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**OVARIAN MORPHOLOGY AND STEROIDOGENESIS IN DOMESTIC FOWL
(*GALLUS DOMESTICUS*): EFFECTS OF AGING, STRAIN,
PHOTOSTIMULATION PROGRAM AND LEVEL OF FEEDING**

BY

MARYLA EWA LUPICKI

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**A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
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DEGREE OF**

MASTER OF SCIENCE

IN

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DEPARTMENT OF ANIMAL SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Ovarian Morphology and Steroidogenesis in Domestic Fowl (*Gallus domesticus*): Effects of Aging, Strain, Photostimulation Program and Level of Feeding** submitted by Maryla Ewa Lupicki in partial fulfilment of the requirements for the degree of Master of Science in Avian Reproductive Physiology.



Dr. Frank E. Robinson, Supervisor



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Dr. R. T. Hardin



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DATE: 1 December, 1993

***"How much better is it to get wisdom than gold!
and to get understanding rather than silver!"***

Proverbs

***For GOD and my two children,
Camille Marie and Marc Daniel for their power.***

ABSTRACT

A study was conducted with 13 groups of female domestic fowl (10 hens per group) varying in age, strain, photostimulation program and level of feeding. Age at sexual maturity, organ weight (heart, liver, abdominal fat pad weight), body composition (carcass fat, protein, water, ash), reproductive organ parameters (oviduct, ovary, ovarian stroma, number and size of large follicles) and estradiol-17 β output from small white follicles (SWF) were examined. Egg-type birds (Single Comb White Leghorn-SCWL) were divided into six treatments based on their age and photostimulation program. Meat-type birds (broiler breeder-BB) were divided into eight groups based on aging, photostimulation program and level of feeding. Classifications of SCWL were based on hens sacrificed: at 19 wks, which is one week prior to photostimulation (SCWL-19); at sexual maturity (SM) when photostimulated at 20 wks (SCWL-P) and non photostimulated (SCWL-NP); at 35 wks of age (SCWL-35); and at 60 wks of age (SCWL-60). Full-fed and feed-restricted BB were sacrificed: at sexual maturity when photostimulated (FF-BB-P; RR-BB-P) and not photostimulated (FF-BB-NP; RR-BB-NP); at 35 wks (FF-BB-35; RR-BB-35) and 60 wks of age (FF-BB-60; RR-BB-60). Birds were killed by cervical dislocation and body organs were removed.

Photostimulation had the greatest effect on RR-BB, followed by FF-BB, followed by SCWL. Age at SM was delayed in these groups by 40.1, 20.2 and 6.2 days respectively. There were significant age, strain and level of feeding effects on BW in all groups. In SCWL and FF-BB, photostimulation did not significantly influence body weight at sexual maturity. Strain and level of feeding had a significant effect on abdominal fat pad weight and carcass fat quantity. Oviduct weight increased with age. In SCWL and RR-BB aging influenced oviduct weight from SM to 60 wks. BB had heavier oviducts than did SCWL. Strain influenced oviduct weight at SM when photostimulated and not photostimulated and at 35 wks

of age. Photostimulation did not significantly influence oviduct weight in SCWL, FF-BB or RR-BB at sexual maturity. Level of feeding influenced oviduct weight in BB at 35 and at 60 wks of age. There was considerable variability in ovary weight. In SCWL total ovary weight increased with age. BB exhibited a significantly higher total ovary weight than SCWL. Photostimulation did not influence total ovary weight. Full feeding resulted in greater ovary development in BB. Ovarian stroma weight was higher at 35 wks, than it was at SM in SCWL, FF-BB and RR-BB. No major differences in stroma weight were observed between 35 and 60 wks in SCWL and RR-BB. BB had significantly higher stroma weight than SCWL. Photostimulated SCWL exhibited significantly higher ovarian stroma weight than non photostimulated birds. Aging did not influence the number of large follicles in SCWL. In FF-BB and RR-BB the number of large follicles decreased significantly with age. BB had more large follicles than SCWL in all age groups. Photostimulation did not influence the number of large follicles in BB.

Small white follicles (SWF) from all treatments were capable of producing estradiol-17 β . Follicles from immature birds, prior to photostimulation (SCWL-19) also had ability to produce estradiol-17 β . This hormone production was stimulated by LH. Estradiol-17 β did not increase further with addition of 10 and 20 ng of LH from that seen with 5 ng of LH. This may indicate that presence of LH receptors in SWF is limited and according to these studies 5 ng of LH could be the maximum amount which stimulates estradiol-17 β production. Furthermore, it can be concluded that ovaries that produce large amounts of estrogen contain follicles that are very LH sensitive and also have a low incidence of atresia. Consequently, the ability to produce estradiol-17 β by SWF is related to the ovarian morphology or "form" of the ovary is related to its "function".

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1. LITERATURE REVIEW

1.1 Introduction

Reproduction in the female chicken is a complex process that involves the entire body. In general, the domestic hen begins to lay eggs at about 22 wks of age (Bahr and Palmer, 1989). During the next 6 to 8 wks egg production increases to 85-90 % of total egg production. This rate of laying can be maintained for several months (Robinson *et al.*, 1990). As the hen gets older egg production may decrease or even stop, depending upon genetic strain, nutrition, environmental conditions and photoperiod (Bahr and Palmer, 1989). It is currently thought that the production of 280 eggs per hen per year has reached a plateau. Opel (1979) suggested that to overcome the current genetic plateau, new selection methods and criteria must be developed based on the present knowledge of the physiological or biochemical basis of egg production. Through the use of radioimmunoassays capable of measuring very small concentrations of hormones in plasma, tissues and incubation media more could be found out about the control of avian reproductive physiology, particularly effects of strain, age, photostimulation program and level of feeding.

1.2 Structure of the Reproductive System

The reproductive system of the domestic fowl consists of a single left ovary and an oviduct, although an occasional functional right ovary and oviduct may be present (Johnson, 1986). At the beginning of embryonic development both ovaries and oviducts are present in the domestic fowl, however by day 10 of incubation regression of the right oviduct begins (Johnson, 1986). Presence of two ovaries in the body cavity of domestic hen is disadvantageous because of the problem of providing enough calcium for the shells of two eggs at once (Taylor, 1970). Birds have some difficulties late in lay in supplying calcium for one egg a day rather than two. Certain

species of wild birds have retained two functional ovaries and oviducts. It is not known how ovulation is controlled in these species, but apparently wild birds do not lay two eggs in one day (Taylor, 1970).

1.2.1 The Hypothalamus

The hypothalamus is an essential component of the endocrine system controlling ovulation. The hypothalamus is a relatively small structure occupying about 3% of the total brain volume (Scanes *et al.*, 1984). The hypothalamus consists of the anterior hypothalamus (including pro-optic nucleus, supra-optic nucleus and paraventricular nucleus) and the posterior hypothalamus including medial posterior and the infundibular nuclear complex (Scanes *et al.*, 1984). The hypothalamus of the hen produces two molecular species of GnRH (I and II). GnRH II is an important physiological regulator of LH, unlike chicken GnRH I which is present in only a minute in the hypothalamus (Sharp, 1980).

1.2.2 The Hypophysis

There is a vascular link between the hypothalamus and the adenohypophysis. The hypophysis secretes hormones which co-ordinate the secretion of other endocrine glands. The hypophysis is divided into the adenohypophysis (anterior lobe) and neurohypophysis (posterior lobe) (Sharp, 1980; Scanes, 1984). The adenohypophysis consists of the pairs distalis and pars tuberalis. The neurohypophysis consists of neural lobe, median eminence (divided into anterior and posterior regions), and infundibular stem. The neural lobe consists of nerve fibres accessing from supra-optic, paraventricular and infundibular nuclei in the hypothalamus. In birds, the pars tuberalis act primarily as a communication channel connecting the median eminence and the pairs distalis via blood vessels. Two histologically different portions of the pairs distalis were distinguished and referred to as the cephalic and caudal lobe. The

candal lobe of the hypophysis is the major site for luteinizing hormone (LH) producing cells. LH in the hen is misnamed because it does not luteinize the follicles after ovulation. In the hen, the functions of LH are to cause follicular rupture, ovulation and to promote steroidogenesis (Burke *et al.*, 1979; Johnson and van Tienhoven, 1980; Robinson and Etches, 1986). Each ovulation is induced by a surge of LH which occurs 6 h before ovulation (Bahr and Palmer, 1989).

1.2.3 The Ovary

During the first 15 wks of life, the ovary of the domestic hen grows slowly and contains only follicles less than 3 mm in diameter (Etches, 1990). The gross morphology of the ovary reflects the reproductive state of the hen (Gilbert, 1971). In the non-laying state, the ovary weighs from 0.3 to 0.5 g (Nalbandov and James, 1949; Amin and Gilbert, 1979). The ovary of the immature bird consists of a mass of small ova, of which at least 2,000 are visible to the naked eye (Pearl and Schoppe, 1921). Only a relatively few of these (200-500) reach maturity and are ovulated within the life time in normal husbandry (Johnson, 1986). In the laying hen the ovary weighs between 40 and 60 g (Romanoff and Romanoff, 1949; Yu *et al.*, 1992b ; Robinson *et al.*, 1993). There are three classes of small follicles: small yellow follicles (SYF, 5 to 10 mm), ten large white follicles (LWF, 2 to 3 mm) and thousands of small white follicles (SWF, 1 mm or less) (Robinson and Etches, 1986). Counts of SWF were reported by Pearl and Schoppe (1921) to be 3,000, and more than 1,000 small white follicles have been removed from the ovary of laying hen (Robinson and Etches, 1986). These small follicles are recruited into the large follicle hierarchy when they reach approximately 10 mm in diameter (Etches, 1990). In the ovary, there is usually only one small follicle recruited into the rank of the large follicles at one time, thus creating a distinct hierarchy of sizes of large follicles (Gilbert, 1971; Zakaria *et al.*, 1984). The ovary of a sexually mature hen usually

has seven to ten large yellow follicles, whose diameters range from 10 to 35 mm (Etches *et al.*, 1983). These follicles are designated as F1 to F10, with the F1 being the largest, most mature follicle which is the next to ovulate. Once this follicle has ovulated, the next largest follicle (F2) will mature and eventually ovulate.

The two populations of follicles differ in the incidence of atresia occurring within them. Atresia is described as a morphological change in granulosa and theca cell activity. Atretic follicles have a crumbling appearance and have a shortening of smooth muscle cells. The large yolky follicles of mature laying birds rarely become atretic, however this is very common fate for small follicles (Gilbert *et al.*, 1983; Etches and Duke, 1984). One of the changes during follicular atresia is a reduction in aromatase activity (Armstrong, 1985). Aromatase activity was detected in the smallest follicle examined and increased as small follicles increased in size. However, once the follicle is recruited into the pool of large yolky follicles (> 10 mm) the aromatase activity decreases rapidly as the follicle approaches ovulation.

The ovary of the hen is a heterogenous tissue containing two predominant cell types in various stages of maturation that are capable of producing progesterone, androgens, estrogens and prostaglandins. Hertelendy *et al.* (1984) strongly suggests that prostaglandins are present in the ruptured follicle and that prostaglandins can induce oviposition of the egg. Plasma concentrations of prostaglandins are higher when there is an egg in the shell gland than shortly after oviposition (Hertelendy and Biellier, 1978). Shortly before oviposition, the prostaglandin concentration of ruptured follicles (post ovulatory follicles) rises sharply. Although this evidence is quite persuasive, it does not mean that the prostaglandins involved in oviposition are produced in the ruptured follicle. Ogasawara and Koga (1978) have presented evidence that indicates that prostaglandins are probably produced in the shell gland and that these prostaglandins are involved in oviposition. It is likely that other hormones such as relaxin, inhibin and oxytocin are produced by avian follicles in

certain stages of maturation (Etches, 1990).

Each follicle consists of concentric layers of tissue that surround the oocyte and yolk including: 1) the oocyte plasma membrane, 2) the perivitelline membrane, 3) granulosa cells, 4) basal lamina which surrounds the granulosa cells, acts as a mechanical filter which does not permit lipoprotein particles to pass through it (Perry *et al.*, 1978), and 5) theca cells (interna and externa) (Gilbert, 1979). Immature follicles contain several layers of granulosa cells, that subsequently become a monolayer in a large, yolky follicles (Etches, 1990). In addition, the theca layer is less developed in immature follicles than it is in large yolky follicles.

1.2.4 The Oviduct

The oviduct of the hen consists of five distinguishable regions: the infundibulum, the magnum, the isthmus, the shell gland (uterus) and the vagina. Subsequent to ovulation, the follicle is dropped into the infundibulum, which is not directly connected to the ovary (North, 1990). The follicle stays in the infundibulum for approximately 15-30 minutes (Warren and Scott, 1935). If the infundibulum fails to receive the follicle, the ovum remains in the body cavity and internal ovulation occurs. During the time the follicle is in the infundibulum, it is fertilized if sperm are present. The ovum next passes to the largest portion of the oviduct, the magnum (in the chicken-33 mm length). The follicle remains in the magnum approximately 2-3 h (Warren and Scott, 1935) and the deposition of the most albumen occurs (North, 1990). The proteins of albumen are synthesized in the magnum from amino-acids removed from the blood. Both inner and outer shell membranes are formed during the 1 to 2 h passage through the isthmus (Warren and Scott, 1935). The developing egg spends most of its time in the shell gland (uterus), taking from 20 to 26 h to pass through (Warren and Scott, 1935). Before deposition of the shell, the developing egg takes up water and salts into the albumen from tubular glands, which

are present in the shell gland (North, 1990). During a period of 15 h the calcification of the egg is completed and the pigment of the shell is formed during the next 5 h (Warren and Conrad, 1942). The vagina has no role in the formation of the egg but, its main function is to aid in oviposition. The egg rotates, allowing oviposition to occur (North, 1990). Transport of the egg through the whole oviduct is mainly accomplished by muscular contractions of the oviduct (Johnson, 1986).

1.3 Ovarian Steroidogenesis and the Ovulatory Cycle

1.3.1 Ovarian Steroid Hormone Production

Steroid hormones are produced by the left ovary in early embryogenesis (Senior and Furr, 1975). After hatching, growth and development of the ovary continue. The production of steroid hormones by the avian follicles depends on their stage of development (Etches and Duke, 1984). All ovarian follicles ranging from the least mature (small white follicles) up to the F1 follicle contain cells which are capable of producing steroid hormones. The small follicles are the major source of androgens and estrogens (Armstrong, 1984; Robinson and Etches, 1986). At the small follicle stage of development, progesterone is not produced either in the presence nor the absence of LH, and has not been detected in the plasma samples taken at the time before the large follicle hierarchy is formed (Williams and Sharp, 1977). After the follicle enters the phase of rapid growth (> 10 mm), it gains the ability to synthesize progesterone (Tilly, 1991). The synthesis and secretion of progesterone by the largest follicles is necessary to potentiate the LH surge. Johnson and van Tienhoven (1980) noticed that without a preovulatory surge of progesterone there was no LH surge.

The various cells of a hen's ovary act in concert to produce progestagens, androgens and estrogens. Pregnenolone can be metabolized to progesterone in both granulosa and theca tissue of large follicles and theca tissue of small white follicles

(Marrone and Hertslendy, 1983). However, the granulosa tissue of the largest follicles is generally accepted to be the major source of progesterone in the hen's ovary (Etches *et al.*, 1981). Injections of LH result in increased progesterone secretion (Shahabi *et al.*, 1975). In large follicles of the hen, the number of LH receptors on the granulosa cells increases as a follicle approaches ovulation (Wells *et al.*, 1983). Consequently, the F1 follicle has been found to contain a greater LH receptor population than that of F2-F4 follicles (Bahr and Johnson, 1984). Also, preovulatory follicles (F2-F3) have greater ability to convert 25-hydroxy-cholesterol to pregnenolone and to convert pregnenolone to progesterone. Among the large follicles, the biosynthesis of estradiol-17 β by theca tissue is maximal in F3 and F4 follicles (Bahr *et al.*, 1983). The granulosa tissue from the large follicles neither contains nor produces detectable levels of estradiol-17 β (Etches and Duke, 1984). On the other hand, the granulosa tissue of avian follicles produces progesterone, which is further metabolized to androgens and estrogens by the theca tissue. According to Porter *et al.* (1989), androgens are produced by theca interna and estrogens by theca externa. The $\Delta 5$ pathway in avian ovarian follicles is predominant in small follicles. However, the $\Delta 4$ pathway becomes functional as the follicle enters the phase of rapid growth (Robinson and Etches, 1986). Synthesis of progesterone, androgens and estrogens in avian follicles shifts from $\Delta 5$ to $\Delta 4$ pathway with increasing follicular maturity (Robinson and Etches, 1986).

1.3.2 Estrogens

Estrogen appears to play a prominent role in avian steroidogenesis. Estradiol-17 β , estrone and estradiol-17 α are the most significant estrogens in the sexually mature hen (Marthier *et al.*, 1966). Estrogen presence in the yellow follicles was first reported in 1940 (Marlow and Richart, 1940). The steroid assayed most commonly to indicate estrogen metabolism is estradiol-17 β . According to Tanig and

Grunder (1984) estradiol-17 β is the major estrogen in the hen while estradiol-17 α is only a minor metabolite. Estradiol output by the F1 follicle is low (Wang and Bahr, 1983; Armstrong, 1984; Robinson and Etches, 1986). Among the large follicles, the biosynthesis of estradiol by theca tissue is maximal in F3 and F4 (Bahr *et al.*, 1983). Small follicles are the major sources of estrogens (Armstrong, 1985) and produce large amounts of estradiol in both the presence and absence of bLH (Robinson and Etches, 1986). During the ovulatory cycle two peaks of estradiol have been reported. The first peak, which has not been well documented was at 22-18 h prior to ovulation. The second peak was at 6-2 h prior to ovulation. There is general agreement that the plasma concentration of estradiol is maximal 6 to 2 h before ovulation (Etches *et al.*, 1984a,b). The preovulatory peaks of LH and estradiol occur almost at the same time, so a cause and effect relationship between these two hormones in the regulation of ovulatory cycle makes a logical sense.

Estrogen has many functions in the domestic fowl. It increases deposition of body fat and stimulates growth of the oviduct (Gilbert, 1971). Estrogen plays an important role in egg production. It acts on the liver to produce the specific yolk lipoprotein. Most of the proteins present in egg albumen are produced in response to estrogens (Gilbert, 1971). Estrogen has been involved in calcium metabolism during the ovulatory cycle (Taylor and Dacks, 1984) and assists in calcium transport across the wall of the shell gland by enhancing formation of calcium binding protein (Guyer *et al.*, 1980). It also plays a role in the deposition of extra calcium in the bones. However, the exact mechanism of that process is not clear. Finally, it highly influences secondary sexual characteristics such as shape, colour and pattern of feathers (Johnson, 1986).

Immature birds have a high plasma concentration of LH because the positive feedback regulation of the hypothalamic-adenohypophyseal-axis has not yet become fully functional (Sharp, 1983). The increase in plasma concentration of LH

stimulates the small follicles to produce dehydroepiandrosterone (DHEA), which serves as a substrate for further synthesis of estrogens. Progesterone is not present yet in circulation. After sexual maturation, the small follicles continue to produce a significant amount of estrogen and androgen in response to LH stimulation.

1.3.3 Fraps's Hypothesis

The ovulatory cycle is a complex physiological process which is regulated by two independent systems (Fraps, 1965). One of them is maturation of the steroidogenic system of the largest follicle (F1) (Johnson, 1990) which determines follicular maturation (Fraps, 1965). Follicular maturity is not governed by the size of the follicle (Etches *et al.*, 1983). It is governed by the ability of the follicle to produce and secrete progesterone in response to LH stimulation (Bahr and Johnson, 1984). LH is the ovulation inducing hormone in avian species (Fraps, 1965). Before recruitment, the ovarian follicles produce large quantities of androstenedione and estrogens through the $\Delta 5$ pathway (Robinson and Etches, 1986). Following recruitment, the yolk filled follicles produce decreasing quantities of estrogens and gain capacity to release progesterone (Etches, 1990) into the circulation through $\Delta 4$ pathway. The surge of progesterone from the F1 follicle induces the release of gonadotrophin-releasing hormone (GnRH) into the hypothalamic pituitary portal system and stimulates LH which in turn causes ovulation of the largest follicles (Etches, 1990). The surge of LH occurs 6 h before ovulation (Bahr and Palmer, 1989) and is required for ovulation to occur (Johnson and van Tienhoven, 1980; Johnson, 1990). Steroidogenesis by small follicles (production of estradiol and androstenedione) is also stimulated by the increase in the plasma concentration of LH that occurs following photostimulation (Etches, 1990).

The second factor is the circadian oscillator in hypothalamus which controls the timing of ovulation. The circadian oscillation marks the open period for LH release

from adenohypophysis in response to LHRH from the hypothalamus (Etches, 1990). For ovulation to occur, the preovulatory follicle must produce sufficient amount of progesterone to stimulate the release of gonadotrophin, and it must do so during a particular period of day. LH is the principal gonadotrophin that stimulates steroid production in the ovary. Therefore, the endocrine control of follicular function is an interaction of GnRH from hypothalamus, the gonadotrophins from the pituitary gland and ovarian steroids from the follicular hierarchy (Etches, 1990).

1.3.4 Sequences in the Ovulatory Cycle

Chickens usually lay one egg per day with days of consecutive egg laying called a "sequence". Sequences are separated by days of non-laying called "pause" (Robinson *et al.*, 1990). Chickens are indeterminate layers, in that the sequence is repeated. The production of an egg, from ovulation to oviposition usually takes from 24 to 28 h (Etches, 1990). Ovipositions in the middle of very long sequences are separated by 24 h or less. Ovulation of each consecutive follicle occurs approximately .5 to 1 h after oviposition, which means that as a sequence progresses, the eggs are laid later each day (Etches, 1990). The first egg of the sequence is laid soon after the "dawn" signal. The terminal egg of a sequence is laid about 6-8 h after dawn. The "prime sequence" is the longest sequence, that is initiated shortly after the onset of lay (Robinson *et al.*, 1990). Young hens lay 15-20 eggs (long sequence) before skipping a day. Old hens lay 3-5 eggs (short sequence) followed by a pause day. The most mature follicle (F1) which becomes a first of sequence egg, takes approximately 40 h to be laid from the time of oviposition of the previous egg. The subsequent eggs in the same sequence are laid 24 h later.

1.4 Aging in the Domestic Fowl

The decrease in egg production with age was observed long ago (Romanoff and Romanoff, 1949) and is one of the major reproductive problems seen in the domestic fowl. The domestic hen begins to lay eggs at approximately 22 weeks of age, and reaches a peak 6 to 8 wks later. At the end of the first year of laying, egg production generally drops to approximately 50-60% of total egg output (Bahr and Palmer, 1989; Etches, 1990). As the hen gets older, egg production may decrease further or cease (Bahr and Palmer, 1989). The decrease in egg production is the result of several physiological changes. First, as the hen gets older, the interval between ovulation and oviposition increases from 24-25 h (young hens) to 26-27 h (old hens) (Bahr and Palmer, 1989). The result is fewer egg in the same period of time (shorter sequences) and consequently decrease in egg production. Also, the preovulatory follicles of young hens (26 wks of age) are ovulated at a smaller size than those of old hens (82 wks) (Williams and Sharp, 1978). The observation that young hens ovulate smaller sized follicles suggests that those follicles acquire a competency to ovulate more quickly. According to Etches *et al.* (1983), follicular maturity is not governed by the size of the follicle. It is governed by the ability of the follicle to produce and secrete progesterone in response to LH stimulation. In the early production cycle sequence length is short. It then reaches a prime mean sequence length at 32 wks of age, and then shortens with hen aging. This indicates there are more pause days at the beginning and the end of the hen's production cycle. Second, sensitivity of the largest follicle of the hen to LH declines with age (Mougdal and Razdan, 1985). Follicles from chickens laying long sequences (15-20 eggs) were more sensitive to LH than follicles from chickens laying short sequences (3-5 eggs) (Bahr and Palmer, 1989). The sensitivity of the preovulatory follicle to LH and its ability to ovulate are important factors in the regulation of ovulation in the hen. Third, young commercial egg producing strains have an average of seven follicles in the follicular hierarchy

(F1- F7), whereas old hens from the same strain have five follicles in the hierarchy (F1-F5) (Robinson *et al.*, 1992). As a result, the follicles of old hens spend a longer time in the rapid growth phase during which time yolk is being deposited, consequently, egg weight of these hens increases. Also, the first of sequence eggs are heavier (40 h in oviduct) and embryos of these eggs are more developed than embryos of subsequent eggs (Bahr and Palmer, 1989).

Although the cause for the decrease in recruitment of small follicles into hierarchy in old hens is not well known (Bahr and Palmer, 1989), several hypotheses can be suggested. First, it is well established that the incidence of atresia in small follicles increases with age, which results in a smaller number of small follicles entering the stage of large follicles (Richards, 1980; Waddington *et al.*, 1985; Bahr and Palmer, 1989). Second, the rate of maturation of the smallest follicles decreases with age (Johnson *et al.*, 1986). It is possible that a decrease in estrogen production by the ovary of old hens may cause a decrease in follicular recruitment and growth (Bahr and Palmer, 1989). Robinson and Etches (1986) observed that the small follicles produce large amounts of estradiol-17 β (approximately 300 ng per follicle) in response to low doses of LH (0 to 6.25 ng). A reduction in the number of small follicles through atresia may result in lower estrogen production (atretic follicles produce less estrogen than healthy follicles) by the ovary and cause a decrease in follicular recruitment and maturation with age (Bahr and Palmer, 1989). A second, but minor source of estrogen in the ovary of the hen is the theca layer in large follicles (Etches and Duke, 1984). Bahr and Palmer (1989) found that theca layer of old hens laying short sequences (3-4 eggs per sequence) contained significantly lower concentrations of estradiol-17 β than theca layer of young hens. Third, old hens are less sensitive to progesterone and may require a stronger preovulatory surge of progesterone to induce the LH surge. Another cause for decreased ovulation rate is internal laying (Bahr and Palmer, 1989) which increased with aging.

One of the major problems associated with age in the domestic hen is the higher incidence of soft-shelled and shell-less eggs (Bahr and Palmer, 1989). Two major endocrine systems: 1α , 25-dihydroxy vitamin D₃, calcium metabolism and the production of sex steroids, namely, estrogen are involved in decrease in egg shell quality. This 1α , 25-dihydroxy vitamin D₃ is synthesized from cholecalciferol in the liver and the kidney and regulates calcium metabolism by increasing absorption of calcium, mobilization of calcium from the bone and may also enhance the transport of calcium into the shell gland in the oviduct. Chickens with thick shells have higher level of 1α , 25-dihydroxy vitamin D₃ than chickens that produce eggs with thin shells (Bahr and Palmer, 1989), because there is a decrease in the synthesis of that vitamin with age. Estrogen is the primary sex steroid involved in the synthesis of 1α , 25(OH)₂D₃. The efficiency of the shell gland in the hen's oviduct in taking up calcium from the blood and depositing it in the shell may also be reduced with age (Bahr and Palmer, 1989). However, the increase in size of the egg with age may also be a cause for thinner shells (Bahr and Palmer, 1989).

1.5 Strain effect (Leghorn vs Broiler Breeder)

Strain has a significant effect on reproductive performance in domestic fowl. Compared with egg-type chickens, broiler breeders lay approximately half as many eggs (Hocking *et al.*, 1987). Egg-type hens are laid in sequences of one or more eggs. Leghorn hens that are laying at high rates lay long sequences. A superior leghorn hen can lay a sequence of 80 eggs when she is at peak production, while a superior broiler breeder hen can lay a sequence of 40 eggs (Robinson *et al.*, 1992). In egg-type hens, which are commonly fed *ad libitum*, the ovary normally contains a single hierarchy of seven to eight large yolk follicles of 10 to 40 mm in diameter and a large population of small follicles (Gilbert, 1971). On the other hand, full-fed broiler breeders hens have an average of 12 large follicles at sexual maturity

(Hocking *et al.*, 1987; Yu *et al.*, 1992b). It seems, that full-fed broiler type birds lose control of the recruitment of follicles into hierarchy of large follicles. Higher number of large follicles in the ovary does not necessarily mean better reproductive performance, as extra follicles does not mean extra eggs. Too many large follicles are associated with double hierarchies and multiple ovulation (Hocking *et al.*, 1987; Yu *et al.*, 1992b). Double or multiple ovulation in chickens may occur if two follicles mature and are ovulated at the same time or if one premature follicle is ovulated along with a mature follicle (Zelenka, *et al.*, 1986). Double hierarchies may lead to multiple ovulation and interfere with normal egg formation (Hocking, 1987). On the other hand, when feed restriction is applied to broiler breeder hens during the rearing only seven large follicles are observed (Hocking *et al.*, 1987; Yu *et al.*, 1992b) in the ovary. That number of follicles is similar to the number of follicles in highly efficient egg-type hens. A combination of feed restriction and aging reduced the average number of large preovulatory follicles in breeder hens to 4.6 at 62 wks of age (Yu *et al.*, 1992b). Also, the incidence of arhythmic sequences is higher in meat-type (broiler breeder) hens than in egg-type (leghorn) hens. Follicular atresia, internal ovulation and internal laying, the production of soft shelled eggs as well as multiple ovulation and oviposition not occurring in sequences result in a decrease in the reproductive performance of broiler breeder hens. These reproductive problems common in broiler breeders can be reduced with feed restriction.

1.6 Photostimulation effect

The physiological control of egg production in the hen involves interaction between circadian rhythm and the physiological systems controlling the rate of follicular maturation (Etches, 1990). As birds approach sexual maturity they become photosensitive. The LH release from the adenohypophysis occurs about 8 h after the

transition from light to dark and results in an increase in concentration of LH in the blood (3-4 ng/ml), a level that is 2-3 times greater than the resting value (Sharp, 1983). Changes in day length play a crucial role in determine the rate and persistency of lay (Sharp *et al.*, 1984). Light (natural or artificial) has a stimulating effect on the pituitary gland. Both duration and intensity of light are important in that process. It has been established (Ogasawa *et al.*, 1962) that day length for domestic fowl should be increased to a minimum of 13 h to stimulate maximum egg production. A photoperiod of 12 h is also stimulatory, but the reproductive response is slow and peak production is depressed (Ogasawa *et al.*, 1962). An increase in day lengths from 13 to 14 h does not improve egg production. Eggs are laid 12 to 18 h after dark signal (Eches, 1990). Ovulation occurs in either the photophase (hours of light) or the scotophase (hours of darkness). The physiological system that translates the light into the signal that allows ovulation to occur has not yet been identified. As little as .25 h of darkness is sufficient to entrain the circadian system regulating the timing of oviposition (Eches, 1990). The most common photoperiod used for mature hens is 14L:10D (North, 1990).

1.7 Feeding effect

It has been reported (Robinson *et al.*, 1991; Yu *et al.*, 1992a, 1992b; Robinson *et al.*, 1993) that restricted feeding during the prebreeding and breeding periods increases the reproductive performance. Broiler breeders require increasing amounts of energy and nutrients to meet the demands for egg production (Hocking *et al.*, 1989). Hens, when fed *ad libitum* during prebreeding and breeding periods may put energy and nutrient intake into growth and fat storage instead of using it for reproductive performance (Ingram and Wilson, 1987; Yu *et al.*, 1992a). In addition, hens that are over-fed appear to lose control of the recruitment of follicles into hierarchy of large follicles (Waren and Conrad, 1940). Full-fed broiler breeder hens

have an average of 12 large follicles at sexual maturity (Hocking *et al.*, 1987; Yu *et al.*, 1992b). When feed-restriction is applied to broiler breeders, approximately seven large follicles are observed in the ovary. The decreased number of follicles in the ovary of restricted broiler breeders may decrease the incidence of double hierarchies and the production of defective eggs (Whitehead and Hocking, 1988). Robinson *et al.* (1991) and Yu *et al.* (1992b) have reported that hens that are feed restricted during breeding lay approximately 40 more eggs than hens that are full-fed. To limit body weight gain and to reduce reproductive complications, feed restriction is routinely applied to the management of female broiler breeders (Summers and Leeson, 1985) during rearing and breeding (Costa, 1981). Feed restriction of broiler breeder females during the rearing period delays sexual maturity (Blair *et al.*, 1976; Robbins *et al.*, 1986; Ingram and Wilson, 1987; Yu *et al.*, 1992b). The sexual maturity is influenced by body weight (Brody *et al.*, 1980), body fat content (Bornstein *et al.*, 1984), photoperiod (Chaney and Fuller, 1975) and age (Brody *et al.*, 1984). All these factors, with the exception of photoperiod and age, are influenced by feed intake. Restricted feeding during the rearing period improves the peak rate of egg production (Blair *et al.*, 1976; Yu *et al.*, 1992b). On the other hand, restricted feeding delays sexual maturity (Blair *et al.*, 1976). Consequently, sexual maturity is progressively delayed by increasing the severity of restricted feeding during the rearing period.

At 18 wks of age, restricted birds had an average body weight of 1.9 kg compared with *ad libitum*-fed birds which had an average body weight of 4.2 kg (Yu *et al.*, 1992a). The percent carcass fat of *ad libitum*-fed birds was almost four times that of restricted birds. According to Yu *et al.* (1992b), body weight was the most important variable in determining the number of large follicles (more than 10 mm) in the ovary of breeders at sexual maturity. The F2 follicle of full-fed breeders had an endocrine profile characteristic of the F1 largest preovulatory follicle, secreting

large amounts of progesterone. The steroidogenetic capability of F2 follicle to secrete a significant amount of progesterone appeared to be a part of mechanism leading to multiple ovulation (Yu *et al.*, 1992b). In addition, the significantly higher production of androstenedione in SWF of full-fed birds was associated with increased incidence of multiple hierarchies in these birds compared with birds that were feed restricted (Yu *et al.*, 1992c).

1.8 Objectives of Research Project

1.8.1 Overall Objectives

The large degree of unexplained variability in reproductive function in egg-type (Single Comb White Leghorn-SCWL) and meat-type (broiler breeder) chickens may be is due in part to a lack of information about the regulation of ovarian function. Some work has been conducted on steroid hormone production from small follicles pooled together for estradiol and androstenedione output (Yu *et al.*, 1992c). From that study individual follicle output was not possible, because it was unclear if changes in steroid output were due to changes in LH sensitivity, changes in the number of follicles producing estradiol or both. The research presented in this project was focused on estradiol-17 β production from individual small white follicles (<1 mm). The influence of the following parameters: aging, strain, photostimulation program, and level of feeding on age at sexual maturity, body organ weights (heart, liver, abdominal fat pad weight), body composition (carcass fat, protein, water, ash) as well as on ovarian morphology (oviduct, ovary, ovarian stroma, number of large follicles) and estradiol-17 β output was examined.

1.8.2 Individual Projects

The total of 130 birds were assigned to 13 treatment groups based on: age, strain, photostimulation program, and level of feeding. The experimental design for

the study is presented in table 1.1. Fifty SCWL (Shaver-288) and 80 broiler breeders (BB-Shaver Starbro) were used in the experiment. Each treatment had 10 replicates. SCWL were divided into 5 groups:

1. Immature hens-19 wk of age (SCWL-19, one week prior to photostimulation 8L:16D)
2. Photostimulated (P) at 20 wks (14L:10D) and killed at sexual maturity (SCWL-P)
3. Non photostimulated (NP) (8L:16D) and killed at sexual maturity (SCWL-NP)
4. Young hens-35wk of age (SCWL-35)
5. Old hens-60wk of age (SCWL-60)

Broiler breeders were divided into eight groups:

1. Full-fed photostimulated at 20 wks (14L:10D) and killed at sexual maturity (FF-BB-P)
2. Feed-restricted photostimulated at 20 wks (14L:10D) and killed at sexual maturity (RR-BB-P)
3. Full-fed non photostimulated (8L:16D) and killed at sexual maturity (FF-BB-NP)
4. Feed-restricted non photostimulated (8L:16D) and killed at sexual maturity (RR-BB-NP)
5. Young hens-35wk of age full-fed (14L:10D) (FF-BB-35)
6. Young hens-35wk of age feed-restricted (14L:10D) (RR-BB-35)
7. Old hens-60wk of age full-fed (14L:10D) (FF-BB-60)
8. Old hens-60wk of age feed-restricted (14L:10D) (RR-BB-60)

In one part of my research I proposed to determine the extent to which morphology of the reproductive tract and the entire carcass is influenced by 1) age of the bird,

2) strain, 3) photoperiod program, 4) and level of feeding. The second objective of the project was to determine if these factors are associated with changes in small white follicles estradiol-17 β output. Four different levels of LH (0 bLH, 5 ng bLH, 10 ng bLH, 20 ng bLH/tube) were used to assess the stimulating effect of LH on estradiol-17 β production. If any of our treatments influence estradiol production or LH sensitivity, the implications of this could be major. Primary breeders of poultry could potentially look for variability in these parameters, to try to identify superior hens, that at the present time are difficult to detect only on the basis of egg production.

TABLE 1.1. Experimental design. Treatment groups according to age classification by type.

Age classification (Aging)	Type		
	SCWL ^a	FF-BB ^b	RR-BB ^c
Immature hens-19wk ¹	SCWL-19		
First egg - P ²	SCWL-P ¹	FF-BB-P ¹	RR-BB-P ¹
First egg - NP ³	SCWL-NP ¹	FF-BB-NP ¹	RR-BB-NP ¹
Young hens-35wk ²	SCWL-35	FF-BB-35	RR-BB-35
Old hens-60wk ²	SCWL-60	FF-BB-60	RR-BB-60

¹ These hens were sacrificed at sexual maturity (first oviposition).

² Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

³ Birds were non photostimulated and controlled by 8L:16D.

^a Egg-type birds; Single Comb White Leghorns.

^b Meat-type birds; Full-fed broiler breeders.

^c Meat-type birds; Restricted broiler breeders.

1.6 LITERATURE CITED

Amin, S. O., and A. B. Gilbert, 1979. Cellular changes in the anterior pituitary of the domestic fowl during growth, sexual maturity and laying. Br. Poult. Sci. 11:451-458.

Armstrong, D. G., 1984. Ovarian aromatase activity in the domestic fowl (*Gallus domesticus*). J. Endocrinol. 100: 81-86.

Armstrong D. G., 1985. Changes in aromatase activity in small ovarian follicles of the domestic fowl (*Gallus domesticus*) during growth and atresia. J. Endocrinol. 105:297-301.

Bahr, J. M., and A. L. Johnson, 1984. Regulation of the follicular hierarchy and ovulation. J. Exp. Zool. 232:495-500.

Bahr, J. M., and S. S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Bahr, J. M., S. C. Wang, and F. O. Calvo, 1983. Steroid concentrations in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of the domestic hen. Biol. Reprod. 29:326-334.

Blair, R., M. M. MacCowan, and M. Bolton, 1976. Effects of food regulation during the growing and laying stages on the productivity of broiler breeders. Br. Poult. Sci. 17:215-223.

Bornstein, S., S. Plavnik, and Y. Lev, 1984. Body weight/or fatness as potential determinants of the onset of egg production in broiler breeder hens. *Br. Poult. Sci.* 25:323-341.

Brody, T. Y., E. M. Soller, I. Nir, and Z. Nitsan, 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. *Br. Poult. Sci.* 21:437-446.

Brody, T. B., P. B. Siegel, and J. A. Cherry, 1984. Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. *Br. Poult. Sci.* 25:245-252.

Burke, W. H., H. Papkoff, P. Licht, and A. Bona-Gallo, 1979. Preparation and properties of luteinizing hormone (LH) subunits from the turkey (*Meleagris gallopavo*) and their recombination with subunits of ovine LH. *Gen. Comp. Endoc.* 37:501-507.

Chaney, L. W., and H. L. Fuller, 1975. The relationship of obesity to egg production in broiler breeders. *Poultry Sci.* 54:200-207.

Costa, M. S., 1981. Fundamental principles of broiler breeder nutrition and the design of feeding programmes. *World's Poult. Sci. J.* 37:177-192.

Etches, R. J., F. Cross, C. E. Duke, 1981. Plasma concentrations of luteinizing hormone, progesterone, testosterone and estradiol in follicular and peripheral venous plasma during the ovulation cycle of the hen. *Adv. Physiol. Sci.* 33:89-98.

Etches, R. J., 1990. The ovulatory cycle of the hen. *Crit. Rev. Poult. Biol.* 2:293-318.

Etches, R. J., H. E. MacGregor, T. F. Morris, and J. B. Williams, 1983. Follicular growth and maturation in the domestic hen (*Gallus domesticus*). *J. Reprod. Fert.* 67: 351-358.

Etches R. J., and C. E. Duke, 1984. Progesterone, androstenedione and oestradiol content of theca and granulosa tissues of the four largest ovarian follicles during ovulatory cycle of the hen. *J. Endocrinol.* 103:71-76.

Etches, R. J., K. W. Cheng, C. E. Anderson-Langmuir, and J. M. John, 1984a. Plasma concentration of luteinizing hormone, progesterone, testosterone and estradiol in follicular and peripheral venous plasma during the ovulation cycle of the hen. *Adv. Physiol. Sci.* 33:89-98.

Etches, R. J., J. B. Williams, and J. Rzasa, 1984b. Effect of corticosterone and dietary changes in the hen on ovarian function, plasma LH and steroids and the response to exogenous LHRH. *J. Reprod. Fert.* 70:121-130.

Fraps, R. M., 1965. Twenty-four-hour periodicity in the mechanism of pituitary gonadotrophin release for follicular maturation and ovulation in the chicken. *Endocrinol.* 77:5-18.

Gilbert, A. B., 1971. The ovary. Pages 1291-1329 in: *Physiology and Biochemistry of the Domestic Fowl*. Vol 3. D. J. Bell, and B. M. Freeman, ed. Academic Press, London, England.

Gilbert, A. B., 1979. Female genital organs. Pages 237-360 in: *Form and Function in Birds*. Vol. 1. A.S. King and J. McLelland, ed. Academic Press, New York, N.Y.

Gilbert, A. B., M. M. Perry, and M. M. Waddington, 1983. Role of atresia in establishing the follicular hierarchy in the ovary of the domestic hen (*Gallus domesticus*). *J. Reprod. Fert.* 69:221-227.

Guyer, R. B., A. A. Grunder, E. G. Buss, and C. O. Clagett, 1980. Calcium binding proteins in serum of chickens: vitellogenin and albumin. *Poult. Sci.* 59:874-879.

Hertelendy, F., M. Yeh, and H. V. Biellier, 1974. Induction of oviposition in the domestic hen by prostaglandins. *Gen. Comp. Endocrinol.* 22:529.

Hertelendy, F., and H. V. Biellier, 1978. Evidence for a physiological role of prostaglandins in oviposition by the hen. *J. Reprod. Fert.* 53:74.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington, 1987. Ovarian follicular structure of White Leghorn fed *ad libitum* and Dwarf and normal broiler breeders fed *ad libitum* or restricted to point of lay. *Br. Poult. Sci.* 28:493-506.

Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30:161-174.

Ingram, D. R., and H. R. Wilson, 1987. *Ad libitum* feeding of broiler breeders prior to peak egg production. *Nutr. Rep.* 36:839-845.

Johnson, A. L, 1986. Reproduction in the female. Pages 403-431 in *Avian Physiology*, 4 th ed. P.D. Sturkie, ed. Springer-Verlag, New York, NY.

Johnson, A. L., 1990. Steroidogenesis and actions of steroids in the hen ovary. *Crit. Rev. Poult. Biol.* 2:319-346.

Johnson, A. L., and A. van Tienhoven, 1980. Plasma concentration of six steroids and LH during the ovulatory cycle of the hen (*Gallus domesticus*). *Biol. Reprod.* 23:386-393.

Leeson, M. S., and J. D. Summers, 1983. Consequences of increased feed allowance for growing broiler breeder pullets as a means of stimulating early maturity. *Poultry Sci.* 62:6-11.

Marlow, H. W., and D. Richaert, 1940. Maturation of ovarian follicles. *Endocrinol.* 26, 531.

Marrone, B. L., and F. Hertelendy, 1983. Steroidogenesis by avian ovarian cells: effects of luteinizing hormone and substrate availability. *Am. J. Physiol.* 244:E487-E493.

Marthur, R. S., P. A. Anastasiadis, and R. H. Common, 1966. Urinary excretion of estrone and of 16-epi-estriol plus 17-epi-estriol by the hen. *Poult. Sci.* 45:946-952.

Mougdal, R. P., and M. N. Razdan, 1985. Induction of ovulation *in vitro* in the hen: Dependency of the response to LH on age and rate of lay. *J. of Endocrinol.*, 106:67-69.

Nalbandov, A. V., and M. F. James, 1949. The blood vascular system of the chicken ovary. *Am. J. Anat.* 85:347-378.

North, M. O., 1990. Pages 36-41 in: *Commercial Chicken Production Manual*. 4th ed. AVI Publishing Company, New York, N.Y.

Ogasawara, F. X., W. O. Wilson, and V. S. Asmudson, 1962. The effect of restricting light during the adolescent period on reproductive performance in turkeys subsequently exposed to a 12, 14 and 20 hour day. *Poultry Sci.* 41:1858-1863.

Ogasawara, T., and O. Koga, 1978. Prostaglandin production by uterus of the hen in relation to spontaneous ovipositions. *Jap. J. Zootech. Sci.* 49:523.

Opel, H., 1979. Major problems in poultry reproduction. In *Beltsville Symposia in Agricultural Research, III. Animal Reproduction* (H. W. Hawk, ed), Allanheld, Osuman & Co., Montclair, N. Y., pp 51-59.

Pearl, R., and W. F. Schoppe, 1921. Studies on the physiology of reproduction in the domestic fowl. XVIII. Further observation on the anatomical basis of fecundity. *J. Exp. Zool.* 34:101-118.

Perry, M. M., A. B. Gilbert, and A. J. Evans, 1978. Electron microscope observation on the ovarian follicle of the domestic fowl during the rapid growth phase. *J. Anat.* 125:481-497.

Porter, T. E., B. M. Hargis, J. L. Silsby, and M. E. El Halawani, 1989. Differential steroid production between theca interna and theca externa cells: a three cells model for follicular steroidogenesis in avian species. *Endocrinology* 125:109-116.

Richards, J. S., 1980. Maturation of ovarian follicles action and interaction of pituitary and ovarian hormones on follicular cell differentiation. *Physiol. Rev.*, 60, 51.

Robbins, K. R., G. C. McGhee, P. Osei, and R. E. Beauchene, 1986. Effect of feed restriction on growth, body composition, and egg production during the breeding season. Poultry Sci. 65:1052-1057.

Robinson F. E. and R. J. Etches, 1986. Ovarian steroidogenesis during follicular maturation in domestic fowl (*Gallus domesticus*). Biol. of Reprod. 35: 1096-1105.

Robinson, F. E., R. T. Hardin, and A. R. Robblee, 1990. Reproductive senescence in domestic fowl: Effects on egg production, sequence length and inter sequence pause length. Br. Poult. Sci. 31:871-879.

Robinson F. E., N. A. Robinson, and T. A. Scott, 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler hens. Can. J. Anim. Sci. 71:549-556.

Robinson N. A., F. E. Robinson, and R. T. Hardin, 1992. Reproductive senescence in egg-type chickens: Effects on egg production, sequence length and inter-sequence pause length. Poultry Science 81st Annual Meeting Abstracts. Poult. Sci. 71:128.

Robinson, F. E., J. L. Wilson, M. W. Yu, G. M. Fasenko, and R. T. Hardin, 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. Poultry Sci. 72:912-922.

Romanoff and Romanoff, A. J., 1949. The Avian Egg. J. Wiley and Sons, Inc., New York.

Scanes, C. G., A. Stockell Hartree, and F. J. Cunningham, 1984. The pituitary gland. In Physiology and Biochemistry of the Domestic Fowl, Vol. 5 (Freeman, ed.), Academic press, London, pp. 39-84.

Senior, B. E., and J. A. Furr, 1975. A preliminary assessment of the source of oestrogen within the ovary of the domestic fowl. J. Reprod. Fertil. 43:241-247.

Shahabi, N. A., J. M. Bahr, and A. V. Nalbandov, 1975. Effect of LH injection on plasma and follicular steroids in the chicken. Endocrinol. 96:969-972.

Sharp, P. J., 1980. Female reproduction. In Avian Endocrinology, (Epple, A. and Stetson, M. H. eds), pp. 435-454. New York and London, Academic Press.

Sharp, P. J., 1983. Hypothalamic control of gonadotrophin secretion in birds. In Recent Progress in Non-Mammalian Brain Research, (Nistico, G. and L. Bolis) pp. 124-164. CRC Press. Boca Raton, FL, USA,

Sharp, P. J., C. MacName, C. R. T. Talbot, R. J. Sterling, and T. R. Hall, 1984. Aspects of neuroendocrine control of ovulation and broodiness in the domestic hen. J. Exp. Zool., 232, 475.

Summers, J. D., and M. S. Lesson, 1985. Poultry Nutrition Handbook. Department of Animal and Poultry Science, Ontario Agricultural College, Guelph, Ontario.

Taylor T. G., 1970. How an egg shell is made. Scientific American, March, Vol. 222, No 3.

Taylor T. G., and C. G. Dacke, 1984. Calcium metabolism and its regulation. In *Physiology and Biochemistry of the Domestic Fowl*, Vol. 5. (B.M. Freeman, ed.), Academic Press, London, pp. 125-170.

Tilly, J. L., K. I. Kowalski, and A. L. Johnson, 1991. Stage of ovarian follicular development associated with the initiation of steroidogenic competence in avian granulosa cells. *Biol. Reprod.* 44:305-314.

Tsang, C. P. W., and A. A. Grunder, 1984. Production, and metabolic fate of estradiol-17 β in the plasma of the laying hen. *Steroids* 43:71-84.

Waddington, D., M. M. Perry, A. B. Gilbert, M. A. Hardie, 1985. Follicular growth and atresia in the ovaries of hen with diminished egg production rates. *J. Reprod. Fert.* 74:399-405.

Wang, S. C., and J. M. Bahr, 1983. Estradiol secretion by theca cells of the domestic hen during the ovulatory cycle. *Biol. Reprod.* 28:618-624.

Warren D. C., and R. M. Conrad, 1940. The production of double yolked eggs in the fowl. *Poultry Sci.* 19:9-17.

Warren D. C., and R. M. Conrad, 1942. Time of pigment deposition in brown-shelled hen eggs and in turkey eggs. *Poultry Sci.* 21:515-520.

Warren, D. C., and H. M. Scott, 1935. The time factor in egg formation. *Poult. Sci.* 14:195-207.

Wells, J. W., M. A. Walker, and J. Culbert, 1983. Effect of luteinizing hormone on progesterone production by the follicular granulosa in the ovarian hierarchy of the domestic fowl (*Gallus domesticus*). Gen. Comp. Endocrinol. 52:265-271.

Whitehead, C., and P. Hocking, 1988. Feeding and managing turkeys and broiler breeder hens. Pages 6-7 in : Science and Poultry Industry, J. Hardcastle, ed. Agriculture and Food Research Council, London, England.

Williams J. B., and P. J. Sharp, 1977. A comparison of plasma progesterone and luteinizing hormone in growing hens from eight weeks of age to sexual maturity. J. Endocrinol., 75:447-448.

Williams, J. B., and P. J. Sharp, 1978. Ovarian morphology and rates of ovarian follicular development in laying broiler breeders and commercial egg-producing hens. Br. Sci. 19:387-395.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. Poultry Sci. 71:1750-1761.

Yu, M. W., F. E. Robinson, and R. J. Etches, 1992c. Effect of feed allowance during rearing and breeding on female broiler breeders. 3. Ovarian steroidogenesis. Poultry Sci. 71:1762-1767.

Yu, M. W., F. E. Robinson, and A. R. Robblee, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poultry Sci. 71:1739-1749.

Zakaria, A. H., H. Sakai and K. Imai, 1984. Time of follicular transportation to the rapid growth phase in relation to the ovulatory cycle of laying hens. Poultry Sci. 63, 1061-1063.

Zelenka, D. J., P. B. Siegel, and H. P. van Krey, 1986. Ovum formation and multiple ovulation in lines of White Plymouth Rocks and their crosses. Br. Poult. Sci. 27:409-414.

2. OVARIAN MORPHOLOGY AND STEROIDOGENESIS IN THE DOMESTIC FOWL: EFFECTS OF AGING.

2.1 Introduction

The gradual reduction in egg production with age was observed a long time ago in the domestic fowl (Romanoff and Romanoff, 1949) and is the result of several physiological changes. As the hen gets older, the interval between ovulation and oviposition increases from 24-25 h (young hens) to 26-27 h (old hens) (Bahr and Palmer, 1989). In the early production cycle sequence length is short. At peak production hens lay very long sequence of 15-20 eggs (Bahr and Palmer, 1989). According to Robinson *et al.* (1990) broiler breeders lay 18-23 eggs before skipping a day at peak production. The term "prime" sequence is associated with the long sequences seen at peak production. There is a strong correlation between length of the prime sequence and total egg production (Robinson *et al.*, 1990). Hens which laid long sequences of eggs also have a reduced number of pauses of greater than 1 day duration. A strong correlation between length of prime sequence and total egg production may have implications in selection for increased egg production (Robinson *et al.*, 1990). Old hens lay 3 to 5 eggs followed by a pause day. The reason for reduced sequence length after the prime sequence is not known, however some suggestions have been proposed. First, sensitivity of ovarian steroidogenesis to LH may declines with age. Follicles from chickens laying long sequences (15 to 20 eggs) were more sensitive to LH than follicles from chickens laying short sequences (3 to 5 eggs) (Bahr and Palmer, 1989). Furthermore, the amount of LH required to induce ovulation *in vitro* is greater in old hens than in young hens (Mougdal and Razdan, 1985). Williams and Sharp (1978) suggested that reduced sensitivity of the hypothalamus to progesterone may be a cause of reduced rate of laying in old hens. Johnson (1986) reported that granulosa cells of the two largest follicles are less sensitive to LH in old hens than in young hens. Also, young commercial egg producing strains have an average of seven follicles in the

follicular hierarchy, whereas old hens from the same strain have five follicles in the hierarchy (Robinson *et al.*, 1992). While the total number of growing follicles decreased with age, the total amount of yolk deposited increased (Gilbert, 1971). Several hypotheses have been proposed to explain that phenomena. It is well established that the incidence of atresia of small follicles increases with age (Waddington *et al.*, 1985; Bahr and Palmer, 1989). Also, the rate of maturation of the smallest follicles decreases with age. According to Bahr and Palmer (1989) a decrease in estrogen production by the ovary of old hens may cause a decrease in follicular growth.

Furthermore, decreased egg output could be due to a condition known as erratic oviposition and defective egg syndrome (EODES) (van Middelkoop, 1972), a term which includes several reproductive problems like follicular atresia, internal ovulation and laying, high incidence of soft shelled or membranous eggs, multiple yolked eggs and oviposition occurring not in sequences. More information is required to understand the physiological changes in the aging hen, especially at the ovarian steroidogenesis level. The sensitivity of the preovulatory follicle to LH and its ability to ovulate are important factors in the regulation of ovulation in the hen and consequently in egg production.

One objective of this work investigated the effect of aging on output of estradiol-17 β from SWF *in vitro* incubated during 3 h in the presence (5, 10, or 20 ng bLH/tube) and absence of bLH. The second objective was to examine the effect of aging on body weight, weight of selected organs (heart, liver and abdominal fat), carcass composition (fat, protein, water, ash) and reproductive organ morphology (oviduct, ovary, stroma, number and size of large follicles).

2.2 Materials and Methods

2.2.1 Stocks and Management

Forty female Single Comb White Leghorns (SCWL-Shaver-288), randomly selected from a flock of 80 birds, and 60 female broiler breeders (BB-Shaver Starbro) randomly selected from a flock of 120 birds, were individually housed in laying cages. The total

of 100 hens were assigned to ten treatment groups of 10 replicates per treatment group (Table 2.1). Egg-type birds (SCWL) were divided into four treatment groups based on the age they were studied. Meat-type birds (BB) were divided into six treatments group based on age and level of feeding. Broiler breeders were randomly assigned to a restricted or *ad libitum* feeding program. Age classifications for SCWL were based on hens sacrificed: at 19 wks one week prior to photostimulation (SCWL-19), at sexual maturity when photostimulated at 20 wks of age (SCWL-P), at 35 wks when photostimulated at 20 wks of age (SCWL-35) and at 60 wks of age when photostimulated at 20 wks of age (SCWL-60). Age classification for full-fed and feed restricted broiler breeders were based on hens sacrificed at: sexual maturity when photostimulated (FF-BB-P; RR-BB-P), at 35 wks (FF-BB-35; RR-BB-35) and at 60 wks of age (FF-BB-60; RR-BB-60).

Full-fed birds were allowed to eat *ad libitum*, while feed-restricted birds were fed daily to maintain body weight as per recommendations of the "breeder" (Yu *et al.*, 1992a). All broiler breeders were fed a standard laying ration (2859 kcal), and 16.5% crude protein. All SCWL hens received a standard commercial diet containing 16% crude protein. The body weight and amount of feed consumed was monitored every week for each bird. Throughout the experiment, hens had free access to water. The laying house was light-controlled and photoperiod was maintained at 14L:10D (14 h light and 10 h dark) for all treatment groups, except SCWL-19. This group was controlled by 8L:16D and was caged in a different house. Lights were on at 7:00 a.m. for all lighting schedules. Birds from each treatment were sacrificed by cervical dislocation 3 to 3.5 h after midsequence oviposition, to ensure that they would not be killed during the preovulatory surge of LH. Experimental birds had access to feed up the time of being killed.

The experimental protocol was approved by the University of Alberta Animal Policy and Welfare Committee.

2.2.2 Carcass Composition

The carcass, heart, liver, abdominal fat pad (including fat adhering to the gizzard and proventriculus), oviduct, ovary, stroma and the large follicles were dissected and weighed individually. The whole carcass, including feathers, was autoclaved at 250°C for 6 hours and homogenized with an industrial blender. While the homogenized samples were being stirred, subsamples were weighed and taken for freeze drying. After freeze drying for 5-6 days, the subsamples were weighed again and ground. Approximately 2 g of the freeze-dried subsample was taken for chemical analyses. Dry matter, ash, total protein, and total lipid were analyzed with standard procedures (Association of Official Analytical Chemists, 1980) for each individual bird. Carcass fat content was analyzed using the Goldfish apparatus with petroleum ether as the solvent. Carcass protein was analyzed using the Kjeldahl procedure. For calculating dry matter a 2 g portion of the freeze-dried sample was taken and dried overnight in an oven adjusted to 110°C. Samples subjected to dry matter analysis were placed in muffle furnace at 550°C and burned overnight to calculate the ash content. All analyses were conducted in duplicates. Samples which had a difference between duplicate larger than 3% were reanalyzed.

2.2.3 Follicular Incubation

The ovary of each bird was quickly removed, dissected and placed into cold Medium 199 (pH=7.2) containing 0.1% bovine serum albumen (BSA)¹, sodiumbicarbonate (NaHCO₃)¹, 10 mM HEPES(N[2-Hydroxyethyl]piperazine-N'-(2-ethanesulfonic acid))¹, Gentamicin (Fisher Scientific) and Medium 199 (BRL Gibco, Catalog No.M0393). The Medium 199 was prepared fresh each time an experiment was conducted. The weight of ovarian stroma and the weight and size of the large follicles (> 1 cm) were recorded. Forty small white follicles (< 1mm) were dissected from each

¹ Sigma Chemical Co.

ovary. Total studies on estradiol output from small white follicles were based on incubation of 4,000 follicles (40 follicles x 100 hens). These follicles were individually placed into 12 x 75 mm borosilicate tubes containing 0.5 mL of cold Medium 199 (pH = 7.2). Four different levels of bovine LH (bLH) were used in the incubation of the follicles. The four dosages of bLH were: 1) 0 bLH; 2) 5 ng bLH per follicle; 3) 10 ng bLH per follicle; 4) 20 ng of bLH per follicle. Each dose of bLH was tested in 10 replicates per bird. The bLH (NIAMDD-LH-B5) was provided courtesy of the National Institute of Diabetes, Digestive, and Kidney Diseases (Bethesda, MD 20892) and the National Hormone and Pituitary Program of the University of Maryland School of Medicine (Bethesda, MD 21201). The incubation of follicles was performed for 3 h in a shaking water bath at 39°C open to air. Following incubation, media were decanted into Ependorf vials (Fisher Scientific, size 1.5 mL) and frozen at -23°C until assayed by radioimmunoassay (RIA) for quantification of estradiol-17 β .

2.2.4 Estradiol-17 β Radioimmunoassay

Estradiol-17 β content was determined by radioimmunoassay of incubation media using an antibody (Rab-A-11, from Dr. N. C. Rawlings, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada) raised in rabbit against estradiol-17 β [1,3,5(10)-Estratrien-3,17-diol:3,17-Dihydroxy 1,3,5,(estratriene)]. Cross-reactions for this antiserum reported by the supplier were as follows: estrone, 8%; estriol, 0.4%; 17 α -estradiol, 0.4%; testosterone, 0.08%; dihydrotestosterone, 0.1%; 5 α -androstan-3 β ,17 β -diol, 0.08%; and cortisone, 0.03%. Androstenedione; 5 α -androstan-3 α ,17 β -diol; progesterone; pregnenolone; cholesterol; corticosterone; and cortisol did not cross-react with this antiserum. Fifty microliters of incubation media were diluted (1:10) with 450 μ l assay buffer (NaH₂PO₄ x H₂O; Na₂HPO₄; NaCl; NaN₃, and .1% gelatin; all chemicals obtained from Sigma). One hundred microliters of diluted sample, 150 μ l of assay buffer (4°C), 200 μ l of Rab A11 antibody at 1/160k final dilution and 100 μ l (20 000 dpm/100 μ l) H-labelled (Du Pont NEN Products, Boston, MA) were added

to each 12 x 75 mm glass tubes. After vortexing, the mixture was incubated at room temperature (20°C) for 1 h and then incubated at 4°C for not less than 24 h to allow the assay components to reach equilibrium. Two hundred microliters of well-mixed, dextran-coated charcoal [Dextran, 0,125 g (Sigma No.D-5251), Carbon Decolouring Alkaline Norit-A 1,25 g (Sigma No.C-5385)]; mixed well in 250 µl assay buffer and cooled to 4°C was added to each assay tube, incubated for 10 min at 4°C and then centrifuged at 1450 x g at 4°C for 10 min. The supernatant from each tube was poured into a 6 ml vial containing 5 ml liquid scintillation fluid (ICN-biomedical, #8824757), mixed thoroughly, and then counted in beta-counter (1600CA Liquid Scintillation Analyzer) for 2 min for each vial. Duplicates tubes were assayed for each sample. The sensitivity of each assay was calculated as the average of Bmax (mean cpm; counts per minute of maximal binding) - 2 x Standard Deviation of maximal binding (Bmax) / average of Bmax. The average sensitivity of the assay was 96% of maximal binding. The intra-assay coefficient of variation was 8.6%. The inter-assay coefficient of variation was 7.71%.

2.2.5 Determination of LH Sensitivity

Determination of LH sensitivity was calculated as a linear relation based on the output of estradiol-17β from follicles incubated with 0 or 5 ng of bLH. As the amount of estradiol-17β stimulated with either 10 or 20 ng of bLH was similar to that stimulated with 5 ng of bLH, it was assumed that 0 and 5 ng of bLH were the most accurate points to describe LH sensitivity. The percent of follicles which responded to LH supplementation (5, 10, 20 ng) based on the production of greater than 1.0 ng of estradiol-17β, was calculated for each treatment group.

2.3 Statistical Analyses

Data were analyzed by one-way analyses of variance (Steel and Torrie, 1980), and significant differences among the means were determined using Duncan's multiple range

test (Steel and Torrie, 1980). Differences among the age classification were determined within each type (Table 2.1) for organ weights, carcass composition and reproductive characteristics.

In estradiol-17 β production, differences among the age classification were determined within type and within each of four dosages of LH (0, 5, 10, 20 ng of LH). The changes in estradiol-17 β production with changes in dosages of LH (Slope) were computed for each hen using only dosages of 0 and 5 ng LH because preliminary analyses indicated essentially a plateau in estradiol-17 β production beginning at 5 ng of LH and continuing at 10 ng and 20 ng of LH. For each hen the percentage of follicles which produced more than 1.0 ng of estradiol-17 β when exposed to 5 ng and more of LH (Percent) was calculated. Data for estradiol-17 β production also were analyzed by one-way analyses of variance and significant differences among the means were determined using Duncan's multiple range test. All statements of significance were based on the 0.05 level of probability.

2.4 Results

The body weight of the SCWL birds increased with age, however there was no significant difference in body weight between sexual maturity (approximately 22 wks) and 35 wks of age (Table 2.2). Heart weight varied slightly with the age. However, there was no significant difference in heart weight between 19 wks and approximately 22 wks of age and between 35 and 60 wks of age. Liver weight increased significantly with age. Liver weight expressed as a percentage of body weight was also significantly influenced by aging. However, there was no significant difference in liver weight between 35 and 60 wks of age. The weight of abdominal fat pad increased with age. However, no significant differences were observed between 19 wks and approximately 22 wks and between 22 and 35 wks. The weight of abdominal fat pad was significantly different between 19, 35 and 60 wks of age (19 wks; 26.59 g; 35 wks; 49.78 g; 60 wks; 69.28 g). On the other hand, there was no difference in fat pad weight between 19 and

22 wks. A similar pattern was observed in total carcass fat weight. The weight of carcass protein was significantly higher in birds at the beginning of production, at peak of production and 60 wks of age than it was for immature birds - 19 wks of age. The percent moisture on a dry matter basis was significantly decreased with age. The percent of body ash, an indicator of bone mineralization, was significantly higher in SCWL-P, SCWL-35 and SCWL-60 than it was in SCWL-19.

Aging had a significant effect on reproductive tract characteristics in SCWL (Table 2.3). The weight of oviduct increased with age from 5.6 g in immature birds to 58.9 g at 60 wks. Total ovary weight significantly increased with each period of study (19 wks-0.73 g; First egg-30.77 g; 35 wks-39.23 g; and 60 wks-49.43 g). Stroma weight was significantly higher at 35 wks and 60 wks (respectively 7.14 g and 7.56 g) than it was at first egg (3.24 g) indicating that weight of the pool of small follicles increased after first egg. However, the number of large follicles did not change with age.

Outputs of estradiol-17 β from SWF incubated with four different levels of LH are presented in table 2.4. There were no significant differences in estradiol-17 β production between the age groups in SCWL. However, estradiol-17 β output from follicles incubated without LH (control group) was significantly higher in laying birds than in immature birds (SCWL-19). The presence of bLH during incubation stimulated estradiol-17 β output in all treatment groups. The highest increase has been observed between control group (0 LH) and group with 5 ng of LH. Increasing the dose of LH to 10 ng or 20 ng per tube did not increase estradiol-17 β output above that produced with 5 ng. Slopes, determined as the linear relations output of estradiol-17 β from follicles incubated with 0 or 5 ng of bLH, were not different between aging groups. Also, the percent of follicles incubated with bLH which produced over 1.0 ng of estradiol-17 β were not significantly different between the groups. On average, 60 % of follicles produced over 1.0 ng of estradiol-17 β in response to 5, 10 and 20 ng of bLH.

In full-fed broiler breeders (Table 2.5) body weight increased with age. However, there was no significant difference in body weight between 35 wks (5,168.2 g) and 60

wks of age (5,098.7 g). Old birds had the largest heart weight (28.09 g) compared to birds at approximately 22 wks and 35 wks of age (16.63 g and 18.14 g respectively). Liver weight significantly increased with age from 93.42 g at sexual maturity (22 wks) to 123.9 g at 60 wks of age. Abdominal fat pad weight was the heaviest in full-fed birds at 35 wks of age (357.72 g). There was no significant difference in fat weight between FF-BB-P and FF-BB-60. Total carcass fat weight was highest in FF-BB-60 (1324.0 g). Also, the amount of protein was the highest in FF-BB-60 (715.32 g). Carcass ash increased with the age, being the highest in FF-BB-60 (154.96 g).

Aging did not significantly influence oviduct weight and total ovary weight (Table 2.6). However, stroma weight was higher at 60 wks (18.41 g) than it was at 22 or 35 wks (respectively 9.46 g and 13.35 g). While the weight of hens increased, the number of large follicles decreased (FF-BB-P; 9.1; FF-BB-35; 7.9; FF-BB-60; 6.3).

Aging influenced estradiol-17 β output in full-fed broiler breeders (Table 2.7). In all LH levels, estradiol-17 β output was the highest in older birds. Again, the presence of LH stimulated estradiol production. There was no significant difference in slope between the treatments in full-fed birds. The percent of follicles incubated with bLH, which produced over 1.0 ng of estradiol-17 β was almost twice as high in FF-BB-35 (64.44%) and FF-BB-60 (78.52%) than it was in FF-BB-P (37.67%).

In restricted broiler breeders there was a significant difference in body weight between 24 wks (RR-BB-P), 35 wks and 60 wks of age (Table 2.8). The heaviest hearts were seen in the oldest birds (RR-BB-60; 15.53 g). On the other hand, restricted broiler breeders at 35 wks had significantly larger liver weight (RR-BB-35; 78.64 g) than restricted broiler breeders at sexual maturity (RR-BB-P; 55.93 g) and at 60 wks (RR-BB-35; 51.57 g). Abdominal fat and carcass fat were significantly higher in RR-BB-35 and RR-BB-60 than in younger birds (Table 2.8). Aging did not influence protein weight. Ash weight decreased with age and a significantly higher amount of ash was observed in birds at sexual maturity (24 wks) and 35 wks of age than in older birds.

Oviduct weight was significantly higher in the oldest birds (94.31 g) compared to

RR-BB-35 and RR-BB-P (Table 2.9). Total ovary weight was higher at 35 wks (70.82 g) than at either 24 wks (47.43 g) or 60 wks (48.54 g) (Table 2.9). Stroma weight increased with age from 6.37 g (RR-BB-P) to 10.1 g at 60 wks. When stroma weight increased, the number of large follicles decreased.

In restricted broiler breeders aging did not influence estradiol-17 β production (Table 2.10). The amount of estradiol-17 β production seen with supplementation of 5 ng of LH was almost three times higher in all aging groups compared to 0 ng of LH. On average, 63% of follicles produced over 1.0 ng of estradiol-17 β when exposed to 5 ng and more of LH in restricted broiler breeders.

2.5 Discussion

Under conditions of aging, SCWL increased in body weight (BW) (Table 2.2). The average BW of SCWL was 1.2 kg at 19 wks and 1.7 kg at 60 wks. Full-fed broiler breeders did not gain a significant amount of weight after sexual maturity (Table 2.5; and Figure 2.1). FF-BB-35 hens were the heaviest (5,168.2 g) compared to other aging groups. The plateau in growth rate after 35 wks of age supports previous findings of Robbins *et al.* (1986) and may have been related to appetite satiation or the physiological limits of gut capacity in these birds. The BW of the feed-restricted broiler breeders increased from sexual maturity to 35 wks of age due to an increased feed allocation. The target and actual BW for these birds did not change significantly from 35 to 60 wks. Liver weight progressively became heavier in SCWL (Table 2.2) and in FF-BB (Table 2.5) with the age. The weight of the liver is likely related to changes in feed intake. We could not say the same about RR-BB (Table 2.8). Liver weight at sexual maturity was significantly lower than liver weight at 35 wks of age. Restricted-feeding may prevent excessive growth of the liver after peak production in BB as reported by Yu *et al.* (1992a). Abdominal fat pad weight was affected by age in SCWL (Table 2.2). SCWL at 60 wks had 43 g more fat than at 19 wks of age. This was mostly due to body weight increase in these birds as fat is positively correlated with the body weight. Fat

pad weight was observed to increase at a relatively constant rate during laying. Full-fed broiler breeders gained significantly more fat pad between sexual maturity and 35 wks than they did after 35 wks. After peak production, the abdominal fat pad weight decreased, which may be related to body weight being decreased in these birds. According to Robbins *et al.* (1986), at 26 wks of age body weight in *ad libitum* fed birds may reach a plateau as a consequence of reduced voluntary food intake. A similar pattern was observed in restricted BB. After 35 wks of age these birds did not gain any significant amount of abdominal fat. In RR-BB, the abdominal fat pad contributed 17.8%, 23.8% and 25.6% of the total carcass fat at sexual maturity, at 35 wks and at 60 wks respectively. Total carcass fat weight was affected by aging in SCWL and FF-BB in the way similar to abdominal fat pad weight.

The oviduct weight increased with the age in SCWL and RR-BB (Figure 2.4). However, oviduct weight in FF-BB was not significantly influenced by age. At the present time the significance of changes in oviduct weight during lay is not known. Further work is needed to clarify if a slight reduction or increase in oviduct weight has any physiological significance. A similar pattern was observed in ovary weight in SCWL (Figure 2.5). Ovary weight from RR-BB reached a peak value at 35 wks and then declined (Table 2.9). The weight of the ovary from FF-BB was already two times that of restricted birds and presumably represents a limit in physiological capacity. Stroma weight in FF-BB increased with aging indicating that the size of pooled small follicles in these birds also increased with aging (Figure 2.6). The number of large follicles did not change with aging in SCWL (Table 2.3 and Figure 2.7). At sexual maturity they had average of 6.2 follicles, at peak production 4.9 and at 60 wks of age 5.9 follicles. This suggests that SCWL are capable of maintaining a consistent number of large follicles. According to Gilbert (1971), in egg-type chickens, the number of large follicles in the ovary is related to the pattern of laying and birds laying in long sequences tend to have more than those laying in short sequences. Waddington *et al.* (1985) also noted the decrease in egg production in egg-type chickens was accompanied

by a corresponding reduction in average sequence length, which may be due to a reduction in follicle number.

Broiler breeders had some depletion of follicles with aging (Figure 2.7). The highest number of follicles (9.1) was observed in FF-BB at sexual maturity. After sexual maturity this number declined to 6.3 follicles at 60 wks of age. Too many large follicles are associated with double hierarchies and multiple ovulation (Hocking *et al.*, 1989; Yu *et al.*, 1992b). The oviduct of the hen can only process one egg per day with a normal shell. Too few large follicles may not sustain daily egg production. Williams and Sharp (1978) observed that young laying hens with a high rate of egg production had more large follicles in the ovary than did old hens of the same strain with lower rates of egg production. Robinson *et al.* (1990) reported that the pause length increased with age and perhaps this is due to insufficient number of follicles late in lay. Also, the decline in the number of large follicles may appear in the form of a decrease in sequence length and increase pauses as showed in previous work (Robinson *et al.*, 1993). According to Hocking *et al.* (1987) and Yu *et al.* (1992b) even though FF-BB have an average of 12 follicles at sexual maturity, they laid significantly fewer eggs than restricted birds. Increased follicular development seen in full-fed broiler breeder hens is not associated with increased egg production. Robinson *et al.* (1990) and Yu *et al.* (1992b) have reported that hens that are feed restricted during breeding lay approximately 40 more eggs than do hens that are full-fed. McDaniel *et al.* (1981) and Robinson *et al.* (1991) observed that *ad libitum* feeding during breeding (18-23 wks) resulted in lower egg production in broiler breeders. In reviewing the literature Bahr and Palmer (1989) reported that the age-related decline in egg production, viewed as the laying of fewer but larger eggs, may be due to an increased incidence of atresia and internal laying, a decrease in recruitment of follicles into the hierarchy, a reduction in shell quality or a decrease in ovulation rate. One result of a decline in ovulation rate is a decrease in sequence length (Robinson *et al.*, 1990).

In restricted BB, the number of large follicles also declined with aging (Figure

2.7). RR-BB started production with an average of 7.4 follicles. At peak production they had 5.8 follicles and at 60 wks 4.4 follicles. According to Hocking *et al.* (1987) and Yu *et al.* (1992b) under feed restriction the number of large follicles in BB is similar to egg-type birds. This observation also follows the work of Williams and Sharp (1978). Feed restriction acts to control the rate of recruitment of large preovulatory follicles into the ovary (Yu *et al.*, 1992b). When feed restriction is applied during the rearing period only, seven large follicles are observed (Hocking *et al.*, 1987; Yu *et al.*, 1992b) in the ovary. A combination of feed restriction and aging reduced the average number of large preovulatory follicles in breeder hens to 4.6 at 62 wks (Yu *et al.*, 1992b). Robinson *et al.* (1990) reported that when restricted broiler breeders were allowed to feed *ad libitum* at the onset of sexual maturity, they developed short sequences of lay with increased number of pause days being observed between sequences. A recent study (Robinson *et al.*, 1990), which characterized sequence length in feed-restricted broiler breeder hens, determined that early in the production cycle, sequence length is short, reaches a prime mean sequence length at 32 wks of age, then quickly shortens with advancing age. This indicates there are more pause days early and late in the hens life than during the middle portion of the laying cycle.

The effects of aging on estradiol-17 β production are presented in tables 2.4, 2.7 and 2.10. This hormone was produced in all aging groups and was stimulated by LH (Figure 2.8; 2.9, 2.10). This observation is with agreement with Robinson and Etches (1986) and Yu *et al.* (1992c). It should be noted that the addition of 5 ng of LH enhanced estradiol-17 β output. However, addition of 10 or 20 ng of LH further did not influence steroidogenesis in SWF. This may indicate that presence of LH receptors in SWF is limited and according to this study 5 ng of LH could be the maximum amount which stimulated estradiol-17 β production. Follicles from immature birds, prior to photostimulation (SCWL-19) also had the ability to produce estradiol-17 β (Table 2.4). Therefore, prior to sexual maturity, which is prior to the time that LH levels become regulated, the ovary can produce some basal estradiol-17 β . Photostimulated SCWL

(SCWL-P) increased the basal output of estradiol-17 β . At sexual maturity SCWL produced more than they did before. In FF-BB and RR-BB, the highest amount of estradiol-17 β was observed in older birds (FF-BB-60 and RR-BB-60).

LH sensitivity (Slope) was not significantly different across the treatments (Table 2.4, 2.7 and 2.10). The percentage of LH responsive follicles was not affected by aging in SCWL and RR-BB (Figure 2.11). An average of 60% follicles from SCWL and 63% follicles from RR-BB produced over 1.0 ng of estradiol-17 β . On the other hand, even though FF-BB did not vary in LH sensitivity (Slope) their percentage of LH responsive follicles significantly increased after sexual maturity (Figure 2.11). At sexual maturity only 37.7% of follicles from FF-BB were steroidogenically active. This indicates that a higher number of SWF were atretic in that group. It is generally accepted that surge of LH is required for ovulation to occur (Johnson, 1990). Egg production rate is reduced as hens age (Bahr and Palmer, 1989). It has been suggested that age-related declines in egg production result from an increased incidence of follicular atresia (Waddington *et al.*, 1985; Palmer and Bahr, 1992), a reduction in rate of follicular maturation (Johnson, 1986), or to alterations in the sensitivity of the hypothalamus to steroid hormone feedback (Williams and Sharp, 1978). According to Robinson *et al.* (1993) the shape of the sequence length curve resembled the shape of the egg production in that the longest sequences occurred at the time of peak egg production. The prime sequence of SCWL hens was about two to threefold greater than that of restricted broiler breeders (Robinson *et al.*, 1992).

From this study we can conclude that the ability to produce estradiol-17 β by SWF is related to the ovary morphology or "form" of the ovary is related to its "function". A good ovary with a high reproductive performance, had a high slope (very LH sensitive) and a high percent (fewer atretic follicles) as was seen in SCWL. On the contrary, a poor ovary had a low slope and a low percent, which indicated a high incidence of atresia (FF-BB). In reproductive performance, the size of the ovary is also very important, since it is negatively correlated with slope. As the ovary became bigger with

age, the follicles were less responsive to LH. It has been well established that more follicles do not necessary mean more eggs (Yu *et al.*, 1992b; Robinson *et al.*, 1993). When fed *ad libitum*, female broiler breeders have an increased incidence of multiple hierarchies of follicles, which leads to multiple ovulation and production of defective eggs (Yu *et al.*, 1992b). Also, full-fed female broiler breeders have in increased incidence of ovarian regression, which results in cessation of egg production Yu *et al.* (1992b).

TABLE 2.1. Experimental design - effect of aging. Treatment groups according to age classification by type.

Age classification (Aging)	Type		
	SCWL ^a	FF-BB ^b	RR-BB ^c
Immature hens-19wk ¹	SCWL-19		
First egg - P ²	SCWL-P ¹	FF-BB-P ¹	RR-BB-P ¹
First egg - NP			
Young hens-35wk ²	SCWL-35	FF-BB-35	RR-BB-35
Old hens-60wk ²	SCWL-60	FF-BB-60	RR-BB-60

¹ These hens were sacrificed at sexual maturity.

² Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

³ Birds were non photostimulated and controlled by 8L:16D.

^a Egg-type birds; Single Comb White Leghorns.

^b Meat-type birds; Full-fed broiler breeders.

^c Meat-type birds; Restricted broiler breeders.

TABLE 2.2. Effect of aging on BW, selected organ weights and carcass composition in full-fed SCWL, killed at 19 wks (SCWL-19), at sexual maturity (SCWL-P), at 35 wks of age (SCWL-35) and at 60 wks of age (SCWL-60).

Variables	Aging				SEM
	SCWL-19	SCWL-P	SCWL-35	SCWL-60	
Number of hens	10	10	10	10	
Average BW, g	1,227.2 ^a	1,492.7 ^a	1,508.0 ^a	1,734.2 ^a	59.13
Heart					
%BW	0.44 ^b	0.41 ^b	0.53 ^a	0.45 ^b	0.02
g	5.36 ^b	6.16 ^b	7.96 ^a	7.81 ^a	0.4
Liver					
%BW	1.61 ^a	2.06 ^b	2.50 ^a	2.71 ^a	0.10
g	19.69 ^d	30.64 ^a	37.50 ^b	46.93 ^a	1.86
Abdominal fat					
%BW	2.15 ^b	2.17 ^a	3.26 ^a	3.92 ^a	0.28
g	26.59 ^c	33.67 ^{ab}	49.78 ^a	69.28 ^a	5.66
Carcass fat					
%BW	6.76 ^b	8.21 ^b	12.02 ^a	14.11 ^a	0.80
g	83.98 ^b	119.75 ^{ab}	181.69 ^{ab}	227.19 ^a	41.84
Carcass protein					
%BW	13.31 ^b	15.11 ^a	14.79 ^a	15.23 ^a	0.30
g	165.46 ^b	214.99 ^a	221.62 ^a	245.1 ^a	16.28
Carcass H₂O					
%BW	78.41 ^a	74.34 ^b	70.87 ^c	68.30 ^d	0.88
g	970.32	1055.88	1059.21	1095.81	52.54
Carcass ash					
%BW	1.87 ^a	2.57 ^a	2.68 ^a	2.48 ^b	0.07
g	23.28 ^b	36.81 ^a	39.93 ^a	40.12 ^a	3.79
Total percent	100.35	100.24	100.36	100.12	

^{a,b,c,d} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.3. Effect of aging on reproductive tract characteristics in full-fed SCWL, killed at 19 wks (SCWL-19), at sexual maturity (SCWL-P), at 35 wks (SCWL-35) and at 60 wks of age (SCWL-60).

Variables	Aging				SEM
	SCWL-19	SCWL-P	SCWL-35	SCWL-60	
Number of hens	10	10	10	10	
Oviduct					
%BW	0.47 ^a	3.26 ^a	3.16 ^a	3.43 ^a	0.12
g	5.62 ^c	48.69 ^b	47.80 ^b	58.94 ^a	2.28
Ovary					
%BW	0.06 ^a	2.03 ^b	2.62 ^a	2.86 ^a	0.16
g	0.73 ^d	30.77 ^a	39.23 ^b	49.43 ^b	2.80
Stroma					
%BW	0.06 ^a	0.22 ^b	0.47 ^a	0.44 ^a	0.02
g	0.73 ^a	3.24 ^b	7.14 ^a	7.56 ^a	0.40
Number of large follicles					
	0.00 ^a	6.2 ^b	4.9 ^b	5.9 ^b	0.46

^{a,b,c,d} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.4. Effect of aging on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed SCWL, killed at 19 wks (SCWL-19), at sexual maturity (SCWL-P), at 35 wks (SCWL-35) and at 60 wks of age (SCWL-60).

Variables	Aging				SEM
	SCWL-19	SCWL-P	SCWL-35	SCWL-60	
Number of hens	10	10	10	10	
E₂ production					
Dose of LH					
0 ng LH	262.03 ^b	454.57 ^a	349.46 ^{ab}	321.31 ^{ab}	56.90
5 ng LH	943.04	1348.94	1201.65	1323.47	191.30
10 ng LH	1263.87	1216.31	1211.87	1366.68	204.70
20 ng LH	1211.76	1306.06	1099.81	1060.43	192.60
Slope ²	136.20	178.87	170.44	200.43	29.50
Percent (%) ³	66.19	64.07	56.33	52.33	8.03

¹ bLH, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{ab} Means within a row with different superscripts are significantly different (P ≤ .05).

TABLE 2.5. Effect of aging on BW, selected organ weights and carcass composition in full-fed photostimulated broiler breeders (BB), killed at sexual maturity (FF-BB-P), at 35 wks of age (FF-BB-35) and at 60 wks of age (FF-BB-60).

Variables	Aging				
	FF-BB-P	FF-BB-35	SEM ¹	FF-BB-60	SEM ²
Number of hens	10	10		9	
Average BW, g	4,446.6 ^b	5,168.2 ^a	139.93	5,098.7 ^a	147.50
Heart					
%BW	0.38 ^b	0.35 ^b	0.03	0.55 ^a	0.03
g	16.63 ^b	18.14 ^b	1.45	28.09 ^a	1.52
Liver					
%BW	2.11	2.34	0.17	2.43	0.18
g	93.42 ^b	120.85 ^{ab}	9.56	123.9 ^a	10.08
Abdominal fat					
%BW	6.21	6.89	0.38	5.90	0.41
g	276.36 ^b	357.72 ^a	24.00	303.5 ^{ab}	25.30
Carcass fat					
%BW	24.89	23.81	0.80	25.3	0.89
g	1102.69 ^b	1221.06 ^{ab}	41.85	1324.0 ^a	46.78
Carcass protein					
%BW	15.03 ^a	13.48 ^b	0.30	13.80 ^b	0.34
g	666.53 ^b	689.33 ^{ab}	16.28	715.32 ^a	18.19
Carcass H ₂ O					
%BW	57.46 ^b	60.33 ^a	0.88	57.78 ^b	0.99
g	2552.6 ^b	3074.9 ^a	52.55	2986.25 ^a	58.75
Carcass ash					
%BW	2.7 ^b	2.6 ^b	0.07	2.97 ^a	0.08
g	119.35 ^a	133.0 ^b	3.79	154.96 ^b	4.24
Total percent	100.08	100.18	0.29	99.85	

¹ SEM given for n=10.

² SEM given for n=9.

^{a,b,c} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.6. Effect of aging on reproductive tract characteristics in full-fed photostimulated broiler breeders (BB) (FF-BB-P), full-fed BB at 35 wks of age (FF-BB-35) and full-fed BB at 60 wks of age (FF-BB-60).

Variables	Aging				
	FF-BB-P	FF-BB-35	SEM ¹	FF-BB-60	SEM ²
Number of hens	10	10		9	
Oviduct					
%BW	1.39	1.47	0.13	1.33	0.14
g	61.50	76.05	6.33	67.62	6.67
Ovary					
%BW	1.67	1.52	0.23	1.97	0.25
g	74.81	79.11	11.68	99.92	12.31
Stroma					
%BW	0.22 ^a	0.26 ^b	0.03	0.36 ^c	0.03
g	9.46 ^b	13.35 ^b	1.68	18.41 ^a	1.77
Number of large follicles	9.1 ^a	7.9 ^a	0.84	6.33 ^b	0.88

¹ SEM given for n=10.

² SEM given for n=9.

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.7. Effect of aging on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed photostimulated broiler breeders (BB) (FF-BB-P), full-fed BB at 35 wks of age (FF-BB-35) and full-fed BB at 60 wks of age (FF-BB-60).

Variables	Aging				
	FF-BB-P	SEM ⁴	FF-BB-35	FF-BB-60	SEM ⁵
Number of hens	10		9	9	
E₂ production					
Dose of LH					
0 ng LH	377.40 ^b	66.7	558.50 ^{ab}	649.67 ^a	70.3
5 ng LH	1021.95 ^b	247.4	1617.43 ^{ab}	1934.58 ^a	260.8
10 ng LH	886.29 ^b	226.0	1773.21 ^a	2108.70 ^a	238.2
20 ng LH	1074.69 ^b	172.9	1493.37 ^{ab}	1890.53 ^a	182.3
Slope ²	128.90	46.5	211.79	257.00	49.0
Percent (%) ³	37.67 ^b	5.9	64.44 ^a	78.57 ^a	6.2

¹ bLH, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

⁴ SEM given for n=10.

⁵ SEM given for n=9.

^{ab} Means within a row with different superscripts are significantly different (P \leq .05).

TABLE 2.8. Effect of aging on BW, selected organ weights and carcass composition in feed-restricted photostimulated broiler breeders (BB), killed at sexual maturity (RR-BB-P), at 35 wks of age (RR-BB-35) and at 60 wks of age (RR-BB-60).

Variables	Aging			SEM
	RR-BB-P	RR-BB-35	RR-BB-60	
Number of hens	10	10	10	
Average BW, g	2,718.3 ^b	3,486.6 ^a	3,405.2 ^a	70.08
Heart				
g BW	0.41 ^a	0.34 ^b	0.45 ^a	0.02
g	11.16 ^b	11.96 ^b	15.53 ^a	0.59
Liver				
%BW	2.07 ^a	2.25 ^a	1.51 ^b	0.10
g	55.93 ^b	78.64 ^a	51.57 ^b	3.75
Abdominal fat				
%BW	2.25 ^b	3.63 ^a	4.43 ^a	0.37
g	61.38 ^b	127.25 ^a	152.99 ^a	13.89
Carcass fat				
%BW	12.92 ^b	15.63 ^{ab}	17.63 ^a	0.80
g	341.63 ^b	534.38 ^a	598.64 ^a	55.51
Carcass protein				
%BW	18.20 ^a	14.81 ^b	13.66 ^c	0.30
g	480.86	502.53	459.23	16.28
Carcass H ₂ O				
%BW	64.99	66.54	66.93	0.88
g	1707.92 ^b	2257.65 ^a	2243.18 ^a	52.55
Carcass ash				
%BW	3.48 ^a	2.71 ^b	2.33 ^c	0.07
g	91.74 ^a	92.02 ^a	78.13 ^b	3.79
Total percent	99.59	99.70	100.54	

^{abc} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.9. Effect of Aging on reproductive tract characteristics in feed-restricted photostimulated broiler breeders (BB), killed at sexual maturity (RR-BB-P), at 35 wks of age (RR-BB-35) and at 60 wks of age (RR-BB-60).

Variables	Aging			SEM
	RR-BB-P	RR-BB-35	RR-BB-60	
Number of hens	10	10	10	
Oviduct				
%BW	2.02 ^b	1.75 ^b	2.77 ^a	0.19
g	54.64 ^b	61.10 ^b	94.31 ^a	6.54
Ovary				
%BW	1.75 ^{ab}	2.03 ^a	1.42 ^b	0.13
g	47.43 ^b	70.82 ^a	48.54 ^b	4.43
Stroma				
%BW	0.23	0.28	0.30	0.02
g	6.37 ^b	9.81 ^a	10.10 ^a	0.75
Number of large follicles	7.4 ^a	5.8 ^b	4.2 ^c	0.45

^{a,b,c} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 2.10. Effect of aging on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from feed-restricted photostimulated broiler breeders (BB) (RR-BB-P), feed-restricted BB at 35 wks of age (RR-BB-35) and feed-restricted at 60 wks of age (RR-BB-60).

Variables	Aging			SEM
	RR-BB-P	RR-BB-35	RR-BB-60	
Number of hens	10	10	10	
E₂ production				
Dose of LH				
0 ng LH	359.96 ^b	515.55 ^{ab}	625.68 ^a	59.28
5 ng LH	1637.04	1630.08	1936.37	246.04
10 ng LH	1600.23	1556.97	1704.20	250.55
20 ng LH	1072.26	1424.67	1271.25	154.32
Slope ²	255.42	222.90	262.14	45.82
Percent (%) ³	61.67	65.00	61.67	8.41

¹ bLH, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{ab} Means within a row with different superscripts are significantly different (P ≤ .05).

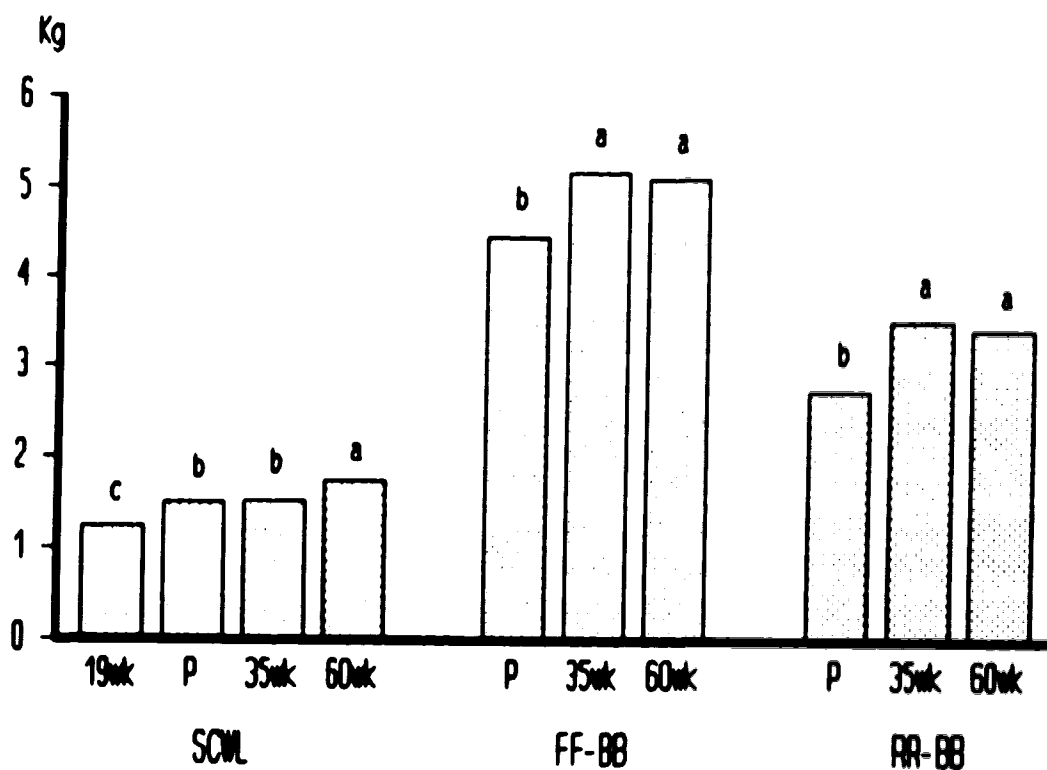


Figure 2.1. The effect of aging on body weight (kg) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (RR-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

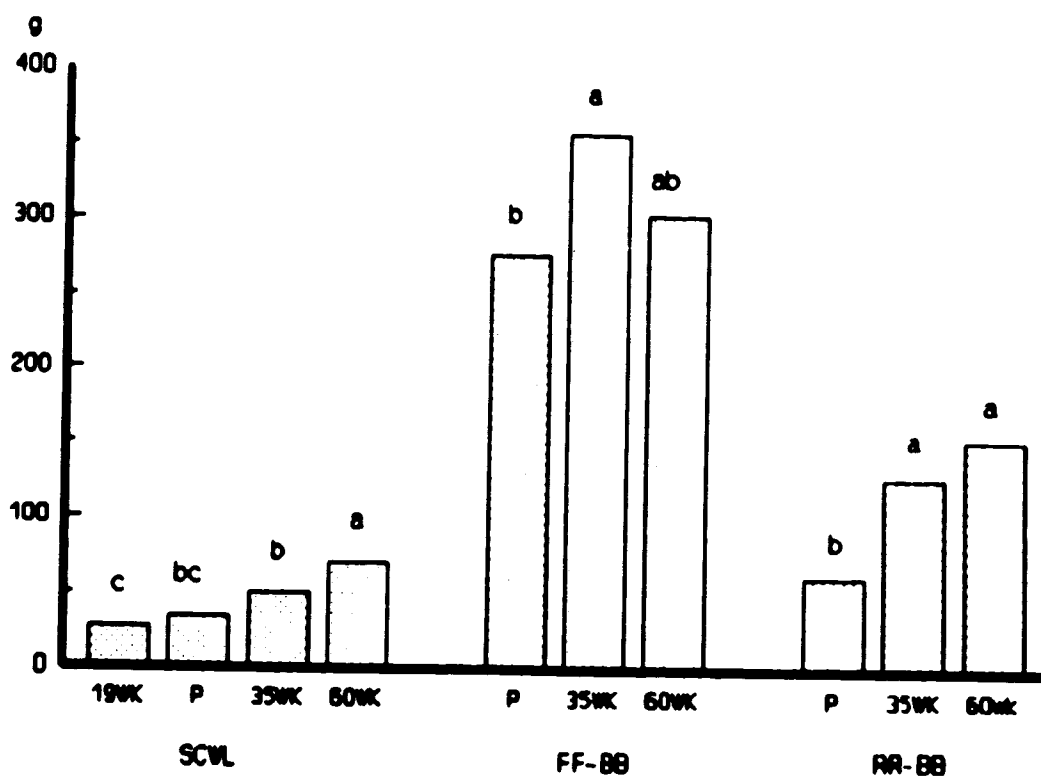


Figure 2.2 The effect of aging on abdominal fat pad weight (g) for three types: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35 wk) and old hens (60 wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

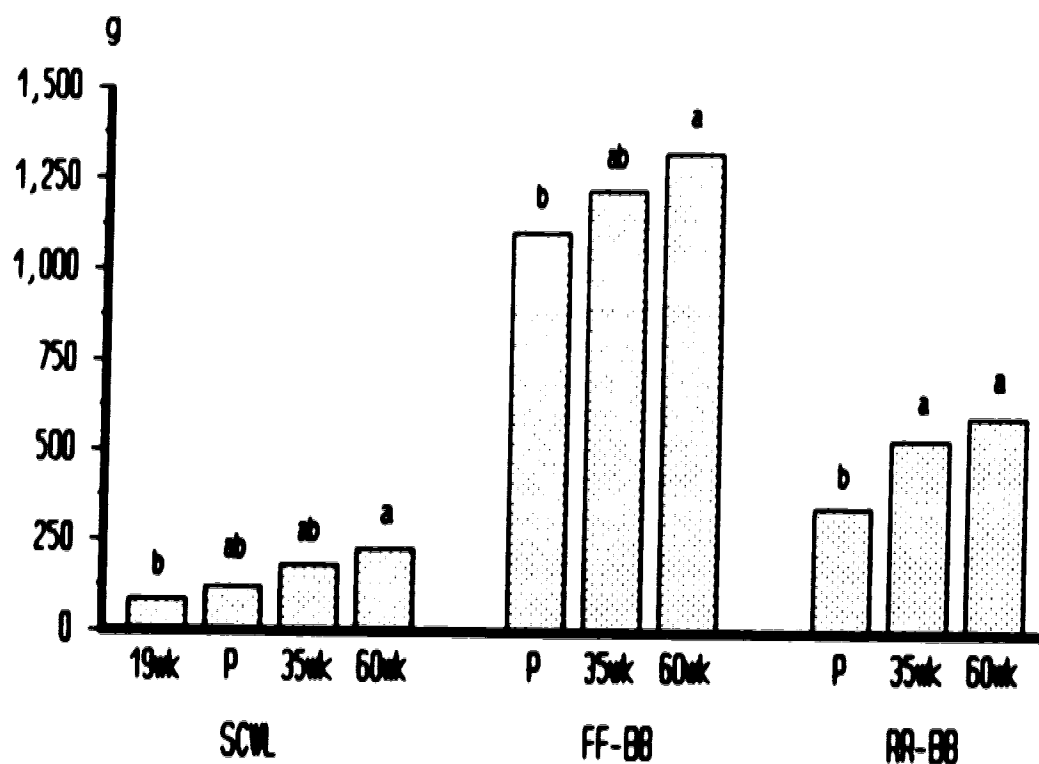


Figure 2.3. The effect of aging on total carcass fat weight (g) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (FF-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

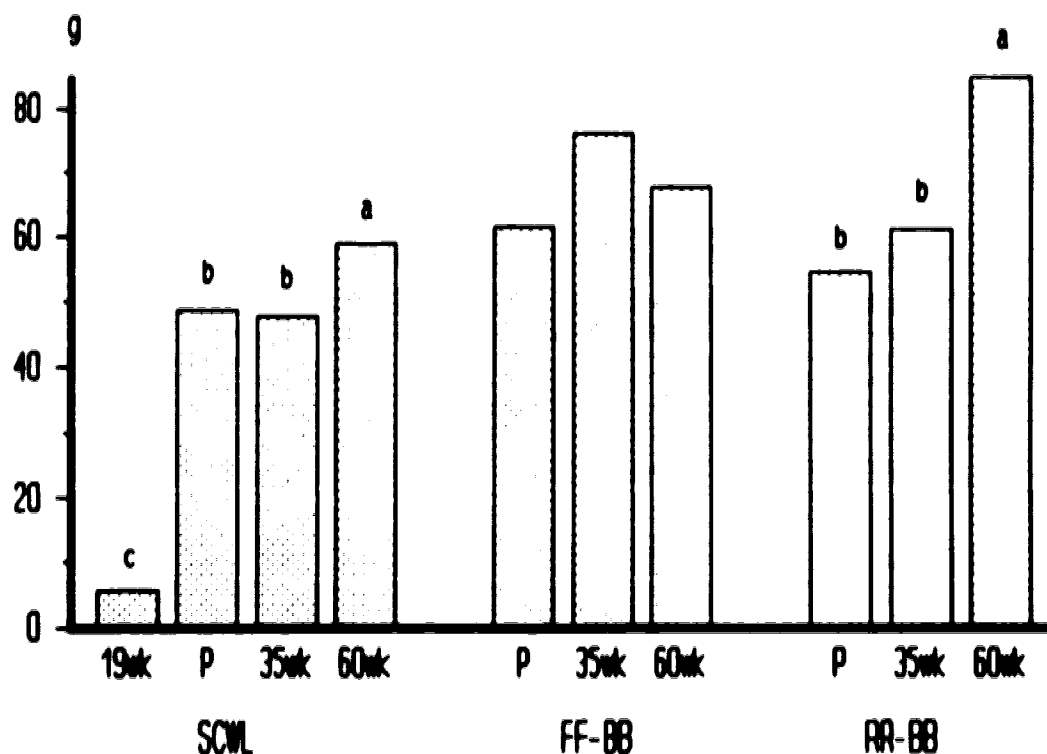


Figure 2.4. The effect of aging on oviduct weight (g) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (FF-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

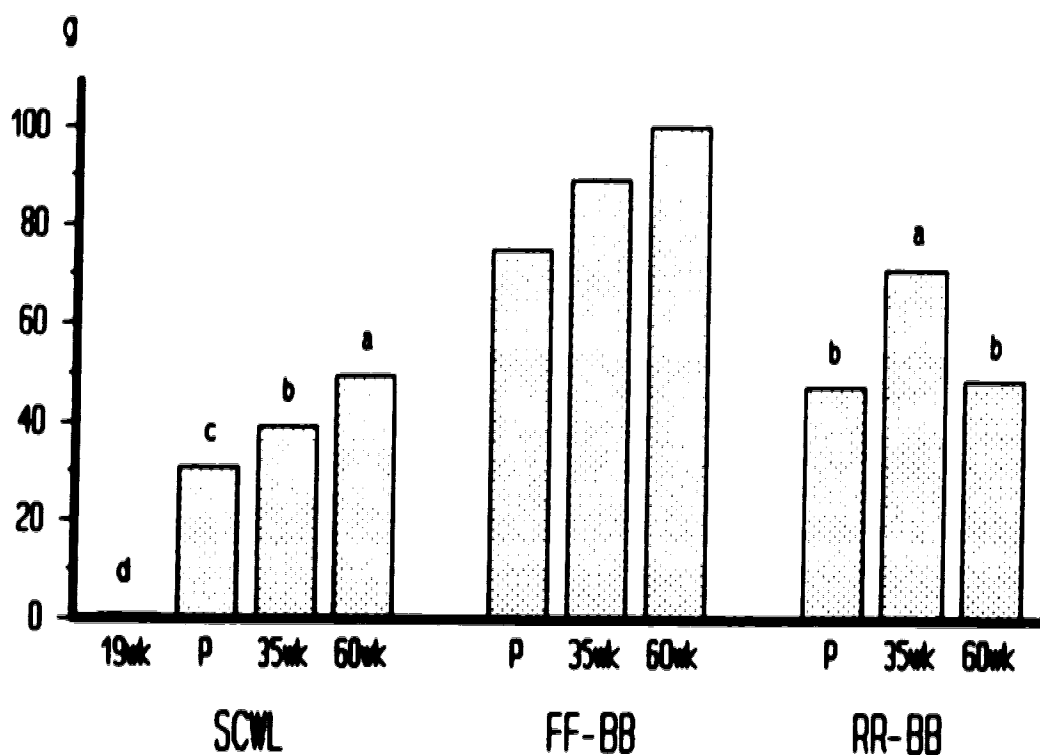


Figure 2.5. The effect of aging on total ovary weight (g) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (RR-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

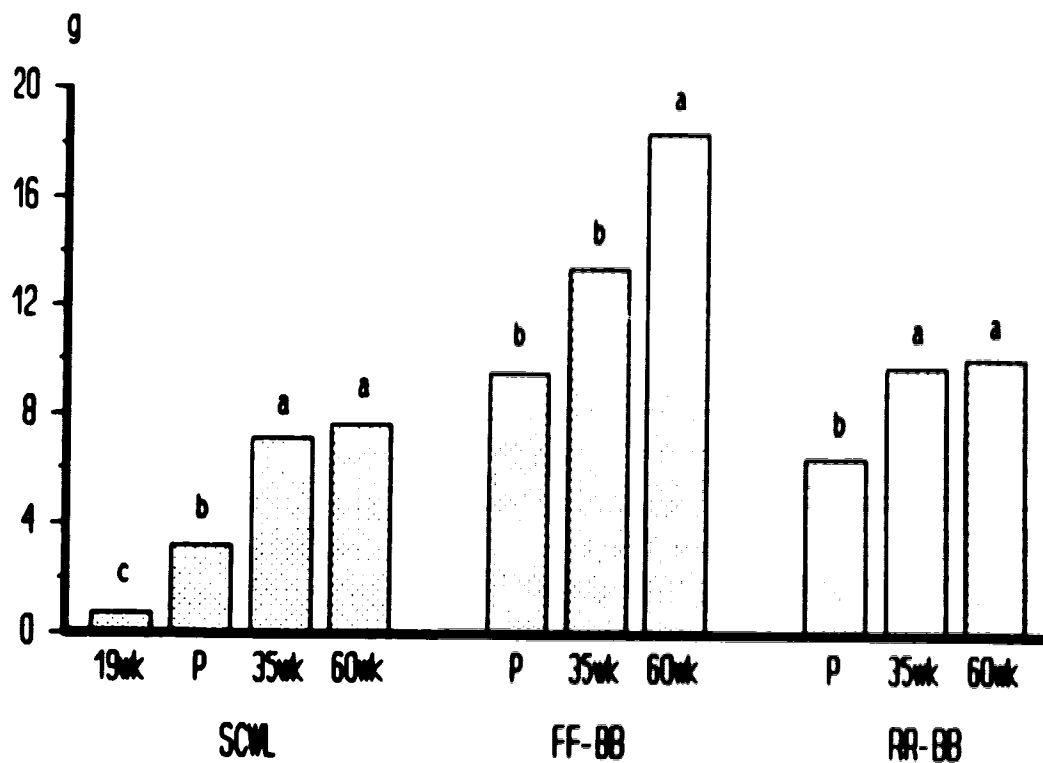


Figure 2.6. The effect of aging on ovarian stroma weight (g) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (RR-BB). Treatments included immature birds (19 wk), first egg photoestimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

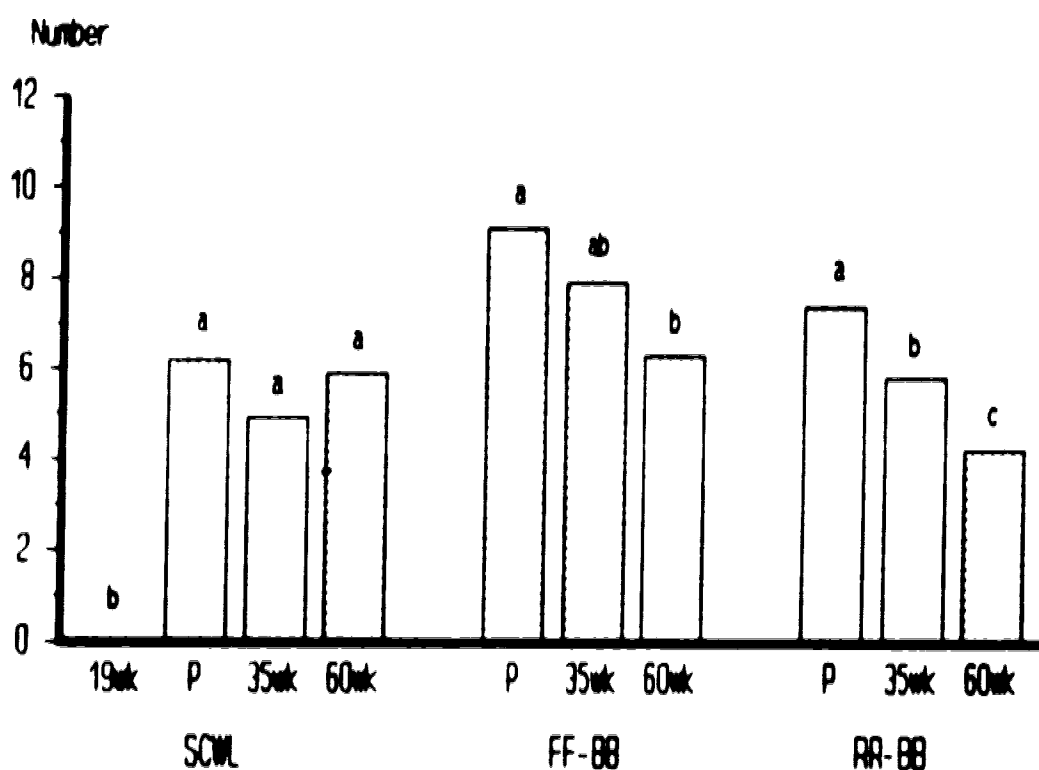


Figure 2.7. The effect of aging on number of large follicles (Number) for three types of birds: Single Comb White Leghorn (SCWL), full-fed (FF-BB) and feed restricted broiler breeders (RR-BB). Treatments included immature birds (19 wk), first egg photostimulated (P), young hens 35 wks (35wk) and old hens 60 wks (60wk) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

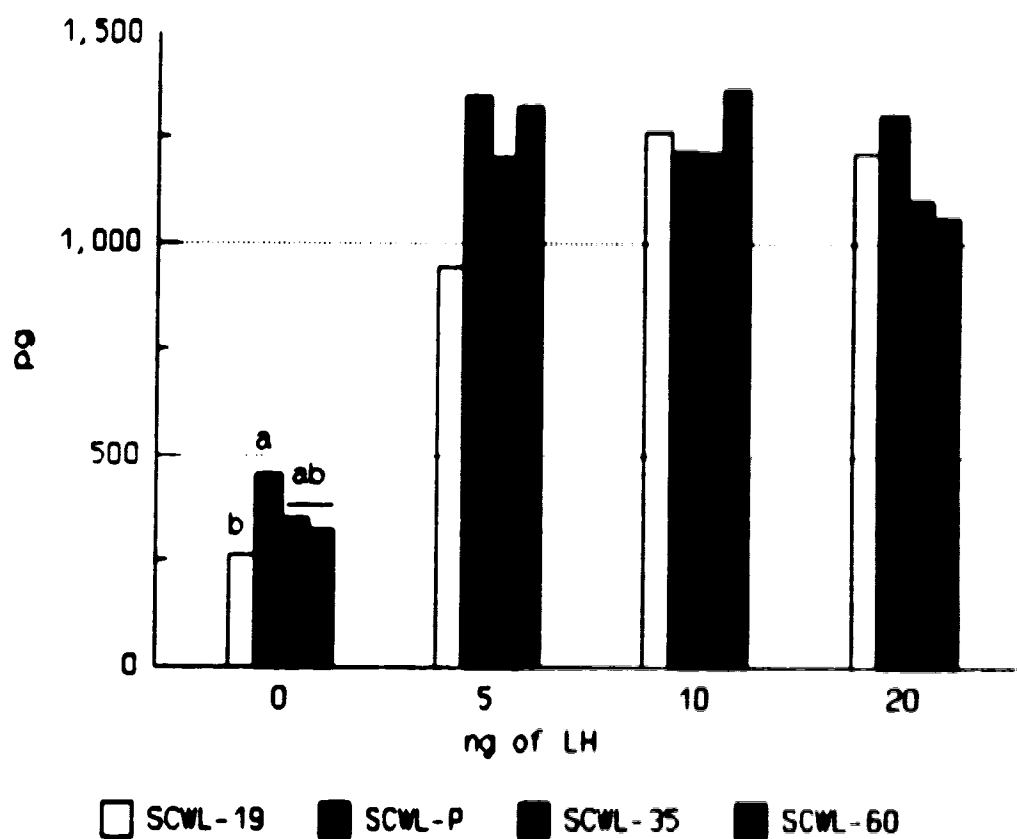


Figure 2.8. The effect of aging on estradiol-17 β output for SCWL. Treatments included immature birds (SCWL-19), first egg photostimulated (SCWL-P), young hens 35 wks (SCWL-35) and old hens 60 wks of age (SCWL-60). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

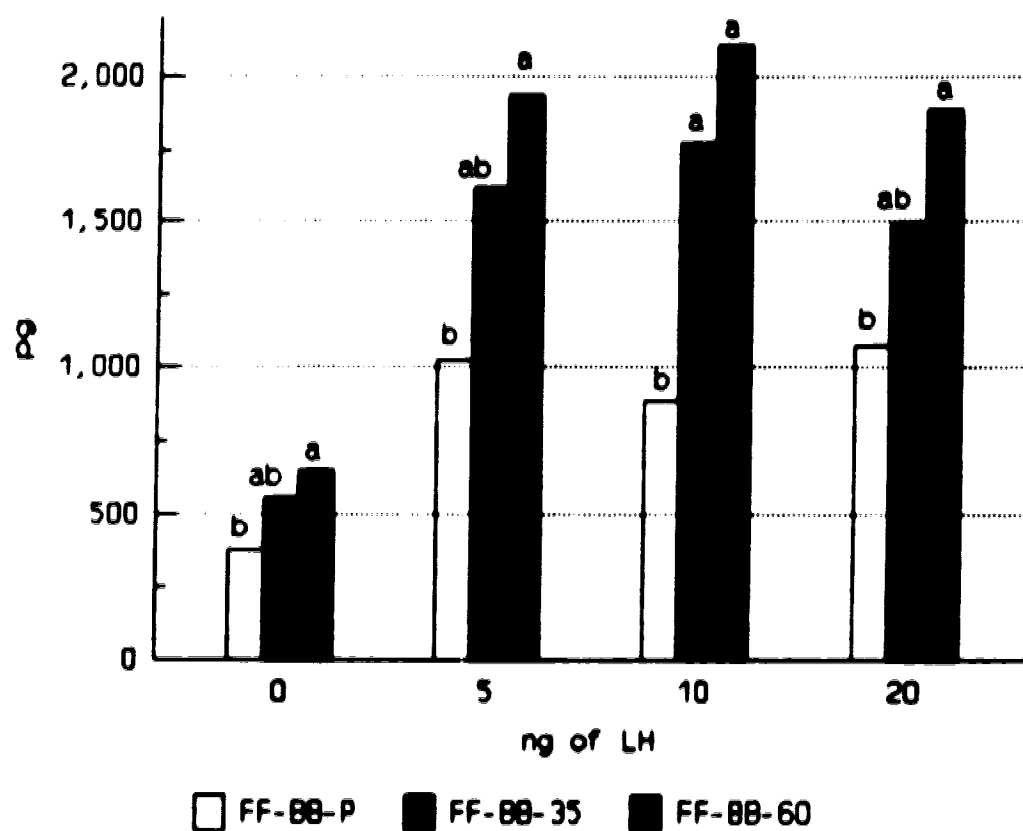


Figure 2.9. The effect of aging on estradiol-17 β output for FF-BB. Treatments included first egg photostimulated birds (FF-BB-P), young hens 35 wks (FF-BB-35) and old hens 60 wks of age (FF-BB-60). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

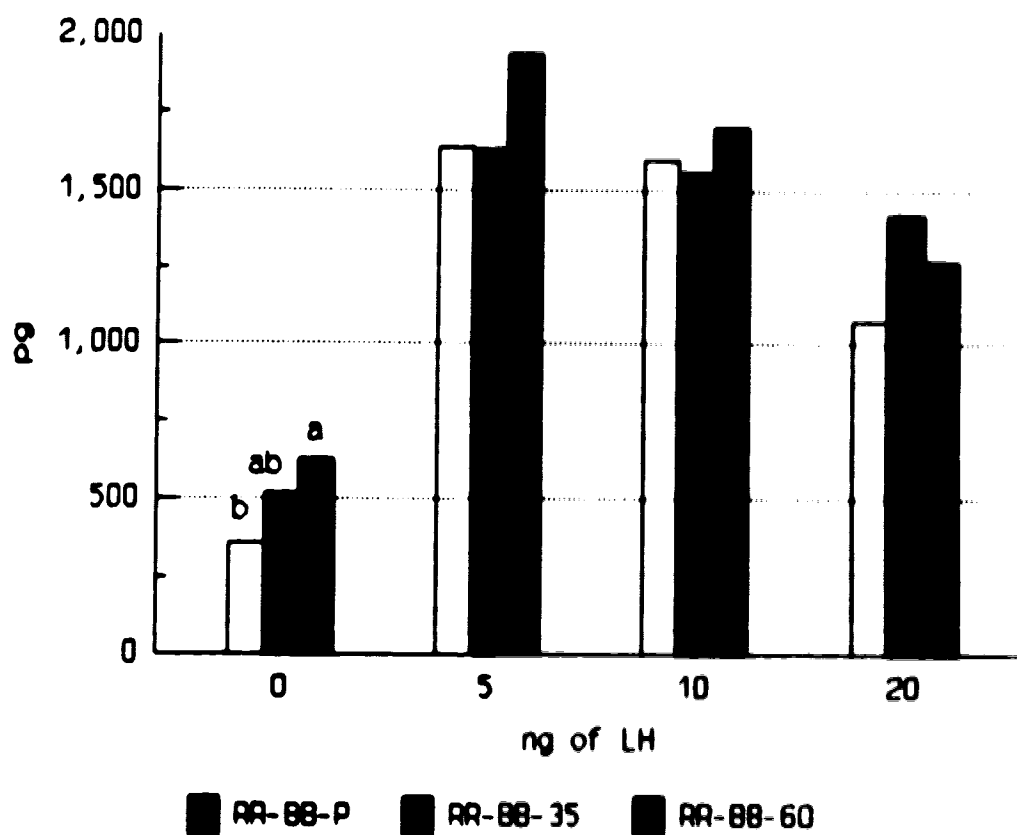


Figure 2.10. The effect of aging on estradiol-17 β output for RR-BB. Treatments included first egg photostimulated birds (FF-BB-P), young hens 35 wks (RR-BB-35) and old hens 60 wks of age (RR-BB-60). Treatments means within a type with different superscript are significantly different ($P \leq 0.05$).

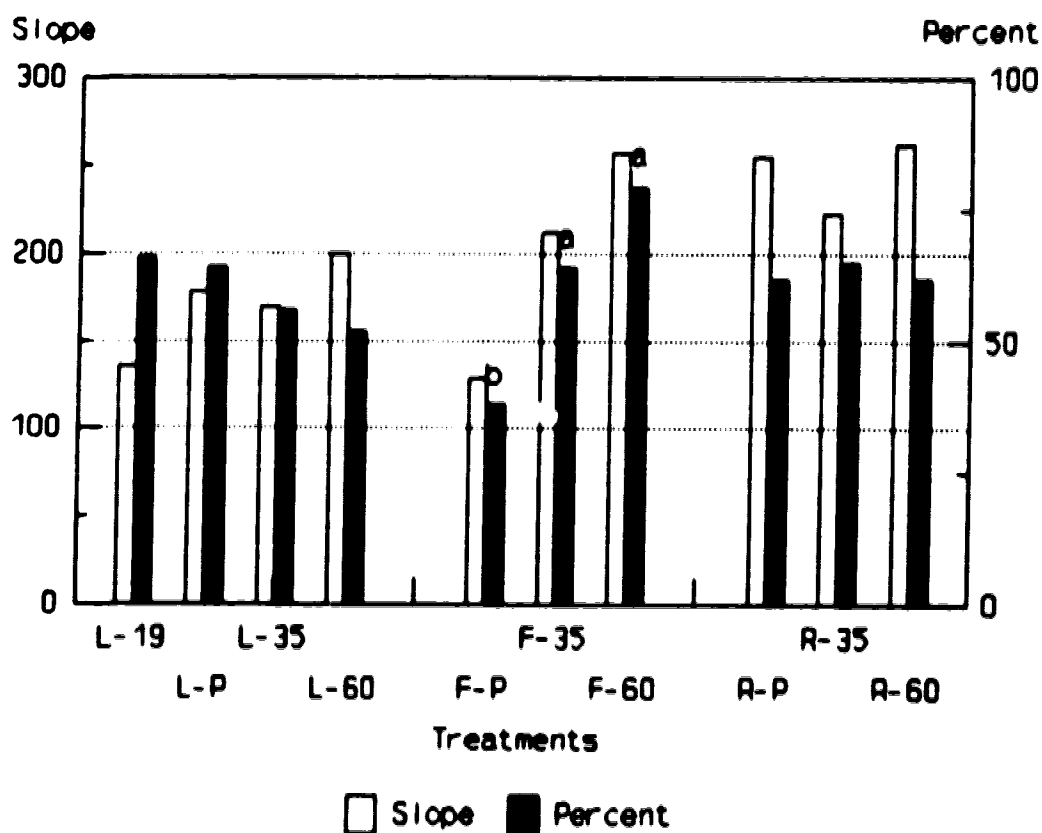


Figure 2.11. The effect of aging on LH sensitivity (Slope) and percent of LH responsive follicles (Percent) for SCWL (L), full-fed BB (F) and restricted BB (R). Treatments included immature birds 19 wks of age (L-19), first egg photostimulated (L-P; F-P; R-P), young hens 35 wks (L-35; F-35; R-35) and old hens at 60 wks of age (L-60; F-60; R-60). Treatments means within a type with different superscript are significantly different ($P \leq 0.05$).

2.6 LITERATURE CITED

Association of Official Analytical Chemists, 1980. Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists, Washington, DC.

Bahr, J. M., and S. S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Gilbert, A. B., 1971. The ovary. Pages 1163-1208 in: Physiology and Biochemistry of Domestic Fowl. Vol 3. D.J. Bell and B. M. Freeman, ed. Academic Press, London, England.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington, 1987. Ovarian follicular structure of White Leghorn fed *ad libitum* and Dwarf and Normal broiler breeders fed *ad libitum* or restricted to point of lay. Br. Poult. Sci. 28:493-506.

Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. Br. Poult. Sci. 30:161-174.

Johnson, A. L., 1986. Reproduction in the female. Pages 403-431 in Avian Physiology, 4 ed. P.D. Sturkie, ed Springer-Verlag, New York, NY.

Johnson, A. L., 1990. Steroidogenesis and actions of steroids in the hen ovary. Crit. Rev. Poult. Biol. 2:319-346.

McDaniel, G. R., J. Brake, and M. K. Eckman, 1981. Factors affecting broiler breeder performance. 4. The interrelationships of some reproductive traits. Poultry Sci. 60:1792-1797.

Mougdal, R. P., and M. N. Razdan, 1985. Induction of ovulation *in vitro* in the hen depending of the response to LH and rate of lay. J. Endocrinol. 106, 67.

Palmer, S. S., and J. M. Bahr, 1992. Follicle stimulating hormone increases serum oestradiol-17 β concentration, number of growing follicles and yolk deposition in aging hens (*Gallus domesticus*) with decreased egg production. Br. Poult. Sci. 33:403-411.

Robbins, K. R., G. C. McGhee, P. Osei, and R. E. Beauchene, 1986. Effect of feed restriction on growth, body composition, and egg production during the breeding season. Poultry Sci. 65:1052-1057.

Robinson F. E. and R. J. Eches, 1986. Ovarian steroidogenesis during follicular maturation in domestic fowl (*Gallus domesticus*). Biol. of Reprod. 35:1096-1105.

Robinson, F. E., R. T. Hardin, and A. R. Robblee, 1990. Reproductive senescence in Domestic fowl: Effects on egg production, sequence length and inter sequence pause length. Br. Poult. Sci. 31:871-879.

Robinson F. E., N. A. Robinson, and T. A. Scott, 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler hens. Can. J. Anim. Sci. 71:549-556.

- Robinson N. A., F. E. Robinson, and R. T. Hardin, 1992. Reproductive senescence in egg-type chickens: Effects on egg production, sequence length and inter-sequence pause length. Poultry Science 81st Annual Meeting Abstracts. Poult. Sci. 71:128.
- Robinson, F. E., J. L. Wilson, M. W. Yu, G. M. Fasenko, 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. Poultry Sci. 72:912-922.
- Romanoff and Romanoff, A. J., 1949. The Avian Egg. J. Wiley and Sons, Inc., New York.
- Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. 2nd ed. Mc Graw-Hill Book Co., Inc., New York, NY.
- Van Middelkoop, J. H., 1972. The relationship between ovulation interval of White Plymouth Rock pullets and the laying of abnormal eggs. Archiv fur Geflugelkunde, 36:223-230.
- Waddington, D., M. M. Perry, A. B. Gilbert, and M. A. Hardie. 1985. Follicular growth and atresia in the ovaries of hen with diminished egg production rates. J. Reprod. Fert. 74:399-405.
- Williams, J. B., and P. J. Sharp, 1978. Ovarian morphology and rates of ovarian follicular development in laying broiler breeders and commercial egg-producing hens. Br. Poult. Sci. 19:387-395.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. Poultry Sci. 71:1750-1761.

Yu, M. W., F. E. Robinson, and R. J. Etches, 1992c. Effect of feed allowance during rearing and breeding on female broiler breeders. 3. Ovarian steroidogenesis. Poultry Sci. 71:1762-1767.

Yu, M. W., F. E. Robinson, and A. R. Robblee, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poultry Sci. 71:1739-1749.

3. OVARIAN MORPHOLOGY AND STEROIDOGENESIS IN THE DOMESTIC FOWL: EFFECTS OF STRAIN.

3.1 Introduction

There are major physiological differences between egg-type (Leghorn) and meat-type (broiler breeder) birds. Leghorn chickens have a more delicate body construction, as they are much smaller birds than broiler breeders are. However, Single Comb White Leghorns lay more eggs than broiler breeders. They can produce more than 300 eggs in the first year of their reproductive life (Cunningham, 1987). In the ovary, they have also smaller and fewer (five to seven) large follicles (Hocking *et al.*, 1987). Laying hens lay eggs in sequences. Leghorn hens that are laying at higher rates laid long sequences of 15 to 20 eggs (Bahr and Palmer, 1989). A superior leghorn hen can lay a sequence of 80 eggs, while a superior broiler breeder hens may lay as many as 40 consecutive days (Robinson *et al.*, 1992). In egg-type birds there is a little evidence of arhythmic sequences. Usually they lay eggs during the day time. Their amount of body fat is usually several times smaller than in broiler breeders, especially in full-fed broiler breeders (Mallard and Douaire, 1988).

Broiler breeders belong to a type of heavy bird. Broiler breeders weight usually 2 to 3 times as much as egg-type birds. Broiler breeders accumulate a large amount of fat when allowed to eat *ad libitum* (Mallard and Douaire, 1988; Robinson *et al.*, 1991; Yu *et al.*, 1992a). Their increased fat accumulation constitutes a loss to both producers and consumers. Finally, fat is negatively correlated with reproductive capability of these birds (Siegel and Dunnington, 1985). Broiler breeder's reproductive performance is not as great as egg-type birds. They lay total of 170 to 180 eggs to 65 wks of age (Robinson *et al.*, 1990). The ovary of full-fed broiler breeders weights almost 1.5 times that of restricted birds and they develop more follicles than do egg-type chickens (Jenp and Clancy, 1968; Hocking *et al.*, 1987). Full-fed broiler breeders have an average of 12 large follicles at sexual maturity (Hocking *et al.*, 1987; Yu *et al.*, 1992b). Higher

numbers of large follicles in the ovary does not necessarily mean better reproductive performance. In broiler types birds many large follicles are associated with double hierarchies and multiple ovulation (Whitehead and Hocking, 1988; Yu *et al.*, 1992b). Conrad and Warren (1940) cited simultaneous development of two or more follicles as the cause of multiple-yolked eggs. Under feed restriction, broiler breeders have similar number of large follicles as do egg-type hens (Williams and Sharp, 1978). Lower numbers of large follicles in the ovary of restricted broiler breeders (Hocking *et al.*, 1989) decrease the incidence of multiple hierarchies of follicles (Whitehead and Hocking, 1988). Overweight broiler breeders do not restrict their egg laying to the day time as normal birds would do, but they lay throughout the night as well (Yu *et al.*, 1992b). Consequently, the incidence of arrhythmic sequences is higher in these birds than in egg-type birds. The presence of two eggs in the oviduct at one time is an obvious indicator of obesity-related problems (Chaney and Fuller, 1975; Yu *et al.*, 1992a, 1992b). Broiler breeders also have higher oviduct and stroma weight compared to egg-type birds (Yu *et al.*, 1992b). At first egg, at *Libin*-fed broiler breeders have about twice as many small follicles between 1 and 8 mm in diameter as do egg type hens (Hocking *et al.*, 1987). Follicular atresia, internal ovulation and internal laying, excessive ovulation, high incidence of soft shelled and membranous eggs are also common problems for overweight broiler breeder hens than for egg-type hens (Clayton, 1972; van Middelloop, 1972).

One of the objectives of the research presented in this section was to examine the effect of strain on body organ weights (heart, liver, abdominal fat), on carcass composition (protein, fat, moisture, ash) and on ovarian development (weight of the oviduct, ovary, stroma and number of large follicles). The second objective was to compare estradiol-17 β output from small white follicles incubated with four doses of bLH (0, 5, 10, 20 ng/follicle) of full-fed and restricted broiler breeders.

3.2 Materials and Methods

3.2.1 Stocks and Management

Forty female Single Comb White Leghorns (SCWL-Shaver-288), randomly selected from a flock of 80 birds, and 40 female broiler breeders (BB-Shaver Starbro), randomly selected from a flock of 120 birds were individually housed in plastic cages at 20 wks of age. The total of 80 birds were assigned to eight treatment groups with 10 birds per treatment (Table 3.1). Egg-type birds (SCWL) and meat-type birds (BB) were further divided into four treatment groups. Classification for SCWL were based on hens sacrificed at: sexual maturity when photostimulated (SCWL-P), non photostimulated (SCWL-NP), 35 wks (SCWL-35) and 60 wks of age (SCWL-60). Full-fed broiler breeders were killed at: sexual maturity when photostimulated (FF-BB-P), non photostimulated (FF-BB-NP), 35 wks (FF-BB-35) and 60 wks of age (FF-BB-60). All the photostimulated birds were exposed to 14L:10D. Non photostimulated hens were maintained at 8L:16D.

SCWL and broiler breeder hens were allowed to eat *ad libitum*. Throughout the experiment birds had free access to water. The photostimulated and non photostimulated hens were sacrificed at sexual maturity (first oviposition). The procedures for managing the birds, collecting samples, chemical analyses, the radioimmunoassay for estradiol-17 β output and determination of LH sensitivity were exactly the same as described in Chapter 2. Studies on estradiol-17 β output were based on incubation of 3,200 individual follicles (40 follicles x 80 birds).

3.3 Statistical analyses

Data were subjected to one-way analyses of variance (Steel and Torrie, 1980). The significant differences between means were evaluated by using Duncan's multiple range test (Steel and Torrie, 1980). Differences among the age classification were determined within each type (Table 3.1) for organ weights, carcass composition and reproductive characteristics. Differences in estradiol-17 β were calculated by using methods

described in Chapter 2. Significance was assessed at the 0.05 level.

3.4 Results

There was a significant difference in body weight at sexual maturity between egg-type (SCWL-P; 1,492.7 g) and meat-type (FF-BB-P; 4,446.6 g) photostimulated birds (Table 3.2). Broiler breeders were 2.5 times heavier than SCWL. However, there was no significant difference in age at sexual maturity between the two strains (SCWL-P; 152.5 days; FF-BB-P; 155.5 days). Heart weight, liver weight, abdominal fat pad weight, protein, water and ash were significantly higher in FF-BB-P than in SCWL-P. There were no significant differences in percent of heart, liver, protein and ash on body weight basis between those two strains. Percent of abdominal fat pad, carcass fat and moisture was significantly higher in FF-BB-P than in SCWL-P on body weight basis.

Strain had a significant effect on reproductive tract characteristics at sexual maturity in full-fed photostimulated SCWL (SCWL-P) and broiler breeders (FF-BB-P). Oviduct, ovary and stroma weight were significantly heavier in FF-BB-P than in SCWL-P (Table 3.3). On a percentage of body weight basis, only oviduct weight was significantly heavier in egg-type birds (3.25%) than in meat-type (1.38%). On the other hand, ovary and stroma weight expressed as a percentage of body weight were not significantly different in these groups. The number of large follicles was significantly higher in broiler breeders than in SCWL.

Table 3.4 gives estradiol-17 β output from follicles of full-fed photostimulated SCWL (SCWL-P) and broiler breeders (FF-BB-P). Strain did not have significant effect on estradiol-17 β output. However, the percent of follicles from SCWL hens incubated with bLH, which produced more than 1.0 ng of estradiol-17 β was almost 1.5 times higher (64.07%) than it was in broiler breeders (37.67%).

Non photostimulated broiler breeders (Table 3.5) were significantly heavier (4,422.4 g) than SCWL (1,502.9 g). Consequently, non photostimulated broiler breeders came into production 17 days after SCWL. All organ weights as well as carcass composition

components were significantly higher in non photostimulated broiler breeders killed at sexual maturity than in SCWL. On the other hand, percent of heart, liver, abdominal fat pad, carcass fat and water on body weight basis were significantly influenced by strain and were higher in SCWL. Nevertheless, there was no difference in percent carcass protein on body weight basis in those two analyzed groups.

Strain also affected reproductive organ weights in non photostimulated birds (Table 3.6). Oviduct, ovary and stroma weight as well as the number of large follicles were significantly different in FF-BB-NP than in SCWL-NP. On the body weight basis, percent of oviduct and ovary was significantly higher in SCWL than in broiler breeders.

Estradiol-17 β outputs from non photostimulated SCWL and broiler breeders are presented in table 3.7. Strain did not have an influence of any estradiol-17 β output.

Full-fed broiler breeders at 35 wks of age were significantly heavier than 35 wks SCWL hens (Table 3.8). Body organ weights as well as body composition were also significantly heavier in full-fed broiler breeders than in SCWL at 35 wks of age. On body weight basis, percent of liver and ash were not affected by strain.

Strains significantly influenced reproductive tract characteristics. Oviduct, stroma and ovary weight were higher in broiler breeders than in SCWL at 35 wks of age (Table 3.9). On body weight basis, percent of above organs were higher in SCWL than in broiler breeders.

Full-fed broiler breeders produced significantly more estradiol-17 β than did SCWL at 35 wks of age during incubation in the absence of bLH and at 20 ng bLH (Table 3.10). The percent of follicles which produced more than 1.0 ng of estradiol-17 β when exposed to 5 ng or more of LH was not affected by strain.

Body weight, heart, liver, abdominal fat pad weight and body composition were higher in full-fed broiler breeders at 60 wks of age than in SCWL (Table 3.11). Also, percent of those parameters on dry matter basis were higher in full-fed broiler breeders than in SCWL.

In birds at 60 wks of age there was no difference in oviduct weight between SCWL

and BB (Table 3.12). On the other hand, ovary (99.92 g) and stroma weight (18.41 g) were significantly higher in broiler breeders than in SCWL (49.43 g and 7.56 g respectively). The number of large follicles did not differ significantly between strains. On a body weight basis, the percent of oviduct and ovary weight were significantly higher in SCWL than in BB.

Follicles from broiler breeders at 60 wks of age produced significantly higher amounts of estradiol-17 β than SCWL birds (Table 5.13). Also the BB group had significantly more follicles which produced 1.0 ng of estradiol-17 β (78.52 %) than SCWL (52.33 %) when incubated with bLH.

3.5 Discussion

The effect of strain on age at sexual maturity was observed only in non photostimulated birds. FF-BB-NP become sexually mature 17 days later than SCWL-NP (Table 3.5). The difference in age at sexual maturity in SCWL-P vs FF-BB-P was only 3 days and was not significant. This indicates the time needed to recruit follicles into the hierarchy and have them reach ovulable size does not differ when birds are photostimulated. However, not only photoperiod (Chaney and Fuller, 1975) regulates sexual maturity. Body weight (Brody *et al.*, 1980, 1984), body fat content (Bornstein *et al.*, 1984) and age (Brody *et al.*, 1980, 1984) are also important. According to Brody *et al.* (1984) the heavier a bird, the sooner the bird will reach sexual maturity. These factors with the exception of photoperiod and age, are influenced by feed intake. According to Pym and Dillon (1974), the severity of feed restriction progressively delays sexual maturity. On the other hand, feed restriction improves peak rate of egg production (Blair *et al.*, 1976) and persistency of egg production (Leeson and Summers, 1983). Although in my experiment I did not focus on egg productivity, study of the reproductive tract characteristics from the restricted birds supports the above phenomena.

There is a strong negative relationship between BW and amount of fat with reproductive efficiency in domestic fowl (Siegel and Dunnington, 1985). The strength

of these relationships is evidenced by the existence of two types of chickens (egg-type and meat-type) that represent opposite extremes in BW and reproductive efficiency. Body weight was significantly different between strains (Figure 3.2). All BB were significantly heavier than SCWL. FF-BB-35 treatment had the heaviest birds with an average of 5,168.3 g (Table 3.8). Older birds (FF-BB-60) were 70 g lighter than the birds at 35 wks of age (Table 3.3 and 3.11). The average body weight for SCWL was approximately 3 times lower (1,500 g) than in BB.

Abdominal fat pad weight and carcass fat weight were significantly influenced by strain (Figure 3.3 and 3.4). Mallard and Douaire (1988), Robinson *et al.*, (1991) and Yu *et al.* (1992a) have reported that broiler breeders accumulate a large amount of carcass fat when allowed to eat *ad libitum*. Carcass fat is positively correlated with BW, the heavier the birds, the more fat they deposited (Siegel and Dunnington, 1985). All BB groups showed a high fat content (Table 3.2, 3.5, 3.8 and 3.11) and had six to ten times more carcass fat than SCWL. At 60 wks of age the broiler breeders had significantly higher amounts of carcass ash than did SCWL (Table 3.11). This may reflect more calcium deposition in a greater number of eggs in SCWL. Full-fed BB have been reported to lay 122 eggs to 62 wks of age (Yu *et al.*, 1992b), whereas SCWL lay about 250 or more eggs in this time period (Robinson *et al.*, 1992).

The examination of ovarian morphology indicated that the oviduct of full-fed BB was significantly heavier than the oviduct of SCWL (Figure 3.5). Total ovary weight was also significantly heavier in BB than in SCWL (Figure 3.6). The weight of the ovary in the mature birds is largely a measure of the number of yellow follicles in the ovary, consequently, the number of large follicles should be higher in BB than in SCWL. The number of large follicles in BB varied from 9.8 (Table 3.6) to 6.3 in FF-BB-60 (Table 3.12). On the other hand, SCWL had fewer large follicles than BB with an average of 5.5 (Figure 3.8). Stroma weight, as an indicator of size of the pool of small follicles, was influenced by strain and was always significantly higher in BB hens (Figure 3.7). In photostimulated and non photostimulated birds stroma weight in BB was

approximately 3 times higher than in SCWL. Large stroma should produce high amounts of estradiol, due to having more follicles. Consequently, small stroma should produce low amounts of estradiol.

Data presented in tables 3.4, 3.7, 3.10 and 3.13 support the work of Robinson and Etches (1986) and Yu *et al.* (1992c) that SWF are steroidogenically active and their estradiol production is stimulated by LH (Figure 3.9). Strain did not have an effect on estradiol-17 β output in photostimulated (Table 3.4) and non photostimulated (Table 3.7) hens, when killed at sexual maturity. At 35 and 60 wks of age BB produced significantly more estradiol-17 β in the absence of LH than SCWL. To date, this is the first data identifying a genetic difference in ovarian steroid production from SWF. Early in lay, the strains did not differ, but at 35 wks (Table 3.10) at 60 wks (Table 3.13) the BB hens had a significantly higher output of estradiol-17 β without LH and also at a high doses of LH. These observations are very interesting in that, high levels of estradiol-17 β may have a negative effect on the ovulatory cycle. These birds also had a significantly higher stroma and number of large follicles, perhaps indicating that estradiol-17 β production may be involved in excessive follicular recruitment. Further study is needed to determine the physiological relationship between estradiol-17 β levels, follicular recruitment and endocrinology of the large follicles.

In this study, estradiol-17 β output was maximally stimulated by 5 ng of LH. The reduction in steroid output by using 10 and 20 ng of LH may be due in part to a limited number of LH receptors on the steroid producing cell of SWF. LH sensitivity (Slope) did not significantly vary across the treatments (Figure 3.10). As ovary weight is negatively correlated with slope (Chapter 6; Table 6.1), we can assume that less follicles would respond to LH. The percentage of LH responsive follicles (Figure 3.10) was affected by strain in photostimulated birds (Table 3.4) and in old birds (Table 3.13). At sexual maturity, only 37.7% follicles from FF-BB were steroidogenically active, while 64.1% of SWF from SCWL were LH responsive. This observation indicates that higher number of SWF were atretic in FF-BB. It should be restated here that BB had

significantly higher ovary weight and stroma weight compared with SCWL. While BB had more follicles, fewer of these follicles were responded to LH and most of them were atretic. At 60 wks of age 78.5% follicles from FF-BB responded to LH stimulation, while 52.3% SWF from SCWL were LH responsive. This indicated a higher level of atresia with age in SCWL than in BB. This can be expanded to suggest that FF-BB have many follicles with more basal output and low levels of atresia. It can be stated that potential estradiol-17 β output for a BB ovary should be much higher than that of SCWL. In studies of large follicles of the hen, Wells *et al.* (1983) suggested that the number of LH receptors on granulosa cells increases as follicles approach ovulation. According to Robinson and Etches (1986), the output of estradiol further increased in LWF and SYF, indicating that these tissues may have more LH receptors.

TABLE 3.1. Experimental design - effect of strain. Treatment groups according to age classification by type.

Age classification (Aging)	Type		
	SCWL ^a	FF-BB ^b	RR-BB ^c
Immature hens-19wk ³			
First egg - P ²	SCWL-P ¹	FF-BB-P ¹	
First egg - NP ³	SCWL-NP ¹	FF-BB-NP ¹	
Young hens-35wk ²			
	SCWL-35	FF-BB-35	
Old hens-60wk ²			
	SCWL-60	FF-BB-60	

¹ These hens were sacrificed at sexual maturity.

² Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

³ Birds were non photostimulated and controlled by 8L:16D.

^a Egg-type birds; Single Comb White Leghorns.

^b Meat-type birds; Full-fed broiler breeders.

^c Meat-type birds; Restricted broiler breeders.

TABLE 3.2. Effect of strain of the bird on age, BW, selected organ weights and carcass composition at sexual maturity in full-fed photostimulated SCWL (SCWL-P) and full-fed photostimulated broiler breeders (BB) (FF-BB-P).

Variables	Strain		SEM
	SCWL-P	FF-BB-P	
Number of hens	10	10	
Age of bird	152.5	155.5	2.18
Average BW, g	4,492.7 ^a	4,446.6 ^a	82.07
Heart			
%BW	0.41	0.38	0.03
g	6.16 ^b	16.63 ^a	0.98
Liver			
%BW	2.06	2.11	0.10
g	30.64 ^b	93.42 ^a	3.33
Abdominal fat			
%BW	2.17 ^a	6.21 ^a	0.30
g	33.67 ^a	276.36 ^a	11.24
Carcass fat			
%BW	8.21 ^b	24.89 ^a	0.80
g	119.75 ^b	1102.69 ^a	41.85
Carcass protein			
%BW	15.11	15.03	0.30
g	214.99 ^b	666.53 ^a	16.28
Carcass H ₂ O			
%BW	74.34 ^a	57.46 ^b	0.88
g	1055.88 ^b	2552.64 ^a	52.55
Carcass ash			
%BW	2.57	2.70	0.07
g	36.81 ^b	119.35 ^a	3.79
Total percent	100.24	100.08	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

Effect of strain of the bird on reproductive tract characteristics at sexual maturity in full-fed photostimulated SCWL (SCWL-P) and full-fed photostimulated broiler breeders (BB) (FF-BB-P).

Variables	Strain		SEM
	SCWL-P	FF-BB-P	
Number of hens	10	10	
Uterus			
%BW	3.25 ^a	1.38 ^b	0.08
g	48.69 ^b	61.50 ^a	2.84
Ovary			
%BW	2.03	1.68	0.19
g	30.77 ^b	74.81 ^a	6.18
Stroma			
%BW	0.22	0.21	0.03
g	3.24 ^b	9.46 ^a	1.09
Number of large follicles	6.20 ^b	9.11 ^a	0.70

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.4. Effect of strain on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed photostimulated SCWL (SCWL-P) and full-fed photostimulated broiler breeders (BB) (FF-BB-P).

Variables	Strain		SEM
	SCWL-P ¹	FF-BB-P	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	454.57	377.40	66.74
5 ng LH	1348.94	1021.95	184.29
10 ng LH	1216.31	886.29	169.44
20 ng LH	1306.06	1074.69	194.03
Slope ²	178.87	128.91	29.29
Percent (%) ³	64.07 ^a	37.67 ^b	7.40

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.5. Effect of strain on age, BW, selected organ weights and carcass composition at sexual maturity in full-fed non photostimulated SCWL (SCWL-NP) and full-fed non photostimulated broiler breeders (BB) (FF-BB-NP).

Variables	Strain		SEM
	SCWL-NP	FF-BB-NP	
Number of hens	10	10	
Age of bird	158.7 ^a	175.7 ^a	5.09
Average BW, g	1,502.9 ^b	4,422.4 ^a	107.04
Heart			
%BW	0.40 ^a	0.34 ^b	0.02
g	6.03 ^b	14.83 ^a	0.56
Liver			
%BW	1.97 ^a	1.74 ^b	0.07
g	29.58 ^b	77.17 ^a	3.64
Abdominal fat			
%BW	2.45 ^b	6.64 ^a	0.33
g	37.18 ^b	294.38 ^a	14.38
Carcass fat			
%BW	8.15 ^b	26.60 ^a	0.80
g	118.37 ^b	1186.33 ^a	41.84
Carcass protein			
%BW	14.23	14.39	0.30
g	204.88 ^b	638.54 ^a	16.28
Carcass H ₂ O			
%BW	75.26 ^a	56.90 ^b	0.88
g	1080.76 ^b	2521.36 ^a	52.55
Carcass ash			
%BW	2.33	2.53	0.07
g	33.51 ^b	112.60 ^a	3.79
Total percent	99.97	100.43	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.6. Effect of strain on reproductive tract characteristics at sexual maturity in full-fed non photostimulated SCWL (SCWL-NP) and full-fed non photostimulated broiler breeders (BB) (FF-BB-NP).

Variables	Strain		SEM
	SCWL-NP	FF-BB-NP	
Number of hens	10	10	
Oviduct			
%BW	3.20 ^a	1.39 ^b	0.11
g	48.07 ^a	61.24 ^a	3.07
Ovary			
%BW	2.25 ^a	1.79 ^b	0.15
g	33.59 ^b	79.77 ^a	5.35
Stroma			
%BW	0.14 ^b	0.22 ^a	0.02
g	2.12 ^b	9.78 ^a	0.82
Number of large follicles	5.6 ^b	9.8 ^a	0.49

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.7. Effect of strain on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed non photostimulated SCWL (SCWL-NP) and full-fed non photostimulated broiler breeders (BB) (FF-BB-NP).

Variables	Aging		SEM
	SCWL-NP	FF-BB-NP	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	535.74	420.30	78.14
5 ng LH	1549.28	1517.27	241.76
10 ng LH	1316.75	1553.57	187.75
20 ng LH	1123.32	1279.21	204.11
Slope ²	202.71	219.39	37.27
Percent (%) ³	55.00	61.66	9.48

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

TABLE 3.8. Effect of strain on BW, selected organ weights and carcass composition in full-fed SCWL (SCWL-35) and full-fed broiler breeders (BB) (FF-BB-35) at 35 wks of age.

Variables	Strain		SEM
	SCWL-35	FF-BB-35	
Number of hens	10	10	
Average BW, g	1,508.0 ^a	5,168.3 ^a	69.23
Heart			
%BW	0.52 ^a	0.35 ^b	0.02
g	7.96 ^b	18.14 ^a	0.64
Liver			
%BW	2.50	2.34	4.69
g	37.50 ^b	120.85 ^a	4.70
Abdominal fat			
%BW	3.26 ^b	6.89 ^a	0.30
g	49.78 ^b	357.73 ^a	13.43
Carcass fat			
%BW	12.02 ^b	23.81 ^a	0.80
g	181.69 ^b	1221.06 ^a	41.84
Carcass protein			
%BW	14.79 ^a	13.48 ^b	0.30
g	221.62 ^b	689.33 ^a	16.28
Carcass H ₂ O			
%BW	70.87 ^a	60.30 ^b	0.88
g	1059.21 ^b	3074.89 ^a	52.55
Carcass ash			
%BW	2.68	2.59	0.07
g	39.93 ^b	133.01 ^a	3.79
Total percent	100.36	100.18	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.9. Effect of strain on reproductive tract characteristics in full-fed SCWL (SCWL-35) and full-fed broiler breeders (BB) (FF-BB-35) at 35 wks of age.

Variables	Strain		SEM
	SCWL-35	FF-BB-35	
Number of hens	10	10	
Oviduct			
%BW	3.17 ^a	1.47 ^b	0.11
g	47.80 ^b	76.05 ^a	3.86
Ovary			
%BW	2.62 ^a	1.73 ^b	0.10
g	39.23 ^b	89.11 ^a	3.67
Stroma			
%BW	0.47 ^a	0.26 ^b	0.02
g	7.14 ^b	13.35 ^a	0.89
Number of large follicles	4.9 ^b	7.9 ^a	0.36

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.10. Effect of strain on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed SCWL (SCWL-35) and full-fed broiler breeders (BB) (FF-BB-35) at 35 wks of age.

Variables	Strain		SEM
	SCWL-35	FF-BB-35	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	349.46 ^b	558.50 ^a	56.11
5 ng LH	1201.65	1617.52	199.70
10 ng LH	1211.87	1773.24	207.11
20 ng LH	1099.81 ^b	1493.32 ^a	111.20
Slope ²	170.44	211.78	39.30
Percent (%) ³	56.33	64.44	6.22

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{a,b} Means within a row with different superscripts are significantly different (P ≤ .05).

TABLE 3.11. Effect of strain on BW, selected organ weights and carcass composition in full-fed SCWL (SCWL-60) and full-fed broiler breeders (BB) at 60 wks of age (FF-BB-60).

Variables	Strain	
	SCWL-60	FF-BB-60
Number of hens	10	9
Average BW, g	1,734.2 ± 130.66 ^b	5,098.7 ± 137.73 ^a
Heart		
%BW	0.45 ± 0.03 ^b	0.55 ± 0.03 ^a
g	7.81 ± 1.11 ^b	28.09 ± 1.17 ^a
Liver		
%BW	2.71 ± 0.18	2.43 ± 0.18
g	46.93 ± 8.73 ^b	123.94 ± 9.20 ^a
Abdominal fat		
%BW	4.43 ± 0.44 ^b	5.90 ± 0.46 ^a
g	69.28 ± 22.43 ^b	303.51 ± 23.64 ^a
Carcass fat		
%BW	14.11 ± 0.80 ^b	25.30 ± 0.90 ^a
g	227.19 ± 41.84 ^b	1323.96 ± 46.78 ^a
Carcass protein		
%BW	15.23 ± 0.30 ^a	13.80 ± 0.34 ^b
g	245.13 ± 16.28 ^a	715.32 ± 18.19 ^b
Carcass H ₂ O		
%BW	68.30 ± 0.88 ^a	57.78 ± 0.99 ^b
g	1095.81 ± 52.54 ^a	2986.25 ± 58.75 ^b
Carcass ash		
%BW	2.48 ± 0.07 ^b	2.97 ± 0.08 ^a
g	40.12 ± 3.79 ^b	154.96 ± 4.24 ^a
Total percent	100.12	99.85

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.12. Effect of strain on reproductive tract characteristics in full-fed SCWL (SCWL-60) and full-fed broiler breeders (BB) at 60 wks of age (FF-BB-60).

Variables	Strain	
	SCWL-60	FF-BB-60
Number of hens	10	9
Oviduct		
%BW	3.43±0.22 ^a	1.33±0.22 ^b
g	58.94±8.21	67.62±8.66
Ovary		
%BW	2.86±0.23 ^a	1.97±0.24 ^b
g	49.43±9.85 ^b	99.92±10.38 ^a
Stroma		
%BW	0.44±0.03	0.36±0.03
g	7.56±1.29 ^b	18.41±1.36 ^a
Number of large follicles	5.9±0.77	6.33±0.81

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 3.13. Effect of strain on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed SCWL (SCWL-60) and full-fed broiler breeders (BB) at 60 wks of age (FF-BB-60).

Variables	Strain	
	SCWL-60	FF-BB-60
Number of hens	10	9
E₂ production		
Dose of LH		
0 ng LH	321.31 \pm 66.00 ^a	649.67 \pm 69.57 ^a
5 ng LH	1323.47 \pm 260.08	1934.58 \pm 274.14
10 ng LH	1366.68 \pm 237.69 ^a	2108.70 \pm 250.55 ^a
20 ng LH	1060.03 \pm 160.12 ^a	1890.50 \pm 168.78 ^a
Slope²	200.43 \pm 44.06	256.98 \pm 46.45
Percent (%)³	52.33 \pm 6.71 ^a	78.52 \pm 7.07 ^a

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{a,b} Means within a row with different superscripts are significantly different (P \leq .05).

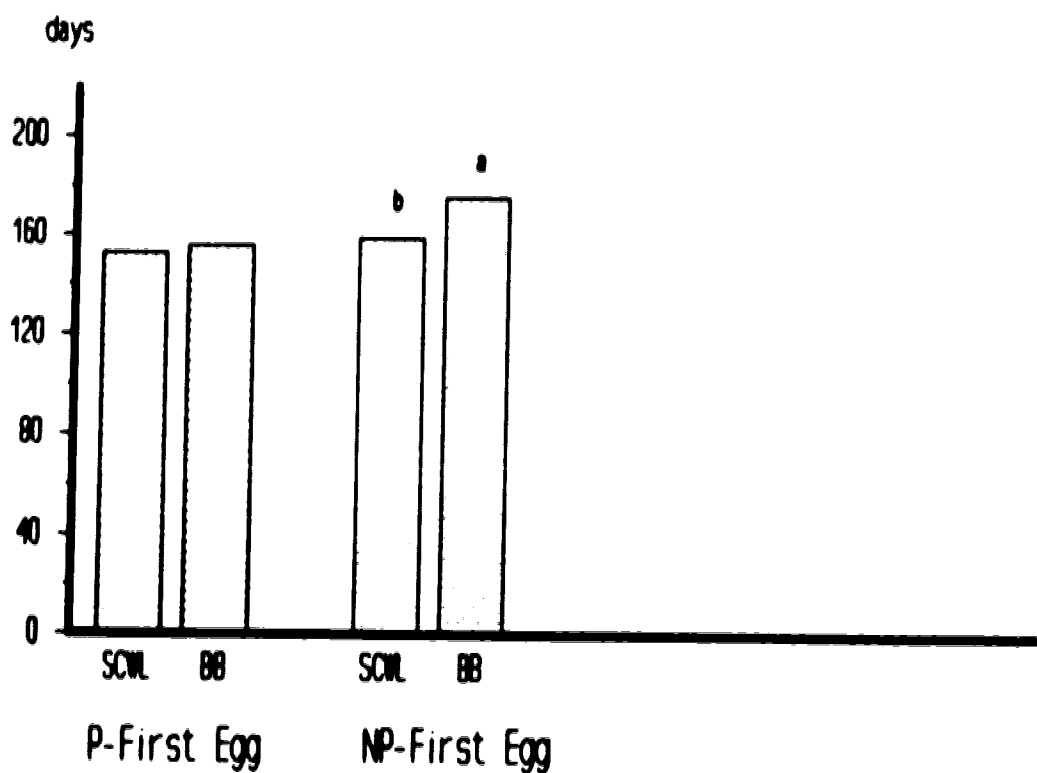


Figure 3.1. The effect of strain on age at sexual maturity (days) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated hens (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

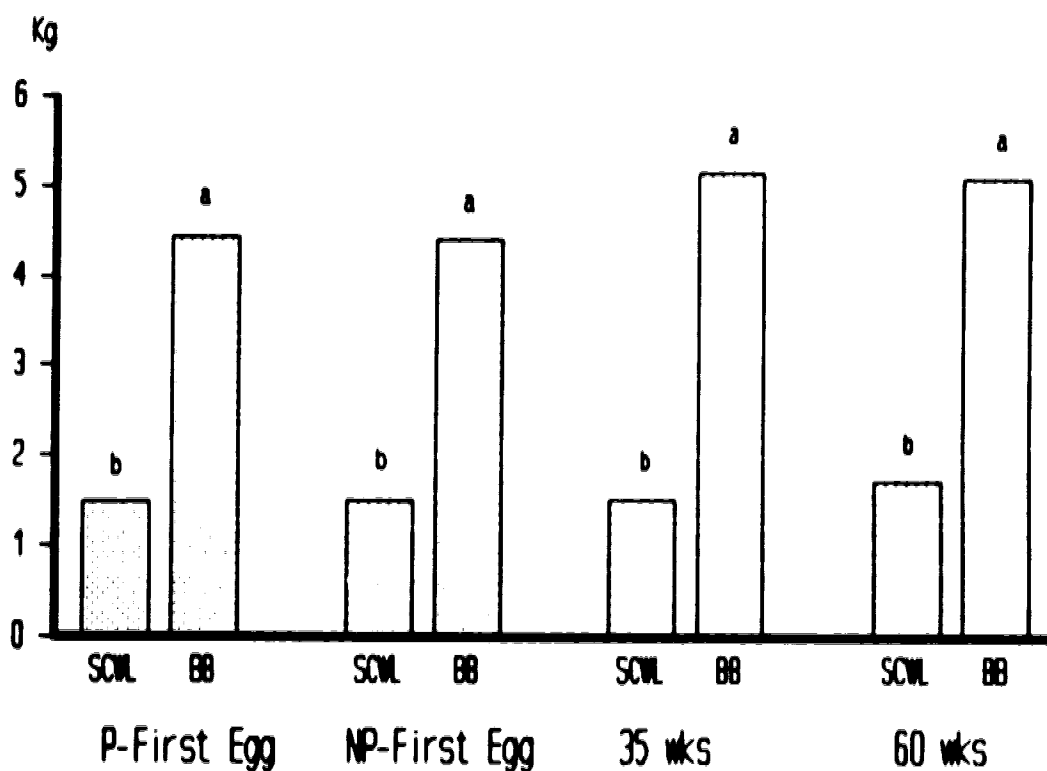


Figure 3.2. The effect of strain on body weight (kg) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

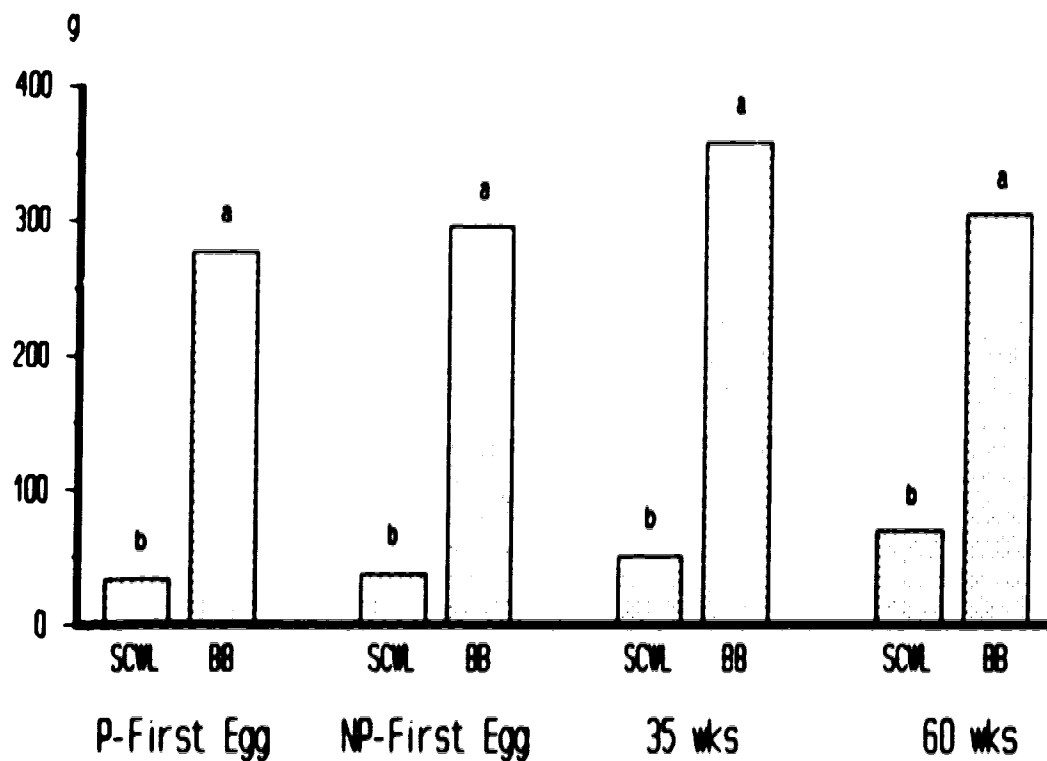


Figure 3.3. The effect of strain on abdominal fat pad weight (g) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

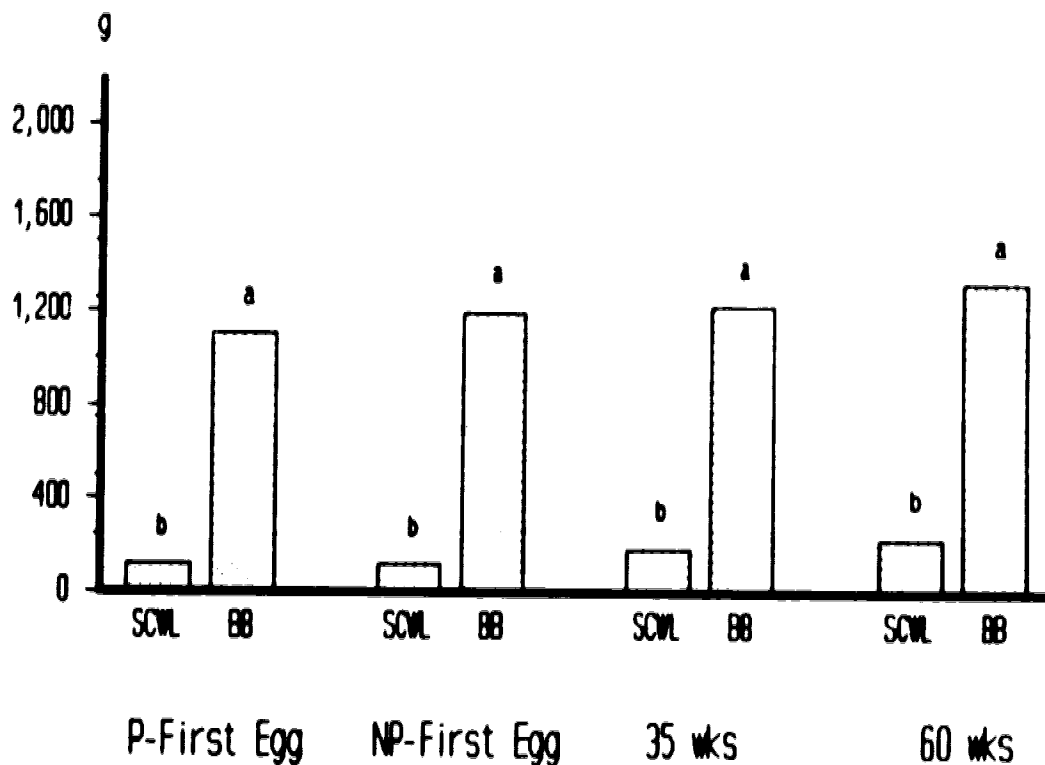


Figure 3.4. The effect of strain on total carcass fat weight (g) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

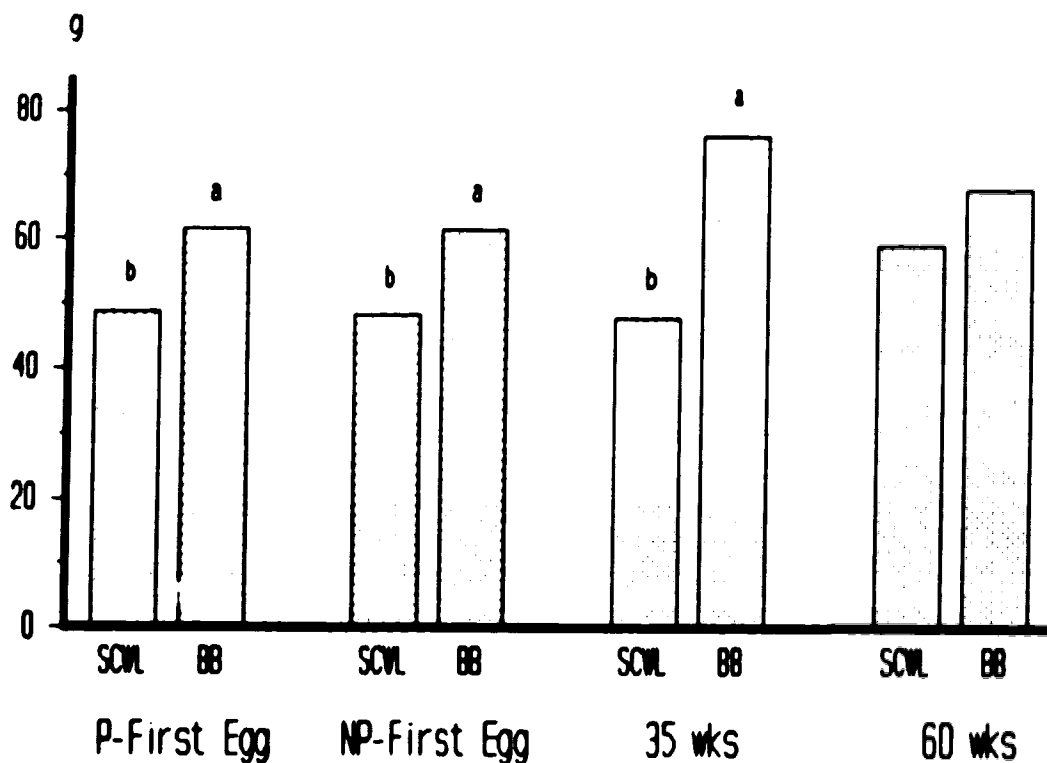


Figure 3.5. The effect of strain on oviduct weight (g) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

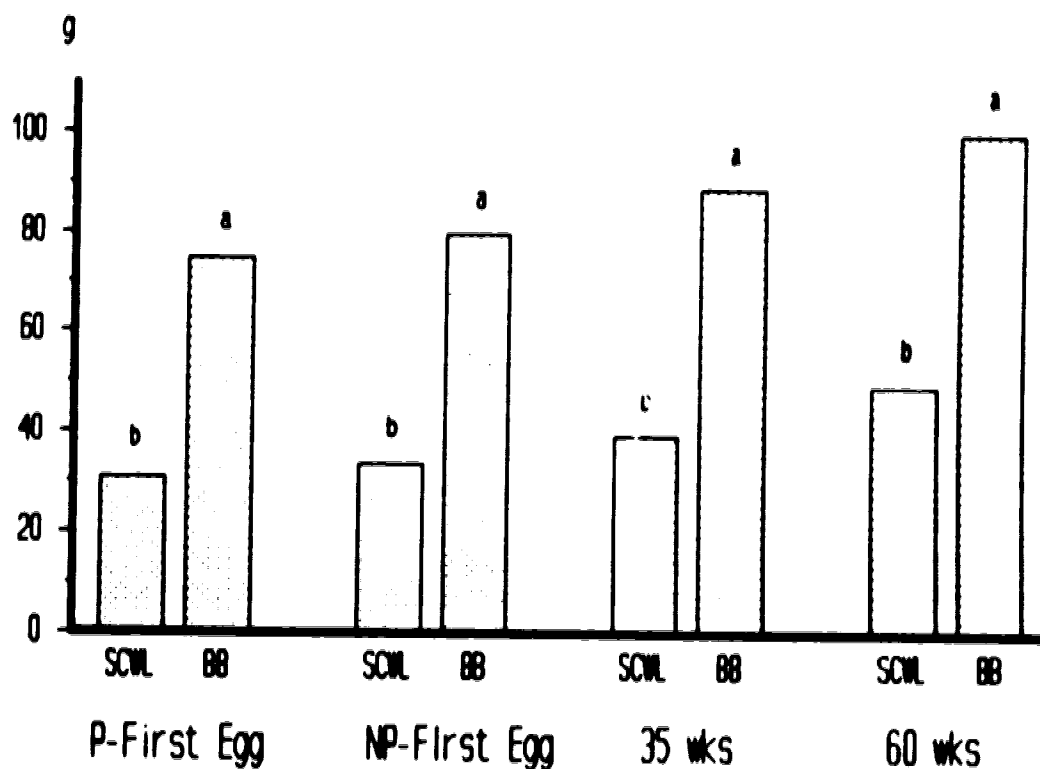


Figure 3.6. The effect of strain on total ovary weight (g) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

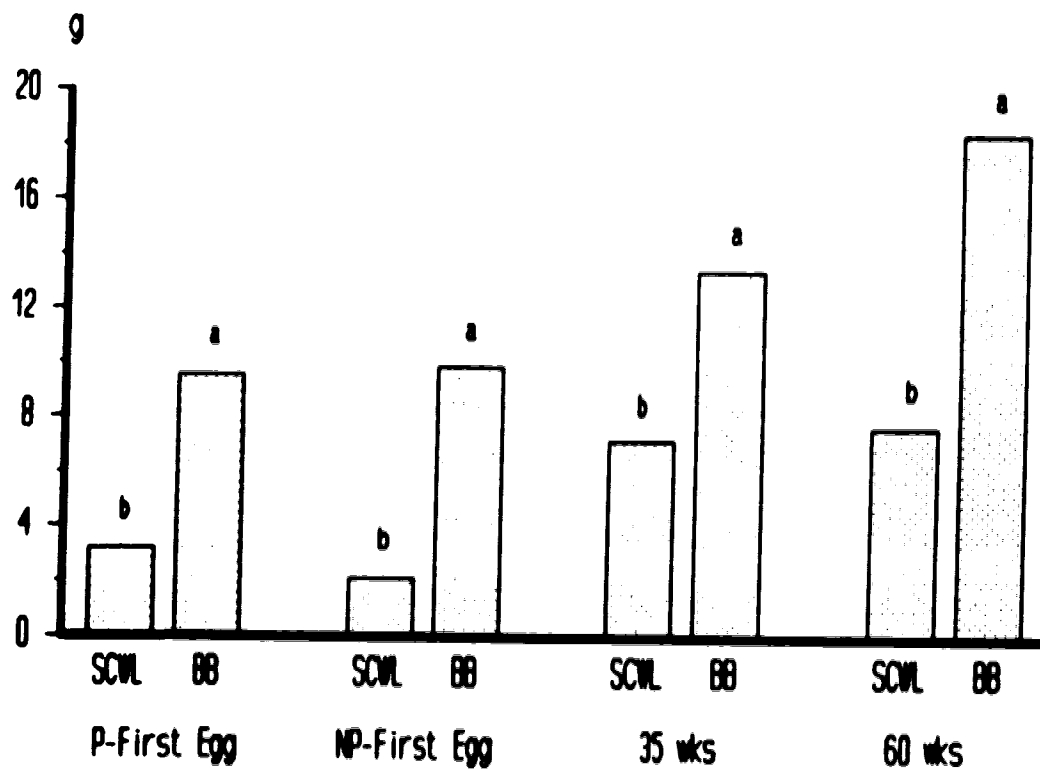


Figure 3.7. The effect of strain on ovarian stroma weight (g) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

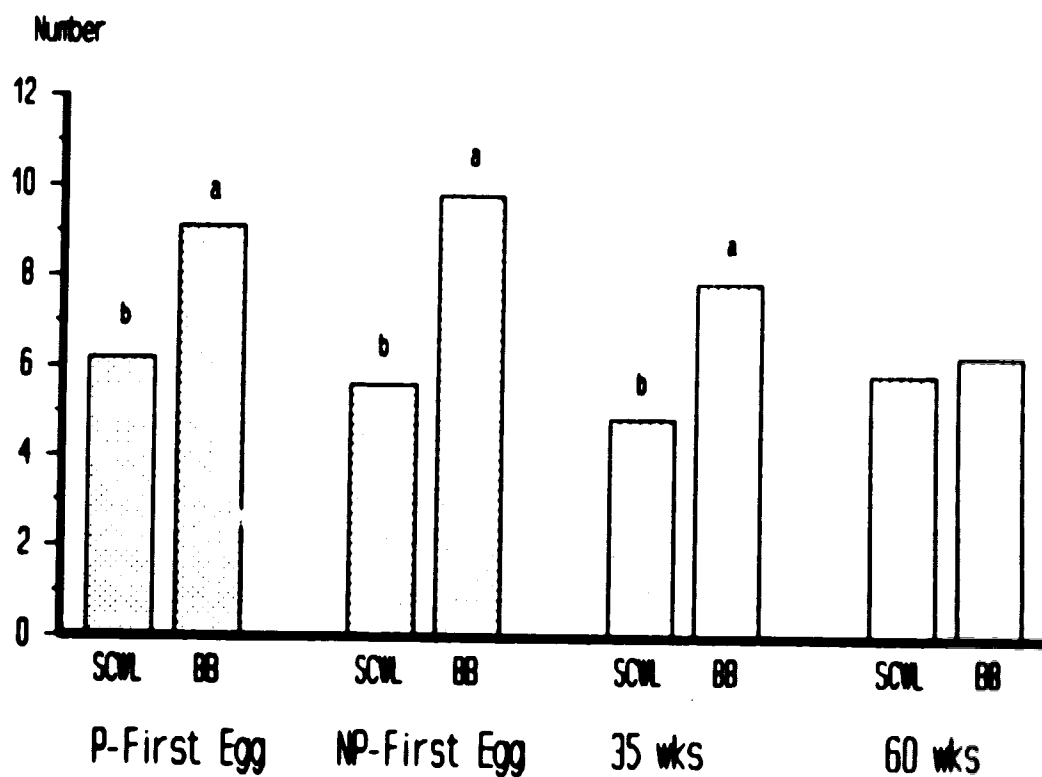


Figure 3.8. The effect of strain on number of large follicles (Number) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

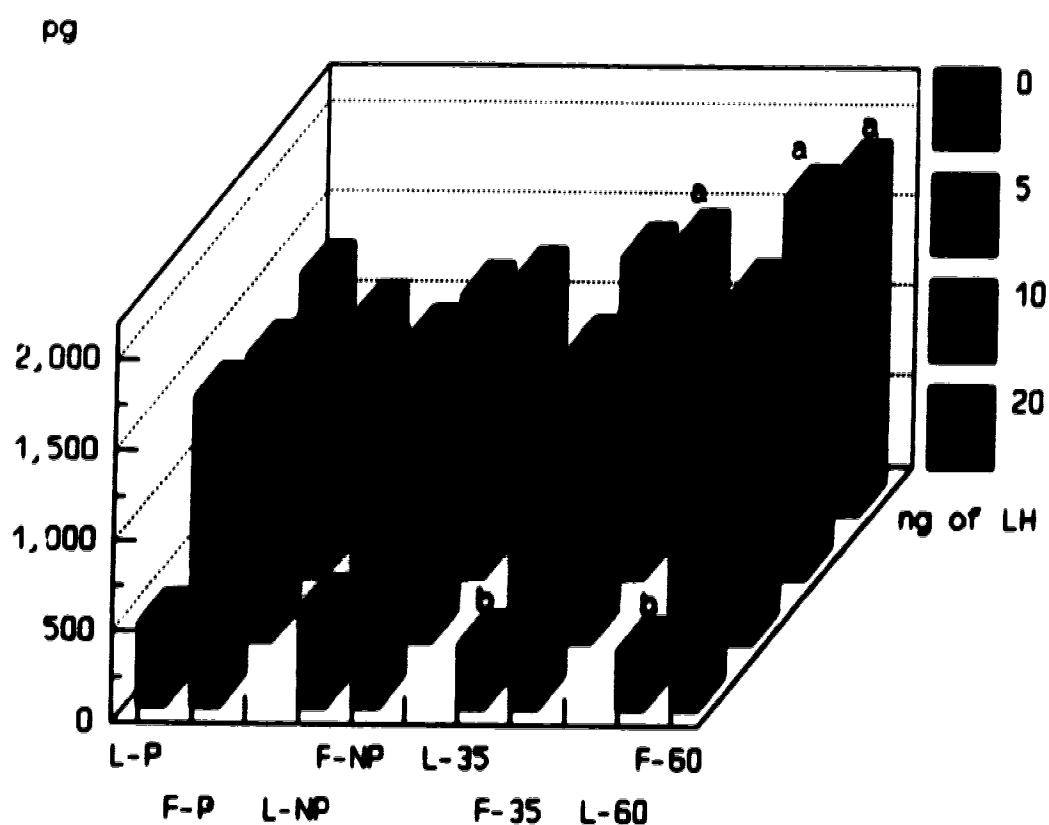


Figure 3.9. The effect of strain on estradiol-17 β output for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

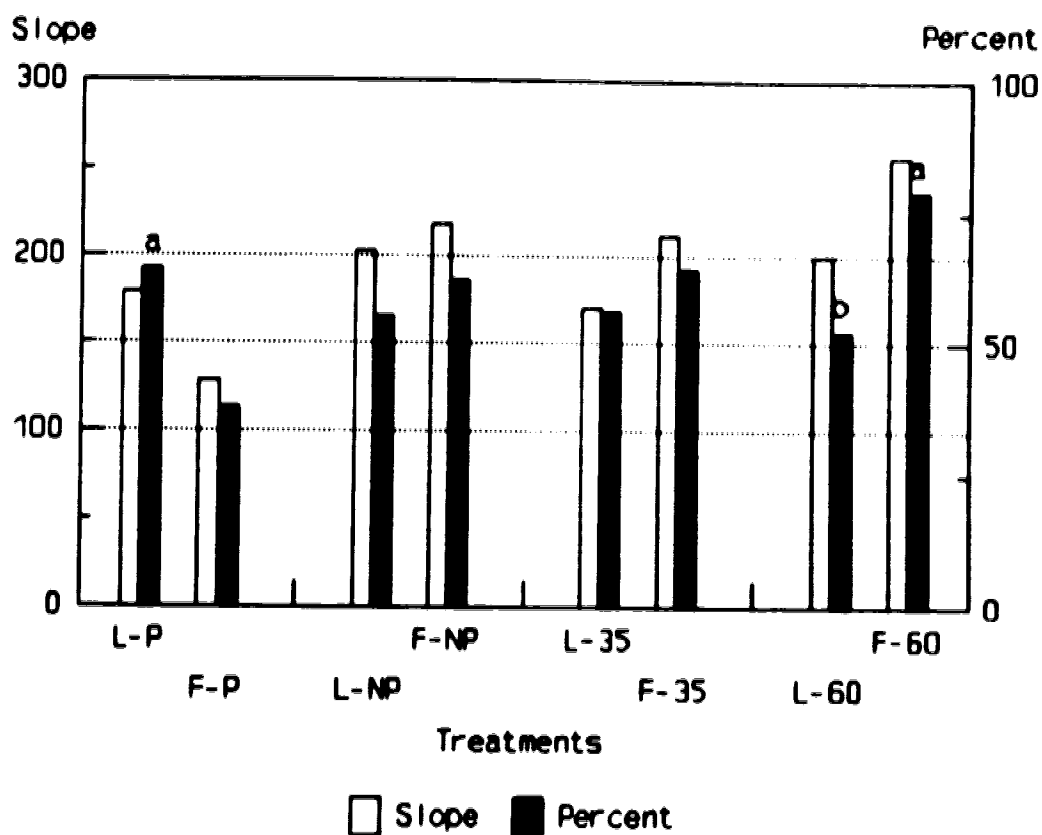


Figure 3.10. The effect of strain on LH sensitivity (Slope) and percent of LH responsive follicles (Percent) for two types of birds: Single Comb White Leghorn (SCWL) and full-fed broiler breeders (BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks), and old hens 60 wks (60wks) of age. Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

3.6 LITERATURE CITED

Bahr, J. M., and S. S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Blair, R., M. M. MacCowan, and W. Bolton, 1976. Effects of food regulation during the growing and laying stages on the productivity of broiler breeders. Br. Poult. Sci. 17:215-223.

Bornstein, S. S. Plavnik, and Y. Lev, 1984. Body weight/or fatness as potential determinants of the onset of egg production in broiler breeder hens. Br. Poult. Sci. 25:323-341.

Brody, T. Y., E. M. Soller, I. Nir, and Z. Nitsan, 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. Br. Poult. Sci. 21:437-446.

Brody, T. B., P. B. Siegel, and J. A. Cherry, 1984. Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. Br. Poult. Sci. 25:245-252.

Chaney, L. W., and H. L. Fuller, 1975. The relationship of obesity to egg production in broiler breeders. Poultry Sci. 54:200-207.

Clayton, G. A., 1972. Effects of selection on reproduction in avian species. J. Reprod. Fertil. Suppl. 15:1-21.

Conrad, R. M., and D. C. Warren, 1940. The production of the double yolked eggs in the fowl. *Poultry Sci.* 19:9-17.

Cunningham, F. J., 1987. Ovulation in the hen: Neuroendocrine control. *Oxford Rev. Reprod. Biol.* 9:96-136.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington, 1987. Ovarian follicular structure of White Leghorn fed *ad libitum* and Dwarf and Normal broiler breeders fed *ad libitum* or restricted to point of lay. *Br. Poult. Sci.* 28:493-506.

Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30:161-174.

Jaap, R. G., and J. A. Clancy, 1968. Reproductive idiosyncrasies of the broiler pullets. Pages 74-79 in : *Proceedings 3rd European Poultry Conference, Vol. II, Jerusalem, Israel.*

Leeson, M. S., and J. D. Summers, 1983. Consequences of increased feed allowance for growing broiler breeder pullets as a means of stimulating early maturity. *Poultry Sci.* 62:6-11.

Mallard, J., and M. Dourais, 1988. Strategies of selection for leanness in meat production. Pages 3-23 in : *Leanness in Domestic Birds. Genetics, Metabolic and Hormonal Aspects.* B. Leclercq and C.C. Whitehead, ed. Butterworths, London, England.

Pym, R. A. E., and J. F. Dillon, 1974. Restricted food intake and reproductive performance of broiler breeder pullets. Br. Poult. Sci. 15:245-259.

Robinson F. E. and R. J. Etches, 1986. Ovarian steroidogenesis during follicular maturation in domestic fowl (*Gallus domesticus*). Biol. of Reprod. 35, 1096-1105.

Robinson, F. E., R. T. Hardin, and A. R. Robblee, 1990. Reproductive senescence in domestic fowl: Effects on egg production, sequence length and inter sequence pause length. Br. Poult. Sci. 31:871-879.

Robinson F. E., N. A. Robinson, and T. A. Scott, 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler hens. Can. J. Anim. Sci. 71:549-556.

Robinson N. A., F. E. Robinson, and R. T. Hardin, 1992. Reproductive senescence in egg-type chickens: Effects on egg production, sequence length and inter-sequence pause length. Poultry Science 81st Annual Meeting Abstracts. Poult. Sci. 71:128.

Siegel, P. B., and E. A. Dunnington, 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in Poultry Genetics and Breeding. W. G. Hill, J. M. Masson, and D. Hewitt, ed. Br. Poult. Sci. Ltd., Longman group, Harlow, England.

Steel, R. G. D., and J. H. Torrie, 1980. Principle and Procedures of Statistics. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Van Middelkoop, J. H., 1972. The relationship between ovulation interval of White Plymouth Rock pullets and the laying of abnormal eggs. *Archiv fur Geflugelkunde*, 36:223-230.

Wells, J. W., M. A. Walker, and J. Culbert, 1983. Effect of luteinizing hormone on progesterone production by the follicular granulosa in the ovarian hierarchy of the domestic fowl (*Gallus domesticus*). *Gen. Comp. Endoc.* 52:265-271.

Williams, J. B., and P. J. Sharp, 1978. Ovarian morphology and rates of ovarian follicular development in laying broiler breeders and commercial egg-producing hens. *Br. Sci.* 19:387-395.

Whitehead, C., and P. Hocking, 1988. Feeding and managing turkeys and broiler breeder hens. Pages 6-7 in : *Science and Poultry Industry*, J. Hardcastle, ed. Agriculture and Food Research Council, London, England.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. *Poultry Sci.* 71:1750-1761.

Yu, M. W., F. E. Robinson, and R. J. Etches, 1992c. Effect of feed allowance during rearing and breeding on female broiler breeders. 3. Ovarian steroidogenesis. *Poultry Sci.* 71:1762-1767.

Yu, M. W., F. E. Robinson, and A. R. Robbles, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. *Poultry Sci.* 71:1739-1749.

4. OVARIAN MORPHOLOGY AND STEROIDOGENESIS IN THE DOMESTIC FOWL: EFFECTS OF PHOTOSTIMULATION PROGRAM.

4.1 Introduction

It is generally assumed that the transition from light to dark is the major environmental clue for the physiological system that entrains the timing of oviposition (Etches, 1990). Light stimulates the release of LH. At about one week of age, there is a peak (about 6 ng/ml) in LH concentration in the chickens (Sharp, 1975). When birds approach sexual maturity they become photosensitive, consequently, the concentrations of LH are higher prior to sexual maturity (first oviposition) than after sexual maturity (Sharp, 1975). A change in the plasma level of LH prior to sexual maturity is accompanied by the appearance of the secondary sexual characteristics such as changing colour of the comb, oviduct development, formation of medullary bone and vitellogenin production by the liver (Johnson, 1986). Ovulation takes place within 6 or 8 hours after the release of the gonadotrophin hormone (LH) produced by the pituitary gland (Bahr and Palmer, 1989; Etches, 1990). The release of LH is related to the time of onset of darkness, and normally occurs between midnight and 8:00 a. m.. Since it takes about 24 hours after ovulation to complete the formation of the egg, the egg is usually laid during the daylight hours (Taylor, 1970).

The first objective of the study was to examine the effect of a photostimulation program (P-14L:10D and NP-8L:16D) on body weight and reproductive organs weight (heart, liver, abdominal fat, oviduct, ovary, stroma, number of large follicles). Carcass composition (protein, fat, moisture, ash) of Single Comb White Leghorns, full-fed and restricted broiler breeders was analyzed. The influence of a lighting program on age at sexual maturity was an important question we tried to answer. The second objective was to examine the effect of lighting program on estradiol-17 β production from small white follicles of Single Comb White Leghorns (SCWL), full-fed and restricted broiler breeders. Also, the determination of LH sensitivity of estradiol-17 β output from follicles

incubated with 0 or 5 ng of bLH was calculated. The percent of follicles which produced > 1.0 ng of estradiol-17 β was analyzed for each treatment.

4.2 Materials and Methods.

4.2.1 Stocks and Management.

Twenty female SCWL (Shaver-288) were randomly selected from a flock of 80 birds. Forty female broiler breeders (Shaver Starbro) were randomly selected from a flock of 120 birds. All birds were individually housed in laying cages at 20 wks of age. The total of 60 birds were assigned to six treatment groups. Each group had 10 replicates (Table 4.1). Egg-type birds (SCWL) were divided into two treatments, based on photostimulation program, and meat-type birds were divided into four treatments, based on photostimulation program and level of feeding. Treatments for SCWL included: photostimulated or non photostimulated at 20 wks of age birds which were sacrificed at sexual maturity (SCWL-P; SCWL-NP). Full-fed broiler breeders were sacrificed at: sexual maturity when photostimulated or non photostimulated (FF-BB-P; FF-BB-NP). Feed-restricted broiler breeders were killed at: sexual maturity when photostimulated or non photostimulated (RR-BB-P; RR-BB-NP respectively). For all photostimulated birds the photoperiod was maintained at 14L:10D. Non photostimulated birds were exposed to 8L:16D.

Full-fed broiler breeders were allowed to eat *ad libitum*, while feed-restricted birds were fed daily to maintain body weight as per the recommendation of breeder (Yu *et al.*, 1992a). Throughout the experiment, all birds had free access to water. All the photostimulated and non photostimulated birds were sacrificed at sexual maturity (first egg). Collection of samples, chemical analyses, radioimmunoassay for estradiol-17 β and determination of LH sensitivity were performed using the same technique which were described in Chapter 2. Studies on estradiol output from small white follicles (< 1 mm) were based on incubation of 2,400 individual follicles (40 follicles x 60 birds).

4.3 Statistical analyses.

Data were statistically analyzed using one-way analyses of variance (Steel and Torrie, 1980). Significance was conducted at 0.05 level. The statements of significance between means were evaluated using Duncan's multiple range test (Steel and Torrie, 1980). Differences among the age classification were determined within each type (Table 4.1) for organ weights, carcass composition and reproductive characteristics. Differences in estradiol-17 β were calculated by using methods which were described in Chapter 2.

4.4 Results

In SCWL, killed at sexual maturity, photostimulation did not influence body weight, organ weight or carcass composition (Table 4.2). The percent of ash was significantly higher in SCWL-P (2.57 g) than in SCWL-NP (2.33 g). Photostimulation advanced age at sexual maturity significantly compared to non photostimulated hens (SCWL-P; 152.5 days; SCWL-NP; 158.7 days). Photostimulated SCWL came into production almost 6 days before non photostimulated SCWL. Photostimulation did not influence oviduct weight, total ovary weight, and number of large follicles (Table 4.3). However, photostimulated birds exhibited higher ovarian stroma weight (SCWL-P; 3.24 g) compared to non photostimulated birds (SCWL-NP; 2.12 g). Also, stroma weight expressed as a percentage of body weight was higher in SCWL-P (0.22%) than in SCWL-NP (0.14%).

The effect of photostimulation program on estradiol-17 β output is presented in table 4.4. Photostimulation did not influence estradiol-17 β production in SCWL-P and SCWL-NP. Also, the percent of follicles which produced over 1.0 ng of estradiol-17 β when exposed to 5 ng and more of LH was not significantly affected by photostimulation program (SCWL-P; 64.1%; SCWL-NP; 55.0%).

Body weight and weight of the heart, abdominal fat pad, total carcass fat, carcass protein, water and ash were not influenced by photostimulation program in full-fed

broiler breeders (Table 4.5). Also, the weight of these components when expressed on a body weight basis were not affected by photostimulation program. Only liver weight was higher in FF-BB-P (93.42 g) than in FF-BB-NP (77.17 g). The same pattern was observed in liver weight expressed as a percentage of body mass. In broiler breeders photostimulation influenced age at sexual maturity significantly. Non photostimulated BB reached sexual maturity 20 days after photostimulated birds (FF-BB-P; 155.5 days; FF-BB-NP; 175.7 days).

No major differences in oviduct, ovary, stroma weight or in number of large follicles were seen in full-fed broiler breeders photostimulated or non photostimulated (Table 4.6).

In full-fed broiler breeders photostimulation did not affect estradiol-17 β production significantly at 0 ng and 5 ng of LH (Table 4.7). However, the follicles from non photostimulated birds which were incubated with 10 ng LH produced almost twice as much estradiol-17 β as photostimulated birds (FF-BB-P; 886.29 pg; FF-BB-NP; 1553.57 pg).

In restricted broiler breeders age at sexual maturity was delayed by 40.1 days, when birds were not photostimulated (Table 4.8). Restricted non photostimulated broiler breeders were significantly heavier (RR-BB-NP; 3,422.9 g) than photostimulated birds (RR-BB-P; 2,718.0 g). Heart weight, liver weight and carcass ash weight were not affected by photostimulation program. Abdominal fat pad, total carcass fat, protein and moisture weight were higher in RR-BB- NP than in RR-BB-P. Heart weight and carcass water, expressed as a percentage of body weight, were not affected by photostimulation program. However, liver weight, carcass protein and ash weight when expressed on a body weight basis were influenced by photostimulation and were higher in restricted photostimulated birds (RR-BB-P).

Weights of the reproductive organs are presented in table 4.9. Oviduct weight, stroma weight and the number of large follicles were not influenced by photostimulation program.

In restricted broiler breeders photostimulation did not influence estradiol-17 β production (Table 4.10). On average, 61 % follicles produced over 1.0 ng of estradiol-17 β in RR-BB-P and 51 % in RR-BB-NP when exposed to 5 ng and more of LH. However, these differences were not significant at 0.05 level.

4.5 Discussion

In all strains, photostimulated birds came into production significantly earlier than non photostimulated BB birds (Figure 4.1; Table 4.11). In SCWL the difference in age at sexual maturity between P and NP birds was only 6 days (Table 4.1). Full-fed photostimulated BB reached sexual maturity 20 days earlier than non photostimulated bird (Table 4.5). Despite coming into production sooner, full-fed broiler breeders lay fewer eggs than do restricted hens (Robbins *et al.*, 1986; Yu *et al.*, 1992 a,b). Full-fed and restricted hens lay eggs in a similar number of sequences, however the prime sequence is shorter in full-fed hens (Robinson *et al.*, 1991). Full-fed broiler hens do not adhere to the models of the ovulatory cycle as strictly as egg-type hens (Eiches, 1990). The delay in sexual maturity in restricted birds was even higher. RR-BB-NP started egg production almost 40 days after RR-BB-P (Table 4.8). The early maturation of full-fed birds is in agreement with previous observation of Robbins *et al.* (1986). From the results, it seems that photostimulation was not required for the initiation of growth of the oviduct or the ovary in full-fed birds. For the onset of egg production, the bird also requires beside light the necessary conditions of body weight (Brody *et al.*, 1980; Dunnington *et al.*, 1983), body fat (Brody *et al.*, 1984) and age (Brody *et al.*, 1980, 1984). There is a considerable variability in modern broiler stocks in the delay of the onset of lay due to feed restriction. Delays have been reported to be 2.5 wks (Yu *et al.*, 1992b), 6.5 wks (O'Sullivan *et al.*, 1991), or 8.1 wks (Katanbaf *et al.*, 1989). It is likely that some of the differences in onset of lay are due to level of restriction or variations in lighting regimens. Katanbaf *et al.* (1989) observed that broiler breeders that are full-fed are dependent upon reaching a critical age to start laying, whereas feed-

restricted hens that commence lay later, are dependent upon attaining critical BW and carcass fat stores. The observation that restricted feeding delays sexual maturity is also in agreement with Pym and Dillon (1974). The comparison of SCWL-NP and RR-BB-NP indicates that restriction in broiler breeders delayed sexual maturity almost 48 days.

Body weight was not affected by photostimulation program in SCWL (Table 4.2) and in full-fed BB (Table 4.5). Even though, FF-BB-NP were 20 days older than FF-BB-P, the difference in body weight between these two groups was not significant (Table 4.5; Figure 4.2). During these 20 days they gained only 24 g. This could indicate that limits to rapid growth were reached by the time of sexual maturity in these birds. RR-BB-NP were significantly heavier than RR-B-P (Table 4.8) possible because during 40 days they gained over 700 g due to continual increases in feed allocation at that time.

The effect of photostimulation program on abdominal fat pad weight and total carcass fat was similar in analyzed groups. No differences in these parameters were observed between P and NP in SCWL and FF-BB (Figure 4.3 and 4.4). Percent of abdominal fat pad and total carcass fat on BW basis were also not significantly different in these treatments. However, in restricted BB abdominal fat pad weight and total body fat expressed as a percentage of BW were significantly higher in NP than in P (Table 4.8; Figure 4.4). This phenomena could be related to the age of the birds in these groups. RR-BB-NP when sacrificed, were 40 days older than RR-BB-P and their body weight was 700 g heavier than RR-BB-P. Also, the amount of fat which is positively correlated with BW were significantly higher in RR-BB-NP birds. One of the interesting effects of photostimulation was a percent carcass ash content. In SCWL and RR-BB, birds that were photostimulated came into production with increase carcass ash content. This difference was also seen in FF-BB on a numeric basis. This may mean that at photostimulation more calcium is deposited in medullary bones than when photostimulation does not occur. Further, photostimulation would appear to increase liver weight. Despite NP birds being older than P birds at sexual maturity, the P birds had significantly higher liver weight in FF-BB and RR-BB than NP birds. This

difference was not significant in SCWL.

No significant differences between photostimulated and non photostimulated hens at sexual maturity were observed in the oviduct weight (Figure 4.5), total ovary weight (Figure 4.6) and number of large follicles (Figure 4.8). The average number of large follicles in SCWL-P was 6.2 (Table 4.3), in FF-BB-P was 9.11 and 7.4 in RR-BB-P. According to Yu *et al.* (1992b), body weight was the most important variable in determining the number of large follicles in the ovary of female broiler breeders. FF-BB-P were much heavier at sexual maturity (4,446.6 g) than RR-BB-P (2,718.0 g), therefore, their number of large follicles was much higher than in RR-BB-P. Hocking *et al.* (1989) reported that the number of large follicles in the ovary at sexual maturity appeared to be positively correlated with incidence of multiple hierarchies and reduced egg production. Photostimulation program only significantly influenced ovarian stroma weight in SCWL. Photostimulated SCWL had significantly higher stroma weight (3.24 g) than SCWL-NP (2.12 g) (Table 4.3). A larger stroma may mean higher estradiol ovarian output since small follicles are the major source of that hormone in hen's ovary (Robinson and Etches, 1986).

The effects of photostimulation on estradiol-17 β production are presented in tables 4.4, 4.7, 4.10 and in figure 4.9. These data substantiate the results which were summarized in chapter II and III. All photostimulated and non photostimulated SWF were capable of producing estradiol-17 β . This observation is also supported by Senior and Furr (1974), Armstrong (1984, 1985), Robinson and Etches (1986) and Yu *et al.* (1992c). However, the results presented in tables 4.4; 4.7 and 4.10 did not demonstrate a significant changes in estradiol-17 β production between P and NP hens. Furthermore, hormone output was stimulated by LH and the plateau was reached with 10 and 20 ng of LH. Estradiol-17 β production by using 5 ng of LH was over 3 times higher than in control group (0 ng of LH) in analyzed treatments. LH sensitivity (Slope) did not vary significantly across the treatments (Table 4.4, 4.7, 4.10 and Figure 4.10). The percentage of LH responsive follicles (Percent) was not affected by photostimulation

program in SCWL or RR-BB (Figure 4.10). An average of 60% follicles from SCWL and 57% follicles from RR-BB produced over 1.0 ng of estradiol-17 β . On the other hand, even though FF-BB did not vary in LH sensitivity (Slope) their percentage of LH responsive follicles (Percent) was influenced by photostimulation program (Figure 4.10). Only 37.7% follicles from FF-BB-P were steroidogenic active. Presumably, incidence of atresia was more common in these follicles. Follicles from FF-BB may be did not respond well to the type of photostimulation program we used. Perhaps, instead of a very rapid-single-step program, a more gradual increase in daylight would have been beneficial in preventing high rate of atresia. When birds took 20 days to come into egg production, they had lower rate of atresia with overall difference in ovary morphology trait. These data support current projects with turkeys that favour more gradual increase in day length at photostimulation compared to a rapid single step program (Robinson, unpublished).

TABLE 4.1. Experimental design - effect of photostimulation program. Treatment groups according to age classification by type.

Age classification (Aging)	Type		
	SCWL ^a	FF-BB ^b	RR-BB ^c
Immature hens-19wk ³			
First egg - P ²	SCWL-P ¹	FF-BB-P ¹	RR-BB-P ¹
First egg - NP ³	SCWL-NP ¹	FF-BB-NP ¹	RR-BB-NP ¹
Young hens-35wk ²			
Old hens-60wk ²			

¹ These hens were sacrificed at sexual maturity.

² Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

³ Birds were non photostimulated and controlled by 8L:16D.

^a Egg-type birds; Single Comb White Leghorns.

^b Meat-type birds; Full-fed broiler breeders.

^c Meat-type birds; Restricted broiler breeders.

TABLE 4.2. Effect of photostimulation program on age, BW, selected organ weights and carcass composition at sexual maturity in full-fed photostimulated (SCWL-P) and non photostimulated SCWL (SCWL-NP).

Variables	Photostimulation program		SEM
	SCWL-P	SCWL-NP	
Number of hens	10	10	
Age of bird	152.5 ^a	158.7 ^a	1.84
Average BW, g	1,492.7	1,502.9	62.72
Heart			
%BW	0.41	0.40	0.02
g	6.16	6.03	0.31
Liver			
%BW	2.06	1.97	0.05
g	30.64	29.58	1.36
Abdominal fat			
%BW	2.17	2.45	0.30
g	33.67	37.18	5.44
Carcass fat			
%BW	8.21	8.15	0.80
g	119.75	118.37	41.85
Carcass protein			
%BW	15.11	14.23	0.29
g	214.99	204.88	16.28
Carcass H ₂ O			
%BW	74.34	75.26	0.88
g	1055.88	1080.76	52.54
Carcass ash			
%BW	2.57 ^a	2.33 ^b	0.07
g	36.81	33.51	3.79
Total percent	100.24	99.97	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 4.3. Effect of photostimulation program on reproductive tract characteristics at sexual maturity in full-fed photostimulated (SCWL-P) and non photostimulated SCWL (SCWL-NP).

Variables	Photostimulation program		SEM
	SCWL-P	SCWL-NP	
Number of hens	10	10	
Oviduct			
%BW	3.25	3.20	0.12
g	48.69	48.07	2.80
Ovary			
%BW	2.03	2.25	0.20
g	30.77	33.59	3.44
Stroma			
%BW	0.22 ^a	0.14 ^b	0.02
g	3.24 ^a	2.12 ^b	0.35
Number of large follicles	6.20	5.60	0.70

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 4.4. Effect of photostimulation program on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed photostimulated SCWL (SCWL-P) and non photostimulated SCWL (SCWL-NP).

Variables	Photostimulation program		SEM
	SCWL-P	SCWL-NP	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	454.57	535.74	75.38
5 ng LH	1348.94	1549.28	220.80
10 ng LH	1216.31	1316.75	190.02
20 ng LH	1306.06	1123.32	187.91
Slope²	178.87	202.71	32.59
Percent (%)³	64.07	55.00	8.40

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

TABLE 4.5. Effect of photostimulation program on age, BW, selected organ weights and carcass composition at sexual maturity in full-fed photostimulated broiler breeders (BB) (FF-BB-P) and non photostimulated BB (FF-BB-NP).

Variables	Photostimulation program		SEM
	FF-BB-P	FF-BB-NP	
Number of hens	10	10	
Age of bird	155.5 ^a	175.7 ^a	4.61
Average BW, g	4,446.6	4,422.4	108.06
Heart			
%BW	0.38	0.34	0.03
g	16.63	14.83	0.95
Liver			
%BW	2.11 ^a	1.74 ^b	0.10
g	93.42 ^a	77.17 ^b	4.16
Abdominal fat			
%BW	6.21	6.64	0.32
g	276.36	294.38	15.33
Carcass fat			
%BW	24.89	26.60	0.80
g	1102.69	1186.33	41.84
Carcass protein			
%BW	15.03	14.39	0.30
g	666.53	638.54	16.28
Carcass H ₂ O			
%BW	57.46	56.90	0.88
g	2552.64	2521.36	52.55
Carcass ash			
%BW	2.70	2.53	0.07
g	119.35	112.60	7.86
Total percent	100.08	100.43	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 4.6. Effect of photostimulation program on reproductive tract characteristics at sexual maturity in full-fed photostimulated broiler breeders (BB) (FF-BB-P) and non photostimulated BB (FF-BB-NP).

Variables	Photostimulation program		SEM
	FF-BB-P	FF-BB-NP	
Number of hens	10	10	
Oviduct			
%BW	1.39	1.39	0.08
g	61.50	61.24	3.03
Ovary			
%BW	1.68	1.79	0.15
g	74.81	79.77	6.65
Stroma			
%BW	0.22	0.23	0.02
g	9.46	9.78	1.15
Number of large follicles	9.11	9.80	0.54

TABLE 4.7. Effect of photostimulation program on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed photostimulated broiler breeders (BB) (FF-BB-P) and non photostimulated BB (FF-BB-NP).

Variables	Photostimulation program		SEM
	FF-BB-P	FF-BB-NP	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	377.40	420.30	69.84
5 ng LH	1021.95	1517.27	208.95
10 ng LH	886.29 ^a	1553.57 ^a	166.89
20 ng LH	1074.69	1279.21	209.76
Slope ²	128.91	219.39	34.42
Percent (%) ³	37.67 ^a	61.66 ^a	8.78

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{a,b} Means within a row with different superscripts are significantly different (P ≤ .05).

TABLE 4.8. Effect of photostimulation program on age, BW, selected organ weights and carcass composition at sexual maturity in restricted photostimulated broiler breeders (BB) (RR-BB-P) and non photostimulated BB (RR-BB-NP).

Variables	Photostimulation program		SEM
	RR-BB-P	RR-BB-NP	
Number of hens	10	10	
Age of bird	166.6 ^b	206.5 ^a	4.61
Average BW, g	2,718.0 ^b	3,422.9 ^a	108.06
Heart			
%BW	0.41	0.37	0.03
g	11.16	12.64	0.90
Liver			
%BW	2.07 ^a	1.57 ^b	0.10
g	55.93	53.61	4.68
Abdominal fat			
%BW	2.25 ^b	3.72 ^a	0.32
g	61.38 ^b	127.10 ^a	14.93
Carcass fat			
%BW	12.92 ^b	16.42 ^a	0.80
g	341.63 ^b	557.37 ^a	41.85
Carcass protein			
%BW	18.20 ^b	16.23 ^b	0.30
g	480.86 ^b	549.67 ^a	16.28
Carcass H ₂ O			
%BW	64.99	64.85	0.88
g	1707.92 ^b	2196.06 ^a	52.55
Carcass ash			
%BW	3.48 ^a	2.65 ^b	0.07
g	91.74	89.82	3.79
Total percent	99.59	100.15	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 4.9. Effect of photostimulation program on reproductive tract characteristics at sexual maturity in restricted photostimulated broiler breeders (BB) (RR-BB-P) and non photostimulated BB (RR-BB-NP).

Variables	Photostimulation program		SEM
	RR-BB-P	RR-BB-NP	
Number of hens	10	10	
Oviduct			
%BW	2.02	1.86	0.08
g	54.64	63.73	3.00
Ovary			
%BW	1.74	1.76	0.15
g	47.43	60.15	6.64
Stroma			
%BW	0.23	0.26	0.02
g	6.37	8.94	1.07
Number of large follicles	7.40	6.60	0.52

TABLE 4.10. Effect of photostimulation program on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from restricted photostimulated broiler breeders (BB) (RR-BB-P) and non photostimulated BB (RR-BB-NP).

Variables	Photostimulation program		SEM
	RR-BB-P	RR-BB-NP	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	359.96	546.44	81.56
5 ng LH	1637.04	1514.94	182.03
10 ng LH	1600.23	1113.10	217.89
20 ng LH	1072.26	870.23	155.76
Slope ²	255.42	193.70	31.63
Percent (%) ³	61.66	51.00	8.04

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

TABLE 4.11. Effect of photostimulation program on age (days) at sexual maturity.

Age classification (Aging)	Type		
	SCWL	FF-BB	RR-BB
First egg - P¹	152.5^b	155.5^b	166.6^b
First egg - NP²	158.7^a	175.7^a	206.5^a

¹ Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

² Birds were non photostimulated and controlled by 8L:16D.

^{a,b} Means within a column with different superscripts are significantly different ($P \leq 0.05$).

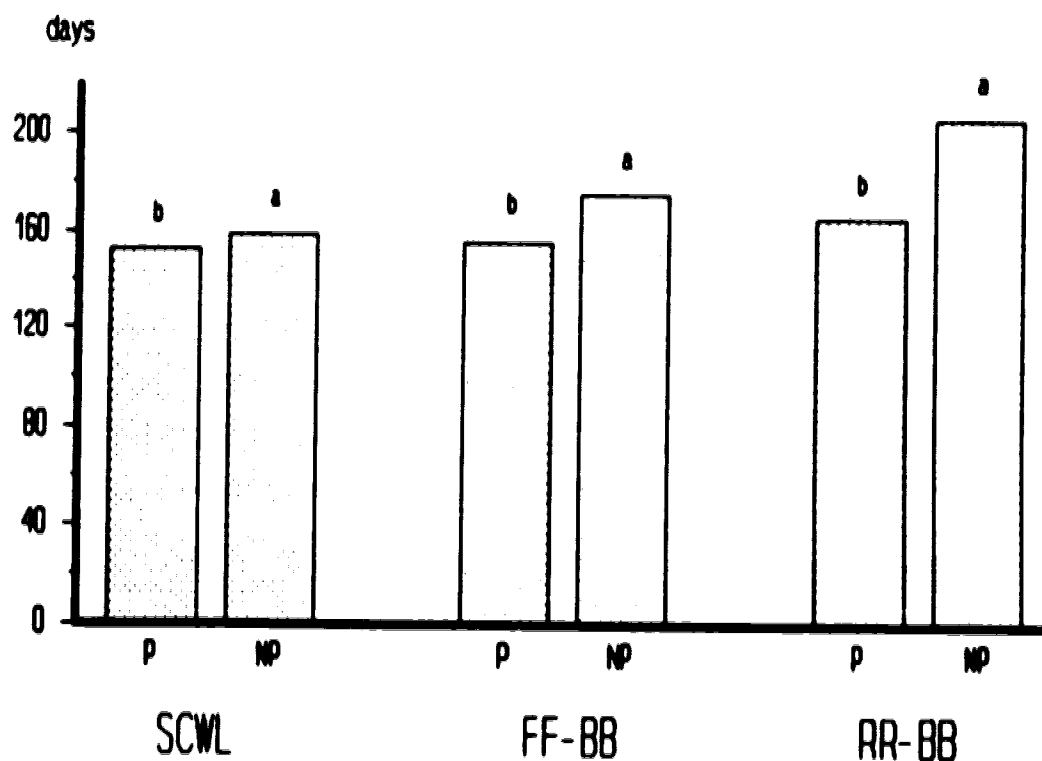


Figure 4.1. The effect of photostimulation program on age at sexual maturity (days) for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

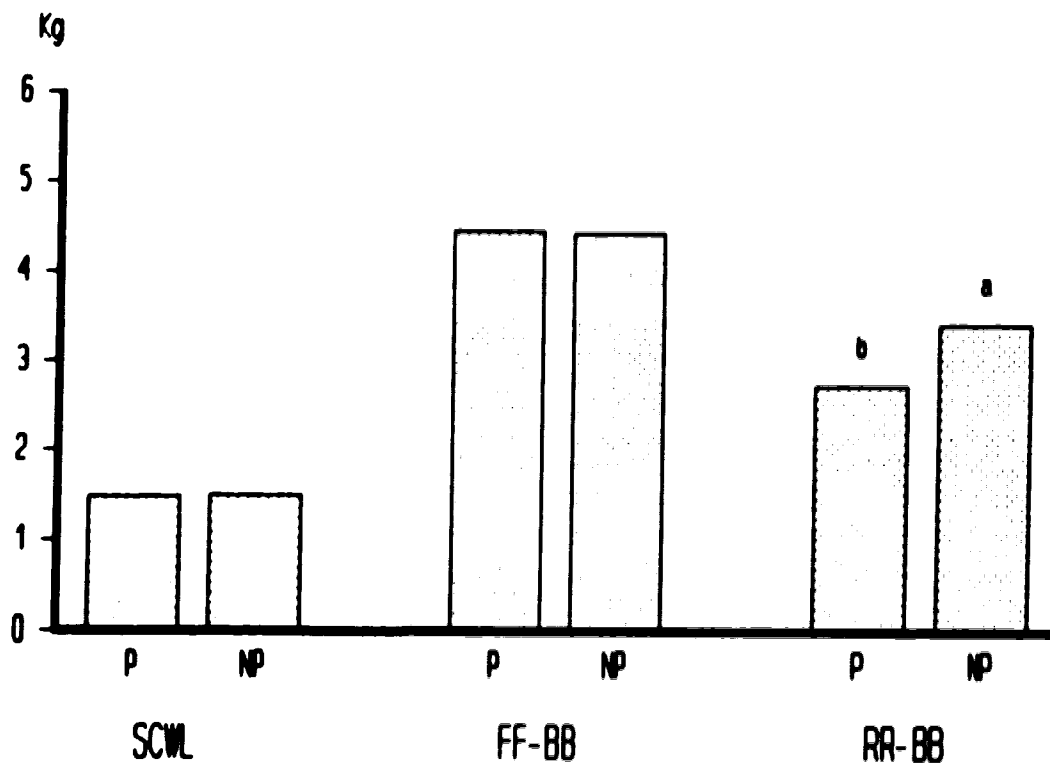


Figure 4.2. The effect of photostimulation program on body weight (kg) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

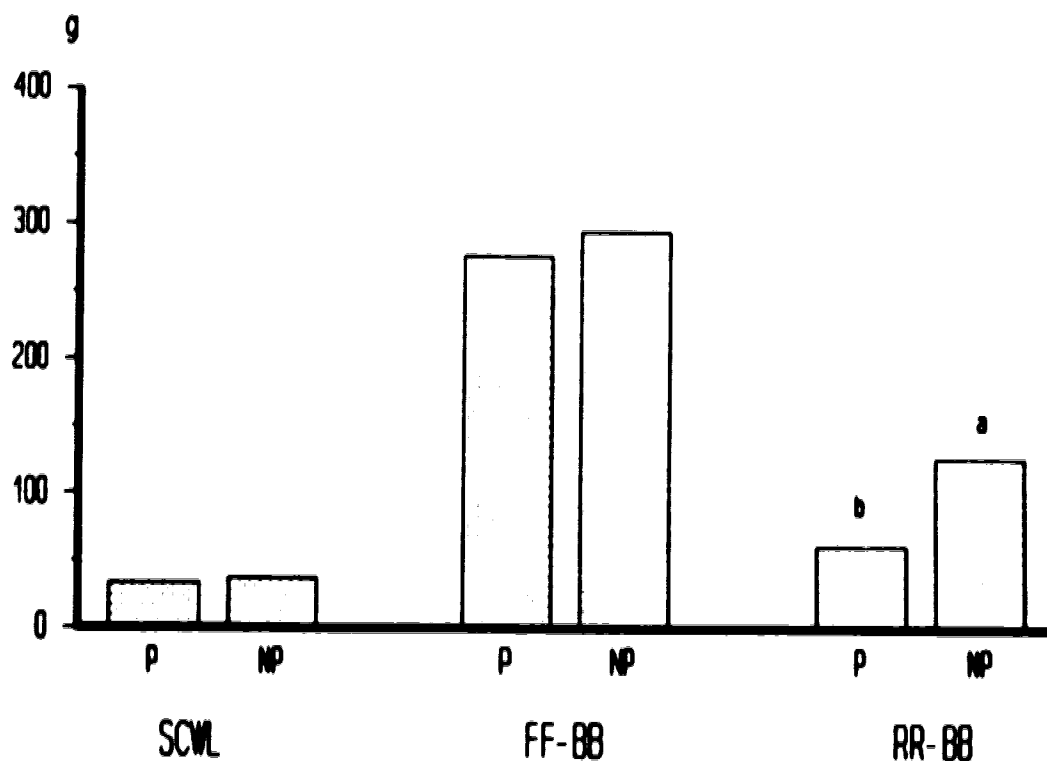


Figure 4.3. The effect of photostimulation program on abdominal fat pad weight (g) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

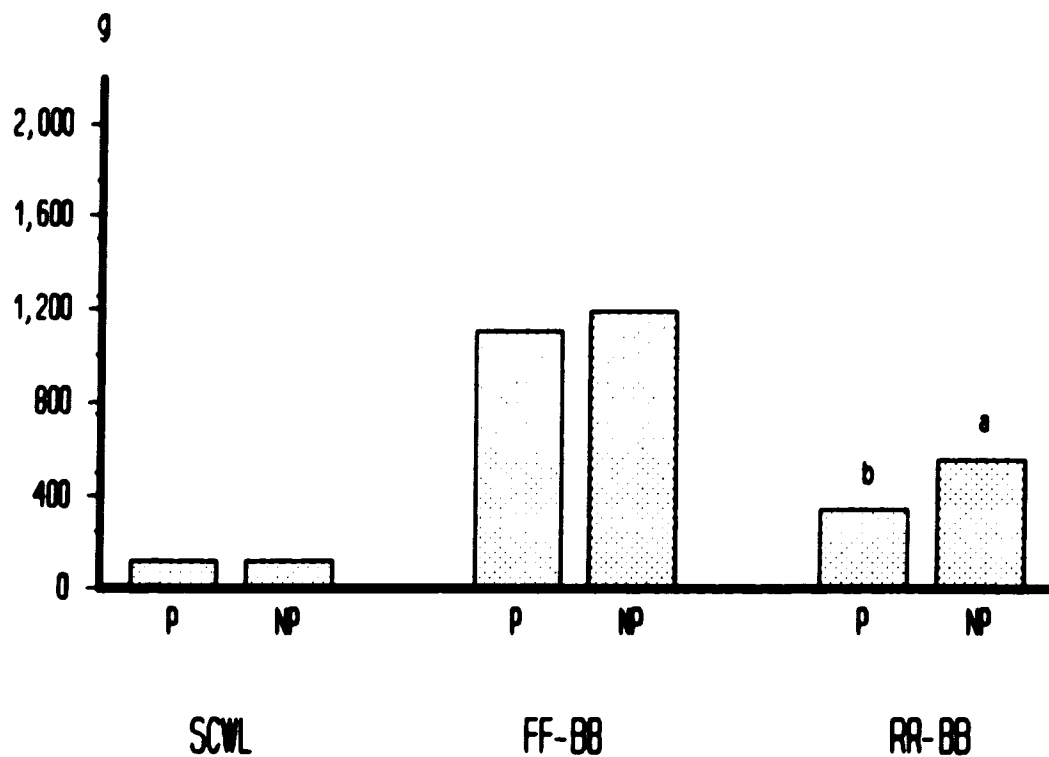


Figure 4.4. The effect of photostimulation program on total carcass fat weight (g) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

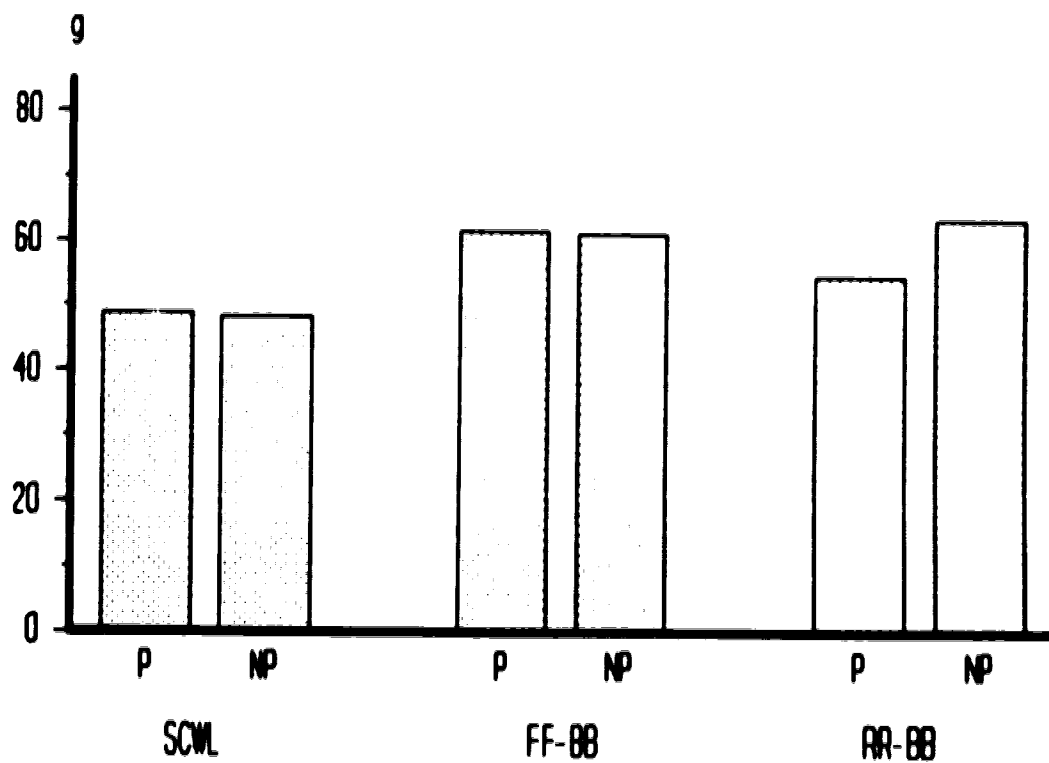


Figure 4.5. The effect of photostimulation program on oviduct weight (g) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP).

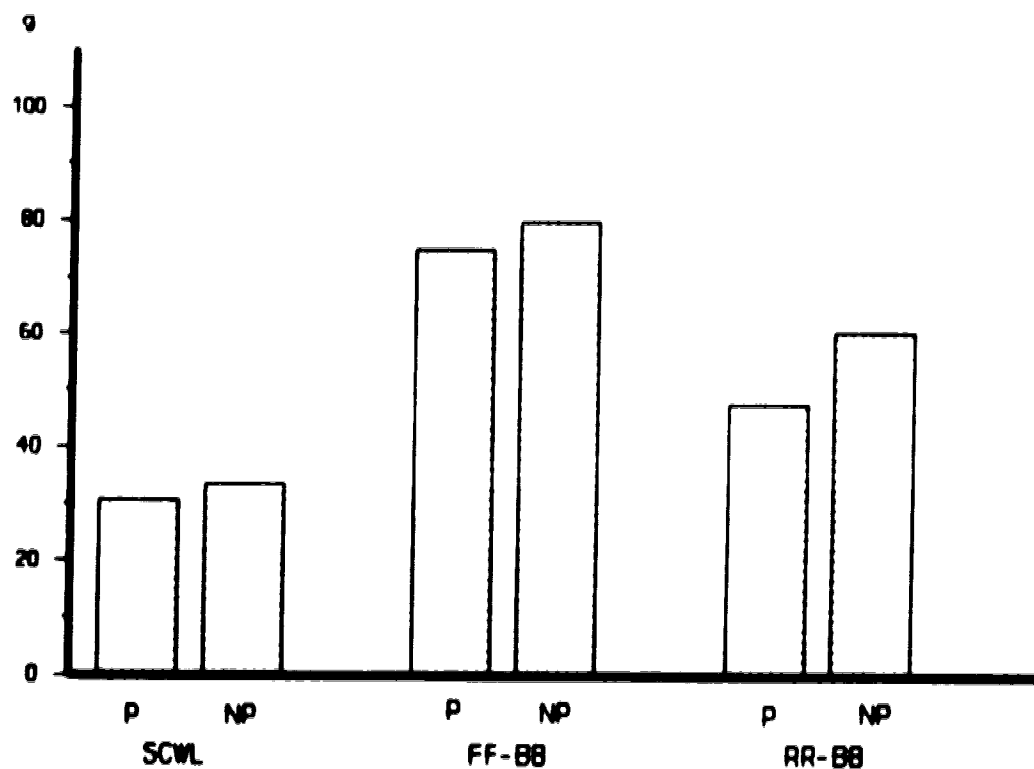


Figure 4.6. The effect of photostimulation program on total ovary weight at sexual maturity (g) for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wk) and old hens 60 wks (60wk).

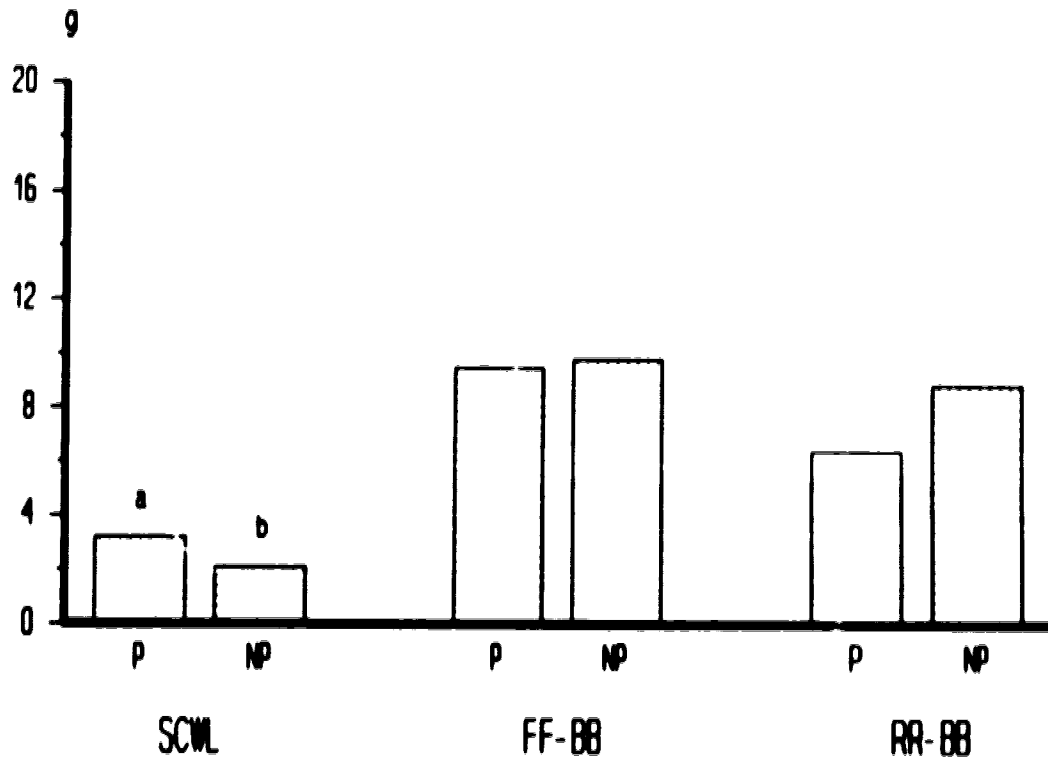


Figure 4.7. The effect of photostimulation program on ovarian stroma weight (g) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

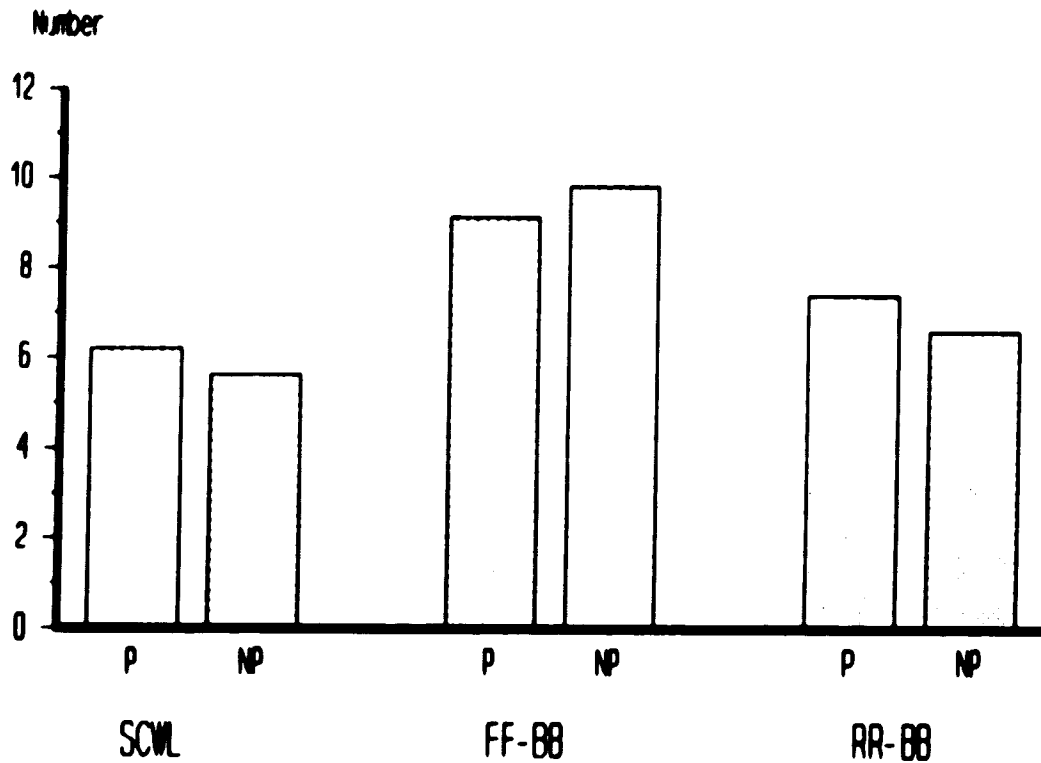


Figure 4.8. The effect of photostimulation program on number of large follicles (Number) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

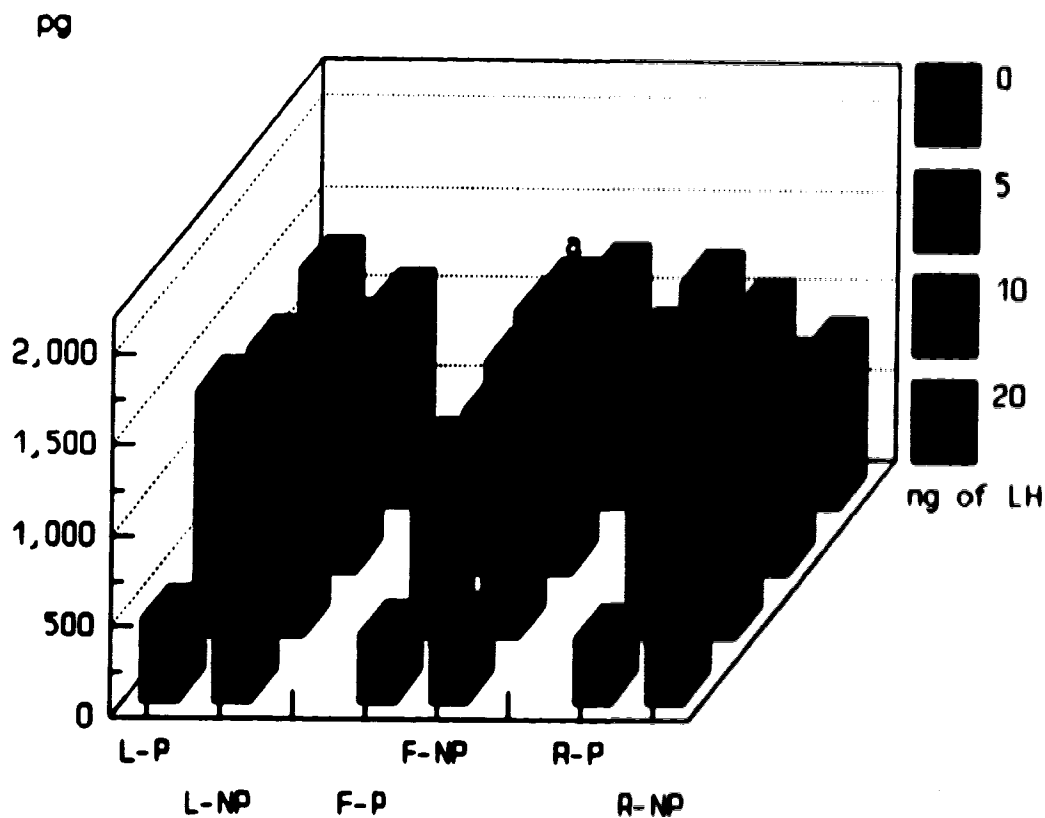


Figure 4.9. The effect of photostimulation program on estradiol-17 β output at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photo stimulated (P) and non photo stimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

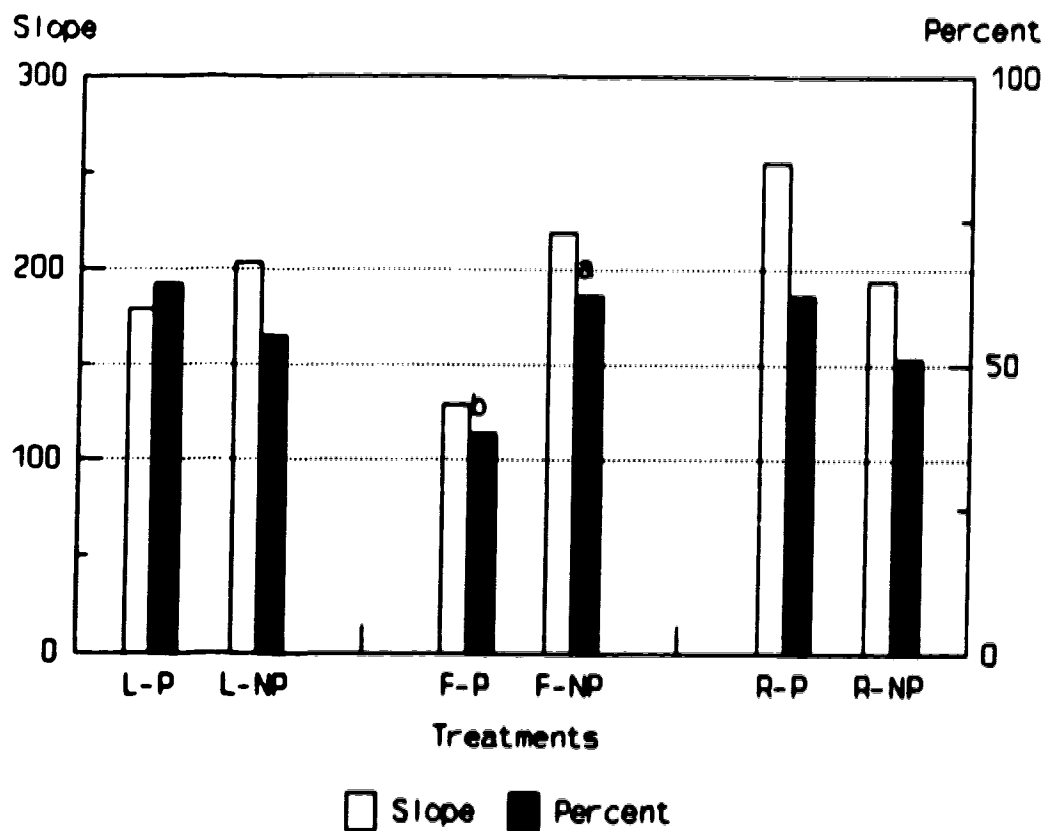


Figure 4.10. The effect of photostimulation program on LH sensitivity (Slope) and percent of LH responsive follicles (Percent) at sexual maturity for three types of birds: Single Comb White (SCWL), full-fed (FF-BB) and feed-restricted broiler breeders (RR-BB). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

4.6 LITERATURE CITED

Armstrong, D. G., 1984. Ovarian aromatase activity in the domestic fowl (*Gallus domesticus*). J. of Endocrinol. 100: 81-86.

Armstrong D. G., 1985. Changes in aromatase activity in small ovarian follicles of the domestic fowl (*Gallus domesticus*) during growth and atresia. J. Endocrinol. 105:297-301.

Bahr, J. M., and S. S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Brody, T. Y., E. M. Soller, I. Nir, and Z. Nitsan, 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. Br. Poult. Sci. 21:437-446.

Brody, T. B., P. B. Siegel, and J. A. Cherry, 1984. Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. Br. Poult. Sci. 25:245-252.

Dunnington, E. A., P. B. Siegel, J. A. Cherry, and M. Soller, 1983. Relationship of age and body weight at sexual maturity in selected lines of chickens. Arch. Geflugelkd. 47:85-89.

Etches, R. J., 1990. The ovulatory cycle of the hen. Crit. Rev. Poult. Biol. 2:293-318.

- Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30:161-174.
- Johnson, A. L., 1986. Reproduction in the female. Pages 403-431 in *Avian Physiology*, 4 th ed. P.D. Sturkie, ed. Springer-Verlag, New York, NY.
- Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel, 1989. Restricted feeding in early and late-feathering chickens. 2. Reproductive responses. *Poultry Sci.* 68:352-358.
- O'Sullivan, N. P., E. A. Dunnington, E. J. Smith, W. B. Gross , and B. P. Siegel, 1991. Performance of early and late feathering broiler breeder females with different feeding regimens. *Br. Poult. Sci.* 32:981-995.
- Pym, R. A. E., and J. F. Dillon, 1974. Restricted food intake and reproductive performance of broiler breeder pullets. *Br. Poult. Sci.* 15:245-259.
- Robbins, K. R., G. C. McGhee, P. Osei, and R. E. Beauchene, 1986. Effect of feed restriction on growth, body composition, and egg production during the breeding season. *Poultry Sci.* 65:1052-1057.
- Robinson F. E. and R. J. Etches, 1986. Ovarian steroidogenesis during follicular maturation in domestic fowl (*Gallus domesticus*). *Biol. of Reprod.* 35:1096-1105.
- Robinson F. E., N. A. Robinson, and T. A. Scott, 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler hens. *Can. J. Anim. Sci.* 71:549-556.

Senior, B. E., and J. A. Furr, 1975. A preliminary assessment of the source of oestrogen within the ovary of the domestic fowl. *J. Reprod. Fertil.* 43:241-247.

Sharp, P. J., 1975. A comparison of variations in plasma luteinizing hormone concentrations in the female domestic chickens from hatch to sexual maturity. *J. Endocrinol.* 67:211.

Steel, R. G. D., and J. H. Torrie, 1980. *Principle and Procedures of Statistics.* 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Taylor T. G., 1970. How an egg shell is made. *Scientific American*, March, Vol. 222, No 3.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. *Poultry Sci.* 71:1750-1761.

Yu, M. W., F. E. Robinson, and R. J. Etches, 1992c. Effect of feed allowance during rearing and breeding on female broiler breeders. 3. Ovarian steroidogenesis. *Poultry Sci.* 71:1762-1767.

Yu, M. W., F. E. Robinson, and A. R. Robblee, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. *Poultry Sci.* 71:1739-1749.

5. OVARIAN MORPHOLOGY AND STEROIDOGENESIS IN THE DOMESTIC FOWL: EFFECTS OF LEVEL OF FEEDING.

5.1 Introduction

Chick production is seriously reduced when hens are overweight. If broiler breeders are given the opportunity to consume as much feed as they like, the rate of egg production will be very low (Siegel and Dunnington, 1985) even though *ad-libitum* birds have significantly heavier ovaries than do feed-restricted birds (Yu *et al.*, 1992b). The weight of the ovary in a mature bird is a measure of the number of yellow follicles and stroma. Research has been reported that the most obvious sign of reproductive disfunction in *ad libitum* feeding was the dramatic increase in the number of large ovarian follicles (Hocking *et al.*, 1987, 1989; Yu *et al.*, 1992b; Robinson *et al.*, 1993). When broiler breeders are over-fed they may have up to 15 large follicles in the ovary, often seen as a double hierarchy. These hens lose control of the recruitment of follicles into hierarchy. It is clear that having more follicles in the ovary does not improve reproductive performance because the oviduct of the hen can only process one egg per day with a normal shell. Also, increased weight of the stroma of full-fed hens reflects increased rates of recruitment of non-hierarchical small white follicles (SWF) into large white (LWF) and small yellow follicles (SYF) following the classification of Robinson and Etches (1986). Restricted feeding in broiler breeders decreases the incidence of double hierarchies and increases egg production (Whitehead and Hocking, 1988; Robinson *et al.*, 1991; Yu *et al.*, 1992b). Also, the oviduct of full-fed birds has been found to be significantly heavier than the oviduct of restricted birds (Yu *et al.*, 1992b). The condition known as "erratic oviposition and defective egg syndrome" (BODES) (van Middelkoop, 1972) is a common condition observed in full-fed broiler breeders. This term describes several reproductive problems including follicular atresia, internal ovulation, internal laying, the production of soft shelled eggs, multiple hierarchies of large follicles and consequently multiple yolk eggs (Hocking *et al.*, 1987; Yu *et al.*,

1992b). In addition, the incidence of erratic laying (night time) was higher in full-fed hens than in restricted hens (Clayton, 1972; Yu *et al.*, 1992b). Erratic laying was significantly correlated with the laying of soft-shelled and membranous eggs and multiple-yolked eggs. "Erratic" laying is probably one of the easiest detectable indicators of a malfunctioning ovary. It would seem that the severity of EODES in commercial broiler breeder hens can be reduced with feed-restriction. In feed-restricted birds severity of restriction, during especially the rearing period increases the delay in sexual maturity (Pym and Dillon, 1974; Robbins *et al.*, 1986; Ingram and Wilson, 1987; and Yu *et al.*, 1992b). Consequently, restricted hens became sexually mature later than full-fed birds.

The source of estrogens is the ovary, in particular, the theca cells of the small follicles (Robinson and Etches, 1986). The synthesis of estrogens by theca cells depends on the maturation of the hypothalamic-adenohypophyseal-ovarian axis. Hypothalamus produces luteinizing hormone releasing hormone (LHRH) which acts on adenohypophysis, influence it to release LH into the general circulation. Rising levels of LH in the circulation stimulates the production of androgens and estrogens. Feed restriction delays the maturation of hypothalamic-adenohypophyseal axis, therefore postponing release of LH and the production of androgens and estrogens from small follicles. Consequently, feed restriction delays sexual maturity.

According to Robinson *et al.* (1991) and Yu *et al.* (1992a) *ad libitum* fed hens produced significantly higher amounts of fat and protein than did the feed restricted hens. *Ad libitum* feeding has also an effect on the incidence of fat metabolism-related mortality (Robinson *et al.*, 1991; Yu *et al.*, 1992a). Ruptured uterus, and hemorrhage of the cloaca as well as distal oviduct are some examples of fat related mortality. Consequently, full-fed hens have higher mortality than feed restricted hens (Robblee *et al.*, 1979).

The first objective of the research presented in this section was to compare body organ weights (heart, liver, abdominal fat pad, total carcass fat, oviduct, ovary, ovarian stroma) as well as a number of large follicles and body composition data (carcass fat,

protein, water and ash) from full-fed and restricted broiler breeders. The second objective was to examine the steroidogenic capabilities (estradiol-17 β output) from small white follicles of female broiler breeders under two different feeding regimens.

5.2 Materials and Methods

5.2.1 Stocks and Management

Eighty female broiler breeders (Shaver-288), randomly selected from a flock of 120 birds were individually housed in laying cages at 20 wks of age (Table 5.1). Each treatment had 10 replicates except full-fed broiler breeders at 60 wks of age (FF-BB-60). One bird from this group died because of fatty liver hemhoragic syndrome. The birds were divided into eight different treatment groups based on feeding regimens. These groups included: full-fed and restricted photostimulated broiler breeders, killed at sexual maturity (FF-BB-P and RR-BB-P); full-fed and restricted non photostimulated broiler breeders, sacrificed at sexual maturity (FF-BB-NP and RR-BB-NP); full-fed and restricted broiler breeders, killed at 35 wks (FF-BB-35 and RR-BB-35); full-fed and restricted broiler breeders, killed at 60 wks (FF-BB-60 and RR-BB-60).

All full-fed birds were allowed to eat *ad libitum*, while feed-restricted birds were fed to maintain body weight as recommended by the breeder (Yu *et al.*, 1992a). Throughout the experiment, birds had free access to water.

All photostimulated birds were exposed to 14L:10D and non photostimulated exposed to 8L:16D. Lights were on at 7:00 a. m. for all lighting schedules. The procedure for collecting samples, chemical analyses and radioimmunoassays were described in section 2. Studies on estradiol-17 β from small white follicles were based on incubation of 3,200 individual follicles (40 follicles from each of 80 birds).

5.3 Statistical analyses

Data were subjected to one-way analyses of variance (Steel and Torrie, 1980). The significant differences between means were evaluated using Duncan's multiple range test (Steel and Torrie, 1980). Differences among the age classification were determined within each type (Table 5.1) for organ weights, carcass composition and reproductive characteristics. Differences in estradiol-17 β were calculated using methods presented in Chapter 2. Significance was assessed at the 0.05 level.

5.4 Results

Full-fed broiler breeders showed an advanced age at sexual maturity (FF-BB-P; 155.5 days; RR-BB-P; 166.6 days). Restricted BB came into production 11 days after full-fed BB. Full-feeding in photostimulated hens had a significant effect on body weight, heart weight, liver weight, abdominal fat pad, total carcass fat, carcass protein, moisture and ash weight (Table 5.2). All body organ weights and carcass compositions were significantly higher in full-fed birds than in restricted fed. On the percentage of body weight basis, full fed birds had larger weight of abdominal fat pad (6.21%) and carcass fat (24.89%) compared to restricted birds (2.26% and 12.92% respectively). However, full-fed birds had lower percent carcass protein, water and ash on body weight basis. The weight of heart and liver expressed as a percentage of body weight, was not significantly different between the two levels of feeding.

Oviduct and stroma weight were not affected by level of feeding (Table 5.3). However, full-fed birds had higher ovary weight (74.81 g) and number of large follicles (9.1) than restricted birds (47.43 g and 7.4) (Table 5.2). The average total estradiol-17 β output from small white follicles during a 3 h incubation is presented in table 5.4. Estradiol-17 β production in SWF from FF-BB-P and RR-BB-P was detectable. Full-fed and restricted photostimulated broiler breeders (FF-BB-P and RR-BB-P) differed significantly in estradiol-17 β production with presence of 5 ng and 10 ng of LH/follicle during incubation. The amount of estradiol-17 β produced by the follicles incubated with

5 ng of LH was twice as much in restricted (RR-BB-P; 1600.23 pg) than in full-fed hens (FF-BB-P; 886.29 pg). Estradiol-17 β production in the control group (0 ng) and 20 ng of LH per tube were not significantly different between full-fed and restricted birds. The maximum production of estradiol-17 β was seen in follicles from restricted birds incubated with 5 ng of LH per tube being 1637.04 pg per follicle.

Full-fed non photostimulated broiler breeders (FF-BB-NP) showed an advanced age at sexual maturity (175.7 days) compared to restricted birds (206.5 days). Also, body weight, liver weight, abdominal fat weight, carcass protein weight, moisture and ash weight were significantly higher in full-fed than were in restricted non photostimulated hens (Table 5.5). Oviduct and stroma weight were not influenced by level of feeding (Table 5.6). However, level of feeding significantly influenced ovary weight and number of large follicles.

Estradiol-17 β output from small white follicles is shown in table 5.7. No significant differences were observed in estradiol-17 β output stimulated with any of the four levels of LH.

At 35 wks of age, level of feeding influenced body weight, heart, liver, abdominal fat, carcass fat, carcass protein, water and ash weight (Table 5.8). These parameters were significantly higher in full-fed hens than in restricted birds. Percent of abdominal fat pad and carcass fat on body weight basis was significantly higher in full-fed than in restricted birds. Percent of heart, liver and ash on body weight basis was not affected by level of feeding. Percent of carcass protein (RR-BB-35; 14.81%) and water (RR-BB-35; 66.54%) on body weight basis were higher in restricted birds than in full-fed (FF-BB-35; 13.48% and 60.30% respectively).

At 35 wks of age, level of feeding influenced reproductive organs weight (Table 5.9). Oviduct, ovary and stroma weight were higher in full-fed broiler breeders than in restricted. However, percentage of these parameters on body weight basis was not influenced by level of feeding. Also, the number of large follicles was higher in full-fed group (7.9) than in restricted (5.8). The total amount of estradiol-17 β produced by

individual follicles during 3 h incubation is presented in table 5.10. No significant differences were observed in all analyzed treatments.

At 60 wks of age, level of feeding significantly influenced body organ weights and carcass composition (Table 5.11). Heart weight, liver weight, abdominal fat pad, carcass fat, carcass protein, water and ash were significantly higher in full-fed hens than in restricted hens. Heart, liver, abdominal fat pad, total carcass fat and ash weight expressed as a percentage of body weight were higher in full-fed than in restricted birds. However, protein weight when expressed as a percentage of body weight was not affected by level of feeding.

At 60 wks of age, level of feeding influenced oviduct, ovary and stroma weight (Table 5.12). Oviduct weight was significantly higher in restricted birds (RR-BB-60; 94.31 g) than in full-fed (FF-BB-60; 67.62 g). However, ovary and stroma weight were higher in full-fed birds. Number of large follicles were not affected by feed allocation at 60 wks of age. Percent of ovary and stroma weight on a body weight basis were not affected by level of feeding.

The follicles incubated with 5 ng of LH showed a very high increase in estradiol-17 β production compared to control group in both levels of feeding (Table 5.13). The amount of estradiol-17 β produced in SWF from full-fed and restricted birds killed at 60 wks with the presence of 5 ng of LH was higher (FF-BB-60; 1934.6 pg; RR-BB-60; 1936.4%) compared to control group ((FF-BB-60; 649.7 pg; RR-BB-60; 625.7 pg). Follicles from full-fed birds incubated with 20 ng of LH produced significantly higher amount of estradiol-17 β than restricted. Percent of follicles from FF-BB-60 incubated with bLH, which produced over 1.0 ng of estradiol-17 β when exposed to 5 ng and more of LH was the highest one in all treatment groups used in the project (78.52%).

5.5 Discussion

Level of feeding significantly influenced age at sexual maturity in full-fed and feed-restricted broiler breeders. Restricted broiler breeders (RR-BB-P; RR-BB-NP) came

into production significantly later than full-fed broiler breeders (FF-BB-P; FF-BB-NP) (Table 5.2 and 5.5; Figure 5.1). These results are different than those of Robinson *et al.* (1991). However, Robinson *et al.* (1991) initiated full-feeding at 22 wks instead of at the time of hatching. In my study, ad libitum-fed hens reached sexual maturity at heavier body weights than feed-restricted hens which is in agreement with Robinson *et al.* (1991) and Yu *et al.* (1992a,b). According to Brody (1980; 1984) not only photostimulation delays age at sexual maturity. Restricted birds must acquire the necessary conditions of body weight, body fat and certain body composition for the onset of egg production. Age at sexual maturity also depends on hepatic synthesis of yolk precursors, which in turn, depends on circulating estrogens (Kudzman *et al.*, 1979). The source of estrogen is the ovary, in particular the theca cells of the small follicles (Robinson and Eiches, 1986). The synthesis of estrogens by theca cells depends on the maturation of the hypothalamic-adenohypophyseal-ovarian axis. Feed restriction delays the maturation of hypothalamic-adenohypophyseal axis, therefore postponing release of LH and the production of steroid hormones in small follicles and consequently age at sexual maturity. The difference in age at sexual maturity between FF-BB-P and RR-BB-P was 11 days and between FF-BB-NP and RR-BB-NP was 31 days.

Level of feeding had a major effect on body weight between full-fed and feed-restricted birds in the analyzed groups (Figure 5.2). Full-fed hens were much heavier than restricted, which supports the work of Yu *et al.* (1992a). In photostimulated birds the difference between FF and RR was 1,730 g (Table 5.2). Non photostimulated FF birds were almost 1,000 g heavier than non photostimulated RR (Table 5.5). Photostimulated birds at peak production (Table 5.8) and at 60 wks of age (Table 5.11) were almost 1,680 g heavier than non photostimulated hens. The weight of the liver was highly responsive to feed intake. This observation is in agreement with Yu *et al.* (1992b) studies. Robinson *et al.* (1993) showed that as few as 7 days of full-feeding of BB hens at 44 wks resulted in change in liver weight from 50.6 g to 139.1 g. Furthermore, the lipid content of the liver increased in this time from 3.15 to 18.3%.

Abdominal fat pad weights in FF and RR were also significantly influenced by level of feeding (Figure 5.3). Full-fed birds had a significantly higher weight of abdominal fat pad than restricted birds. The highest amount of abdominal fat pad was noticed in FF-BB-35 (357.7 g) and in old hens (303.5 g). This was mostly due to increase in body weight in these birds, since fat is positively correlated with the body weight (Siegel and Dunnington, 1985). The weight of fat pad, expressed as a percentage of BW, was the highest in FF-BB-35 (6.89%). Total carcass fat weight (Figure 5.4) was also influenced by feeding allowance. Also, carcass fat weight when expressed as a percentage of BW was the highest in FF-BB-60 (25%). Overall in BB, the abdominal fat pad contributed to about 18 to 26% of the total carcass fat. Carcass protein, water content and ash were significantly higher in feed restricted groups than in full-fed groups.

The examination of ovarian morphology indicated that the oviduct weight, expressed as a percentage of BW, was significantly higher in RR-BB-P, RR-BB-NP, and RR-BB-60 (Table 5.3, 5.6 and 5.12). On the other hand, at 35 wks oviduct weight, expressed as a percentage of BW, was not significantly affected by level of feeding (Table 5.9). Even though, ovary and stroma weight expressed as a percentage of BW were not affected by level of feeding, number of large follicles was significantly higher in full-fed hens than in restricted. According to Yu *et al.* (1992b) body weight was the most important variable determining the number of large follicles. The highest number of large follicles were observed in FF-BB-NP (9.8), followed by FF-BB-P (9.1), FF-BB-35 (7.9) and FF-BB-60 (6.33). The number of large follicles in restricted treatments was significantly lower and ranged from 7.4 in RR-BB-P to 4.2 in RR-BB-60. Considerable research indicates *ad libitum* feeding during rearing results in an increase in the number of ovarian follicles (Hocking *et al.*, 1987, 1989). Despite this increase the pool of ovulable follicles and egg output is reduced in *ad libitum*-fed hens compared to feed-restricted hens. According to Robinson *et al.* (1991) hens restricted from 22-62 wks of age produced 40.4 more eggs than *ad libitum*-fed hens. The length of the prime sequence was longer for the feed-restricted hens (24 days) compared to the *ad libitum*-fed

sequence was longer for the feed-restricted hens (24 days) compared to the *ad libitum*-fed hens (15 days). Also, full-fed hens had a higher number of pauses of 11 or more days duration than the restricted birds. It cannot be definitely stated whether the reduced sequence length seen in full-fed hens is a result of a reduction or an increase in rate of follicular maturation. A reduction in rate of follicular maturation may be associated with increase incidence of atresia, internal ovulation, multiple ovulation or internal laying (Robinson *et al.*, 1991; Yu *et al.*, 1992b). Consequently, egg production rate is reduced in full-fed birds compared to feed-restricted hens (Robbins *et al.*, 1986; Hocking *et al.*, 1987, 1989; Robinson *et al.*, 1991; Yu *et al.*, 1992b)

The data presented in tables 5.4, 5.7, 5.10, 5.13 and figure 5.9 support Robinson and Eiches (1986) and Yu *et al.* (1992c) conclusion that steroidogenesis in SWF was stimulated by LH. The output of estradiol-17 β from SWF in response to 5 ng of bLH was almost three times higher than in control group (0 LH). Furthermore, the plateau in estradiol-17 β output was reached when SWF were stimulated with 10 and 20 ng of LH. Level of feeding did not significantly influence estradiol-17 β output in FF-BB-NP vs RR-BB-NP (Table 5.7), FF-BB-35 vs RR-BB-35 (Table 5.10) and in FF-BB-60 vs RR-BB-60 (Table 5.13). Only level of feeding in photostimulated birds at sexual maturity (Table 5.4) influence estradiol-17 β production (Figure 5.9). According to Yu *et al.* (1992c) no significant difference was observed in estrogen output from SWF between FF and RR feeding, although androstenedione was higher in full-fed birds. Yu *et al.* (1992c) pooled together small follicles and analyzed estradiol-17 β output. Consequently it was not clear if changes in steroid output were due to changes in LH sensitivity, changes in the number of follicles producing estradiol-17 β or both. Yu *et al.* (1992c) also incubated follicles with 25 ng of bLH. It is not clear if the work of Yu *et al.* (1992c) truly establish steroidogenic potential with physiological normal dosages of LH. From this study, we know that 5 ng of bLH was the maximum amount of 4 dosages, which stimulated estradiol-17 β production (positive slope).

LH sensitivity (Slope) did not significantly vary across treatments with the exception

of birds studied at sexual maturity that were photostimulated (FF-BB-P vs RR-BB-P) (Table 5.4; Figure 5.10). Restricted photostimulated at sexual maturity hens were more LH sensitive and produced significantly higher amount of estradiol-17 β . The percent of LH responsive follicles (Percent) was also affected by level of feeding in the same groups (Table 5.4; Figure 5.10). Only 37.85 % of follicles from FF-BB-P responded with production over 1.0 ng of estradiol-17 β . This indicates that higher numbers of SWF were atretic in that group. According to Hocking *et al.* (1987), follicular atresia is a common observation in full-fed BB. Steroidogenesis work from my study supports this statement. The reason that this phenomena of increased atresia was not seen in other groups (NP, 35 wks, 60 wks) may be related to both aging and the rate of development at sexual maturity. When FF-BB are photostimulated rapidly, poor quality follicles are produced which are both less LH sensitive and have a higher incidence of atresia than restricted birds. This may indicate an important interaction between body weight, age and photostimulation program.

TABLE 5.1. Experimental design - effect of feeding. Treatment groups according to age classification by type.

Age classification (Aging)	Type		
	SCWL ^a	FF-BB ^b	RR-BB ^c
Immature hens-19wk¹			
First egg - P²		FF-BB-P¹	RR-BB-P¹
First egg - NP³		FF-BB-NP¹	RR-BB-NP¹
Young hens-35wk²			
		FF-BB-35	RR-BB-35
Old hens-60wk²			
		FF-BB-60	RR-BB-60

¹ These hens were sacrificed at sexual maturity.

² Birds were photostimulated at 20 wks of age and photoperiod was maintained at 14L:10D.

³ Birds were non photostimulated and controlled by 8L:16D.

^a Egg-type birds; Single Comb White Leghorns.

^b Meat-type birds; Full-fed broiler breeders.

^c Meat-type birds; Restricted broiler breeders.

TABLE 5.2. Effect of level of feeding on age, BW, selected organ weights and carcass composition at sexual maturity in photostimulated full-fed broiler breeders (BB) (FF-BB-P) and feed-restricted BB (RR-BB-P).

Variables	Level of feeding		SEM
	FF-BB-P	RR-BB-P	
Number of hens	10	10	
Age of the bird	155.5 ^a	166.6 ^a	2.40
Average BW, g	4,446.6 ^a	2,718.3 ^b	88.12
Heart			
%BW	0.38	0.41	0.03
g	16.63 ^a	11.16 ^b	1.18
Liver			
%BW	2.11	2.07	0.10
g	93.42 ^a	55.93 ^b	3.94
Abdominal fat			
%BW	6.21 ^a	2.26 ^b	0.32
g	276.36 ^a	61.38 ^b	13.13
Carcass fat			
%BW	24.89 ^a	12.92 ^b	1.13
g	1102.69 ^a	341.63 ^b	55.51
Carcass protein			
%BW	15.03 ^a	18.20 ^a	0.48
g	666.53 ^a	480.86 ^b	21.80
Carcass H ₂ O			
%BW	57.46 ^b	64.99 ^a	1.27
g	2552.64 ^a	1707.92 ^b	66.49
Carcass ash			
%BW	2.70 ^b	3.48 ^a	0.07
g	119.35 ^a	91.74 ^b	3.79
Total percent	100.08	99.99	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.3. Effect of level of feeding on reproductive tract characteristics at sexual maturity in photostimulated full-fed broiler breeders (BB) (FF-BB-P) and feed restricted BB (RR-BB-P).

Variables	Level of feeding		SEM
	FF-BB-P	RR-BB-P	
Number of hens	10	10	
Oviduct			
%BW	1.39 ^b	2.02 ^a	0.08
g	61.50	54.64	2.92
Ovary			
%BW	1.68	1.75	0.15
g	74.81 ^a	47.43 ^b	6.98
Stroma			
%BW	0.22	0.23	0.02
g	9.46 ^a	6.37 ^b	1.30
Number of large follicles	9.1 ^a	7.4 ^b	0.57

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.4. Effect of level of feeding on output of estradiol-17 β (pg/follicles) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected at sexual maturity from photostimulated full-fed broiler breeders (BB) (FF-BB-P) and feed-restricted BB (RR-BB-P).

Variables	Level of feeding		SEM
	FF-BB-P	RR-BB-P	
Number of hens	10	10	
E₂ production			
Dose of LH			
0 ng LH	377.40	359.96	67.49
5 ng LH	1021.95 ^b	1637.04 ^a	172.26
10 ng LH	886.29 ^b	1600.23 ^a	188.75
20 ng LH	1074.69	1072.26	153.34
Slope²	128.91 ^b	255.42 ^a	32.76
Percent (%)³	37.67 ^b	61.67 ^a	6.41

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

^{a,b} Means within a row with different superscripts are significantly different (P ≤ .05)

TABLE 5.5. Effect of level of feeding on age, BW, selected organ weights and carcass composition at sexual maturity in non photostimulated full-fed broiler breeders (BB) (FF-BB-NP) and feed-restricted BB (RR-BB-NP).

Variables	Level of feeding		SEM
	FF-BB-NP	RR-BB-NP	
Number of hens	10	10	
Age of the bird	175.7 ^a	206.5 ^a	4.61
Average BW, g	4,422.4 ^a	3,422.9 ^b	108.06
Heart			
%BW	0.34	0.37	0.03
g	14.83	12.64	0.95
Liver			
%BW	1.74	1.57	0.10
g	77.17 ^a	53.61 ^b	4.16
Abdominal fat			
%BW	6.64 ^a	3.72 ^b	0.32
g	294.38 ^a	127.10 ^b	15.32
Carcass fat			
%BW	26.60 ^a	16.42 ^b	0.80
g	1186.33 ^a	557.37 ^b	41.85
Carcass protein			
%BW	14.39 ^b	16.23 ^a	0.30
g	638.54 ^a	549.67 ^b	16.28
Carcass H ₂ O			
%BW	56.90 ^b	64.85 ^a	0.88
g	2521.36 ^a	2196.06 ^b	52.55
Carcass ash			
%BW	2.53	2.65	0.07
g	112.60 ^a	89.82 ^b	3.79
Total percent	100.43	100.15	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.6. Effect of level of feeding on reproductive tract characteristics at sexual maturity in non photostimulated full-fed broiler breeders (BB) (FF-BB-NP) and feed restricted BB (RR-BB-NP).

Variables	Level of feeding		SEM
	FF-BB-NP	RR-BB-NP	
Number of hens	10	10	
Oviduct			
%BW	1.39 ^a	1.87 ^a	0.08
g	61.24	63.73	3.03
Ovary			
%BW	1.79	1.76	0.15
g	79.77 ^a	60.15 ^b	6.65
Stroma			
%BW	0.22	0.26	0.02
g	9.78	8.94	1.07
Number of large follicles	9.8 ^a	6.6 ^b	0.52

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.7. Effect of level of feeding on output of estradiol-17 β (pg/follicles) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected at sexual maturity from non photostimulated full-fed broiler breeders (BB) (FF-BB-NP) and feed-restricted BB (RR-BB-NP).

Variables	Level of feeding		SEM
	FF-BB-NP	RR-BB-NP	
Number of hens	10	10	10
E₂ production			
Dose of LH			
0 ng LH	420.30	546.44	56.42
5 ng LH	1517.27	1514.94	227.92
10 ng LH	1553.57	1113.10	269.22
20 ng LH	1279.21	870.23	156.61
Slope ²	219.39	193.70	43.66
Percent (%) ³	61.66	51.00	8.04

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

TABLE 5.8. Effect of level of feeding on BW, selected organ weights and carcass composition in full-fed broiler breeders (BB) (FF-BB-35) and feed-restricted BB (RR-BB-35) at 35 wks of age.

Variables	Level of feeding		SEM
	FF-BB-35	RR-BB-35	
Number of hens	10	10	
Average BW, g	5,168.2 ^a	3,486.6 ^b	69.23
Heart			
%BW	0.35	0.34	0.02
g	18.14 ^a	11.96 ^b	0.64
Liver			
%BW	2.34	2.25	0.11
g	120.85 ^a	78.64 ^b	4.70
Abdominal fat			
%BW	6.89 ^a	3.63 ^b	0.29
g	357.73 ^a	127.25 ^b	13.43
Carcass fat			
%BW	23.81 ^a	15.63 ^b	0.80
g	1221.06 ^a	534.38 ^b	41.84
Carcass protein			
%BW	13.48 ^a	14.81 ^a	0.30
g	689.33 ^a	502.53 ^b	16.28
Carcass H ₂ O			
%BW	60.30 ^a	66.54 ^a	0.88
g	3074.89 ^a	2257.65 ^b	52.55
Carcass ash			
%BW	2.60	2.71	0.07
g	133.01 ^a	92.02 ^b	3.79
Total percent	100.18	99.70	

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.9. Effect of level of feeding on reproductive tract characteristics in full-fed broiler breeders (BB) (FF-BB-35) and feed-restricted BB (RR-BB-35) at 35 wks of age.

Variables	Level of feeding		SEM
	FF-BB-35wk	RR-BB-35wk	
Number of hens	10	10	
Oviduct			
%BW	1.47	1.75	0.11
g	76.05 ^a	61.10 ^b	3.86
Ovary			
%BW	1.73	2.03	0.10
g	89.11 ^a	70.82 ^b	3.67
Stroma			
%BW	0.26	0.28	0.02
g	13.35 ^a	9.81 ^b	0.89
Number of large follicles	7.9 ^a	5.8 ^b	0.36

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.10. Effect of level of feeding on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed broiler breeders (BB) (FF-BB-35) and feed-restricted BB (RR-BB-35) at 35 wks of age.

Variables	Level of feeding	
	FF-BB-35	RR-BB-35
Number of hens	9	10
E₂ production		
Dose of LH		
0 ng LH	558.5 \pm 59.47	515.6 \pm 56.42
5 ng LH	1617.5 \pm 240.25	1630.1 \pm 227.92
10 ng LH	1773.2 \pm 283.78	1557.0 \pm 269.22
20 ng LH	1493.3 \pm 165.08	1424.7 \pm 156.61
Slope²	211.8 \pm 46.02	222.9 \pm 43.66
Percent (%)³	64.4 \pm 7.79	65.0 \pm 7.39

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

TABLE 5.11. Effect of level of feeding on BW, selected organ weights and carcass composition in full-fed broiler breeders (BB) (FF-BB-60) and feed-restricted BB (RR-BB-60) at 60 wks of age.

Variables	Level of feeding	
	FF-BB-60	RR-BB-60
Number of hens	9	10
Average BW, g	5,098.7±137.73 ^a	3,410.0±130.66 ^b
Heart		
%BW	0.55±0.03 ^a	0.45±0.03 ^b
g	28.09±1.17 ^a	15.53±1.11 ^b
Liver		
%BW	2.43±0.19 ^a	1.51±0.18 ^b
g	123.94±9.20 ^a	51.57±8.73 ^b
Abdominal fat		
%BW	5.90±0.46 ^a	4.43±0.44 ^b
g	303.51±23.64 ^a	152.99±22.43 ^b
Carcass fat		
%BW	25.30±0.90 ^a	17.63±0.80 ^b
g	1323.96±46.78 ^a	598.64±41.85 ^b
Carcass protein		
%BW	13.80±0.34	13.66±0.30
g	715.32±18.19 ^a	459.23±16.28 ^b
Carcass H ₂ O		
%BW	57.78±0.99 ^a	66.93±0.88 ^a
g	2986.25±58.75 ^a	2243.18±52.55 ^b
Carcass ash		
%BW	2.97±0.08 ^a	2.33±0.07 ^b
g	154.96±4.24 ^a	78.13±3.79 ^b
Total percent	99.85	100.54

^{a,b} Means within a row with different superscripts are significantly different (P ≤ .05).

TABLE 5.12. Effect of level of feeding on reproductive tract characteristics in full-fed broiler breeders (BB) (FF-BB-60) and feed-restricted BB (RR-BB-60) at 60 wks of age.

Variables	Level of feeding	
	FF-BB-60	RR-BB-60
Number of hens	9	10
Oviduct		
%BW	1.33±0.24 ^b	2.77±0.22 ^a
g	67.62±8.66 ^b	94.31±8.21 ^a
Ovary		
%BW	1.97±0.24	1.42±0.23
g	99.92±10.38 ^a	48.54±9.85 ^b
Stroma		
%BW	0.37±0.03	0.30±0.03
g	18.41±1.36 ^a	10.10±1.29 ^b
Number of large follicles	6.33±0.81	4.20±0.77

^{a,b} Means within a row with different superscripts are significantly different ($P \leq .05$).

TABLE 5.13. Effect of level of feeding on output of estradiol-17 β (pg/follicle) from small white follicles in vitro during a 3h incubation period in the presence and absence of bLH¹. Follicles were collected from full-fed broiler breeders (BB) (FF-BB-60) and feed-restricted BB (RR-BB-60) at 60 wks of age.

Variables	Level of feeding	
	FF-BB-60	RR-BB-60
Number of hens	9	10
E₂ production		
Dose of LH		
0 ng LH	649.7 \pm 67.65	625.68 \pm 64.18
5 ng LH	1934.6 \pm 337.95	1936.37 \pm 320.61
10 ng LH	2108.7 \pm 267.91	1704.24 \pm 254.16
20 ng LH	1890.5 \pm 189.62 ^a	1271.25 \pm 179.89 ^a
Slope ²	257.0 \pm 62.11	262.14 \pm 58.93
Percent (%) ³	78.52 \pm 8.53	61.67 \pm 8.10

¹ bLH-5, NIAMDD.

² Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

³ Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

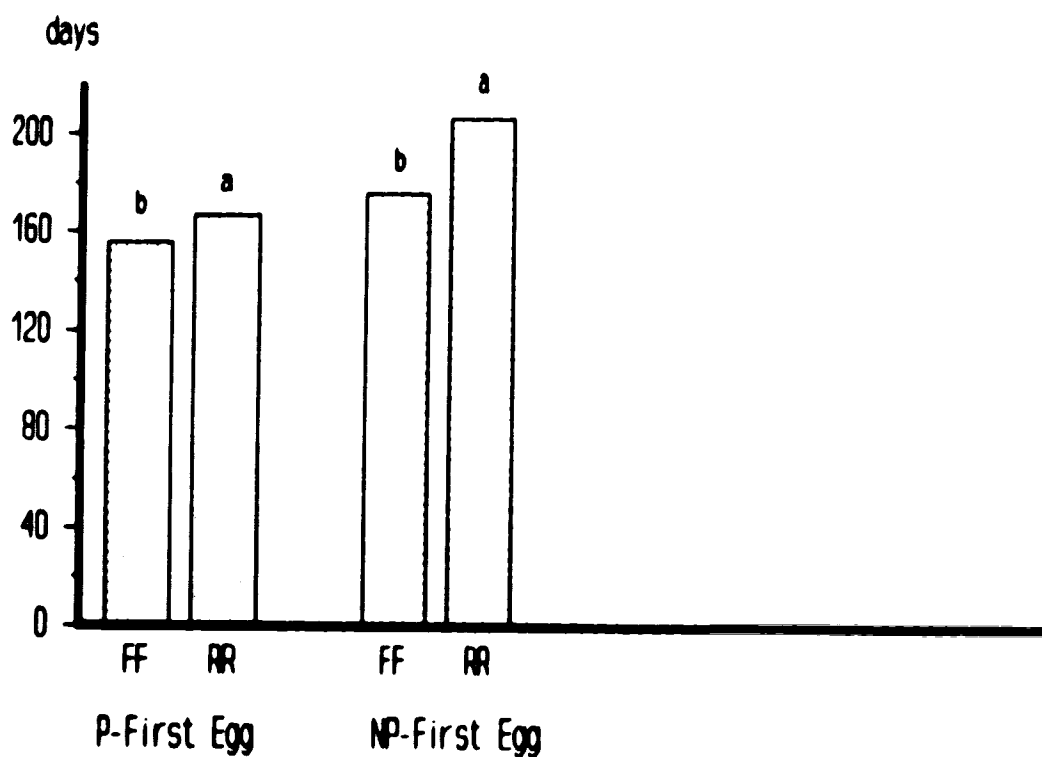


Figure 5.1. The effect of level of feeding on age at sexual maturity (days) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

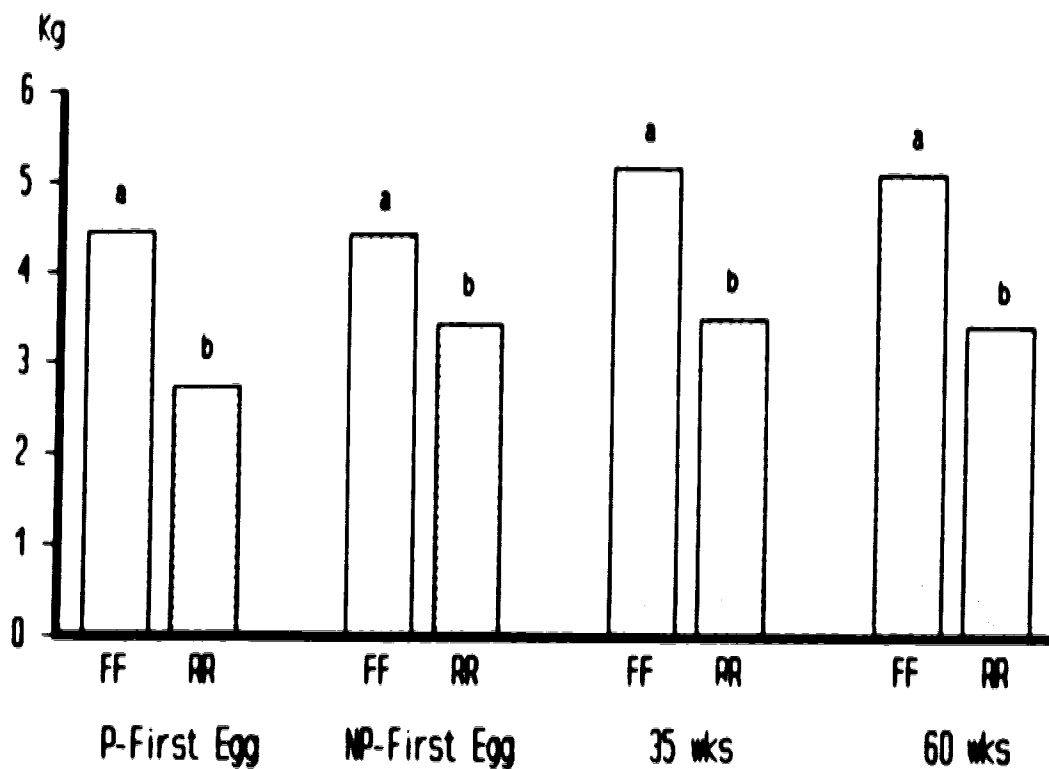


Figure 5.2. The effect of level of feeding on body weight (kg) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

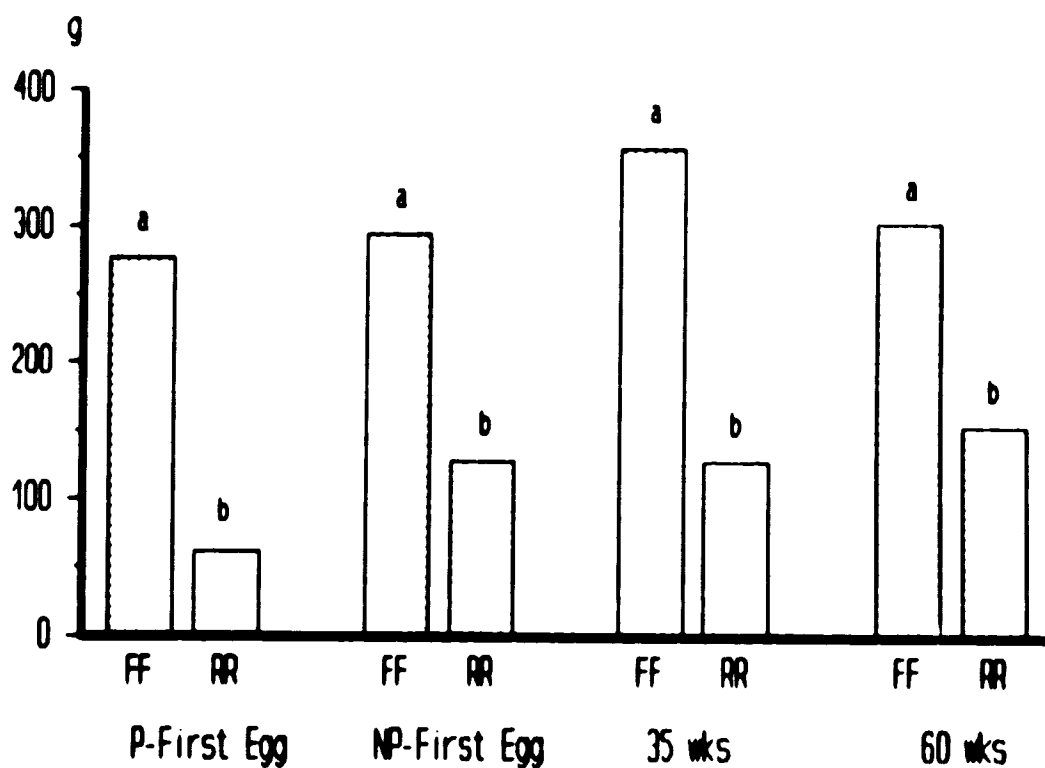


Figure 5.3. The effect of level of feeding on abdominal fat pad weight (g) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

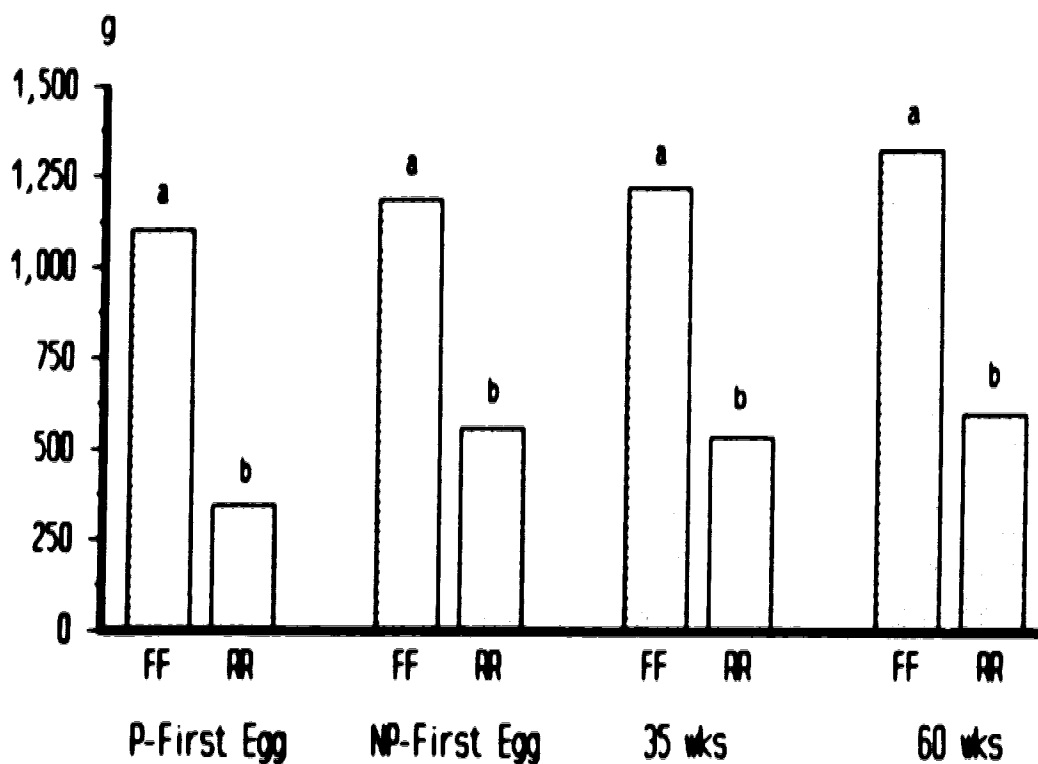


Figure 5.4. The effect of level of feeding on total carcass fat weight (g) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

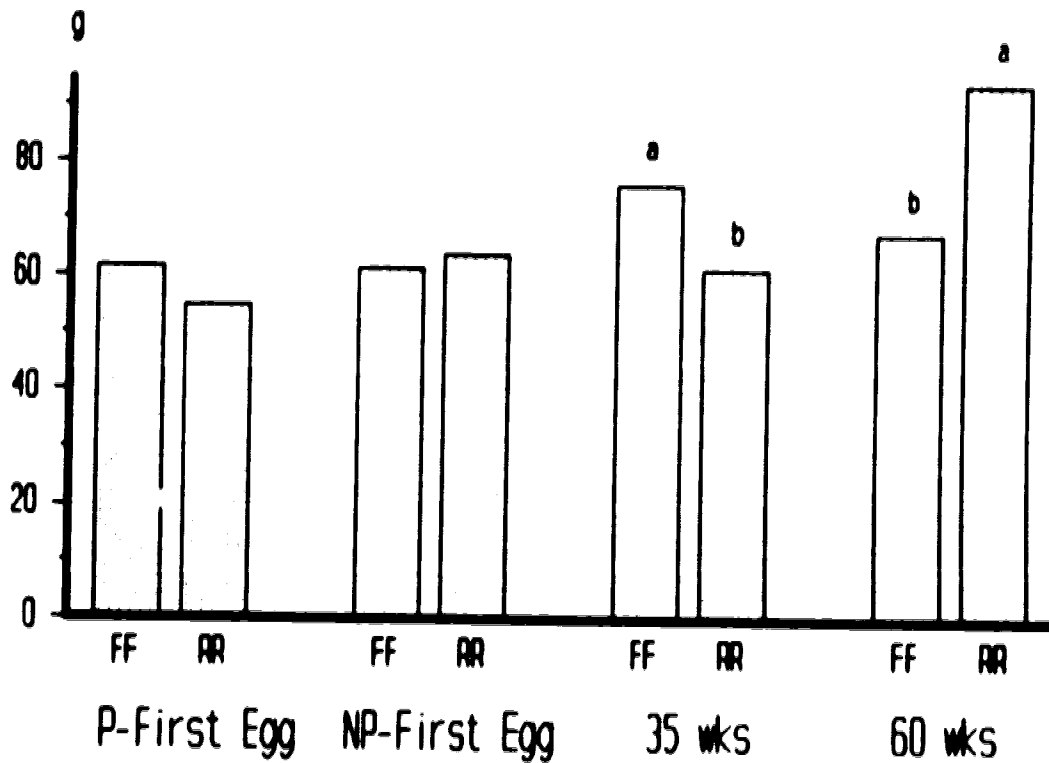


Figure 5.5. The effect of level of feeding on oviduct weight (g) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

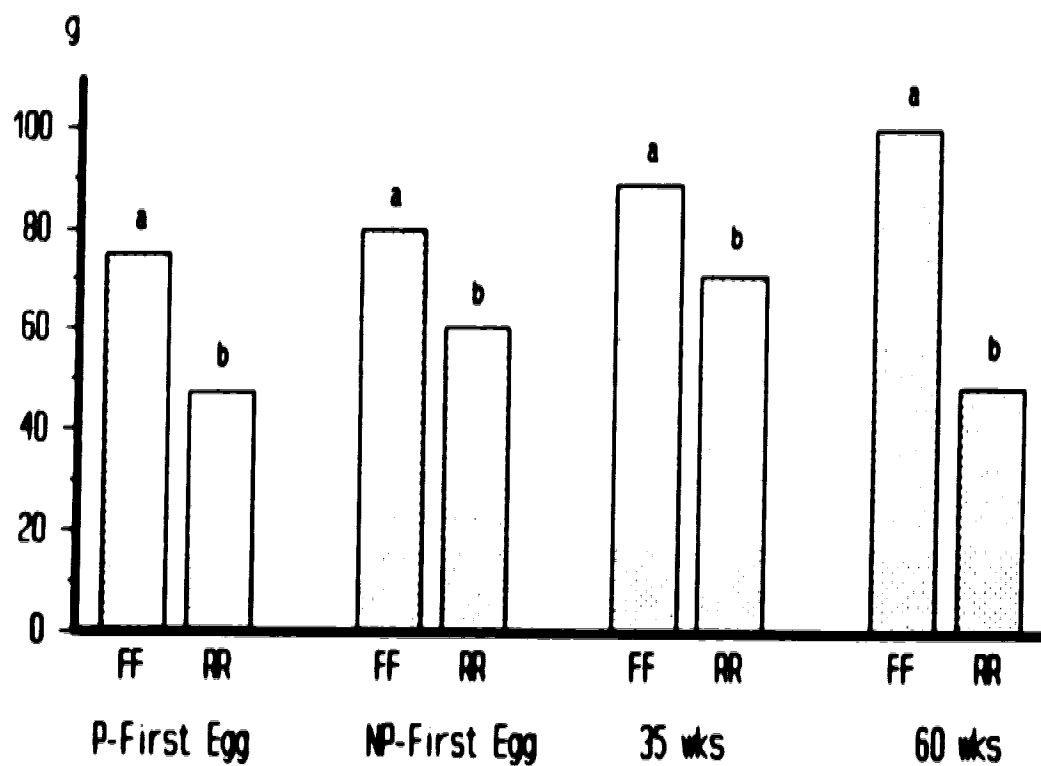


Figure 5.6. The effect of level of feeding on total ovary weight (g) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

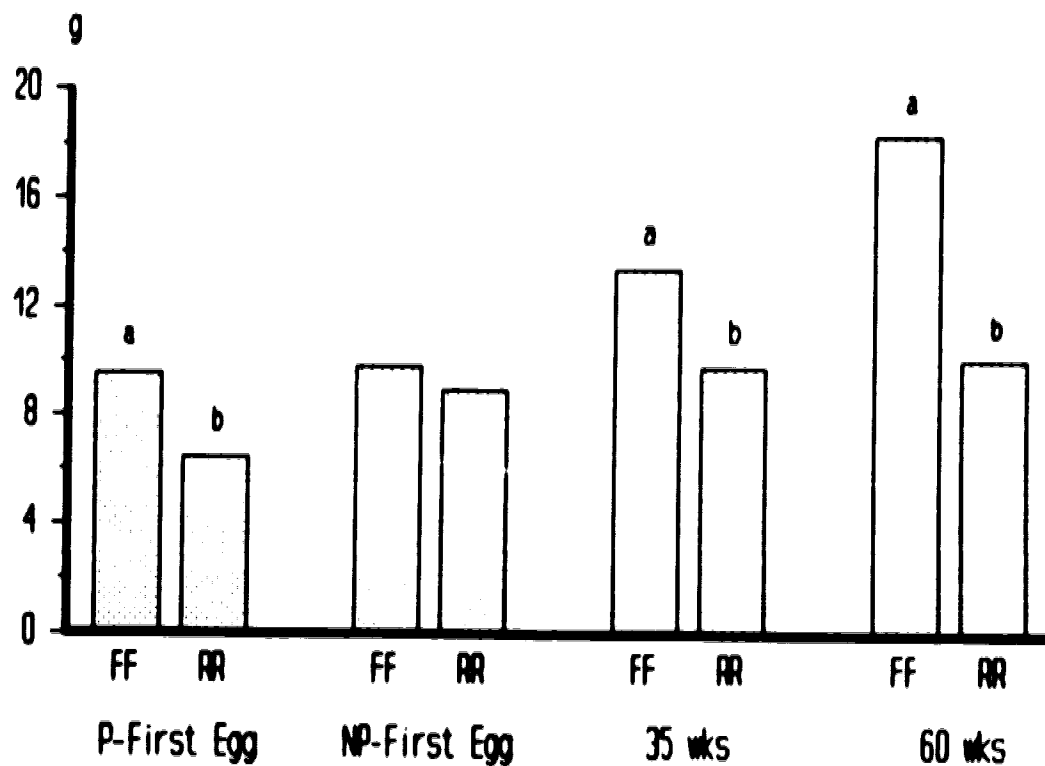


Figure 5.7. The effect of level of feeding on ovarian stroma weight (g) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wks) and old hens 60 wks (60wks). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

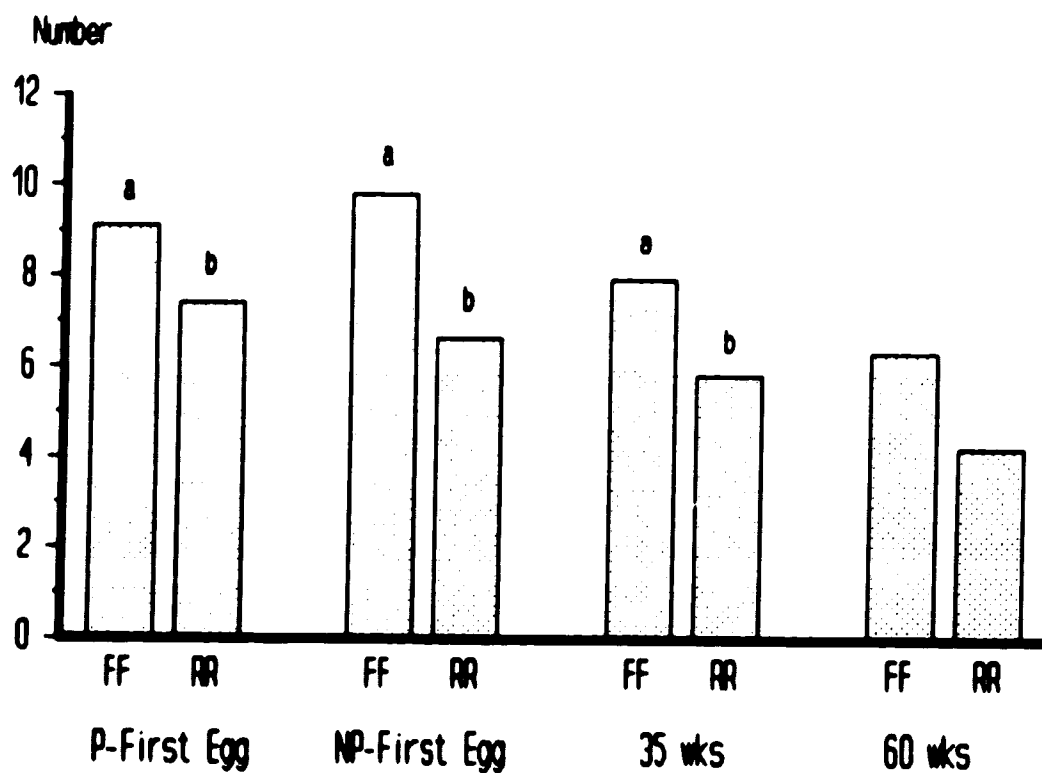


Figure 5.8. The effect of level of feeding on number of large follicles (Number) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wk) and old hens 60 wks (60wk). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

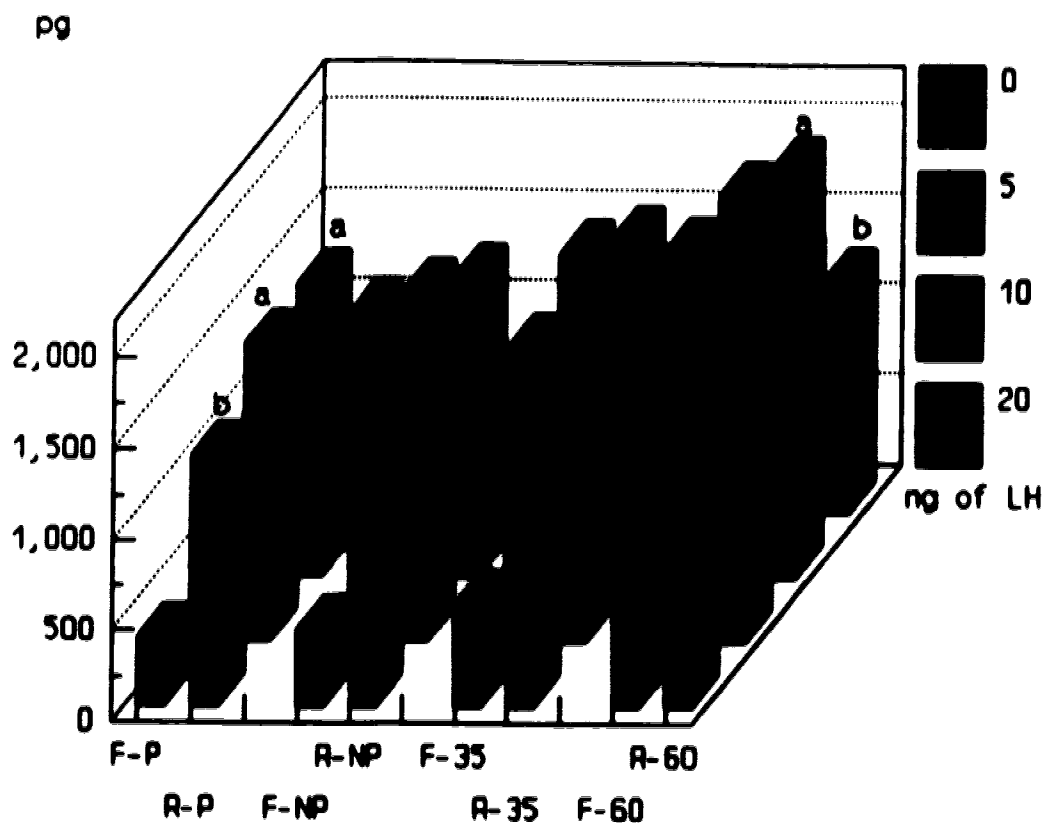


Figure 5.9. The effect of level of feeding on estradiol-17 β output for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wk) and old hens 60 wks (60wk). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

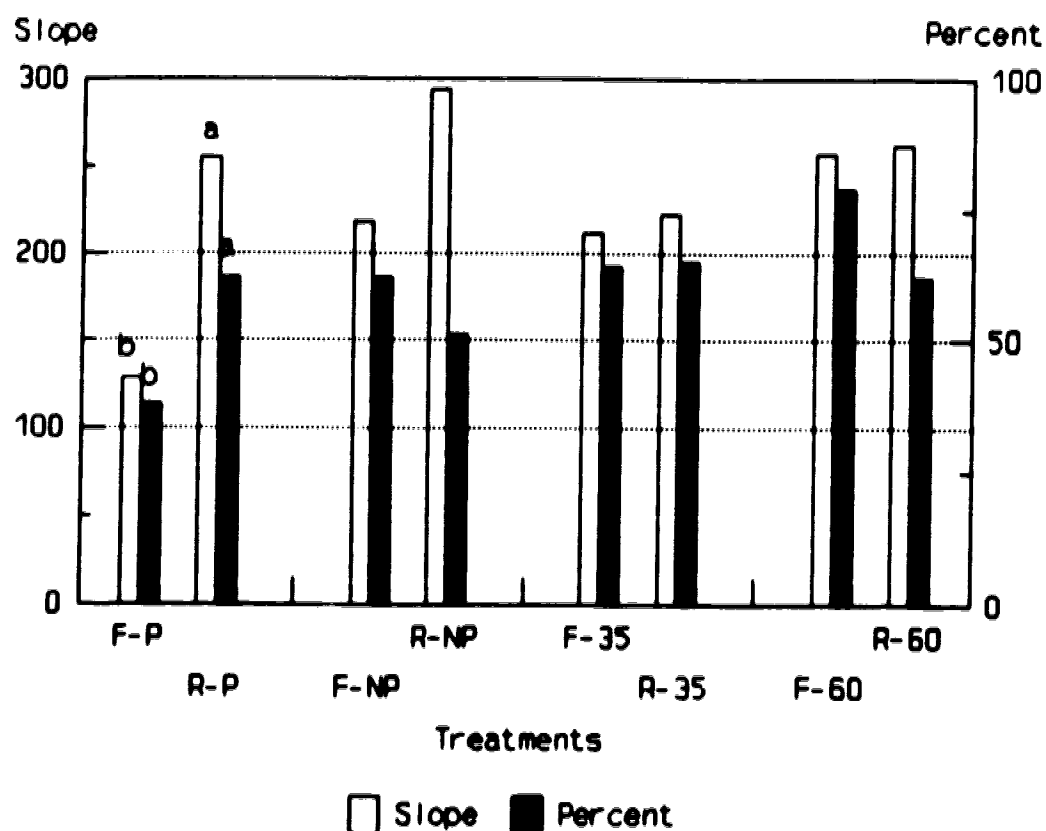


Figure 5.10. The effect of level of feeding on LH sensitivity (Slope) and percent of LH responsive follicles (Percent) for two types of birds: full-fed (FF) and feed-restricted broiler breeders (RR). Treatments included first egg photostimulated (P) and non photostimulated (NP), young hens 35 wks (35wk) and old hens 60 wks (60wk). Treatment means within a type with different superscripts are significantly different ($P \leq 0.05$).

5.6 LITERATURE CITED

Brody, T. Y., E. M. Soller, I. Nir, and Z. Nitsan, 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. *Br. Poult. Sci.* 21:437-446.

Brody, T. B., P. B. Siegel, and J. A. Cherry, 1984. Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. *Br. Poult. Sci.* 25:245-252.

Clayton, G. A., 1972. Effects of selection on reproduction in avian species. *J. Reprod. Fertil. Suppl.* 15:1-21.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington, 1987. Ovarian follicular structure of White Leghorn fed *ad libitum* and Dwarf and Normal broiler breeders fed *ad libitum* or restricted to point of lay. *Br. Poult. Sci.* 28:493-506.

Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30:161-174.

Ingram, D. R., and H. R. Wilson, 1987. *Ad libitum* feeding of broiler breeders prior to peak egg production. *Nutr. Rep. Int.* 36:839-845.

Kudzma, D. J., J. B. Swaney, and E. N. Ellis, 1979. Effects of estrogen administration on the lipoproteins of the children. *Biochim. Biophys. Acta* 572:257-268.

Pym, R. A. E., and J. F. Dillon, 1974. Restricted food intake and reproductive performance of broiler breeder pullets. *Br. Poult. Sci.* 15:245-259.

Robbins, K. R., G. C. McGhee, P. Osei, and R. E. Beauchene, 1986. Effect of feed restriction on growth, body composition, and egg production during the breeding season. *Poultry Sci.* 65:1052-1057.

Robblee, A. R., D. R. Clandinin, K. Darlington, and G. R. Milne, 1979. The effects of restricted feeding and energy content of the ration on the performance of broiler breeding chickens. *Can. J. Anim. Sci.* 59:539-544.

Robinson F. E., and R. J. Etches, 1986. Ovarian steroidogenesis during follicular maturation in domestic fowl (*Gallus domesticus*). *Biol. of Reprod.* 35:1096-1105.

Robinson, F. E., R. T. Hardin, N. A. Robinson, and B. J. Williams, 1991. The influence of egg sequence position on fertility, embryo viability and embryo weight in broiler breeders. *Poultry. Sci.* 70:760-765.

Robinson, F. E., J. L. Wilson, M. W. Yu, G. M. Fasenko, and R. T. Hardin, 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. *Poultry Sci.* 72:912-922.

Siegel, P. B., and E. A. D. Dunnington, 1985. Reproductive complications associated with selection for broiler growth. Pages 59-71 in: *Poultry Genetics and Breeding*. W. G. Hill, M. Manson, and D. Hewitt, ed. British Poultry Science Ltd., Edinburgh, Scotland.

Steel, R. G. D., and J. H. Torrie, 1980. Principle and Procedures of Statistics. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Van Middelkoop, J. H., 1972. The relationship between ovulation interval of White Plymouth Rock pullets and the laying of abnormal eggs. Archiv fur Geflugelkunde, 36:223-230.

Whitehead, C., and P. Hocking, 1988. Feeding and managing turkeys and broiler breeder hens. Pages 6-7 in : Science and Poultry Industry, J. Hardcastle, ed. Agriculture and Food Research Council, London, England.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. Poultry Sci. 71:1750-1761.

Yu, M. W., F. E. Robinson, and R. J. Etches, 1992c. Effect of feed allowance during rearing and breeding on female broiler breeders. 3. Ovarian steroidogenesis. Poultry Sci. 71:1762-1767.

Yu, M. W., F. E. Robinson, and A. R. Robbles, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poultry Sci. 71:1739-1749.

6. GENERAL DISCUSSION

This study was initiated to examine the influence of aging, strain, photostimulation program and level of feeding on age at sexual maturity, body organs weight, body composition, ovarian morphology and estradiol-17 β production by SWF. I tried to examine if ability to produce estradiol-17 β by SWF is related to the ovarian morphology or if "form" of the ovary is related to its "function".

According to Brody *et al.* (1984) the heavier the bird, the less sexual maturity is delayed. Not only photoperiod affects sexual maturity (Chaney and Fuller, 1975). Body weight (Brody *et al.*, 1980, 1984), body fat content (Bornstein *et al.*, 1984) and age (Brody *et al.*, 1980, 1984) are also important. These factors with the exception of photoperiod and age, are influenced by feed intake. According to Pym and Dillon (1974), the severity of feed restriction progressively delays sexual maturity. On the other hand, feed restriction improves peak rate of egg production (Blair *et al.*, 1976) and persistency of egg production (Leeson and Summers, 1983). It is now apparent that SCWL are physiologically competent to reach sexual maturity and come into production at a significantly younger age than do broiler breeders. In SCWL, FF-BB and RR-BB photostimulation advanced age at sexual maturity significantly compared to non photostimulated birds (Chapter 3). FF-BB-NP came into production 20 days later than FF-BB-P (175.7; 155.5 days). RR-BB-NP laid their first egg 40 days after RR-BB-P hens did. FF-BB came into production at a younger age than did RR-BB hens.

There is a strong negative relationship between body weight, amount of fat and reproductive efficiency in domestic fowl (Siegel and Dunnington, 1985). There were significant age, strain and level of feeding effects in body weight comparisons. BB were significantly heavier than SCWL. The reduction in growth rate of FF-BB after peak production may have been related to appetite satiation. This observation supports Robbins *et al.* (1986) and Yu *et al.* (1992a) studies in which they reported that the appetite of feed restricted birds remain uniformly high, whereas among *ad libitum*-fed

birds, some reduce voluntary feed intake late in production. The excessive amount of body fat may result in reduced feed intake in these birds. Strain and level of feeding had the most significant effects on carcass fat quantity.

The examination of ovarian morphology indicates that it may be easier for BB than for SCWL to put energy into stroma weight and number of large follicles. Too many large follicles in FF-BB are associated with double hierarchies and multiple ovulation (Hocking *et al.*, 1989; Yu *et al.*, 1992b). Increased follicular development seen in full-fed broiler breeder hens is not associated with increased egg production (Robinson *et al.*, 1991; Yu *et al.*, 1992b). In chickens, ovulation is possibly regulated energetically at both the ovary and the hypothalamo-hypophyseal complex. It is likely that feed-restriction delays the maturation of that complex, thus postponing age at sexual maturity (Chapter 5). Female broiler breeders should be feed restricted to reduce body weight, body fat and ovary weight.

In Table 6.1 correlation of ovarian morphology and dosages of LH with estradiol-17 β production is presented for a data set consisting of all 13 groups of studied hens. No correlation between stroma weight and number of large follicles with slope (LH sensitivity) was observed. Negative correlation was observed between ovary weight and slope. This correlation was not particularly strong (-0.21) but is very significant (0.03). No correlation between stroma weight and number of large follicles with percent of follicles incubated with bLH which produced more than 1 ng of estradiol-17 β was noticed. Furthermore, a very strong correlation was observed between percent and slope and between percent and estradiol-17 β output. These correlation coefficients indicate that where follicles produce estradiol-17 β , those that produce greatest amount are those which are the most sensitive to LH and have a greater likelihood of having fewer atretic follicles. Therefore, it can be concluded that ovaries that produce lots of estrogen, contain follicles that are very LH sensitive and also have a low incidence of atresia (High percentage).

The results of this study could be expanded by a series of future studies using 1,

2, 3 and 4 ng of LH per tube to observe the pattern of estradiol-17 β production between 0 and 5 ng of LH. We do not know now, if there is a linear relationship between 0 and 5 ng of LH. Also, the period of time required for LH to increase its own receptor population may be greater than three hours (time of incubation used in this study). Further examination in this area is also required.

Table 6.1. Correlation for ovarian morphology and dosages of LH with estradiol 17- β production.

Variables	Correlation with estradiol-17 β production			
	Slope ¹		Percent ²	
	r	p	r	p
Ovary weight	-0.21	0.03	-0.72	0.45
Stroma weight	-0.03	0.72	0.03	0.72
Number of l. follicles	-0.15	0.12	-0.17	0.08
E ₂ production				
0 ng LH	0.31	0.00	0.61	0.00
5 ng LH	0.96	0.00	0.75	0.00
10 ng LH	0.79	0.00	0.79	0.00
20 ng LH	0.48	0.00	0.80	0.00
Slope ²			0.65	0.00

¹ Slope determined as the linear relations output of E₂ from follicles incubated with 0 or 5 ng of bLH.

² Percent of follicles incubated with bLH, which produced > 1.0 ng of E₂.

6.1 LITERATURE CITED

- Blair, R. M. M. MacCowan, and W. Bolton, 1976. Effects of food regulation during the growing and laying stages on the productivity of broiler breeders. *Br. Poult. Sci.* 17:215-223.
- Kurman, S. S. Plavnik, and Y. Lev, 1984. Body weight/or fatness as potential determinants of the onset of egg production in broiler breeder hens. *Br. Poult. Sci.* 25:323-341.
- Brody, T. Y., E. M. Soller, I. Nir, and Z. Nitsan, 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. *Br. Poult. Sci.* 21:437-446.
- Brody, T. B., P. B. Siegel, and J. A. Cherry, 1984. Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. *Br. Poult. Sci.* 25:245-252.
- Chaney, L. W., and H. L. Fuller, 1975. The relationship of obesity to egg production in broiler breeders. *Poultry Sci.* 54:200-207.
- Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert, 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30:161-174.
- Leeson, M. S., and J. D. Summers, 1983. Consequences of increased feed allowance for growing broiler breeder pullets as a means of stimulating early maturity. *Poultry Sci.* 62:6-11.

- Pym, R. A. E., and J. F. Dillon, 1974. Restricted food intake and reproductive performance of broiler breeder pullets. *Br. Poult. Sci.* 15:245-259.
- Robbins, K. R., G. C. McGhee, P. Osei, and R. E. Beauchene, 1986. Effect of feed restriction on growth, body composition, and egg production during the breeding season. *Poultry Sci.* 65:1052-1057.
- Robinson F. E., N. A. Robinson, and T. A. Scott, 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler hens. *Can. J. Anim. Sci.* 71:549-556.
- Siegel, P. B., and E. A. Dunnington, 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in *Poultry Genetics and Breeding*. W. G. Hill, J. M. Manson, and D. Hewitt, ed. *Br. Poult. Sci. Ltd., Longman group, Harlow, England.*
- Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt, 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. *Poultry Sci.* 71:1750-1761.
- Yu, M. W., F. E. Robinson, and A. R. Robbles, 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. *Poultry Sci.* 71:1739-1749.