Potential Role of Thyroid Hormones and Prolactin in the Programming of Photorefractoriness in Turkey Hens

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ABSTRACT The domestic turkey hen is a seasonal breeder, requiring a period of short days to establish photosensitivity and a long day length to initiate egg production. The reproductive season is then limited by the onset of photorefractoriness (PR), which causes a decline, and then termination, of egg laying. In passerine birds, PR is programmed early in the reproductive season by the presence of thyroid hormones and a long photoperiod. High circulating prolactin (PRL) is thought to hasten the onset of PR. In a prior study, we reported that hens destined to have PR exhibited lower levels of thyroxine (T4) and PRL at certain points (weeks) following photostimulation than did hens destined to remain photosensitive (PS), a result opposite to what might be expected. The present study was conducted to further explore the possible relationship between circulating hormone levels and subsequent PR in the commercial turkey hen at times (days) closer to photostimulation than our previous study. Plasma levels of triiodothyronine (T3), T4, and PRL were compared in 2 subpopulations of hens identified retrospectively after 50 wk of egg production: A group of 17 hens that exhibited PR (mean onset = 27 wk of photostimulation) and a group of “good” layers that remained PS (mean production = 210 eggs/50 wk). Results showed no differences between groups in plasma T3 or T4 levels or in the T3:T4 ratio at −6, 0, 1, 3, and 7 d from photostimulation. Plasma PRL levels were significantly higher at 8 and 9 wk after photostimulation in hens that remained PS vs. those that became PR. We conclude that thyroid hormone levels around the time of photostimulation either are not actively related to programming of subsequent PR in turkeys or programming for PR in the turkey hen occurs later in the reproductive cycle than in passerine birds. We further conclude that hens that exhibit PR tend to have lower circulating PRL levels early in the reproductive season than hens that remain PS and lay at a relatively high rate.

Key words: turkey, photorefractoriness, prolactin, thyroid hormone

INTRODUCTION

Seasonal breeding in wild bird populations is stimulated by increasing day length in the winter and spring, resulting in the production of young during the optimum time for survival. As the season progresses, the breeder becomes refractory to the previously stimulatory long days so that reproduction ceases and the gonads regress well before fall and winter. Short, natural day lengths then reestablish photosensitivity for the next breeding season.

The commercial turkey hen retains many of the seasonal breeding characteristics of its wild ancestors. Therefore, the year-round production of hatching eggs requires photoperiod control to provide, first, a short day length to establish photosensitivity in the young hen, followed by a long day length to initiate egg laying at sexual maturity. However, like its wild cousins, the hen becomes refractory to continued photostimulation such that egg laying declines and then ceases. Nicholls et al. (1988) and Wilson (1997) suggested that both the stimulation of reproduction and subsequent photorefractoriness (PR) are programmed by the interaction of thyroid hormones and long day length early in photostimulation. This model, developed using starlings and tree sparrows, suggests that PR is programmed by the interaction of thyroxine (T4) and long days (Nicholls et al., 1988; Wilson and Reinert, 1999; Dawson, 2001; Wilson, 2001; Mishra et al., 2004), and that programming occurs as early as 1 d (Dawson, 2001) but within a few weeks of long day exposure. Prolactin (PRL), once suspected of directly inducing PR (Dawson and Goldsmith, 1983), is now thought to accelerate the onset of PR through inhibition of the secretion of luteinizing hormone (Dawson and Sharp, 1998; Sharp and Blache, 2003). Little is known of similar mechanisms for the programming of PR in turkeys, although Lien and Siopes (1989a) reported that hens require the presence of thyroid hormones for photoinduced ovarian development. These authors also compared thyroid hormones...
and PRL between photosensitive (PS) and PR turkey hens throughout an egg laying cycle from 2 wk of photostimulation (Lien and Siopes, 1989b).

The turkey hen presents an attractive model for studying the programming of PR, because there exists within a flock a range of PR responses from individuals that become PR early in the reproductive cycle to those that remain PS for extended periods. This allows for retrospective comparisons of physiological parameters between PS and PR hens. In a prior study (Proudman and Siopes, 2002), we compared circulating thyroid hormone and PRL levels at 0, 1, 2, 8, and 14 wk following photostimulation in turkey hens that did or did not subsequently exhibit PR. This study revealed lower plasma T4 levels 1 and 2 wk after photostimulation in hens destined to exhibit PR than in flockmates that remained PS. Similarly, we observed lower PRL levels during the egg production cycle in hens destined to become PR. These results were opposite to what might be expected based upon current theories as noted above. However, it was also possible that the hormone measurements were not done early enough after photostimulation. Dawson (2001) reported as little as 1 long day is needed to program PR. The present study was conducted to further explore the possible relationship between circulating hormone levels and subsequent PR in the commercial turkey hen by examining thyroid hormones and PRL within days of photostimulation.

MATERIALS AND METHODS

Birds

Thirty-seven female parent-line BUTA (British United Turkeys of America, Lewisburg, WV) strain roaster turkeys were raised from 1 d of age following the guidelines of the primary breeder. Birds were raised on a 14L:10D photoperiod until 18 wk of age and then on a 6L:18D photoperiod until 30 wk of age. At 29 wk of age, hens were moved to laying pens in 2 rooms with independent light control of sodium vapor lamps set to provide 50 lx at bird height. Electronic time clocks with battery backup provided precise control of the light-dark cycle. Birds were photostimulated at 30 wk of age (April 23) with a photoperiod of 18L:6D (lights on at 0400 h). Egg production and nesting activity were monitored by trap-nesting, with the nests checked and hens expelled 5 times per day. Any hens that did not lay consistently in the trap nests thereafter or hens that became broody were removed from the experiment. Eighteen weeks after photostimulation, the photoperiod in 1 room was reduced to 13L:11D and then returned to 18L:6D after 3 wk. This photoperiod change identified hens that were relatively PR (rPR) or absolutely PR (aPR) (Proudman and Siopes, 2002). That is, hens that ceased laying on 13L:11D and returned to lay after resumption of 18L:6D were deemed to be rPR, whereas those that had not returned to lay 4 wk after return to 18L:6D were deemed to be aPR. Classification of a hen as aPR was confirmed by examination of the ovary by necropsy. At 25 wk after photostimulation, the rPR hens that returned to lay, as well as many of the hens that had continued to lay on constant 18L:6D, were consolidated into 1 room with an 18L:6D photoperiod. This consolidation was necessary because of space limitations, and it allowed us to continue the experiment until 50 wk after photostimulation to identify hens with high production potential. Hens that spontaneously ceased laying on an 18L:6D photoperiod were killed, and the ovary was examined by necropsy. Those hens with regressed ovaries were deemed to be aPR. Hens observed at necropsy to have abnormal ovaries or reproductive tracts were excluded from the experiment.

Blood Sampling and Hormone Assays

Five-milliliter blood samples were collected into heparinized tubes from all hens via venipuncture of the ulnar vein in the morning (0700 to 1100 h) at 6 d prior to photostimulation, on the day of photostimulation, and 1, 3, and 7 d after photostimulation. Additional blood samples were similarly collected at 7, 8, and 9 wk following photostimulation. Plasma was separated by centrifugation and stored at −80°C until analysis. Based on egg production and necropsy records, hens that had become aPR during the experiment were identified, and their plasma samples collected between −6 and +7 d of photostimulation were assayed for triiodothyronine (T3) and T4. Samples collected at 7, 8, and 9 wk were assayed for PRL. An additional 10 hens were classified as “good” layers based on high total egg production over the entire 50 wk and continued high egg production (at least 40%) during the final 30 d of the experiment. Plasma samples from these hens were assayed for T3, T4, and PRL as described for the aPR hens. Prolactin was measured using the homologous RIA of Proudman and Opel (1981). Thyroxine and T3 were measured using commercial kits (Diagnostic Products Corp., Los Angeles, CA) with modifications for turkey plasma (Siopes, 1997).

Statistical Analysis

Treatment effects were evaluated by ANOVA using the GLM procedures of the SAS Institute Inc. (1990). Repeated-measures analysis was applied to the hormone data. When group (PS vs. PR) or time × group effects were found, differences between means of the 2 groups within times were assessed using ANOVA. Statements of statistical significance were based on P ≤ 0.05.

RESULTS

Mean flock hen-day egg production declined to just 12% after 50 wk of photostimulation. Seventeen hens became aPR, whereas 10 hens maintained a high rate of egg production (as defined previously) throughout the
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Figure 1. Average weekly hen-day egg production (%) of turkey hens during a 50-wk reproductive season (C). Flock size was reduced from n = 122 to n = 70 at 25 wk (arrow). Average weekly egg production of hens that became absolutely photorefractory during the reproductive cycle (C; n = 17) is contrasted with a subpopulation of hens that remained photosensitive and were classified as “good” layers (▲; n = 10).

experiment (Figure 1). Both the mean and the median time of onset of aPR was 27 wk from photostimulation. This onset was delayed from what was expected and reported by Siopes (2002) to be 18 wk from photostimulation for spring-lit Nicholas breeder hens. It is likely the difference was due to a strain effect. The reduction in photoperiod to 13L:11D at 18 wk caused 10 hens to cease production. Three of these were rPR and resumed production after return to 18L:6D, whereas 7 were aPR and ended their reproductive season. Two of the hens that were rPR at 18 wk remained PS throughout the experiment, and 1 of these laid 192 eggs despite being out of production for 1 mo because of the photoperiod change. The third rPR hen later became aPR. Mean egg production of all hens that became aPR was 91 eggs, whereas that of the “good” layers was 210 eggs in 50 wk.

Plasma T3 and T4 levels (Figure 2) and the T3:T4 ratio (Figure 3), measured around the time of photostimulation, did not differ significantly between hens destined to become aPR and those that remained PS and continued to lay at a high rate for 50 wk. Plasma PRL levels, measured during peak egg production, were significantly higher at 8 and 9 wk after photostimulation in hens that remained PS than in those that subsequently became aPR (Table 1).

DISCUSSION

We have examined thyroid hormones relative to PR within days before, at, and immediately after photostimulation. We extended this comparison through several weeks postlighting in a previous report (Proudman and Siopes, 2002). In the present experiment, we were able to identify subpopulations of turkey hens that differed markedly in their seasonal egg production and onset of PR. One group exhibited a sharp decline in egg production after 18 wk of photostimulation and ceased laying despite a continued stimulatory photoperiod. A second group remained PS for a long reproductive season that extended for nearly a year (Figure 1). The former group had been programmed to become PR, although at a slower rate than would be typical of wild birds and

Figure 2. Plasma concentrations (mean ± SEM) of triiodothyronine (T3) and thyroxine (T4) measured around the time of photostimulation (d 0) of turkey hens that subsequently became absolutely photorefractory during a 50-wk production cycle (C; n = 17) or that remained photosensitive and were classified as “good” layers (▲; n = 10). A significant difference for T4 occurred by time but not between groups of hens. Repeated measures ANOVA Pr > F: group (G) = 0.92; time (T) = 0.002; G × T = 0.83. For plasma T3 Pr > F: G = 0.30; T = 0.13; and G × T = 0.68.

Figure 3. Mean (±SEM) ratio of plasma concentrations of triiodothyronine (T3) and thyroxine (T4) measured around the time of photostimulation (d 0) of turkey hens that subsequently became absolutely photorefractory during a 50-wk production cycle (C; n = 17) or that remained photosensitive and were classified as “good” layers (▲; n = 10). Pr > F: group (G) = 0.32; time (T) = 0.06; and G × T = 0.53.
slightly slower than expected for turkey breeder hens. The latter group was clearly not programmed to become PR within a typical reproductive year. Curiously, 2 hens ceased laying when tested with a reduced, but still stimulatory, photoperiod at 18 wk (a response that we interpret to indicate the presence of rPR) but remained PS for 50 wk. Relative PR is generally thought to be a lesser form of PR that leads to aPR (Follett and Nicholls, 1984; Nicholls et al., 1988; Bentley et al., 1997; Proudman and Siopes, 2002), yet these 2 individual hens show that this may not always be the case and suggest that the presence of rPR may not be an unequivocal indicator that programming for aPR has occurred. Alternatively, these 2 hens may have just had an unusually long delay to the expression of aPR that may be characteristic of this roaster strain turkey. Certainly, the mean onset of aPR was delayed in this strain of turkey, as noted earlier.

The prevailing model for programming of PR in sparrows, starlings, and other passerines holds that thyroid hormones are required for the photoperiodic system to function, and 1 of these hormones (probably T4) interacts with long days early during photostimulation to set in motion the mechanisms (i.e., programming) that subsequently constrain the breeding season (Nicholls et al., 1988; Wilson, 1997; Wilson and Reinert, 2000; Dawson et al., 2001; Wilson, 2001). Generally, this process (programming) has been reported to occur within a few weeks postlighting. Indeed, Dawson (2001) presented evidence to suggest that both photostimulation and PR in European starlings may even be initiated during the first long photoperiod. In the design of the present experiment, we focused sampling on the period immediately preceding and following photostimulation and retrospectively compared the circulating T3 and T4 levels of hens that later exhibited PR with those of hens that remained PS for an extended reproductive season. We reasoned that if circulating thyroid hormone levels around the time of photostimulation are actively related to programming for PR in the turkey, then we should observe a difference in thyroid hormone levels between these 2 groups. Our results clearly show that both T3 and T4 were present, and at similar levels, during the period of initial exposure to long days in hens that subsequently became aPR as well as in those that were not programmed for PR. Therefore, either a change in thyroid hormone is not required or the hormone signal for PR programming occurs later. Proudman and Siopes (2002) reported similar plasma T3 and lesser T4 levels 1 and 2 wk postlighting in turkey hens that became PR than in hens remaining PS. However, it is also possible that thyroid hormones have a permissive rather than active role in programming aPR (Bentley, 1997). That is, the presence of T4 allows or permits other neural processes to occur that result in PR. Bentley et al. (1997) reported that changes in plasma T4, such as those occurring when starlings are switched from short to long days, are not required for the induction of PR. Thyroid hormones only need be present for PR to be induced, and this was interpreted to suggest a permissive rather than active role in inducing PR. In short, our present results suggest that long-term photosensitivity for turkey hens is not associated with either lower or higher thyroid hormone levels at or within days of photostimulation. Apparently, thyroid hormones only need be present. Our previous study extends this time to 2 wk postlighting.

Our studies fail to support a role for PRL in initiating or hastening the onset of PR in the laying turkey hen. Peak flock egg production typically occurs 3 to 6 wk after photostimulation, and plasma PRL concentrations reach a peak at about the same time. If high PRL levels hasten the decline in egg production and the onset of PR, one might expect higher PRL levels following peak production in hens destined to become aPR vs. those destined to remain PS. Instead, our results revealed the opposite. Plasma PRL levels were higher at 8 and 9 wk following photostimulation in hens destined to remain PS and be highly productive. This result confirms our earlier study (Proudman and Siopes, 2002) demonstrating higher circulating PRL levels at 8 and 14 wk in hens that remained PS. Lien and Siopes (1989b) also reported lower PRL levels in PR than PS turkey hens from weekly observations over an entire lay cycle. Proudman (1998) has previously shown that high-producing hens exhibit moderate PRL levels that were either maintained or were declining after 6 wk of photostimulation. High PRL levels, which are clearly antagonadal (El Halawani and Rozenboim, 1993; Proudman, 1995), were associated with incubation behavior, whereas hens destined to become PR exhibited either moderate or low PRL levels during and after peak egg production (Proudman, 1998). This suggests dissociation between broodiness and PR.

Although our studies demonstrate that the domesticated turkey hen exhibits PRL profiles relative to PR that appear to be at variance to studies using other (wild) avian species, we cannot make direct comparisons. Most studies of PR in passerine birds have been conducted using males. In these species, both sexes exhibit PR,

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<th>Table 1. Mean (±SEM) plasma prolactin concentrations after 7, 8, or 9 wk of photostimulation of hens that subsequently became photorefractory during a 50-wk production cycle (n = 17) or that remained photosensitive and were classified as “good” layers (n = 10)</th>
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<td><strong>Weeks of photostimulation</strong></td>
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a,bDifferent superscripts indicate significant differences between groups within weeks (P ≤ 0.05).
whereas this may not be the case for the turkey (Proudman and Siopes, 2005). Further, the breeding season of passerines is relatively short, whereas that of the turkey has been extended by selective breeding. Therefore, programming for PR may be altered or delayed in commercial strains of turkeys. Further studies will focus on establishing when programming for PR occurs in the turkey hen.

REFERENCES


