size and the cellularity of adipose tissue.

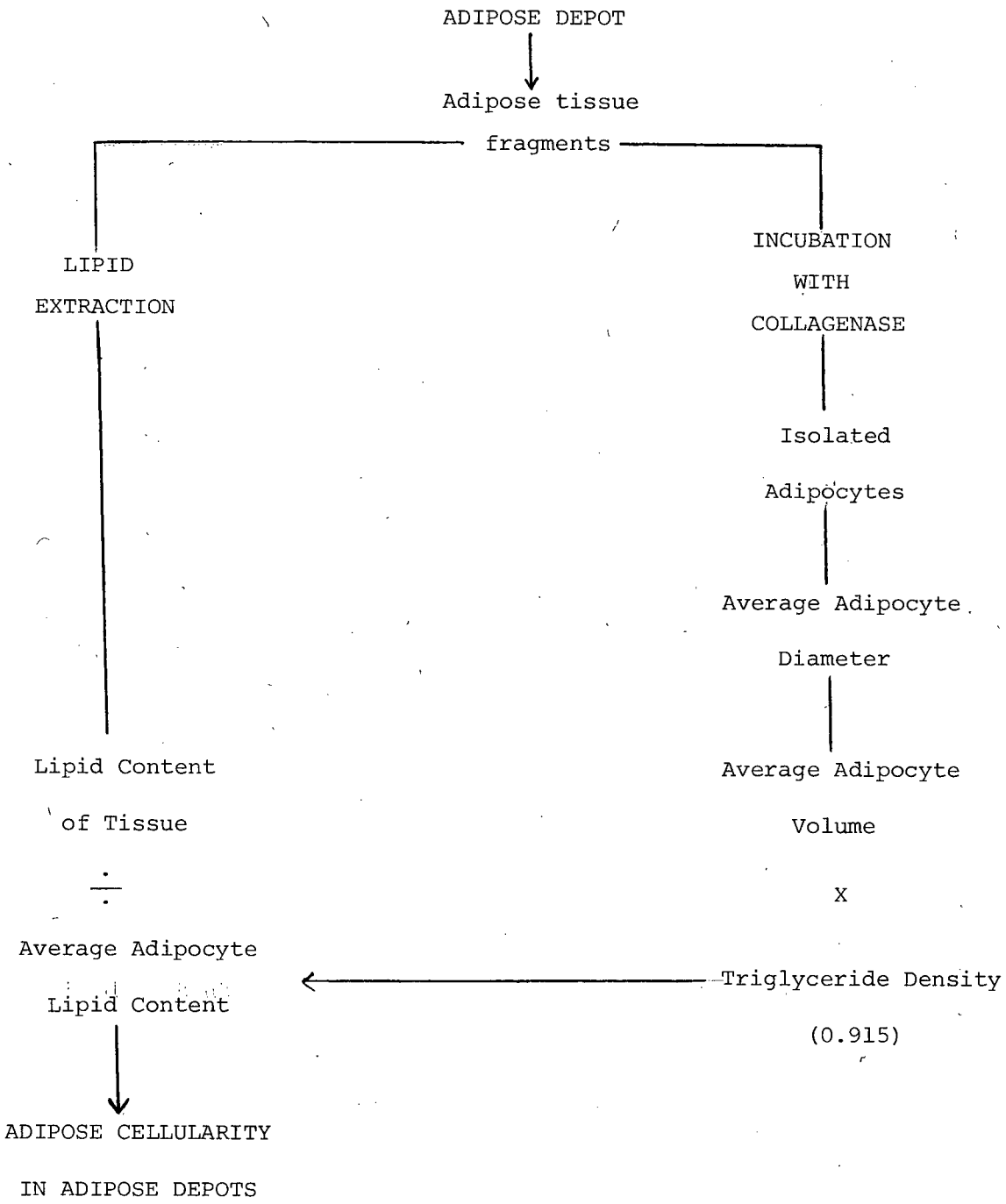


Table I. Composition of experimental diet.

Ingredients	%
Wheat	31.0
Corn	31.0
Oats	20.0
Soybean meal	12.8
Dehydrated cereal grass	2.0
Iodized salt	0.5
Limestone	1.2
Calcium phosphate	1.5
Micronutrients	*

* per kg of diet: manganese sulfate 150 mg, zinc oxide 62 mg, choline chloride 1320 mg, riboflavin 3 mg, vitamin B 12 13.2 mcg, vitamin A 4400 I.U., vitamin D_3 440 I.C.U., Amprol** 125 mg.

** Amprol (coccidiostat) supplied courtesy of Merck, Sharp and Dohme Canada Ltd.

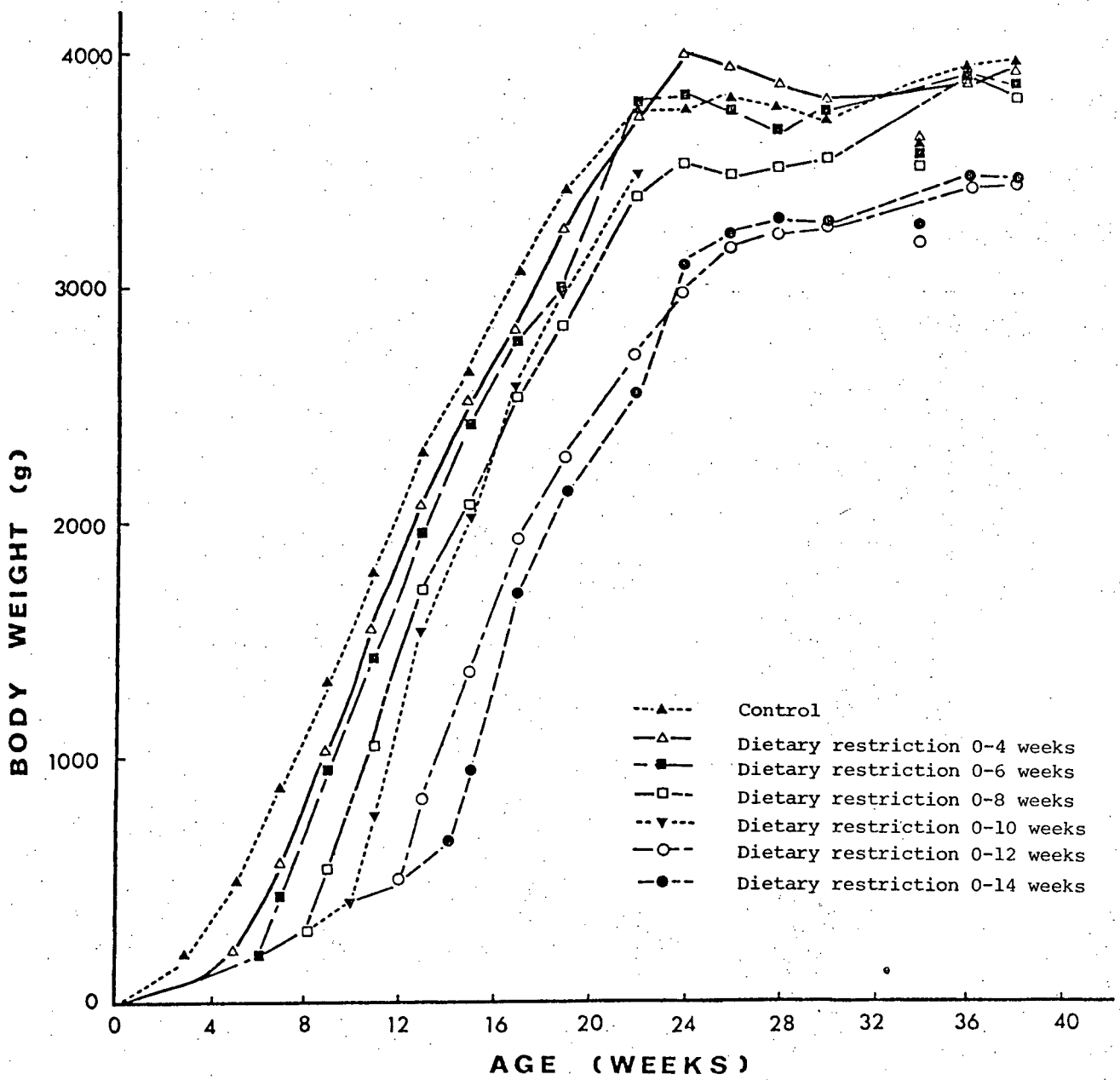
RESULTS AND DISCUSSION

I. The effects of early dietary restriction on growth rate and mature body weights of male and female broiler-type birds

The body weights of the birds subjected to dietary restriction and of the birds fed ad libitum from day 2 (controls), are shown in Table II and in Figures 2 and 3. The results indicate that restriction of feed intake severely reduced growth rate in both male and female birds. In birds subjected to feed restriction until 14 weeks of age, the body weights of the male birds were 19% of the body weights of their control counterparts; and the body weights of the female birds were only 27% of the body weights of their control counterparts.

At 14 weeks of age the body weights of the control males were significantly greater than the body weights of the control females. The greater growth rate of male birds fed ad libitum (control) resulted in a difference in body weights appearing at week 5. It was interesting to note that male and female birds subjected to dietary restriction had similar body weights during the period of dietary restriction (Table II). The similarity in the body weights between male and female birds placed on dietary restriction was surprising, since male birds normally have a greater growth rate (Edwards et al., 1973); and were therefore expected to be able to eat more and have a greater body weight than the slower growing females. The growth rates of the female and male birds following

Figure 2. Growth rates of female broiler-type birds subjected to dietary restriction for different periods of time.



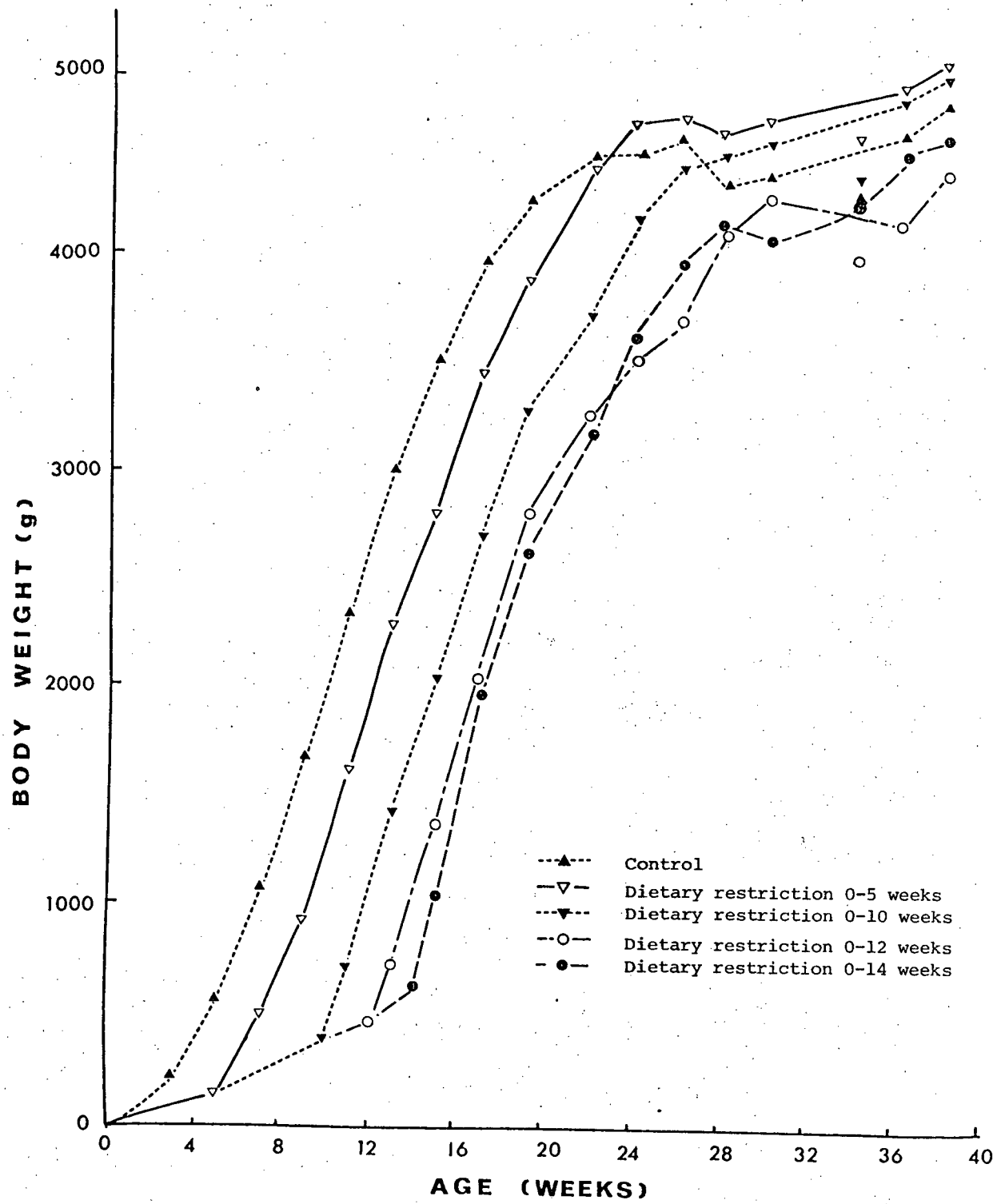


Figure 3. Growth rates of male broiler-type birds subjected to dietary restriction for different periods of time.

Table II. The effects of dietary restriction on the body weights of male and female broiler-type chickens to 14 weeks of age.

Period of Dietary Restriction (weeks)	Age of Birds (weeks)	Average Body Weights of Birds Subjected to Dietary Restriction From 0-14 Weeks (g) Males	Females	Average Body Weights of Control Birds (g) Males	Females
0-2	2	$54^{a}\pm8(51)$*	$54^{a}\pm7(73)$*	$99^{b}\pm9(12)$*	$103^{b}\pm16(9)$*
0-3	3	$72^{a}\pm12(43)$	$71^{a}\pm10(67)$	$202^{b}\pm27(12)$	$190^{b}\pm38(9)$
0-4	4	$104^{a}\pm19(37)$	$103^{a}\pm15(57)$	$342^{b}\pm58(12)$	$320^{b}\pm63(9)$
0-5	5	$139^{a}\pm26(34)$	$141^{a}\pm21(45)$	$565^{c}\pm84(12)$	$489^{b}\pm86(9)$
0-6	6	$185^{a}\pm41(27)$	$187^{a}\pm28(37)$	$816^{c}\pm114(12)$	$679^{b}\pm98(9)$
0-7	7	$259^{a}\pm58(27)$	$255^{a}\pm42(29)$	$1074^{c}\pm126(12)$	$887^{b}\pm105(9)$
0-8	8	$289^{a}\pm63(27)$	$290^{a}\pm47(29)$	$1281^{c}\pm161(12)$	$1077^{b}\pm91(9)$
0-9	9	$339^{a}\pm82(21)$	$343^{a}\pm59(24)$	$1674^{c}212(12)$	$1333^{b}\pm126(9)$
0-10	10	$396^{a}\pm104(21)$	$415^{a}\pm75(24)$	$2030^{c}\pm217(12)$	$1591^{b}\pm120(9)$
0-11	11	$414^{a}\pm96(13)$	$433^{a}\pm65(21)$	$2330^{c}\pm269(12)$	$1792^{b}\pm116(9)$
0-12	12	$481^{a}\pm128(13)$	$508^{a}\pm80(21)$	$2695^{c}\pm331(12)$	$2057^{b}\pm123(9)$
0-13	13	$575^{a}\pm167(7)$	$584^{a}\pm81(10)$	$2997^{c}\pm353(12)$	$2294^{b}\pm133(9)$
0-14	14	$642^{a}\pm195(7)$	$667^{a}\pm107(10)$	$3296^{c}\pm278(10)$	$2498^{b}\pm127(9)$

* Number of birds

abcd Values with the same superscript for body weights are not significantly different at $P < 0.05$.

the different periods of dietary restriction are illustrated in Figure 2 and Figure 3 respectively.

Figure 2 illustrates the growth rates of female birds subjected to dietary restriction. The data from treatments 2, 3 and 5 were omitted from this graph for simplification. The final body weights at 38 weeks of these omitted treatments (2, 3 and 5) are given in Table IIIa.

Figure 3 illustrates the growth rates of male birds subjected to dietary restriction. Treatments 2 & 3 and treatment 7 have been similarly omitted. The final body weights at 38 weeks of these omitted treatments (2, 3 & 7) are given in Table IIIb. The data from treatments 4 and 6 were omitted from all results because these treatments only contained one bird at the end of the experimental period.

At 33 weeks of age the birds in all treatments were accidentally left without feed for 3-4 days. The birds were unable to regain the resultant weight loss by 34 weeks of age and so the body weight at this time, although recorded on Figures 2 & 3, were not included as points on the growth curves. The body weights of the birds in the various experimental treatments by 36 weeks of age had returned to values similar to those observed before 33 weeks. At 38 weeks of age the body weights seemed unaffected by the period of feed deprivation which occurred at 33 weeks of age. Investigations by Douglas and associates (1978), demonstrated that feed deprivation of up to 72 hours at 65 weeks of age had no effect on final body weights of White Leghorn pullets following refeeding. These findings, coupled with the data in Figures 2 & 3, indicate

Table IIIa. Effects of early dietary restriction on body weights of female broiler-type chickens at 38 weeks of age.

	T R E A T M E N T S *								
	1	2	3	4	5	6	7	9	10
Average Body Weights (g)	Control	Feed Restriction (0-2 weeks)	Feed Restriction (0-3 weeks)	Feed Restriction (0-4 weeks)	Feed Restriction (0-5 weeks)	Feed Restriction (0-6 weeks)	Feed Restriction (0-8 weeks)	Feed Restriction (0-12 weeks)	Feed Restriction (0-14 weeks)
	(7)***	(5)	(6)	(9)	(6)	(6)	(4)	(6)	(7)
Mean	3980[b]	3954[b]	3976[b]	3953[b]	3882[b]	3873[b]	3833[b]	3453[a]	3543[a]
S.D.	±254	±278	±470	±449	±337	±341	±331	±344	±202

Table IIIb. Effects of early dietary restriction on body weights of male broiler-type chickens at 38 weeks of age.

	T R E A T M E N T S **							
	1	2	3	5	7	8	9	10
Average Body Weights (g)	Control	Feed Restriction (0-2 weeks)	Feed Restriction (0-3 weeks)	Feed Restriction (0-5 weeks)	Feed Restriction (0-8 weeks)	Feed Restriction (0-10weeks)	Feed Restriction (0-12 weeks)	Feed Restriction (0-14 weeks)
	(8)***	(5)	(5)	(5)	(3)	(5)	(2)	(3)
Mean	4704[a]	4691[a]	4880[a]	4735[a]	4375[a]	4841[a]	4382[a]	4546[a]
S.D.	±424	±530	±635	±413	±899	±504	±1524	±482

* Treatment 8 had only 1 bird (data not included)

** Treatments, 4 and 6 had only 1 bird (data not included)

*** Number of birds

ab Values with the same superscript for each body weight are not significantly different at $P < 0.05$.

that the final body weights at 38 weeks of age were unaffected by the earlier feed deprivation occurring at 33 weeks.

The effects of the early dietary restriction on the mature body weights of the female and male birds at 38 weeks are given in Tables IIIa and IIIb respectively. Dietary restriction in female birds, as previously mentioned, retarded growth during the period when restriction was practised (Table II). After ad libitum feed was instituted, however, the female birds in treatments 2-8 were able to compensate during the period of dietary rehabilitation, and at 38 weeks of age achieved body weights similar to the body weights of the control birds (Table IIIa). A certain degree of caution must be exercised in interpretation because the data from treatment 8 were not included in the results. Therefore it cannot be conclusively shown that these birds restricted from 0-10 weeks of age were unaffected by the dietary restriction. However, the body weights of these birds at 22 weeks of age (data from 3 birds), Figure 2, were similar to the body weights of treatment 7 (feed restricted from 0-8 weeks). This indicated that the birds were able to compensate following dietary restriction and achieve body weights similar to other treatments in which the birds fully compensated for the early dietary restriction. It seems likely, therefore, that the birds restricted from 0-10 weeks (treatment 8), were also able to fully compensate for the early growth restriction.

The female birds in treatments 9 and 10 had body weights at 38 weeks of age which were significantly lower than the body weights of the female control birds (Table IIIa). The significant reduction in the

reduced body weight of those birds restricted from 0-12 and from 0-14 weeks implies that the early dietary restriction had a permanent effects on the development of these birds, in contrast to the effect of restriction for lesser periods of time. These results support the hypothesis of McCance (1976), that there are "critical periods of growth" during an animal's life which determine the ultimate size and composition of the animal. The results of this experiment demonstrate that female broiler-type birds subjected to dietary restriction for up to 10 weeks of age, when fed ad libitum, were able to compensate fully, showed "catch-up" growth and attained a body size similar to the fully nourished control birds. However, when the dietary restriction was extended from 0-12 or from 0-14 weeks of age, despite early compensatory growth, the female birds were not able to achieve their full genetic size, indicative of the control birds. The existence of "critical periods of growth", beyond which complete compensation is not possible, has also been observed in pigs and rats (McCance, 1976).

The early dietary restriction did not appear to reduce the final body weights of male birds, Figure 3, Table IIIb. The male birds restricted from 0-12 and 0-14 weeks of age seemed to compensate during the rehabilitation period and at 38 weeks, achieve body weights which were similar to the body weights of the control birds. However, the small number of birds in either treatment 9 or treatment 10 prevents a definite conclusion as to whether early dietary restriction had any permanent effect on the mature body weights of male birds. The high

mortality, 67%, in male birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age (Table V) indicates that this dietary restriction had an adverse effect on the mortality of these birds. The possibility exists that the dietary restriction imposed in treatments 9 & 10 caused an increase in male mortality, with the result that only those birds which were able to fully compensate for the early dietary restriction were able to survive.

In female birds, Figure 2, growth rates following dietary restriction were constant until 22 weeks of age (treatments 2-8) or 24 weeks of age (treatments 9 & 10). In the former case the inflection point for the change in growth rates in treatments 2-6 occurred between 3500 and 4000 g, and in treatment 7 between 3400-3500 g. The inflection in the growth curves correspond to the times at which the birds reached sexual maturity as judged by the onset of lay, Table IX. Treatments 2-7 came into lay at 18-22 weeks corresponding to their inflection points; whereas treatments 9 & 10 came into lay between 22-24 weeks of age corresponding to their inflection points.

A striking difference in the nature of the inflection points was observed between those birds unaffected permanently by dietary restriction and those birds permanently affected by dietary restriction. Those birds, apparently unaffected permanently by dietary restriction (treatments 2-7), had a very sharp change in their growth rate at 20-24 weeks of age whereas those birds permanently affected by the dietary restriction (treatments 9 & 10) had a very gradual alteration of their growth

rate. The sharp change in growth rate of those birds unaffected by dietary restriction was similar to the change in growth rate at sexual maturity observed in the control birds (treatment 1). The sharp change in growth rate at sexual maturity of the female broiler-type birds, fully-nourished or apparently unaffected permanently by the dietary restriction is not characteristic of other strains of chickens (McCance, 1960; Lister et al., 1966). The sharp change in the rate of growth at sexual maturity observed in the broiler-type chickens, may therefore be characteristic of the faster growth rate of female broiler-type birds.

In male birds the change in growth rates at sexual maturity (22 weeks), was not as pronounced as it was in females (Figure 3). The inflection in the growth curves of male birds subjected to dietary restriction occurred at higher body weights: 4500-5000 g for treatments 2-8, and 4000-4500 g for treatments 9 and 10. Dietary restriction had a pronounced effect on the age at which the inflection in growth rate occurred. The more severe dietary restriction (treatments 9 & 10) delayed the occurrence of the inflection points to 28-30 weeks of age; compared with the inflection point at 20 weeks of age for the control birds, and 24 weeks of age for those birds restricted from 0-5 weeks of age. The difference in the shape of the inflection points between male (gradual), and female birds (sharp), fully nourished or permanently unaffected by dietary restriction, may result in the female birds, from the sudden onset of of egg production and the physiological processes associated with this newly acquired reproductive condition.

A comparison between the rates of growth of birds following termination of the dietary restriction is given in Tables IVa and IVb.

The growth rate was calculated from the slope of the lines, m, in Figure 2 and Figure 3 respectively, where $y = mx + b$. The units are in grams gained per day.

In female birds, Figure 2, the growth rate on all treatments was fairly constant below 3000 g. The rate of growth following dietary restriction was accordingly investigated between two levels of body weight: 700 to 1700 and 1700 to 3000 g (Table IVa). The growth rates for birds restricted for the longest period of time tended initially to be the greatest as evidenced by the larger slope. The control birds grew at an average growth rate of 32 g/day between 700 and 1700 g of body weight, whereas those birds on dietary restriction from 0-10, 0-12 and 0-14 weeks of age grew at a rate of 49, 39 and 50 g/day respectively, for the same gain in weight. However, this initial greater growth rate in birds subjected to dietary restriction was only temporary, and between the body weights of 1700 and 3000 g the rates of growth in these birds decreased and were similar to that of the control birds. In fact the average growth rate in the birds restricted from 0-12 and from 0-14 weeks of age was eventually lower than that of the control birds (Figure 2, Table IVa) resulting in mature body weights significantly lower than the controls (Table IIIa).

Growth rates following dietary restriction in the male birds followed a similar pattern to those observed in the female birds (Table IVb). In this Table, growth rates of male birds following dietary restriction were calculated for 2 levels of body weight from 700 to 1700 g

Table IVa. Growth rate, (m)*, of female broiler-type chickens following periods of dietary restriction.

Body Weight Gain (g)	Average Growth Rate, (m), for gain in body weight						
	Control (9)** g/day	Feed Restriction (0-4 weeks) (11) g/day	Feed Restriction (0-6 weeks) (8) g/day	Feed Restriction (0-8 weeks) (5) g/day	Feed Restriction (0-10 weeks) (3) g/day	Feed Restriction (0-12 weeks) (8) g/day	Feed Restriction (0-14 weeks) (8) g/day
700-1700	32	37	36	42	49	39	50
1700-3000	30	32	29	27	33	23	27

Table IVb. Growth rate, (m)*, of male broiler-type chickens following periods of dietary restriction.

Body Weight Gain (g)	Average Growth Rate, (m), for gain in body weight				
	Control (12)** g/day	Feed Restriction (0-5 weeks) (6) g/day	Feed Restriction (0-10 weeks) (8) g/day	Feed Restriction (0-12 weeks) (5) g/day	Feed Restriction (0-14 weeks) (5) g/day
700-1700	39	44	51	44	60
1700-4000	41	39	34	27	33

* (m) is the rate of growth (weight gain per unit time), representing the slope of the lines in Figure 1 and Figure 2 respectively, where $y = mx + b$

** Number of birds.

and from 1700 to 4000 g of body weight. Male birds achieved heavier body weights and so the latter body weight interval was extended to 4000 g instead of the 3000 g limit established in the growth rate for female birds.

The growth rates of males following dietary restriction were initially greater than those of the control birds for the gain in weight between 700 and 1700 g. Birds restricted from 0-8, 0-12, and from 0-14 weeks had growth rates of 51, 44 and 60 g/day respectively compared to the growth rate of 39 g/day of the control birds, for the gain in weight between 700 and 1700 g. However, this greater growth rate for birds subjected to dietary restriction was only temporary and the growth rate decreased and became similar to, or less than, the growth rate of the control birds for the gain in weight between 1700 and 4000 g (Table IVb). These results confirm the earlier observations of McCance (1960) and Lister et al. (1966), who demonstrated that after a prolonged period of undernutrition early in life (0-6 months), both male and female birds initially grew at a faster rate than the fully nourished male and female birds, respectively, for the same gain in weight. Lister and associates also showed that following the prolonged period of undernutrition, the birds took a similar length of time to achieve their final body weights as did the fully nourished controls (Lister et al., 1966).

II. The effects of early dietary restriction on the mortality of male and female broiler-type birds

The effects of dietary restriction on mortality are presented in Tables V and VI. The total number of deaths occurring throughout the experimental period are shown in Table V. Mortality was summarized according to different periods of time throughout the experimental period. The periods were selected according to the periods of dietary restriction and the growing stages of the birds. Mortality was calculated for the first two weeks of dietary restriction; for the remainder of the period of dietary restriction (weeks 3-14); for the growing period terminating with sexual maturity (weeks 15-24); and for the period of time from sexual maturity to the termination of the experiment (weeks 25-38).

Mortality was highest during the first two weeks of dietary restriction reaching 18% during the first week and 20% by the end of the second week. Birds subjected to dietary restriction had higher mortality than did the control birds. The birds subjected to dietary restriction during the first week were severely affected and were not able to survive further feed restriction. The severity of the dietary restriction during the first week was indicated by the fact that when feed was withheld on day 5, there was a considerable increase in the number of deaths, accounting for 29 of the total 42 deaths for the entire first week (data not shown).

Chicks are able to survive for periods of time after hatching without feed, provided water is supplied ad libitum. Periods of up to

Table V. Mortality* in male and female broiler-type chickens subjected to dietary restriction for different periods of time.

Treatments	Total Number of Birds	Number of deaths (male and female)				
		Week 1	Week 2	Week 3-14	Weeks 15-24	Weeks 25-38
Control	23	0	0	1	5	0
Feed Restriction:**						
0-2,0-3, & 0-4 wks	72	8	15	3	8	7
0-5, & 0-6 wks	54	12	12	2	4	6
0-8, & 0-10 wks	49	11	11	4	4	5
0-12, & 0-14 wks	58	11	9	5	6	9
Total mortality in birds placed on dietary restriction	233	42	47	14	22	27
Percent mortality in birds placed on dietary restriction		18%	20%	6%	9%	12%

* Mortality includes birds which were cropbound.

** Data from treatments were combined to increase sample size.

72 hours posthatch without feed (water ad libitum) have been found to have no effect on mortality (March and Hansen, 1977). In this regard, periods of feed and water deprivation up to 48 hours in duration posthatch have been found to have no effect on final body weights, mortality, carcass quality, or feed conversion (Conners et al., 1971; Proudfoot, 1975). During the first few days posthatch the chick relies on the lateral thoracic adipose depot as an energy source for survival (Langslow and Lewis, 1972). The lateral thoracic adipose depot is quickly depleted following hatch and by the 8th day contains only 40% of the lipid content which was present on day 1 (Langslow and Lewis, 1972).

If feed is withheld for longer periods of time, 72 hours or 120 hours, mortality rises to 18% (as earlier mentioned) or 27% (March and Hansen, 1977), respectively. There also appears to be a genetic difference in the ability of birds to survive without feed following hatching. When feed was withheld for 120 hours after hatch, broiler-type birds suffered 27% mortality in contrast to White Leghorns who only suffered 6% mortality (March and Hansen, 1977).

Following the first two weeks of dietary restriction the death rate decreased to 6% for the rest of the period of dietary restriction. Following 14 weeks of age the death rate was between 9 and 12% for the duration of the experimental period. The mortality in birds subjected to early dietary restriction was greater than the mortality in the control birds during the first few weeks of life and for the rest of the experimental period, Table V.

Table VI compares the mortality between male and female birds

from 2 to 38 weeks of age. The data on the mortality in birds prior to two weeks was omitted in order to gain a clearer picture of the levels of mortality in birds during the subsequent growing period. The mortality in birds placed on early dietary restriction increased with the increase in the severity of the dietary restriction (Table VI). The mortality in birds placed on dietary restriction from 0-12 and from 0-14 weeks of age was 53% and was greater than the mortality in the control birds (26%). The mortality in birds subjected to dietary restriction from 0-8 and 0-10 weeks (48%); from 0-5 and 0-6 weeks (40%); and from 0-2, 0-3 and 0-4 weeks (37%) was also greater than the control birds never placed on dietary restriction (26%). The mortality in both male and female birds was increased in birds subjected to dietary restriction, although it cannot be stated whether or not one sex was affected to a greater extent than the other.

Cropbound birds were killed and included in the figures for mortality. Cropbound birds were killed because the eating habits of these birds would not reflect the eating habits of a normal population of birds. Furthermore, the growth rate and physiological state of cropbound birds would be altered and would be different from birds without bound crops. Therefore, the data from cropbound birds were omitted from the results on growth rates and body weight.

The occurrence of cropbound birds in birds subjected to dietary restriction is presented in Table VII. Dietary restriction early in life appeared to increase the incidence of cropbound birds. The occurrence of cropbound birds seemed to increase in a similar fashion in both male and female birds subjected to dietary restriction without evidence of a sex difference.

Table VI. A comparison of total mortality* between male and female broiler-type chickens from 2 to 38 weeks of age.

Treatments	Total No.of Birds (male and female) at 2 weeks of age	TOTAL MORTALITY (male and female) % Total Mortality	No.of Males	% Mortality in Males	No.of Females	% Mortality in Females
Control	23	26%	13	38%	10	10%
Feed Restriction:**						
0-2, 0-3, and 0-4 weeks	49	37	18	39	31	35
0-5, and 0-6 weeks	30	40	12	50	18	33
0-8 and 0-10 weeks	27	48	16	50	11	45
0-12 and 0-14 weeks	38	53	15	67	23	43
Total mortality in birds subjected to dietary restriction	144	44	61	51	83	39

* Mortality includes birds which were cropbound.

** Data from treatment were combined to increase sample size.

Table VII. The effects of early dietary restriction on the occurrence of cropbound birds between 2 and 38 weeks of age.

Treatments	Total No.of Birds (male and female) at 2 weeks of age	BIRDS BECOMING CROPBOUND (male and female) Birds Becoming Cropbound	No. of Males	No. of Cropbound Males	No. of Females	No. of Cropbound Females
Control	23	2	13	1	10	1
Feed Restriction:*						
0-2, 0-3, and 0-4 weeks	49	5	18	1	31	4
0-5 and 0-6 weeks	30	4	12	1	18	3
0-8 and 0-10 weeks	27	6	16	2	11	4
0-12 and 0-14 weeks	38	2	15	2	23	0
Total cropbound birds in birds subjected to dietary restriction	144	17	61	6	83	11
Percent cropbound birds in birds subjected to dietary restriction	144	11.8%	61	9.8%	83	13.2%
Percent cropbound birds in control	23	8.7%	13	7.7%	10	10.0%

* Data from treatments were combined to increase sample size.

Feeding management of birds may affect the susceptibility of birds to become cropbound by promoting conditions favourable to the growth of undesirable microorganisms in the crop and/or the failure of desirable microorganisms to attach themselves to the crop wall (Brooker and Fuller, 1975; Fuller and Brooker, 1974).

The major cause of mortality in all birds was the development of leg problems. Perosis or slipped-tendon accounted for 43% of the leg problems. The incidence of leg weakness in the experimental population is presented in Table VIII. Early dietary restriction increased the development of leg weakness in birds subjected to dietary restriction to 18.7% compared with 13% in the control population. The occurrence of leg weakness in the control birds was high, 13%, and reflects the rearing problem in broiler-type birds in the poultry industry. Female birds placed on early dietary restriction seemed more susceptible to leg weakness than the males. Leg weakness in female birds placed on dietary restriction increased from 0% in the control birds to 14.5% in those birds placed on dietary restriction; whereas the occurrence of leg weakness in male birds subjected to dietary restriction was similar to the occurrence of leg weakness in the control population, 23.1% and 24.6% respectively. However, caution must be employed when evaluating the sex difference in the susceptibility of birds to leg-weakness following periods of dietary restriction since relatively few birds were involved. McCance (1960) found that during the period of rehabilitation following early dietary restriction, the rehabilitated birds developed varying degress of leg weakness.

Table VIII. The effects of early dietary restriction on the occurrence of birds developing leg weakness* between 2 and 38 weeks of age.

Treatments	Total No.of Birds (male and female) at 2 weeks of age	BIRDS DEVELOPING LEG WEAKNESS (male and female) Birds Developing Leg Weakness	No.of Males	Males Developing Leg Weakness	No.of Females	Females Developing Leg Weakness
Control	23	3	13	3	10	0
Feed Restriction:**						
0-2, 0-3, and 0-4 weeks	49	6	18	2	31	4
0-5 and 0-6 weeks	30	8	12	5	18	3
0-8 and 0-10 weeks	27	6	16	4	11	2
0-12 and 0-14 weeks	38	7	15	4	23	3
Total birds with weak legs in birds subjected to dietary restriction	144	27	61	15	83	12
Percent leg weakness in birds subjected to dietary restriction	144	18.7%	61	24.6%	83	14.5%
Percent leg weakness in control birds	23	13.0%	13	23.1%	10	0%

* Perosis or slip-tendon accounted for 43% of the birds developing leg weakness.

** Data from treatments were combined to increase sample size.

III. The effects of early dietary restriction on egg production from 24 to 38 weeks of age

Dietary restriction delayed sexual maturity in female birds subjected to early dietary restriction, Table IXa. Female birds subjected to the longest periods of dietary restriction, restricted from 0-8, 0-12 and 0-14 weeks of age, had their sexual maturity delayed the longest -- 22, 22.5 and 24 weeks of age respectively. Birds which were never subjected to dietary restriction (controls) or restricted from 0-2 weeks of age reached sexual maturity at 18.5 and 19 weeks of age respectively.

Egg production was measured from 24 to 38 weeks of age. However, as mentioned earlier, feed was accidently withheld for several days at 33 weeks of age, resulting in lower subsequent egg production. Therefore, data for egg production during weeks 33, 34 and 35, were omitted from the calculations of average egg production (Table IXa). At 36 weeks of age and thereafter, egg production in all treatments was similar to the levels of egg production occurring before 33 weeks of age. Douglas and associates (1978) showed that food deprivation up to 72 hours in duration, although initially reducing egg production, had no effect on subsequent egg production or egg weight, once egg production returned to the levels occurring before the food deprivation. Therefore, egg production from 36 to 38 weeks of age was included in the calculation of average egg production (Table IXa).

Table IXa. The effects of early dietary restriction on egg production between 24 and 38 weeks of age.

Treatments		Age of Birds at Onset of Lay (weeks)	Egg Production* from 24-38 weeks of age (%)
Control	(7)***	18.5	30.4
Feed Restriction:**			
0-2 weeks	(5)	19.0	49.5
0-3 weeks	(6)	21.5	35.0
0-4 weeks	(9)	22.5	34.0
0-5 weeks	(6)	20.0	35.9
0-6 weeks	(6)	21.0	32.8
0-8 weeks	(4)	22.0	24.5
0-12 weeks	(6)	22.5	41.5
0-14 weeks	(7)	24.0	27.1

* Egg production was calculated on hen-day basis.

** Treatment 8 (feed restriction 0-10 weeks) had only 2 birds, (data not included)

*** Number of birds

Egg production calculated on a hen-day basis seemed unaffected by the early dietary restriction (Table IXa). Birds placed on dietary restriction from 0-14 weeks of age had a lower total egg production from 24 weeks to 38 weeks of age. However, these birds came into lay later than all other treatments and therefore the total egg production was expected to be lower than the other treatments. Toward the end of the experimental period, weeks 35-38, the egg production in birds placed on feed restriction from 0-14 weeks of age were similar to the other treatments (data not shown). The egg weights of birds between 36 and 38 weeks of age indicated that egg size was not affected by the early dietary restriction (Table IXb). Egg weights were only measured on those birds whose body weight seemed to be adversely affected by the early dietary restriction. The reproductive capacity of the birds did not appear to have been affected by the dietary restriction imposed during the growing period.

Other investigators have shown that milder forms of dietary restriction during the growing period delay the onset of sexual maturity; have no effect on mature egg size although the dietary restriction may reduce the percentage of small eggs laid at the beginning of the production cycle; and increase subsequent egg production through a higher peak-lay and a slower rate of decline thereafter (Fuller and Dunahoo, 1962; Gowe et al., 1960; Strain et al., 1965).

Table IXb. The effects of early dietary restriction on egg weights between 36 and 38 weeks of age.

Treatments		No. of Eggs	Average Egg Weight (g)
Control	(7)*	45	$60.6^{a} \pm 7.9$
Feed Restriction:			
0-2 weeks	(5)*	45	$59.4^{a} \pm 4.7$
0-12 weeks	(6)	32	$62.4^{a} \pm 7.5$
0-14 weeks	(7)	70	$60.5^{a} \pm 5.3$

* Number of birds

IV. The effects of early dietary restriction on the mature body weight, proportion of tissues, cellularity of adipose tissue and adipocyte size of broiler-type chickens

A. Mature body weight and proportion of tissues of female broiler-type birds subjected to early dietary restriction

The growth curves for the female birds (Figure 1), indicated that at 38 weeks of age, those birds which had feed consumption restricted from 0-12 and from 0-14 weeks of age had significantly lower mature body weights (Table IIIa). Therefore, an investigation was undertaken to determine whether the reduction in body weight of these birds could be attributed specifically to a reduction in the amount of adipose tissue or was due to a reduction in the weights of other organs or tissues as well. Since restriction of feed intake from 0-12 and from 0-14 weeks of age were the only treatments to cause significant reductions in body weights, only the birds subjected to these treatments were investigated. Birds restricted from 0-2 weeks of age had body weights similar to those of the control birds and were investigated to determine whether mild dietary restriction early in life had adverse effects on body composition.

The results of this investigation are shown in Table X & XI. The birds which had feed consumption restricted from 0-2 weeks of age were similar to the control birds in all parameter studied, Table X. The proportion of tissues in birds subjected to dietary restriction from 0-2 weeks of age correspond to those of the controls, Table XI. Therefore,

Table X. The effects of early dietary restriction on body weight, weights of selected organs and length of the tibiotarsus in broiler-type female chickens at 40-43 weeks of age.

	Treatment 1 Control Mean ± (7)*	Treatment 2 Feed Restriction (0-2 weeks) Mean ± (5)*	Treatment 9 Feed Restriction (0-12 weeks) Mean ± (6)*	Treatment 10 Feed Restriction (0-14 weeks) Mean ± (7)*
Body weights (g)	3813^{b} ±240	3926^{b} ±346	3410^{a} ±304	3403^{a} ±212
M. pectoralis major (g)	197.8^{a} ±35.5	210.4^{a} ±39.2	159.7^{a} ±34.6	169.4^{a} ±20.0
Liver (g)	61.4^{a} ±8.8	65.6^{a} ±15.2	62.6^{a} ±11.1	57.0^{a} ±10.0
Ovary (g)	82.3^{a} ±14.2	72.0^{a} ±10.7	87.4^{a} ±10.5	77.9^{a} ±16.2
Oviduct (g)	64.9^{a} ±4.0	63.8^{a} ±5.7	64.8^{a} ±5.6	67.8^{a} ±6.3
Tibiotarsus (g)	13.8^{b} ±1.7	13.9^{b} ±1.7	11.8^{a} ±1.6	10.9^{a} ±1.2
Tibiotarsus length (cm)	12.6^{a} ±0.5	12.4^{a} ±0.4	11.8^{a} ±1.0	11.9^{a} ±0.6
Retroperitoneal adipose depot (g)	243.8^{a} ±65.2	246.0^{a} ±120.3	226.9^{a} ±60.6	198.2^{a} ±24.7
M. sartorius adipose depot (g)	9.0^{b} ±1.7	9.1^{b} ±4.6	6.3^{ab} ±2.4	5.3^{a} ±0.5

* Number of birds

ab Values with the same superscript for each parameter are not significantly different $P < 0.05$.

Table XI. Effects of early dietary restriction on body weight, relative weights of selected organs and length of the tibiotarsus in broiler-type female chickens at 40-43 weeks of age.

	Treatment 1 Control Mean ± (7)*	Treatment 2 Feed Restriction (0-2 weeks) Mean ± (5)*	Treatment 3 Feed Restriction (0-12 weeks) Mean ± (6)*	Treatment 4 Feed Restriction (0-14 weeks) Mean ± (7)*
Body weights (g)	3813^{b} ±240	3926^{b} ±346	3410^{a} ±304	3403^{a} ±212
M.pectoralis major (%)	5.2^{a} ±0.7	5.4^{a} ±0.9	4.6^{a} ±0.6	5.0^{a} ±0.4
Liver (%)	1.6^{a} ±0.2	1.7^{a} ±0.4	1.8^{a} ±0.2	1.7^{a} ±0.3
Ovary (%)	2.1^{a} ±0.3	1.8^{a} ±0.3	2.6^{a} ±0.5	2.3^{a} ±0.5
Oviduct (%)	1.7^{a} ±0.1	1.6^{a} ±0.2	1.9^{b} ±0.3	2.0^{b} ±0.2
Tibiotarsus (%)	0.36^{a} ±0.04	0.35^{a} ±0.04	0.34^{a} ±0.4	0.32^{a} ±0.03
Tibiotarsus length(cm)	12.6^{a} ±0.05	12.4^{a} ±0.04	11.8^{a} ±1.0	11.9^{a} ±0.6
Retroperitoneal adipose depot (%)	6.4^{a} ±1.9	6.2^{a} ±2.8	6.4^{a} ±1.7	5.8^{a} ±0.6
M. sartorius adipose depot (%)	0.24^{a} ±0.04	0.23^{a} ±0.1	0.18^{a} ±0.07	0.16^{a} ±0.01

* Number of birds.

ab Values with the same superscript for each parameter are not significantly different $P < 0.05$.

dietary restriction from 0-2 weeks of age did not seem to have any permanent effect on the body composition of mature female birds.

At 40-43 weeks of age, the average body weights of those birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age were significantly less than the average body weight of the control birds and those birds placed on feed restriction from 0-2 weeks of age, Table X. The average weights of the tissues and organs selected for examination, with the exception of the tibiotarsus and the M. sartorius depot, were not significantly affected by the dietary restriction from 0-12 or from 0-14 weeks of age. The weights of the M. pectoralis major, liver, ovary, oviduct and retroperitoneal adipose depot were not significantly different from the controls. Although the length of the tibiotarsus was unaffected by dietary restriction, the dry weight ofthe tibiotarsus was significantly reduced in birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age. In addition, the wet weight of the M. sartorius adipose depot was also significantly reduced in birds restricted from 0-14 weeks of age. Since the tibiotarus and the M. sartorius adipose depot were the only tissues examined that were significantly reduced by dietary restriction, suggests that these tissues and organs were more sensitive to the adverse effects of early dietary restriction. Investigations by Pratt and McCance (1961) and Lister et al. (1966) have also demonstrated that bone development is sensitive to early dietary restriction. Lister et al. (1966) also showed that there was a sex difference in the sensitivity of bone development to early dietary restriction. The dimensions of the femur in female pullets restricted to 140-170 g of body weight at 6 months of age, upon rehabilitation, were able to achieve dimensions similar

to those of the controls. In contrast, the dimensions of the femurs in cockerels, similarly restricted, upon rehabilitation were unable to achieve the same stature of the femurs of the control birds (Lister et al., 1966).

The retroperitoneal adipose depot seemed unaffected permanently by the early dietary restriction. Although the weights of the abdominal adipose depot appeared to be lower in birds having feed restriction from 0-14 weeks, this reduction in depot size was not significant ($P < 0.05$). Dietary restriction from 0-14 weeks of age seemed to permanently limit the development of the M. sartorius adipose depot since full development of the depot was not achieved despite 26-29 weeks of rehabilitation following dietary restriction, Table X. Therefore, it seems likely that different adipose depots may show different responses to dietary restriction and one particular adipose depot may not be representative of all adipose tissue in the body. Investigations by Lemonnier (1972) and Walkley et al. (1978) have also demonstrated considerable variability in the response of different adipose depots to dietary manipulations.

The reduction in growth caused by the dietary restriction from 0-12 and from 0-14 weeks of age, probably represented permanent stunting since the birds remained significantly lighter in weight when the experiment was terminated at between 40 and 43 weeks of age. The lighter weights appeared to be due to slight reduction in the growth of all tissues since the proportional weights of the M. pectoralis major, liver,

tibiotarus, ovary, and retroperitoneal and M. sartorius adipose depots were similar in the restricted and the controls, Table XI.

The relative proportional weight of the oviduct increased in sexually mature birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age, Table XI. The increase in the ratio of oviduct:body weight in the sexually mature birds should be expected in birds with significantly lowered body weights, if egg size and egg production were unaltered by dietary restriction. Table XI indicates that the relative proportion of both ovary and oviduct increased with the reduction in body weight observed in treatments 9 and 10, although the increase was only statistically significant for the oviducts of birds.

B. The effects of age and early dietary restriction on adipocyte diameter in the retroperitoneal adipose depot

The development of adipose tissue is characterized by hyperplasia and hypertrophy. In the avian species, as in other species, hyperplasia initially occurs followed by hypertrophy. Adipocytes continue to increase in size after adipocyte hyperplasia has terminated. Therefore, a measurement of adipocyte diameter would provide an assessment of adipose tissue development.

To assess the ongoing process of adipose development in birds following various periods of dietary restriction, adipocyte diameter was determined in the retroperitoneal adipose depot at 17-19 weeks of

age. The results of this investigation are shown in Table XIIa, b and c.

The average adipocyte diameter in the birds in treatment 1 (control) serves as an indicator of normal adipocyte development. While removing fragments of adipose tissue from the anesthetized birds, 2 birds in treatment 1 and 1 bird in treatment 9 were adversely affected by the anesthesia and died during the biopsy operation (Table XIIa). In the period of time between the biopsy of adipose tissue and the termination of the experiment at 40 weeks of age, 1 bird in treatment 9 and 1 bird in treatment 10 died of causes unrelated to the biopsy procedure (leg weakness and prolapse, respectively). Furthermore, the adipose biopsy procedure did not affect subsequent growth rate, final body weight, proportion of selected tissues or organs, including the adipose depots, or adipocyte diameter or cellularity (data not shown). In fact, upon removal of the retroperitoneal adipose depots at 40-43 weeks of age there was no evidence of scar tissue on the abdominal wall or in the retroperitoneal adipose depot.

Table XIIb shows that birds subjected to feed restriction from 0-14 weeks of age had an average adipocyte diameter which was significantly smaller than the adipocyte diameter of the control birds, regardless of the age at which the adipocyte diameters were measured. Dietary restriction from 0-14 weeks of age, therefore, retarded adipocyte hypertrophy at 17-19 weeks of age to such an extent that recovery to normal adipocyte size (judged by adipocyte diameters of the control birds), was not complete by 40-43 weeks of age.

Table XIIa. The effects of early dietary restriction on adipocyte diameter in the retroperitoneal depot of female broiler-type chickens at different ages.

Bird Number	Average Adipocyte Diameter (μm)	Dispersion*** Around the Average Adipocyte Diameter (μm)	C.V.	Average Adipocyte Diameter (μm)	Dispersion Around the Average Adipocyte Diameter (μm)	C.V.
	Average Adipocyte Diameter at 17-19 Weeks of Age			Average Adipocyte Diameter at 40-43 Weeks of Age		
TREATMENT 1: (Control):						
805	98.1	13.9	0.14	105.6	11.7	0.11
8024	84.7	18.2	0.21	96.9	12.2	0.12
8015	89.4	14.5	0.16	*		
8019	101.3	15.2	0.15	*		
8013				104.7	11.8	0.11
8028				100.9	11.3	0.11
8023				115.6	12.6	0.11
8025				136.2	10.7	0.08
8012				99.1	11.0	0.11
$\bar{x}$	93.4	15.4	0.16[a]	108.4	11.6	0.11
σ	±7.7	±1.9	±0.03	±13.7	±0.1	±0.01
TREATMENT 9 (Feed Restriction: 0-12 weeks):						
8044	87.4	14.9	0.17	126.0	12.9	0.10
8176	78.7	12.5	0.16	78.5	9.8	0.13
8178	75.2	10.7	0.14	**		
8050	102.5	11.5	0.11	*		
8180	92.8	10.7	0.12	97.3	8.8	0.09
8173				90.1	9.3	0.10
8049				89.0	9.5	0.11
8046				106.2	10.8	0.10
$\bar{x}$	87.3	12.0	0.14	97.8	10.2	0.11
σ	±11.0	±1.8	±0.02	±16.6	±1.5	±0.02
	Cell Diameter at 17-19 Weeks of Age			Cell Diameter at 40-43 Weeks of Age		
TREATMENT 10 (Feed Restriction 0-14 weeks):						
8059	74.7	18.9	0.25	100.2	8.4	0.08
8064	76.5	15.2	0.19	104.7	9.8	0.09
8150	60.8	9.9	0.16	**		
8154	79.8	11.9	0.15	91.9	9.9	0.11
8111	62.4	9.7	0.15	90.9	8.1	0.08
8153				98.1	9.6	0.10
8065				93.6	8.4	0.09
8115				84.7	11.7	0.13
$\bar{x}$	70.8	13.1	0.18	94.9	9.4	0.10
σ	±8.6	±3.9	±0.04	± 6.6	±1.3	±0.02

* Died during biopsy for retroperitoneal adipose tissue

** Died later in experimental period of causes unrelated to biopsy

*** Dispersion refers to the standard deviation of the average adipocyte diameter of an adipose depot. The dispersion around the average adipocyte diameter measures the variation in adipocyte diameters, within an adipose depot, around the average adipocyte diameter.

$\bar{x}$ Mean cell diameter per treatment

σ Standard deviation of treatment means

C.V. Coefficient of variation ($\sigma/\bar{x}$)

Table XIIb. Analysis of variance* showing main effects on adipocyte diameter (μm).

Age of Bird At Time of Measurement	TREATMENT 1 Control	TREATMENT 9 Feed Restriction 0-12 weeks	TREATMENT 10 Feed Restriction 0-14 weeks	Effects of Age At Time of Measurement
17-19 weeks	93.4±7.7	87.3±10.9	70.8±8.6	83.2^{a}±13.1
40-43 weeks	108.4±13.7	97.8±16.6	94.9±6.6	100.5^{b}±13.5
Effect of Treatment	103.0^{b}±13.7	93.1^{ab}±14.7	84.9^{a}±14.3	93.4±15.7

ab Values with the same superscript for cell diameter are not significantly different at $P < 0.05$

* There was no significant interaction, appendix, Table VII(A).

Table XIIc. Analysis of variance showing the variations in homogeneity of adipocyte diameter in the retroperitoneal depot of broiler-type chickens with age and dietary restriction.

Age of Birds	TREATMENT			
	Control	Restricted (0-12 weeks)	Restricted (0-14 weeks)	Age
	Avg CV[1] of mean adipocyte diameter	Avg CV of mean adipocyte diameter	Avg CV of mean adipocyte diameter	Avg CV of mean adipocyte diameter
18 weeks	0.16	0.14	0.18	0.16[b]
42 weeks	0.11	0.11	0.10	0.10[a]
Treatment, Avg of mean adipocyte diameter	0.13[a]	0.12[a]	0.13[a]	

1 Coefficient of variation

ab Average values with the same superscript are not significantly different, $P < 0.05$. There was no significant interaction between treatment effect and the age of the birds when the adipocytes were measured, appendix, Table VIII(A).

The reduction in size of adipocytes in the retroperitoneal adipose depot of birds placed on early dietary restriction from 0-14 weeks of age is an important consideration because cell size may determine the metabolic activity of the adipose tissue (Salans et al., 1968).

Adipocyte hypertrophy was not complete at 17-19 weeks of age, but was shown to continue in the retroperitoneal adipose depot to between 40 and 43 weeks of age, regardless of the dietary restriction, Table XIIb. These findings are in agreement with Pfaff and Austic (1976) who found that in White Leghorn pullets, adipocyte hyperplasia was complete in the retroperitoneal adipose depot by 12-16 weeks of age, after which time adipose tissue enlargement was characterized by adipocyte hypertrophy.

The average adipocyte diameter, as described earlier, was determined from the frequency distribution of adipocyte diameters. The frequency distribution of retroperitoneal adipocytes into size classes according to treatment at 17-19 weeks, (18 weeks) and at 40-43 weeks, (42 weeks) of age are shown in Figure 4. The difference in the retroperitoneal adipocyte class sizes between 18 (17-19) weeks of age and 42 (40-43) weeks of age was due to the different methods of determining adipocyte diameter employed at the time of analysis. Adipocytes greater than 96 μm were present in greater proportion in the retroperitoneal depot of the control birds and the birds restricted for only 2 weeks, than in birds restricted for 14 weeks.

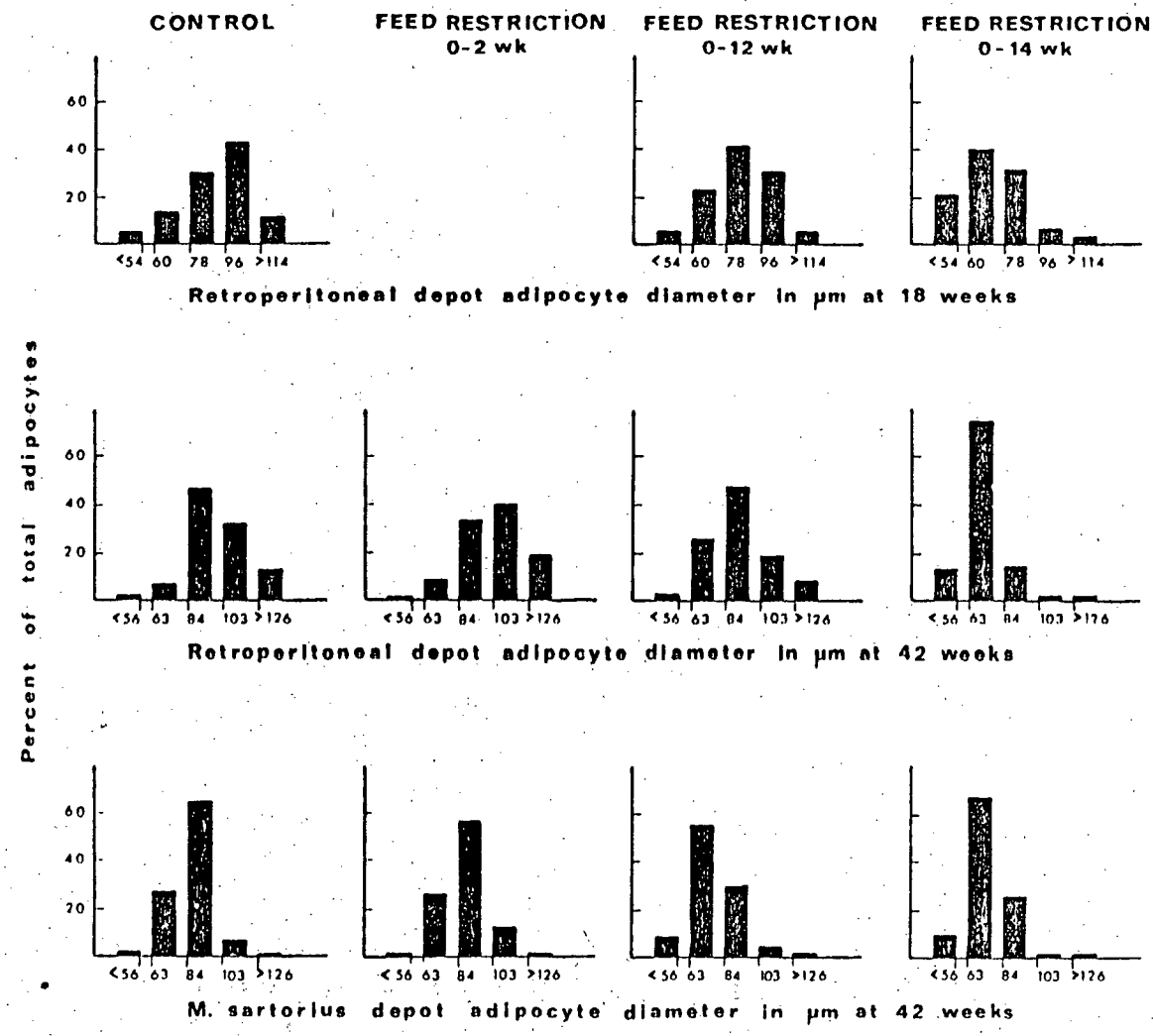


Figure 4. Adipocyte diameter distribution at different ages, for the retroperitoneal and the M. sartorius depots of broiler-type chickens subjected to varying degrees of dietary restriction.

This was true for birds at 18 weeks (40-43 weeks) and at 42 weeks (40-43 weeks) of age. Birds subjected to dietary restriction for 12 and 14 weeks had a larger proportion of cells less than 78 μm in diameter at 18 weeks and at 42 weeks of age.

It was stated above that adipocytes less than 24 μm in diameter were not considered in the distribution. It was found however, that many of the adipocyte preparations from the tissue taken by biopsy at 17-19 weeks of age contained clumps of 10 to 100 small adipocytes less than 30 μm in diameter in association with fragments of connective tissue. Estimates of the numbers of these clumped cells were made when the larger adipocytes were being measured. The small adipocytes were placed in classes of 6-12 μm and 18-30 μm in diameter as shown in Table XIII. These small clumped adipocytes appeared in greatest numbers in the most severely restricted birds. Small clumped adipocytes were not apparent in the cell preparations examined when the birds were 40-43 weeks of age.

It is apparent from Table XIII, that at 17-19 weeks of age, adipocyte hyperplasia was continuing in birds subjected to dietary restriction from 0-14 weeks of age, as shown by the number of small adipocytes found in association with tissue fragments. Since similar numbers of small adipocytes were not found in the control birds at this time, it can be assumed that adipocyte hyperplasia in the control birds was not occurring at the same rate. Other investigators have shown that early dietary manipulation will prolong the period of adipocyte hyperplasia, although adipocyte multiplication continues

Table XIII. Estimates of the numbers of small adipocytes less than 30 μm in diameter remaining in clumps following collagenase treatment of the retroperitoneal adipose tissue biopsy samples taken between 17-19 weeks of age. The numbers noted were expressed in terms of 600 adipocytes measured/bird.

Treatment	Bird No.	Number of Small Adipocytes Diameter 6-12 μm	Diameter 18-30 μm
1 (control)	1	0	0
	2	0	0
	3	0	0
	4	8	0
3 (Feed Restriction 0-12 weeks)	1	0	0
	2	190	0
	3	190	60
	4	0	0
	5	0	0
4 (Feed Restriction 0-14 weeks)	1	100	70
	2	410	70
	3	110	90
	4	120	30
	5	170	40

at a reduced rate during the period of dietary manipulation (Knittle and Hirsch, 1968; Pfaff and Austic, 1976).

In addition to showing the treatment effects on the average adipocyte diameter of the retroperitoneal depot, Table XIIa also illustrates the dispersion of adipocyte diameters around the average adipocyte diameter. The average adipocyte diameter (described earlier) was determined as the average adipocyte diameter obtained from two frequency distributions consisting of 300-400 cells/frequency distribution. Calculation of the average adipocyte diameter from a frequency distribution permits the calculation of the standard deviation of the average adipocyte diameters around the average adipocyte diameter. Expressing the dispersion of adipocyte diameters in terms of the coefficient of variation permits a comparison of the relative dispersion between two population means. A comparison of the dispersion of adipocyte diameters around the average adipocyte diameter between treatments and ages is shown in Table XIIc. The distribution of the coefficient of variation was found to be normal and the effects of dietary restriction and age on the coefficient of variation was performed by analysis of variance (Table VIII(A), Appendix). The results demonstrate that the dispersion around the average adipocyte diameter is significantly greater in the retroperitoneal adipose depot measured at 17-19 weeks of age than in the retroperitoneal depot measured at 40-43 weeks of age. The greater relative dispersion in the retroperitoneal depot of younger birds implies that there are a greater number of adipocytes in different stages of development than are in the retroperitoneal depot of older birds. Looking at the situation in terms of the older birds, the lower degree of dispersion

in the adipocytes of the retroperitoneal depot implies that there is a greater proportion of adipocytes achieving similar sizes. This observation suggests that the enlarging adipocytes may have a limit for cell expansion, since during hypertrophy, as the adipocytes get closer to their maximal size, the relative dispersion in the size of the cell would decrease. The maximal cell size attained by adipocytes in the avian species, may be characteristic for the species, and more importantly may be an important regulator of the cells metabolic activities.

Di Girolamo et al. (1971) found that adipocyte hyperplasia during the growing period of four mammalian species, rat, hamster, guinea pig, and dog, led to adipocyte populations which were more homogeneous in size. The increase in homogeneity of adipocyte diameters associated with adipocyte enlargement in mammalian and avian species, indicates that this may be a biological variable common to adipose tissue from all species.

C. The effects of early dietary restriction on the average adipocyte diameter and the adipose tissue cellularity in the retroperitoneal and the M. sartorius depots of female birds at 40-43 weeks of age.

The effects of early dietary restriction on the average adipocyte diameter in the retroperitoneal and the M. sartorius depots of female birds at 40-43 weeks of age are shown in Tables XIVa and XIVb. The results indicate that there was considerable

interdepot variation in the average adipocyte diameter between the retroperitoneal and M. sartorius adipose depots, Table XIVb. The average adipocyte diameter was significantly less in the M. sartorius depot than in the retroperitoneal depot, regardless of treatment. The relatively larger adipocyte diameters associated with the retroperitoneal adipose depot implies that this depot has a greater tendency to incorporate and to store lipid than the M. sartorius depot. The difference in the size of adipocytes between the retroperitoneal and the M. sartorius adipose depots was visually apparent as shown in Figures 5 & 6 and Figures 7 & 8. Figures 5 & 6 demonstrate the difference in adipocyte diameters between the retroperitoneal and M. sartorius depots in a control bird, while Figures 7 & 8 demonstrate the difference in adipocyte diameters between the two depots in a bird subjected to dietary restriction from 0-14 weeks of age.

Similar interdepot variation in the size of adipocytes has been observed in rats and man (Bjurulf, 1959; Lemmonier, 1972; Johnson and Hirsch, 1972; Salans et al., 1971; Brook, 1971; Di Girolamo et al., 1971). Investigations of adipose tissue cellularity in meat animals have also shown variability in the size and number of adipocytes among the adipose depots within the animal. (Allen, 1976; Hood and Allen, 1977).

Dietary restriction was shown to reduce the average adipocyte diameter in the retroperitoneal and M. sartorius adipose depots of birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age, Table IVb. The reduction in the average adipocyte diameter in those birds subjected to dietary restriction from 0-12 weeks

of age was surprising because a similar reduction was not earlier observed in the previous analysis, Table XIIb. The difference in the results between these two analyses, Table XIIb and Table XIVb respectively, is explained in the former analysis by the fact that only one adipose depot was observed, resulting in a smaller number of observations. As a consequence of the relatively few numbers of observations, the differences in adipocyte diameters between the birds in treatment 9 (restricted 0-12 weeks) and the control birds were not large enough to produce statistical significance. In the present analysis, Table XIVb, the effects of dietary restriction were analyzed on two adipose depots, resulting in an increased sample size and differences in adipocyte diameters between birds of different treatments which were statistically significant. Since there was no significant interaction between the effect of the depot and the effect of dietary restriction, Table IX(A), (Appendix); these results indicate that dietary restriction reduced the average adipocyte diameter in those birds subjected to the dietary restriction from 0-12 and from 0-14 weeks of age. Knittle and Hirsch (1968), demonstrated that early dietary restriction reduced the average adipocyte size in the epididymal adipose depot in rats. Subsequent experiments have shown that exercise in addition to food restriction early in life (Oscai et al., 1974), and protein restriction imposed immediately after weaning (Lau et al., 1976), may cause a permanent reduction in the size of adipocytes in the epididymal adipose depots or parametrial and retroperitoneal adipose depots of rats, respectively. Similar

Table XIVa. The effects of early dietary restriction on the average adipocyte diameter of the retroperitoneal and M. sartorius depots of female broiler-type chickens at 40-43 weeks of age.

	Average Adipocyte Diameter in the M.sartorius Adipose Depot			Average Adipocyte Diameter in the Retroperitoneal Adipose Depot		
Bird Number	Average Adipocyte Diameter (µm)	Dispersion* Around the Average Adipocyte Diameter (µm)	C.V.	Average Adipocyte Diameter (µm)	Dispersion Around the Average Adipocyte Diameter (µm)	C.V.
TREATMENT 1 (Control):						
8013	88.9	9.9	0.11	104.7	11.8	0.11
8028	92.3	10.5	0.11	100.9	11.3	0.11
8023	96.2	9.5	0.10	115.6	12.6	0.11
8024	82.8	9.3	0.11	96.9	12.2	0.12
805	95.6	7.9	0.08	105.6	11.7	0.11
8025	96.1	11.8	0.12	136.2	10.7	0.08
8012	83.9	9.4	0.11	99.1	11.0	0.11
$\bar{x}$	90.8	9.8	0.11	108.4	11.6	0.11
σ	±5.7	±1.2	±0.01	±13.7	±0.7	±0.01
TREATMENT 2 (Feed Restriction: 0-2 weeks):						
8010	102.7	10.7	0.10	127.0	10.6	0.08
8107	85.9	9.0	0.10	100.0	8.8	0.09
8003	77.2	8.7	0.11	90.7	8.7	0.09
8006	100.6	11.0	0.11	119.8	11.0	0.09
8009	98.0	8.5	0.08	125.1	14.0	0.11
$\bar{x}$	92.9	9.6	0.10	112.6	10.6	0.09
σ	±10.9	±1.2	±0.01	±16.1	±2.1	±0.01

Cont'd.....

Bird Number	Average Adipocyte Diameter (μm)	Dispersion Around the Average Adipocyte Diameter (μm)	C.V.	Average Adipocyte Diameter (μm)	Dispersion Around the Average Adipocyte Diameter (μm)	C.V.
	Average Adipocyte Diameter in the M. sartorius Adipose Depot			Average Adipocyte Diameter in the Retroperitoneal Adipose Depot		
TREATMENT 9 (Feed Restriction: 0-12 weeks):						
8173	75.3	9.8	0.13	90.1	9.3	0.10
8044	101.6	9.9	0.09	126.0	12.9	0.10
8180	75.0	9.5	0.12	97.3	8.8	0.09
8049	77.1	10.1	0.13	89.0	9.5	0.11
8176	70.8	9.1	0.13	78.5	13.6	0.17
8046	92.4	9.4	0.10	106.2	10.8	0.10
$\bar{x}$	82.0	9.6	0.12	97.8	10.8	0.11
σ	±12.1	±0.4	±0.02	±16.6	±2.0	±0.03
TREATMENT 10 (Feed Restriction: 0-14 weeks):						
8064	85.7	7.9	0.09	104.7	9.8	0.09
8111	70.7	9.2	0.13	90.9	8.1	0.08
8059	81.6	9.1	0.11	100.2	8.4	0.08
8153	80.4	9.1	0.11	98.1	9.6	0.10
8154	70.4	9.6	0.13	91.9	9.9	0.11
8065	82.6	8.8	0.10	93.6	8.4	0.09
8115	76.9	9.4	0.12	84.7	11.7	0.13
$\bar{x}$	78.3	9.0	0.11	94.9	9.4	0.10
σ	±5.9	±0.5	±0.01	±6.6	±1.3	±0.02

* Dispersion refers to the standard deviation of the average adipocyte diameter of an adipose depot. The dispersion around the average adipocyte diameter measures the variation in adipocyte diameters, within an adipose depot, around the average adipocyte diameter.

$\bar{x}$ Mean per treatment

σ Standard deviation of treatment means.

C.V. Coefficient of variation ($\sigma/\bar{x}$)

Table XIVb. Analysis of variance* of the data showing the main effects of early dietary restriction and adipose depot on the average adipocyte diameter (μm).

Adipose Depot		TREATMENT 1 Control	TREATMENT 2 Feed Restriction (0-2 weeks)	TREATMENT 9 Feed Restriction (0-12 weeks)	TREATMENT 10 Feed Restriction (0-14 weeks)	Effect of Adipose Depot
M. sartorius depot		90.8	92.9	82.0	78.3	85.6^{a}
		±5.7	±10.9	±12.1	±5.9	±10.2
Retroperitoneal depot		108.4	112.6	97.8	94.9	102.9^{b}
		±13.7	±16.1	±16.6	±6.6	±14.6
Effects of Treatment	$\bar{x}$	99.6^{b}	102.8^{b}	89.9^{a}	86.6^{a}	94.3
	σ	±13.6	±16.6	±16.1	±10.5	±15.2

ab Values with the same superscript for adipocyte diameter in different adipose depots or for different treatments are not significantly different at $P < 0.05$.

* There was no significant interaction, appendix, Table IX(A).

Table XIVc. Analysis of variance showing the variations in homogeneity of adipocyte diameter in the retroperitoneal and M. sartorius depot of broiler-type chickens with depot and dietary restriction.

Age of Birds	Control	Restricted (0-2 weeks)	Restricted (0-12 weeks)	Restricted (0-14 weeks)	Depots
	Avg. CV[1] of mean adipocyte diameter	Avg. CV of mean adipocyte diameter	Avg. CV of mean adipocyte diameter	Avg. CV of mean adipocyte diameter	Avg. CV of mean adipocyte diameter
Retroperitoneal adipose depot	0.11	0.09	0.11	0.10	0.10[a]
M. sartorius adipose depot	0.11	0.10	0.12	0.11	0.11[a]
Treatment, Avg. of mean adipocyte diameter	0.11[a]	0.10[a]	0.11[a]	0.10[a]	

1 Coefficient of variation

ab Average values with the same superscript are not significantly different $P < 0.05$. There was no significant interaction between treatment effect and the depot examined, appendix, Table X(A).

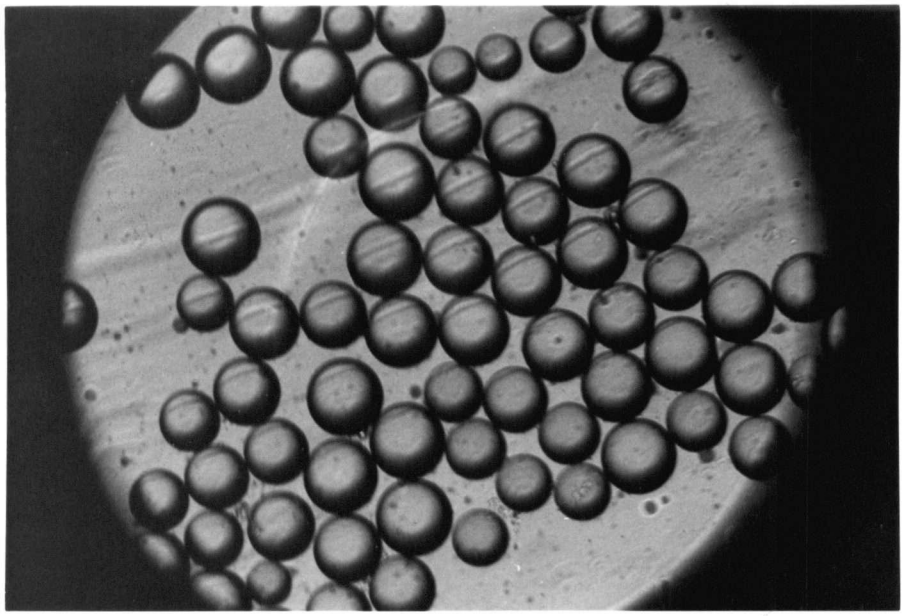


Figure 5. Photomicrograph of adipocytes isolated from the retroperitoneal adipose depot of a control bird (69X). Bird number 8023.

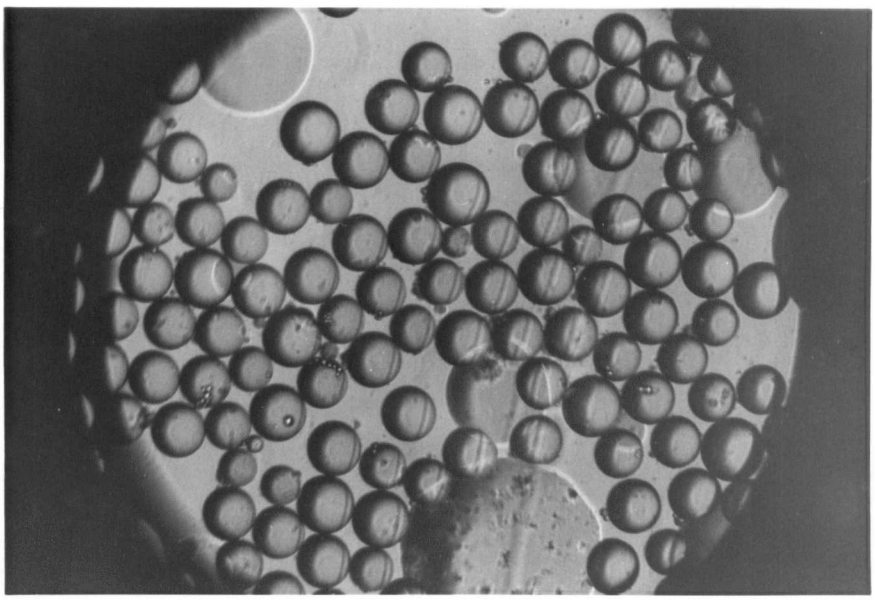


Figure 6. Photomicrograph of adipocytes isolated from the M. sartorius adipose depot of a control bird (69X). Bird number 8023.

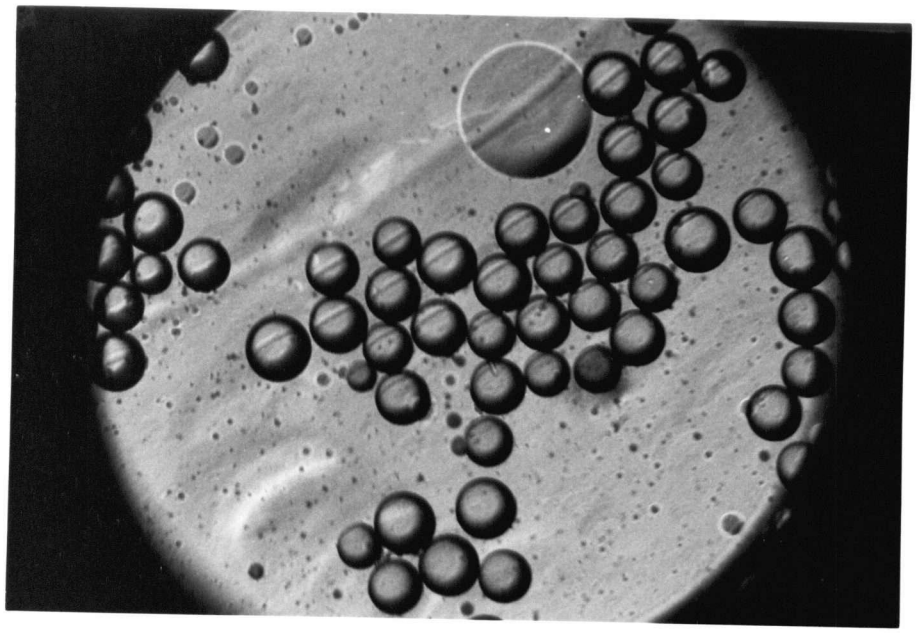


Figure 7. Photomicrograph of adipocytes isolated from the retroperitoneal adipose depot of a bird subjected to dietary restriction from 0-14 weeks of age (67X). Bird number 8154.

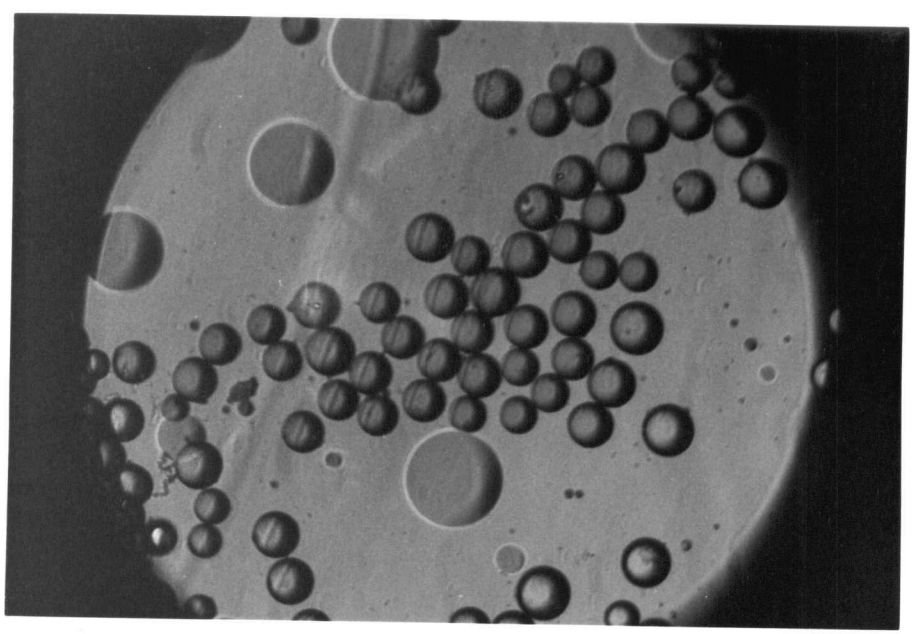


Figure 8. Photomicrograph of adipocytes isolated from the M. sartorius adipose depot of a bird subjected to dietary restriction from 0-14 weeks of age (67X). Bird number 8154.

investigations in meat animals have shown that dietary restriction early in life has reduced adipocyte size in lambs (Haugebak et al., 1974), and pigs (Lee et al., 1973b).

The dispersion of adipocyte diameters around the average adipocyte diameter is similar in all treatments studied, Table XIVc. The similarity in the degree of dispersion of adipocyte diameters around the average adipocyte diameter between treatments and between depots implies that the adipocytes have reached their maximum attainable size for the physiological conditions present in the birds at the time of measurement.

The average adipocyte diameter was used to determine the average adipocyte volume according to Goldrick's formula: adipocyte volume = $\pi/6\ (3\sigma^2 + \bar{x}^2)\bar{x}$, where $\bar{x}$ and σ are the average adipocyte diameter and standard deviation respectively (Goldrick, 1967). The average adipocyte number per adipose depot was then determined by dividing the average lipid content per adipose depot by the average adipocyte volume (as previously described). The results of these calculations are shown in Table XVa. The effect of early dietary restriction on the adipocyte cellularity of the retroperitoneal and the M. sartorius adipose depots are shown in Table XVb.

The retroperitoneal adipose depot responded differently to the early dietary restriction than did the M. sartorius depot, Table XVb. The adipocyte cellularity of the M. sartorius depot was unaffected by early dietary restriction. Although there was a permanent reduction in the amount of adipose tissue in the M. sartorius depot, Table X, a corresponding reduction in the adipocyte cellularity was not observed. The reduction in the M. sartorius depot is accounted

for by the reduction in the adipocyte diameter, Table XIVb, and therefore the amount of lipid per cell.

Early dietary restriction from 0-12 and from 0-14 weeks of age seemed to increase the number of adipocytes in the retroperitoneal adipose depot, Table XVb. The amount of adipose tissue in the retroperitoneal depot, however, seemed unaffected by the early dietary restriction imposed upon the birds from 0-12 and from 0-14 weeks of age, Table X. In the previous discussion dietary restriction was shown to decrease the average diameter of adipocytes in the adipose depots of birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age, Table XIVb. The decreased adipocyte diameter in the retroperitoneal adipose depot would explain the ability of the adipocyte cellularity to increase without a corresponding increase in the amount of adipose tissue.

The effect of early dietary restriction on the adipocyte cellularity of the retroperitoneal adipose depot indicates that early dietary restriction may affect adipose development by imposing a limit on the size which adipocytes may attain. Although the mechanism by which early dietary restriction may impose a limit on adipocyte size in unknown; the results of this experiment indicate that dietary restriction early in life reduced adipocyte diameter in both the retroperitoneal and M. sartorius adipose depots, resulting in an increase in adipocyte cellularity in the former and a decreased lipid content in the latter. Knittle and Hirsch (1968) were able to reduce both adipocyte diameter and adipocyte cellularity in the adipose

Table XVa. The effects of early dietary restriction on the total lipid, average adipocyte diameter, average adipocyte volume* and adipose cellularity in the adipose depots of female broiler-type chickens at 40-43 weeks of age.

Bird Number	M. sartorius adipose depot				Retroperitoneal adipose depot			
	Total Lipid (g)	Average Adipocyte Diameter (μm)	Average Adipocyte Volume (pl)**	Average Adipocyte Number ($X10^6$)	Total Lipid (g)	Average Adipocyte Diameter (μm)	Average Adipocyte Volume (pl)	Average Adipocyte Number ($X10^6$)
TREATMENT 1 (control):								
8013	9.4	88.9	382.2	27.0	230.1	104.7	625.1	402.3
8028	6.8	92.3	427.5	17.3	170.5	100.9	559.0	333.3
8023	9.0	96.2	479.4	20.4	252.9	115.6	838.9	329.5
8024	5.3	82.8	309.2	18.6	212.5	96.9	499.2	465.2
805	8.2	95.6	466.8	19.1	192.2	105.6	640.5	328.0
8025	8.9	96.1	486.0	20.0	340.2	136.2	1348.8	275.7
8012	6.7	83.9	321.2	22.7	149.5	99.1	528.3	309.2
$\bar{x}$	7.8	90.8	410.3	20.7	221.1	108.4	720.0	349.0
σ	±1.5	±5.7	±74.1	±3.2	±63.1	±13.7	±299.0	±63.7
TREATMENT 2 (Feed Restriction: 0-2 weeks):								
8010	15.1	102.7	586.4	28.2	381.6	127.0	1095.4	380.7
8009	8.0	98.0	504.4	17.4	239.9	125.1	1064.8	246.2
8006	8.2	100.6	602.9	14.8	306.3	119.8	924.6	362.0
8170	5.5	85.9	343.4	17.6	99.6	100.6	545.1	199.7
8003	3.7	77.2	250.3	16.0	125.2	90.7	402.1	340.2
$\bar{x}$	8.1	92.9	457.5	18.8	230.5	112.6	806.4	305.8
σ	±4.3	±10.9	±154.8	±5.4	±119.3	±16.1	±314.6	±78.7

Cont'd.....

Bird Number	M. sartorius adipose depot: Total Lipid (g)	Average Adipocyte Diameter (μm)	Average Adipocyte Volume (pl)	Average Adipocyte Number ($X10^6$)	Retroperitoneal adipose depot: Total Lipid (g)	Average Adipocyte Diameter (μm)	Average Adipocyte Volume (pl)	Average Adipocyte Number ($X10^6$)
TREATMENT 9 (Feed Restriction 0-12 weeks):								
8173	4.0	75.3	235.4	18.4	164.7	90.1	395.6	455.0
8044	7.4	101.6	565.2	14.3	295.1	126.0	1081.7	298.2
8180	5.6	75.0	231.8	26.5	212.1	97.3	494.8	468.5
8049	5.6	77.1	252.3	24.2	154.3	89.0	382.3	441.1
8176	2.0	70.8	195.5	11.2	131.9	78.5	277.7	479.6
8046	7.5	92.4	426.4	19.2	261.8	106.2	640.4	442.6
$\bar{x}$	5.3	82.0	317.8	19.0	203.3	97.8	546.4	430.8
σ	±2.1	±12.1	±146.0	±5.8	±64.7	±16.6	±290.2	±66.6
TREATMENT 10 (Feed Restriction 0-14 weeks):								
8064	4.7	85.7	338.5	15.1	206.9	104.7	616.6	366.7
8111	4.5	70.7	195.1	25.3	168.1	90.9	403.2	455.7
8059	4.3	81.6	295.2	15.9	162.5	100.2	538.1	330.1
8153	4.6	80.4	282.6	17.9	167.8	98.1	508.9	360.4
8154	4.3	70.4	193.3	24.1	182.0	91.9	420.5	472.9
8065	5.2	82.6	305.3	18.6	206.7	93.6	439.6	513.8
8115	4.1	76.9	249.1	18.1	153.2	84.7	337.1	496.7
$\bar{x}$	4.5	78.3	265.6	19.3	178.2	94.9	466.3	428.0
σ	±0.4	±5.9	±55.6	±3.9	±21.3	±6.6	±94.0	±73.9

* Average adipocyte volume was calculated from the average adipocyte diameter according to the Goldrick's formula, Adipocyte volume = $\pi/6(3\sigma^2 + \bar{x}^2)\bar{x}$, (Goldrick, 1967).

** Adipocyte volume units are picoliters (pl).

Table XVb. The effects of early dietary restriction on the adipocyte cellularity of the M. sartorius and retroperitoneal adipose depots of female birds at 40-43 weeks of age.

Adipose Depot	TREATMENT 1 Control	TREATMENT 2 Feed Restriction (0-2 weeks) $\bar{x}$	σ	TREATMENT 9 Feed Restriction (0-12 weeks) $\bar{x}$	σ	TREATMENT 10 Feed Restriction (0-14 weeks) $\bar{x}$	σ
	($X10^6$ cells)	($X10^6$ cells)		($X10^6$ cells)		($X10^6$ cells)	
M. sartorius depot	$20.7^a \pm 3.2$	$18.8^a \pm 5.4$		$19.0^a \pm 5.8$		$19.3^a \pm 3.9$	
Retroperitoneal depot	$349.0^a \pm 63.7$	$305.8^a \pm 78.7$		$430.8^b \pm 66.6$		$428.0^b \pm 73.9$	

ab Values with the same superscript for adipocyte number are not significantly different as $P < 0.05$.

tissue of rats by early dietary restriction. In this laboratory, however, early dietary restriction was shown only to decrease adipocyte diameter in the depots of broiler-type birds and was unable to decrease adipocyte cellularity. The increase in the number of observable adipocytes in the retroperitoneal adipose depot of birds subjected to dietary restriction from 0-12 and 0-14 weeks of age suggests that the retroperitoneal adipose depot may respond differently to dietary manipulation than the M. sartorius depot, and therefore may not be indicative of other adipose depots in the body (Pfaff and Austic, 1976).

Although the results of this experiment indicate that there was an increase in the adipocyte cellularity of the retroperitoneal depot in birds restricted from 0-12 and from 0-14 weeks of age; interpretation of these results must be viewed with caution because the actual number of adipocytes in the adipose tissue is unknown. The microscopic technique designed to determine adipocyte cellularity in this experiment only measured observable adipocytes greater than 25 μm and therefore was unable to detect the existence of preadipocytes in the stromal-vascular portion of the adipose tissue (Greenwood and Hirsch, 1974). The possibility exists therefore, that the increase in adipocyte cellularity of the retroperitoneal depot of birds subjected to dietary restriction from 0-12 and from 0-14 weeks of age, reflected the filling of preadipocytes rather than in increase in the total number of adipocytes.

Microscopic determination of adipocyte diameters permitted morphological inspection of the adipose tissue. There was, as mentioned earlier, an increase in the proportion of small adipocytes associated with the retroperitoneal adipose tissue of birds subjected to dietary restriction from 0-14 weeks of age, Figure 4. In addition there was a greater number of adipocytes less than 30 μm in diameter associated with retroperitoneal adipose tissue fragements of birds restricted from 0-14 weeks of age than of the control birds, Table XIII. It seems likely therefore, that the apparent increased adipocyte cellularity of the retroperitoneal depot could have resulted from the observed increased numbers of newly formed adipocytes associated with this depot.

It is not known how adipocyte multiplication is controlled. Greenwood and Hirsch (1974) have suggested that the rate at which preformed adipocytes fill with lipid is determined by the amount of lipid presented to the tissue and may provide a feedback signal to regulate cell proliferation in the growing rat. The increase in the number of retroperitoneal adipocytes in the birds in the present experiment which were subjected to feed restriction until they were 12 or 14 weeks old may have resulted from the shift in the feeding pattern rather than in the daily nutrient intake. The periodic surge in the amount of fat available for accumulation by adipocytes in birds given feed for only 30 minutes per day may have had a stimulatory effect on adipocyte proliferation despite the fact that the total amount of fat available per day for storage in the depots was small.

The liver is the primary site for lipid synthesis in the chicken in contrast to the adipose tissue as the important site for synthesis in the rat. However, the adaptation of the rat to periodic hyperphagia may be pertinent. Kazdova et al. (1968) found that rats adapt to a single 2-hr meal per day by acceleration of the rate of RNA synthesis in the adipocytes of the parametrial adipose tissue compared to that in rats fed ad libitum. The authors consider that the enhanced RNA and protein synthesis in the adipose tissue of meal-fed rats is associated with de novo synthesis of enzymes involved in adaptive hyperlipogenesis. Although the rate of filling of avian adipocytes is not associated with lipogenesis in these cells, birds may nevertheless adapt to periodic hyperphagia and periodic release of newly synthesized lipid from the liver to the circulation by an increase in adipocyte multiplication or in the number of the adipocytes which fill with lipid.

BIBLIOGRAPHY

Allen, C.E. 1976. Cellularity of adipose tissue in meat animals, Fed. Proc. 35: 2302-2307.

Anderson, D.B. and R.G. Kauffman. 1973. Cellular and enzymatic changes in porcine adipose tissue during growth. J. Lipid Res. 14: 160-168.

Anderson, D.B., R.G. Kauffman, and L.L. Kastenschmidt. 1972. Lipogenic enzyme activities and cellularity of porcine adipose tissue from various anatomical locations. J. Lipid Res. 13: 593-599.

Ball, E.G. and R.L. Jungas. 1964. III. Hormone and cellular metabolism. Some effects of hormones on the metabolism of adipose tissue. Recent Progr. Hormone Res. 20: 183-214.

Balnave, D. 1973. A review of restricted feeding during growth of laying-type pullets. Wlds. Poultry Sci. J. 29: 354-362.

Balnave, D. 1976. The effect of low-protein grower diets on the subsequent response of pullets to quantitative food restriction during lay. Br. Poultry Sci. 17: 145-150.

Bartov, I., S. Borstein and B. Lipstein. 1974. Effect of calorie to protein ratio on the degree of fatness in broilers fed on practical diets. Br. Poultry Sci. 15: 107-117.

Bartov, I. and S. Bornstein. 1976. Effects of degree of fatness in broilers on other carcass characteristics: relationship between fatness and the composition of carcass fat. Br. Poultry Sci. 17: 17-27.

Bell, D.J. and P.D. Sturkie. 1965. In: Avian Physiology edited by Sturkie, P.D.) Ithaca: Comstock, 1965. p.32.

Bell, E.T. 1909. On the occurrence of fat in the epithelium, cartilage, and muscle fibers of the ox. II. On the histogenesis of adipose tissue of the ox. Am. J. Anat. 9: 401-438.

Benson, J.D. and A. Bensadoun. 1977. Response of adipose tissue lipoprotein lipase to fasting in the chicken and the rat -- a species difference. J. Nutr. 107: 990-997.

Berg, L.R. and G.E. Bearse. 1958. Protein and energy studies with developing White Leghorn pullets. Poultry Sci. 37: 1340-1346.

Berg, L.R. 1959. Protein, energy and method of feeding as factors in the nutrition of developing White Leghorn pullets. Poultry Sci. 38: 158-165.

Best, C.H. and J. Campbell. 1936. Anterior pituitary extracts and liver fat. J. Physiol. (Lond.) 86: 190-203.

Bezrak, A.B.L. and Z. Harris. 1937. The effect of sympathectomy on the fatty deposit in connective tissue. Quart. J. Exptl. Physiol. 27: 1-16.

Biely, J. and B. March. 1957. Fat studies in poultry. 7. Fat and nitrogen retention in chicks fed diets containing different levels of fat and protein. Poultry Sci. 36: 1235-1240.

Bixler, E.G., G.F. Combs and C.S. Shaffner. 1968. Effect of protein level on carcass composition of turkeys. Poultry Sci. 47: 261-266.

Björntorp, P., M. Karlsson, H. Pertoft, P. Petterson, L. Sjostrom and U. Smith. 1978. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. J. Lipid Res. 19: 316-324.

Björntorp, P. and L. Sjöström. 1971. Number and size of adipose tissue fat cells in relation to metabolism and human obesity. Metabolism 20: 703-713.

Bjurulf, P. 1959. Atherosclerosis and body-build. Acta. Med. Scand. Supp. 349: 25-43.

Bolton, W., R. Blair and D.W. Night. 1970. Egg production of light and medium hybrids given diets varying in energy level during the chick, rearing and laying stages. Br. Poultry Sci. 11: 53-56.

Brambalia, S. and F.W. Hill. 1966. Comparison of neutral fat and free fatty acids in high lipid-low carbohydrate diets for the growing chicken. J. Nutr. 88: 84-92.

Bray, G.A. 1969. Studies on the composition of adipose tissue from genetically obese rats. Proc. Soc. Expl. Biol. Med. 131: 1111-1114.

Bremer, J.L. 1938. The protoplasmic films of the fat cell, the wall of the pulmonary alveolus, and renal glomerulus. Anat. Record 70: 263-281.

Brobeck, J.R. 1946. Mechanisms of the development of obesity in animals with hypothalamic lesions. Physiol. Rev. 26: 541-559.

Brook, C.G.D. 1971. Composition of human adipose tissue from deep and subcutaneous sites. Br. J. Nutr. 25: 377-380.

Brooker, B.E. and R. Fuller. 1975. Adhesion of lactobacilli to the chicken crop epithelium. J. Ultrastructure Res. 52: 21-31.

Butcher, R.W., J.G.T. Sneyd, C.R. Park, and E.W. Sutherland. 1966. Effect of insulin on adenosine 3', 5'-monophosphate in the rat epididymal fat pad. J. Biol. Chem. 241: 1651-1653.

Cameron, G.R. and R.D. Seneviratne. 1947. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Chaney, L.W. and H.L. Fuller. 1975. The relation of obesity to egg production in broiler breeders. Poultry Sci. 54: 200-208.

Clark, E.R. and E.L. Clark. 1940. Microscopic studies of the new formation of fat in living adult rabbits. Am. J. Anat. 67: 255-286.

Clarkson, T.B., J. Stanton Kink Jr., and N.H. Warnock. 1957. A comparison of the effect of gallogen and sulfarlem on the normal bile flow of the cockerel. Am. J. Vet. Res. 55: 187-190.

Cohn, C. and D. Joseph. 1962. Influence of body weight and body fat on appetite of "normal" and lean and obese rats. Yale Biol. Med. 34: 598-607.

Combs, G.F. 1968. Amino acid requirement of broilers and laying hens. Proc. Maryland Nutrition Conference for Feed Manufacturers, 86-96.

Combs, G.F., E.H. Boseard, G.R. Childs and D.L. Blamberg. 1964. Effect of protein level and amino acid balance on voluntary energy consumption and carcass composition. Poultry Sci. 43: 1309.

Conners, J.K., H.W. Burton and R.V. Brynes. 1971. The influence on subsequent performance of time between hatching of broiler chickens and their access to feed and water. Aust. Vet. J. 47: 551-552.

Cunningham, D.C.and W.D. Morrison. 1976. A dietary energy and fat content as factors in the nutrition of developing egg strain pullets and young hens. 1. Effect on several parameters and body composition at sexual maturity. Poultry Sci. 55: 85-97.

Cunningham, D.C. and W.D. Morrison. 1977. Diet energy and fat content as factors in the nutrition of developing egg strain pullets and young hens. 4. Effect on growth, hepatic lipogenic enzyme activity and body chemical composition of White Leghorn pullets from hatch to 20 weeks of age. Poultry Sci. 56: 1792-1805.

Deaton, J.W. and J.H. Quisenberry. 1963. Effect of calorie restriction during the growing period on the performance of egg type replacement stock. Poultry Sci. 42: 608-613.

Deaton, J.W., L.F. Kubena, T.C. Chen and F.N. Reece. 1974. Factors influencing the quantity of abdominal fat in broilers. 2. Cage versus floor rearing. Poultry Sci. 53: 574-576.

Dickerson, J.W.T. and R.A. McCance. 1960. Severe undernutrition in growing and adult animals. 3. Avian skeletal muscle. Br. J. Nutr. 14: 331-338.

Di Girolamo, M., S. Mendlinger and J.W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. Am. J. Physiol. 221: 850-858.

Di Girolamo, M. and Daniel Rudman. 1968. Variations in glucose metabolism and sensitivity to insulin of the rats adipose tissue, in relation to age and body weight. Endocrinology. 82: 1133-1141.

Dogliotti, G.C. 1928. Z. Zellforsch viii, 222. Cited from Cameron, G.R. and R.D. Seneviratne, 1948. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Dole, V.P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35: 150-154.

Dole, V.P. 1965. Energy storage. In: Handbook of physiology, Section 5: Adipose tissue (edited by Renold, A.E. and G.F. Cahill Jr.) Washington, D.C.: Am. Physiol. Sci. 1965 p.417-431.

Donaldson, W.E., G.F. Combs and G.L. Romoser. 1956. Studies on energy levels in poultry rations. 1. The effect of calorie-protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35: 1100-1105.

Donaldson, W.D., G.F. Combs and G.L. Romoser. 1958. Studies on energy levels in poultry rations. 3. Effect on calorie-protein ratio of the ration in growth, nutrient utilization and body composition of poults. Poultry Sci. 37: 614-619.

Douglas, C.R., R.H. Harms and W.G. Nesbeth. 1978. Performance of laying hens as influenced by length of time without feed. Poultry Sci. 57: 968-970.

Duncan, H.J. and R.H. Common. 1967. Glucose oxidation by liver slices from the domestic fowl. Activity of the phosphogluconate-oxidative pathway. Can. J. Biochem. 45: 979-989.

Edwards, H.M. Jr., R. Denman, A. Abou-Ashour and Denis Nugara. 1973. Carcass composition studies 1. Influence of age, sex and type of dietary fat supplementation on total carcass and fatty acid composition. Poultry Sci. 52: 934-948.

Enesco, M. and C.P. Leblond. 1962. Increase in cell numbers as a factor in the growth of the organs and tissues of the young male rat. J. Embryol. Exptl. Morphol. 10: 530-562.

Evans, A.J. 1972a. In vitro lipogenesis in the liver and adipose tissue of the female Aylesburg duck at different ages. Br. Poultry Sci. 13: 595-602.

Evans, A.J. 1972b. Fat accretion during postembryonic growth in the domestic duck, with additional data from the Mallard, Physiol. Zool. 45(1): 167-177.

Farrell, D.J. 1974. Effects of dietary energy concentration on utilization of energy by broiler chickens and on body composition determined by carcass analysis and predicted using tritium. Poultry Sci. 15: 25-41.

Favarger, P. 1965. Relative importance of different tissues in one synthesis of fatty acids. In: Handbook of physiology, Section 5: Adipose tissue (edited by Renold, A.E. and G.F. Cahill, Jr.) Washington, D.C.: Am. Physiol. Soc. 1965. p.19-25.

Feller, D.D. 1954. Metabolism of adipose tissue. 1. Incorporation of acetate carbon into lipides by slices of adipose tissue. J. Biol. Chem. 206: 171-180.

Flemming, W. 1871. Arch. mikr. Anat. vii 32, 328, cited from Cameron G.R. and R.D. Seneviratne, 1947. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Folch, J., M. Lees and G.H. Solane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497-509.

Foot, N.C. 1912. Beitr. path. Anat. liii, 446 cited from Cameron G.R. and R.D. Seneviratne, 1947. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Fraps, G.S. 1943. Relation of the protein, fat and energy of the ration to the composition of chickens. Poultry Sci. 22: 421-424.

Freeman, B.M. 1967. Some effects of cold on the metabolism of the fowl during the perinatal period. Comp. Biochem. Physiol. 20: 179-193.

Frohlich, J., A. Vost and C.H. Hollenberg. 1972. Organ culture of rat white adipose tissue. Biochim. Biophys. Acta. 280: 579-587.

Fuller, H.L. and W.S. Dunahoo. 1962. Restricted feeding of pullets 2. Effect of duration and time of restriction on three-year laying house performance. Poultry Sci. 41: 1306-1314.

Fuller, R. and B.E. Brooker. 1974. Lactobacilli which attach to the crop epithelium of the fowl. Am. J. Clin. Nutrition. 27: 1305-1312.

Gersh, I. and M.A. Still. 1945. Blood vessels in fat tissue. Relation to problem of gas exchange. J. Explt. Med. 81: 219-232.

Goldrick, R.B. 1967. Morphological changes in the adipocyte during fat deposition and mobilization. Am. J. Physiol. 212(4): 777-782.

Goodridge, A.G. 1964. The effect of insulin, glucagon and prolactin on lipid synthesis and related metabolic activity in migratory and non-migratory finches. Comp. Biochem. Physiol. 13: 1-26.

Goodridge, A.G. 1968a. Metabolism of glucose-U-^{14}C *in vitro* in adipose tissue from embryonic and growing chicks. Am. J. Physiol. 214: 897-901.

Goodridge, A.G. 1968b. Lipolysis *in vitro* in adipose tissue from embryonic and growing chicks. Am. J. Physiol. 214: 902-907.

Goodridge, A.G. 1968c. Citrate-cleavage enzyme, "malic" enzyme certain and dehydrogenases in embryonic and growing chicks. Biochem. J. 108: 663-666.

Goodridge, A.G. 1968d. The effect of starvation and starvation followed by feeding on enzume activity and the metabolism of U-^{14}C-glucose in liver from growing chicks. Biochem. J. 108: 667-673.

Goodridge, A.G. and E.G. Ball. 1966. Lipogenesis in the Pigeon: in vitro studies. Am. J. Physiol. 211: 803-808.

Goodridge, A.G. and E.G. Ball. 1967. Lipogenesis in the Pigeon: in vivo studies. Am. J. Physiol. 213: 245-249.

Gordon, R.S. Jr. and A. Cherkes. 1956. Unesterified fatty acid in human blood plasma. J. Clin. Invest. 35: 206-212.

Gous, R.M. and W.J. Stielau. 1976. Growth and laying performance of light-hybrid pullets subjected to quantitative food restriction. Br. Poultry. Sci. 17: 487-498.

Gowe, R.S., A.S. Johnson, R.D. Crawford, J.H. Downs, A.T. Hill, W.F. Mountain, J.R. Pelletier and J.H. Strain. 1960. Restricted versus full-feeding during the growing period for egg production stock. Br. Poultry Sci. 1: 37-41.

Greenwood, M.R.C. and J. Hirsch. 1974. Postnatal development of adipocyte cellularity in the normal rat. J. Lipid Res. 15: 474-483.

Griffiths, L., S. Leeson and J.D. Summers. 1977. Fat deposition in broilers: effect of dietary energy to protein balance, and early life caloric restriction on productive performance and abdominal fat pad size. Poultry Sci. 56: 638-646.

Gross, R.J. 1966. Hypertrophy versus hyperplasia Science 153: 1615-1620.

Hammar, J.A. 1895. Arch. mikr. Anat. xlv., 512 cited from Cameron, G.R. and R.D. Seneviratne, 1947. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Harms, R.H. and P.W. Waldrup. 1962. The effect of supplemental lysine and methionine in low protein laying diets. Poultry Sci. 41: 1648.

Harshaw, H.M. 1936. Effect of diet, range, and fattening on the physical and chemical composition of cockerels. J. Agr. Res. 53: 357-368.

Harshaw, H.M. 1938. The effect of fattening at different ages on the composition of cockerel. Poultry Sci. XVII: 163-169.

Haugebak, C.D., H.B. Hedrick and J.M. Asplund. 1974. Adipose tissue accumulation and cellularity in growing and fattening lambs. J. Anim. Sci. 39: 1016-1025.

Hausberger, F.X. 1938. Arch. path. Anat. cocii, 640 cited from Cameron, G.R. and R.D. Seneviratne, 1947. Growth and repair in adipose tissue, J. Path. Bact. 59: 665-676.

Hausberger, F.X., S.W. Milstein and R.J. Rutman. 1954. The influence of insulin on glucose utilization on adipose and hepatic tissue in vitro. J. Biol. Chem. 208: 431-438.

Hausberger, F.X. 1965. Effect of dietary and endocrine factors on adipose tissue growth. In: Handbook of Physiology. Section 5. Adipose Tisue (edited by Renold, A.E. and G.F. Cahill Jr.) Washington, D.C.: Am. Physiol. Soc. 1965. p.519-528.

Hazelwood, R.L. 1965. In: Avian Physiology (edited by Sturkie, P.D.) Ithaca: Comstock, 1963, p.313.

Hazelwood, R.L. 1971. Endocrine control of avian carbohydrate metabolism. Poultry Sci. 50: 9-18.

Hepburn, J.S. and R.C. Holder. 1922. Rations for feeding poultry in the packing house. U.S. Dept. Agri. Bull. 1052: 24.

Herberg, L., W. Doppen, E. Major and F.A. Gries. 1974. Dietary-induced hypertrophic-hyperplastic obesity in mice. J. Lipid Res. 15: 580-585.

Heuser, G.R., L.C. Norris and J.H. Bruckner. 1945. Pasture experiments with growing pullets. Bull. Cornell Univ. Agric. Exp. Stn. 823.

Hill, R.W. and L.M. Dansky. 1954. Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. Poultry Sci. 33: 112-119.

Hirsch, J. 1972. Can we modify the number of adipose cells. Postgrad. Med. 51(5): 83-86.

Hirsch, J. and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. J. Lipid Res. 9: 110-117.

Hirsch, J. and P.W. Han. 1969. Cellularity of rat adipose tissue: Effects of growth, starvation and obesity. J. Lipid Res. 10: 77-82.

Hirsch, J. and J.L. Knittle. 1970. Cellularity of obese and non-obese human adipose tissue. Fed. Proc. 29: 1516-1523.

Hirsch, J., J.L. Knittle and L.B. Salans. 1966. Cell lipid content and cell number in obese and non-obese human adipose tissue. J. Clin. Invest. 45: 1023.

Hollenberg, C.H. and A. Vost. 1968. Regulation of DNA synthesis in fat cells and stromal elements from rat adipose tissue. J. Clin. Invest. 47: 2485-2498.

Hoolenberg, C.H., A. Vost and R.L. Patten. 1970. Regulation of adipose mass: control of fat development and lipid content. Recent Progr. Hormone Res. 26: 463-503.

Hood, R.L. and C.E. Allen. 1973. Cellularity of bovine adipose tissue. J. Lipid Res. 14: 605-610.

Hood, R.L. and C.E. Allen. 1977. Cellularity of porcine adipose tissue -- effects of growth and adiposity. J. Lipid Res. 18: 275-283.

Houpt, T.R. 1958. Effects of fasting on blood sugar levels in baby chicks of varying ages. Poultry Sci. 37: 1452-1459.

Hubbard, R.W. and W.T. Matthew. 1971. Growth and lipolysis of rat adipose tissue. Effect of age, body weight and food intake. J. Lipid Res. 12: 286-293.

Husbands, D.R. 1972. The distribution of lipoprotein lipase in tissues of the domestic fowl and the effects of feeding and starving. Br. J. Poultry Sci. 13: 85-90.

Inglis, K. 1927. The so-called interscapulary gland and tumors arising therein. J. Antat. 61: 452-466.

Janse, G.R., C.F. Hutchison and M.E. Zanetti. 1966. Studies on lipogenesis *in vivo*. Effect of dietary fat or starvation on conversion of ^{14}C-glucose into fat and turnover of newly synthesized fat. Biochem. J. 99: 323-332.

Johnston, D.W. 1971. The absence of brown adipose tissue in birds. Comp. Biochem. Physiol. 40A: 1107-1108.

Johnson, P.R. and J. Hirsch. 1972. Cellularity of adipose depots in six strains of genetically obese mice. J. Lipid Res. 13: 2-11.

Johnson, P.R., J.S. Stern, M.R.C. Greenwood, L.M. Zucker and J. Hirsch. 1973. Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat. J. Nutrition 103: 738-743.

Johnson, P.R., L.M. Zucker, J.A.F. Cruce and J. Hirsch. 1971. Cellularity of adipose depots in the genetically obese Zucker rat. J. Lipid Res. 12: 706-714.

Jull, M.A. and W.A. Maw. 1923. Determination of the dressed, drawn and edible percentages of various kinds of domestic birds. Sci. Agr. 3: 329-338.

Kari, R.R., J.H. Quisenberry and J.W. Bradley. 1977. Egg quality and performance as influenced by restricted feeding of commercial caged layers. Poultry Sci. 56: 1914-1919.

Kazdova, L., T. Braun, P. Fabry and R. Poledne. 1968. Enhanced RNA and protein synthesis in adipose tissue of rats adapted to periodic hyperphagia. Can. J. Physiol. Pharmacol. 46: 903-906.

Von Kölliker, A. 1886. Anat. Anzeig i, 206 cited from Cameron, G.R. and R.D. Seneviratne. 1947. Growth and repair in adipose tissue. J. Path. Bact. 59: 665-676.

Knittle, J.L. 1972. Maternal diet as a factor in adipose tissue cellularity and metabolism in the young rat. J. Nutr. 102: 427-434.

Knittle, J.L. and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: Cellularity and metabolism. J. Clin. Invest. 47: 2091-2098.

Köhler, A. 1900. Hoppe-Seyler's Ztschr. Physiol. Chem. 31: 479-519, cited from Harshaw, H.M., 1936. Effect of diet, range, and fattening on the physical and chemical composition of cockerels. J. Agr. Res. 53: 357-368.

Korn, E.D. and T.W. Quigley. 1957. Lipoprotein lipase of chicken adipose tissue. J. Biol. Chem. 226: 833-839.

Kubena, L.F., B.D. Lott, J.W. Deaton, F.N. Reece and J.D. May. 1972. Body composition of chicks as influenced by environmental temperature and selected dietary factors. Poultry Sci. 51: 517-522.

Kubena, L.F., J.W. Deaton, T.C. Chen and F.N. Reece. 1974. Factors affecting the quantity of abdominal fat in broilers. 1. Rearing temperature, sex, age or weight, and dietary choline chloride and inositol supplementation. Poultry Sci. 53: 211-214.

Langslow, D.R. 1971. The anti-lipolytic action of prostaglandin E., on isolated chicken fat cells. Biochem. Biophys. Acta. 239: 33-37.

Langslow, D.R. 1972. The development of lipolytic sensitivity in the isolated fat cells of Gallus Domesticus during the fetal and neonatal period. Comps. Biochem. Physiol. 43B: 689-701.

Langslow, D.R., E.J. Butler, C.W. Hales and A.W. Pearson. 1970. The response of plasma insulin, glucose and NEFA to various hormone nutrients and drugs in the domestic fowl. J. Endocrinology 46: 243-260.

Langslow, D.R. and R.J. Lewis. 1972. The compositional development of adipose tissue in Gallus Domesticus. Comp. Biochem. Physiol. 438B: 681-688.

Lau, H.C., E. Flaim and S.J. Ritchey. 1976. Changes in body weights gain and adipose tissue cellularity in protein restricted and rehabilitated rats. Nutr. Rep. Int. 14: 32-42.

Laurell, S. 1956. Plasma free fatty acids in diabetic acidosis and starvation. Scand. J. Clin. Lab. Invest. 8: 81-82.

Lavau, M., C. Susini, J. Knittle, S. Blanchet-Hirst and M.R.C. Greenwood. 1977. A reliable photomicrographic method for determining fat cell size and number: Application to dietary obesity. Proc. Soc. Exp. Biol. Med. 156: 251-256.

Lee, A.R. 1911. Fattening Poultry. U.S. Dept. Agr. Bur. Anim. Indus. Bull. 140: 60.

Lee, P.J.W., A.L. Gulliver and T.R. Morris. 1971. Review article. A quantitative analysis of the literature concerning the restricted feeding of pullets. Br. Poultry Sci. 12: 413-437.

Lee, Y.B. and R.G. Kauffman. 1974. Cellular and enzymatic changes with animal growth in porcine intramuscular adipose tissue. J. Anim. Sci. 38: 532-537.

Lee, Y.B., R.G. Kauffman and R.H. Grummer. 1973a. Effect of early nutrition on the development of adipose tissue in the pig. 1. Age constant basis. J. Anim. Sci. 37: 1312-1318.

Lee, Y.B., R.G. Kauffman and R.H. Grummer. 1973b. Effect of early nutrition on the development of adipose tissue in the pig. II. Weight constant basis. J. Anim. Sci. 37: 1319-1325.

Lemonnier, D. 1972. Effect of age, sex, and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. J. Clin. Invest. 51: 2907-2915.

Lepkovsky, S. 1973. Hypothalamic-adipose tissue interrelationships. Fed. Proc. 36: 1705-1708.

Lepkovsky, S., A. Chari-Briton, R.M. Lemmon, R.C. Ostwald and M.K. Dimick. 1960. Metabolic and anatomic adaptations in chickens "trained" to eat their daily food in two hours. Poultry Sci. 39: 385-389.

Lepkovsky, S., M.K. Dimick, F. Furuta, W. Snapir; R. Park, W. Narita and K. Komatsu. 1967. Response of blood glucose and NEFA to fasting and to injection of insulin and testosterone in chickens. Endocrinology 81: 1001-1006.

Lepkovsky, S. and F. Furuta. 1971. The role of homeostasis in adipose tissues upon regulation of food intake of White Leghorn cockerels. Poultry Sci. 50: 573-577.

Lepkovsky, S. and M. Yasuda. 1966. Hypothalamic lesions, growth rate and body composition of male chickens. Poultry Sci. 45: 582-588.

Leveille, G.A. 1967. In vivo fatty acid synthesis in adipose tissue and liver of meal-fed rats. Proc. Soc. Exp. Bio. Med. 125: 85-88.

Leveille, G.A. 1969. In vitro hepatic lipogenesis in the hen and chick. Comp. Biochem. Physiol. 28: 431-437.

Leveille, G.A., E.K. O'Hea and K. Chakrabarty. 1968. In vivo lipogenesis in the domestic chicken. Proc. Soc. Exp. Biol. Med. 128: 398-401.

Leveille, G.A., D.R. Romos, Y.Y. Yeh, and E.K. O'Hea. 1975. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanism. Poultry Sci. 54: 1075-1093.

Li, J.C.R. 1966. Statistical inference I. Michigan: Edwards Brothers. Inc. 1966. p.252.

Lillie, R.J. and C.A. Denton. 1966. Effect of nutrient restriction on White Leghorns in the grower and subsequent layer periods. Poultry Sci. 45: 810-818.

Lipstein, B., S. Bornstein and I. Bartov. 1975. The replacement of some of the soybean meal by the first-limiting amino acids in practical broiler diets. 3. Effects of protein concentrations and amino acid supplementations in broiler finisher diets on fat deposition in the carcass. Br. Poultry Sci. 16: 627-635.

Lister, D., T. Cowen and R.A. McCance. 1966. Severe undernutrition in growing and adult animals 16. The ultimate results of rehabilitation: Poultry. Br. J. Nutr. 20: 633-639.

Luther, L.W., W.W. Abbott and J.R. Couch. 1976. Low lysine, low protein, and skip-a-day restriction of summer and winter reared broiler breeder pullets. Poultry Sci. 55: 2240-2247.

McCance, R.A. 1960. Severe undernutrition in growing and adult animals 1. Production and general effects. Br. J. Nutr. 14: 59-73.

McCance, R.A. and E.M. Widdowson. 1962. Nutrition and growth. Proc. Roy. Soc. London Ser. B. 156: 326-337.

McCance, R.A. 1976. Symposium on "Nutrition and Growth". Critical periods of growth. Proc. Nutr. Soc. 35: 309-314.

McCullough, A.W. 1944. Evidence of the macrophagal origin of adipose cells in the white rat as shown by studies on starved animals. J. Morphol. 75: 193-201.

March, B.E. and G. Hansen. 1977. Lipid accumulation and cell multiplication in adipose bodies in White Leghorn and broiler-type chicks. Poultry Sci. 56: 886-894.

Martinsson, A. 1968. Methods of isolation and characterization of human subcutaneous fat cells. Acta. Morphol. Neer. Scand. 7: 41-50.

Mason, J.V. and W.E. Donaldson. 1972. Fatty acid synthesizing systems in chick liver: influences of biotin deficiency and dietary fat. J. Nutr. 102: 667-672.

Maximow, S. 1927. Handbuch der mikroskopischen Anatomie des Menschen, v. Moeillendorf. 12/I: 232 cited from Wells, H.G., 1940. Adipose tissue, a neglected subject. J.A.M.A. 114: 2178-2183.

Montemurro, D.G. and J.A.F. Stevenson. 1957. Body composition in hypothalamic obesity derived from estimations of body specific gravity and extracellular fluid volume: Metabolism 6(1): 161-168.

Napolitano, L. 1965. The fine structure of adipose tissues. In: Handbook of Physiology, Section 5, Adipose tissue (edited by Renald, A.E. and G.F. Cahill Jr.) Washington, D.C.: Am. Physiol. Soc. 1965 p.109-124.

Novikoff, M. and J. Biely. 1945. Observations on two methods of feeding chickens from one day old to twelve months of age. Poultry Sci.24: 245-251.

Oscai, L.B., C.N. Spirakis, C.A. Wolff and R.J. Beck. 1972. Effects of exercise and of food restriction on adipose tissue cellularity. J. Lipid Res. 13: 588-592.

O'Hea, E.K. and G.A. Leveille. 1968. Lipogenesis in isolated adipose tissue of the domestic chick (Gallus Domesticus) . Comp. Biochem. Physiol. 26B: 111-120.

O'Hea, E.K. and G.A. Leveille. 1969a. Lipid biosynthesis and transport in the domestic chick (Gallus Domesticus). Comp. Biochem. Physiol. 30B: 149-159.

O'Hea, E.K. and G.A. Leveille. 1969b. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J. Nutr. 99: 338-344.

Pace, Nello and E.N. Rathbun. 1945. Studies on body composition.III. The body water and chemically combined nitrogen content in relation to fat content. J. Biochem. 158: 685-692.

Palmer, W.K. and C.M. Tipton. 1973. Influence of hypophysectomy and training on the size of isolated fat cells. Am. J. Physiol. 224: 1206-1209.

Pearce, J. 1968. The effect of dietary fat on lipogenic enzymes in the liver of the domestic fowl. Biochem. J. 109: 702-704.

Pearce, J. 1971a. An investigation of lipogenic and glycolytic enzyme activity in the liver of sexually immature and mature domestic fowl. Biochem. J. 123: 717-719.

Pearce, J. 1971b. Carbohydrate metabolism in the domestic fowl. Proc. Nutr. Soc. 30: 254-259.

Pearce, J. 1972a. Effect of diet and also physiological state on some enzymes of carbohydrate metabolism in the liver of the domestic fowl. Biochem. J. 130: 21-22p.

Pearce, J. 1972b. Changes in the activities of the lipogenic enzymes ATP-citrate lyase and the "Malic" enzyme, in the liver of the female domestic fowl (Gallus Domesticus) from four weeks of age to adulthood. Comp. Biochem. Physiol. 42B: 721-724.

Peckham, S.C., C. Entenman and H.W. Carroll. 1962. The influence of a hypercaloric diet on gross body and adipose tissue composition in the rat. J. Nutr. 77: 187-197.

Pepper, W.F., S.L. Slinger, J.D. Summers and J.D. McConachie. 1966. Effect of restricted feeding and debeaking on the reproductive performance of heavy type breeders. Poultry Sci. 45: 1387-1391.

Pfaff, Jr. F.E. and R.E. Austic. 1976. Influence of diet on the abdominal fat pad in the pullet. J. Nutri. 106: 443-450.

Pfeiffer, L. 1887. Ztschr. Biol. 23: 340-380 cited from Harshaw, H.M., 1936. Effect of diet, range and fattening on the physical and chemical composition of cockerels. J. Agri. Res. 53: 357-368.

Portis, B. 1924. Role of omentum of rabbits, dogs and guinea-pigs in antibody production. J. Infect. Dis. 34: 159-185.

Poznanski, W.J. and I. Rvan. 1973. Human fat cell presursors: Morphological and metabolic differentiation in culture. Lab. Invest. 29: 570-577.

Pratt, C.W.M. and R.A. McCance. 1961. Severe undernutrition in growing and adult animals 6. Changes in the long bones during the rehabilitation of cockerels. Br. J. Nutr. 15: 121-129.

Proudfoot, F.G. 1975. The response of broilers to delays between hatching and feeding under intermittent lighting treatments. Poultry Sci. 54: 405-408.

Raheja, K.L., J.G. Snedecor and R.A. Freedland. 1971. Activities of some enzymes involved in lipogenesis, gluconeogenesis, glycolysis and glycogen metabolism in chicks (Gallus Domesticus) from day of hatch to adulthood. Comp. Biochem. Physiol. 39B: 237-246.

Rakow, I., G. Beneke and C. Vogt. 1971. Changes in collagen content of white adipose tissue in starved-refed and obese mice. Beitr. Pathol. 144: 377-388.

Randle, P.J. 1963. Endocrine control of metabolism. Am. Rev. Physiol. 25: 291-324.

Rasmussen, A.T. 1923. The so-called hibernating gland. J. Morphol. 38: 147-192.

Reh, H. 1953. Arch. Pathol. Anat. Physiol. 324: 234-242 cited from Goldrick, R.B. 1976. Morphological changes in the adipocyte during fat deposition and mobilization. Am. J. Physiol. 212: 777-782.

Renold, A.E. and G.F. Cahill, Jr. 1965. Metabolism of isolated adipose tissue: A summary. In: Handbook of physiology, Section 5: Adipose tissue (edited by Renold, A.E. and Cahill, G.F. Jr.) Washington, D.C. Am. Physiol. Soc. 1965. p.483-490.

Renold, A.E., O.B. Crofford, W. Stauffacher and B. Jeanrenaud. 1965. Hormonal control of adipose tissue metabolism, with special reference to the effects of insulin. Diabetologia 1: 4-12.

Renold, A.E., A.I. Winegrad, B. Jeanrenaud and D.B. Martin. 1960. Suggested importance of adipose tissue as a site of insulin action and as a major site of metabolic interrelations between carbohydrates and fats in the mechanism of insulin action. Oxford: Blackwell Scientific publications Ltd. 1960. p.153.

Robinson, G., R.W. Butcher, and E.W. Sutherland. 1967. Adenyl cyclase as an adrenergic receptor. Am. N.J. Acad. Sci. 139: 703-723.

Rodbell, M. 1964a. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biochem. 239: 375-380.

Rodbell, M. 1964b. Localization of lipoprotein lipase in fat cells of rat adipose tissue. J. Biochem. 239: 753-755.

Rodbell, M. 1970. The fat cell in mid-term: Its past and future. In: Adipose Tissue. Regulation and metabolic functions (edited by Levine, R. and E.F. Pfeiffer). Germany: Georg Thieme Verlag Stuttgart. 1970. p.1-3.

Romsos, D.R. and G.A. Leveille. 1974. Effect of dietary fructose on *in vitro* and *in vivo* fatty acid synthesis in the rat. Biochim. Biophys. Acta. 360: 1-11.

Rudman, D. and M. Di Girolamo. 1967. Comparative studies on the physiology of adipose tissue. Advan. Lipid Res. 5: 35-117.

Ryzyllo, E., W. Szostak. 1978. Effect of overnutrition and undernutrition on cellularity of rat epididymal fat pad. Mat. Med. Pol. 8(4): 371-378.

Salans, L.B., E.S. Horton and E.A.H. Sims. 1971. Experimental obesity in man: Cellular character of adipose tissue. J. Clin. Invest. 50: 1005-1011.

Salans, L.B., J.L. Knittle and J. Hirsch. 1967. The role of adipose cell enlargement in the carbohydrate in tolerance of human obestiy. J. Clin. Invest. 46: 1112.

Salans, L.B., J.L. Knittle and J. Hirsch. 1968. The role of adipose cell size and adipose insulin sensitivity in the carbohydrate intolerance of human obesity. J. Clin. Invest. 47: 153-165.

Schemmel, R., O. Mickelsen and L. Fisher. 1973. Body composition and fat depot weights of rats as influenced by ration fed dams during lactation and that fed rats after weaning. J. Nutri. 103: 477-487.

Schneider, A.J., B.B. Bohren and V.L. Anderson. 1955. The effect of restricted feeding on several genetically controlled characters in the fowl. Poultry Sci. 34: 691-702.

Schoenheimer, R. and D. Rittenberg. 1937. Deuterium as an indicator in the study of intermediate metabolism IX. The conversion of stearic acid into palmitic acid in the organism. J. Biol. Chem. 120: 155-165.

Scott, M.L., F.W. Hill, E.H. Parsons Jr., J.H. Bruckner and E. Dougherty. III. 1959. Studies on duck nutrition. 7. Effect of dietary energy-protein relations upon growth, feed utilization and carcass composition in market ducklings. Poultry Sci. 38: 497-507.

Simon, G. 1965. Histogenesis. In: Handbook of physiology, Section 5, Adipose tissue (edited by Renold, A.E. and G.F. Cahill, Jr.) Washington, D.C. Am. Physiol. Soc. 1965. p.101-107.

Sims, E.A., R.F. Goldman, C.M. Gluck, E.S. Horton, P.C. Kelleher and D.W. Rowe. 1968. Experimental obesity in man. Trans. Ass. Am. Physiol. 81: 153-170.

Singsen, E.P., J. Nagel, S.G. Patrick and L.D. Matterson. 1965. The effect of lysine deficiency on growth characteristics, age at sexual maturity and reprodutive performance of meat type pullets. Poultry Sci. 44: 1467-1473.

Steinbaum, E.A. and N.E. Miller. 1965. Obesity from eating elicited by daily stimulation of hypothalamus. Am. J. Physiol. 208: 1-5.

Strain, J.H., R.S. Gowe, R.D. Crawford, A.T. Hill, S.B. Slen and W.F. Mountain. 1965. Restricted feeding of growing pullets. 1. The effect on the performance trait of egg production stock. Poultry Sci. 44: 701-716.

Strandberg, J. 1915. Hygiea 77: 372 cited from Wells, H.G. 1940. Adipose tissue a neglected subject. J.A.M.A. 114: 2179-2183.

Summers, J.D., W.F. Pepper, S.J. Slinger and J.D. McConachie. 1967. Feeding meat type pullets and breeders. Poultry Sci. 46: 1158-1164.

Summers, J.D., S.J. Slinger and G.C. Ashton. 1965. The effect of dietary energy and protein on carcass composition with a note on method for estimating carcass composition. Poutry Sci. 43: 501-509.

Temperton, H. and F.J. Dudley. 1941. The control of mash consumption during rearing. Harper Adams Util. Poultry J. 26(2): 33-36.

Therricault, D.G. and D.B. Mellin. 1971. Cellularity of adipose tissue in cold exposed rats and the caroligenic of norepinephrine. Lipids 6: 486-491.

Toldt, C. 1870. Sitzber, Akad Wiss Wien. Math. Naturwiss Kl. 62: 455 cited from Renold, A.E. and G.F. Cahill, Jr. (editors). Handbook of physiology. Section 5: Adipose tissue. Washington, D.C.: Am. Physiol. Soc. 1965. p.87.

Tuerkisher, E. and E. Wertheimer. 1942. Glycogen and adipose tissue. J. Physiol. (London) 100: 385-409.

Walkley, S.U., C.E. Hunt, R.S. Clements and J.R. Lindsey. 1978. Description of obesity in the PBB/Ld mouse. J. Lipid Res. 19: 335-341.

Wasserman, F. 1926. Z. Zellforsch, u. mikroskop. Anat 3:235 cited from Wertheimer, E. and B. Shapiro. 1948. The physiology of adipose tissue. Physiol. Rev. 28: 451-464.

Wasserman, F. 1965. The development of adipose tissue. In; Handbook of Physiology, Section 5, Adipose tissue (edited by Renold, A.E. and G.F. Cahill Jr.) Washington, D.C. : Am. Physiol. Soc. p. 87-100.

Watson, N.A. 1975. Reproductive activity of broiler hens subjected to restricted feeding during rearing. Br. Poultry. Sci. 16: 259-262.

Wells, H.G. 1940. Adipose tissue, a neglected subject. J.A.M.A., 114: 2177-2183.

Wertheimer, E. and B. Shapiro. 1948. The physiology of adipose tissue. Physiol. Rev. 28: 451-464.

Wertheimer, E. and E. Shafrir. 1960. Influence of hormones on adipose tissue as a center of fat metabolism. Recent Progr. Hormone Research 16: 467-496.

Winegrad, A.I. and A.E. Renold. 1958. Studies on rat adipose tissue in vitro. I. Effect of insulin on the metabolism of glucose, pyruvate and acetate. J. Biol. Chem. 233: 267-272.

Winick, M. and A. Noble. 1965. Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Develop. Biol. 12: 451-466.

Winick, M. and A. Noble. 1966. Cellular response in rats during malnutrition at various ages. J. Nutr. 89: 300-310.

Yates, J.D. and P.J. Schaible. 1963. Skip-feeding and energy level of the ration for developing Leghorn-type pullets. Feedstuffs Minneap. 35(46): 18-19.

Yeh, Y.Y.and G.A. Leveille. 1969. Effect of dietary protein on hepatic lipogenesis in the growing chick. J. Nutri. 98: 356-366.

Yeh, Y.Y. and G.A. Leveille. 1970. Hepatic fatty acid synthesis and plasma free fatty acid levels in chicks subjected to short periods of food restriction and refeeding. J. Nutri. 100: 1389-1398.

Yeh, Y.Y., G.A. Leveille and J.H. Wiley. 1970. Influence of dietary lipid on lipogenesis and on the activity of malic enzyme and citrate cleavage enzyme in liver of the growing chick. J. Nutri. 100: 917-924.

Zar, J.H. 1974. Biostatistical Analysis. Englewood Cliffs, N.J.: Prentice-Hall Inc. 1974.

Zing, W., A. Angel and M.D. Steinberg. 1961. Studies in the number and volume of fat cells in adipose tissue. Proc. Canad. Fed. Biol. Soc. 4: 68.

Zing, W., A. Angel and M.D. Steinberg. 1962. Studies on the number and volume of fat cells in adipose tissue. Canad. J. Biochem. Physiol. 40: 437-442.

APPENDIX TABLES I - XI

Table I(A). Statistical analysis comparing the body weights to 14 weeks of age, of male and female broiler-type chickens subjected to early dietary restriction.

i. Analysis of variance

a. 2 weeks of age

Source of variation	df	MS	F	P
Among groups	3	13011.3	185.17	$P < 0.05$
Within groups	141	70.3		

b. 3 weeks of age

Source of variation	df	MS	F	P
Among groups	3	93040.5	362.80	$P < 0.05$
Within groups	127	256.4		

c. 4 weeks of age

Source of variation	df	MS	F	P
Among groups	3	302240.0	352.29	$P < 0.05$
Within groups	111	875.9		

d. 5 weeks of age

Source of variation	df	MS	F	P
Among groups	3	798830.0	446.69	$P < 0.05$
Within groups	95	1788.3		

e. 6 weeks of age

Source of variation	df	MS	F	P
Among groups	3	1746600.0	490.48	$P < 0.05$
Within groups	81	3560.3		

f. 7 weeks of age

Source of variation	df	MS	F	P
Among groups	3	2810400.0	487.68	P < 0.05
Within groups	73	5762.8		

g. 8 weeks of age

Source of variation	df	MS	F	P
Among groups	3	4274900.0	596.37	P < 0.05
Within groups	75	7168.2		

h. 9 weeks of age

Source of variation	df	MS	F	P
Among groups	3	6919451.6	511.80	P < 0.05
Within groups	62	13518.6		

i. 10 weeks of age

Source of variation	df	MS	F	P
Among groups	3	10167460.4	641.60	P < 0.05
Within groups	62	15847.2		

j. 11 weeks of age

Source of variation	df	MS	F	P
Among groups	3	12486611.8	571.10	P < 0.05
Within groups	50	21864.0		

k. 12 weeks of age

Source of variation	df	MS	F	P
Among groups	3	16718566.8	516.70	P < 0.05
Within groups	51	32354.9		

l. 13 weeks of age

Source of variation	df	MS	F	P
Among groups	3	14859792.4	289.90	P < 0.05
Within groups	34	51265.8		

m. 14 weeks of age

Source of variation	df	MS	F	P
Among groups	3	19036362.4	494.46	P < 0.05
Within groups	34	38499.0		

ii. Individual degree of freedom contrast (multiple range)

a. 2 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	54	54	99	103
Conclusion ($P < 0.05$)	a	a	b	b

b. 3 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	71	72	190	202
Conclusion ($P < 0.05$)	a	a	b	b

c. 4 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	103	104	320	342
Conclusion ($P < 0.05$)	a	a	b	b

d. 5 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	139	141	489	565
Conclusion ($P < 0.05$)	a	a	b	c

e. 6 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	185	187	679	816
Conclusion ($P < 0.05$)	a	a	b	c

f. 7 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	255	259	887	1074
Conclusion ($P < 0.05$)	a	a	b	c

g. 8 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	289	290	1077	1281
Conclusion ($P < 0.05$)	a	a	b	c

h. 9 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	339	343	1333	1674
Conclusion ($P < 0.05$)	a	a	b	c

i. 10 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	396	415	1591	2030
Conclusion ($P < 0.05$)	a	a	b	c

j. 11 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	414	433	1792	2330
Conclusion ($P < 0.05$)	a	a	b	c

k. 12 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	481	508	2057	2695
Conclusion ($P < 0.05$)	a	a	b	c

l. 13 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	575	584	2294	2997
Conclusion ($P \leq 0.05$)	a	a	b	c

m. 14 weeks of age

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, in grams body weight)	642	667	2498	3296
Conclusion ($P \leq 0.05$)	a	a	b	c

Table II(A). Statistical analysis comparing the body weights of female broiler-type chickens at 38 weeks of age, previously subjected to early dietary restriction.

i. Analysis of variance

Source of variation	df	MS	F	P
Among groups	8	244810.0	2.01	$P > 0.05$
Within groups	47	121990.0		

ii. Individual degree of freedom contrast (multiple range)

Ranks of sample means (i)	1	2	3	4	5	6	7	8	9
Ranked sample means ($\bar{X}_i$, in grams body weight)	3453	3543	3833	3873	3882	3953	3954	3976	3980
Conclusion ($P \leq 0.05$)	a	a	b	b	b	b	b	b	b

Table III(A). Statistical analysis comparing the body weights of male broiler-type chickens at 38 weeks of age, previously subjected to early dietary restriction.

i. Analysis of variance

Source of variation	df	MS	F	P
Among groups	7	122090.0	0.34	$P > 0.05$
Within groups	28	360630.0		

ii. Individual degree of freedom contrast (multiple range)

Ranks of sample means (i)	1	2	3	4	5	6	7	8
Ranked sample means ($\bar{X}_i$, in grams body weight)	4375	4382	4546	4691	4704	4735	4841	4880
Conclusion ($P < 0.05$)	a	a	a	a	a	a	a	a

Table IV(A). Statistical analysis comparing the egg weights of broiler-type chickens between 36 and 38 weeks of age, previously subjected to early dietary restriction.

i. Analysis of variance

Source of variation	df	MS	F	P
Among groups	3	99.3	2.59	P > 0.05
Within groups	188	38.4		

Table V(A). Statistical analysis comparing the body weight, weights of selected organs and length of the tibiotarsus in female broiler-type chickens at 40-43 weeks of age, previously subjected to early dietary restriction.

i. Analysis of variance

a. Body weight (g)

Source of variation	df	MS	F	P
Among groups	3	441210.0	5.95	$P < 0.05$
Within groups	21	74146.0		

b. M. pectoralis major (g)

Source of variation	df	MS	F	P
Among groups	3	3281.6	3.01	$P > 0.05$
Within groups	21	1090.2		

c. Liver (g)

Source of variation	df	MS	F	P
Among groups	3	77.4	0.63	$P > 0.05$
Within groups	21	123.1		

d. Ovary (g)

Source of variation	df	MS	F	P
Among groups	3	238.0	1.31	$P > 0.05$
Within groups	21	181.2		

e. Oviduct (g)

Source of variation	df	MS	F	P
Among groups	3	18.69	0.64	$P > 0.05$
Within groups	21	29.17		

f. Tibiotarsus (g)

Source of variation	df	MS	F	P
Among groups	3	14.3	5.93	$P < 0.05$
Within groups	21	2.4		

g. Tibiotarsus length (cm)

Source of variation	df	MS	F	P
Among groups	3	0.9746	2.18	$P > 0.05$
Within groups	21	0.4472		

h. Retroperitoneal adipose depot (g)

Source of variation	df	MS	F	P
Among groups	3	3191.3	0.64	$P > 0.05$
Within groups	21	5091.5		

i. M. sartorius adipose depot (g)

Source of variation	df	MS	F	P
Among groups	3	22.8	3.61	$P < 0.05$
Within groups	21	6.3		

ii. Individual degree of freedom contrast (multiple range)

a. Body weight (g)

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, grams of body weight)	3403	3410	3813	3926
Conclusion ($P \leq 0.05$)	a	a	b	b

b. Tibiotarsus (g)

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, grams of tissue weight)	10.9	11.8	13.8	13.9
Conclusion ($P \leq 0.05$)	a	a	b	b

c. M. sartorius adipose depot (g)

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, grams of tissue weight)	5.3	6.3	9.0	9.1
Conclusion ($P \leq 0.05$)	a	ab	b	b

Table VI(A). Statistical analysis comparing the relative weights of selected organs and length of the tibiotarsus in broiler-type female chickens at 40-43 weeks of age, previously subjected to early dietary restriction. Calculations were performed on "arcsin a" transformed data.

i. Analysis of variance

a. Body weight (g)

Source of variation	df	MS	F	P
Among groups	3	441210.0	5.95	$P < 0.05$
Within groups	21	74146.0		

b. M. pectoralis major (%)

Source of variation	df	MS	F	P
Among groups	3	0.8966	1.28	$P > 0.05$
Within groups	21	0.7013		

c. Liver (%)

Source of variation	df	MS	F	P
Among groups	3	0.2712	0.68	$P > 0.05$
Within groups	21	0.4003		

d. Ovary (%)

Source of variation	df	MS	F	P
Among groups	3	1.91	3.03	$P > 0.05$
Within groups	21	0.63		

e. Oviduct (%)

Source of variation	df	MS	F	P
Among groups	3	0.8233	6.0	$P < 0.05$
Within groups	21	0.1371		

f. Tibiotarsus (%)

Source of variation	df	MS	F	P
Among groups	3	5.34	1.72	P > 0.05
Within groups	21	3.09		

g. Tibiotarsus length (cm)

Source of variation	df	MS	F	P
Among groups	3	0.9746	2.18	P > 0.05
Within groups	21	0.4472		

h. Retroperitoneal adipose depot (%)

Source of variation	df	MS	F	P
Among groups	3	1.09	0.23	P > 0.05
Within groups	21	4.67		

ii. M. sartorius adipose depot (%)

Source of variation	df	MS	F	P
Among groups	3	0.36	2.42	P > 0.05
Within groups	21	0.15		

iii. Individual degree of freedom contrast (multiple range)

a. Body weight (g)

Ranks of sample means (i)	1	2	3	4
Ranked sampled means ($\overline{X}_i$, grams of body weight)	3403	3410	3810	1926
Conclusion ($P < 0.05$)	a	a	b	b

b. Oviduct (%)*

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\overline{X}_i$, % of body weight)	7.332	7.491	7.937	8.113
Conclusion ($P < 0.05$)	a	a	b	b

* values are arcsin $\sqrt{a}$

Table VII(A). Statistical analysis comparing the average adipocyte diameter in the retroperitoneal adipose depot of female broiler-type chickens at different ages.

i. Analysis of variance

Source of variation	df	MS	F	P
Among Treatments	2	892.8	6.72	$P < 0.05$
Among Ages	1	2233.0	16.81	$P < 0.05$
Interaction	2	133.6	1.01	$P > 0.05$
Error	28	132.8		

ii. Student Newman-Keuls multiple range:

a. Treatment effect

Ranks of sample means (i)	1	2	3
Ranked sample means ($\bar{X}_i$, average adipocyte diameter, um)	84.9	93.1	103.0
Conclusion ($P < 0.05$)	a	ab	b

b. Age effect

Ranks of sample means (i)	1	2
Ranked sample means ($\bar{X}_i$, average adipocyte diameter, um)	83.2	100.5
Conclusion ($P < 0.05$)	a	b

Table VIII(A). Statistical analysis comparing the average coefficient of variation of the mean adipocyte diameter (relative dispersion) in the retroperitoneal adipose depot of broiler-type chickens with age and dietary restriction.

i. D'Agostino's assessment for normality (Zar, 1974, p.82)

SS = 0.0496

$D = T/\sqrt{n^3 SS}$; where $T = \Sigma (i - \frac{n+1}{2})X_i$

D = 0.2661

Since D is neither < 0.2609 nor > 0.2873, at $P < 0.01$; (Table D.26, Zar, 1974), do not reject H_o; i.e. the coefficient of variation came from a normally distributed population.

ii. Analysis of variance

Source of variation	df	MS	F	P
Among Treatments	2	0.655×10^{-3}	1.03	$P > 0.05$
Among Ages	1	26.955×10^{-3}	42.24	$P < 0.05$
Interaction	2	1.847×10^{-3}	2.89	$P > 0.05$
Error	28	0.638×10^{-3}		

iii. Student Newman-Keuls multiple range

a. Treatment effect

Ranks of sample means (i)	1	2	3
Ranked sample means ($\bar{X}_i$, average adipocyte diameter, um)	0.12	0.13	0.13
Conclusion ($P < 0.05$)	a	a	a

b. Age effect

Ranks of sample means (i)	1	2
Ranked sample means ($\bar{X}_i$, average adipocyte diameter, um)	0.10	0.16
Conclusion ($P < 0.05$)	a	b

Table IX(A). Statistical analysis comparing the average adipocyte diameter between the retroperitoneal and M. sartorius adipose depots of female broiler-type chickens at 40-43 weeks of age, previously subjected to early dietary restriction.

i. Analaysis of variance

Source of variation	df	MS	F	P
Among Treatments	3	722.9	5.60	$P < 0.05$
Among Depots	1	3723.9	28.90	$P < 0.05$
Interaction	2	8.1	0.06	$P > 0.05$
Error	28	129.0		

ii. Student Newman-Keuls multiple range

a. Treatment effect

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, average adipocyte diameter, um)	86.6	89.9	99.6	102.8
Conclusion ($P < 0.05$)	a	a	b	b

b. Depot effect

Ranks of sample means (i)	1	2
Ranked sample means ($\bar{X}_i$, average adipocyte diameter um)	85.6	102.9
Conclusion ($P < 0.05$)	a	b

Table X(A). Statistical analysis comparing the average coefficient of variation of the mean adipocyte diameter (relative dispersion), in the retroperitoneal and M. sartorius adipose depots of broiler-type chickens with depot and dietary restriction.

i. D'Agostino's assessment for normality (Zar, 1974, p.82)

SS = 0.0150

$D = T/\sqrt{n^3\ SS}$; where $T = \Sigma\ (i - \frac{n+1}{2})X_i$

D = 0.2672

Since D is neither $<$ 0.2655 nor $>$ 0.2874 at $P < 0.01$, (Table D.26, Zar, 1974), do not reject H_o i.e. the coefficient of variation came from a normally distributed population.

ii. Analysis of variance

Source of variation	df	MS	F	P
Among Treatments	3	$6.0497\ X10^{-4}$	2.10	$P > 0.05$
Among Depots	1	$5.7800\ X10^{-4}$	2.01	$P > 0.05$
Interaction	3	$1.7614\ X10^{-4}$	0.61	$P > 0.05$
Error	42	$2.8802\ X10^{-4}$		

Table XI(A). Statistical analysis comparing the average adipocyte cellularity in the retroperitoneal and M. sartorius adipose depots of female broiler-type chickens at 40-43 weeks of age, previously subjected to early dietary restriction.

i. Analysis of variance

a. Retroperitoneal adipose depot

Source of variation	df	MS	F	P
Among groups	3	21939.9	4.42	$P < 0.05$
Within groups	21	4958.1		

b. M. sartorius adipose depot

Source of variation	df	MS	F	P
Among groups	3	3.63	0.17	$P > 0.05$
Within groups	21	21.01		

ii. Individual degree of freedom contrast (multiple range)

Ranks of sample means (i)	1	2	3	4
Ranked sample means ($\bar{X}_i$, adipocyte cellularity $X10^6$)	305.8	349.0	430.8	428.0
Conclusion ($P < 0.05$)	a	a	b	b