

REPRODUCTIVE ASSESSMENT OF TWO GENETIC STRAINS OF BROILER BREEDER  
MALES REARED ON FOUR DIFFERENT FEED INTAKE REGIMENS

by

BRITTANY MCKINNON STEVENS

(Under the Direction of Adam J. Davis)

ABSTRACT

The reproductive development of 2 meat strains (Cobb 500 and Cobb MX) of broiler breeder males provided different levels of total feed intake during rearing was evaluated. Six hundred day old cockerels from each strain were divided among 4 feeding treatments (full fed; and concave, standard, and convex restricted feed levels) with each treatment and strain housed separately. The concave and convex feeding treatments were 5-10% above and below standard strain guidelines. All cockerels were full fed to 3 weeks of age, with the 3 feed restricted treatments implemented at 22 days and continued to 20 weeks of age. At 21 weeks of age 44 cockerels from each restricted feeding treatment and strain were moved to individual cages, photostimulated and fed to maintain target body weight. Total testes weight relative to total body weight was greater in the full fed cockerels than the restricted fed birds after 8 weeks of age. There were no differences in relative testes weight between any of the feed restricted birds. For the entire rearing period, the overall relative testes size was greater for the MX males. Regardless of the feeding regimen total testosterone concentrations reached detectable levels 2 to 4 weeks earlier in the MX males than the Cobb 500 males. The ontogeny of detectable plasma

testosterone levels was also negatively correlated with total feed intake. The results suggest that sexual maturation in the MX strain may occur at a faster rate than in the Cobb 500 strain.

INDEX WORDS: Testosterone, Testes Development, Inhibin

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## Chapter 1

### **REPRODUCTIVE ENDOCRINOLOGY OF BROILER BREEDER ROOSTERS**

#### ***1.1. Structure of the Testis***

In avian species paired internal testes are located along the dorsal body wall below the spine and cranial to the kidneys. This internal location means that spermatogenesis occurs at 41° C in avian species as opposed to the scrotal temperature of about 25° C in other livestock animals. Because spermatogenesis occurs at an elevated temperature in birds, there is a higher mortality rate for the developing sperm, but this increased rate of mortality is negated by the higher synthetic capacity of birds to produce sperm per weight of testes compared to other farm animals (Bearden and Fuquay 1984; Howarth, 1995). The sensitivity to high temperatures also results in decreased semen quality and fertility in roosters exposed to heat stress (McDaniel *et al.*, 1995, 1996; Jiang *et al.*, 1999; Karaca *et al.* 2002). In addition to the higher synthetic capability, avian species also lack the accessory reproductive organs such as the bulbourethral glands, seminal vesicles and prostate which are common in mammalian species. The absence of the secretions from these accessory organs results in avian semen being much less voluminous and more concentrated than semen produced by mammalian species (Howarth, 1995)

Each individual testis is composed primarily of two types of tissue: the interstitial tissue and seminiferous epithelium. The interstitial tissue is dispersed in the spaces between the seminiferous tubules and contains blood and lymphatic vessels, nerves, and leydig cells. Thin concentric layers of myoepithelial cells (also called myoid cells or peritubular contractile cells),

fibroblasts, and connective tissue overlie the basal lamina separating the interstitial and seminiferous epithelium (Rothwell and Tingari. 1973).

The seminiferous epithelium is composed of spermatogonia and sertoli cells which completely line the circumference of each seminiferous tubule. The seminiferous epithelium within the seminiferous tubules is compartmentalized into basal and adluminal regions via tight junctions between adjacent sertoli cells. The sertoli cells are large cells that project from the basal lamina to the lumen of the seminiferous tubule. In the basal region the sertoli cells surround the spermatogonia. As they extend towards the lumen, adjacent sertoli cells lining the circumference of the seminiferous tubule contact one another and form tight junctions. The tight junctions between all the sertoli cells lining the seminiferous tubule gives rise to the blood testis barrier (Osman *et al.*, 1980; Bergmann and Schidelmeyer, 1987; Pelletier 1990). The adluminal region of the seminiferous tubule consists of the portion of the sertoli cells below the tight junctions until they terminate at the lumen of the seminiferous tubule and consists of all of the spermatogonia which are actively undergoing spermatogenesis and are embedded within crypts of the sertoli cells. The blood testes barrier protects developing sperm from toxins and mutagens as well as from the immune system which would not recognize the developing sperm as self and would mount an immune response to destroy them (Howarth, 1995).

Fully developed sperm are released into the lumen of the seminiferous tubules. The seminiferous tubule network merges into the rete testis followed by the efferent ducts, connecting ducts, the epididymal duct (commonly referred to as the epididymis in mammalian species) and finally the deferent duct (or vas deferens in mammalian species). The caudal end of each deferent duct expands into the receptacle of the deferent duct which opens into the cloaca via a papilla. The papilla of the paired deferent ducts is located next to the rudimentary phallus.

The phallus of the rooster is very small and is not a true intromittent phallus. Instead the phallus nonprotrudens is located ventro-medially in the cloaca on the ventral lip of the vent. When erected with lymph fluid the rooster phallus is heart-shaped in structure and with a median groove that facilitates the flow of semen to the outside of the vent for copulation (Nishiyama, 1955).

### **1.2. LHRH, FSH, and LH**

Gonadotropin releasing hormone, or GnRH, is released from the hypothalamic median eminence in response to environmental and physiological cues (Contijoch *et al.*, 1992; Advis and Contijoch, 1993), and it initiates the reproductive cycle by stimulating the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. The key cues that stimulate GnRH production in the domestic rooster are adequate photostimulation (greater than 12 hours), attaining a body weight to support reproduction, and having adequate nutrition to support reproduction. In avian species, GnRH is specifically referred to as luteinizing hormone releasing hormone (LHRH) because experimentally only LH release from the anterior pituitary has been proven to be stimulated by GnRH (Krishnan *et al.*, 1993; Proudman *et al.*, 2006). There are three forms of GnRH (GnRH I, GnRH II, and 1GnRH III), however only GnRH I appears to control LH secretion *in vivo* in avian species (Katz *et al.*, 1990; Sharp *et al.*, 1990; Wilson *et al.*, 1990a, 1990b, 1991; Millam *et al.*, 1998).

LH is released from the anterior pituitary in a pulsatile manner (Wilson and Sharp, 1975; Bacon *et al.*, 1991; Vizcarra *et al.*, 2004). The Leydig cells of the testes are LH sensitive (Brown *et al.*, 1975, 1977; Ishii and Furuya, 1975; Ishii and Yamamoto, 1976). The Leydig cells have

the enzymes necessary for steroidogenesis and quickly respond to LH stimulation by producing androgens (Maung and Follett, 1977; Johnson 1996).

The sertoli cells are the primary testicular target of FSH (Brown *et al.*, 1975, 1977; Ishii and Furuya, 1975; Ishii and Yamamoto, 1976). Although FSH stimulates sertoli cell growth and function, its actions are potentiated by testosterone (Brown and Follett, 1977) and the androgen receptor (Dornas *et al.*, 2008; Gonzalez-Moran *et al.*, 2008) and androgen binding protein are present in Sertoli cells.

### ***1.3. Leydig Cell Function***

The primary function of the Leydig cells is the production of androgens primarily androstenedione and testosterone. Androstenedione is the common end product of both the  $\Delta 4$  and  $\Delta 5$  steroidogenesis pathways, and it can be metabolized into testosterone, which then can be converted to estrogen by aromatase. In addition, testosterone can be converted by 5-alpha-reductase to 5-alpha-dihydrotestosterone, a very potent androgen. The androgens stimulate the expression of secondary sexual characteristics such as plumage, muscle mass and testis size as well as modulate the expression of a variety of behaviors in avian species such as aggression, territoriality, and copulation (Murton and Westwood, 1977).

Testosterone in the adult male is necessary for spermatogenesis, maintenance of the excurrent ducts and behavioral aspects such as crowing, territorial aggressiveness and mating behavior. Testosterone also inhibits apoptosis of germ cells. Conversion of testosterone to 5-alpha-dihydrotestosterone at the pituitary inhibits LH production. Similarly, at the hypothalamus estrogen produced from testosterone inhibits GnRH production (Davies *et al.*, 1980 a, b; Wilson

*et al.*, 1983). Thus, the negative feedback regulation from the testes to the brain to control testosterone production is accomplished indirectly.

In addition to its role in regulating testosterone production, estrogen has several other important roles in male reproduction. It is involved in the growth, differentiation and function of male reproductive tissues and plays vital roles in spermatogenesis (Howarth, 1995). Estrogen also plays a pivotal role in the development of the specialized feathers in male birds to attract females (Sturkie, 1976).

#### ***1.4. Sertoli Cell Function***

Sertoli cells sequester postmeiotic germ cells within the adluminal compartment of the seminiferous tubule which protect them from toxins and immunological attack. Because the developing sperm cells are protected by the Sertoli cell derived blood testes barrier, the Sertoli cells must provide a microenvironment in which spermatogenesis occurs. Therefore, they produce androgen binding protein which binds testosterone and sequesters it near developing spermatocytes for their normal growth and maturation. The Sertoli cells transfer nutrients to the developing sperm cells, and remove the debris from damaged spermatocytes or degenerating germ cells. They regulate the number of developing sperm through Fas ligand mediated apoptosis of germ cells. The Sertoli cells also produce inhibin which provides a negative feedback loop to the brain to limit the production of FSH. During embryonic development the Sertoli cells are the source of anti-Mullerian hormone, which leads to the degradation of the Mullerian duct in males for proper sexual differentiation. Finally, Sertoli cells secrete the fluid which suspends spermatozoa within the lumen of the seminiferous tubules (Kirby and Froman, 2000). Given the vital functions Sertoli cells play in sperm development it is not surprising that

the number of Sertoli cells present in the testis is proportional to testicular size and thus sperm production (Etches, 1996).

## ***1.5. Other Key Hormones Involved in Testes Function***

### ***1.5.1. Activin and Inhibin***

Activin and inhibin are two closely related dimeric glycoprotein hormones that share a common protein subunit. Activin is composed of a combination of two distinct but similar  $\beta$  subunit proteins and exists in three different forms: activin A (consisting of two  $\beta_A$  subunits), activin B (two  $\beta_B$  subunits), and activin AB (one  $\beta_A$  subunit and one  $\beta_B$  subunit). Inhibin is composed of one  $\alpha$  subunit protein and one  $\beta$  subunit protein and exists as either inhibin A (with one  $\alpha$  and one  $\beta_A$  subunit) or inhibin B ( $\alpha$  and  $\beta_B$ ). Activin and inhibin not only modulate FSH secretion from the pituitary but also have many other reproductive functions in mammalian species as reviewed by Halvorson and DeCherney (1996), Mather *et al.*, (1997), Woodruff (1998, 2002), Welt *et al.*, (2002), and Phillips and Woodruff (2004).

Follistatin, a soluble binding protein, binds activin and inhibin through their common  $\beta$ -subunit (Nakamura *et al.*, 1990). However, follistatin binds inhibin with less affinity than it does with activin (Shimonaka *et al.*, 1991; Krummen *et al.*, 1993). As reviewed by Michel *et al.* (1993), many of the biological actions of activin seem to be neutralized by the binding of activin with follistatin, but binding of activin by follistatin may not neutralize all biological activities of activin (Mather *et al.*, 1993). Changes in the bioactivity of inhibin once bound to follistatin have not been characterized.

The mRNA expression and protein expression of the inhibin and activin subunits as well as follistatin have been reported in roosters (Davis and Johnson 1998; Lovell *et al.*, 2000;

Onagbesan *et al.*, 2004). The mRNA for all of the inhibin and activin subunits is expressed throughout the prepubertal period of testicular development (Davis and Johnson 1998; Onagbesan *et al.*, 2004). Plasma inhibin A concentrations in cockerels are detectable at 5 weeks of age and show a rapid but transient rise from 14 to 18 (onset of sexual maturity) weeks of age and then decline thereafter to low levels in the adult (Lovell *et al.*, 2000). In contrast, plasma inhibin B concentrations are undetectable before 16 weeks of age but then increase progressively through 25 weeks of age in parallel with increasing plasma FSH and testosterone concentrations (Lovell *et al.*, 2000). The increased plasma concentrations of inhibin B seen in the rooster after sexual maturation is consistent with the increase in plasma inhibin B levels seen in human males after puberty and may indicate that plasma inhibin B levels in roosters may be positively correlated with sperm production as it is in humans (Andersson *et al.*, 1998; Byrd *et al.*, 1998). Circulating levels of activin A are not detectable in rooster plasma (Lovell *et al.*, 2000), but that does not negate the possibility that activin A and B may play critical autocrine and paracrine roles in the testes.

### **1.5.2. Leptin**

Leptin, a protein hormone synthesized and secreted by adipose tissue in mammals, regulates food intake and energy expenditure as reviewed by Friedman and Halaas (1998) and Houseknecht and Portocarrero (1998), and influences the onset of reproductive puberty and gonad steroidogenesis as reviewed by Smith *et al.* (2002), Ebling (2005), Zieba *et al.*, (2005), and Budak *et al.* (2006). In avian species a putative leptin protein is produced in the liver in response to feeding (Taouis *et al.*, 1998; Ashwell *et al.*, 1999; Kochan *et al.*, 2006). The biology of the chicken leptin receptor has also been fairly well characterized (Horev *et al.*, 2000; Ohkubo

*et al.*, 2000). Leptin's role in avian reproduction is not well investigated, but based on preliminary reports in hens it may provide an endocrine mechanism that allows nutritional status to influence reproduction (Ohkubo *et al.*, 2000; Paczoska-Eliasiewicz *et al.* 2003; Cassy *et al.* 2004).

### **1.5.3. Ghrelin**

In mammals, the hormone ghrelin is produced by the stomach. Ghrelin production is increased when there is a negative energy balance and many of its physiological actions involve increasing feed intake and influencing metabolism as reviewed by Korbonits *et al.* (2004), Van der Lely *et al.* (2004), and Ueno *et al.*, 2005. Ghrelin has been found to suppress LH secretion in mammals and may provide a mechanism by which insufficient caloric intake depresses reproduction (Furuta *et al.*, 2001; Fernandez-Fernandez *et al.*, 2004; Vulliamoz *et al.*, 2004; Tena-Sempere, 2005). In chickens, ghrelin mRNA expression is highest in the proventriculus (Kaiya *et al.*, 2002; Richards *et al.*, 2006). Plasma ghrelin levels increase when chicks are fasted and after refeeding return to baseline levels (Kaiya *et al.*, 2007). Plasma ghrelin levels also increase in broiler breeder hens that are fasted (Freeman, 2008). The mRNA for the ghrelin receptor has been detected in the theca and granulosa cells from hierarchical and nonhierarchical follicles and the mRNA expression of the ghrelin receptor is down-regulated by FSH and LH in cultured granulosa cells (Freeman, 2008).

### **1.5.4. Thyroid Hormone**

In mammalian species, thyroid hormones (T3 and T4) are well established as regulators of metabolism, but there is emerging evidence that they may be involved in regulating

reproduction as well. Elevated levels of thyroid hormone can delay sexual maturity, alter gonadotropin release, and increase sex hormone binding globulin production such that steroid hormone activity is altered (Fitko and Szelezyngier, 1994; Doufas and Mastorakos, 2000). Low levels of thyroid hormones are also associated with decreased androgen production (Doufas and Mastorakos, 2000). In avian species, thyroid hormones help regulate body temperature (Danforth and Burger, 1984) and growth and maturation (Bouvet *et al.*, 1987). The role of thyroid hormones in regulating reproduction in avian species has not been examined extensively. Exogenous thyroid hormone will stimulate testicular growth in quail (Follett and Nicholls, 1985; Yoshimura *et al.*, 2003). In addition, T4 concentrations are elevated and T3 concentrations are depressed during molting (Brake *et al.*, 1979; Lien and Siopes, 1989; Davis *et al.*, 2000). The elevated T4 levels during molting are interesting given that feed restricted broiler breeder hens (Bruggeman *et al.*, 1997), male chicks food deprived for about one day (Buyse *et al.*, 2000), and male quail food deprived for three days (Kobayashi and Ishii, 2002) all have reduced plasma concentrations of T3. Therefore, more research is needed to determine the potential roles thyroid hormones play in avian reproduction.

### ***1.6. Spermatogenesis and Testicular Development***

Spermatogenesis, the formation of a spermatozoa from a spermatogonia (germ cell) is 4 to 6 times more rapid in avian species than humans and other farm animals (de Reviere, 1968; Takeda 1969; Howarth 1995). In general, spermatogenesis in avian species commences with the mitosis of a spermatogonia into two daughter cells. One of these daughter cells remains quiescent and thus renews the original spermatogonia. The other daughter cell undergoes mitosis to yield two more spermatogonia which both undergo mitosis to yield 4 B spermatogonia which

move to adluminal compartment of the seminiferous tubule. The B spermatogonia undergo another mitotic division to yield 8 primary spermatocytes. Two subsequent meiotic divisions yield 16 secondary spermatocytes and 32 spermatids, respectively. The spermatids undergo cellular structural transformations (spermiogenesis) to give rise to spermatozoa that are released (spermiation) into the lumen of the seminiferous tubule.

Testicular development in avian species can be categorized into 3 phases. In the prepubertal phase the growth of the testes is slow and is linearly correlated with age and body weight. Spermatogonia are increasing in number by mitosis and at the end of this phase primary spermatocytes may be present. In the domestic rooster this phase typically spans from 0 to 10 to 14 weeks of age. The pubertal phase is next and it spans in domestic roosters from 10-14 to 20-24 weeks of age. During this phase there is rapid sertoli cell growth and thus rapid testicular growth. The first spermatozoa are produced. Sexual maturity is the final phase and during this phase testes are at maximum size and activity and the quality of spermatozoa produced is at its maximum (de Reviere and Williams, 1984).

Wild avian species are seasonal breeders and display a cyclic reduction of fertility which is accompanied by regression of the testes. The ancestors of domestic roosters were also seasonal breeders, but over time they were selected and managed for continuous breeding. For roosters, fertility peaks at about 32 weeks of age and subsequently declines until fertility is very low by 65 to 70 weeks of age. The decline in fertility is concomitant with a reduction in the number of spermatozoa in the ejaculate (Lake 1989; Sexton *et al.*, 1989). The reduction in fertility in aged domestic male fowl is also associated with an age-related decline in testicular FSH, LH and androgen receptors (Ottinger *et al.*, 1997, 2002; Gonzalez-Moran *et al.*, 2008) and an increase in the estrogen receptor alpha isoform and the progesterone receptor (Gonzalez-

Moran *et al.*, 2008). In addition, the density and morphology of the Leydig cells in aged roosters is altered such that less androgen is produced which is likely responsible for the impaired release of spermatozoa from Sertoli cells observed in aging roosters (Rosenstrauch *et al.*, 1994, 1998)

### ***1.7. Summary***

The cellular structure of the avian testes mirrors that of other domestic farm animals. However, avian testes are located internally and thus spermatogenesis occurs at the core body temperature of the male bird. Male birds also lack most of the accessory sex organs found in other farm animals. In most bird species reproduction is seasonal and the testes regress in males when they are not in the breeding season. Through genetic selection, controlled photostimulation and providing the nutrient and energy intake to support reproduction, reproductive seasonality has mostly been eliminated in commercially farmed poultry species. However, there still is a decline in male fertility as the sexually mature male ages.

## Chapter 2

### FEED RESTRICTION AND REPRODUCTION

#### *2.1. The Need for Feed Restriction*

Today's broiler breeders have been genetically selected for many generations for maximum meat yield and fast and efficient growth (Renema and Robinson, 2004). Unfortunately, this genetic selection has been associated with a loss of reproductive efficiency (Rappaport and Soller, 1966; Reddy 1996; Barbato 1999; Renema and Robinson, 2004). Furthermore broiler breeders differ from layers, in that they will consume feed beyond their nutritional requirement. Layers, conversely, will consume the appropriate feed amounts to meet their nutritional needs (Renema and Robinson, 2004). Broiler breeders are known to consume to the full capacity of the gastrointestinal tract. The assumption is that the selection for rapid growth in broilers has resulted in them having an eating behavior being controlled more by satiety mechanisms (preprandial) from the hypothalamus rather than hunger mechanisms (postprandial; Bokkers and Koene, 2003).

While the nearly insatiable appetite of broilers supports their rapid growth rate and allows them to reach a market weight of 2 to 2.5 kilograms in 6 weeks, this appetite and rapid growth rate are problematic for optimal reproductive performance in the genetically similar parent stocks of broilers. Optimum reproductive efficiency in broilers is dependent on attaining an ideal body weight to support reproduction, consuming a nutritionally adequate diet, and being photostimulated. In the United States, broiler breeder cockerels are reared on corn/soybean meal diets which are the same type of diets used to feed commercial broilers that reach market size in

6 weeks. The ideal body weight for reproduction is similar to market size, but the optimum sensitivity to photostimulation for reproduction in broilers does not occur until about 16 to 20 weeks of age (Ingkasuwan and Ogasawara, 1966; Sharp and Gow, 1983).

Testicular development is very sensitive to stimulation by increasing photoperiod lengths. A photoperiod of 12 to 14 hours is sufficient to stimulate maximum plasma LH and FSH concentrations, and testicular growth as reviewed by Sharp (1993) and Etches (1996). However, chicks are born photorefractory and acquire sensitivity to light only after they have been exposed to short day lengths (Etches, 1996). The duration of the exposure to short days before photosensitivity is acquired is not well researched, but it appears to be 8-12 weeks as reviewed by Etches (1996). Cockerels that are not photostimulated will still reach sexual maturity and produce sperm based on attaining a body weight to support reproduction and consuming a diet that meets the energy demands of reproduction. Domestic fowl reared on a daily, continuous lighting schedule that provides either 6 hours or 22 hours of light will still reach sexual maturity at about 21 weeks of age (Johnson, 2000).

Because maximum hatching egg production is the goal for broiler breeder farmers, they must manage their broiler breeder pullets so ideal body weight for the onset of reproduction and maximum egg size is achieved at about 20 to 21 weeks of age and matches the acquisition of photosensitivity for reproduction. As a result, cockerels have been managed to fit the same time schedule so their sexual maturity coincides with egg production by the hens. However, sexual maturity in cockerels could probably be achieved at an earlier age closer to the acquisition of photosensitivity at 8-12 weeks of age without a loss in male fertility based on research conducted in turkey toms (Etches *et al.*, 1993).

To prevent broiler breeder cockerels from growing too quickly and becoming obese and reproductively unfit by 20 to 21 weeks of age when they are placed with pullets and photostimulated for reproduction, their dietary intake is severely restricted. Typically, feed allocations are 60-80 percent less during the rearing period and 25-50 percent less during the laying period than what the breeder pullets/hens would consume *ad libitum* (Renema and Robinson, 2004). For the larger-bodied cockerels the amount of feed restriction is equal or greater than that imposed on the pullets.

## ***2.2. Quantitative Feed Restriction***

There are two basic approaches to feed restricting broiler breeders, a severe quantitative restriction of the typical energy dense corn and soybean meal based diet or a qualitative restriction in which the nutrient density of the diet is diluted so that more feed can be fed at a given time. Typically for the quantitative restriction feeding programs, the corn/soybean meal diet that is used is so nutrient dense that the amount of feed available on daily basis during the rearing period is not enough to be distributed to all of the available feeder space of the rearing facility. Therefore, the cockerels are typically fed on a skip a day basis so that the feed allotment for two days can be combined. By combining two days worth of feed, the amount of feed that is fed is large enough that it can be distributed to all the available feeder space, which reduces the competition among the birds and helps ensure that the less aggressive birds also get their portion of feed. The reduction in competition for food results in improved flock body weight uniformity (Bartov *et al.*, 1988), which leads to better overall flock performance because their nutrient and management requirements are similar. However, even with this management technique there is still variation in testes size and development across the flock of sexually mature males (Vizcarra

*et al.*, 2000). Once the males are placed with females and are actively breeding, the amount of feed needed to support fertility and their health allows the total volume of feed to be sufficient so that the roosters can be placed on an everyday feeding schedule.

### ***2.3. Qualitative Feed Restriction***

In an effort to decrease the severity of the feed restriction associated with quantitative feed restriction programs, researchers have developed and tested qualitative feed restriction programs. The goal of the qualitative feed restriction programs is to increase the amount of feed available to be fed through the use of feed diluents or by utilizing less nutrient dense feed ingredients. Research in this area has almost been exclusively targeted towards broiler breeder pullets and hens. Zuidhof *et al.*, (1995) fed broiler breeder hens standard rearing and laying diets from 0 to 56 weeks of age or the standard diets diluted with either 15% or 30% with ground oat hulls. The birds fed the diluted diets had better flock body weight uniformity and decreased stress levels than the birds fed the standard undiluted diets. The better stress levels and flock uniformity values probably resulted from the increased availability of feed. In fact, during the lay period the average time until all the feed was consumed was 264, 349, and 489 minutes for the standard diet, and the standard diet diluted with 15 or 30 percent oat hulls, respectively. Hens fed the diet with the 15% ground oat hulls had the highest egg production and chick production of all the treatments through 56 weeks of age. Egg production for the birds fed 30 percent ground oat hulls was equivalent to the control birds. Tolkamp *et al.*, (2005) also utilized oat hulls (400 g/kg of diet) as a dietary diluent, but they combined its use with Ca propionate, an appetite suppressant. By adding both oat hulls and Ca propionate to the diet, Tolkamp *et al.*, were able to feed the diet *ad libitum* to broiler breeder pullets during the rearing phase. Even

though the pullets were fed *ad libitum* and consumed 6.54 kg/bird more feed than the pullets fed a control diet lacking the oat hulls and Ca propionate, they gained body weight at an equivalent rate to the control birds and had equal subsequent reproductive performance.

Lordelo et al. (2004) utilized a different approach to qualitative feed restriction. Instead of diluting the diet with “filler” such as oat hulls, they reared broiler breeder pullets from 2 to 18 weeks of age with either a standard corn/soybean meal diet or a diet in which the soybean meal was replaced with cottonseed meal, which has a lower nutrient density than soybean meal. The birds that were fed the cottonseed meal diet had to be fed a greater amount of feed in order to achieve the same body weight as those fed the diet containing soybean meal. In particular, the birds could be fed more of the corn/cottonseed meal diet without increasing body weight gain compared to the birds fed the corn/soybean meal diet because of the very low levels of total and available lysine in cottonseed meal relative to soybean meal. As a result of being provided more feed than the birds fed the diet containing soybean meal, the birds fed the corn/cottonseed meal diet had significantly better flock body weight uniformity.

#### ***2.4. Decreasing Fertility of Male Broiler Breeders***

Despite feed restriction programs controlling broiler breeder body weight, fertility rates of male broiler breeders continue to decline. Geneticists continue to select for growth and yield characteristics rather than reproductive traits (Reddy and Sadjadi, 1990; Brillard 2004). To maintain acceptable fertility rates, the female to male ratio has decreased in breeder flocks, and the replacement of males over the life cycle of the breeding hens has increased as has the use of sex separate feeding programs (Hammerstedt, 1999). However, increasing the number of males relative to females in a flock is limited by the need to maintain the social structure of the flock

and thus fertility levels. Replacing old males in the breeder flock with younger males (spiking) while normally sustaining flock fertility at high levels, can disrupt the flock social behavior to the point of reducing fertility and it increases the cost of hatched chicks. Thus these management practices are not long term solutions to the problem of declining fertility in male broiler breeders and point to the need of including male fertility in the genetic selection of broiler breeder stocks (Hammerstedt, 1999; Brillard, 2004).

The decline in fertility is especially severe in broiler breeder males as the later stage of the reproductive cycle commences around 50 weeks of age (Kirk *et al.*, 1980; Walsh and Brake 1997). Although as discussed earlier there is an age related decrease in fertility in all male species, the fact that fertility can be maintained by artificial insemination at the end of the broiler breeder production cycle, suggests that the decline in fertility seen commercially with natural mating is associated with the physical condition of the males (Brillard and McDaniel, 1986). A decline in mating activity and efficiency is largely attributed to the heavy body weight and poor body condition of older males (Duncan *et al.*, 1990; Hocking, 1990). Older, overweight males are more likely to have difficulty in achieving cloacal contact with hens (Soller *et al.*, 1965; Hocking and Duff, 1989; Hocking *et al.*, 1989). In addition, older males often have fertility issues due to a higher prevalence of leg problems and lameness (Duff and Hocking, 1986, Hocking and Duff 1989; Hocking *et al.*, 1989) that are related to excessive male body weight (Hocking and Bernard, 2000; Hocking and Robertson, 2000).

### ***2.5. The Timing and Degree of Feed Restriction for Optimum Fertility***

While the necessity of feed restricting broiler breeders is widely recognized by poultry scientists and producers, the degree of feed restriction as well as the timing and duration of feed restriction during a broiler breeder's life cycle is not agreed upon. The primary focus of this

research has been with broiler breeder pullets and hens (Pym and Dillon 1974; McDaniel *et al.*, 1981; Robbins *et al.*, 1986 and 1988; Robinson *et al.*, 1991; and Yu *et al.*, 1992a, b; Bruggeman *et al.*, 1999; Gibson *et al.*, 2006). Although the research has yielded conflicting results the overall consensus is that some degree of feed restriction should occur in both the rearing and breeding periods for optimum reproductive performance.

There is a perception that under restricting males during rearing such that early sexual precocity occurs, results in a more rapid decline in reproductive performance as the roosters age (Brillard, 2004). On the other hand, there is growing evidence that the decline in male fertility in late production is not simply the result of males becoming overweight but rather due to over restriction or a metabolizable energy deficiency (Buckner *et al.*, 1986; Sexton *et al.*, 1989 a, b; Cerolini *et al.*, 1995; Bramwell *et al.*, 1996, Romero-Sanchez *et al.*, 2008). Poor fertility after 45 weeks of age could also be reversed by increasing male feed allocation (Cerolini *et al.*, 1995; Romero-Sanchez *et al.*, 2007a, b). More research is needed in male breeders to determine the degree and timing of feed restriction for the development and maintenance of optimum fertility levels in these birds.

## **2.6. Welfare Issues**

Millman, *et al.*, (2000) reported that broiler breeder males exhibit extremely high levels of aggression which varies across genetic strains but is not associated with feed restriction. Males fed *ad libitum*, displayed the most male to male and male to female aggression and sexual behavior was not affected by feeding regimen (Millman, *et al.*, 2000). However, *ad libitum* feeding of broiler breeders is thought to be detrimental to the welfare of the birds because it leads to obesity which results in higher mortality (Katanbaf *et al.*, 1989; Bruggeman *et al.*, 2005) and

more joint problems as reviewed by Renema and Robinson (2004). But, *ad libitum* feeding reduces stress (Kubikova *et al.*, 2001). Broiler breeder hens that are feed restricted have increased abnormal behaviors related to stress, including more time spent pecking at feeders, less time sitting, more time pacing, and less time preening (Kubikova *et al.*, 2001). Plasma corticosterone levels may also indicate the amount of stress a bird is experiencing as higher levels of corticosterone are indicative of higher stress levels in avian species. Of course, corticosterone levels may also be high when measured in experimental birds due to the stress of handling and/or removal of blood by venipuncture, but Etches (1976) reported that bird handling or removal of blood should not affect plasma corticosterone levels based on an experiment that compared corticosterone levels in samples collected from birds through vein puncture or through a previously placed catheter. Broiler breeder hens that are feed restricted according to the breeder's guidelines have higher corticosterone levels than birds fed *ad libitum*, fed twice the amount of the restricted birds, or fed a qualitatively restricted diet diluted with 30% hardwood dust (Kubikova *et al.*, 2001). Thus, feed restricted birds may be stressed because they may be hungry, bored, or stressed by the increased competition amongst their flock mates when feed is given to them.

Utilizing a qualitative feed restriction program to feed birds may be better for animal welfare compared to quantitative feed restriction programs. Sandilands *et al.* (2005) noted lower cortisol levels, less object picking (less signs of boredom), and less feed motivation in qualitatively restricted birds compared to birds quantitatively restricted throughout their lives. Zuidhof *et al.* (1995) also reported that broiler breeders fed a qualitatively restricted diet implemented during the rearing and lay periods spend less time at the water source and that their heterophil:lymphocyte ratios suggest a lower stress level at 12 weeks of age compared to a

standard quantitatively restricted diet implemented during the rearing and lay periods. Kubikova *et al.* (2001) also reported that hens which were qualitatively feed restricted had lower corticosterone levels than hens that were quantitatively feed restricted, but they had higher corticosterone levels than hens fed *ad libitum*.

## **2.7. Summary**

Restricting feed intake is necessary in male broiler breeders to prevent them gaining too much body weight which can be detrimental to fertility. Feed restriction methods fall into two categories, qualitative and quantitative. In quantitative feed restriction programs small amounts of a typical commercial broiler diet are fed, while in qualitative restriction programs the nutrient content of the typical diet is diluted so that more of it can be fed. Although the best timing and the degree to which feed needs to be restricted is not well defined for optimum male fertility, in most commercial settings in the United States broiler breeders are restricted during both the rearing and breeding periods. Typically some form of a quantitative skip a day feed restriction program is used during the rearing phase followed by a quantitative every day feed restriction program during the breeding/egg production period. Much more research is needed to determine the best feed restriction protocol that maximizes fertility in male broiler breeders.

### **Chapter 3**

#### **STATEMENT OF PURPOSE**

Genetic selection for meat yield and fast and efficient growth in broilers is associated with a loss of reproductive efficiency. To maintain acceptable male fertility rates the broiler breeder industry has increased the number of males housed with females and replaces aging males with younger more fertile males during the breeding production cycle. However, these management practices are costly and are also ultimately limited by the need to maintain the behavioral social structure of the flock. Thus, these management practices are not long term solutions to the problem of declining fertility in male broiler breeders and point to the need of including male fertility in the genetic selection of broiler breeder stocks. Broiler breeder males are heavily feed restricted throughout their life to prevent them from obtaining a body weight which is too large for effective mating or having too much body fat which can reduce spermatogenesis. Although defining proper feed restriction protocols in female broiler breeders has received a lot of research attention, the same is not true for male broiler breeders. More research is needed in male breeders to determine the degree and timing of feed restriction for the development and maintenance of optimum fertility levels in these birds. Therefore, the purpose of the current research is to compare the reproductive development of a newly developed parent line (Cobb MX) of broiler breeder males relative to its progenitor (Cobb 500), to determine how different levels of feed restriction affect reproductive development in both strains and to determine if plasma inhibin-B concentrations in roosters are correlated with sperm mobility values and thus may be an indicator of fertility.

## Chapter 4

### MATERIALS AND METHODS

#### 4.1. Experiment 1

Four environmentally controlled rooms were used to rear the cockerels for this experiment. The rooms measured  $7.32 \times 9.14$  m and had pine shavings for litter. A nylon mesh partition was placed down the middle of each room to form two equal sized pens for a total of eight pens across the 4 rooms. At one day of age 560 Cobb 500 fast-feathering and 560 Cobb MX cockerels were randomly divided among four rooms so that each room contained a pen of 140 Cobb 500 cockerels and a pen of 140 Cobb MX cockerels. The temperature of each room was maintained at  $32.2^{\circ}\text{C}$  for the first week, and then decreased by approximately  $2.8^{\circ}\text{C}$  every week thereafter until a target temperature of  $21^{\circ}\text{C}$  was reached. From one to three days of age the chicks were given 24 hours of light per day and then from 4 to 21 days of age the amount of light was decreased from 24 to 8 hours per day. The 8 hour per day lighting schedule was then maintained until the conclusion of the experiment at 28 weeks of age. All the cockerels were beak trimmed at the hatchery and were maintained on an industry standard vaccination program throughout the experiment. Feed was distributed in each pen by automatic chain feeders and the birds were given *ad libitum* access to water from nipple drinkers. All cockerels were wing-banded for identification purposes. All animal procedures were approved by the Animal Care and Use Committee of the University of Georgia.

All birds were fed a standard starter corn/soy diet (Table 4.1) *ad libitum* from 0 to 3 weeks of age and a developer diet (Table 4.1) from 3 through 20 weeks of age. At 3 weeks of age each of the four rearing rooms was assigned one of the following feed restriction treatment:

**Table 4.1.** Composition of the experimental diets (Experiments 1 and 2).

Ingredient	Diet		
	Starter <sup>1</sup>	Developer <sup>2</sup>	Layer <sup>3</sup>
	-----% of diet-----		
Corn	62.95	57.82	70.87
Soybean meal, 49% CP	22.24	12.78	19.20
Poultry fat	0.00	0.50	0.59
Wheat middlings	10.53	25.00	0.00
Limestone	1.16	1.11	6.63
Deflourinated phosphate	1.75	1.56	1.85
Sodium chloride	0.54	0.35	0.30
Vitamin premix <sup>4</sup>	0.50	0.50	0.30
DL-Methionine	0.15	0.20	0.08
L-Lysine	0.10	0.09	0.10
Trace mineral premix <sup>5</sup>	0.08	0.08	0.08
Calculated analysis <sup>6</sup>			
M. E. (kcal/kg)	2,865.00	2,866.00	2,921.00
Crude protein (%)	18.00	15.00	15.00
Lysine (%)	1.00	0.75	0.84
Calcium (%)	0.91	1.03	3.25
Methionine and cystine (%)	0.73	0.70	0.63
Available phosphorus (%)	0.45	0.44	0.45

<sup>1</sup>Starter diet was fed to 3 weeks of age.

<sup>2</sup>Developer diet was fed from 3 through 28 weeks of age in Experiment 1 and from 3 through 20 weeks of age in Experiment 2.

<sup>3</sup>Layer diet was fed from 21 to 60 wk of age (Experiment 2).

<sup>4</sup>Vitamin premix provided the following per kg of diet: vitamin A, 5,510 IU; vitamin D<sub>3</sub>, 1,100 IU; vitamin E, 11 IU; vitamin B<sub>12</sub>, 0.01 mg; riboflavin, 4.4 mg; niacin, 44.1 mg; d-pantothenic acid, 11.2 mg; choline, 191.3 mg; menadione sodium bisulfate, 3.3 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

<sup>5</sup>Trace mineral premix provided the following in mg per kg of diet: Mn, 60; Zn, 50; Fe, 30; I, 1.5; and Se 0.5.

<sup>6</sup>Calculated analysis was based on Dale (2001).

full fed, standard restricted, concave restricted and convex restricted. The standard feed restriction program followed the feeding recommendations of the primary breeder for each genetic line. The concave feed restriction program provided the cockerels with 3 to 13 percent less feed than the standard feed restriction program from 4 through 20 weeks of age (Table 4.2) while the convex feeding program provided the cockerels 2 to 13 percent more feed than the standard feed restriction program from 4 through 20 weeks of age (Table 4.2). Although the newly developed Cobb MX meat strain is related to the Cobb 500 strain, it is a larger bird and thus the Cobb MX cockerels were fed 3.5 to 5.7 percent more feed from 3 to 21 weeks of age (Table 4.2). From 3 through 20 weeks of age the cockerels that were on the standard, concave and convex feed restriction programs were fed on a skip a day basis. After the rearing period concluded at the end of 20 weeks of age, the cockerels from each feed restriction program were fed to maintain their target body weight until the experiment ended at 28 weeks of age.

A random selection of 25 percent of the birds from each pen was weighed every week to monitor body weight progression of the cockerels on all the feeding programs. Over half of the mortality in the full fed cockerels was associated with leg problems leading to immobility (data not shown). In addition, there was also a high incidence (about 30% of the mortality) of sudden deaths in the full fed cockerels, which may have been linked to heat attacks. In addition, based on the weight of the cockerels fed on the standard feed restricted program, weekly feed allocations were determined so that the body weight gain of these cockerels matched the recommended guidelines of the primary breeder. All the cockerels were weighed at 7, 11, 15 and 20 weeks of age for subsequent statistical analyses of body weight between the different genetic strains and feeding programs. All mortality was recorded on a daily basis throughout the experiment.

#### ***4.1.1 Testes and blood collection***

Testes were collected at 6, 8, 11, 13, 15, 18, 21, 25, 26 and 28 weeks of age from 5 cockerels from each genetic strain on each feed restriction program except at 6 and 8 weeks of age when testes were collected only from cockerels from the full fed and standard feed restriction programs. The 5 cockerels chosen for each combination of genetic strain and feed restriction program was based on the cockerels having body weights that reflected the body weight profile of the given treatment pen. The selected cockerels were weighed and then killed by cervical dislocation. The testes were quickly removed from each bird, cleaned of any connective tissue, weighed and then placed in Davidson's Fixative. Testis that weighed over 15 grams were sliced horizontally on the top and bottom at a depth of about one-third their total height to ensure complete penetration of the Davidson's Fixative. Collected testes from each cockerel remained in the Davidson's Fixative solution for 24 hours, were rinsed with 70 percent (v/v) ethanol and then stored in 70 percent ethanol for subsequent further processing.

Blood samples were collected from a minimum of 5 cockerels from each genetic strain and feed restriction combination every other week from 6-28 weeks of age, except at 6 and 8 weeks of age when blood samples were only collected from cockerels from the full fed and standard feed restriction programs. The cockerels chosen for sampling from each combination of genetic strain and feed restriction program was based on the cockerels having body weights that reflected the body weight profile of the given treatment pen. Blood was collected from the brachial vein of each cockerel, immediately placed into individual glass Vacutainer tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ) containing EDTA as an anticoagulant, and stored on ice. Samples were then centrifuged at  $1,000 \times g$  at  $4^{\circ}\text{C}$  for 10 minutes. Plasma was collected from each sample and frozen at  $-80^{\circ}\text{C}$  for subsequent testosterone analysis.

**Table 4.2.** Feed allotments per cockerel per day of cockerels reared on four different feed restriction programs from 2 to 20 weeks of age (Experiment 1)<sup>1</sup>.

Age (week)	Feed restriction program and genetic strain							
	Standard Cobb 500	Standard Cobb MX	Concave Cobb 500	Concave Cobb MX	Convex Cobb 500	Convex Cobb MX	Full fed Cobb 500	Full fed Cobb MX
3	55	58	55	58	55	58	160	155
4	55	58	53	56	56	59	157	152
6	55	58	53	56	58	61	274	263
7	56	59	54	57	60	62	235	164
8	58	61	54	57	63	65	310	292
9	60	63	55	58	66	69	233	262
10	62	65	56	59	69	72	294	262
11	64	67	57	60	72	75	329	318
12	66	69	58	61	74	78	331	325
13	68	71	59	62	76	80	338	449
14	70	73	61	64	78	82	328	349
15	72	75	63	66	80	84	415	472
16	74	77	65	68	82	86	389	329
17	77	80	68	71	85	89	314	297
18	81	84	72	75	88	92	453	365
19	86	89	79	82	93	97	459	377
20	93	97	86	89	98	102	513	404

<sup>1</sup>The weekly feed allotment values for the full fed cockerels fluctuate from week to week

because they reflect the amount of feed added to the automatic feeder system. There were weeks when less feed was added than the previous week because feed was left over from the previous week. This experiment was also conducted during the summer and there were times when the capacity of the evaporative cooling system to keep the rearing room temperature in the thermal neutral comfort zone of the very large body weight cockerels on the full fed treatment was exceeded and thus feed consumption decreased.

#### ***4.1.2 Seminiferous tubule diameter and epithelium height***

A cross section of each testis that had been stored in 70 percent ethanol was cut processed, and embedded in paraffin. A 4-5 micron cross section was then sectioned from the paraffin block and stained with Hematoxylin and Eosin. Samples were then examined by light microscopy. One stained testis cross section for each of the 5 cockerels from each genetic strain and feed restriction program combination was used to determine seminiferous tubule diameter and seminiferous epithelium height at 22, 24, 26 and 28 weeks of age. A total of 5 representative seminiferous tubules per slide were used to determine seminiferous diameter and epithelial height. The diameter of each of the 5 tubules was determined by averaging a top to bottom and a side to side measurement taken directly across the center of the tubule from the outer most edges of the tubule. The calculated diameter for each of the 5 tubules was then averaged to obtain a value for each cockerel. The epithelial height measurement for each tubule was based on averaging three individual measurements that were taken from the outer most edge of the seminiferous tubule to the luminal opening of the tubule. The calculated epithelial height for each of the 5 tubules was then averaged to obtain a value for each cockerel. Morphological indices were determined using computer-aided light microscopy (10x magnification of the objective lens) image analysis.

#### ***4.2. Experiment 2***

At twenty one weeks of age, 88 cockerels (44 Cobb 500 and 44 Cobb MX) from each of the standard, concave and convex feeding programs were removed from the rearing pens (Experiment1) and placed into individual suspended battery cages measuring 56 cm high by 56 cm long and 41 cm wide. Each cage was equipped with a nipple drinker and a trough feeder.

The 44 cockerels selected for each genetic strain and feed restriction program combination were chosen so that they reflected the body weight distribution profile of the entire population of cockerels for each treatment at 20 weeks of age in Experiment 1. The cockerels were photostimulated at 22 weeks of age by providing 14 hours of light per day. The cockerels were fed a standard laying diet (Table 4.1) that would normally be used when roosters are housed with broiler breeder females during egg production. From 22 through 65 weeks of age the roosters were fed on a daily basis to maintain target body weight established in Experiment 1 for each genetic strain and feed restriction program (Table 4.3). All of the roosters were individually weighed at 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 34, 36, 38, 40, 42, 45, 50, 55 and 60 weeks of age to monitor the body weight progression of the roosters on all the feeding programs. In addition, the weight of the roosters fed on the standard feed restricted program was used to determine weekly feed allocations so that the body weight gain of these roosters matched the recommended guidelines of the primary breeder.

Blood samples were taken from 20 of the 44 roosters in each treatment group at 23.5, 25.5, 27.5, 31, 35, 41, 46, 51, 56, 61 and 65 weeks of age. The 20 roosters for each treatment group were selected based on body weight such that the body weight profile was the same for this subset of birds as the whole population and that all statistical differences in body weight for the roosters that existed among the genetic strains and feeding programs at 22 weeks for the whole population also existed for this population subset. The same 20 roosters for each treatment were used for blood sampling throughout the experiment. Blood samples were collected between 1100 and 1330 hours at each collection date. Blood was collected and processed as previously described for Experiment 1.

Semen samples were collected from all of the roosters at 46, 51, 56, 61 and 65 weeks of age for sperm mobility determinations. Sperm mobility was determined as previously described (Froman and Feltmann 1998, Froman *et al.*, 1999).

**Table 4.3.** Feed allotments per rooster per day of cockerels reared on three different feed restriction programs from 21 to 65 weeks of age (Experiment 2).

Age (week)	Feed restriction program and genetic strain					
	Standard Cobb 500	Standard Cobb MX	Concave Cobb 500	Concave Cobb MX	Convex Cobb 500	Convex Cobb MX
	------(g)-----					
21	93	97	86	89	98	98
22 – 23	96	100	90	93	100	103
24 – 28	99	102	93	96	102	105
29 – 30	99	102	98	100	99	102
31 – 32	99	102	98	100	99	101
33	98	101	97	99	98	101
34 – 35	98	100	98	100	98	100
36 – 65	100	102	100	102	100	102

#### 4.3. Total testosterone RIA

Plasma total testosterone was determined by RIA using the Coat-A-Count Total Testosterone Kit (Diagnostic Products Corporation, Los Angeles, CA) following the manufacturer's protocol. The RIA samples were counted with a Wallac Wizard 1470 gamma counter (Perkin-Elmer, Waltham, MA). The mean interassay and intraassay CV for both assays were less than 10.

#### ***4.4. Two-Site Dimeric Inhibin-A and Inhibin-B ELISAs***

Based on sperm mobility measurements obtained at 46, 51, 56, and 61 weeks of age from the roosters in Experiment 2, a high and low sperm mobility group of roosters were selected. Nineteen low mobility roosters were selected based on having sperm mobility absorbance values less than 0.186 when measured at 46, 51, 56 and 61 weeks of age. The overall (46 to 61 weeks of age) mean  $\pm$  SEM sperm mobility absorbance value at 550 nm for the 19 roosters was  $0.085 \pm 0.006$  with a range of 0.036 to 0.150. Twenty high mobility roosters were selected based on having sperm mobility absorbance values greater than 0.4 when measured at 46, 51, 56 and 61 weeks of age. The overall (46 to 61 weeks of age) mean  $\pm$  SEM sperm mobility absorbance value at 550 nm for the 20 roosters was  $0.556 \pm 0.014$  with a range of 0.454 to 0.691. To determine if plasma inhibin levels are correlated with sperm mobility a two-site dimeric inhibin-A, 96-well plate ELISA kit (DSL-10-28100) and a two-site dimeric inhibin-B, 96-well plate ELISA kit (DSL-10-84100) (Diagnostic Systems Laboratories Inc., Webster, Texas) were utilized to measure dimeric inhibin A and B respectively, in plasma samples obtained at 61 weeks of age from the low and high mobility roosters. Procedures for the dimeric ELISAs were performed following the manufacturer's protocols. The plates were analyzed with a Victor 3 plate reader (Perkin-Elmer, Waltham, MA).

#### ***4.5. Statistical analyses***

Data were subjected to ANOVA using the General Linear Model procedure with genetic strain and feed restriction program as factors. Tukey's multiple-comparison procedure (Neter *et al.*, 1990) was used to detect significant differences among the different feed restriction programs. All statistical procedures were done with the Minitab Statistical Software package

(Release 13, State College, PA). Differences were considered significant when  $P$ -values were < 0.05.

## Chapter 5

### RESULTS

#### *5.1 Experiment 1*

The body weight profiles (Figure 5.1) based on weighing a random sample of 25 percent of the cockerels in each feed restriction program and genetic strain combination during the rearing period from 3 through 20 weeks of age indicated the rapid body weight gain for the full fed cockerels relative to the feed restricted cockerels. When all of the cockerels were weighed at 11, 15 and 20 weeks of age the body weights of the cockerels fed on the four different feed restriction programs all differed (Table 5.1). Given that the feed restricted Cobb MX males were fed 3.5 to 5.7 percent more than the Cobb 500 males it is not surprising that overall these cockerels weighed more at 7, 15 and 20 weeks of age (Table 5.1). However, this difference in body weight between the two genetic strains was not observed in the full fed cockerels or in the convex restricted cockerels at 7, 11, 15 and 20 weeks of age (Table 5.1). The difference in body weight between the full fed and feed restricted cockerels was maintained in the 5 cockerels used for testes collection for each treatment at 21, 25, 26 and 28 weeks of age (Table 5.2)

For both genetic strains of cockerels the percent cumulative mortality for the full fed cockerels through 20 weeks of age was more than double that of the cockerels in any of the other feed restriction programs (Table 5.3). The overall mortality across treatments through 20 weeks of age was 7% higher for the Cobb 500 genetic strain than the Cobb MX strain of cockerels (Table 5.3).

Testes growth and development occurred at an earlier age in the full fed cockerels than any of the other feed restriction programs (Tables 5.4 – 5.7). Testes weight relative to body

weight was significantly greater in the full fed cockerels compared to the standard fed cockerels from 8 to 25 weeks of age (Tables 5.4 and 5.5). Testes weight in the cockerels from the

**Figure 5.1.** Body weight of two genetic strains of broiler breeder cockerels reared on four different feed restriction programs from 4 to 20 weeks of age. The body weight profile of the cockerels from all four feed restriction programs (A) and the body weight profile of the cockerels excluding the cockerels from the full fed feeding program (B). Abbreviations: std = standard feed restriction, con = concave feed restriction program, cvx = convex feed restriction program, ff = full fed feed restriction program, 500 = Cobb 500 genetic strain, and MX = Cobb MX genetic strain.



**Table 5.1.** Body weight of two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		7	11	15	20
		------(g)-----			
Standard	Cobb 500	1212 ± 12	1660 ± 19	2006 ± 30	2531 ± 42
Standard	Cobb MX	1250 ± 12*	1735 ± 20*	2139 ± 32*	2669 ± 48*
Concave	Cobb 500	1188 ± 12	1553 ± 17	1840 ± 28	2264 ± 38
Concave	Cobb MX	1221 ± 11*	1645 ± 16*	1954 ± 29*	2448 ± 39*
Convex	Cobb 500	1233 ± 13	1783 ± 23	2241 ± 35	2798 ± 50
Convex	Cobb MX	1283 ± 13*	1811 ± 21	2303 ± 29	2901 ± 39
Full fed	Cobb 500	2589 ± 31	5488 ± 46	6139 ± 87	7295 ± 155
Full fed	Cobb MX	2658 ± 30	5405 ± 45	6367 ± 82	7258 ± 108
Standard		1231 ± 8 <sup>bc</sup>	1698 ± 14 <sup>c</sup>	2074 ± 22 <sup>c</sup>	2600 ± 32 <sup>c</sup>
Concave		1205 ± 8 <sup>c</sup>	1599 ± 12 <sup>d</sup>	1898 ± 20 <sup>d</sup>	2359 ± 28 <sup>d</sup>
Convex		1258 ± 9 <sup>b</sup>	1797 ± 15 <sup>b</sup>	2274 ± 23 <sup>b</sup>	2853 ± 32 <sup>b</sup>
Full fed		2625 ± 21 <sup>a</sup>	5443 ± 32 <sup>a</sup>	6256 ± 60 <sup>a</sup>	7275 ± 93 <sup>a</sup>
	Cobb 500	1534 ± 26	2466 ± 71	2803 ± 80	3132 ± 87
	Cobb MX	1592 ± 27*	2577 ± 68	2925 ± 79*	3262 ± 81*

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-d</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 150$  cockerels for each feeding program and genetic strain combination,  $n = 300$  cockerels for each feeding program and  $n = 600$  cockerels for each genetic strain.

**Table 5.2.** Body weight of two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic Strain	Age (week)			
		21	25	26	28
		------(g)-----			
Standard	Cobb 500	2510 ± 132	3580 ± 223	3186 ± 239	3687 ± 362
Standard	Cobb MX	2718 ± 299	3592 ± 196	3707 ± 255	3660 ± 287
Concave	Cobb 500	2220 ± 295	3289 ± 203	3140 ± 254	3176 ± 279
Concave	Cobb MX	2337 ± 257	3506 ± 215	3204 ± 217	3351 ± 196
Convex	Cobb 500	2767 ± 334	3857 ± 278	3646 ± 378	3709 ± 244
Convex	Cobb MX	2929 ± 271	3721 ± 306	3701 ± 237	3865 ± 342
Full fed	Cobb 500	6692 ± 720	7778 ± 515	7320 ± 132	7638 ± 442
Full fed	Cobb MX	6872 ± 291	7614 ± 274	7402 ± 267	7293 ± 354
Standard		2614 ± 158 <sup>b</sup>	3586 ± 140 <sup>b</sup>	3447 ± 186 <sup>b</sup>	3674 ± 218 <sup>b</sup>
Concave		2278 ± 185 <sup>b</sup>	3398 ± 144 <sup>b</sup>	3172 ± 158 <sup>b</sup>	3264 ± 163 <sup>b</sup>
Convex		2848 ± 204 <sup>b</sup>	3789 ± 196 <sup>b</sup>	3674 ± 211 <sup>b</sup>	3787 ± 200 <sup>b</sup>
Full fed		6782 ± 367 <sup>a</sup>	7696 ± 276 <sup>a</sup>	7361 ± 141 <sup>a</sup>	7465 ± 281 <sup>a</sup>
	Cobb 500	3547 ± 463	4652 ± 430	4323 ± 418	4552 ± 440
	Cobb MX	3714 ± 440	4608 ± 415	4504 ± 403	4398 ± 370

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ; P < 0.05.

<sup>1</sup>Values are means ± SEM, n = 5 cockerels for each feeding program and genetic strain combination, n = 10 cockerels for each feeding program and n = 20 cockerels for each genetic strain.

**Table 5.3.** Cumulative mortality of two genetic strains of broiler breeder cockerels reared on four different feed restriction programs from 3 to 20 weeks of age (Experiment 1).

Age (week)	Feed restriction program and genetic strain								Feed restriction program				Genetic strain	
	Stan- dard Cobb 500	Stan- dard Cobb MX	Con- cave Cobb 500	Con- cave Cobb MX	Con- vex Cobb 500	Con- vex Cobb MX	Full fed Cobb 500	Full fed Cobb MX	Stan- dard	Con- cave	Con- vex	Full fed	Cobb 500	Cobb MX
	-----%-----													
1	1	1	1	0	3	2	3	1	1	1	2	2	3	2
2	1	1	1	0	3	2	4	1	1	1	3	3	3	2
3	3	1	1	0	3	2	5	2	2	1	3	4	4	2
4	3	1	2	0	3	2	6	3	2	1	3	4	5	2
5	3	2	2	0	3	2	6	3	3	1	3	4	5	2
6	5	4	2	0	4	2	9	5	5	1	3	7	7	4
7	5	4	2	0	4	2	10	5	5	1	3	8	7	4
8	9	7	2	0	7	2	15	9	8	1	4	12	11	6
9	9	7	2	0	7	3	17	9	8	1	5	13	12	6
10	9	7	3	0	7	3	19	9	8	1	5	14	13	6
11	12	11	6	4	11	6	23	13	12	5	8	18	18	11
12	13	11	6	4	11	6	27	17	12	5	8	22	19	12
13	16	14	9	7	16	9	33	24	15	8	13	29	25	18
14	17	15	9	7	16	9	34	28	16	8	13	31	26	20
15	21	19	15	11	19	13	40	37	20	13	16	38	32	26
16	22	21	17	13	19	13	49	43	22	15	16	46	35	30
17	22	23	17	13	19	13	52	49	23	15	16	51	37	33
18	26	27	22	17	23	16	67	59	26	19	20	63	46	39
19	26	28	24	17	24	16	67	60	27	21	20	64	47	40
20	26	28	24	18	25	16	71	63	27	21	21	67	49	42

**Table 5.4.** Testes weight as a percent of body weight during the early rearing period for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic Strain	Age (week)				
		6 <sup>2</sup>	8 <sup>2</sup>	11	13	15
-----[total testes weight (g)/total body weight (g)]*100-----						
Standard	Cobb 500	0.011 ± 0.001	0.013 ± 0.001	0.014 ± 0.002	0.014 ± 0.002	0.015 ± 0.003
Standard	Cobb MX	0.013 ± 0.001	0.014 ± 0.001	0.012 ± 0.001	0.014 ± 0.002	0.010 ± 0.001
Concave	Cobb 500			0.016 ± 0.002	0.012 ± 0.001	0.013 ± 0.002
Concave	Cobb MX			0.012 ± 0.001	0.014 ± 0.002	0.013 ± 0.001
Convex	Cobb 500			0.016 ± 0.002	0.013 ± 0.002	0.011 ± 0.001
Convex	Cobb MX			0.012 ± 0.002	0.014 ± 0.001	0.021 ± 0.003*
Full fed	Cobb 500	0.018 ± 0.002	0.014 ± 0.001	0.032 ± 0.006	0.042 ± 0.016	0.091 ± 0.019
Full fed	Cobb MX	0.021 ± 0.003	0.034 ± 0.006	0.036 ± 0.010	0.146 ± 0.064	0.298 ± 0.029*
Standard		0.014 ± 0.001	0.012 ± 0.001 <sup>b</sup>	0.013 ± 0.001 <sup>b</sup>	0.014 ± 0.001 <sup>b</sup>	0.012 ± 0.001 <sup>b</sup>
Concave				0.014 ± 0.001 <sup>b</sup>	0.013 ± 0.001 <sup>b</sup>	0.013 ± 0.001 <sup>b</sup>
Convex				0.014 ± 0.001 <sup>b</sup>	0.014 ± 0.001 <sup>b</sup>	0.016 ± 0.002 <sup>b</sup>
Full fed		0.018 ± 0.002	0.020 ± 0.001 <sup>a</sup>	0.034 ± 0.005 <sup>a</sup>	0.094 ± 0.035 <sup>a</sup>	0.194 ± 0.038 <sup>a</sup>
	Cobb 500	0.016 ± 0.001	0.015 ± 0.002	0.017 ± 0.002	0.020 ± 0.005	0.032 ± 0.009
	Cobb MX	0.017 ± 0.002	0.017 ± 0.002	0.020 ± 0.004	0.047 ± 0.020	0.085 ± 0.029*

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age, P < 0.05.

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ; P < 0.05.

<sup>1</sup>Values are means  $\pm$  SEM, n = 5 cockerels for each feeding program and genetic strain combination, n = 10 cockerels for each feeding program and n = 20 cockerels for each genetic strain.

<sup>2</sup>At 6 and 8 weeks of age, testes samples were only collected from the standard and full fed restricted treatments.

**Table 5.5.** Testes weight as a percent of body weight during the late rearing period for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)				
		18	21	25	26	28
-----[total testes weight (g)/total body weight (g)]*100-----						
Standard	Cobb 500	0.013 ± 0.001	0.015 ± 0.002	0.519 ± 0.081	0.416 ± 0.102	0.861 ± 0.047
Standard	Cobb MX	0.012 ± 0.002	0.024 ± 0.007	0.434 ± 0.091	0.872 ± 0.069*	0.902 ± 0.094
Concave	Cobb 500	0.012 ± 0.000	0.034 ± 0.016	0.257 ± 0.046	0.625 ± 0.044	0.649 ± 0.134
Concave	Cobb MX	0.014 ± 0.001	0.024 ± 0.004	0.562 ± 0.147	0.771 ± 0.112	0.861 ± 0.087
Convex	Cobb 500	0.017 ± 0.003	0.040 ± 0.014	0.537 ± 0.073	0.669 ± 0.022	0.684 ± 0.101
Convex	Cobb MX	0.014 ± 0.002	0.029 ± 0.009	0.510 ± 0.119	0.849 ± 0.119	0.888 ± 0.104
Full fed	Cobb 500	0.563 ± 0.098	0.567 ± 0.155	0.759 ± 0.037	0.632 ± 0.022	0.777 ± 0.041
Full fed	Cobb MX	0.661 ± 0.033	0.760 ± 0.018	0.810 ± 0.088	0.874 ± 0.093*	0.680 ± 0.026
Standard		0.012 ± 0.001 <sup>b</sup>	0.019 ± 0.004 <sup>b</sup>	0.477 ± 0.059 <sup>b</sup>	0.644 ± 0.095	0.882 ± 0.050
Concave		0.013 ± 0.001 <sup>b</sup>	0.029 ± 0.008 <sup>b</sup>	0.410 ± 0.089 <sup>b</sup>	0.698 ± 0.062	0.755 ± 0.083
Convex		0.015 ± 0.002 <sup>b</sup>	0.034 ± 0.008 <sup>b</sup>	0.524 ± 0.066 <sup>b</sup>	0.759 ± 0.064	0.786 ± 0.076
Full fed		0.572 ± 0.061 <sup>a</sup>	0.664 ± 0.088 <sup>a</sup>	0.785 ± 0.046 <sup>a</sup>	0.766 ± 0.065	0.734 ± 0.030
	Cobb 500	0.151 ± 0.059	0.164 ± 0.064	0.518 ± 0.050	0.583 ± 0.036	0.743 ± 0.045
	Cobb MX	0.155 ± 0.060	0.209 ± 0.075	0.579 ± 0.062	0.841 ± 0.047*	0.841 ± 0.045

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means  $\pm$  SEM, n = 5 cockerels for each feeding program and genetic strain combination, n = 10 cockerels for each feeding program and n = 20 cockerels for each genetic strain.

standard, concave and convex feeding programs did not start increasing until 21 weeks of age, but once the weight of the testes started to increase the rate of increase was quicker than had been seen in the full fed cockerels (Tables 5.4 and 5.5). Testes weight was consistently and sometimes significantly (week 15 and 25) greater for the Cobb MX males than the Cobb 500 males (Tables 5.4 and 5.5). Based on seminiferous tubule diameter and seminiferous epithelium height, seminiferous tubule development was more advanced in the full fed cockerels at 21 weeks of age than in the cockerels from the other feed restriction programs (Table 5.6). Once seminiferous epithelium height no longer differed between the cockerels fed on the different feed restriction programs at 26 and 28 weeks of age the seminiferous epithelium height in Cobb MX males was greater than in Cobb 500 males (Table 5.7). Seminiferous tubule diameter was smaller in the Cobb MX standard feed restricted roosters than in the corresponding Cobb 500 males (Table 5.7).

Total plasma testosterone concentrations were below detectable levels (20 ng/dL) in the cockerels from both genetic strains and across all the feed restriction programs at 6 weeks of age (Table 5.8). Full fed Cobb MX cockerels started to have detectable plasma testosterone levels at 8 weeks of age followed by full fed Cobb 500 cockerels at 12 weeks of age (Table 5.8). MX cockerels from the standard, concave and convex feed restriction programs had detectable levels of plasma total testosterone at 22 weeks of age (Table 5.9). Cobb 500 cockerels from the standard and convex feed restriction programs had detectable testosterone levels at 24 weeks of age (Table 5.9). For the Cobb 500 cockerels on the concave feed restriction program testosterone was first detected at 28 weeks of age (Table 5.9).

**Table 5.6.** Seminiferous tubule diameter and epithelium height at 21 and 25 weeks of age in two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		21		25	
		Diameter	Height	Diameter	Height
		------( $\mu\text{m}$ )-----			
Standard	Cobb 500	24 $\pm$ 1	10 $\pm$ 1	111 $\pm$ 7	26 $\pm$ 1
Standard	Cobb MX	26 $\pm$ 4	11 $\pm$ 2	102 $\pm$ 13	27 $\pm$ 3
Concave	Cobb 500	31 $\pm$ 6	13 $\pm$ 2	82 $\pm$ 6	25 $\pm$ 2
Concave	Cobb MX	27 $\pm$ 3	12 $\pm$ 1	91 $\pm$ 10	23 $\pm$ 2
Convex	Cobb 500	33 $\pm$ 7	14 $\pm$ 2	103 $\pm$ 9	29 $\pm$ 3
Convex	Cobb MX	30 $\pm$ 5	12 $\pm$ 1	102 $\pm$ 10	27 $\pm$ 2
Full fed	Cobb 500	111 $\pm$ 18	32 $\pm$ 5	133 $\pm$ 4	33 $\pm$ 2
Full fed	Cobb MX	125 $\pm$ 3	33 $\pm$ 0	133 $\pm$ 6	35 $\pm$ 1
Standard		25 $\pm$ 2 <sup>b</sup>	11 $\pm$ 1 <sup>b</sup>	106 $\pm$ 8 <sup>b</sup>	26 $\pm$ 1 <sup>b</sup>
Concave		29 $\pm$ 3 <sup>b</sup>	12 $\pm$ 1 <sup>b</sup>	86 $\pm$ 5 <sup>b</sup>	24 $\pm$ 1 <sup>b</sup>
Convex		32 $\pm$ 4 <sup>b</sup>	13 $\pm$ 1 <sup>b</sup>	103 $\pm$ 6 <sup>b</sup>	28 $\pm$ 2 <sup>b</sup>
Full fed		116 $\pm$ 11 <sup>a</sup>	32 $\pm$ 3 <sup>a</sup>	133 $\pm$ 3 <sup>a</sup>	34 $\pm$ 1 <sup>a</sup>
	Cobb 500	49 $\pm$ 9	17 $\pm$ 2	106 $\pm$ 5	28 $\pm$ 1
	Cobb MX	45 $\pm$ 9	15 $\pm$ 2	107 $\pm$ 6	28 $\pm$ 1

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means  $\pm$  SEM,  $n = 5$  cockerels for each feeding program and genetic strain combination,  $n = 10$  cockerels for each feeding program and  $n = 20$  cockerels for each genetic strain. For each individual cockerel the diameter and height values are based on measurements from 5 duplicate tubules from a testicular cross section.

**Table 5.7.** Seminiferous tubule diameter and epithelium height at 26 and 28 weeks of age in two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		26		28	
		Diameter	Height	Diameter	Height
		------( $\mu\text{m}$ )-----			
Standard	Cobb 500	96 $\pm$ 6	26 $\pm$ 2	132 $\pm$ 2	35 $\pm$ 1
Standard	Cobb MX	111 $\pm$ 5	31 $\pm$ 2	114 $\pm$ 3*	32 $\pm$ 2
Concave	Cobb 500	106 $\pm$ 5	30 $\pm$ 2	106 $\pm$ 9	27 $\pm$ 2
Concave	Cobb MX	130 $\pm$ 10*	36 $\pm$ 2*	124 $\pm$ 6	36 $\pm$ 2*
Convex	Cobb 500	109 $\pm$ 5	30 $\pm$ 1	110 $\pm$ 8	31 $\pm$ 2
Convex	Cobb MX	112 $\pm$ 5	30 $\pm$ 3	127 $\pm$ 5	37 $\pm$ 1*
Full fed	Cobb 500	126 $\pm$ 3	33 $\pm$ 2	128 $\pm$ 6	33 $\pm$ 2
Full fed	Cobb MX	123 $\pm$ 5	33 $\pm$ 0	141 $\pm$ 8	38 $\pm$ 4
Standard		104 $\pm$ 4 <sup>b</sup>	29 $\pm$ 2	123 $\pm$ 3 <sup>ab</sup>	33 $\pm$ 1
Concave		118 $\pm$ 6 <sup>ab</sup>	33 $\pm$ 2	115 $\pm$ 6 <sup>b</sup>	32 $\pm$ 2
Convex		111 $\pm$ 3 <sup>ab</sup>	30 $\pm$ 1	119 $\pm$ 5 <sup>b</sup>	34 $\pm$ 1
Full fed		124 $\pm$ 3 <sup>a</sup>	33 $\pm$ 1	134 $\pm$ 5 <sup>a</sup>	35 $\pm$ 2
	Cobb 500	109 $\pm$ 3	30 $\pm$ 1	119 $\pm$ 4	31 $\pm$ 1
	Cobb MX	119 $\pm$ 4*	33 $\pm$ 1*	127 $\pm$ 3	36 $\pm$ 1*

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means  $\pm$  SEM,  $n = 5$  cockerels for each feeding program and genetic strain combination,  $n = 10$  cockerels for each feeding program and  $n = 20$  cockerels for each genetic strain. For each individual cockerel the diameter and height values are based on measurements from 5 duplicate tubules from a testicular cross section.

**Table 5.8.** Total plasma testosterone concentration during the early rearing period for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)											
		6		8		10		12		14		16	
		ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>
Standard	Cobb 500	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Standard	Cobb MX	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Concave	Cobb 500	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Concave	Cobb MX	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Convex	Cobb 500	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Convex	Cobb MX	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5	n.d.	0/5
Full fed	Cobb 500	n.d.	0/5	n.d.	0/5	n.d.	0/5	25 ± 0	1/10	83 ± 19	8/10	84 ± 19	13/15
Full fed	Cobb MX	n.d.	0/5	29 ± 6	3/5	39 ± 18	2/5	51 ± 10	5/10	140 ± 25	10/10	167 ± 31*	15/15
Standard		n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10
Concave		n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10
Convex		n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10	n.d.	0/10
Full fed		n.d.	0/10	29 ± 6	3/10	39 ± 18	3/10	47 ± 10	6/20	114 ± 18	18/20	128 ± 19	28/30
	Cobb 500	n.d.	0/20	n.d.	0/20	n.d.	0/20	25 ± 0	1/25	83 ± 19	9/25	84 ± 19	13/30
	Cobb MX	n.d.	0/20	29 ± 6	3/20	39 ± 18	3/20	51 ± 10	5/25	140 ± 25	10/25	167 ± 31*	15/30

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age, P < 0.05.

<sup>1</sup>Values are means ± SEM.

<sup>2</sup>The number of roosters for a given feed restriction program and /or genetic strain with plasma total testosterone concentrations above the lowest RIA standard concentration of 20 ng/dL.

**Table 5.9.** Total plasma testosterone concentration during the late rearing period for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 1).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)											
		18		20		22		24		26		28	
		ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>
Standard	Cobb 500	n.d.	0/5	n.d.	0/10	n.d.	0/5	41 ± 3	2/5	56 ± 0	1/5	125 ± 28	4/5
Standard	Cobb MX	n.d.	0/5	n.d.	0/10	20 ± 0	1/5	31 ± 3	2/5	50 ± 22	3/5	303 ± 88	4/5
Concave	Cobb 500	n.d.	0/5	n.d.	0/10	n.d.	0/5	n.d.	0/5	n.d.	0/5	101 ± 0	1/5
Concave	Cobb MX	n.d.	0/5	n.d.	0/10	27 ± 5	2/5	472 ± 0	1/5	189 ± 91	3/5	423 ± 266	5/5
Convex	Cobb 500	n.d.	0/5	n.d.	0/15	n.d.	0/10	76 ± 39	3/5	161 ± 83	2/5	171 ± 44	5/5
Convex	Cobb MX	n.d.	0/5	n.d.	0/15	53 ± 18	5/10	163 ± 94	4/5	247 ± 79	2/5	261 ± 77	5/5
Full fed	Cobb 500	149 ± 13	23/25	199 ± 33	13/15	189 ± 58	9/10	218 ± 65	5/5	250 ± 65	5/5	473 ± 21	5/5
Full fed	Cobb MX	196 ± 17*	23/25	173 ± 28	14/15	228 ± 62	8/10	395 ± 89	5/5	328 ± 33	5/5	398 ± 70	5/5
Standard		n.d.	0/10	n.d.	0/10	20 ± 0	1/10	37 ± 3 <sup>b</sup>	4/10	51 ± 15 <sup>b</sup>	4/10	214 ± 55 <sup>b</sup>	8/10
Concave		n.d.	0/10	n.d.	0/10	27 ± 5 <sup>b</sup>	2/10	472 ± 0	1/10	189 ± 91 <sup>a</sup>	3/10	369 ± 224 <sup>ab</sup>	6/10
Convex		n.d.	0/10	n.d.	0/10	53 ± 18 <sup>b</sup>	5/20	125 ± 55 <sup>b</sup>	7/10	204 ± 53 <sup>a</sup>	4/10	216 ± 44 <sup>b</sup>	10/10
Full fed		172 ± 14	46/50	186 ± 24	27/30	209 ± 41 <sup>a</sup>	17/20	307 ± 59 <sup>a</sup>	10/10	289 ± 37 <sup>a</sup>	10/10	435 ± 37 <sup>a</sup>	10/10
	Cobb 500	149 ± 13	23/40	199 ± 33	13/30	189 ± 58	9/30	140 ± 41	10/20	204 ± 49	8/20	255 ± 45	15/20
	Cobb MX	196 ± 17*	23/40	173 ± 28	14/30	135 ± 39	16/30	263 ± 54*	12/20	219 ± 39	14/20	349 ± 73	19/20

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-d</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

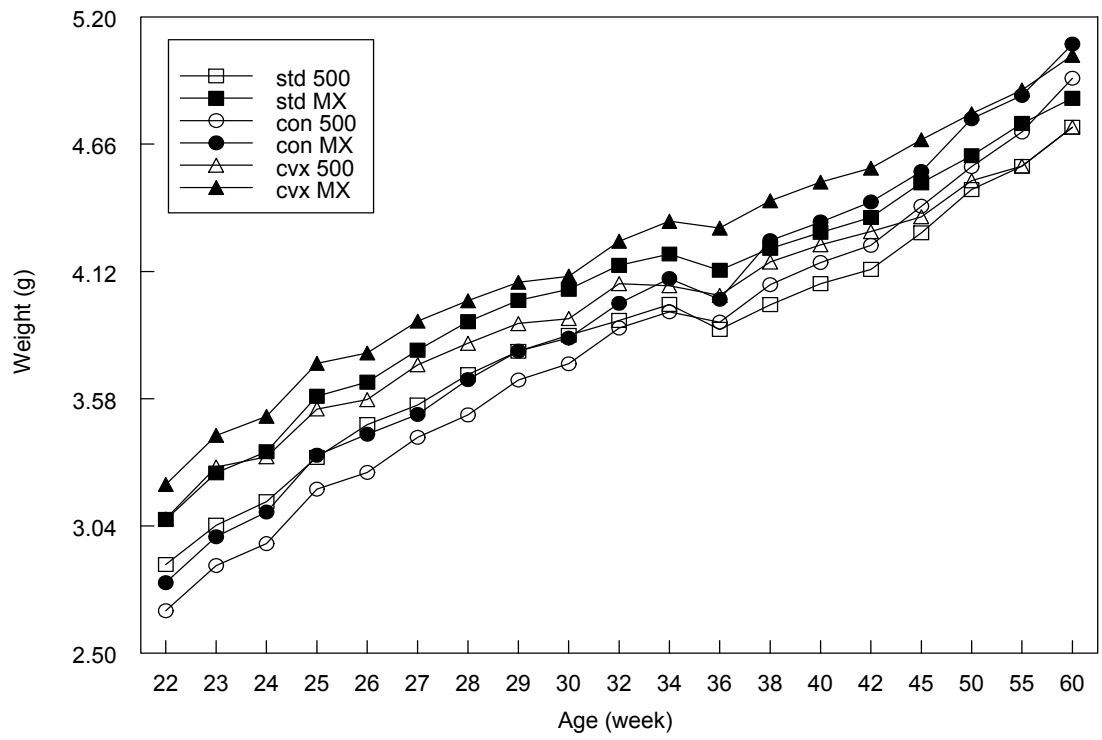
<sup>1</sup>Values are means ± SEM.

<sup>2</sup>The number of roosters for a given feed restriction program and /or genetic strain with plasma total testosterone concentrations above the lowest RIA standard concentration of 20 ng/dL.

## ***5.2 Experiment 2***

The roosters were photostimulated at 22 weeks of age and fed from this point to the end of the experiment to maintain the relative differences in body weight established during the rearing phase through the differing feed restriction programs. Overall differences in body weight were maintained throughout much of the experiment (Figure 5.2). In particular, the difference between the body weight of the concave and convex roosters was maintained through 36 weeks of age (Tables 5.10-5.13). Cobb MX roosters which were fed 2 to 5 percent more feed than the Cobb 500 roosters weighed more than the Cobb 500 roosters throughout the entire experiment (Tables 5.10 – 5.14). Cumulative mortality through 65 weeks of age was more than double for the Cobb 500 versus the Cobb MX roosters that were reared on the concave feed restriction program (Table 5.15). Overall the Cobb 500 roosters had a livability of 85 percent versus 92 percent for the Cobb MX roosters (Table 5.15).

Plasma total testosterone at 23.5 weeks of age or a week and a half after photostimulation was higher in the roosters that had been reared on the convex feed restriction program compared to the roosters fed on the concave feed restriction program (Table 5.16). Total plasma testosterone at 23.5 weeks of age for the roosters fed on the standard breeder feed restriction guidelines was intermediate to the roosters from the other two feed restriction programs (Table 5.16). Plasma total testosterone levels peaked and remained high for the roosters from all three feed restriction programs in the nine weeks (22 to 31 weeks of age) following photostimulation (Table 5.16). Subsequently, plasma total testosterone values declined in the roosters from all three feeding regimens (Tables 5.17 and 5.18). More importantly, there was a sharp decline in the percentage of roosters with detectable levels of plasma total testosterone from 90 percent at 25.5 weeks of age to less than 50 percent at 41, 46 and 51 weeks of age (Table 5.16 and 5.17).



**Figure 5.2.** Body weight from 22 through 60 weeks of age of two genetic strains of broiler breeder cockerels reared on four different feed restriction programs. Abbreviations: std = standard feed restriction, con = concave feed restriction program, cvx = convex feed restriction program, 500 = Cobb 500 genetic strain, and MX = Cobb MX genetic strain.

**Table 5.10.** Body weight of two genetic strains of broiler breeder roosters from 22 to 25 weeks of age reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		22	23	24	25
		------(g)-----			
Standard	Cobb 500	2876 ± 67	3043 ± 67	3143 ± 67	3331 ± 65
Standard	Cobb MX	3067 ± 71*	3265 ± 71*	3355 ± 68*	3590 ± 66*
Concave	Cobb 500	2680 ± 58	2872 ± 58	2965 ± 58	3196 ± 55
Concave	Cobb MX	2799 ± 62	2994 ± 62	3099 ± 60	3340 ± 57
Convex	Cobb 500	3070 ± 75	3290 ± 72	3333 ± 70	3536 ± 68
Convex	Cobb MX	3216 ± 76	3424 ± 76	3505 ± 72	3730 ± 71*
Standard		2970 ± 50 <sup>a</sup>	3152 ± 50 <sup>b</sup>	3249 ± 49 <sup>b</sup>	3461 ± 48 <sup>b</sup>
Concave		2740 ± 43 <sup>b</sup>	2934 ± 43 <sup>c</sup>	3033 ± 42 <sup>c</sup>	3268 ± 40 <sup>c</sup>
Convex		3142 ± 54 <sup>a</sup>	3356 ± 52 <sup>a</sup>	3419 ± 51 <sup>a</sup>	3633 ± 50 <sup>a</sup>
	Cobb 500	2874 ± 41	3068 ± 40	3148 ± 40	3355 ± 38
	Cobb MX	3068 ± 43*	3223 ± 43*	3319 ± 41*	3553 ± 40*

\* Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-c</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 44$  roosters for each feeding program and genetic strain combination,  $n = 88$  roosters for each feeding program and  $n = 132$  roosters for each genetic strain.

**Table 5.11.** Body weight of two genetic strains of broiler breeder roosters from 26 to 29 weeks of age reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		26	27	28	29
		------(g)-----			
Standard	Cobb 500	3407 ± 63	3553 ± 65	3682 ± 63	3781 ± 63
Standard	Cobb MX	3650 ± 66*	3786 ± 65*	3907 ± 67*	3997 ± 68*
Concave	Cobb 500	3267 ± 59	3416 ± 60	3511 ± 60	3659 ± 56
Concave	Cobb MX	3429 ± 59*	3513 ± 60	3661 ± 55	3782 ± 54
Convex	Cobb 500	3576 ± 66	3724 ± 66	3815 ± 64	3899 ± 64
Convex	Cobb MX	3774 ± 69*	3909 ± 68*	3996 ± 68*	4074 ± 65
Standard		3530 ± 47 <sup>b</sup>	3673 ± 47 <sup>b</sup>	3797 ± 48 <sup>a</sup>	3891 ± 47 <sup>a</sup>
Concave		3349 ± 43 <sup>c</sup>	3465 ± 43 <sup>c</sup>	3587 ± 41 <sup>b</sup>	3722 ± 39 <sup>b</sup>
Convex		3675 ± 49 <sup>a</sup>	3816 ± 48 <sup>a</sup>	3906 ± 48 <sup>a</sup>	3987 ± 46 <sup>a</sup>
	Cobb 500	3419 ± 38	3567 ± 38	3672 ± 37	3782 ± 36
	Cobb MX	3619 ± 39*	3738 ± 40*	3856 ± 39*	3952 ± 37*

\* Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-c</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 44$  roosters for each feeding program and genetic strain combination,  $n = 88$  roosters for each feeding program and  $n = 132$  roosters for each genetic strain.

**Table 5.12.** Body weight of two genetic strains of broiler breeder roosters from 30 to 36 weeks of age reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		30	32	34	36
		------(g)-----			
Standard	Cobb 500	3849 ± 59	3912 ± 61	3980 ± 60	3874 ± 68
Standard	Cobb MX	4044 ± 68*	4146 ± 71*	4194 ± 73*	4125 ± 78*
Concave	Cobb 500	3728 ± 57	3880 ± 59	3949 ± 61	3905 ± 65
Concave	Cobb MX	3837 ± 53	3985 ± 52	4090 ± 55	4002 ± 102
Convex	Cobb 500	3920 ± 65	4068 ± 78	4059 ± 73	4018 ± 77
Convex	Cobb MX	4100 ± 65*	4248 ± 62	4333 ± 62*	4304 ± 60*
Standard		3949 ± 46 <sup>a</sup>	4032 ± 48 <sup>ab</sup>	4091 ± 49 <sup>ab</sup>	4004 ± 53 <sup>ab</sup>
Concave		3784 ± 39 <sup>b</sup>	3934 ± 39 <sup>b</sup>	4020 ± 41 <sup>b</sup>	3954 ± 61 <sup>a</sup>
Convex		4010 ± 47 <sup>a</sup>	4158 ± 51 <sup>a</sup>	4197 ± 50 <sup>a</sup>	4163 ± 51 <sup>b</sup>
	Cobb 500	3835 ± 35	3956 ± 39	3997 ± 38	3934 ± 41
	Cobb MX	3995 ± 37*	4128 ± 37*	4207 ± 38*	4146 ± 48*

\* Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 44$  roosters for each feeding program and genetic strain combination,  $n = 88$  roosters for each feeding program and  $n = 132$  roosters for each genetic strain.

**Table 5.13.** Body weight of two genetic strains of broiler breeder roosters from 38 to 45 weeks of age reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)			
		38	40	42	45
		------(g)-----			
Standard	Cobb 500	3979 ± 68	4068 ± 69	4129 ± 71	4284 ± 72
Standard	Cobb MX	4218 ± 80*	4286 ± 82*	4349 ± 82*	4496 ± 81*
Concave	Cobb 500	4064 ± 70	4158 ± 70	4231 ± 68	4397 ± 73
Concave	Cobb MX	4251 ± 53*	4330 ± 53*	4415 ± 51*	4544 ± 50
Convex	Cobb 500	4160 ± 72	4233 ± 73	4289 ± 76	4352 ± 78
Convex	Cobb MX	4420 ± 58*	4499 ± 58*	4558 ± 57*	4679 ± 59*
Standard		4102 ± 54 <sup>b</sup>	4180 ± 55 <sup>b</sup>	4242 ± 55 <sup>b</sup>	4392 ± 55
Concave		4159 ± 45 <sup>ab</sup>	4248 ± 44 <sup>ab</sup>	4326 ± 43 <sup>ab</sup>	4473 ± 44
Convex		4292 ± 48 <sup>a</sup>	4366 ± 49 <sup>a</sup>	4422 ± 49 <sup>a</sup>	4513 ± 52
	Cobb 500	4069 ± 41	4154 ± 41	4217 ± 42	4343 ± 43
	Cobb MX	4297 ± 38*	4373 ± 39*	4440 ± 38*	4519 ± 48*

\* Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 44$  roosters for each feeding program and genetic strain combination,  $n = 88$  roosters for each feeding program and  $n = 132$  roosters for each genetic strain.

**Table 5.14.** Body weight of two genetic strains of broiler breeder roosters from 50 to 60 weeks of age reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)		
		50	55	60
		------(g)-----		
Standard	Cobb 500	4468 ± 81	4566 ± 79	4732 ± 76
Standard	Cobb MX	4611 ± 75	4748 ± 71	4855 ± 74
Concave	Cobb 500	4565 ± 77	4712 ± 71	4940 ± 76
Concave	Cobb MX	4768 ± 52*	4867 ± 57	5085 ± 61
Convex	Cobb 500	4505 ± 78	4567 ± 78	4732 ± 94
Convex	Cobb MX	4790 ± 54*	4890 ± 48*	5037 ± 51*
Standard		4541 ± 55	4660 ± 54	4796 ± 53 <sup>b</sup>
Concave		4670 ± 47	4791 ± 46	5017 ± 49 <sup>a</sup>
Convex		4646 ± 50	4728 ± 49	4889 ± 55 <sup>ab</sup>
	Cobb 500	4511 ± 45	4614 ± 44	4797 ± 48
	Cobb MX	4722 ± 36*	4835 ± 35*	4992 ± 37*

\* Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM,  $n = 44$  roosters for each feeding program and genetic strain combination,  $n = 88$  roosters for each feeding program and  $n = 132$  roosters for each genetic strain.

**Table 5.15.** Cumulative mortality from 21 to 65 weeks of age of two genetic strains of broiler breeder cockerels that had been reared on four different feed restriction programs (Experiment 2).

Age (week)	Feed restriction program and genetic strain						Feed restriction program			Genetic strain	
	Stan- dard Cobb 500	Stan- dard Cobb MX	Con- cave Cobb 500	Con- cave Cobb MX	Con- vex Cobb 500	Con- vex Cobb MX	Stan- dard	Con- cave	Con- vex	Cobb 500	Cobb MX
	-----%-----										
21	0	0	0	0	0	0	0	0	0	0	2
22	0	2	0	0	0	2	1	0	1	0	2
23	0	2	0	0	0	2	1	0	1	0	2
24	0	2	0	0	2	2	1	0	2	1	2
25	0	2	0	0	2	2	1	0	2	1	2
26	5	2	5	2	2	2	3	3	2	4	2
27	5	2	5	2	2	2	3	3	2	4	2
28	5	2	5	2	2	2	3	3	2	4	2
29	5	2	7	2	2	2	3	5	2	5	2
30	5	2	9	2	2	2	3	6	2	5	2
31	5	2	14	2	2	2	3	8	2	7	2
32	5	2	14	2	2	2	3	8	2	7	2
33	5	2	14	2	2	2	3	8	2	7	2
34	5	2	14	2	2	2	3	8	2	7	2
35	5	2	14	2	2	2	3	8	2	7	2
36	5	2	14	7	2	2	3	10	2	7	4
37	5	5	14	9	2	2	5	11	2	7	5
38	5	5	14	9	2	5	5	11	3	7	6
39	5	5	14	9	2	5	5	11	3	7	6
40	5	5	14	9	7	7	5	11	7	8	7
41	5	5	14	9	7	7	5	11	7	8	7
42	5	5	14	9	7	7	5	11	7	8	7
43	5	5	14	9	7	7	5	11	7	8	7
44	5	5	14	9	7	7	5	11	7	8	7
45	5	5	14	9	7	7	5	11	7	8	7
46	5	5	14	9	7	7	5	11	7	8	7
47	5	5	14	9	7	7	5	11	7	8	7
48	7	5	14	9	7	7	6	11	7	9	7
49	7	5	14	9	9	7	6	11	8	10	7
50	7	7	14	9	11	7	7	11	9	11	8
51	11	7	14	9	11	7	9	11	9	12	8
52	11	7	14	9	11	7	9	11	9	12	8
53	11	7	14	9	11	7	9	11	9	12	8

54	11	7	16	9	11	7	9	13	9	13	8
55	11	7	16	9	11	7	9	13	9	13	8
56	11	7	18	9	11	7	9	14	9	14	8
57	11	7	20	9	11	7	9	15	9	15	8
58	11	7	20	9	11	7	9	15	9	15	8
59	11	7	20	9	11	7	9	15	9	15	8
60	11	7	20	9	11	7	9	15	9	15	8
61	14	7	20	9	11	7	10	15	9	15	8
62	14	7	20	9	11	7	10	15	9	15	8
63	14	7	23	11	11	7	10	17	9	16	8
64	14	7	23	11	11	7	10	17	9	16	8
65	14	7	23	11	11	7	10	17	9	16	8

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**Table 5.16.** Total plasma testosterone concentration from 23.5 to 31 weeks of age for two genetic strains of broiler breeder cockerels reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)							
		23.5		25.5		27.5		31	
		ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	Ng/dL	n <sup>2</sup>
Standard	Cobb 500	167 ± 29	12/20	197 ± 21	17/20	186 ± 26	17/20	146 ± 29	15/20
Standard	Cobb MX	218 ± 33	16/20	273 ± 30*	20/20	295 ± 38*	18/20	198 ± 38	14/20
Concave	Cobb 500	130 ± 24	17/20	198 ± 25	17/20	239 ± 27	16/20	225 ± 36	16/19
Concave	Cobb MX	160 ± 26	19/20	246 ± 26	19/20	213 ± 23	18/20	207 ± 20	20/20
Convex	Cobb 500	154 ± 22	18/20	226 ± 34	16/20	182 ± 25	18/20	185 ± 28	17/19
Convex	Cobb MX	269 ± 36*	16/20	324 ± 29*	19/20	191 ± 30	20/20	228 ± 37	18/20
Standard		197 ± 23 <sup>ab</sup>	28/40	239 ± 20	37/40	242 ± 25	35/40	171 ± 24	29/40
Concave		146 ± 18 <sup>b</sup>	36/40	223 ± 18	36/40	225 ± 18	34/40	215 ± 19	36/39
Convex		208 ± 23 <sup>a</sup>	34/40	279 ± 23	35/40	187 ± 20	38/40	207 ± 23	35/39
	Cobb 500	149 ± 14	47/60	207 ± 15	50/60	201 ± 15	51/60	186 ± 18	48/58
	Cobb MX	213 ± 18*	51/60	281 ± 17*	58/60	231 ± 19	56/60	212 ± 18	52/60

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM.

<sup>2</sup>The number of roosters for a given feed restriction program and /or genetic strain with plasma total testosterone concentrations above the lowest RIA standard concentration of 20 ng/dL.

**Table 5.17.** Total plasma testosterone concentration from 35 to 51 weeks of age for two genetic strains of broiler breeder cockerels reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)							
		35		41		46		51	
		Ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	Ng/dL	n <sup>2</sup>
Standard	Cobb 500	164 ± 32	8/20	138 ± 39	10/20	135 ± 21	9/20	189 ± 35	5/19
Standard	Cobb MX	139 ± 24	12/20	192 ± 111	6/20	174 ± 27	18/20	250 ± 57	7/20
Concave	Cobb 500	117 ± 22	10/19	58 ± 13	6/19	144 ± 24	9/19	147 ± 30	5/19
Concave	Cobb MX	166 ± 20	15/19	217 ± 37*	10/19	125 ± 31	8/18	129 ± 24	9/18
Convex	Cobb 500	155 ± 40	7/19	108 ± 30	6/19	87 ± 34	4/19	102 ± 37	7/18
Convex	Cobb MX	178 ± 32	12/20	120 ± 47	10/20	166 ± 33	11/19	263 ± 46*	12/19
Standard		149 ± 19	20/40	158 ± 44	16/40	135 ± 14	18/40	224 ± 36	12/39
Concave		146 ± 16	25/38	157 ± 37	16/38	135 ± 19	17/37	136 ± 18	14/37
Convex		170 ± 25	19/39	116 ± 30	16/39	145 ± 27	15/38	204 ± 37	19/37
	Cobb 500	140 ± 17	26/58	108 ± 21	22/58	130 ± 15	22/58	141 ± 21	17/56
	Cobb MX	161 ± 15	39/59	187 ± 33*	24/59	161 ± 18	37/57	217 ± 27*	28/57

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM.

<sup>2</sup>The number of roosters for a given feed restriction program and /or genetic strain with plasma total testosterone concentrations above the lowest RIA standard concentration of 20 ng/dL.

The percentage of roosters with detectable plasma total testosterone levels increased to over 70 percent at 56, 61 and 65 weeks of age (Table 5.18). Plasma total testosterone concentrations were consistently and sometimes significantly (week 23.5, 25.5 and 51 of age) greater for the Cobb MX males than the Cobb 500 males (Tables 5.16 and 5.17). Overall (23.5 through 65 weeks of age) plasma total testosterone concentration was greater in the Cobb MX males than the Cobb 500 males (Table 5.18).

For the roosters which were fed on the standard feed restriction protocol, sperm mobility was less in the Cobb MX roosters than the Cobb 500 roosters at each week of age it was measured (Table 5.19). There were no differences in sperm mobility between on the Cobb MX and 500 genetic strains of roosters that were reared on the concave or convex feed restriction programs (Table 5.19). The roosters fed on a convex feed restriction program had less sperm mobility at 46 and 51 weeks of age than the roosters fed on the concave feed restriction protocol (Table 5.19).

Inhibin-A was not detected in any of the plasma samples collected at 61 weeks of age from the high and low sperm mobility roosters (data not shown). There were no differences in plasma inhibin-B concentrations between the Cobb 500 and MX strains of roosters and between roosters with high and low sperm mobility or reared on different feed restriction programs (Table 5.20).

**Table 5.18.** Total plasma testosterone concentration from 56 to 65 weeks of age and overall (23.5 to 65 weeks of age) for two genetic strains of broiler breeder cockerels reared on three different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic strain	Age (week)							
		56		61		65		23.5 to 65	
		ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>	Ng/dL	n <sup>2</sup>	ng/dL	n <sup>2</sup>
Standard	Cobb 500	157 ± 18	15/18	141 ± 27	14/18	127 ± 27	13/20	156 ± 12	20/20
Standard	Cobb MX	121 ± 20	15/20	120 ± 16	15/20	146 ± 27	15/20	198 ± 12*	20/20
Concave	Cobb 500	78 ± 15	14/19	152 ± 32	11/18	123 ± 17	13/20	151 ± 8	20/20
Concave	Cobb MX	141 ± 32*	13/18	172 ± 25	17/18	151 ± 20	17/20	183 ± 14*	20/20
Convex	Cobb 500	108 ± 20	10/17	76 ± 18	10/17	96 ± 13	12/20	142 ± 14	20/20
Convex	Cobb MX	160 ± 36	13/19	135 ± 40	10/18	121 ± 33	14/20	211 ± 16*	20/20
Standard		132 ± 14	30/38	130 ± 15	29/38	137 ± 19	28/40	177 ± 9	40/40
Concave		109 ± 18	27/37	164 ± 19	28/36	139 ± 14	30/40	167 ± 8	40/40
Convex		138 ± 23	23/36	105 ± 22	20/35	110 ± 18	26/40	177 ± 12	40/40
	Cobb 500	116 ± 11	39/54	126 ± 16	35/53	116 ± 12	38/60	150 ± 6	60/60
	Cobb MX	135 ± 17	41/57	144 ± 15	42/56	140 ± 15	46/60	197 ± 8*	60/60

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>1</sup>Values are means ± SEM.

<sup>2</sup>The number of roosters for a given feed restriction program and /or genetic strain with plasma total testosterone concentrations above the lowest RIA standard concentration of 20 ng/dL.

**Table 5.19.** Sperm mobility at 46, 51, 56, 61 and 65 weeks of age for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 2).<sup>1</sup>

Feed restriction program	Genetic Strain	Age (week)				
		46	51	56	61	65
-----absorbance (550 nm)-----						
Standard	Cobb 500	0.314 ± 0.026	0.394 ± 0.020	0.389 ± 0.028	0.453 ± 0.026	0.340 ± 0.030
Standard	Cobb MX	0.246 ± 0.022*	0.268 ± 0.028*	0.247 ± 0.028*	0.304 ± 0.028*	0.217 ± 0.025*
Concave	Cobb 500	0.313 ± 0.025	0.350 ± 0.028	0.291 ± 0.021	0.420 ± 0.034	0.345 ± 0.033
Concave	Cobb MX	0.308 ± 0.023	0.299 ± 0.026	0.334 ± 0.023	0.396 ± 0.035	0.325 ± 0.038
Convex	Cobb 500	0.250 ± 0.024	0.260 ± 0.021	0.331 ± 0.028	0.339 ± 0.029	0.294 ± 0.025
Convex	Cobb MX	0.239 ± 0.040	0.260 ± 0.029	0.303 ± 0.028	0.421 ± 0.032	0.282 ± 0.035
Standard		0.278 ± 0.017 <sup>ab</sup>	0.327 ± 0.019 <sup>a</sup>	0.314 ± 0.021	0.371 ± 0.021	0.271 ± 0.020
Concave		0.311 ± 0.017 <sup>a</sup>	0.323 ± 0.019 <sup>a</sup>	0.313 ± 0.016	0.407 ± 0.024	0.334 ± 0.025
Convex		0.246 ± 0.021 <sup>b</sup>	0.260 ± 0.018 <sup>b</sup>	0.317 ± 0.020	0.379 ± 0.022	0.288 ± 0.022
	Cobb 500	0.295 ± 0.015	0.334 ± 0.014	0.337 ± 0.015	0.401 ± 0.018	0.325 ± 0.017
	Cobb MX	0.270 ± 0.015	0.275 ± 0.016*	0.294 ± 0.015*	0.373 ± 0.019	0.274 ± 0.019*

\*Significantly different from the corresponding value for the Cobb 500 strain at a given age,  $P < 0.05$ .

<sup>a-b</sup>Feeding program values for a given week without a common superscript differ;  $P < 0.05$ .

**Table 5.20.** Plasma inhibin-B concentrations of roosters at 61 weeks of age with low or high sperm mobility values and for two genetic strains of broiler breeder cockerels reared on four different feed restriction programs (Experiment 2).<sup>1</sup>

Sperm mobility		n	Inhibin-B (pg/mL)
Low <sup>2</sup>		19	7.99 ± 1.50
High <sup>3</sup>		20	7.34 ± 1.63
Feed restriction program	Genetic strain		
Standard	Cobb 500	6	5.96 ± 2.74
Standard	Cobb MX	10	6.19 ± 2.37
Concave	Cobb 500	5	6.44 ± 3.20
Concave	Cobb MX	6	7.70 ± 1.32
Convex	Cobb 500	5	8.86 ± 4.38
Convex	Cobb MX	7	11.18 ± 2.54
Full fed	Cobb 500		
Full fed	Cobb MX		
Standard		16	6.11 ± 1.75
Concave		11	7.13 ± 1.54
Convex		12	10.21 ± 2.25
Full fed			
	Cobb 500	23	8.10 ± 1.36
	Cobb MX	16	7.02 ± 1.87

<sup>1</sup>Values are means ± SEM.

<sup>2</sup>Low mobility roosters were selected based on having sperm mobility absorbance values less than 0.186 when measured at 46, 51, 56 and 61 weeks of age. The overall (46 to 61 weeks of age) mean ± SEM sperm mobility absorbance value at 550 nm for the 19 roosters was 0.085 ± 0.006 with a range of 0.036 to 0.150.

<sup>3</sup>High mobility roosters were selected based on having sperm mobility absorbance values greater than 0.4 when measured at 46, 51, 56 and 61 weeks of age. The overall (46 to 61 weeks of age) mean ± SEM sperm mobility absorbance value at 550 nm for the 20 roosters was 0.

## Chapter 6

### DISCUSSION

#### ***6.1.1. Genetic strain comparison***

One of the goals of the current research was to evaluate the reproductive capability of the newly developed Cobb MX male versus its progenitor the Cobb 500 male. Overall the Cobb MX male is as good as or better than the Cobb 500 male based on the current research findings. The Cobb MX males have a quicker onset of sexual maturity based on testes development and testosterone production than the Cobb 500 male. Livability is also better for the Cobb MX male than the Cobb 500 male. However, sperm mobility tended to be better for the Cobb 500 males than the MX males, but the sperm mobility values obtained for the Cobb MX and 500 males are both considered average and would not be expected to vary much in fertility (Froman *et al.*, 1999).

#### ***6.1.2. Feed restriction and testes development***

The value of feed restriction in improving reproduction of broiler breeders hens is well established. Feed restriction of broiler breeder hens decreases body weight gain (Robbins *et al.*, 1986; Katanbaf *et al.*, 1989; Bruggeman *et al.*, 1999, 2005; Onagbesan *et al.*, 2006), delays the onset of sexual maturity (Robbins *et al.*, 1986; Yu *et al.*, 1992a; Heck *et al.*, 2004; Bruggeman *et al.*, 2005; Hocking and Robertson, 2005; Onagbesan *et al.*, 2006), and decreases mortality (Robbins *et al.*, 1986; Katanbaf *et al.*, 1989; Heck *et al.*, 2004; Bruggeman *et al.*, 2005). In addition, feed restriction during the rearing and laying periods reduces the number of large

follicles on the ovary of broiler breeder hens (Hocking *et al.*, 1987, 1989; Heck *et al.*, 2004; Hocking and Robertson, 2005). More important, broiler breeder hens that have been feed restricted produce more eggs (Yu *et al.*, 1992; Heck *et al.*, 2004; Bruggeman *et al.*, 2005; Onagbesan *et al.*, 2006), lay longer sequences (Robinson *et al.*, 1991), persist in lay longer (Fattori *et al.*, 1991), lay fewer abnormal eggs, and have fewer multiple ovulations in a single day (Fattori *et al.*, 1991; Yu *et al.*, 1992a; Heck *et al.*, 2004) compared with fully fed broiler breeder hens.

Given all of the benefits of feed restricting broiler breeder pullets and hens, it is not surprising that broiler breeder males have been feed restricted following the same protocols of the females. However, there has not been much research to support these restriction protocols in males and biologically the role of nutrition in male reproduction differs from that of females in both avian species and mammalian species. Egg production in female birds and pregnancy in mammals is energy costly and sufficient fat deposits are required to support the demands of reproduction. However, the acquisition of excess fat in female poultry or mammalian species is associated with a significant decrease reproductive ability. In mammalian females the hormone leptin, which is produced by fat cells, is the key regulator involved in initiating puberty when nutrient stores are sufficient to support reproduction and repressing reproduction when energy stores are in extreme excess and the reproductive fitness of the individual is compromised (Smith *et al.* 2002; Ebling 2005; Zieba *et al.*, 2005; and Budak *et al.* 2006).

In contrast, leptin is less important in regulating male reproduction in mammalian species because the energy costs associated with sperm production are minimal. In fact the levels of leptin in male mammalian species are negatively correlated with testosterone levels because testosterone, unlike estrogen, inhibits the synthesis of leptin by fat cells and thus provides

protection against reproductive failure as males become overweight. Ultimately in very obese mammalian males the levels of leptin reach a threshold where it starts to inhibit testosterone production. Thus roosters, like their male mammalian counterparts, may not have reproductive capability degraded with the addition of fat below obesity levels. Furthermore in the current research the full fed males did not become obese. Despite weighing 6 to 7 kg by 20 weeks of age, the full fed males appeared to have no greater body fat deposits than the restricted birds when the animals were killed for testes collection. The full fed males were very large bodied, muscular and prone to leg problems which would have made them useless for natural mating. In addition, the full fed males had over a 50% cumulative mortality rate which was more than double that of the restricted birds. However, the lack of fat accumulation in these birds may suggest that roosters may not have to be restricted to the degree that broiler breeder hens are. The onset of testosterone production was delayed as the degree of feed restriction increased. In addition, based on our research the convex restriction program besides delaying the onset of reproduction and increasing mortality had no detected benefit in reproductive fitness at as the roosters aged to 65 weeks of age. Thus our results may indicate that the severity of current feed restriction programs in male broiler breeders could be lessened without hurting fertility. This agrees with other research that has suggested that broiler breeder males are over feed restricted during the end of the broiler breeder production cycle (Buckner *et al.*, 1986; Sexton *et al.*, 1989 a, b; Cerolini *et al.*, 1995; Bramwell *et al.*, 1996, Romero-Sanchez *et al.*, 2008).

Obtaining a critical body weight and having the energy to support reproduction was enough to initiate testes development in the full fed broiler breeder cockerels at 8 to 11 weeks of age in the absence of any photostimulation. It is interesting to note that testes development in the full fed cockerels is the slow and steady progression in testes size and rate of testosterone

production through 25 weeks of age. In contrast, testes development was incredibly rapid in the feed restricted birds essentially occurring in a 3 week period from 25 to 28 weeks of age. None of the birds in Experiment 1 were photostimulated so testes development was based on other factors. However, factors other than obtaining a certain body weight were clearly involved since there is no correlation between the full fed and feed restricted cockerels and the body weights which testes development occurred.

The results suggest that a chronological reproductive maturation unrelated to acquiring photosensitivity and/or obtaining a certain body weight has to be at work to explain the slowness of the sexual maturation in the full fed males and the quickness of maturation in the feed restricted males. While body weight and having the metabolizable energy to support reproduction initiated reproductive development in the full fed males these factors were not enough to allow maximum testes development which occurred later when they reached 25 weeks of age which also coincided with the massive reproductive development in the feed restricted males. Further research is needed to explore what biological event or maturation occurred when all of the cockerels reached about 25 weeks of age and reproductive capability exploded in the feed restricted birds and peaked in the full fed birds. The results would argue that attempting to lessen the amount of time for developing sexually mature males from the current 24 to 25 weeks of age to 14 or 15 weeks of age by allowing more rapid growth and earlier photostimulation would yield roosters with less than optimum fertility. This increase in maturation rate has become more appealing because roosters flocks are now routinely grown for spiking (male replacement) purposes. Cutting the amount of time for the production of these replacement flocks would have financial benefits.

### **6.1.3. Plasma testosterone**

The plasma testosterone values obtained in the current research are similar to those obtained previously in broiler breeder males (Vizcarra *et al.*, 2004; 2010). The variability seen in the plasma testosterone concentration over time once sexual maturity was reached was also reported in a previous research report that examined plasma testosterone concentrations over time (Vizcarra *et al.*, 2010). Beyond establishing the functionality of steroidogenesis in the Leydig cells and the maturation progression of the testes as cockerels approach reproductive maturity, plasma testosterone levels in sexually mature male broiler breeders vary over a wide range and have no predictive value in determining a rooster's fertility (Bowling *et al.*, 2003).

In the current research, the production of testosterone was used to monitor testes development. The differences in plasma testosterone concentrations between the genetic strains and feed restriction programs were often actually greater than what the reported mean values indicate. Throughout the experiment, plasma testosterone values for individual roosters were often below the assay detection limit of 20 ng/dL. But, without a numeric value these very low testosterone concentrations could not be included in the overall treatment mean which results in misleading and inflated mean values of plasma testosterone. Regretfully the roosters with testosterone concentrations below the detection limit were often proportionally greater for the Cobb 500 males and the most feed restricted birds. To help better indicate the situations where this could be skewing the results, the number of cockerels or roosters with detectable testosterone concentrations out of the total number examined for each strain or feed restriction program was presented in our results with the mean testosterone concentrations.

#### **6.1.4. Plasma inhibin-B**

Beyond establishing the functionality of steroidogenesis in the Leydig cells and the maturation of the testes, plasma testosterone levels in male broiler breeders that are within a wide normal range have absolutely no predictive value in determining a rooster's fertility (Bowling *et al.*, 2003). Because broiler breeders breed through natural mating rather than artificial insemination and because of the millions of parent broiler breeders present in the United States at one time, there is no routine evaluation of semen quality or fertility. At the grandparent and great grandparent level there is more interest in evaluating male fertility at a degree greater than visualization of semen color, ejaculate volume and sperm concentration. However, finding a reliable, quick, and user friendly indicator of fertility has been elusive for poultry. The sperm mobility assay (Froman and Feltmann 1998; Froman *et al.*, 1999) is the best test because it accurately and reliably indicates rooster fertility, but the procedure for the analysis is somewhat complex and time consuming.

In all mammalian species studied, except rams in which inhibin-A is the predominate inhibin form found in blood, inhibin-B is the detectable form of inhibin found in the plasma. Therefore, in many male mammalian species, especially humans, plasma inhibin-B levels is gaining favor as an indicator for fertility (Anawalt *et al.*, 1996; Burger and Robertson 1997; Klingmuller and Haidl, 1997; Andersson *et al.*, 1998; Byrd *et al.*, 1998; Pierik *et al.*, 1998; Groome and Evans 2000; Hayes *et al.*, 2001) because the ELISA assay is fairly easy to conduct and quickly allows for the testing of a multitude of samples. However, our results indicate that there is no correlation between plasma inhibin-B concentrations and sperm mobility which is highly correlated with fertility in poultry. The fact that inhibin-A was not detectable in rooster plasma samples in the current research while inhibin-B was detectable is in agreement with a

previous report (Lovell *et al.*, 2000) and indicates that the lack of a relationship between fertility and plasma inhibin-B levels was not related to a faulty assay. Lovell *et al.*, (2000) reported that plasma inhibin A concentrations in cockerels are detectable at 5 weeks of age and show a rapid but transient rise from 14 to 18 weeks of age at the onset of sexual maturity and then decline thereafter to low levels in the adult. In contrast, plasma inhibin B concentrations are undetectable before 16 weeks of age but then increase progressively through 25 weeks of age in parallel with increasing plasma FSH and testosterone concentrations.

#### **6.1.5. Summary**

Although further research is needed in which the fertility of the roosters from both genetic strains is compared throughout the breeding production cycle with hens in a floor pen study, based on greater testes weights and lower mortality the Cobb MX males appear reproductively superior to the Cobb 500 roosters. Plasma inhibin-B concentrations do not appear to predict fertility in male broiler breeders as it does in mammalian species. Further research is needed to determine what factors control testes development in broiler breeders over time beyond, light, body weight and energy intake. Understanding these factors will allow for better feed restriction protocols to be developed and for the potential to improve fertility in male broiler breeder.

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