

**THE PHOTOPERIODIC RESPONSE OF MALE
BROILER BREEDERS**

by

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PREFACE

The experimental work described in this thesis was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from January 2003 to December 2009, under the supervision of Professor Rob Gous.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

NC Tyler (Candidate)

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DECLARATION 1 - PLAGIARISM

I, Nicola Claire Tyler, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis

For all publications, I, Nicola Tyler, carried out all the experimental work and wrote up the papers for publication under the supervision and guidance of my supervisor, Prof Rob Gous, the co-author, and in the case of Publication 3, after discussion with Dr Peter Lewis.

Publication 1

Tyler, N.C. and Gous, R.M. (2008) The effect of constant photoperiod on testis weight and the use of comb area to predict testis weights in broiler breeder males. *South African Journal of Animal Science* 38(2):153-158.

Publication 2

Tyler, N.C. and Gous, R.M. (2009) The effect of age at photostimulation of male broiler breeders on testes growth and the attainment of sexual maturity. *South African Journal of Animal Science* 39(3):169-175.

Publication 3

Submitted to British Poultry Science:

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ABSTRACT

The expression of photorefractoriness, a feature of seasonal breeding in birds, is important in production species, where egg production and fertility are affected by the photoperiod. Years of selection in meat-type birds have reduced the potential reproductive function, and the environmental manipulation of such genotypes is essential to maximise productivity. While it is known that egg-type pullets no longer exhibit photorefractoriness, and that female broiler breeders do, there is not much information about the response of male broiler breeders to photoperiod. Such information is important when designing lighting programmes for breeding stock, and this study aimed to gain more insight into the male response to photoperiod. A series of experiments was designed in order to achieve this. In the first the response of male broiler breeders to rearing on constant photoperiods was measured, and in the second the effects of age at photostimulation on age at sexual maturity were assessed. The response to an increase in photoperiod during the production cycle was investigated in the third trial. Evidence of photorefractoriness in males was observed, but this was not the case in all birds, possibly due to high variation in some of the fertility traits measured. It seems possible that males do not respond to photoperiod to the same extent as females. Unexpected observations of a response in some birds to early photostimulation, and the high variation observed prompted another experiment in which the heritability of the response to early photostimulation was measured as a potentially revolutionary method of selection, whilst checking that this response is not negatively linked to broiler growth rates. Left and right testis weight data, collected in the above experiments, were analysed for asymmetry. There was no consistent response in testes asymmetry to photoperiod, or evidence that asymmetry in testis weights is as a result of the imposition of genetic or environmental stress. The strong correlation found between testes dimensions and weight suggests that predictions of testis weights could be made through ultrasonics or laparoscopy

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1. GENERAL INTRODUCTION

The result of years of selection for growth traits in broilers has greatly reduced the time to slaughter and improved feed conversion efficiency, but the greater the selection pressure for these economic traits, the greater the potential reduction in reproductive function (Amann, 1999), which is a concern in broiler breeders that are required to be fertile but are heavily selected for production traits. While selection indices for broilers do consider fertility, progress via this route is considerably slower than that achievable through manipulation of environmental factors such as photoperiod and feeding schedules which can also enhance the phenotypic expression of fertility traits in broiler breeders.

Photorefractoriness, the state of being refractory (insensitive) to the stimulus of light, is a feature of seasonal breeding in birds. It ensures that wild birds do not try to rear offspring in unfavourable conditions, and this is achieved in two ways: juveniles are prevented from breeding in the same season as they hatch (juvenile photorefractoriness), and fertility in adults decreases after exposure to long days (adult photorefractoriness). Seasonal breeders are photorefractory when they hatch and require exposure to short days before this is dissipated, thereby preventing them from breeding late in the breeding season in which they hatched. Egg-type pullets appear not to exhibit juvenile (Lewis *et al.*, 1996) or adult (Morris *et al.*, 1995) photorefractoriness as is the case with some tropical species, such as the Spotted Antbird (Beebe *et al.*, 2005), where cues for optimal breeding originate from environmental factors other than light. However, photorefractoriness has been reported in most species, for example the Chuckar Partridge (Siopes and Wilson, 1978), the Black-headed Bunting (Kumar and Tewary, 1984), turkeys (Siopes, 2002), and more recently, broiler breeders (Lewis *et al.*, 2003).

Truly seasonal species exhibit the absolute form of photorefractoriness, where sexual maturation does not occur unless the birds are exposed to short days, such as in the Chuckar Partridge (Siopes and Wilson, 1978). Some domesticated species such as turkeys and broiler breeders exhibit the relative form of photorefractoriness (Lewis *et al.*, 2003; Siopes and Proudman, 2003), where rearing on short days allows, but is not essential for, the dissipation of photorefractoriness.

The development of photorefractoriness is said to be due to the inhibitory influences of photorefractoriness exceeding photoperiod drive (Siopes, 2001), the onset of which is probably initiated when the bird is first exposed to a long photoperiod (Dawson, 2001) and gradually the ratio of stimulatory to inhibitory factors decreases until egg production ceases. Although the overall flock response may be classified as relative photorefractoriness, turkey hens in a flock have been shown to exhibit varying degrees of photorefractoriness (Siopes and Proudman, 2003). Thus, near the end of a typical laying period a flock will consist of a variable mix of birds that are still photosensitive and laying, that could have shown spontaneous recovery of photosensitivity during the laying period, and others that are photorefractory, and have ceased to lay (Siopes, 2001).

Research directed towards the measurement of the response of broiler breeder females to light has only recently gathered momentum, with the results indicating that female broiler breeders, unlike commercial laying hens, exhibit photorefractoriness (Lewis *et al.*, 2003; Gous and Cherry, 2004; Lewis *et al.*, 2004). However, there is virtually no literature addressing the response of broiler breeder males to light, partly because fertility is harder to quantify in the male but mainly because it was believed that broiler breeders responded to light in the same way as did commercial laying hens, so the need to study their response was not recognised. While it is expected that the male and female response should be similar, this cannot be guaranteed, so it is necessary to assess the male response also in an effort to maximise fertility in both males and females. In most countries of the world, currently, male and female broiler breeders are reared under the same lighting conditions, whether in light-tight or open housing. Primary breeding companies (Aviagen, Cobb-Vantress and Hubbard) currently (management manual accessed online 2009 (Aviagen, 2009; Cobb-Vantress, 2009; Hubbard, 2009) recommend long (14 h) daylengths in rearing when light-tight rearing facilities are unavailable, and photoperiods in lay that are known to accelerate the onset of photorefractoriness in females (Lewis and Gous, 2006a). These strategies are not conducive for maximising fertility, and may account partly for the relatively poor fertility achieved with broiler breeders particularly towards the end of the laying period (Lewis, 2009), which was evident from Lewis *et al.* (2007c) where increases in photoperiod during the laying period had an adverse effect on rate of lay.

This study was aimed at gaining more insight into the response of male broiler breeders to photoperiod. With a better understanding of the expression and dissipation of photorefractoriness in the male, and by comparing the results obtained in this lighting study with the responses reported with female broiler breeders, it should be possible to define lighting programmes that will ensure that the fertility exhibited by males is close to their potential.

2. LITERATURE REVIEW

2.1 INTRODUCTION

Seasonal breeding in birds allows optimal survival of offspring, and because most species of birds fly, it is also advantageous to minimise the mass of the reproductive organs outside of the breeding season (Dawson and Sharp, 2007), especially in those species that migrate. Also, because good quality plumage is important for survival in birds, but is energetically costly, it is necessary for moult and replacement of plumage to occur during a period of reproductive inactivity (Dawson and Sharp, 2007). Photorefractoriness is the mechanism which causes seasonality in birds, and is expressed in the juvenile form to prevent breeding in the same season as hatch, and in the adult form after prolonged exposure to otherwise stimulatory photoperiods to terminate breeding.

For years the attempts to prevent seasonal breeding have been of interest to poultry producers, as well as breeders of other bird species and those requiring birds for song or breeding plumage, where periods of refractoriness prevent the desired attributes from being expressed. The concept of photorefractoriness has long been known. In a review of seasonal reproduction in birds, Burger (1949) summarised that “long days effect a persistence of the refractory phase, and short days favor its dissipation” but it was also stated that the mechanisms of refractoriness were poorly understood. This was confirmed later by Hamner (1968) who stated that “short days allow termination of photorefractoriness, but continuous light and LD 18:6 maintain refractoriness”.

With the selection of laying hens for high egg production, it appears as though there has been a concomitant loss in photorefractoriness. It was shown that egg-type pullets do not show photorefractoriness towards the end of lay (Morris *et al.*, 1995). While the rate of lay does decline with age, this is not a photoperiodic effect (Morris *et al.*, 1995), but has been attributed to a reduction in the rate of recruitment or yellow-yolky follicles, and towards the end of the year also to an increased incidence of follicular atresia, internal ovulation and the production of membraneous or soft shelled eggs (Williams and Sharp, 1978). Ageing of the reproductive tract and ovary probably also contribute to the reduction in the rate of lay.

Broiler breeders, however, still appear to exhibit photorefractoriness (Lewis *et al.*, 2004), and because the research reported in this thesis involves male photorefractoriness, previous research that has addressed this phenomenon is reviewed here, as are other impacts of lighting on fertility. A thorough understanding of these factors is essential if the fertility of broiler breeder males is to be maximised.

2.2 PERCEPTION OF LIGHT IN BIRDS

Birds perceive light, not only through retinal receptors, but also via extraretinal photoreceptors. This was made clear when the testis weights of ocular-enucleated House Sparrows responded in a similar manner to those of sighted birds, where testis weights increased in response to an increase in light, and were larger than in sighted birds which remained on short photoperiods (Menaker and Keats, 1968). Siopes & Wilson (1974) also showed that maturation of both male and female Japanese quail is not dependent on the eyes. The onset of sexual maturation, measured by the onset of cloacal gland foam formation, was actually advanced in blinded males compared to controls (Siopes and Wilson, 1974) and genetically blind laying hens produced significantly more eggs and were more efficient in converting feed into eggs than sighted controls, thought to be due to both reduced stress and activity lowering maintenance requirements (Ali and Cheng, 1985).

The route by which light reaches the extraretinal photoreceptor in the House Sparrow is preferentially through the dorsal surface of the head. When this route was suppressed using Indian ink injections under the skin of the head, there was not enough light penetration to cause a response in testis weight to an increase to a 16-h photoperiod, at low light intensity (7-14 lux). It was therefore assumed that retinal light perception has no impact on reproductive cycles as retinal perception would have been the same in both groups (Menaker *et al.*, 1970).

It was also concluded that the extraretinal photoreceptor is not the pineal gland, as there was no effect of pinealectomy on testis recrudescence in the House Sparrow (Menaker *et al.*, 1970), but it was later mentioned (Menaker, 1971) that the pineal may be one component of a multiple extraretinal photoreceptor. Likewise, starlings, after

pinealectomy, still exhibited photoinduced changes in prolactin secretion (associated with refractoriness) and therefore the pineal was considered not to have an effect on the onset, and dissipation of, photorefractoriness (Chakraborty, 1995). In Japanese Quail, pinealectomy had no effect on the rate of sexual maturation in both males and females (Siopes and Wilson, 1974). However, in turkey hens there was a significant delay in the onset of egg production after pinealectomy (Siopes and Underwood, 1987).

However, studies in the chicken do show the pineal to be directly photosensitive (Robertson and Takahashi, 1988) and that individual chick pineal cells possess photoreceptive, circadian clock and melatonin-synthesising abilities (Nakahara *et al.*, 1997), indicating the link between photoreception and control of circadian rhythms, although the pineal is not the sole extraretinal photoreceptor. This would have confounded the results from earlier experiments reporting the pineal not to be involved in photoreception.

Thus, although the pineal and eyes do have an influence over certain reproductive parameters, there is an extraretinal, extrapineal photoreceptive mechanism in operation (Siopes and Underwood, 1987). This has been shown to involve avian encephalic photoreceptors. Davies & Follet (1975a) demonstrated the role of the anterior tuberal hypothalamus (infundibular nuclear complex) in the photoinduced induction of gonadal development in the Japanese Quail, located more precisely within the preoptic area (Davies and Follett, 1975b). Opsin (the protein found in all photopigments) and gonadotropin-releasing hormone (GnRH) neurons and/or their axons were found in abundance in the lateral septum, the median eminence and the preoptic area through microscopy (Saldanha *et al.*, 2001). A direct interaction between brain photoreceptors and the reproductive axis was also demonstrated with the observation of opsin-positive synapses on GnRH dendrites in the septum (Saldanha *et al.*, 2001).

Two types of photoreceptor cells are found in the retina; rods, responsible for scotopic vision, and cones, responsible for vision in photopic conditions (Lewis and Morris, 2006). Four kinds of cone pigments have been identified (Okano *et al.*, 1989; Bowmaker *et al.*, 1997) namely chicken green, chicken blue, chicken violet and iodopsin (Yen and Fager, 1984) as well as the pigment rhodopsin in the rods (Fager and Fager, 1982; Okano *et al.*, 1989). The ratio of pigments of rhodopsin:red:green:blue:violet was found to be

49:40:5:5:1 (Okano *et al.*, 1989), with the mean maximal absorbance in chickens being about 571 (red), 507 (green) and 453 (blue) and 418 (violet) nm (Bowmaker *et al.*, 1997). The complement of photoreceptor classes was highly consistent across species (Bowmaker *et al.*, 1997), with the turkey showing very similar maximal absorbance values of 564, 505, 460 and 420 nm respectively (Hart *et al.*, 1999). The violet sensitive cone contains a transparent oil droplet (which can change the effective spectral sensitivity) which displayed no significant absorbance above 330 nm, suggesting that turkeys have considerable sensitivity to wavelengths in the ultraviolet-A (315 to 400 nm) range (Hart *et al.*, 1999). It has been shown that broilers can also “see” in the ultraviolet-A range (Prescott and Wathes, 1999) and because their spectral sensitivity is different to humans it is probable that chickens perceive colour differently to humans, and also that perceived luminosity is greater than in humans (Prescott and Wathes, 1999). Plumage may also be more readily visible in fluorescent light (Lewis and Morris, 1998).

Light signals via the retinal pathway can impact circadian rhythmicity (Zawilska *et al.*, 2004a) as more current evidence suggests that pineal serotonin N-acetyltransferase (an important enzyme in the melatonin biosynthetic pathway) activity is suppressed at low light intensities (4 lux). It is unlikely that such low light intensity can stimulate the pineal and/or encephalic photoreceptors through the skull. The suppression of melatonin has also been shown as a direct result of activation of D1-Dopamine receptors in the retina as administration of an antagonist to these retinal receptors did not suppress melatonin production (Zawilska *et al.*, 2004b). Therefore, despite extraretinal photoreceptors, the eye may still be the primary site of light reception at low intensities, and is therefore relevant in controlled environment houses, where illuminance is sometimes used to control bird behaviour (Lewis and Morris, 2006).

2.3 THE ENDOCRINE RESPONSE TO PHOTOPERIOD

Transfer of the light signal influences the neurons synthesising and secreting GnRH-I, which regulates the secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary, which, in turn, control the development of the gonads (Sharp, 1993). A second GnRH (GnRH-II) was isolated and determined in chicken hypothalamic extract (Miyamoto *et al.*, 1984), which was shown to play a part in regulating social interactions in House sparrows through the neural activity of GnRH-II

functioning as a neuromodulator or neurotransmitter for reproductive behaviours (Stevenson *et al.*, 2008). Control of gonadotropin release was also discovered, in quail, to be due to an inhibitory peptide in the hypothalamus termed gonadotropin-inhibitory hormone (GnIH) (Tsutsui *et al.*, 2000).

Other hormones implicated in the expression of photoperiodic control of reproduction include thyroid hormones and prolactin. Photostimulation induces the expression of the gene which encodes the enzyme type 2 iodothyronine deiodinase, which catalyses the reaction of thyroxine (T_4) to 3, 5, 3 –triiodothyronine (T_3) (Yoshimura *et al.*, 2003). Both the infusion of T_3 and the inhibition of the conversion of T_4 to T_3 inhibited testicular growth (Yoshimura *et al.*, 2003). Administration of T_4 to male starlings caused premature testes regression compared to control birds (Goldsmith and Nicholls, 1984b). Removal of the thyroid gland of photorefractory starlings kept on long daylengths resulted in testicular growth, albeit not immediately, but the testes of those kept intact remained small, and thus photorefractoriness was terminated in the same manner as if the birds had been subjected to short daylengths (Dawson *et al.*, 1985). Removal of the thyroid gland of photosensitive starlings prevents the induction of photorefractoriness altogether (Bentley *et al.*, 1997), and, while it suggested that thyroxine causes the development of photorefractoriness in European starlings (Goldsmith and Nicholls, 1984b), it was also suggested that thyroxine doesn't actively drive the photorefractory response but is rather a permissive factor (Bentley *et al.*, 1997).

Proudman (1998) showed that plasma prolactin levels rise following long day photostimulation. Although plasma prolactin levels may vary throughout the photophase and scotophase, it was shown that measurements of daytime levels accurately reflect the reproductive state of turkey hens, where levels are moderate in good layers and low in those that are photorefractory (Proudman, 1998).

Plasma prolactin concentrations were significantly elevated in response to thyroxine administration in male starlings (Goldsmith and Nicholls, 1984b). Although prolactin is not required for the eventual onset of photorefractoriness, it was suggested that it may mediate the onset (Proudman and Siopes, 2005). There is an association between high concentrations of circulating prolactin and the onset of photorefractoriness and the induction of the postnuptial moult in starlings (Dawson and Goldsmith, 1983; Dawson

and Sharp, 1998). The inverse association was also observed where starlings on 11 h, while exhibiting a slight increase in gonadotrophins and slow gonadal maturation, showed no increase in prolactin levels and these birds did not become photorefractory (Dawson and Goldsmith, 1983).

However, it was pointed out that even if prolactin does cause photorefractoriness, the neural or neuroendocrine processes underlying the phenomenon may not always be receptive to prolactin (Nicholls *et al.*, 1984), and although prolactin may hasten gonadal regression during the onset of photorefractoriness in starlings, it does not itself cause photorefractoriness (Dawson and Sharp, 1998). Sharp *et al.* (1998) found that increased prolactin is associated with increased vasoactive intestinal polypeptide (VIP). Immunisation against VIP in starlings, which would suppress prolactin secretion, during a photoinduced breeding cycle, was effective in slowing, but not preventing, the rate of development of photorefractoriness after transfer to long days. Because there were no significant differences in the levels of T₄, the possible delay in photorefractoriness due to hypothyroidism was negated (Dawson and Sharp, 1998). A comparison of short- and long-day breeders demonstrated that the annual cycle of increase in plasma LH leads that of prolactin in both (Sharp and Blache, 2003). Birds, such as the Emu, that are short-day seasonal breeders show the same response in plasma prolactin, with an increase corresponding with the time of photorefractoriness (Malecki *et al.*, 1998). LH and testosterone concentrations are high in autumn and winter, while prolactin is low, which induces sexual behaviour and reproductive function at this time (Malecki *et al.*, 1998). This was explained by Malecki *et al.* (1998) as either dissipation of photorefractoriness due to the decrease in daylength at this time, or due to an endogenous component based on seasonal change in photoperiod causing an annual rhythm to coincide hatching, after a 56 d incubation period, with spring.

Confounding effects of testicular hormones on prolactin secretion have also been observed. Sreekumar and Sharp (1998) reported that both testicular hormones and photostimulation stimulate prolactin secretion in bantam cockerels. While gonadal hormones may cause a negative feedback inhibition on the hypothalamus resulting in a drop in GnRH and gonadotrophins, this is not the only cause of photorefractoriness; castrated birds still exhibit photorefractoriness. Furthermore, because after testes regression, photorefractoriness is maintained while birds are exposed to long days,

gonadal hormones cannot be implicated in this response (Goldsmith and Nicholls, 1984a). In a review by Nicholls *et al.* (1988), it was concluded that the differences between avian species, in terms of whether or not gonadal steroids have an influence on photorefractoriness, is probably only in the degree to which gonadal steroids further reduce levels of gonadotrophins, already reduced as a result of photorefractoriness.

The castration response, however, may be species dependent. Nicholls *et al.* (1988) reviewed the castration response of Japanese Quail, grouse and mallards, where Japanese Quail show a small castration response on short day lengths, grouse show a clear castration response, which increases with photostimulation and the castration response in mallards is maximal. It was suggested that differences between species might be due to quantitative variations in magnitude of castration response, physical limits on GnRH secretion rate and completeness of hypothalamic inactivity during the refractory state, although the processes underlying photorefractoriness may be identical and steroid independent.

2.4 ILLUMINATION & PHOTOPERIODIC EFFECTS ON REPRODUCTION

The perception of light has an impact on circadian (daily) and circannual (yearly) rhythms, important in birds, and the effect of illumination and photoperiod, mainly on seasonal reproduction, will be considered.

2.4.1 ILLUMINATION

A light intensity of 2 lux is sufficient in layer strain pullets to allow full photoperiod response to stimulation (Lewis *et al.*, 1999) and to induce lay in Chukar Partridges (Siopes and Wilson, 1978), although in blinded Chukar Partridges this was reduced to 1.5 lux, possibly due to more light penetration after ocular enucleation (Siopes and Wilson, 1978). However, the threshold rearing illuminance for maximum egg production in broiler breeders lies between 10 and 40 lux (Lewis *et al.*, 2007b), thought to be a consequence of restricted feeding (Lewis *et al.*, 2007b).

Rearing illuminance (10 to 100 lux), and transfer to open-sided houses with the same illuminance in lay, had no impact on the onset of adult photorefractoriness, as evidenced

by similar egg production in the final 2 months of lay (Lewis *et al.*, 2007b), and Lewis *et al.* (2009) concur with current recommendations of 10 to 20 lux during rearing and 30 to 60 lux in lay. However, no evidence to support the addendum recommendation by some primary breeding companies, that provision of 100 to 150 lux might enhance egg numbers, was found (Lewis *et al.*, 2009).

A drop in light intensity can have the same effect as a drop in photoperiod. Chukar Partridges that were photorefractory were made photosensitive by decreasing light intensity from 100 to 4 lux, while remaining on 16-h photoperiods (Siopes and Wilson, 1978), although egg production was not as good as in the control birds that had a period of short days (8L:16D), and it was suggested that a light intensity of less than 1 lux is required to terminate photorefractoriness completely when birds remain on long days. Termination of photorefractoriness was thought to be due to the light stimulus being below the threshold of photoreceptors mediating the photosexual response, however there were variable degrees of termination of photorefractoriness, thought to be due to variability in the photoreceptors or a difference in the transmittance of light to the receptors (Siopes and Wilson, 1981).

A significantly superior terminal egg production was found from broiler breeders exposed to Arcadia bird lamps providing UV-A light at 10 lux compared to those only exposed to 10 lux of warm white light (Lewis *et al.*, 2007b). The birds would have perceived the Arcadia bird lamps as 20 % brighter than that from the white light lamps, but because egg production of birds exposed to ultraviolet light was also significantly higher than those exposed to 40 and 100 lux of white light, the significantly better terminal egg production was attributed to wavelength rather than illuminance (Lewis *et al.*, 2007b).

2.4.2 PHOTOPERIOD

It is important to remember that, because broiler breeders are feed restricted, there is an effect of body weight on acquisition of photosensitivity, where birds become photosensitive earlier on an accelerated growth curve (Dunn and Sharp, 1990; Lewis *et al.*, 2007a). However, because of the lack of interaction between growth curve and lighting treatment, the extent to which each affects age at sexual maturity can be predicted independently (Ciacciariello and Gous, 2005).

It has also been reported that, while seasonal birds rely mostly on photoperiodic cues, various supplementary cues result in the fine tuning to time reproductive success. An interesting report (Davis, 1945) describes how, when Australian Silver Gulls were moved to the National Zoological Park in Washington, USA in 1922, they retained the timing of breeding season of the southern hemisphere for two seasons (which entailed winter courtship and nest-building), and only after this shifted it to the spring and summer. However, in 1943, the descendants of the original stock reverted to timing of the breeding season to that seen in the southern hemisphere, which lead to hatching during a snow storm! However, this is mostly not the case. While there is some evidence the non-photoperiodic cues can affect the timing of photorefractoriness, no evidence was found that they could influence the time of the end of photorefractoriness (Dawson and Sharp, 2007). This review will focus primarily on the photoperiodic cues affecting fertility.

2.4.2.1 Constant photoperiod

While egg-type pullets show no evidence of juvenile photorefractoriness (Lewis *et al.*, 1996), female broiler breeders have been shown to exhibit juvenile photorefractoriness, where dissipation occurs faster with the provision of short days. Female broiler breeders on a constant 16-h photoperiod matured three weeks later than those on a constant 11-h photoperiod (Lewis *et al.*, 2003). Photorefractoriness was also dissipated at different rates when birds were reared on different constant photoperiods, with those on 10 or 11 h maturing significantly earlier than those on 12, 13, 14 or 16 h (Lewis *et al.*, 2004). Gous and Cherry (2004) showed that maintaining broiler breeders on 17 h led to a delay of 27 d in sexual maturity compared to those reared on 8 h and photostimulated at 19 weeks.

While age at sexual maturity in broiler breeder females is accelerated by exposure to short days, but not dependent upon them (Lewis, 2006), some species will show long delays in sexual maturity, or only achieve sexual maturity after having been exposed to short days. With the exception of one bird, Chukar Partridges held on 16L:8D did not become sexually mature even after more than 2 years (Siopes and Wilson, 1978), and Woodard (1980) showed in Red-legged Partridges that the first bird to lay an egg after being on a 16-h photoperiod was at 475 d, as opposed to the first bird to lay an egg at 257 d from those provided an 8-h photoperiod.

The response of males to photoperiod is more ambiguous. When male Delaware cockerels were provided with constant 1, 3, 9 or 13 h light from hatch there was no significant difference in the average age of onset of semen production, although growth was significantly slower in males on the 1 and 3-h photoperiods (Parker and McCluskey, 1965). However, the range of photoperiods is lacking considering the critical daylength of 9.5 h in chickens (Lewis *et al.*, 2008) and with advances made in genetic selection, these dated results are unlikely to be representative of what occurs in the modern broiler breeder. Renden *et al.* (1991) showed that the pattern of response in maturity of male broiler breeders to photoperiod was cubic, where males on 4- or 8-h photoperiods matured earlier than those on 2, 16 or 24 h. If birds were photorefractory, an advance in maturity in birds on the 2-h treatment would also have been expected, but body weight was positively related to light duration at 1 week of age and may have affected the delay in maturity of birds on the 2-h treatment. The delay in maturity seen in birds on 16 and 24 h compared to 8 h is similar to that seen in females (Lewis *et al.*, 2003).

After starlings were castrated (to avoid possible confounding effects of testicular hormones) a reproductive cycle was induced, and, after completion of moult, photoperiods of either 1, 5, 8 or 11 h were provided to determine the rate of reacquisition of photosensitivity by measurement of LH (Boulakoud and Goldsmith, 1994). Those on 1, 5 or 8 h showed a faster rate, implying that juvenile photorefractoriness is dissipated at a faster rate if birds are reared on photoperiods of 8 h or less, although there is no advantage in utilising photoperiods less than 8 h.

Short days dissipate photorefractoriness in the Black-headed Bunting, but only when the short days are provided on a 24h and 48h cycle. Birds that were given short days on a 12h (6L:6D), 36h (6L:30D) and 60h (6L:54D) remained photorefractory, with estimated testis weights after photostimulation similar to the control group kept on 15L:9D. This suggests that it is not just the short days that dissipate photorefractoriness, but short days coinciding with a particular phase of the circadian rhythm (Kumar and Tewary, 1984), which was also implied by Hamner (1968).

2.4.2.2 Age at photostimulation

Evidence of juvenile photorefractoriness in broiler breeder females is also shown by a delay in maturity when photostimulation occurs before photosensitivity is achieved. Birds photostimulated at 67 d as opposed to 124 d matured nearly 7 weeks later (Lewis *et al.*, 2003). Further work to determine the time required on short days for maximal dissipation of photorefractoriness to occur was investigated in female broiler breeders, and it was shown that birds photostimulated earlier than 14 weeks had not yet dissipated photorefractoriness (Ciacciariello and Gous, 2005). Birds transferred from an 8-h to a 16-h photoperiod, before achieving photosensitivity, mature as if they have been exposed to the 16-h photoperiod from day old (Lewis *et al.*, 2007a).

Photostimulation, in starlings, after two weeks did not provide enough time for the acquisition of photosensitivity, while photostimulation at 4, 6 or 10 week resulted in a positive effect on testis development (Boulakoud and Goldsmith, 1995). However, 4-week photostimulation, while allowing for the acquisition of photosensitivity, resulted in the fastest onset of photorefractoriness, and it was suggested that there is a progressive development in the ability to exhibit a maximal response to photostimulation (Boulakoud and Goldsmith, 1995).

2.4.2.3 Degree of photostimulation

No evidence of adult photorefractoriness was observed in egg-type pullets (Morris *et al.*, 1995), however, superior egg production in broiler breeder females was observed after photostimulation to 11 or 12 h as opposed to photostimulation to 16 h (Ciacciariello and Gous, 2005; Lewis and Gous, 2006b; Lewis *et al.*, 2007c). There was no compensatory increase in egg weight associated with the decline in rate of lay (Lewis *et al.*, 2007c) as occurs in egg-type pullets. The inferior egg production is caused partly by an advance in the age at which individual birds become photorefractory on longer days, but may also be due to a higher demand for maintenance (Lewis and Morris, 2006).

Research in broiler breeders has shown that there is a critical and a saturation daylength after transfer from a non-stimulatory rearing photoperiod. The critical daylength is the shortest photoperiod needed to stimulate LH release and the saturation daylength is the

shortest photoperiod needed to stimulate maximum LH release (Dunn and Sharp, 1990). In dwarf broiler breeders the critical daylength was < 10.5 h and the saturation daylength was between 12.75 and 15.25 h (Dunn and Sharp, 1990). Lewis *et al.* (2008) refined this, after examining a greater range of photoperiods, in broiler breeders, to be about 9.5 and 13 h, although the minimum photoperiod to achieve a significant change in either LH or advance in age at first egg is between 11 and 11.5 h (Lewis *et al.*, 2008). Male broiler breeders exposed to the same lighting treatments showed no difference in the age at sexual maturity, but semen concentration was greater in males, at the corresponding female age at sexual maturity, on treatments ≤ 11.5 h (McPhail and Tyler, 2008).

A reduction in fertility of meat-type males was observed on a 2-h photoperiod compared to either 6, 14 or 15.5 h, and, due to a reduction in both energy consumption and aggressive behaviour, it was considered advantageous to use a 6-h photoperiod (Proudfoot, 1981). However, in this experiment, males were provided 17 h light/d for the first week and thereafter light was reduced by 15 min a week, resulting in a photoperiod of 13 h at 119 d. Males were only transferred to lighting treatments at 120 d, so this does not give an indication of the dissipation rate of juvenile photorefractoriness and because 13 h is known to be about the saturation daylength (Lewis *et al.*, 2008), the response in sexual maturity, while not reported, may have been the same, and any effects on fertility could be due to the photoperiod used after 120 d.

Chen *et al.* (2007) reported that the age at first egg was significantly advanced in pullets transferred to 17L:7D at 20 weeks after rearing on 8L:16D compared to an increase from the same rearing photoperiod to 13L:11D and 11L:13D. However, the age at first egg of these particular birds was 144.8 ± 3.2 days, and considering the process of yolk formation takes about 7 to 8 days, the age at first egg response could not have been due to light, as yolk formation would have started before photostimulation. This could have been due to body weight, as birds were weighed before being randomly assigning to pens, but treatment allocation was not blocked for weight. Lewis *et al.* (2008) showed that there is no further advance in age at first egg of broiler breeders with photostimulation above about 13 h.

Perhaps a misconception about providing a longer photostimulatory daylength for broiler breeders is the concern of a possible lack of maximal response, because despite

recommendations not to photostimulate to more than 14 h (Lewis and Gous, 2006a; bb) current Breeder Company recommendations still advocate the provision of light up to 16 h (management manuals accessed online 2009). In fact, long daylengths afford no advantage but, due to the earlier onset of adult photorefractoriness, are actually counterproductive.

2.4.2.4 Absolute and relative photorefractoriness

Hamner (1968) proposed the hypothesis that the refractory period in the House Finch is composed of absolute refractoriness and relative refractoriness. The absolute refractive period was described as the absence of gonadal growth when exposed to even continuous light, which was said to be followed by a state of relative refractoriness, in which birds gradually regain photosensitivity. As the daylength shortens, approaching winter, it was proposed that there is continual temporal readjustment of the timing mechanism so that progressively shorter and shorter days become photoperiodically stimulatory (Hamner, 1968).

This hypothesis refers to the reacquisition of photosensitivity after the expression of absolute photorefractoriness and Nicholls *et al.*, (1988) depicted this diagrammatically (Figure 2.1). Birds exhibiting absolute photorefractoriness are in a state where inhibitory influences override the stimulatory influence of photoperiodic drive. Under short days, photoperiodic drive is reduced, while the inhibitory effect slowly declines. After a period of time, if birds were returned to long days, the photoperiodic drive would be greater than the inhibitory influences, until the inhibitory process is minimal and full photosensitivity is achieved. During the period where the inhibitory effect is gradually reducing, and photoperiodic drive would be greater than inhibitory influences, before full photosensitivity is achieved, the bird is considered to be relatively photorefractory.

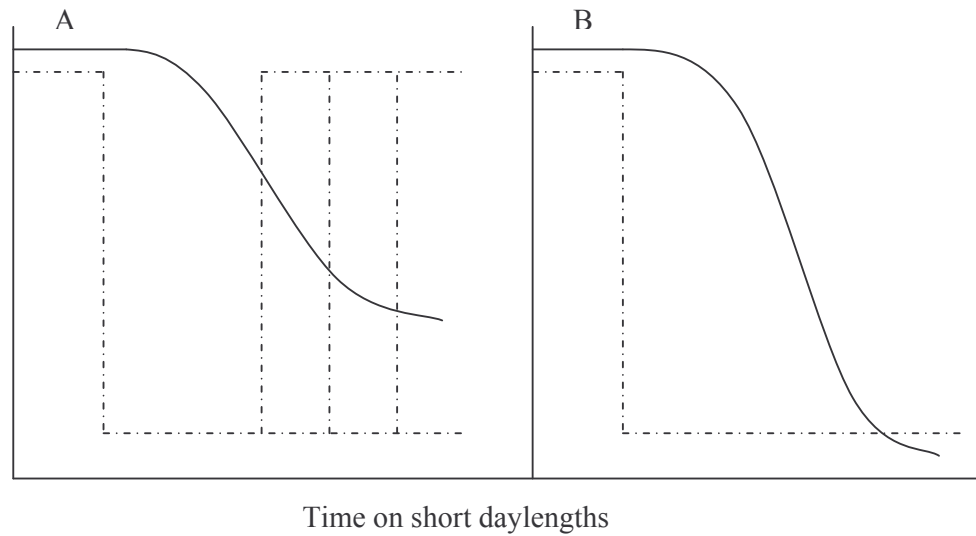


Figure 2.1: Diagrammatic representation of the termination of photorefractoriness to long days, redrawn from Nicholls *et al.* (1988), where the relationship between inhibitory influences (—) and the stimulatory influence of photoperiodic drive (----) determines the timing of photorefractoriness. A species such as the White-crowned Sparrow (A), where, upon exposure to short days, there is a gradual reacquisition of photosensitivity, an increase in photoperiodic drive would be greater than the inhibitory influences, and B, a species such as the starling, that shows gonadal development spontaneously after a period of exposure to short days without an increase in photoperiodic drive.

Nicholls *et al.*, (1988) contrasted the above response with species in which the inhibitory effects are markedly reduced so that even the minimal photoperiodic drive of short days allows spontaneous gonadal development, and this results in the concept of different species showing either absolute or relative photorefractoriness. Sharp (1996) describes different genotypes as having different strengths of the inhibitory input to GnRH-I neurones (activated by increasing daylength) and different rates of development. The development of absolute photorefractoriness is due to a strong photoinduced inhibitory input to GnRH-I neurones that eventually overrides the photoinduced stimulatory input, whereas a weaker photoinduced inhibitory input may have no noticeable effect on reproductive activity until daylength begins to decrease, which weakens the stimulatory input, allowing it to be overridden by the inhibitory input, resulting in relative photorefractoriness. This means that reproductive activity can be stimulated in birds showing relative photorefractoriness by further increasing the daylength, whereas birds showing absolute photorefractoriness will not respond to an increase in daylength (Sharp, 1996).

Lewis (2009) compared typical rates of egg production for truly-seasonal, relatively photorefractory and non-photorefractory species of poultry (Figure 2.2). Those that are truly seasonal show gonadal regression and remain infertile until the following spring. For economical reasons, the production cycle in brown-egg hybrids and Pekin Ducks is stopped, but it is clear that egg production would continue for some time, regardless of photoperiod, and these species are therefore considered non-photorefractory. Turkeys and broiler breeders, that show relative photorefractoriness have a faster rate of decline of egg production than non-photorefractory species, but often pause, but spontaneously return, to production. The Japanese Quail is a species also thought to exhibit only the relative form of photorefractoriness (Robinson and Follett, 1982). Refractoriness was said not to be absolute, since birds with regressing gonads can be photostimulated by an increased daylength, but gonadal regression does occur under daylengths that were once stimulatory.

The hypothesis of Hamner (1968) describes the period of relative photorefractoriness to be after gonadal regression until the reacquisition of photosensitivity, whereas other authors (Robinson and Follett, 1982; Follett and Nicholls, 1984; Nicholls *et al.*, 1988; Proudman and Siopes, 2002; Siopes and Proudman, 2003; Dawson and Sharp, 2007) refer to the period of relative photorefractoriness as the period between acquisition of photosensitivity to the time of gonadal regression. Siopes (2001) describes the turkey hen as becoming absolutely photorefractory when the inhibitory influences of photorefractoriness exceed photoperiodic drive and result in the cessation of egg production, but prior to this, during the gradual decline in egg production from peak performance, the turkey hen is said to show relative photorefractoriness. Similarly, the absolute photorefractory condition was said probably to be due to the culmination of an inhibitory process that develops gradually and progressively from the time the birds first experience long days (Nicholls *et al.*, 1984). Birds may first become relatively photorefractory, when laying would cease only if photoperiod is reduced, and then become absolutely photorefractory, when laying will cease despite a long daylength (Siopes and Proudman, 2003). Thus, the transient stage in the development of absolute photorefractoriness may be analogous to relative photorefractoriness (Dawson and Sharp, 2007), and it is thought that perhaps all birds pass through a stage of relative photorefractoriness (Dawson and Sharp, 2007).

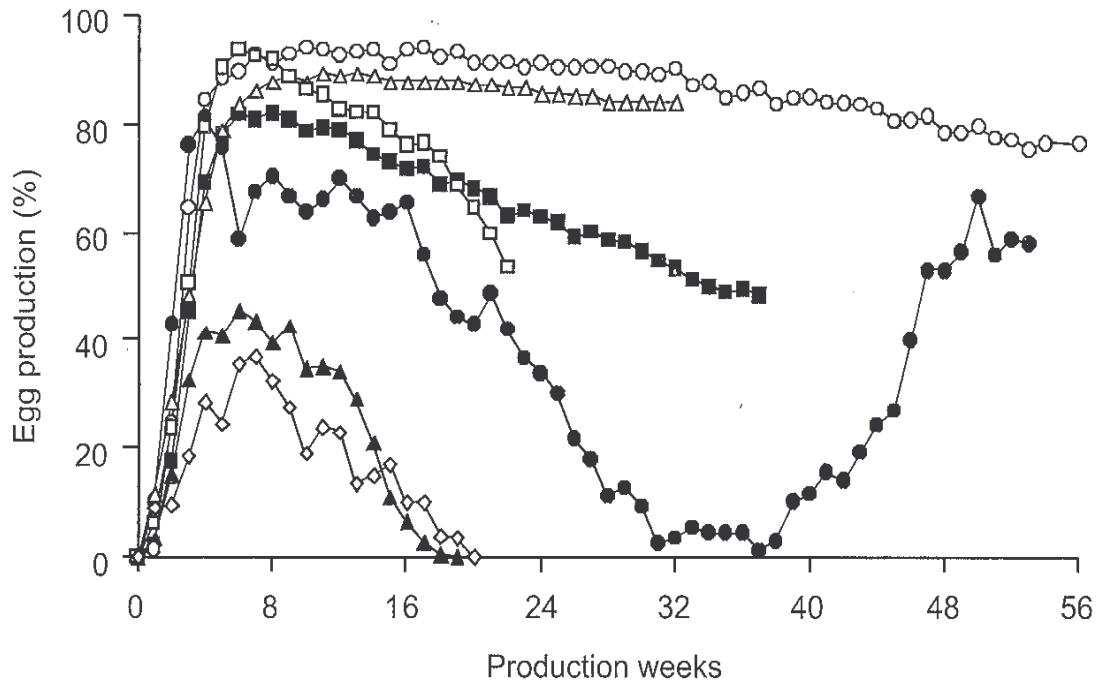


Figure 2.2 Mean weekly rates of egg production for brown-egg hybrids (o), Pekin Ducks (Δ), Muscovies (\square), broiler breeders (\blacksquare), domestic turkeys (\bullet), Emden Geese (\blacktriangle) and Chukar Partridges (\diamond), from Lewis (2009).

Siopes and Proudman (2003) showed that if turkey hens are exposed for two weeks to a lower photoperiod (but kept above the critical daylength at 11.5L:12.54D), egg production drops significantly below that of the untreated controls (maintained on 16L:8D), and in some cases ceases completely. However, within four weeks of re-exposure to the stimulatory daylength of 16L:8D, there was a robust return in egg production to at least the level of the untreated control group. Thus, there is a progressive increase in the rate of expression of absolute photorefractoriness in turkey hens, confirming that relative photorefractoriness is a lesser form of, and precedes, absolute photorefractoriness (Siopes and Proudman, 2003).

The Spotted Antbird (a tropical species) is thought not to show absolute photorefractoriness (Beebe *et al.*, 2005). Light increases given at the end of the breeding season in birds with regressed gonads during moult stimulated testis growth in both males given a 1 h increase in light from 12 to 13 h and birds given an increase to 22 h of light. In male Spotted Antbirds the small increase in light from 12 to 13 h stimulated testis growth,

including that in hatch-year males, who, if they did exhibit absolute photorefractoriness, would not be able to breed in the year in which they hatch.

MacDougall-Shackleton *et al.* (2006) showed that the two criteria used to indicate absolute photorefractoriness (that gonads will spontaneously regress when held on long days, and once gonads have regressed, that birds are unresponsive to very long, and even continuous, days) are not necessarily a unitary phenomenon, and one of these criterion for photorefractoriness does not necessarily imply the other.

2.4.2.5 Mechanism/models for photorefractoriness

Hamner (1968) stated that as day length shortens there is a continual temporal readjustment of the timing mechanism, by changing the duration of the two phases of the same circadian photoperiodic clock which times gonadal growth in the spring, so that shorter days become stimulatory. Robinson & Follett (1982) proposed that in the quail, the photoperiodic mechanism of time measurement changes its properties as a result of the birds photoperiodic history, so that the critical daylength is altered such that after experiencing the long days of summer, the critical photoperiod is increased.

Storey & Nicholls (1976) proposed that photorefractoriness is caused by a form of exhaustion of the reproductive neuroendocrine or endocrine system due to previous secretory activity. It was suggested that very long photoperiods would result in a highly stimulatory input which would force a high level of secretory activity with a rapid progression towards an exhausted state, whereas short photoperiods would allow a gradual recovery.

Sharp (1993) proposed that the physiological state of the bird at the time modulates the response, and that as egg production progresses in pullets, daylength should be increased progressively, just sufficiently to balance the progressive development of photorefractoriness. However, Morris (2004) argued that pullets do not appear to exhibit photorefractoriness and that quantitative predictions of the age at first egg on different lighting patterns cannot be made from this.

In European Starlings, Falk & Gwinner (1988) suggested that the development of photorefractoriness is more dependent on photoperiodic cues occurring later in the season, but more recent evidence (Dawson, 2001) showed that the mechanism causing photorefractoriness in European Starlings is initiated during the first long photoperiod so that the acquisition of photosensitivity may also be associated with the start of photorefractoriness.

In some birds, there is a reported rate of dissipation of photorefractoriness inversely proportional to the photoperiod (Dawson and Goldsmith, 1983; Nicholls *et al.*, 1988). The period of commencement of testicular growth to the onset of photorefractoriness and gonadal regression in the Border Canary was suggested to be inversely related to the duration of the photostimulatory photoperiod (Storey and Nicholls, 1976). However, in broiler breeders, the rate of dissipation of photorefractoriness is not a simple linear relationship (Lewis *et al.*, 2004).

Lewis *et al.* (2003) suggested that the rate of development of adult photorefractoriness in broiler breeder females is inversely related to the rate at which the juvenile form is dissipated due to the earlier sexual maturity and more persistent egg production from birds photostimulated at 124 d, compared with birds reared and maintained on a constant 16-h photoperiod, or photostimulated at 67 d (where birds responded as if reared on 16 h). The faster the rate of development of adult photorefractoriness, the fewer eggs will be produced in a season. An inverse relationship was identified between the onset of photorefractoriness and the total egg production in turkeys (Siopes, 2001), but the early onset of photorefractoriness was not related to prior rate of lay (Siopes, 2001).

A slower rate of development of photorefractoriness is associated with a more plastic variation in the timing of reproduction (Hahn *et al.*, 2004). Absolute photorefractoriness completely prevents inappropriately timed late summer and autumn reproductive attempts but may be costly because it restricts timing of breeding and therefore reduces potential reproductive flexibility (Hahn and MacDougall-Shackleton, 2008). Thus relative photorefractoriness was thought to confer some of the advantages of absolute photorefractoriness such as the reduction of breeding in unfavourable conditions, without eliminating the possibility of breeding should unusually favourable conditions be encountered (Hahn and MacDougall-Shackleton, 2008). Therefore it was suggested that

relative photorefractoriness should only evolve, or be maintained, when the cost of missing some legitimate reproductive opportunities is outweighed by the benefit of not trying to breed at inopportune times (Hahn and MacDougall-Shackleton, 2008).

It also appears that, while birds are very sensitive to photoperiod changes, the control of timing of breeding in birds is not related to the absolute photoperiod, but rather the response is due to the change in shape of the photoperiod (Dawson, 2007).

2.4.2.6 Population differences in response to light (or genetic control)

The flock response to the time of photostimulation can be variable, despite uniformity in the flock body weight targets. If broiler breeder females are photostimulated at younger than 19 weeks of age, sexual development is delayed in a proportion of birds, while advanced in others, despite uniform, and on target, body weights (Lewis and Morris, 2006). Thus, when a flock is photostimulated after the first bird becomes photoresponsive, but before the whole flock has achieved photosensitivity, a bimodal frequency distribution of age at maturity will result from the advance in maturity of some birds, but a delay in others (Lewis, 2009).

Variability in the response to light has also been observed in turkey hens. Not all turkey hens become photorefractory (Siopes, 2001), and within a group exposed to a short photoperiod (for two weeks) during lay, the varied response allowed classification into groups of non-responders, partial responders and full responders (Siopes and Proudman, 2003). The egg production of non-responders did not differ from the untreated controls left on 16L:8D. Partial responders were those whose egg production declined by more than one standard deviation of the control group level, and full responders ceased egg production for at least 7 days following the light treatment.

Differences in photoresponsiveness between turkey breeder hens, shown by differences in egg production, were not reflected in circulating levels of either LH or PRL (Siopes and Proudman, 2003), suggesting that the most photosensitive hens can maintain egg production with levels of LH low enough to terminate egg production in other hens, although the authors do not preclude the possibility that alterations in the preovulatory LH

surge may account for the differences in photoresponsiveness (Siopes and Proudman, 2003).

Proudman (1998) showed that plasma prolactin levels rise following long day photostimulation. Although plasma prolactin levels may vary throughout the photophase and scotophase, it was shown that measurements of daytime levels accurately reflect the reproductive state of turkey hens, where levels are moderate in good layers and low in those that are photorefractory (Proudman, 1998).

In White Guineas given an increase in light before the breeding season in an attempt to obtain hatching eggs at an earlier age, egg production was initiated earlier than those not given the light increase, but the time taken to reach 50 % production was lengthened (Davis and Penquite, 1942). This indicates the variability in those that responded early, whereas the initiation in egg production was much more uniform, but later, in those not given a precocious increase in light. While most Chukar Partridges remained photorefractory when held on 16L:8D, one bird did lay a few eggs without any previous short day exposure, inferring that the potential for genetic selection against photorefractoriness exists (Siopes and Wilson, 1978). Whether this would result in concomitant selection for egg production traits and against meat and growth traits needs to be verified, and the selection for egg production in egg-type hybrids inadvertently resulted in the loss of photorefractoriness.

2.4.2.7 Gender differences in response to photoperiod

While photorefractoriness in the male avian species has been demonstrated in species such as starlings (Dawson and Sharp, 1998) and bantams (Sreekumar & Sharp, 1998), there is a dearth of information regarding the expression of photorefractoriness in broiler breeder males. Gender differences in the response to photoperiod have been suggested in the Spotted Antbird (Beebe *et al.*, 2005) where follicle size in females did not show the same increase as testis size in males given an increase in light at the end of the breeding season. This could have been due to the small number of females tested, but could be that females cannot respond to a small increase in light in the same manner as males (Beebe *et al.*, 2005). Although it seems unlikely that reproductive control mechanisms would differ between the genders, as it would render the adaptation in males useless if the females

could not breed, it could enable males to be fertile by the time females became reproductively active, especially due to the higher reproductive cost to females. It appears that the lack of photorefractoriness is an evolutionary adaptation to allow flexibility at the end of the breeding season in species living in tropical areas, where favourable conditions may persist (Beebe *et al.*, 2005). Common Weaver males, also a tropical species, do not exhibit photorefractoriness, as sexual activity was maintained throughout the duration of the experiment (15 months), without testes regression or loss in breeding condition occurring (Thapliyal and Saxena, 1964).

Therefore, it cannot be assumed that because broiler breeder females exhibit photorefractoriness the males will too. While photorefractoriness is observed in female turkeys (Siopes, 2002), whether male turkeys exhibit photorefractoriness is questionable. Male turkeys maintained under constant long days exhibit precocious semen production (Polley *et al.*, 1962; Noirault *et al.*, 2006). An increase in both LH and testosterone (T) was also observed at about 18 weeks of age in males kept on both 16L:8D or 6L:16D from 10 until 29 weeks of age (Yang *et al.*, 1998b). The turkey toms responded positively to early photostimulation (although lighting treatment for the first 10 weeks is unknown, but assumed to be 6L:16D) where 13 birds on the 16L:8D treatment were producing semen by 25 weeks of age whereas only 4 out of 14 males were producing semen in the 6L:16D treatment group. If turkey toms exhibit juvenile photorefractoriness it would be expected that the 6-h photoperiod should allow faster dissipation of photorefractoriness than the 16-h photoperiod and that there would be no response to 10 week photostimulation, which was not the case. Proudman & Siopes (2005) also found no evidence of photorefractoriness in turkey toms, because plasma prolactin levels were found to be low in turkey toms following photostimulation, unlike the increase observed in turkey females, thought to be associated with the onset of photorefractoriness (Proudman, 1998). Thyroid hormones did not differ between females and males in this study, but the levels varied to a greater extent in males with an apparent 2- to 3-w cyclical interval in restricted males, while full-fed males showed less variation.

From the literature it is clear that a clearer understanding of the male broiler breeder response to photoperiod is required in order to better interrogate the efficiency of current lighting programmes, especially considering the concern for the decline in fertility with greater selection pressure for meat traits in broiler breeders, and given that each male in

the selection process has an impact on more offspring than each female. The following experiments were designed in order to gain a better understanding of the male breeder response to photoperiod.

3. THE EFFECT OF CONSTANT PHOTOPERIOD ON TESTIS WEIGHT, AND THE USE OF COMB AREA TO PREDICT TESTIS WEIGHTS IN BROILER BREEDER MALES¹

3.1 INTRODUCTION

Broiler breeder pullets have been shown to exhibit both juvenile and adult photorefractoriness, a condition in which there is no photosexual response to an otherwise stimulatory daylength (Lewis *et al.*, 2003; Gous and Cherry, 2004). This condition is evident in meat-type hybrids, while selection for early maturity and high egg production in laying-type pullets has led to the disappearance of this phenomenon in these birds. It appears that the photoperiodic response of male and female meat-type hybrids is similar (Parker and McCluskey, 1965; Renden *et al.*, 1991; Lewis *et al.*, 2004); however, it would be beneficial to determine if this is the case, in order to maximise fertility of both males and females in the design of lighting programmes.

While female fertility may be quantitatively measured by oviposition, male fertility is more difficult to assess. Wilson *et al.* (1988) report a, positive ($P < 0.001$) ($r = 0.79$) correlation between number of spermatozoa per ejaculate and testes weight. Brillard & de Reviers (1981) showed, in guinea fowl, a strong, positive relationship ($r = 0.84$) between number of ejaculated spermatozoa and total testis weight; however, because the testes are internal, this measurement is only possible with the use of ultrasound (Hofbauer and Krautwald-Junghanns, 1999). Many assessments of semen quality have been used in an attempt to quantify male fertility, such as volume (Brown and McCartney, 1983), concentration (Ansah *et al.*, 1983; Bilgili and Renden, 1984), morphology and mobility (Froman *et al.*, 1999; Froman and Feltmann, 2000; Donoghue *et al.*, 2003). However, this requires training of males, as well as the fact that collecting semen is not necessarily representative of semen quality during natural mating. Wilson *et al.* (1988) reported that 25 % of the males used in their study that exhibited spermatogenic activity, based on

¹ Tyler, N.C. and Gous, R.M. (2008) The effect of constant photoperiod on testis weight and the use of comb area to predict testis weights in broiler breeder males. *South African Journal of Animal Science* 38(2):153-158 (Appendix 1).

visual appraisal of seminiferous tubular size and maturity of cell types, failed to ejaculate semen.

Spermatogenesis relies on testosterone, which can also be measured in the blood as an indicator of fertility (Schanbacher *et al.*, 1974; Rozenboim *et al.*, 1993). However, an external, highly correlated, non-invasive indicator of testis size would be a valuable measurement. Testosterone is also responsible for the development of secondary sexual characteristics, such as comb development (Zeller, 1971). There is literature to suggest that birds with large ornaments are expressing higher reproductive fitness. It has been shown in red jungle fowl that, as well as favouring new females or females with high promiscuity, males also favour females with large ornaments, by preferentially allocating sperm to these individuals, which are thought to signal superior maternal investment (Pizzari *et al.*, 2003). In males, comb size had a significant ($P < 0.05$) effect on male-female proximity (Graves *et al.*, 1985), where females exposed to unfamiliar males stayed closer to males with larger combs. Parker & Ligon (2002) found that crowing, a secondary sexual characteristic, occurred more frequently in larger-combed males, and females preferred males with larger, brighter combs (Zuk *et al.*, 1995). This paper investigates the relationship between comb size and testis weight in male broiler breeders, and the effects of constant photoperiods on fertility.

3.2 MATERIALS AND METHODS

Day old male Ross broiler breeders were given 48h light and thereafter placed on one of six constant photoperiods; 8, 10, 12, 14, 16 or 18h. Each photoperiod was replicated twice, starting with 50 birds in each light-tight room. Lights were set to come on at 07:00 for all treatments.

Birds received ad-libitum feed for the first three weeks, and thereafter were fed a commercial breeder ration to follow the growth curve recommended by the primary breeder. Each bird was weighed on a weekly basis, and feed was allocated to a room based on the average body weight of birds in that room.

Males were trained for semen collection, starting at 19 weeks.

One or two birds from each room, depending on the stocking density and mortality rate, were slaughtered weekly from 71d to 232d, and the remaining birds at 467d. Before slaughter, a digital photograph was taken of the left and right side of the bird, with a linear scale being included in each photograph. The area of the comb was measured with image analysis technology (McGary *et al.*, 2003).

After slaughter, both the left and right testes were removed, weighed, and their dimensions recorded.

A general ANOVA of the average testis weight (left and right testis weights were not significantly different) and comb area for the six photoperiods was performed. A logistic regression of average testis weight until 232d and comb area was fitted using GenStat 6th edition (2006)

3.3 RESULTS AND DISCUSSION

A positive ($P < 0.001$) relationship between average testis weight and comb area was found, with 69 % of the variance in average testis weight being accounted for by comb area (Figure 3.1), suggesting that comb area provides a valid indicator of testis weight. There appear to be a few outliers as comb size and testis weight increase. Thus, it appears possible that weak individuals may try to signal dominance by displaying well-developed ornaments. However, a behavioural mechanism has been reported, in which dominant acting males (those with large combs and a higher incidence of crowing) show aggression towards like individuals, which allows “punishment of cheaters” (Parker and Ligon, 2002). A negative relationship between comb size and circulating lymphocytes has also been identified (Zuk *et al.*, 1995), inferring a cost to the immune system by maintenance of secondary sexual characteristics. In females, the relationship between comb size and plasma oestradiol concentration was small, but the change in comb size was a good indicator of age at sexual maturity (Joseph *et al.*, 2003).

Other non-invasive measurements are possible. Sperm penetration of the perivitelline membrane over the region of the germinal disc can be assessed (Bakst and Howarth, 1977; Wishart, 1997; Hazary *et al.*, 2000; McGary *et al.*, 2003) and is positively correlated with egg fertility ($r = 0.89$, $P < 0.0001$) (Bramwell *et al.*, 1995). Behavioural

traits could also be used as a non-invasive indicator of fertility. Wing flapping, in particular, was correlated with dominance and fertility (Jones and Mench, 1991; Leonard and Zanette, 1998). However, measuring comb size would practically be easier than observing behaviour.

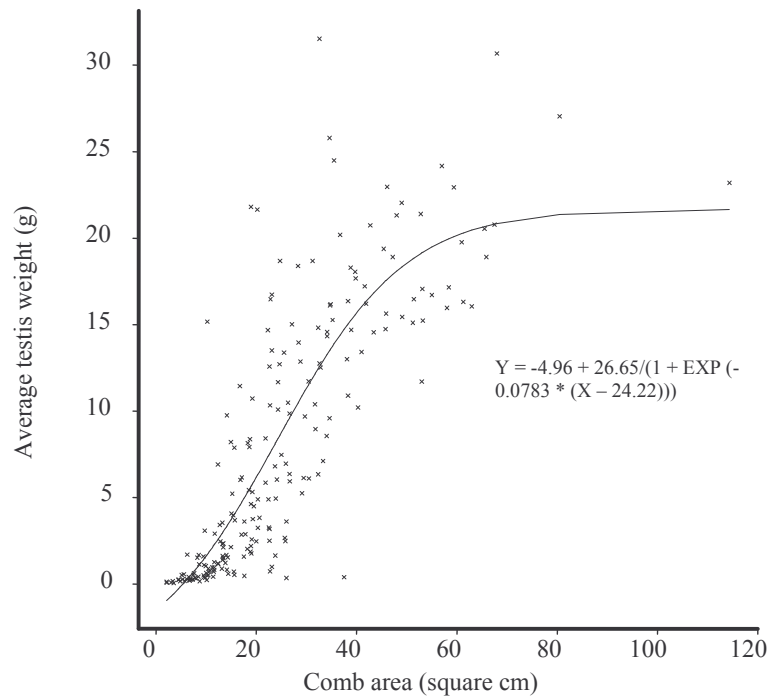


Figure 3.1 Logistic regression of average testis weight and comb area

Both juvenile and adult photorefractoriness were evident in this experiment, with photoperiod having an effect ($P < 0.001$) on average testis weight over the entire slaughter period. Photoperiods of 8 and 10 h resulted in significantly higher testis weights, whilst any photoperiod over 14 h reduced this weight. Average testis weights at 165 d (Figure 3.2) show evidence of juvenile photorefractoriness ($n=4$ for all photoperiods except $n=3$ for 18h) where birds were not responsive to the stimulatory photoperiods. Average testis weights from images of birds not slaughtered at 165 d were predicted using the relationship established in Figure 3.1, and the only testis weights above 10 g were predicted from birds on the 8, 10 and 12 h photoperiods.

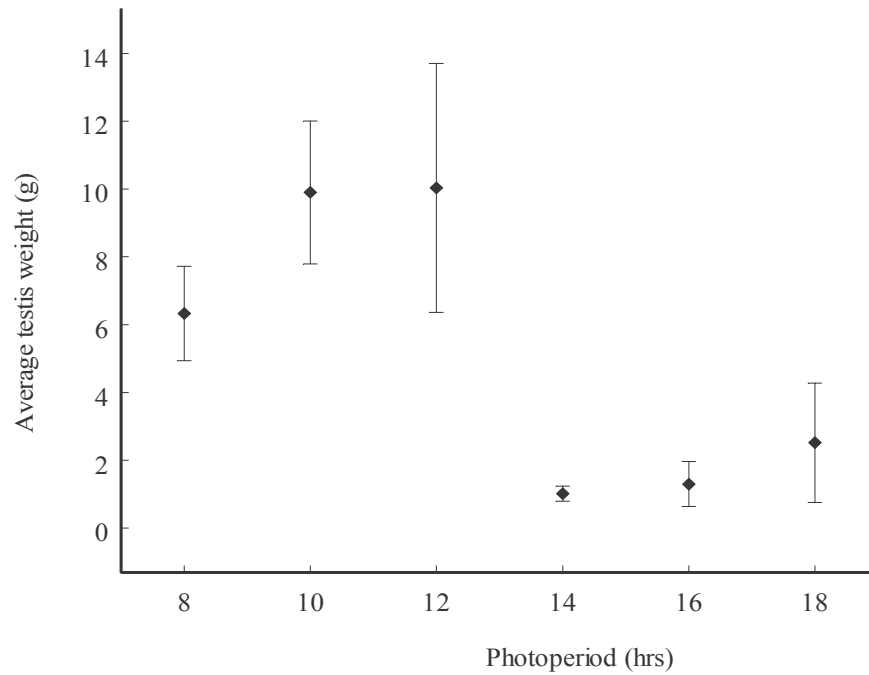


Figure 3.2 Ave testis weights at 165d of birds on different constant photoperiods (n=4 for each treatment, except 18 h, n=3).

As the birds aged, juvenile photorefractoriness was dissipated, and photoperiod had less impact on average testis weight by 189 d (Figure 3.3).

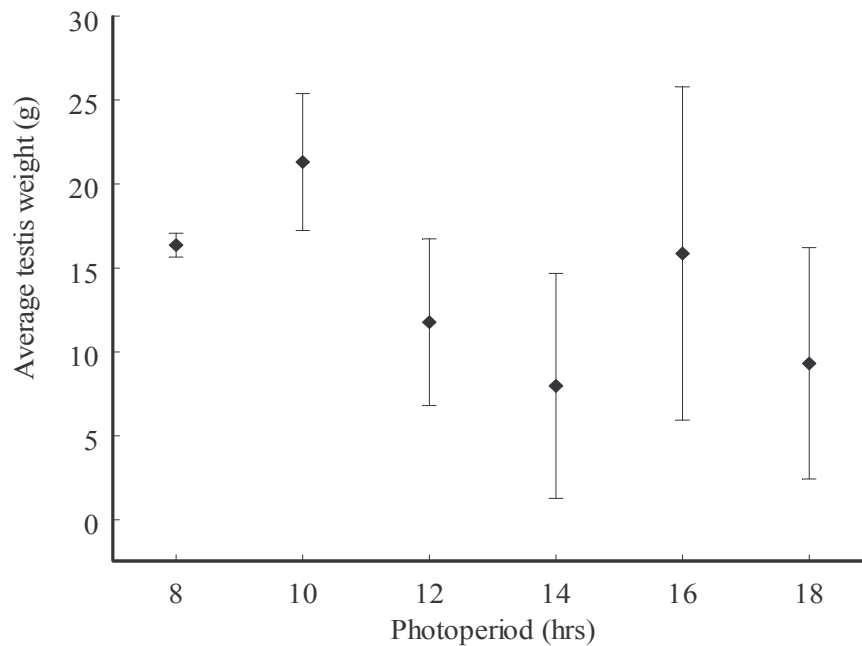


Figure 3.3 Average testis weights at 189 d of birds on different constant photoperiods (n = 2 for all treatments, except 10 h, n = 4)

Evidence of photorefractoriness was again observed in the adult form at the end of the experiment with birds on longer photoperiods showing a decline in average testis weight (Figure 3.4). Regression of the testes, especially in seasonal breeders, occurs due to regulated changes in number and viability of Sertoli cells, spermatogonia and spermatids (Thurston and Korn, 2000). Thus, longer photoperiods were no longer stimulatory.

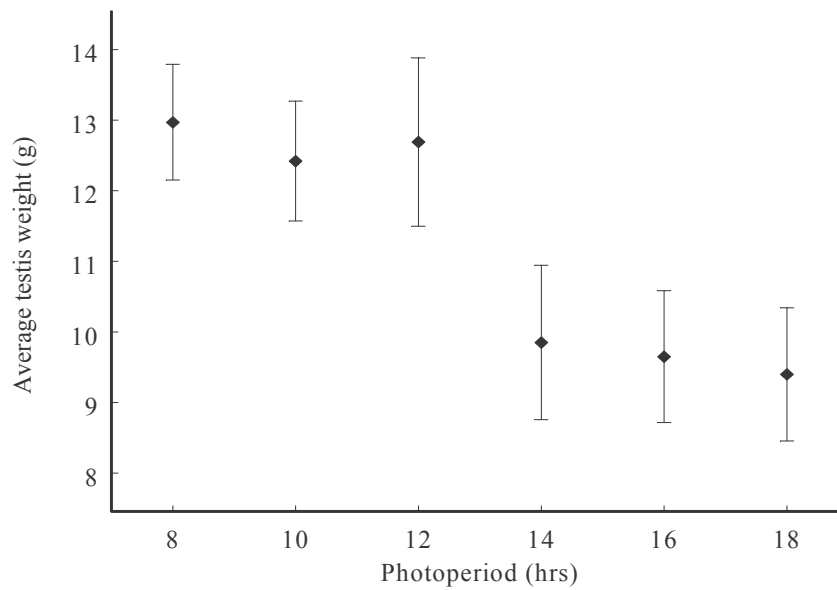


Figure 3.4 Average testis weights at 467 d of birds on different constant photoperiods (n = 22 for 8, 12, 18 h, n = 23 for 16 h, n = 24 for 10 and 14 h)

3.4 CONCLUSIONS

The results provide strong evidence that male broiler breeders exhibit both juvenile and adult photorefractoriness. The response to photoperiod appears to be similar to that of females, where maturity was most delayed in pullets maintained on constant 13 and 14 h photoperiods (Lewis *et al.*, 2004). Therefore, these results suggest that the response of males and females to constant photoperiods is similar.

Comb size can be used as an indicator of testis weight in the early stages of growth leading to sexual maturity, which may be useful in selection of males and also provides a non-invasive measurement in research looking at differences in fertility on birds on different treatments.

4. THE EFFECT OF AGE AT PHOTOSTIMULATION OF MALE BROILER BREEDERS ON TESTES GROWTH AND THE ATTAINMENT OF SEXUAL MATURITY ²

4.1 INTRODUCTION

Evidence of juvenile photorefractoriness has been found in broiler breeders reared on different constant daylengths, with the age at sexual maturity (ASM) being delayed in birds reared on long daylengths in both females (Lewis *et al.*, 2004) and males (Tyler and Gous, 2008). This suggests that photorefractoriness is dissipated at different rates, and that dissipation could occur at a faster rate when broiler breeders are exposed to short daylengths for a period of time. The length of exposure to short daylengths and the age at which birds acquire photosensitivity was previously investigated in female broiler breeders by Lewis *et al.* (2007a). Females photostimulated from 8 to 16 h light/d at 69, 76, 83 or 97 d of age did not respond to photostimulation, and dissipation of photorefractoriness was delayed so that birds matured at the same rate - as if they had been maintained on long days from hatch, as the delay in ASM was similar to the delay seen in females reared on 16h light (Lewis *et al.*, 2004). An advance in ASM was observed when females were photostimulated at 111 and 125 d, but a bimodal distribution was observed when the females were photostimulated at 111 d, as the ASM in some had been advanced while others matured as if reared on 16h light/d (Lewis *et al.*, 2007a), suggesting that there is some genetic variation in the acquisition of photosensitivity. By 124 d the entire population had become photosensitive. Robinson *et al.* (1996) also found a significant delay in ASM when broiler breeders were photostimulated at 160 d compared to 120 d, but the number of days from photostimulation to sexual maturity was lower in the later-stimulated pullets, indicating that this may not be a photo-induced response. Proudfoot *et al.* (1984), however, found no significant difference in days to 50 % production of broiler breeder females photostimulated at 112 or 140 d.

² Tyler, N.C. and Gous, R.M. (2009) The effect of age at photostimulation of male broiler breeders on testes growth and the attainment of sexual maturity. *South African Journal of Animal Science* 39(3):169-175 (Appendix 2).

Dunn *et al.* (1990) suggested that the neuro-endocrine pathways involved in the transmission of photoperiodic information are functional at 3 weeks of age in dwarf female broiler breeders. Photostimulation at 3 weeks of age, in feed-restricted birds, resulted in significantly higher plasma luteinising hormone (LH) concentrations compared to non-photostimulated control birds. However, this may not translate to a different ASM as Lewis *et al.* (2005) concluded that changes in plasma gonadotrophins were not particularly useful as predictors of ASM, although a photoperiodic response curve, where a plot of the photo-induced increase in plasma LH against a range of photoperiods after photostimulation, is useful in predicting the critical and saturation daylengths (Lewis *et al.*, 2008).

The aim of the experiment reported here was to determine the response of male broiler breeders to different ages at photostimulation, through testes development and asymmetry as well as age at first semen production and serum testosterone concentration, and to determine whether flock fertility may be maximised if males are reared on the same lighting programme as females.

4.2 MATERIALS AND METHODS

Four hundred Ross broiler breeder males were randomly allocated to 12 light-tight rooms (32 per room) at 56 d of age after having been reared on a constant 8-h photoperiod. A 60W incandescent lamp, located 1.8m above the floor, was used to provide a mean illuminance in each room of 47 ± 1.8 lux. The birds were photostimulated to 14 h at 56, 77, 98, 119, 147 and 161 d of age, each treatment being replicated in two rooms. A controlled feeding programme recommended by the primary breeding company (Aviagen, 2007) was used throughout the growing period, where the average weight of birds in each room was used to allocate feed to keep body weights on the target growth curve. This was in an attempt to minimise the effect of body weight, as there is an expected interaction between body weight and acquisition of photosensitivity - where birds become photosensitive earlier when on an accelerated growth curve (Dunn and Sharp, 1990; Lewis *et al.*, 2007a). From 123 to 224 d of age, 1 or 2 birds from each room were sacrificed weekly, and the left and right testis were removed and weighed. This time period was to ensure that the range in testis development would be recorded for each

treatment. The remaining birds were trained to produce semen using a modified method of Lake (1957), and the age at which birds first produced semen was recorded.

After slaughter, the shanks and wings were removed and frozen for later measurement. After thawing, the bones were boiled to facilitate cleaning, and the following measurements were taken, with vernier callipers, from at least 6 birds per treatment: shank length (SL), shank diameter (SD) at the narrowest point and humerus length (HL). Relative asymmetry (RA) was defined as the ratio of the absolute value of asymmetry (L-R) divided by the value for the size of the bilateral trait as expressed in the equation: $RA = (|L - R| / [(L + R)/2]) * 100$ (Yang *et al.*, 1998a).

Blood was collected from 3 males in each room at 123, 130, 137, 158, 168, 197, 224 and 351 d of age, the serum extracted and frozen for later analysis. After thawing, the serum samples were analysed for testosterone using an enzyme immunoassay kit (DSL-10-4000 Active ® Testosterone enzyme immunoassay kit of Diagnostic Systems Laboratories, Inc. 445 Medical Center Blvd. Webster, Texas 77598-4217 USA) which has been validated for use in poultry (Mohan *et al.*, 2002).

Bilateral traits and the left (L) and right (R) testis weights were subjected to a Student's *t*-test to determine whether these bilateral measurements differed significantly one from the other overall, and within each treatment. As the output from the testes is a result of the production from both testes, the average testis weight was used in further analyses. The data for L-R of each bilateral trait were tested for normality using the NORMTEST procedure in Genstat 11th edition (2008). Regression analysis was performed for L-R testis weight, L-R testis weight RA, L-R SL, L-R SL RA, L-R SD, L-R SD RA, L-R HL and L-R HL RA.

A Gompertz growth curve was fitted to the average testis weights, measured in sampled birds over time, for each treatment, to determine the point of inflexion - the age at most rapid testis growth (t^*). Two outliers in the 77 d photostimulation treatment were removed to enable a Gompertz curve to be fitted. Genstat 11th edition (2008) was used for all statistical analyses.

Ethical approval by the University of KwaZulu-Natal Ethics Committee was obtained prior to the start of the experiment.

4.3 RESULTS AND DISCUSSION

There was no significant difference between the earliest and latest mean age at first semen production of males on all treatments (Table 4.1). Males photostimulated at 56 and 77 d produced semen as early as 133 d. Males photostimulated at 161 d had a mean age at first semen production earlier than the age at photostimulation (Table 4.1). Thus, in this case, testis development and spermatogenesis had preceded photostimulation, the time from the start of spermatogenesis to the appearance in the ejaculate being between 13 and 15 d (de Reviers, 1968). Therefore, it would appear that the age at photostimulation had no influence on the mean age when semen production was first observed.

Table 4.1 Mean (\pm s.e) age at first semen production (d) of males photostimulated at different ages

Age at photostimulation (d)	Mean age at first semen production (d)	s.e.
56	167	3.1
77	165	2.2
98	164	2.2
119	170	1.4
147	172	1.7
161	164	4.7

The age at which testis growth was most rapid (t^*) was earlier, in all cases, than the predicted age at 50 % production in females, which was calculated as a more accurate indicator of sexual maturity in females than age at first egg, as not all pullets lay on consecutive days after the first egg (Lewis *et al.*, 2007a) (Figure 4.1). It appears as if males and females exhibit a similar response to photostimulation when using t^* as an indicator of sexual maturity, rather than the age at which semen is first observed or produced, although t^* is a measure of testis development, rather than sexual maturity. Although it may be expected that males would naturally reach sexual maturity before their female counterparts, the apparent earlier development may not necessarily be

accompanied by the production of functional spermatozoa during the rapid growth phase - this aspect possibly being delayed until the testes have reached their mature weight.

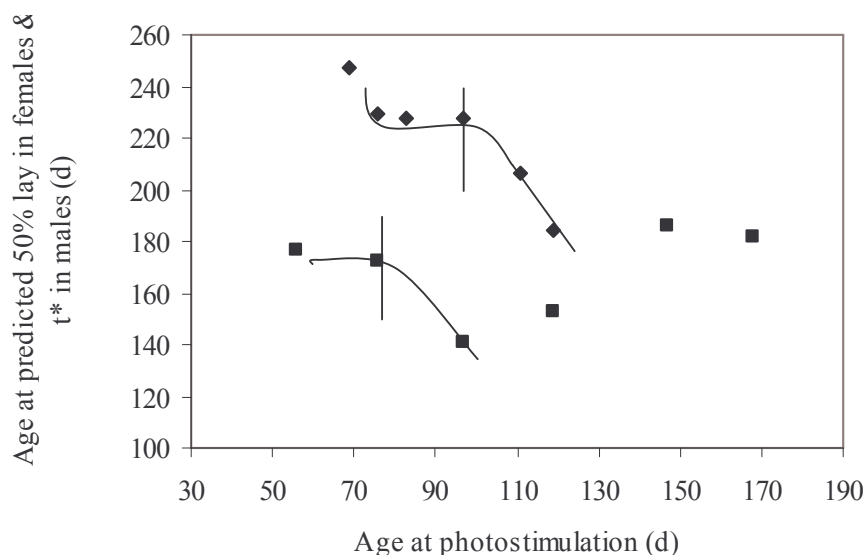


Figure 4.1 Estimated age at 50 % production of female broiler breeders (♦) (Lewis *et al.*, 2007) and the age at which testis growth was most rapid in male broiler breeders (■) photostimulated at different ages, with curves fitted by eye.

A difference in the pattern of response to photostimulation of males compared to females is that t^* increases with photostimulation after 98 d (Figure 4.1). This is probably due to the initiation of the rapid growth phase before photostimulation. Therefore, regardless of age at photostimulation, testis growth will still be initiated, and these points are not in response to age at photostimulation, but rather to spontaneous maturation. In female broiler breeders, photostimulated after 130 d, the response curve appears similar to that seen in males in Figure 4.1, where age at sexual maturity is delayed (Ciacciariello, 2003) and is thought to be due to spontaneous maturation and not as a result of lighting treatment. Because t^* was similar for males photostimulated at 56, 77, 147 and 161 d, it appears that photostimulation at 56 and 77 d was too early to elicit a photosexual response in most males. These males thus responded as if reared on 16 h, while those photostimulated at 147 and 161 d initiated sexual development close to or before photostimulation.

For male broiler breeders across all treatments, the left testis weight was heavier than the right in 75.6 % of the cases (Figure 4.2), which concurs with most reports (Hocking, 1992; Wolanski *et al.*, 2004) and shows directional asymmetry (Møller, 1994). Møller (1994) also showed that, in barn swallows and House sparrows, the greater the directional asymmetry, in the form of a higher volume of the left testis compared to the right, the greater the phenotypic quality, as measured by the expression of the secondary sexual characteristics. Although this would be an added advantage in birds that have flight requirements, in this study, directional asymmetry was interpreted as a reliable indicator of developmental stability, as two large testes would have a higher immuno-suppressive cost of androgen production (Zuk *et al.*, 1995) and the right testis was thought to play a more compensatory role, with symmetry resulting from an increased right testis, indicating a lower performance of the left testis.

In chickens, McGary *et al.* (2003) found no overall relationship between degree of fluctuating asymmetry of the secondary sexual traits (wattle length, width and area) and male fertility potential, as measured by macroscopic assessment of the germinal disc and sperm penetration of the perivitelline membrane. However greater dorsal pelvic width asymmetry in one strain was associated with lower estimated fertility, but it is not clear how pelvic width was considered to be a bilateral trait.

This is in contrast with other studies measuring morphological traits, which suggest that fluctuating asymmetry, as a measure of developmental stability, is a reflection of genetic or environmental stress, and has been suggested, and used, to measure genetic stress (Yang *et al.*, 1998a; Yang and Siegel, 1998) and welfare and environmental stress (Møller *et al.*, 1999; Yalcin *et al.*, 2001) in poultry.

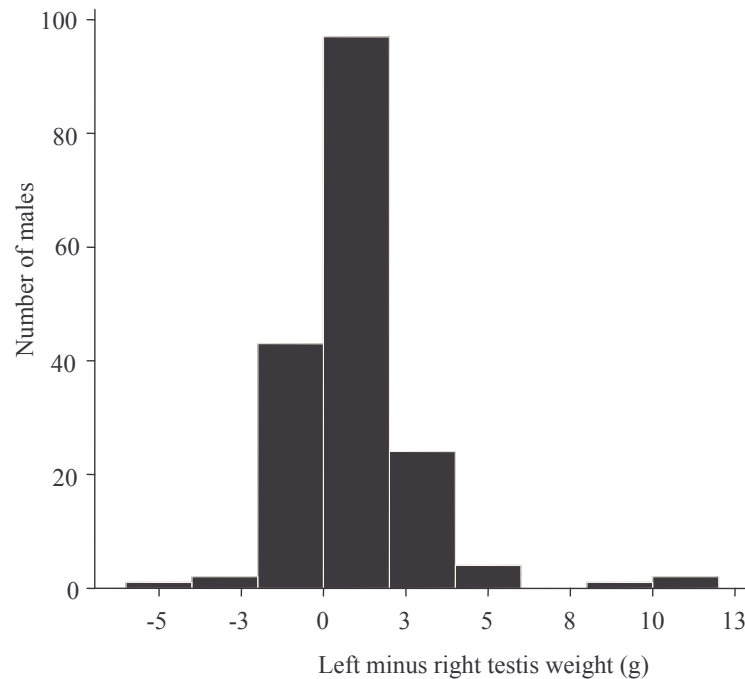


Figure 4.2 Directional asymmetry in testis weight of broiler breeder males photostimulated at different ages

The L and R testis weights were shown by the *t*-test to be significantly different for males on each treatment, except for those birds photostimulated at 161 d, where the L and R testis weights were the same. The other bilateral traits measured also showed directional asymmetry, except for HL, which showed a skew distribution. There was no effect of photoperiodic treatment on the degree of RA of SL, SD or HL. Therefore it is hard to determine whether the directional asymmetry observed in testis weights is an adaptation advantageous to fertility, or whether all treatments other than photostimulation at 161 d impose developmental stress on the birds. This could also be the result of spontaneous testis development, rather than development in response to light.

Average testis weights clearly demonstrated that some birds respond to early stimulation while others on the same treatment remain photorefractory. Some birds photostimulated at 56 and 77 d recorded average testis weights above 2g at 123 d of age, while the testis weights of birds photostimulated at 98 d and later remained below 2g until 144 d. However, after 189 d, all males photostimulated at 98 d and later had an average testis weight >10g, while the weights of those photostimulated at 56 and 77 d ranged from 1.61 to 15.58 g. Thus, early photostimulation induced a photosexual response in some birds,

while causing a more prolonged delay in others. Later photostimulation resulted in a more uniform response. This suggests that the variation in the genetic composition of broiler breeders is large, where some birds respond to very early light stimulation, while others remain photorefractory. While such early stimulation would not be recommended in practice, it would be useful to capitalise on the genetics of such birds as a means of eliminating photorefractoriness in broiler breeders.

While the males were fed to reach the target body weights recommended by the breeder, there was a normal distribution in the body weight of birds within a treatment, and there may also be an environmental effect of the response to light based on the degree of body reserves to allocate to testis development.

There was no photoperiodic effect on serum testosterone concentration which, from 123 to 224 d, remained below 6 ng/ml for males on all treatments, except for 2 birds sampled at 137 d that recorded concentrations of 9.6 ng/ml (photostimulated at 77 d) and 7.5 ng/ml (photostimulated at 98 d). However, at 351 d, near the end of the production cycle, the same variation in response to photoperiod observed when measuring t^* was also evident (Figure 4.3), with birds photostimulated earlier than 119 d exhibiting either high or low serum testosterone concentrations, contrasting with a more even spread among males photostimulated at 119 d and later. It would be interesting to know whether early responders initiated gonadal regression earlier or later than non-responders towards the end of the season, as this could inform breeding companies of the value of selecting for responders among males.

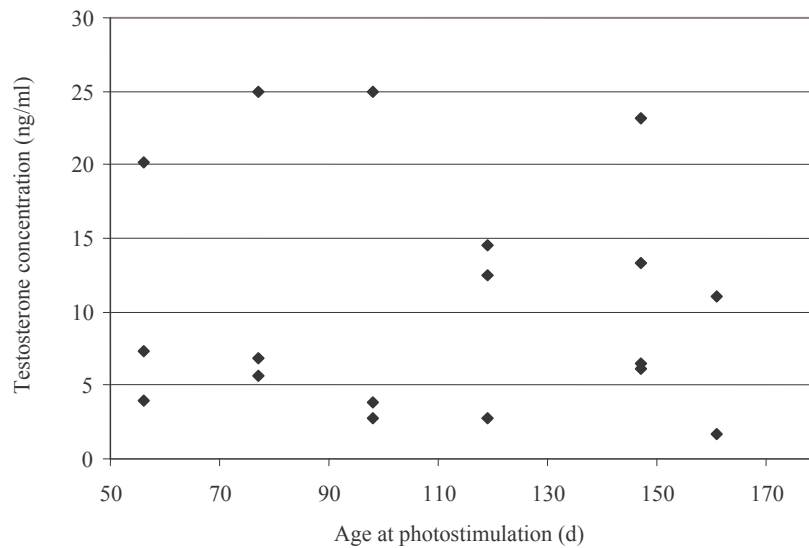


Figure 4.3 Serum testosterone concentrations of males at 315 d following photostimulation at different ages

4.4 CONCLUSIONS

Where male and female broiler breeders are reared on the same lighting programme, males are likely to attain sexual maturity before females, which is necessary to maximise fertility. All broiler breeder males should respond to photostimulation at 98 d or later, while some respond to earlier photostimulation. Such responders could be selected and utilised in a breeding programme to eliminate seasonality in broiler breeders. Such a genotype would be useful in production systems using open-sided houses, where short daylengths cannot be applied during the rearing period, resulting, at present, in differences in fertility between in-season and out-of-season flocks.

5. SELECTION OF BROILER BREEDERS AGAINST PHOTOREFRACTORINESS

5.1 INTRODUCTION

Due to divergent selection pressures in egg-type hybrids and broiler breeders, and with the greater potential reduction in reproductive function with selection pressure for performance traits (Amann, 1999), fertility traits are expectedly different in these types of chicken. Broiler breeders exhibit a faster rate of decline in egg production (Lewis, 2009), exacerbated by exposure to longer daylengths in the laying period, where inferior egg production was observed after photostimulation to 16 h as opposed to 11 or 12 h (Lewis *et al.*, 2007c). The drop in egg production was not associated with any compensatory increase in egg weight, indicating a different mechanism behind the cause in the decline in rate of lay to that of egg-type pullets (Morris *et al.*, 1995). This is probably because broiler breeders still exhibit photorefractoriness, the condition in seasonal breeding birds, which, in the juvenile form, prevents breeding in the same season as hatch, and in the adult form, after prolonged exposure to otherwise stimulatory photoperiods, terminates breeding. Different species express different degrees of photorefractoriness. Absolute photorefractoriness is expressed in species such as the partridge (Siopes and Wilson, 1978) where exposure to short days is essential for photorefractoriness to be dissipated. Relative photorefractoriness is seen in species such as broiler breeders, where maturity is delayed but not prevented with no previous exposure to short daylengths (Lewis *et al.*, 2004) and turkeys, which spontaneously return to lay despite remaining on a long daylength (Siopes, 2005).

There is some evidence that turkey toms do not exhibit photorefractoriness (Proudman and Siopes, 2005; Noirault *et al.*, 2006), and that some broiler breeder males did respond to early (8 or 11 week) photostimulation (Tyler and Gous, 2009). Broiler breeder females also show a bimodal response to early photostimulation, despite body weight uniformity (Lewis and Morris, 2006), indicating genetic variation in the photosexual response and therefore that the possibility exists that selection against photorefractoriness may be successful.

While both the female and male influence egg fertility, the male component is of greater importance in a commercial breeding operation as many females are fertilised by a single male, and, in a sire line, an individual male can have a major influence, through male descendants, on the efficiency of the multiplication pyramid (Wolc *et al.*, 2009).

Therefore the aim of this experiment was to determine the extent to which this reproductive characteristic is heritable. Responders and non-responders were identified in a small population of male broiler breeders and these were mated with both egg-type hybrids and broiler breeder females. Age at maturity of the offspring was monitored following photostimulation at 8 weeks.

5.2 MATERIALS AND METHODS

Ross 788 broiler breeder males ($n = 150$) were obtained at day old and reared on a litter floor in a light-tight facility. Birds were given 2 d continuous illumination and then provided with an 8-h photoperiod. At 8 weeks of age the photoperiod was abruptly increased to 14 h. The birds were full-fed to 24 d and thereafter control fed to achieve the target body weight of 3.04 kg at 20 weeks recommended by the breeding company (Aviagen, 2007).

From 20 weeks of age, birds were trained to produce a semen sample. At 25 weeks 20 'responders' (R) were identified by means of three characteristics as having had the greatest response to early photostimulation: they had produced a semen sample, their combs were reddened, and they had a comb area in excess of 10 cm^2 , as measured through image analysis (Rasband, 2006) of a digital photograph of the head taken against a ruler. A group of 20 'non-responders' (NR) were subsequently chosen as those yet to produce a semen sample, without reddening of the comb, and with a comb area of $< 10 \text{ cm}^2$. These birds were placed in individual cages (60 cm wide x 44 cm deep x 60 cm high) on a litter floor.

Once all birds had reached sexual maturity, semen samples were collected from 8 NR and 8 R each that had consistently produced the greatest volume. The semen from each of these was used to inseminate a maximum of 5 egg-type hybrid hens (assumed to be 'responders') and 5 broiler breeder hens each week for a period of 5 weeks. The hens

were housed in individual cages and eggs from each female were identified, labelled and collected. During the first 3 weeks of artificial insemination the females were allowed to adapt to the procedure and then, for the following 2 weeks, eggs were collected, stored in a cold room and set in an incubator. The hatcher baskets were modified with partitions, such that eggs from each female could be kept separate on hatching. Once hatched, chicks were placed in cages and marked to identify parentage.

Chicks were weighed individually at 9, 16 and 21 d of age. All chicks were fed a commercial broiler starter ration *ad lib* until 24 d. Continuous light was provided for 2 d, and thereafter an 8-h photoperiod was provided. At 24 d chicks were transferred from cages to a light-tight facility on a litter floor, and kept on an 8-h photoperiod. Feed restriction was implemented at this time to achieve the target body weight of 3.04 kg at 20 weeks recommended by the breeding company (Aviagen, 2007). The photoperiod was abruptly increased at 8 weeks to 14 h. At 17 weeks of age males and females were identified and separated. Females were placed in individual cages on a litter floor and the age at first egg (AFE) recorded. Males remained in the pens and when they appeared to be reaching sexual maturity they were killed by severing the jugular vein after electrical stunning. The left and right testis were then removed and weighed. Males from the broiler breeders were sampled at 141, 164 and 168 d, while those from the egg-type hybrids were sampled at 138, 145, 146 and 152 d.

Because it was evident that the testes had not reached maturity by the time the birds were killed, a Gompertz equation was used to predict the age at most rapid testis growth (t^*) using the age at killing and testis weights measured here. The parameter values used were those derived from testis weight data at different ages by Tyler and Gous (2009) for the treatment in which broiler breeder males were photostimulated at 8 weeks. The form of Gompertz equation used was:

$$Y = A + C * (\text{EXP}(-\text{EXP}(-B(X-M))), \text{ g}$$

where:

Y = mean testis weight, g

X = age, d

A (constant) = 1.25

C (mature weight) = 7.37, g

B (rate of maturing) = 0.28 /d

$$M = t^*, d$$

Mean testis weight (Y) at different ages (X) was substituted in the above equation, rearranged (see below), to predict t^* in cases where the mean testis weight was > 1.25

$$M = X - (\ln(\ln((Y - A)/C)))/B$$

A general ANOVA was performed to determine differences in the AFE of female offspring from both NR and R maternity and paternity. A Shapiro-Wilk test for normality was carried out on the AFE, and because the data were not normally distributed a histogram of the frequency of birds at each AFE was plotted. The data, as anticipated, followed a bimodal distribution, so separate distributions were fitted to the data to separate the offspring into R and NR groups using Minitab (2007). The responder distribution was fitted for offspring from broiler breeders that had an AFE < 210 d and offspring from egg-type hybrids that had an AFE < 190 d. These data were also subjected to a general ANOVA to determine if there were differences in the AFE within this group of birds with different paternity. The predicted t^* values from males whose full-sib sisters were included in the responder distribution were also subjected to a general ANOVA to determine if there was any difference in this age in birds with different parentage. Within each family, the mean AFE and mean predicted t^* of average male testis weight were correlated and also subjected to linear regression analysis using Genstat (2008).

The mean testis weight was log transformed to follow a normal distribution, after a Shapiro-Wilk test for normality indicated that these data were not normally distributed. A Students 't' test was performed on the log-transformed testis weights to determine if there were any significant differences from males with different paternity from both broiler breeder and egg-type hybrid maternity, using Genstat (2008).

The weights of the female offspring at 21 d, included in the responder distribution, were subjected to a general ANOVA to determine if there were any differences between birds with paternal NR or R from maternal egg-type hybrids and broiler breeders, using Genstat (2008).

Ethical approval by the University of KwaZulu-Natal Ethics Committee was obtained prior to the start of the experiment.

5.3 RESULTS

As expected, females from maternal egg-type hybrids had a significantly advanced AFE compared with those from maternal broiler breeders (173 vs. 214 d) (Table 5.1). While there was no difference in the AFE of offspring from NR or R paternity and egg-type hybrid maternity (172 vs. 174 d), there was a significant advance in the AFE from offspring with R paternity compared to NR paternity, both with broiler breeder maternity (207 vs. 221 d).

The frequency of AFE followed a bimodal distribution (Figure 5.1). When the responder normal distribution was fitted, although it included offspring from both NR and R paternity (Figure 5.2), there was a significant ($P < 0.05$) advance in AFE in the female offspring with R paternity as opposed to NR paternity with both maternal egg-type hybrids and broiler breeders (Table 5.2). The difference between the AFE in females of R paternity from maternal egg-type hybrids and broiler breeders became non-significant between females included in the responder distribution. The ratio of NR:R male parents of the birds included in the responder distribution was 1:4.5 from those with broiler breeder maternity and 1:3.3 from birds with egg-type hybrid maternity.

Table 5.1 Mean age at first egg \pm standard error of the mean (sem) and number of observations making up the mean (n) of female offspring

	Mean age at first egg (d)		
	Maternal egg-type hybrid	Maternal broiler breeder	<i>P</i> value
Paternal responder	173 \pm 1.90 (88)	207 \pm 4.79 (80)	<0.001
Paternal non-responder	174 \pm 1.62 (62)	221 \pm 5.14 (64)	<0.001
<i>P</i> value	0.43	0.05	

Table 5.2 Mean age at first egg \pm sem (n) of female offspring from different parentage within the responder distribution

	Mean age at first egg (d)		
	Maternal egg-type hybrid	Maternal broiler breeder	<i>P</i> value
Paternal responder	167 \pm 1.44 (75)	170 \pm 3.10 (40)	0.23
Paternal non-responder	172 \pm 1.40 (55)	180 \pm 2.91 (27)	0.004
<i>P</i> value	0.03	0.04	

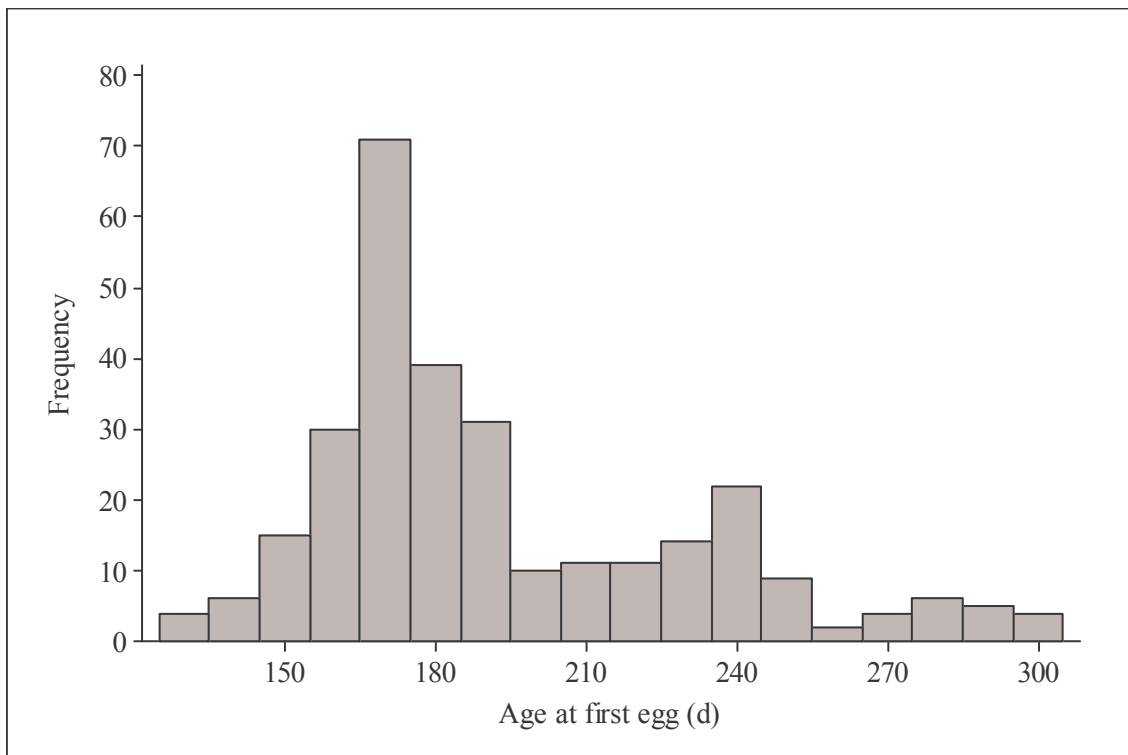


Figure 5.1 Frequency distribution of the age at first egg in female offspring with R and NR paternal parentage and broiler breeder or egg-type hybrid maternal parentage that had laid an egg by 300 d

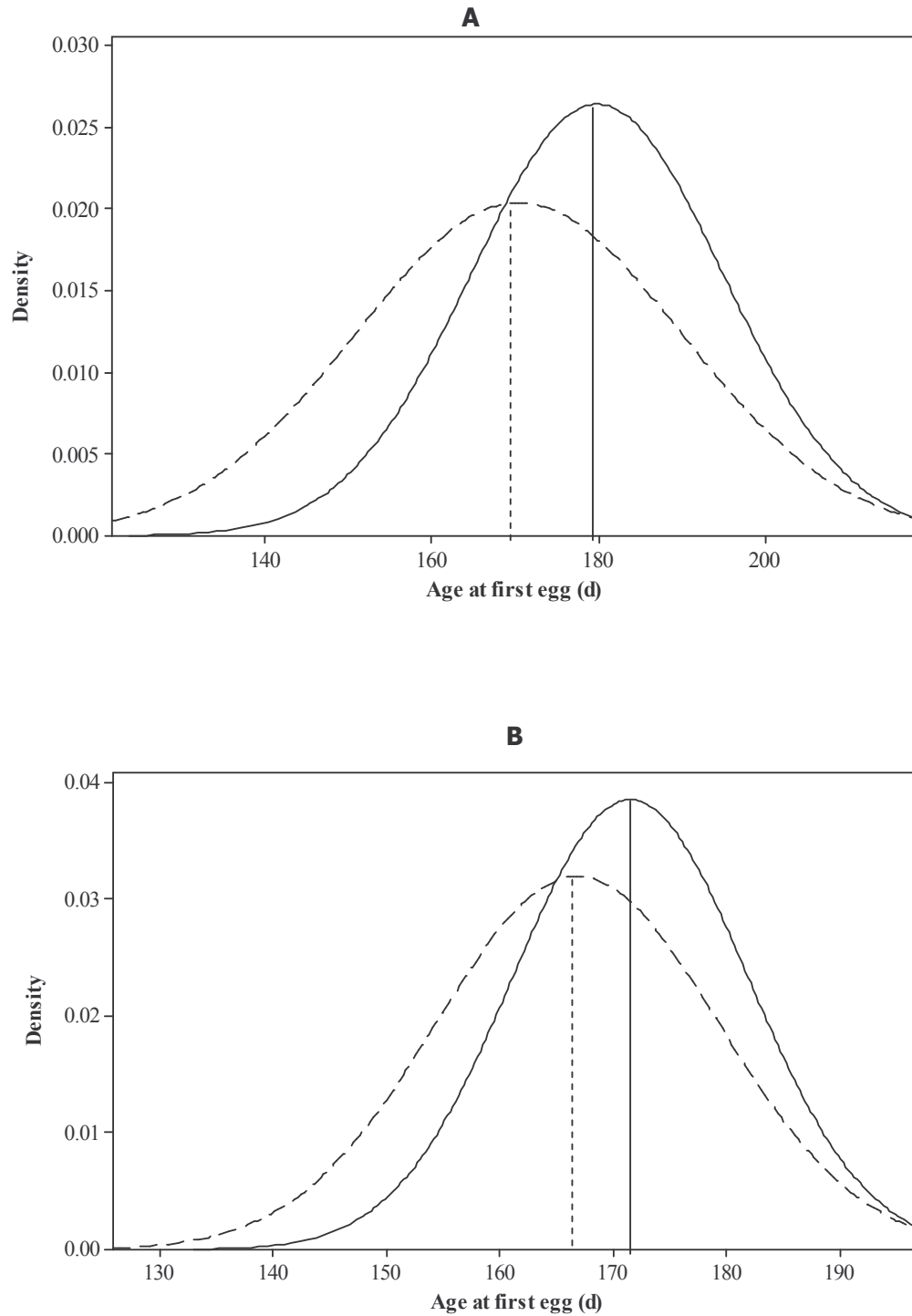


Figure 5.2 Frequency distribution of the mean age at first egg in female offspring from responder and non-responder parentage. — Paternal non-responders, ----- Paternal responders. Maternal parents in graph A were broiler breeders, and in graph B, egg laying hybrids.

The mean testis weight of birds with different paternity showed no significant differences at any age (Table 5.3), and there was no advance in the predicted t^* of males with R or NR paternity with either broiler breeder or egg-type hybrid maternity (Table 5.4).

Table 5.3 Mean testis weight \pm sem (n) of offspring from NR and R paternity and broiler breeder and egg-type hybrid maternity, slaughtered at different ages.

Age (d)	Mean testis weight (g)		
	Paternal non-responder	Paternal responder	P value
<i>Maternal broiler breeder</i>			
141	0.19 \pm 0.01 (4)	0.17 \pm 0.02 (10)	0.75
164	0.78 \pm 0.35 (6)	2.50 \pm 0.79 (10)	0.10
168	1.05 \pm 0.25 (5)	1.16 \pm 0.46 (13)	0.52
<i>Maternal egg-type hybrid</i>			
138	1.14 \pm 0.52 (11)	1.10 \pm 0.45 (11)	0.90
145	1.57 \pm 0.27 (32)	1.32 \pm 0.22 (31)	0.50
146	1.27 \pm 0.40 (5)	1.54 \pm 0.48 (6)	0.74
152	1.10 \pm 0.19 (25)	1.69 \pm 0.27 (40)	0.08

Table 5.4 Mean predicted $t^* \pm$ sem (n) of male offspring whose full sib sisters were included in the responder distribution from different parentage

	Mean predicted t^* (d)		
	Maternal egg-type hybrid	Maternal broiler breeder	P value
Paternal responder	152 \pm 1.06 (31)	171 \pm 6.66 (5)	<0.001
Paternal non-responder	151 \pm 1.19 (11)	170 (1)	<0.001
P value	0.32	0.83	

The mean AFE for each dam family was highly correlated (0.81) with t^* predicted from the mean testis weight when the males were sampled (Table 5.5). The age at most rapid testis growth could therefore be predicted from AFE using the linear regression:

$$\text{Predicted } t^* = 117.6 (\pm 5.03) + 0.21 (\pm 0.03) \text{ AFE } (R^2 = 0.64).$$

Table 5.5 Mean age at first egg and predicted age at most rapid testis growth (t^*) \pm sem (n) of offspring from NR and R paternity and broiler breeder and egg-type hybrid maternity

Paternal NR/R	Paternal no	Maternal type	Maternal no	females			males		
				Mean AFE	sem	n	Mean predicted t^*	sem	n
NR	6	broiler breeder	56	218		1			
			58	170	9.5	2			
			59	245		1			
			60	222	0	2			
		egg-type hybrid	56	173	4.3	4			
			57	170	1	2	146	2.5	2
			58	183	16.5	2			
			59	176	3.9	5			
	11	broiler breeder	42	238	3.6	3			
			43	249		1			
			44	229		1			
			45	273	0	2			
	49	broiler breeder	51	263	15.2	4			
			52	233	4	2			
			54	261	23.5	2			
			55	199	18.1	6			
		egg-type hybrid	51	190		1			
			52	177	5.0	3			
			53	162		1	155		1
			54	183	0.5	2			
	54	broiler breeder	48	182	4.0	6			
			49	184	10.5	2			
			50	181	3.6	8			
		egg-type hybrid	47	170	7.8	5	153.5	2.5	2
			48				149	3.4	4
			49	160		1	150		1
			50	207		1	150		1
	68	broiler breeder	71	273		1			
			72	250	21.6	3			
			73	231	8	4			
	78	egg-type hybrid	42	169	2.7	3			
			43	189.5	1.5	2			
			45	168	6.5	2			
			46	168	12.5	3			
	95	broiler breeder	76	283	5.0	2			
			78	252		1	177		1
			79	240	21.1	3	170		1
			80	230		1			
	123	broiler breeder	66	302		1			
			68	230	6.7	3			
		egg-type hybrid	63	187		1	150	2.4	3
			66	171	5.5	2			
			67				144		1
			68	167	4.3	3			
			69	175	3.1	8	152		1
			70	171	4.5	5	152		1
R	14	broiler breeder	16	187.6	8.9	7			
			17	152	2.8	4			
			19	232		1			

		20	238	0.5	2		
	egg-type hybrid	16				148	1
		17				140	1
		18	173	2.4	3	163	1
		19	188	6.4	4	153	2.2
		20	176	9.1	3		4
33	broiler breeder	11	229	9.0	3	173	1
		12	188	30.1	4	162	1
		13	195	22.5	5		
		14	215		1	163	1
		15	204		1		
	egg-type hybrid	11	149	4.1	8	142	6.0
		13	151	17.5	2	145	0.5
		14	174	4.3	7	157	7.0
		15	177	4.3	7	152	0.0
39	broiler breeder	36	278		1	171	1
		40	250		1	175	1
	egg-type hybrid	37	170	2.5	2	154	1
62	broiler breeder	2	188	12.6	5		
		3	236		1		
		5	222	14.6	4		
	egg-type hybrid	1	171	4.9	4		
		3	180	12.0	2	150	1
		4	192		1	155	1
85	broiler breeder	26	205		1	169	2.5
		27	261	31	2		
		28				188	1
	egg-type hybrid	27	166		1		
		28	171	2.2	5	153	3.0
		30	210		2		
118	broiler breeder	34	193		1		
		35	217		1		
	egg-type hybrid	31	211		1	154	2.1
		32	177	8.5	2		
		33	181	14.6	3	154	1.5
		34	162	6.6	4		2
		35	158		1	152	5.5
150	broiler breeder	21	189	15.7	7		
		22	272	17.5	2		
		23	272	25.0	3		
		24	184	14.6	6		
		25	176	1.5	2		
	egg-type hybrid	22	176	5	6		
		23	163	5.7	5	153	0
192	broiler breeder	6	240	15.4	4		
		7	190	10.6	8	163	1.0
		8	217		1		
		9	296		1	167	1
		10	250		1		
	egg-type hybrid	6	170	4.8	4	145	1
		8	167	4.3	5	153	1
		9	197	14.1	4	157	1
		10	152	8	2	147	4.0

There was no significant difference in the 21 d body weight of female chicks of NR or R paternity that were included in the responder distribution with broiler breeder maternity, although the 21 d body weights were significantly higher in female offspring with R and egg-type hybrid parentage (Table 5.6).

Table 5.6 Mean 21 d body weight \pm sem (n) of female offspring of different parentage
Mean body weight at 21 d (g)

	Maternal egg-type hybrid	Maternal broiler breeder
Paternal responder	367 \pm 4.61 (74)	427 \pm 20.8 (40)
Paternal non-responder	349 \pm 8.15 (50)	412 \pm 16.2 (24)
P value	0.04	0.60

5.4 DISCUSSION

Females with maternal egg-type hybrid maternity were expected to have an advanced AFE compared with those of broiler breeder maternity, due to the years of divergent selection pressures applied to these different types. But, interestingly, this difference became non-significant when considering the AFE in female offspring within only the responder distribution. Also, despite selection pressures for egg production in egg-type hybrids, the AFE was still advanced in birds included in the responder distribution with egg-type hybrid maternity and R, as opposed to NR, paternity. This, together with the 14 d advance in AFE in birds with R, as opposed to NR paternity, and broiler breeder maternity overall, and the 10 d increase in those in the responder distribution, suggests the possibility for progress in selection against photorefractoriness in male broiler breeders.

Although the males were sampled too early to observe any differences in testes weight from NR or R parentage, the strong relationship between the predicted t^* and the AFE in full sibs, indicates that an advance in AFE in female offspring would also lead to an advance in the age of testis development in the male offspring. However, this is quite subjective considering no advance in t^* was observed in birds with R compared to NR paternity.

It is acknowledged that selection in broiler breeders needs to be balanced to improve performance traits while maintaining reproductive potential (Wolc *et al.*, 2009). A concern when selecting against photorefractoriness is that progress in this area may result in the selection of a more egg-type bird, and a possible loss in broiler growth rates. However, when the female 21 d body weights, which were later included in the responder distribution, were compared between groups with NR and R paternity, no significant differences were observed in those with broiler breeder maternity and the weights were actually heavier in birds with R compared to NR paternity and egg-type hybrid maternity, indicating that progress in selection against photorefractoriness could be made without detrimental effects on broiler growth rates.

These findings should be useful to the broiler breeder industry, where poorer production in out-of-season flocks occurs in open-sided houses, because of the considerable delay in age at sexual maturity resulting from photorefractoriness. If progress could be made in selecting against photorefractoriness, sexual maturity in broiler breeders reared on long days (>14 h) would not be delayed, and egg production and fertility would not decline as rapidly towards the end of the laying period. This would enable birds to perform closer to their potential thereby maximising the number of fertile hatching eggs.

6. REPRODUCTIVE STATUS IN BROILER BREEDER MALES IS MINIMALLY AFFECTED BY A MID-CYCLE INCREASE IN PHOTOPERIOD³

6.1 INTRODUCTION

Photorefractoriness is the inability of seasonal-breeding animals to respond sexually to an otherwise stimulatory photoperiod and is the mechanism that limits reproductive activity to a time of the year when conditions for successfully rearing offspring are likely to be most favourable. All seasonal breeding birds are hatched photorefractory and this juvenile form of the condition, which, in truly seasonal breeders, prevents reproduction in the first year after hatch (Lewis, 2009), is dissipated by exposure to an adequate period of short days. When truly seasonal breeding birds are maintained on long days from soon after hatch, sexual maturation is either prevented or markedly delayed; for example, when Red-legged Partridge were maintained on 16-h photoperiods, none had laid an egg before 68 weeks of age and more than half were still infertile 3 years later (Woodard *et al.*, 1980). In contrast, maturity is not prevented, but only delayed, when species such as broiler breeders, that show only the relative form of photorefractoriness are maintained on long days (Lewis *et al.*, 2003; Gous and Cherry, 2004; Lewis *et al.*, 2004).

After a prolonged exposure to long days, the laying cycle of all seasonal-breeding birds is terminated by the onset of adult photorefractoriness. However, the timing of its development is thought to be initiated by the transfer to long days which induced sexual maturation (Dawson, 2001; Siopes and Proudman, 2009). Adult photorefractoriness in females, as indicated by a more rapid decline in rate of lay or cessation of egg-laying, is advanced when birds are either maintained on long days from soon after hatch (Lewis *et al.*, 2004) or transferred from a neutral to a highly stimulatory daylength (Sharp *et al.*, 1992; Ciccone *et al.*, 2005; Lewis *et al.*, 2007c). An earlier onset of photorefractoriness was also indicated when males maintained on long days had lower testes weights at 67 weeks of age than others kept on constant short days (Tyler and Gous, 2008).

This paper reports the findings of an investigation designed to test the hypothesis of Tyler and Gous (2008) that the smaller testes of the birds maintained on long-days were indeed

³ Submitted to British Poultry Science

a consequence of an earlier onset of adult photorefractoriness. Broiler breeder males were transferred from 8-h to 12-h photoperiods at 20 weeks of age and then given a further increase at 40 weeks to a photoperiod of between 14 and 22 h, with controls kept on 12 h. Reproductive status was assessed by measuring semen concentration and comb area at various ages between 42 and 60 weeks, and testicular weight and testosterone concentration at 60 weeks.

6.2 MATERIALS AND METHODS

Ross 788 broiler breeder males were reared together on 8-h photoperiods following an initial 24 h of continuous illumination. At 15 weeks, 8 birds were randomly allocated to individual cages (60 cm wide x 44 cm deep x 60 cm high) on a litter floor in each of 12 light-tight rooms and maintained on 8-h photoperiods. At 20 weeks, after sufficient exposure to short days to dissipate juvenile photorefractoriness, all birds were abruptly transferred to a 12-h photoperiod. At 40 weeks of age, the photoperiod in pairs of rooms was abruptly increased to either 14, 16, 18, 20 or 22 h, or maintained on 12 h as controls (6 photoperiods x 2 replicates x 8 birds = 96 birds). A 60W white incandescent lamp, located 1.8m above the floor, was used to provide a mean illuminance of 47 ± 1.8 lux at bird-head-height (approximately 40 cm). The birds were control-fed to achieve a mean body weight of 3.04 kg at 20 weeks, as recommended by the primary breeding company (Aviagen, 2007).

Birds were trained to produce semen on a weekly basis from 15 weeks of age using a modified method of Lake (1957), and samples collected for semen counts at 44, 46, 48, 51, 54, 56, and 58 weeks. A 50 μ l sample of semen was diluted in 8 ml of an eosin/nigrosine solution and the concentration of live sperm/ml calculated with a haemocytometer assuming 90 % normal, motile sperm. Four days before the photoperiod was increased at 40 weeks and at 42, 43, 46, 51, 54, 56 and 58 weeks, the left and right side of the head was photographed against a ruler with a digital camera and the comb area calculated using image analysis of the side with the clearer picture (Rasband, 2006).

At 60 weeks of age, the birds were electrically stunned, the jugular vein severed, a 1-2 ml blood sample collected spun at 3000 rpm for 15 min, and serum collected and stored at -20°C for subsequent determination of testosterone concentration; gelatinous or

haemolysed samples were discarded (sample n = 10, 10, 12, 10, 9, 11 for 12-, 14-, 16-, 18-, 20- and 22-h treatments respectively). An ELISA method was used with an enzyme immunoassay kit (DSL-10-4000 Active ® Testosterone enzyme immunoassay kit of Diagnostic Systems Laboratories, Inc. 445 Medical Center Blvd. Webster, Texas 77598-4217 USA) validated for use in poultry (Mohan *et al.*, 2002). The testes were excised and weighed individually, and the presence or absence of semen in the vas deferens recorded.

A stepwise regression and an ANOVA using a general linear model were performed to determine the influence of age and photoperiodic treatment on semen concentration, with significant differences identified using a Students 't' test. Data for samples taken at 51, 54, 56 and 58 weeks were not normally distributed and were log transformed prior to analysis. Actual comb areas were subjected to an ANOVA using a general linear model and regressed on age, whilst changes in area between 51 and 58 weeks were regressed on age and equality of slopes tested for the various groups. Mean testosterone concentrations at 60 weeks for birds held on a photoperiod of between 12 and 20 h only were regressed on photoperiod; a subjective examination of the data prior to the regression had suggested that the 22-h data be excluded. The combined testes weights were subjected to an ANOVA using a general linear model. Genstat 11th edition (2008) was used for all statistical analyses.

Ethical approval by the University of KwaZulu-Natal Ethics Committee was obtained prior to the start of the experiment.

6.3 RESULTS

Sample sizes varied from one collection time to another because, despite all birds having responded to semen training before the experiment started, some individuals, though not necessarily the same ones each time, failed to produce semen (Figure 6.1). At the end of the experiment, there were 5, 1, 2, 5, 3 and 5 birds from the 12, 14, 16, 18, 20 and 22 h treatments, respectively, which had no semen in their vas deferens. Neither photoperiod nor age contributed significantly to semen concentration, and there was no significant influence of photoperiod on semen concentration at any of the collection ages (Table 6.1).



Figure 6.1. Semen production(■) and mortality (■) at 44, 46, 48, 51, 54, 58 and 60 weeks for individual birds transferred to different photoperiods at 40 weeks of age. At 60 weeks this was measured by the presence or absence of semen in the vas deferens.

Table 6.1. Mean \pm sem (n) semen concentration, comb area, and combined testes weight of broiler breeders transferred from 8 to 12 h at 20 weeks and either maintained on 12 h or further transferred to 14, 16, 18, 20 or 22 h at 40 weeks.

Age (weeks)	Photoperiod from 40 to 60 weeks (h)							P value
	12	14	16	18	20	22		
Semen concentration (million live sperm/ml)								
44	2369 ± 247 (10)	2358 ± 271 (12)	2519 ± 324 (13)	2697 ± 275 (12)	2224 ± 309 (13)	2209 ± 336 (11)	0.867	
46	2438 ± 275 (7)	2808 ± 394 (8)	2196 ± 293 (9)	2266 ± 373 (11)	1903 ± 399 (10)	2008 ± 349 (9)	0.745	
48	2556 ± 313 (11)	2556 ± 447 (9)	2515 ± 266 (11)	2674 ± 304 (15)	2679 ± 379 (11)	2124 ± 298 (11)	0.937	
51	2896 ± 406 (9)	1626 ± 355 (9)	2147 ± 547 (7)	2696 ± 398 (8)	2967 ± 558 (7)	2258 ± 235 (11)	0.222	
54	1908 ± 398 (9)	1017 ± 403 (8)	1790 ± 478 (7)	1244 ± 365 (10)	1756 ± 458 (9)	1384 ± 336 (10)	0.818	
56	2168 ± 599 (7)	1854 ± 315 (9)	2304 ± 283 (9)	1845 ± 474 (8)	2147 ± 358 (11)	1463 ± 288 (10)	0.791	
58	2804 ± 986 (5)	2542 ± 656 (8)	2434 ± 844 (4)	3381 ± 368 (7)	2577 ± 511 (7)	1461 ± 187 (7)	0.263	
Comb area (cm ²)								
39	46.8 ± 4.6 (12)	50.0 ± 7.2 (9)	47.3 ± 4.2 (15)	35.5 ± 8.5 (8)	42.2 ± 4.1 (5)	44.7 ± 3.8 (15)	0.604	
42	58.4 ± 2.6 (15)	69.2 ± 3.3 (16)	69.0 ± 3.5 (16)	65.6 ± 5.4 (16)	61.9 ± 3.4 (16)	54.6 ± 4.7 (16)	0.286	
43	63.5 ± 2.7 (15)	72.1 ± 3.5 (15)	68.9 ± 3.2 (15)	66.3 ± 4.4 (16)	64.7 ± 3.4 (15)	59.9 ± 4.8 (15)	0.427	
46	68.9 ± 2.5 (15)	76.5 ± 4.3 (14)	79.0 ± 3.3 (14)	72.1 ± 3.8 (15)	73.9 ± 4.1 (15)	69.6 ± 4.3 (15)	0.748	
51	69.3 ± 3.3 (15)	73.6 ± 3.8 (16)	80.3 ± 3.3 (16)	75.3 ± 4.0 (16)	72.9 ± 3.8 (14)	67.0 ± 4.4 (16)	0.341	
54	68.2 ± 3.2 (15)	73.2 ± 2.8 (14)	77.8 ± 3.3 (16)	74.7 ± 4.7 (15)	68.1 ± 3.4 (15)	62.7 ± 4.5 (16)	0.291	
56	71.0 ± 3.4 (15)	79.5 ± 2.6 (15)	83.3 ± 3.5 (15)	71.6 ± 4.1 (16)	72.2 ± 3.8 (15)	68.2 ± 4.6 (16)	0.064	
58	71.4 ± 3.6 (15)	77.9 ± 3.3 (14)	82.2 ± 4.0 (16)	73.6 ± 4.6 (15)	70.8 ± 4.1 (14)	64.8 ± 5.1 (15)	0.620	

Comb area generally increased with age, but no photoperiodic effects were observed (Table 6.1). Rates of comb growth between 51 and 58 weeks were similar for all treatment groups.

Changes in comb area in the period from 4 d prior to light transfer at 40 weeks to the end of the study at 60 weeks were not significantly affected by photoperiod, whilst the test for equality of slopes for the various regressions of change in comb area on age between 51 and 58 weeks were not significantly different from birds on different photoperiods.

Mean plasma testosterone concentration at 60 weeks decreased linearly with photoperiod, with the exception of the 22 h treatment (Figure 6.2), and was described by the equation:

$$y = 5.43 - 0.2x \quad (P < 0.001, R^2 = 0.987, \text{res df} = 3)$$

where y = testosterone concentration (ng/ml) and x = photoperiod (h).

The testes weight of birds on 22 h was significantly lower than for birds on all other treatments, apart from the intermediate value for 18-h birds (Table 6.2).

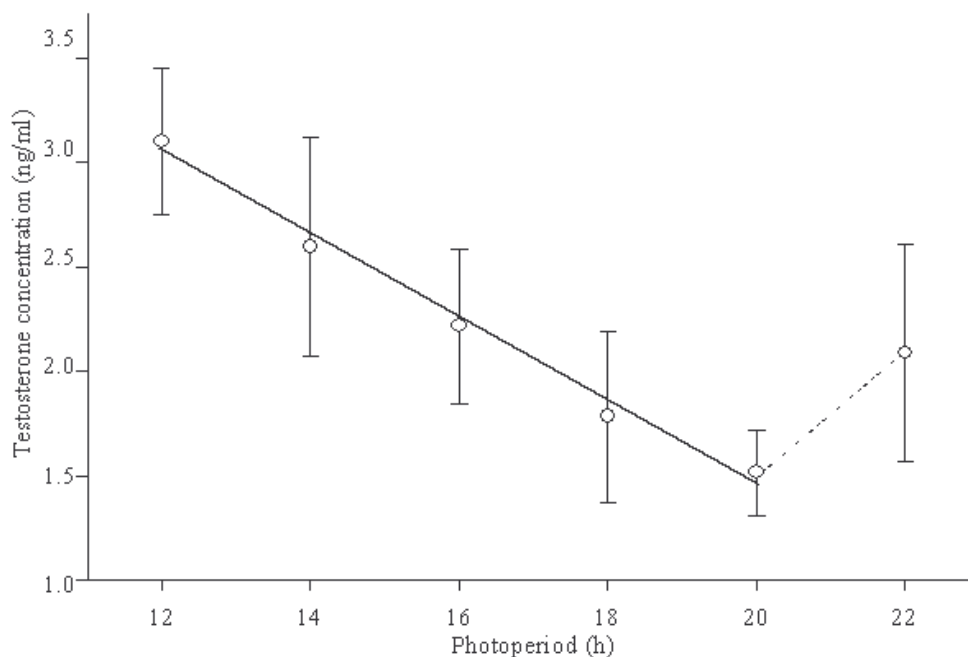


Figure 6.2. Mean \pm sem testosterone concentration at 60 weeks of age for male broiler breeders transferred from 8 to 12 h at 20 weeks and either maintained on 12 h or further transferred to 14, 16, 18, 20 or 22 h at 40 weeks

Table 6.2. Mean \pm sem (n) left and right testis weights and combined testes weight at 60 weeks for broiler breeders transferred from 8 to 12 h at 20 weeks and either maintained on 12 h or further transferred to 14, 16, 18, 20 or 22 h at 40 weeks.

Photoperiod from 40 to 60 weeks	Left testis weight (g)	Right testis weight (g)	Combined testes weight (g)
12	12.0 \pm 1.1 (15)	13.0 \pm 1.4 (15)	25.0 \pm 2.5 (15)
14	11.8 \pm 0.9 (15)	14.0 \pm 0.9 (15)	25.8 \pm 0.9 (15)
16	11.5 \pm 1.4 (16)	12.0 \pm 1.3 (16)	23.5 \pm 2.6 (16)
18	10.0 \pm 1.1 (16)	11.00 \pm 1.3 (16)	20.9 \pm 2.5 (16)
20	11.3 \pm 1.0 (16)	13.2 \pm 1.3 (16)	24.4 \pm 2.3 (16)
22	7.8 \pm 1.0 (16)	8.7 \pm 1.1 (16)	16.5 \pm 2.0 (16)
<i>P</i> value			0.037
Pooled SED			3.19
Res df			88

6.4 DISCUSSION

The lack of a semen sample from some males at each age and the large individual fluctuations in semen concentration, but with no overall effect of photoperiod, suggests that the observed responses were to factors other than light. The differences in semen concentration may reflect variations in individual birds' threshold photoperiod for inducing adult photorefractoriness, as suggested by Siopes and Proudman (2009) for turkey females. Whilst photoperiodic influences have been found to impact semen concentration (McPhail and Tyler, 2008), the lack of a significant effect of photoperiod on semen concentration may not have been observed due to the large within-treatment variation. However, these results are consistent with the absence of a photoperiodic effect on comb area or comb growth.

Contrastingly, the decrease in mean plasma testosterone concentration at 60 weeks with increasing photoperiod, except for the unexplained value of the 22 h treatment (Figure 2), does suggest a more rapid onset of photorefractoriness when broiler breeders are transferred to longer photoperiods at 40 weeks. There is evidence in broiler breeder males that ultra-long photoperiods, such as 22 h, are only mildly stimulatory (Renden *et al.*, 1991) and, as a consequence, less inductive of adult photorefractoriness; this may explain

the higher testosterone concentration of this group at 60 weeks. Although this group had the lowest testes weight (Table 2), the lack of a correlation between testosterone and testes weight has been observed in broiler breeders (Zemen *et al.*, 1986) and turkeys (Noirault *et al.*, 2006), and testosterone concentration is more likely to be related to LH concentration (Sharp *et al.*, 1977) than testes weight. Testosterone has been shown to be essential for comb growth and castration to cause dramatic comb shrinkage (Parker and Ligon, 2007), however, if comb regression lags the decrease in testosterone concentration, this could be the explanation for the disparity between the size of the combs and testosterone concentrations in this study. There were three possible responses to the photoperiodic transfers made at 40 weeks: a positive response, a negative response or no response. Sharp (1993) hypothesised that the ideal way to initiate and sustain sexual maturation in domestic hens might be to transfer them initially to a mildly stimulatory photoperiod and to provide further increments when required to balance the progressive decline in LH release associated with the onset of photorefractoriness (Sharp *et al.*, 1992; Ciccone *et al.*, 2005). However, Lewis *et al.* (2007c) showed that further photostimulation of broiler breeders to 16 h for birds given an initial increase from 8 to 11 h at 20 weeks not only had minimal effect on plasma LH concentration but accelerated the age-related decline in egg production, relative to controls held on 11 h from 20 weeks. The observed drop in egg production was not associated with any compensatory increase in egg weight (Lewis *et al.*, 2007c), as observed in egg-type pullets by (Morris *et al.*, 1995), which led Lewis *et al.* (2007c) to conclude that the onset of adult photorefractoriness had been advanced. The current findings suggest that secondary photostimulation during the breeding cycle, at least when provided at 40 weeks, has neither a positive nor a negative effect on reproductive status and, as a corollary, the development of adult photorefractoriness in broiler breeder males. If the timing of the onset of adult photorefractoriness is initiated simultaneously with rapid gonadal maturation, as hypothesised by Dawson (2001) from studies of male European Starlings, then transfers to a more stimulatory daylength 20 weeks after an initial transfer to a mildly stimulatory daylength would not be expected to affect the development of photorefractoriness. Whilst the current findings seem to contradict those of Siopes and Proudman (2009), that turkey females initially transferred from 8 to 12 h increased their rate of lay following secondary photostimulation given 24 weeks after initial photostimulation, the suggestion of Falk and Gwinner (1988) that, in European Starlings, more emphasis is placed on photoperiodic cues occurring later in the season, and the observation of Stokkan *et al.* (1982) that further

increases in photoperiod following an initial transfer to a mildly stimulatory photoperiod results in a transient rise in reproductive function in Willow Ptarmigan (a galliform species closely related to domestic fowl), they do support the conclusion of Siopes and Proudman (2009) that adult photorefractoriness in turkey females is fully programmed by 20 weeks of exposure to long days and therefore unlikely to be influenced by photoperiodic increases given after this stage.

The apparent difference in sexual response to secondary photostimulation between broiler breeder males (minimal response) and females (negative response) has also been observed in turkeys, where prolactin, a hormone associated with the onset of adult photorefractoriness, increases after photostimulation in turkey hens (Proudman, 1998; Proudman and Siopes, 2005) but remains low in turkey toms and with no ensuing onset of adult photorefractoriness (Proudman and Siopes, 2005). However, the evidence for adult photorefractoriness in broiler breeder males from the constant photoperiod study of Tyler and Gous (2008) remains compelling, with testis weights being significantly higher at 67 weeks in birds maintained from soon after hatch on short daylengths than those of birds kept on long daylengths.

In most commercial broiler breeder operations males and females are kept together, so secondary light increases during the breeding cycle are not to be recommended because of their negative effect on egg production (Lewis and Gous, 2006b; Lewis *et al.*, 2007c). However, the data gathered in this study provide minimal evidence that photoperiodic increments given to broiler breeder males in mid-cycle will either advance the onset of photorefractoriness or accelerate the decline in fertility.

7. TESTES ASYMMETRY

7.1 INTRODUCTION

Most animals are bilaterally symmetric, and belong to the group Bilateria. Most bilateral animals have an identical shape on either side, and this attribute permits streamlining, favours the formation of a central nerve centre, contributes to cephalisation, and facilitates active movement in organisms (Wikipedia, 2010). Bilateral symmetry has often been used as an indicator of developmental stability, with any asymmetry indicating the effects of a stressor e.g. (Yang and Siegel, 1998). Interestingly though, asymmetry in the avian testes has been suggested to be indicative of individuals with high phenotypic quality (Møller, 1994) although this suggestion has been criticised by (Palmer, 1996). In the course of the project reported in this thesis, large numbers of testes from males of different ages, having been subjected to different treatments, have been dissected and measured thus providing material that could be used to corroborate or refute the above suggestion.

Deviations from symmetry in bilateral traits have been classified as directional asymmetry (e.g. the mammalian heart), antisymmetry (e.g. a human population with left- or right-handed individual but few ambidextrous individuals) or fluctuating asymmetry, where there is random deviation from perfect symmetry resulting in equal mean development on each side (Van Valen, 1962). These types of asymmetry were represented diagrammatically (Figure 7.1) by Palmer (1996). Fluctuating asymmetry describes a specific pattern of bilateral variation where the frequency distribution of right minus left (R-L) has a mean of zero and follows a normal distribution, and offers one measure of developmental precision, which refers to how closely a structure approaches its ideal for a particular genotype and growth environment (Palmer, 1996). Developmental noise arises when random variation during growth tends to cause a structure to depart from its ideal for a particular genotype and environment, so that asymmetries arise because developmental noise affects the right and left sides of a bilateral pair of structures independently. Other factors, such as differential gene activity or differential use of e.g. limbs, may also cause the right side to differ from the left, and if the mean of the R-L distribution departs from zero, directional asymmetry results, or if the shape of the distribution departs from normality antisymmetry results (Palmer, 1996). Developmental

stability is the buffering of the disruptive effects of developmental noise during growth to ensure potential development (Palmer, 1996). Developmental noise refers exclusively to non-genetic phenomena while developmental stability does appear to have a genetic basis and both may independently influence the frequency distribution of asymmetrical development (Palmer and Strobeck, 1992).

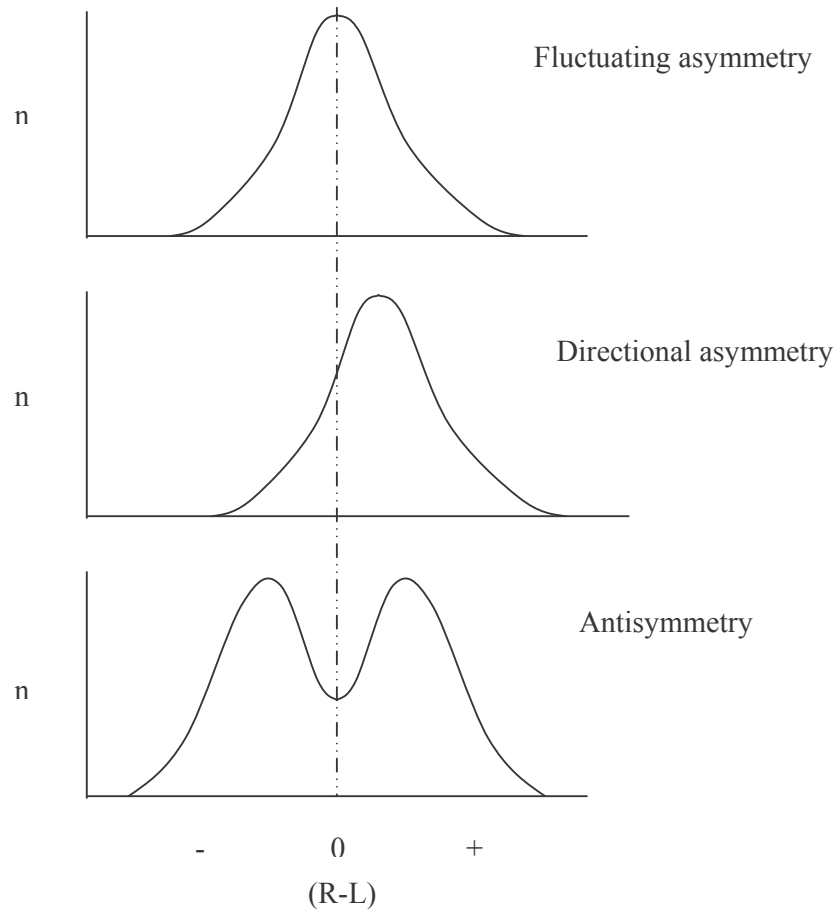


Figure 7.1 Illustration of the three commonly observed patterns of deviation from bilateral asymmetry from Palmer (1996)

Relative asymmetry, which has been defined as the ratio of the absolute value of asymmetry divided by the value for the size of the bilateral trait, so that; $RA = (|L - R| / [(L + R)/2]) \times 100$, has been suggested as a valid measurement of developmental stability (Yang *et al.*, 1997). Other studies suggest that fluctuating asymmetry, used as a measure of developmental stability, is a reflection of genetic or environmental stress

(Yang *et al.*, 1998a; Yang and Siegel, 1998; Møller *et al.*, 1999; Yalcin *et al.*, 2001) in poultry, and many authors have used asymmetry in bilateral traits as an indicator of welfare in chickens (Campo and Dávila, 2008; Dennis *et al.*, 2008; Campo and Prieto, 2009), as well as across plant (Henríquez *et al.*, 2009) and other animal species (Viguié *et al.*, 2009).

The reproductive tract is considered a bilateral trait, with left and right gonad development. Gonadal development in the male is symmetrical with the development of the reproductive tract on both sides (testes and vas deferens), but in most female birds, only the left ovary and oviduct develops from the embryonic Müllerian ducts. This is presumably as an adaptation to reduce weight and aid in flight, due to the space and investment that would be required from development of both oviducts and ovaries in comparison to the relatively smaller oviducts and ovaries of mammals. Various authors do report observations of two ovaries, two oviducts, or both ovaries and oviducts in avian species, for example in the Pekin Duck (Kamar and Yamani, 1962) and the Peregrine Falcon (White, 1969). In the Kiwi, paired ovaries occur consistently, although only the left oviduct is functional (Kinsky, 1971), and an observation of a White Leghorn pullet with a functional left and right oviduct has been reported (Bickford, 1965), but generally only the left ovary develops in avian species. This appears to be under genetic control, with hereditary persistent right oviduct development in an inbred line of chickens thought to be controlled by two pairs of autosomal recessive major genes, and numerous loci that have minor effects (Wakamatsu *et al.*, 2000). Embryonic exposure to excess oestrogen also resulted in right oviduct retention as well as structural abnormalities (Berg *et al.*, 2001).

7.2 TESTES ASYMMETRY

Despite the development of two testes in avian males, there are reports of a lack of symmetry between the left and right testis. The left testis was significantly heavier than the right in 52 to 55 week old turkeys (Burke, 1973) and Zebra Finches (Birkhead *et al.*, 1998) and significantly larger in Barn Swallows and House Sparrows (Møller, 1994). Although Hocking (1992) reported the proportion of domestic fowl with a heavier left testis to be 0.669, he found no significant difference between the left and right testes, and Kimball *et al.* (1997) found 10-month-old Red Junglefowl to have a larger right testis, but

by 12 months of age the difference was not significant (in a different sample of birds). The left testis has been described as larger or heavier than the right in 48 and 63-week-old broiler breeders (Wolanski *et al.*, 2004), in White Leghorns up to 179 d (Latimer, 1924) and in Pied Flycatchers (Silverin, 1975), but these reports did not state whether or not these differences were significant. The opposite was found by Law and Kosin (1958) where the right testis of turkeys was significantly heavier than the left. Therefore there is some inconsistency in the reports of testes asymmetry.

Directional asymmetry was found by Tyler and Gous (2009) in broiler breeders (Figure 4.2), where the left testis was significantly heavier than the right in birds photostimulated at 56, 77, 98, 119 and 147 d, but not in birds photostimulated at 161 d. Birds on this latter treatment attained sexual maturity before being photostimulated and it was suggested that the lack of asymmetry could be due to spontaneous, rather than light-stimulated, testis development. However, when looking at the left minus right testis weight at each age, there was no apparent difference in the degree of asymmetry across treatments (Table 7.1), although the number of birds sampled at each age is low.

Directional asymmetry in testes weights was also found, but in the opposite direction, in 60-week-old broiler breeders reared on 8 h, photostimulated to 12 h at 20 weeks, and provided a light increase at 40 weeks to 14, 16, 18, 20 or 22 h, with a control group remaining on 12 h, where the right testis was heavier than the left (Figure 7.2). The left and right testis weights were significantly different ($P < 0.05$) for males on the 12-hour control and for those further photostimulated to 18 and 20 h, while those photostimulated to 22 h were approaching a significant difference ($P = 0.052$). This could be as a result of the timing of the testis weight measurement. If greater development of the left testis during the breeding season reflects a higher selective advantage (Møller, 1994) and causes directional asymmetry, then it is also possible that the left testis could undergo greater regression at the end of the breeding season, which may cause directional asymmetry in the opposite direction. However, this would mean that males on treatments showing no differences in left and right testis weight should then exhibit a greater left testis weight than those on treatments with differences between left and right testis weight, which was not the case; there was no significant difference in left, right or average testis weights across treatments, and no response in testis weight to increases in photostimulation at 40 weeks.

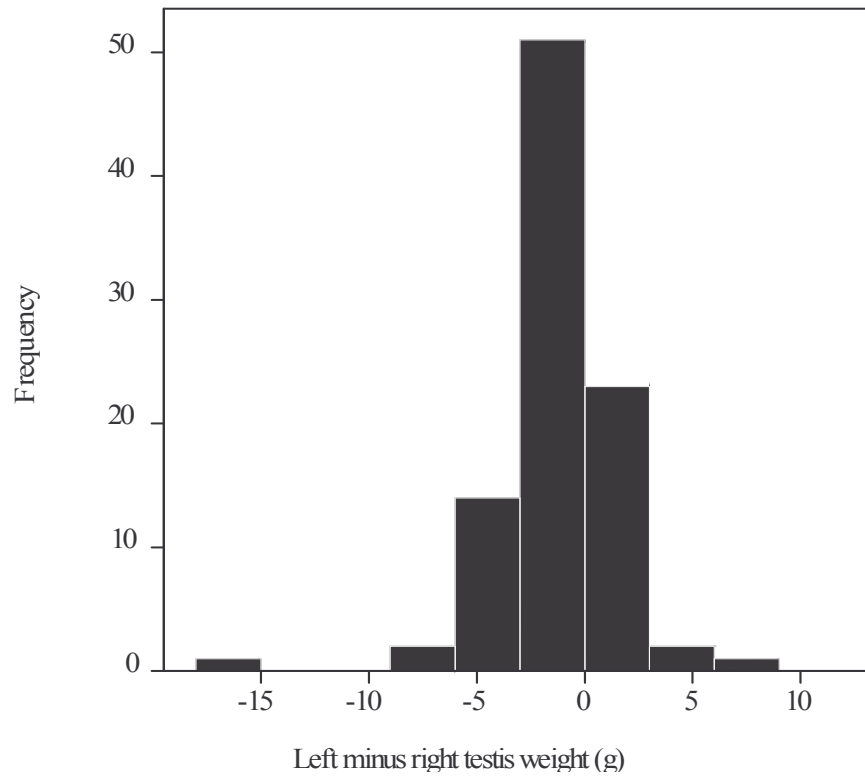


Figure 7.2 Directional asymmetry in 60-week-old broiler breeder males photostimulated at 20 weeks and maintained on 12 h or provided a further photoperiodic increase at 40 weeks to 14, 16, 18, 20 or 22 h.

Møller (1994) suggested that testes asymmetry is indicative of high quality males, as the degree of directional asymmetry increased with the size of secondary sexual character measured (tail length in swallows and badge size in sparrows), although Birkhead *et al.* (1998), in questioning the differences observed in these experiments, attributed it to the low R^2 seen in the relationship between testes asymmetry and secondary sexual characters from Møller (1994). Birkhead *et al.* (1998) found no relationship between beak colour and song rate (as secondary sexual characters) and relative testes asymmetry in Zebra finches.

Directional asymmetry has also been observed in the testes of broiler breeders that were reared and maintained on constant photoperiods; the overall directional asymmetry showed the left testis to be larger than the right (Figure 7.3). There was no consistent relationship with the testis asymmetry and age observed (Table 7.2), although a linear regression of left minus right testis weight showed constant photoperiod to have a

significant effect on the slope ($P = 0.02$) and constant term ($P < 0.001$) of birds sampled at ≥ 175 d compared to those sampled at < 175 d, where shorter photoperiods, which resulted in earlier testis development, also resulted in a greater degree of asymmetry (Figure 7.4). This may have coincided with greater testis weight recordings, been a result of greater phenotypic quality with greater left testis maturation, or the result of greater developmental instability with the faster testis maturation on the shorter photoperiods. McGary *et al.*, (2003) found no relationship between the degree of fluctuating asymmetry of secondary sexual traits (wattle length, width and area) and male fertility potential in chickens, but testis weights were not measured in that trial.

Table 7.1 The difference between the left and right testis weight \pm sem (n) of birds photostimulated at different ages

Age at photostimulation (weeks)	Mean L-R testis weight (g) \pm sem (n)					
	8	11	14	17	20	23
Age (d)						
17	0.55 ^a (1)	2.08 ^a \pm 1.72 (2)	0.05 ^a \pm 0.04 (2)	0.00 ^a \pm 0.00 (2)	-0.01 ^a \pm 0.01 (2)	-0.04 ^a \pm 0.05 (2)
18	-0.08 ^a \pm 0.03 (2)	0.58 ^a \pm 0.41 (2)	0.07 ^a \pm 0.24 (2)	0.05 ^a \pm 0.04 (2)	0.05 ^a \pm 0.09 (2)	-0.07 ^a \pm 0.07 (2)
19	0.70 ^a \pm 0.08 (2)	0.57 ^a \pm 0.02 (2)	1.02 ^a \pm 0.93 (2)	0.03 ^a \pm 0.09 (2)	0.08 ^a \pm 0.02 (2)	-0.02 ^a \pm 0.03 (2)
20	0.05 ^a \pm 0.02 (2)	0.34 ^a \pm 0.43 (2)	0.56 ^a \pm 0.43 (2)	0.26 ^a \pm 0.29 (2)	-0.02 ^a \pm 0.05 (2)	0.20 ^a \pm 0.21 (2)
21	0.23 ^a \pm 0.11 (2)	0.82 ^a \pm 0.01 (2)	2.03 ^a \pm 2.02 (2)	0.08 ^a \pm 0.02 (2)	-0.01 ^a \pm 0.12 (2)	0.11 ^a \pm 0.12 (2)
22	0.07 ^a \pm 0.06 (3)	0.43 ^a \pm 0.29 (2)	2.03 ^a \pm 1.11 (2)	1.56 ^a \pm 1.39 (2)	-0.01 ^a (1)	0.88 ^a \pm 0.42 (2)
24	-0.01 ^a \pm 0.04 (2)	-0.03 ^a \pm 0.28 (2)	3.85 ^a \pm 4.22 (2)	1.19 ^a \pm 0.85 (2)	0.75 ^a \pm 0.43 (2)	0.50 ^a \pm 1.78 (2)
25	0.03 ^a \pm 0.11 (2)	2.45 ^b \pm 2.38 (4)	-2.10 ^a \pm 1.06 (2)	0.18 ^a \pm 0.31 (2)	0.05 ^a \pm 0.90 (2)	2.32 ^b \pm 0.06 (2)
26	3.05 ^a \pm 0.82 (2)	2.23 ^a (1)	0.47 ^a \pm 0.27 (3)	2.96 ^a \pm 0.12 (2)	0.35 ^a \pm 0.79 (3)	-1.86 ^a \pm 3.39 (2)
27	1.45 ^a \pm 0.96 (2)	-0.14 ^a \pm 0.53 (2)	6.06 ^a \pm 4.88 (2)	1.55 ^a \pm 0.09 (2)	1.41 ^a \pm 0.39 (2)	2.28 ^a \pm 1.61 (2)
28	0.08 ^a (1)	0.93 ^a \pm 0.48 (2)	3.36 ^a (1)	1.79 ^a \pm 1.47 (2)	2.09 ^a \pm 1.18 (2)	2.94 ^a \pm 2.61 (2)
29			1.53 ^a \pm 1.22 (3)	0.71 ^a \pm 0.55 (3)	0.77 ^a \pm 0.50 (9)	1.06 ^a \pm 1.00 (2)
30	0.15 ^a \pm 0.52 (2)	0.30 ^a \pm 0.02 (2)	0.66 ^a (1)	-1.23 ^a (1)		
32	0.77 ^a \pm 0.40 (6)	1.85 ^a \pm 1.39 (8)	0.89 ^a \pm 0.89 (3)	1.64 ^a \pm 1.23 (4)		
Within a row, means without a common superscript differ significantly ($P < 0.05$)						

Within a row, means without a common superscript differ significantly ($P < 0.05$)

Table 7.2 The difference between the left and right testis weight \pm sem (n) of birds reared and maintained on constant photoperiods of 8, 10, 12, 14, 16 and 18 h at different ages

Constant photoperiod (h)	Age (d)	Mean L-R testis weight (g) \pm sem (n)					
		8	10	12	14	16	18
71	71	0.01 ^a \pm 0.01 (2)	-0.01 ^a (1)	0.01 ^a \pm 0.02 (2)	-0.01 ^a \pm 0.02 (2)	0.01 ^a \pm 0.02 (2)	0.01 ^a \pm 0.01 (2)
78	78	0.00 ^a (1)	0.00 ^a (1)	0.00 ^a \pm 0.00 (2)	0.03 ^a \pm 0.03 (2)	0.01 ^a \pm 0.01 (2)	0.02 ^a (1)
92	92	0.02 ^a \pm 0.01 (2)	0.01 ^a \pm 0.01 (2)	0.01 ^a \pm 0.04 (2)	0.03 ^a \pm 0.01 (2)	0.07 ^a \pm 0.07 (2)	0.07 ^a (1)
99	99	0.02 ^a \pm 0.02 (4)	0.02 ^a \pm 0.04 (4)	0.05 ^a \pm 0.03 (4)	0.01 ^a \pm 0.01 (4)	0.06 ^a \pm 0.03 (4)	0.00 ^a \pm 0.02 (3)
106	106	0.04 ^a \pm 0.01 (2)	0.00 ^a \pm 0.02 (2)	0.02 ^a \pm 0.01 (2)	0.06 ^a \pm 0.06 (2)	0.03 ^a \pm 0.02 (2)	0.04 ^a (1)
113	113	0.05 ^a \pm 0.01 (2)	0.10 ^a \pm 0.03 (2)	0.02 ^a \pm 0.04 (2)	0.04 ^a \pm 0.05 (2)	0.06 ^a \pm 0.05 (2)	0.04 ^a (1)
119	119	0.03 ^b \pm 0.02 (2)	-0.21 ^a (1)	0.03 ^b \pm 0.01 (2)	0.06 ^b \pm 0.03 (2)	0.03 ^b \pm 0.03 (3)	0.12 ^b (1)
126	126	0.04 ^a \pm 0.03 (2)	0.03 ^a \pm 0.03 (4)	0.02 ^a \pm 0.02 (2)	0.07 ^a (1)	0.03 ^a \pm 0.02 (2)	0.05 ^a \pm 0.03 (2)
133	133	0.09 ^{ab} \pm 0.09 (2)	0.36 ^b \pm 0.03 (2)	0.09 ^{ab} \pm 0.03 (2)	0.09 ^{ab} \pm 0.03 (2)	-0.07 ^a \pm 0.1 (2)	0.05 ^a (1)
140	140	0.09 ^a \pm 0.07 (2)	0.09 ^a \pm 0.01 (2)	-0.02 ^a \pm 0.04 (2)	0.03 ^a \pm 0.03 (2)	0.07 ^a \pm 0.02 (2)	0.10 ^a \pm 0.02 (2)
147	147	-0.18 ^a \pm 0.12 (2)	0.34 ^c \pm 0.03 (2)	0.15 ^{bc} \pm 0.10 (2)	0.02 ^{ab} \pm 0.02 (2)	0.09 ^b \pm 0.01 (2)	0.05 ^b \pm 0.04 (2)
154	154	0.03 ^a \pm 0.03 (2)	-0.39 ^a \pm 0.42 (2)	0.16 ^a \pm 0.09 (2)	0.07 ^a \pm 0.09 (2)	0.07 ^a (1)	0.00 ^a (1)
161	161	0.70 ^a \pm 0.37 (2)	0.22 ^a \pm 0.65 (2)	0.36 ^a \pm 0.47 (2)	0.07 ^a \pm 0.13 (2)	0.12 ^a \pm 0.01 (2)	-0.12 ^a \pm 0.03 (2)
165	165	0.51 ^a \pm 0.44 (4)	0.47 ^a \pm 1.00 (4)	0.71 ^a \pm 0.39 (4)	0.09 ^a \pm 0.05 (4)	0.27 ^a \pm 0.18 (4)	0.45 ^a \pm 0.33 (3)
168	168	0.41 ^a \pm 0.21 (4)	0.36 ^a \pm 0.48 (3)	-0.08 ^a \pm 0.35 (4)	0.11 ^a \pm 0.08 (4)	0.13 ^a \pm 0.08 (4)	0.06 ^a \pm 0.06 (4)
173	173	0.40 ^a \pm 0.24 (3)	0.30 ^a \pm 0.18 (4)	0.17 ^a \pm 0.13 (4)	0.03 ^a \pm 0.07 (4)	0.18 ^a \pm 0.11 (4)	0.14 ^a \pm 0.14 (4)
175	175	1.58 ^{ab} \pm 1.17 (4)	3.48 ^b \pm 1.29 (4)	0.46 ^a \pm 0.62 (4)	0.60 ^a \pm 0.70 (4)	-0.64 ^a \pm 0.41 (4)	0.17 ^a \pm 0.04 (3)
180	180	-0.31 ^a \pm 0.95 (4)	2.89 ^b \pm 1.70 (3)	0.20 ^a \pm 0.63 (4)	0.12 ^a \pm 0.11 (4)	0.27 ^a \pm 0.17 (4)	-0.31 ^a \pm 0.05 (3)
182	182	1.35 ^a \pm 0.83 (4)	1.37 ^a \pm 0.59 (4)	1.32 ^a \pm 0.96 (5)	1.28 ^a \pm 1.37 (3)	0.58 ^a \pm 0.40 (4)	-0.17 ^a \pm 0.43 (3)
189	189	0.55 ^a \pm 0.58 (6)	2.58 ^a \pm 0.93 (5)	1.56 ^a \pm 0.90 (4)	2.10 ^a \pm 2.10 (2)	2.62 ^a \pm 1.64 (4)	0.81 ^a \pm 0.67 (5)
196	196	1.47 ^a \pm 0.66 (4)	0.31 ^a (1)	2.00 ^a \pm 1.15 (3)	3.81 ^a \pm 0.26 (2)	0.68 ^a \pm 0.83 (3)	2.93 ^a \pm 1.09 (3)
467-470	467-470	1.66 ^c \pm 0.25 (22)	0.85 ^{ab} \pm 0.25 (24)	1.49 ^{bc} \pm 0.35 (22)	0.73 ^a \pm 0.21 (24)	0.88 ^{ab} \pm 0.28 (23)	0.36 ^a \pm 0.20 (22)

Within a row, means without a common superscript differ significantly ($P < 0.05$)

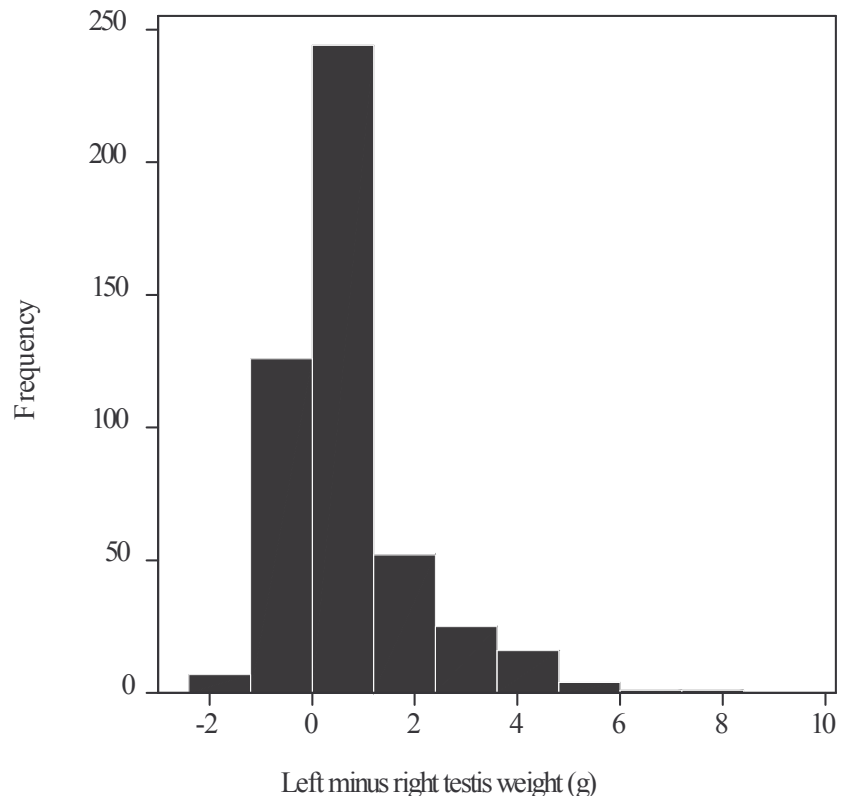


Figure 7.3 Directional asymmetry in broiler breeder males at various ages (10 to 60 weeks) reared and maintained on constant photoperiods of 8, 10, 12, 14, 16 and 18 h.

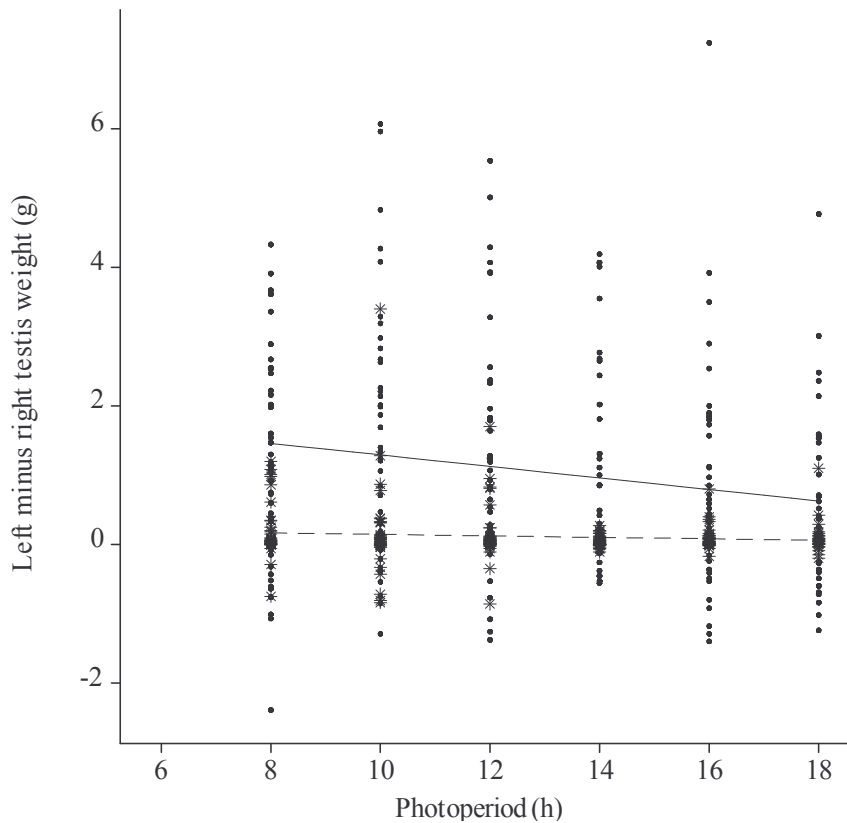


Figure 7.4 Linear regression of the effect of photoperiod on the left minus right testis weight of birds sampled at < 175 d of age (---) or at ≥ 175 d of age (—) ($R^2 = 0.16$)

Møller's (1994) hypothesis was thought to be consistent with the view that two large testes would be costly to produce and maintain, implying an adaptive advantage of directional asymmetry, maintained by directional selection, and that the left testis is functional, while the right plays a more compensatory role. The cost of two large testes include the greater immuno-suppressive effect of testosterone (Zuk *et al.*, 1995), and greater testicular mass which could affect flight performance. However, the lack of a relationship between testosterone and testes weight (Zemen *et al.*, 1986; Noirault *et al.*, 2006) could negate this cost, and flight could also be adversely affected by asymmetrical testes in the event that it could affect balance. Also, as suggested by Kimball *et al.* (1997), avian species which are primarily terrestrial may exhibit differences in the degree of testicular asymmetry to more aerial species due to weaker selection pressure.

Birkhead *et al.* (1998) considered that because there was no significant relationship between left testis mass and relative testis symmetry ($(L-R)/0.5(L+R)$) there was no evidence that the right testis serves a compensatory role, as suggested by Møller (1994),

whose hypothesis was based on a significant relationship between directional asymmetry and secondary sexual characters.

Ishimaru *et al.* (2008) reported morphological asymmetry of the chick gonad cortex (which contributes significantly to ovarian development) just before sex differentiation, with the right cortex becoming thinner and the left becoming thicker. It was also reported that the initial differential expression of some genes was responsible for asymmetric ovarian development, such that cell proliferation is accelerated in the left cortex and decelerated in the right, as well as the loss of responsiveness to oestrogen of the right cortex. It would be interesting to know if there is any genetic involvement in the asymmetric development in the testes of the male chick. Birkhead *et al.* (1998) argue that asymmetry in the male reproductive system could be a by-product of selection for asymmetry in the female reproductive tract, but that equally, asymmetry in the female reproductive tract could be a consequence of selection for asymmetry in the male system, and that because there are cases where no asymmetry in testis size is reported, that a non-adaptive explanation such as this is not adequate to explain testis asymmetry.

7.3 CORRELATION OF TESTES WEIGHT AND DIMENSIONS

Strong, significant ($P < 0.001$) correlations were observed between the left testis weight and left testis length and width (0.94 and 0.93 respectively) and between the right testis weight and right testis length and width (0.94 and 0.94 respectively). These measurements were recorded in 800 broiler breeders at different ages ranging from 10 to 67 weeks and over various photoperiodic treatments, indicating the stability of the relationship.

If testes dimensions could be measured in the live bird, the high correlation between testes weight and dimensions would allow an accurate estimation of testis weight to be made. A strong relationship ($R^2 = 0.89$) between testes weight and daily sperm output has been reported (Amann, 1999), and in another study was significantly correlated to sample fertility (0.65) and flock fertility (0.61) in one broiler breeder strain but not another (McGary *et al.*, 2002). However, because the testes in avian species are internal, it is difficult to record dimensions. One method is through ultrasonography, and although it has been considered impractical due to the presence of air sacs and pneumatized bones, Hofbauer and Krautwald-Junghanns (1999) reported transcutaneous ultrasonography to be

a useful tool in examining the urogenital tract of pet birds, mainly belonging to the Psittaciformes, Columbiformes and Passeriformes species, and sonographic visualisation of the testes was possible in sexually active quail.

The use of a prostate probe inserted into the cloaca of broiler breeders resulted in observation of one or both testes in 88 % of roosters, and the measurement of testis diameter. However, mortality did occur in some birds, due to faeces compaction by the probe, leading to intestinal rupture (Wilson, 2006). However, Hildebrandt *et al.* (1995), using intracloacal ultrasonography in raptors, could identify the female reproductive tract, but identified males only through the lack of vaginal structure. Laparoscopy also allows visualisation and measurement of the gonads, but requires anaesthesia and the risk of injury to the bird.

7.4 CONCLUSION

There appears to be a lack of consistency in the display and/or the direction of testes asymmetry in broiler breeders, perhaps due to the lack of selection pressure that has been applied to birds of flight. There is also no clear evidence that asymmetry is as a result of the imposition of genetic or environmental stress.

The strong positive correlation between testes dimensions and weight suggests that it might be possible to predict testes weight, and thus fertility, from their dimensions. If the techniques of ultrasonics or laparoscopy could be further developed it would be possible to monitor male fertility throughout the breeding period thereby determining at what stage and under what circumstances fertility declines in a breeding flock.

8. GENERAL DISCUSSION

The aim of this study was to gain an understanding of the male broiler breeder response to photoperiod. To this end, the response of males to constant daylengths, different ages at photostimulation and further increases in photoperiod at 40 weeks was investigated.

When broiler breeder males were reared and maintained on constant photoperiods, juvenile photorefractoriness was evident, in that birds maintained on constant long days had lower testis weights, implying a delayed age at sexual maturity. Later in the cycle there were no differences in testis weight, but the birds on constant long days appeared also to exhibit adult photorefractoriness with the presentation of significantly lower testis weights at 66 weeks of age compared to birds on shorter photoperiods. This response appears to be similar to that seen in broiler breeder pullets, where those maintained on constant 13 and 14-h photoperiods showed delayed maturity (Lewis *et al.*, 2004).

When broiler breeder males were photostimulated at different ages, there was also evidence presented of the exhibition of juvenile photorefractoriness. When photostimulation was at 14 weeks or later it appeared that all birds had achieved photosensitivity and were able to respond to photostimulation. Most birds photostimulated early had not yet achieved photosensitivity (i.e. were still photorefractory), although there were some birds that did respond to early photostimulation, indicating a probable high degree of variation in this trait, and therefore the possibility of selection against photorefractoriness. This led to an experiment to investigate whether selection against photorefractoriness in broiler breeders is possible, without compromising growth traits in their offspring. This experiment showed the potential to do this, but because of the quantitative nature of photorefractoriness, progress in this area could require time. In the F₁ generation there were no negative effects of selection for birds that respond to early photostimulation on broiler growth rate.

However, evidence for photorefractoriness in broiler breeders was not so clear in an experiment where birds were provided a further photoperiod increase at 40 weeks, because this did not appear to advance the onset of adult photorefractoriness. This could, however, be due to the mechanisms that cause adult photorefractoriness to be fully programmed by 20 weeks of exposure to long days as seen in female turkeys (Siopes and

Proudman, 2009), and therefore the photoperiodic increase provided at 40 weeks may have had no influence on the timing of the onset of adult photorefractoriness.

Asymmetry in bilateral traits has been used as an indication of the degree of imposition of a genetic or environmental stress (Yang *et al.*, 1998a; Yang and Siegel, 1998; Møller *et al.*, 1999; Yalcin *et al.*, 2001). Birds reared and maintained on constant long daylengths showed less asymmetry in testis weights than birds on shorter days, suggesting that longer photoperiods, while delaying sexual maturity, also allow greater developmental stability, although this was only evident after 175 d of age. Although directional asymmetry in testis weights was observed in birds on all three photoperiod-response experiments, the direction of asymmetry was not consistent. The strong correlation between testis weight and dimensions may allow the possible future use of ultrasound or the endoscopic viewing to calculate testis dimensions, which would allow for the continuous monitoring of testis weight of the same males, which would give a more accurate estimation of the effects of various treatments in a research environment. Part of this work examined the use of comb area to predict testis weight, which was found to be accurate during the developmental stages (Tyler and Gous, 2008) but not at the end of the cycle (McPhail and Tyler, 2008), as was found with commercial laying hens where comb height did not predict the oviduct or ovary weight when the birds were in lay (Burgess, 1986). Therefore the use of ultrasound or endoscopic viewing may provide a more accurate assessment throughout the cycle.

These results indicate that broiler breeder males exhibit photorefractoriness, as do female broiler breeders, but the variation in exhibition of this phenomenon shows a greater degree of variability than is observed in female broiler breeders, and may allow for the selection against photorefractoriness, without a concomitant loss in broiler growth rates.

Therefore, there is no reason for broiler breeder males and females to be reared on different lighting programmes. The ideal programme during rearing would be on a short (8-h) photoperiod. Broiler breeders should not be photostimulated before 14 weeks in males as most will not have achieved photosensitivity by that age. Photostimulation at 20 weeks, which is generally recommended in the Industry, should have no detrimental effects on male fertility. The photostimulatory photoperiod used at this age should be less than 14 h as the onset of adult photorefractoriness has been shown to be faster in birds on

14-h photoperiods compared to those on 12 or 13h photoperiods (Lewis *et al.*, 2010). A decline in fertility is likely to occur earlier in the breeding period on longer photoperiods. The saturation daylength in broiler breeder females is 13 h (Lewis *et al.*, 2008), and therefore there is no advance in maturity when the photostimulatory photoperiod is above this. McPhail and Tyler (2008) found no difference in the age at sexual maturity of male broiler breeders when photostimulated to different photoperiods, but semen concentration was higher at the corresponding female age at sexual maturity when birds were on photoperiods ≤ 11.5 h. Currently many primary breeding companies recommend a mid-cycle increase in photoperiod. Although no evidence for an advance in the onset of adult photorefractoriness by providing a mid-cycle photoperiod increase was found in broiler breeder males, this practice would not be recommended because of the negative effect on egg production (Lewis and Gous, 2006b; Lewis *et al.*, 2007c).

This work has contributed to the understanding of the male broiler breeder response to photoperiod, and has allowed recommendations for lighting programmes that coincide with optimum lighting schedules for broiler breeder females. This should be useful to the Industry, who are still using recommendations based on requirements for commercial laying hens, despite many publications since 2003 indicating that broiler breeder females exhibit photorefractoriness which has a negative consequence on egg production.

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