The effect of constant photoperiod on testis weight and the use of comb area to predict testis weights in broiler breeders males

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Abstract

From the literature, it is evident that broiler breeder pullets exhibit photorefractoriness, and while it is assumed that male broiler breeders respond in a similar manner to females, it would be beneficial to determine if this is the case. This would enable lighting programmes to be designed that will ensure maximum fertility of both males and females. Male broiler breeders were housed in light-tight rooms and given constant photoperiods of 8, 10, 12, 14, 16 or 18 h/d. One or two birds were sampled from each room weekly from 71 to 232 d, and the remaining birds at 467 d, and testis weights measured. There was no significant difference between left and right testis weights, so the average was used in the analyses. Digital images of the head were recorded prior to slaughter and of four birds in each room weekly. The area of the comb was measured using image analysis software. The relationship between comb area and average testis weight was determined by regression analysis, with a logistic curve being best at fitting the data. A positive, significant relationship was observed with 69% of the variance in average testis weight being accounted for by comb area. Thus, comb area could provide a reasonable, non-invasive technique to assess fertility. At 165 d, average testis weights showed strong evidence that males exhibit juvenile photorefractoriness in a manner similar to females. Males on shorter photoperiods matured faster than those on photoperiods of 14 h and longer. As the birds aged, the effects of photorefractoriness wore off, but at the end of the experiment adult photorefractoriness was again evident, with birds on 14 h and longer having smaller average testis weights, and therefore, no longer responding to the stimulatory photoperiod.

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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 & 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 & 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) reported 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 & Krautwald-Junghanns, 1999). Many assessments of semen quality have been used in an attempt to quantify male fertility, such as volume (Brown & McCartney, 1983), concentration (Anash *et al*., 1983; Bilgilli & Renden, 1984), morphology and mobility (Froman *et al*., 1999; Froman & 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 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.

Materials and Methods

Day old male Ross broiler breeders were given 48 h light and thereafter placed on one of six constant photoperiods: 8, 10, 12, 14, 16 or 18 h. 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. Males were trained for semen collection, starting at 19 weeks.

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.

One or two birds from each room, depending on the stocking density and mortality rate, were slaughtered weekly from 71 d to 232 d, and the remaining birds at 467 d. 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 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* (Lawes Agricultural Trust, 2002)

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 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 & 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 & 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 & Mench, 1991; Leonard & Zanette, 1998). However, measuring comb size would practically be easier than observing behaviour.

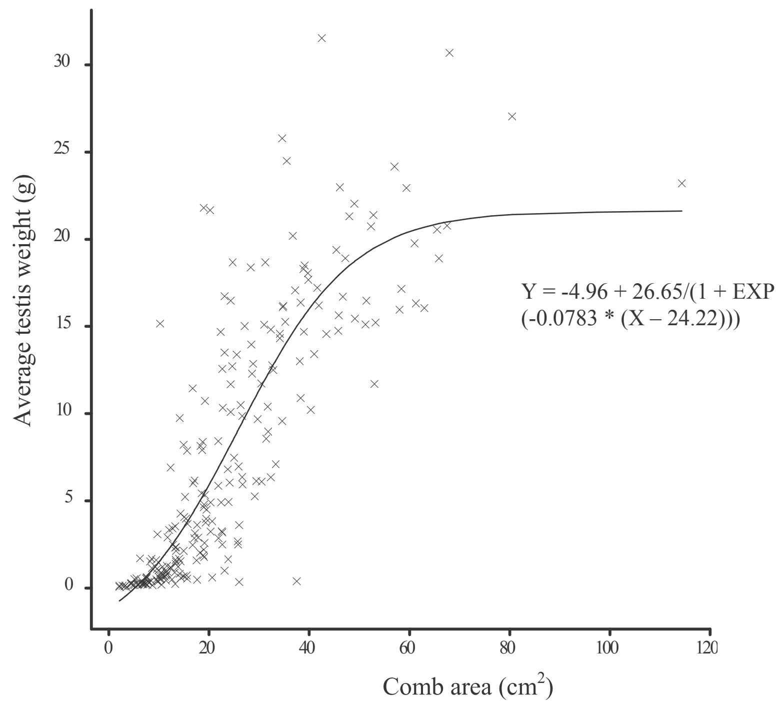

Figure 1 Logistic regression of average testis weight and comb area.

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 2) showed evidence of juvenile photorefractoriness (n = 4 for all photoperiods except n = 3 for 18 h) where birds were not responsive to the stimulatory photoperiods. Average testis weights from images of birds not slaughtered at 165d were predicted using the relationship established in Figure 1, and the only testis weights above 10 g were predicted from birds on the 8, 10 and 12 h photoperiods.

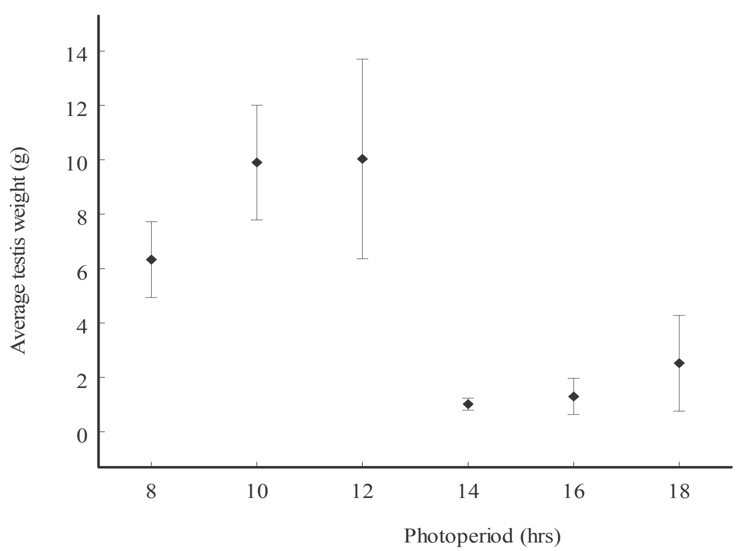

Figure 2 Ave testis weights at 165d of birds on different constant photoperiods (n = 4 for each treatment, except 18 hr, n = 3).

As the birds aged, juvenile photorefractoriness was dissipated, and photoperiod had less impact on average testis weight by 189 d (Figure 3).

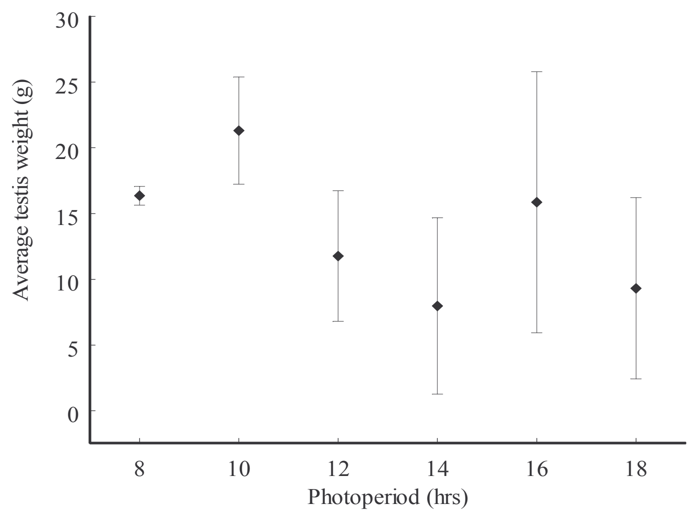

Figure 3 Average testis weights at 189d of birds on different constant photoperiods (n = 2 for all treatments, except 10 hrs, n = 4).

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 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 & Korn, 2000). Thus, longer photoperiods were no longer stimulatory.

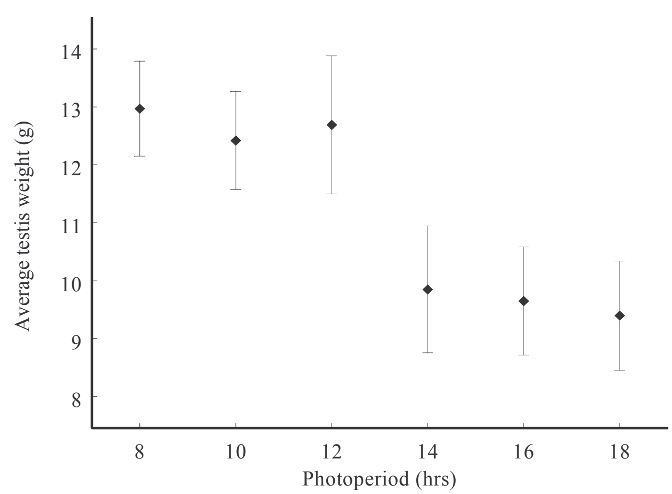

Figure 4 Average testis weights at 467 d of birds on different constant photoperiods (n = 22 for 8, 12, 18 h, n = 23 for 16 h, n = 24 for 10 and 14 hrs).

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.

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