Transient Hypothyroidism Reinitiates Egg Laying in Turkey Breeder Hens: Termination of Photorefractoriness by Propylthiouracil

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ABSTRACT
A study was conducted to determine the effects of transient hypothyroidism induced by propylthiouracil (PTU) on termination of photorefractoriness and reinitiation of lay in turkey breeder hens. The PTU was given for 6- or 8-wk periods via the feed and at various doses to yearling hens that had been continuously exposed to long photoperiods [16 h light (L):8 h dark (D)] for at least 25 wk.

There was a dose-dependent cessation of lay as well as deletion of thyroxine (T4) and triiodothyronine (T3) during the treatment period. Hens receiving 0.1% PTU or more had little or no circulating thyroid hormones after 2 wk of treatment. Furthermore, resumption of a normal rate and duration of egg laying occurred following withdrawal of the PTU, without any changes in photoperiod. However, this effect only occurred in those hens that had received PTU doses of 0.1% or more and only when the treatment had been given for greater than 6 wk. The resumption of normal levels of egg laying occurred in the absence of a typical preceding molt. Body weights, livability, and fertility and hatchability of eggs from these hens were similar to those of controls. Clearly, turkey hens can be effectively recycled by pharmacological manipulation of the thyroid gland and the results are supportive of thyroid hormone(s) involvement in maintaining photorefractoriness in turkey hens.

(Key words: thyroid, photorefractoriness, propylthiouracil, photoperiod, turkey)

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INTRODUCTION
Photorefractoriness in birds refers to a physiological condition in which there is spontaneous gonadal regression with extended exposure to long day lengths and the bird is nonresponsive to photoperiods that were previously stimulatory. In nature it is a primary mechanism by which seasonal reproduction is normally terminated at an appropriate time of year. Thus, photorefractoriness is a major mechanism restricting continuous egg and semen production. Seasonality and avian breeding cycles, including photorefractoriness, have recently been discussed by Sharp (1996).

It is generally accepted that photorefractoriness is normally terminated, and photosensitivity initiated, with exposure to natural or artificial short photoperiods (generally < 12 h/d). Turkey hens have a short-day requirement during the immediate prelay period due to the presence of photorefractoriness (Siopes, 1989). Therefore, hens must be exposed to short days to terminate photorefractoriness and make the hens photosensitive so that they can respond to long photoperiods by laying eggs.

There exists a good amount of literature supporting a function for thyroid hormones in initiating and maintaining photorefractoriness in birds (Nicholls et al., 1988; Dawson, 1989b; Wilson and Reinert, 1993, 1995). It appears that the presence of both long days and thyroid hormones are required for initiating and maintaining photorefractoriness. A direct approach for studying thyroid involvement with photorefractoriness has been thyroidectomy under appropriate physical and physiological conditions, and observation of subsequent reproductive performance and development of photorefractoriness.

The direct approach to studying photorefractoriness by thyroidectomy is not suited for turkey hens because they require the presence of the thyroid gland to initiate and maintain ovarian function; that is, thyroid hormones are essential for reproductive activity in turkey hens (Lien and Siopes, 1989a). In the absence of egg production, a direct and functionally complete evaluation of photorefractoriness cannot be made. Therefore, the results presented herein were obtained using the approach of transient, pharmacological manipulation of thyroid hormones.

If thyroid hormones are responsible for maintaining photorefractoriness in long photoperiods, then their removal should terminate photorefractoriness and allow turkey hens to regrow their ovaries and resume egg laying, even with continuous exposure to long pho-
toperiods. This effect is, in fact, supported by reports with avian species such as the starling and quail (Nicholls et al., 1988), and was possible because thyroid hormones are not essential for gonadal growth in these species and thus termination of photorefractoriness by thyroidec-tomy can be followed by state of gonadal development. To accomplish this response with a turkey, chemical thyroidec-tomy with an antithyroid substances has a distinct advantage over surgical thyroidec-tomy in that it is readily reversible with removal of the antithyroid substance. Chemical thyroidec-tomy allows thyroid hormone to be transiently removed but also to be reinstated as needed for critical developmental stages.

The hypothesis for the present study was that thyroid hormones are important in maintaining photorefractoriness in turkey hens. The objective was then to alter photorefractoriness in adult turkey breeder hens main-tained on long day lengths by depressing thyroid hormone levels. In the process of accomplishing this objective, yearling hens would be recycled into a second lay period without a preceding exposure to short day lengths by thyroid hormone manipulation alone. This objective has not been accomplished previously.

**MATERIALS AND METHODS**

**General**

Experiments were started in consecutive fall seasons with adult Large White turkey breeder hens that were at the end of their 1st yr of reproductive activity. In brief, the first two experiments were primarily to determine a dose-response for an antithyroid compound on plasma thyroid hormone levels and to estimate effective duration of treatment. The third experiment evaluated transient, pharmacologically induced hypothyroidism on terminating photorefractoriness and inducing a subsequent return to normal egg laying. All of the yearling hens used in these experiments had been maintained in floor pens within a light-controlled building and exposed to 16 h of incandescent light per day [16 h light (L):8 h dark (D)] at a mean intensity level of 40 lx for a minimum of 25 wk. End of lay hens such as these are generally regarded as photorefractory and Lien and Siopes (1989b) have reported that photorefractory hens can be identified by spontaneous cessation of lay after about 18 wk exposure to long day lengths.

For the present studies, all of these hens were continued in the same facility and light schedule except as noted below, by experiment. The building was not temperature controlled but was insulated and the rooms were mechanically ventilated. Feed and fresh water were provided for *ad libitum* intake throughout the study.

During the treatment period, a mash feed was given that was calculated to contain 12% protein, 0.85% calcium, and 3,084 kcal of metabolizable energy/kg of feed. After 6 or 8 wk of treatment (short photoperiods or antithyroid substance in the feed) all hens were given a pelleted layer ration calculated to contain 16% protein, 3.5% calcium, and 2,970 kcal of metabolizable energy/kg of feed. This ration was given to the end of each experiment.

Blood sampling by venipuncture was done during the treatment period to obtain plasma for assay of thyroid hormones and assessment of the antithyroid treatment. Plasma concentration of thyroxine (T4) and triiodothyro-nine (T3) were measured by radioimmunoassay as detailed in a concomitant paper by the author using kits<sup>2</sup> and validated for turkey plasma. In brief, the sensitivity of the assay was 0.6 and 0.5 ng/mL for T4 and T3, respectively. Accuracy was 106.3 and 105.9% for 2.0 and 8.0 ng/mL of T4 and 98.6 and 103.0% for 1.0 and 4.0 ng/mL of T3. The intra-assay coefficient of variation was 6.0% for T4 and 4.2% for T3, whereas the inter-assay coefficient was 7.8 and 5.3% for T4 and T3, respectively.

### Experiment 1

This experiment was done to evaluate dose-response of the antithyroid agent 6-n-propyl-2-thiouracil<sup>3</sup> (PTU) on plasma thyroid hormones. In addition, this experiment served as a preliminary study for induced transient hypothyroidism on terminating photorefractoriness in hens continuously exposed to long photoperiods. The PTU was given in the feed. There were six treatment groups: short-day control, no PTU (SD-Cont); long-day control (LD-Cont); long-day + 0.0001% PTU (0.0001 PTU); long-day + 0.01% PTU (0.01 PTU); long-day + 0.2% PTU (0.2 PTU); and long-day + 1% PTU (1.0 PTU). Short-days provided 8 h of incandescent light/d (8L:16D) whereas long-days provided 16 h of light/d (16L:8D). Exposure to appropriate short photoperiods is the typical means of terminating photorefractoriness and making turkey hens photosensitive so that they may resume egg laying when subsequently exposed to long photoperiods (Siopes, 1989). Continued exposure of hens to long photoperiods promotes and maintains photorefractoriness and thus inhibits egg production.

Each treatment group consisted of eight hens. After 8 wk of treatment, all hens were continued on a long daily photoperiod of 16L:8D and standard layer ration for 14 wk. Data were collected for plasma thyroid hormone concentration, BW, livability, and cumulative egg production per hen.

### Experiment 2

The purpose of the experiment was to estimate how long the PTU treatment needs to be applied to terminate photorefractoriness. There were three treatment groups with six hens in each: short-day controls (SD-Cont), which received short photoperiods for 8 wk and no PTU; long-
day controls (LD-Cont), which were continuously given long day lengths without PTU; and, long-days + 1% PTU (PTU), which received 1% PTU as per Experiment 1 but for 6 wk only. Short and long day lengths were as for Experiment 1 as was post-treatment conditions. Blood was obtained from all hens for evaluation of thyroid hormone concentration at the end of the treatment period to confirm that PTU had eliminated T4 and T3. Cumulative eggs per hen to 14 wk post-treatment was also obtained.

**Experiment 3**

This experiment was based on results of the first two experiments and was performed to further evaluate induced, transient hypothyroidism on terminating photorefractoriness and on the degree of effectiveness in reinstating photosensitivity and egg laying. That is, how effective is controlled hypothyroidism in recycling a turkey breeder hen? There were four treatment groups as follows: short-day control (Control); long-days + 1.0% PTU (1% PTU); long-days + 0.5% PTU (0.5% PTU); and long-days + 0.1% PTU (0.1% PTU). The treatments were applied for 8 wk and consisted of four replicate pens with five hens per pen. The short-days (SD) and long-days (LD) were as in Experiment 1. After 8 wk of treatment, all hens were maintained on long photoperiods (16L:8D) and a standard layer ration. Data were collected for BW, feed intake, livability, plasma concentrations of thyroid hormones, molt, days to resume lay, egg production, fertility, and hatchability.

For fertility evaluations, pooled semen was used to artificially inseminate all hens twice, 4 d apart. Evaluations were done at 10 wk post-treatment. Semen was diluted 1:1 with semen extender prior to insemination with 0.03 mL per hen. Eggs were collected daily for 7-d periods, biweekly, for 8 wk after the final insemination, stored in a cold room at 12.8°C and about 75% relative humidity, and set in an incubator biweekly. Eggs were candled for the presence of an embryo after 14 d of incubation and all candled infertile eggs were broken-out for visual verification of the absence of an embryo.

Statistical analyses were done by one-way analysis of variance using the General Linear Models procedure of the SAS Institute (1990). With the exception of BW and thyroid hormone concentrations, data analyses were by replicate pen. The least squares option was used to estimate significant differences among treatment means. Statement of statistical significance are based on $P \leq 0.05$ unless otherwise specified.

**RESULTS**

**Experiment 1**

At the end of the 8-wk treatment period, BW were similar among treatment groups and ranged from 11.0 to 11.8 kg. Only one hen died during the experiment and that was in the 0.01% PTU treatment group. Plasma concentrations of the thyroid hormones at the end of the 8-wk treatment period are presented in Figure 1. Both T4 and T3 concentrations were similar between the LD and SD-Cont groups. Plasma T4 and T3 were significantly reduced by 0.2% PTU treatment and the 1% PTU treatment resulted in nondetectable levels in the plasma.

Cumulative egg production for 14 wk post-treatment is presented in Figure 2. The SD-Cont group responded in a typical manner producing 50 eggs per hen, more than twice that in the LD-Cont group. The PTU treatments at 0.2 and 1.0% dose levels caused resumption of lay. Egg production was dose-dependent in the PTU treatment groups with no effect at 0.01 and 0.0001% PTU and the PTU 1% group produced the most eggs.

**Experiment 2**

At the end of the 6-wk treatment period 1% PTU had reduced plasma T3 to nondetectable levels and T4 was barely detectable (mean = 0.6 ng/mL; 3/6 = nondetectable). This result was consistent with the results of Experiment 1. Control groups had normally expected levels of T4 and T3, which were very similar to those presented for Experiment 1 in Figure 1. As presented in Figure 3, numbers of eggs produced per hen by the PTU treatment group remained low and were less than half of
FIGURE 2. Mean egg production during a 14-wk post-treatment period for hens previously treated for 8 wk with doses of propylthiouracil (PTU) in the feed ranging from 0.0001 to 1.0% (Experiment 1). These hens were exposed continuously to a [16 h light (L):8 h dark (D)] photoperiod. Controls consisted of hens exposed to either 8L:16D (SD) or 16L:8D (LD) photoperiods and fed no PTU. There were seven to eight hens in each treatment group.

FIGURE 3. Mean egg production during a 14-wk post-treatment period for hens previously treated for 6 wk with [16 h light (L):8 h dark (D)] plus 1% propylthiouracil (PTU) in the feed (Experiment 2). Both short-day (SD = 8L:16D) and long-day (LD = 16L:8D) control groups are included and each of the groups contained six hens.

FIGURE 4. Mean plasma thyroxine (T4) and triiodothyronine (T3) concentrations at the end of an 8-wk treatment period with propylthiouracil (PTU) in the feed at dosages ranging from 0 (control) to 1% (Experiment 3). ND = nondetectable: that is, T4 and T3 levels less than 0.6 and 0.5 ng/mL, respectively.

that of the SD-Cont group by 14 wk post-treatment. Clearly, 6 wk of PTU treatment is insufficient to induce resumption of lay (recycle) in turkey hens.

**Experiment 3**

Livability was excellent during the experiment, with only one hen dying in the treatment groups. As presented in Table 1, BW from the 1% PTU treatment group was less than those of the control group at the end of the treatment period, but by 8 wk post-treatment (peak egg laying) all groups had similar BW. Feed intake during the treatment period was reduced from that of controls in all of the PTU treatment groups (Table 1).

At the start of treatments, mean plasma T4 and T3 (n = 8 each) were 5.7 and 2.0 ng/mL, respectively. By 2 wk of treatment T4 of all PTU treatment groups were barely at, or were below detectable levels and remained nondetectable to the end of the treatment period. Plasma T3 followed a very similar pattern except that levels remained barely detectable (0.5 to 1.0 ng/mL). As a consequence, plasma T4 and T3 levels are presented in Figure 4 only for the end of treatments. Clearly, all PTU treatments eliminated plasma T4 whereas plasma T3 concentration was significantly reduced in a dose-dependent manner. Control T4 and T3 concentrations were similar to those measured at the start of treatments.

Upon removal of PTU treatments, resumption of egg laying occurred in all hens of all treatment groups but was significantly delayed in the 0.5 and 1.0% PTU treatment groups (Figure 5). Mean days to first egg was 22.8, 25.0, 28.8, and 31.0 for controls, 0.1, 0.5, and 1.0% PTU, respectively. Figure 5 also shows the rate of egg production by hens of each treatment group. Eggs per hen for a 24-wk post-treatment period ranged from 70 to 92 for the PTU treatments and was 84 eggs per hen for the controls. Both the mean fertility and hatch of fertile eggs were similar among treatment groups and this is presented in Table 2. Overall mean percentage fertility ranged from 53 to 66% among treatments and hatchability ranged from 41 to 53%.

**DISCUSSION**

From results of the present study, it can be seen that sufficient dose and duration of treatment with the
TABLE 1. Mean body weights and feed intake of birds during and after treatment with propylthiouracil (PTU) in the feed at doses between 0.1 and 1.0%, Experiment 3. 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weights</th>
<th>Feed intake</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of</td>
<td>8 wk post-</td>
<td>g/kg</td>
</tr>
<tr>
<td></td>
<td>treatments</td>
<td>treatment</td>
<td>BW/d</td>
</tr>
<tr>
<td></td>
<td>(kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD-control, (no PTU)</td>
<td>11.1 a,c</td>
<td>11.6</td>
<td>24.3 a</td>
</tr>
<tr>
<td>LD + 1.0% PTU</td>
<td>9.7 b,d</td>
<td>11.9</td>
<td>21.2 b</td>
</tr>
<tr>
<td>LD + 0.5% PTU</td>
<td>10.4 a,d</td>
<td>11.3</td>
<td>21.3 b</td>
</tr>
<tr>
<td>LD + 0.1% PTU</td>
<td>11.5 c</td>
<td>11.6</td>
<td>21.3 b</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.52</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Probability</td>
<td>0.002</td>
<td>NS</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1There were 20 hens per treatment group and the root mean square error (RMSE) and probability were from ANOVA.
2SD and LD = short days [8 h light (L):16 D dark (D)] and long days (16L:8D), respectively.
3Mean for the 8-wk treatment period.

antithyroid compound PTU depletes plasma T4 and T3 concentrations and causes cessation of lay in yearling turkey hens. Furthermore, resumption of a normal rate and duration of egg laying occurred following withdrawal of the PTU but only at PTU doses of 0.1% or more and only when the treatment was given for greater than 6 wk. This study is the first demonstration of the recycling of turkey hens into an additional normal egg laying period without manipulation of photoperiod, that is, without exposure to short photoperiods during the prelay period. This response was almost certainly not due to changes in BW or a reproductive rest per se. Although this response can occur in chickens, as exemplified by conventional methods of induced molting, induced reproductive rest by hormones or feed restriction without associated use of short day lengths does not result in improved subsequent egg production in turkey hens (Lien and Siopes, 1993a; Siopes, unpublished results).

Because the hens had been maintained continuously on long photoperiods to promote maintenance of photorefractoriness, a reasonable explanation for the recycle response is that photorefractoriness was terminated by the PTU-induced, transient hypothyroidism. This response allowed resumption of photosensitivity and the hens then responded to the existing long photoperiods by resuming egg laying. This response was similar to that reported in starlings (Dawson et al., 1985). Photorefractoriness in starlings maintained on long photoperiods was terminated by thyroidectomy, which caused the photosensitive birds to undergo gonadal development.

FIGURE 5. The effect of an 8-wk treatment period with 0.1 to 1% propylthiouracil (PTU) in the feed on reinitiating (recycling) egg laying (percentage hen-day egg production) by turkey hens maintained continuously on long daily photoperiods of 16 h light (L):8 h dark (D) (Experiment 3). Control hens (C) were given short photoperiods (8L:16D) and no PTU during the treatment period and 16L:8D thereafter.
TABLE 2. Mean percentage fertility and hatchability for turkey eggs from propylthiouracil (PTU)-treated hens artificially inseminated twice during the 10th wk of photostimulation, Experiment 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks postinsemination (%) Fertile</th>
<th>Weeks postinsemination (Hatch of fertile eggs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>SD Control</td>
<td>85.3</td>
<td>77.0</td>
</tr>
<tr>
<td>1.0% PTU</td>
<td>85.7</td>
<td>87.5</td>
</tr>
<tr>
<td>0.5% PTU</td>
<td>88.3</td>
<td>66.0</td>
</tr>
<tr>
<td>0.1% PTU</td>
<td>87.8</td>
<td>82.0</td>
</tr>
<tr>
<td>RMSE</td>
<td>13.1</td>
<td>10.3</td>
</tr>
<tr>
<td>Probability</td>
<td>0.98</td>
<td>0.07</td>
</tr>
</tbody>
</table>

1The number of eggs at each set ranged from 325 to 405.
2RMSE = root mean square error.

The present experimental design strongly implicates thyroid hormones in the maintenance of photorefractoriness but does not address thyroid hormone function under normal conditions. For example, there is never an absence of T4 or T3 in normal birds. How normal levels of thyroid hormones participate in regulating photorefractoriness remains to be determined; however, because the time component and effects for hypothyroid-induced, as well as short-day-induced, termination of photorefractoriness are so similar, they may utilize the same processes. Of particular interest in this regard has been the implication of effects on the photoperiodic time-measuring system that allows a bird to distinguish between short and long photoperiods (Follett et al., 1988; Dawson, 1989a,b).

Although previous research addressing thyroid hormone relationship to photorefractoriness has been done in turkeys (Lien and Siopes, 1989b, 1993a,b) a cause and effect relationship remained equivocal. However, as reviewed by Nicholls et al. (1988), involvement of thyroid hormone(s) in the initiation and maintenance of avian photorefractoriness has been adequately documented (Dawson, 1989b; Wilson and Reinert, 1993, 1995).

Because either the absence of long photoperiods (presence of short photoperiods) or the absence of thyroid hormones (by PTU treatment) resulted in termination of photorefractoriness in turkey hens it appears that both long days and thyroid hormones are required to sustain photorefractoriness. Nicholls et al. (1988) clearly indicated that both long photoperiods and thyroid hormones are required for photorefractoriness in starlings and that the dependence on thyroid hormones for photoperiodic processes varies among species. That the absence of T4 and T3 in the presence of long photoperiods was associated with the subsequent resumption of as typical a reproductive performance as that induced by light manipulation suggests thyroid hormones could be as important as light in sustaining photorefractoriness in turkeys.

That effective treatment with PTU required an exposure time in excess of 6 wk demonstrates an important time component in the process. It is quite likely more than coincidental that this time requirement is similar to that for short photoperiods to terminate photorefractoriness (Siopes, 1989), which suggests a common mechanism. Nicholls et al. (1988) have also noted a similar relationship for the time taken after thyroidectomy for starlings to recover photosensitivity. In American tree sparrows, photosensitivity recurred between 9 and 12 wk after thyroidectomy (Wilson and Reinert, 1995). It is notable that extending the PTU treatment of turkeys from 8 to 10 wk duration did not change the response (results not presented). Likewise, both 8 and 10 wk of exposure to short photoperiods has similar effects on terminating photorefractoriness and allowing initiation of subsequent lay in turkey hens (Siopes, 1989).

Only the short-day control hens underwent a typical molt during the treatment period. The PTU-treated hens had a distinctly different appearance from controls at the end of the treatment period. The controls were all fully feathered with new, smooth, white feathers, whereas all PTU hens had old and rough-appearing feather covering; many hens exhibiting bare spots on the neck, wings, and abdomen. A useful indicator for completeness of molting in turkeys is the loss of the number 10 primary remiges. The percentage of hens with number 10 primaries at the end of the treatment period was 2.9% for controls and 25 to 35% for the PTU-treated hens. Clearly, a typical molt did not occur in the PTU treatment groups. This result is consistent with the
reports by Lien and Siopes (1989a) for molting in thyroidectomized turkey hens and Wilson and Reinert (1993) for tree sparrows thyroidectomized prior to photostimulation. This lack of molt is interesting because these groups of hens had normal subsequent egg production and illustrates that the degree of molting is not tightly coupled to photorefractoriness or to subsequent egg production. For good egg production to occur, there must be complete termination of photorefractoriness and this is a neural process. Dawson (1991) has also documented that molting can be independent from reproduction and that starlings do not have to be photorefractory to molt.

From the results it is clear that the objectives were achieved. For the first time, termination of photorefractoriness and recycling of turkey hens into an additional normal egg laying period occurred without exposure to short photoperiods, by treatment with an anti-thyroid compound. This response is consistent with thyroid hormone(s) involvement in maintaining photorefractoriness in turkey hens but how this occurs under normal conditions remains to be determined.

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REFERENCES


