Supplementary Dim Light Differentially Influences Sexual Maturity, Oviposition Time, and Melatonin Rhythms in Pullets

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ABSTRACT The addition of two 3-h periods of very dim light, one before and one after a normal 8-h photoperiod, advances sexual maturity in pullets by about a week. This trial tested the hypothesis that dim light given before a short day of normal intensity is linked to form a more stimulatory day length and that dim light given after it is photosexually ignored. Pullets were reared from 2 d of age on 8-h photoperiods. From 10 wk, they were continued on 8-h photoperiods, transferred to 16 h, or given an 8-h period of dim light (0.09 lx) immediately before or after the main 8-h photoperiod. The bright/dim and dim/bright groups matured at the same age, thus disproving the hypothesis tested. Both groups matured 1 wk earlier than the 8-h controls but 5 wk later than birds transferred to 16-h photoperiod. Oviposition time was similar for 8-h controls and bright/dim hens and delayed by 3 h for 16-h birds, but phase advanced by 2.4 h for dim/bright hens. Plasma melatonin rhythm was phase-advanced by about 5 h in the dim/bright hens and retarded by about 5 h in the bright/dim hens, suggesting a 13-h subjective day. However, these treatments were not regarded as fully stimulatory, as a transfer to a normal 13-h photoperiod at this age advances maturity by 5 to 6 wk. These findings show that the addition of a period of dim light to a normal nonstimulatory photoperiod differentially affects the clocks that control sexual maturation, plasma melatonin concentration, and oviposition time.

(Key words: sexual maturity, oviposition time, melatonin, dim light, phase setting)

INTRODUCTION

When domestic fowl are exposed to a photoperiod composed partly of bright light and partly of dim light, the dim phase may be linked to the bright phase and perceived by the bird as forming an extended day (Bhatti et al., 1988), or it may be treated as part of the night (Morris and Owen, 1966). How the dim phase is interpreted depends for some physiological functions on the absolute intensity of light (Morris and Owen, 1966) and for other responses on the ratio of the intensities in the dim and bright light (Morris, 1973). Observations of locomotor activity and cloacal protrusion area growth in Japanese quail indicate that their interpretation of dim light depends on whether it is alternated with a bright photoperiod or with darkness (Meyer et al., 1988). When linked to a bright photoperiod, the dim light was treated as night, but when linked to darkness it was regarded as day. The interpretation of dim light within a schedule containing bright light, dim light, and darkness may also depend on the position of the dim light relative to the bright light. When very dim light (0.14 lx) was given from 10 wk of age for 3 h, before and after a bright (8.7 lux) 8-hour photoperiod, egg laying in domestic fowl began 10 d earlier than in short-day (8 h) controls but 20 d later than in birds exposed to 14 h of bright light (Lewis et al., 1999). The 10-d advance in mean age at first egg (AFE) was similar (7.8 d) to that reported for birds transferred from 8- to 10-h bright photoperiods at 9 wk of age by Lewis et al. (1997). These data suggest that one of the periods of dim light was being registered and the other ignored, with the birds interpreting the system as an 11-h day, or that the influence of one or both of the two dim phases caused a shift in the biological clock such that part of the photoinducible phase was illuminated by the 8 h of bright light.

This paper reports the findings of a trial that tested these hypotheses with an 8-h photoperiod of very dim light (0.09 ± 0.005 lx; an illuminance well below the 0.9 to 1.7 lx threshold intensity needed to activate the photoperiodic mechanism in pullets; Lewis et al., 1999) before or after an 8-h main photoperiod of bright illuminance (7.0 ± 0.46 lx). Oviposition times and plasma melatonin...
concentrations were also studied to assess the bird’s response to the location of the dim phase of light within a mixed-intensity lighting regime. The use of bright as a description of a photoperiod in this trial is solely to differentiate it from the dim phase and is not an absolute description of the illuminance.

MATERIALS AND METHODS

One hundred forty ISA-Brown (brown-egg hybrids) were reared on a litter floor from 0 to 5 wk in one of four ostensibly identical controlled environment rooms and given an 8 h light (L):16 h darkness (D) lighting regime. At 5 wk, the birds were randomly distributed among all four rooms (35 per room). At 10 wk, 30 birds within each room were transferred to individual birdcages arranged on three tiers, and the lighting regime within a room was changed to one of the treatments described in Table 1. The temperature was maintained at a constant 20 ± 1°C from 5 wk in all rooms. All treatments were applied at 10 wk because the maximum advance in sexual maturity is obtained when pullets are photostimulated at about this age (Lewis et al., 1997).

Illumination was from white incandescent lamps, operated at full voltage, and shielded to produce the required illuminance without altering the spectral characteristics of the light. Typically, the mean illuminance on the three tiers was 4.4 ± 0.17 lx (bottom tier), 7.0 ± 0.38 lx (middle tier), and 9.6 ± 0.46 lx (top tier) during the bright photoperiods and 0.06 ± 0.004 lx (bottom tier), 0.09 ± 0.005 lx (middle tier), and 0.12 ± 0.006 lx (top tier) during the very dim photoperiods. Mean intensities within each room during the various photoperiods are given in Table 1 and show that all birds received light above the 1.7-lx maximum threshold for full sexual stimulation during the bright photoperiod and well below the 0.9-lx minimum threshold for sexual stimulation during the very dim photoperiods.

Feeding, egg collection, and routine servicing were only carried out during the bright photoperiod. Individual oviposition times were recorded by continuous observation over 48 h during the twenty-fifth week (when the birds in all rooms were sexually mature), using a small pencil flashlight to see eggs during the hours of darkness. Profiles of plasma melatonin concentration are useful indicators of how a bird interprets day and night. Accordingly, blood samples were taken from a brachial vein at 0700, 0900, 1200, 1500, 1700, 2000, 2300, and 0100 h at 24 wk of age for determination of plasma melatonin concentration by radioimmunoassay, using a standard procedure for human plasma that has been adapted and validated for use with chicken plasma (Fraser et al., 1983).

Data on AFE and oviposition time were grouped according to room and subjected to ANOVA, and means were compared using a pooled SEM and Student’s t-test. Plasma melatonin concentration data were grouped to room or sampling time, and each grouping was separately subjected to an ANOVA. Means were compared among lighting treatments at the same sampling time or among sampling times with the same treatment by using a pooled SEM and a Tukey test. Whereas it is acknowledged that the use of an ANOVA on all four sets of room data is not strictly valid, because responses to lighting treatment were confounded with room effects, the differences among the treatments were of such a magnitude that it is considered useful to include the analysis in the assessment of responses (Tables 2 and 3).

RESULTS

Mean AFE were similar for the two groups given the extra 8-h dim light period from 10 wk, whether administered before or after the main 8-h photoperiod. However, both groups matured about 1 wk earlier than the constant 8-h controls but almost 5 wk later than the birds transferred from 8 to 16 h of bright light at 10 wk (Table 2).

In the constant 8-h controls, mean oviposition time was 3 h earlier, individual oviposition times were more widely spread, and significantly more eggs were laid before dawn than in birds transferred from 8- to 16-h bright photoperiods at 10 wk (Table 2, Figure 1, a and d). When the 8-h dim period occurred after the bright light phase, the mean time of lay, the proportion of eggs laid before the bright photoperiod, and the distribution of individual ovipositions were not significantly different from those for birds maintained on 8-h bright photoperiods (Table 2, Figure 1, a and b). However, when 8 h of dim light was provided before the bright 8-h photoperiod, the mean time of lay was, significantly, 5.6 h later than 8-h controls and 2.6 h later than birds receiving 16 h of normal light (Figure 1c). This group also laid a significantly higher proportion of their eggs before the start of the bright photoperiod than the other three treatments (Table 2, Figure 1c), although the distribution of individual ovipositions about the mean was similar to the constant 8-h and bright/dim photoperiod groups (Figure 1a, b, and c).

<table>
<thead>
<tr>
<th>Treatment (h)</th>
<th>0800–1600 h</th>
<th>1600–2400 h</th>
<th>0000–0800 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>8L:16D</td>
<td>7.0 ± 0.46 lx</td>
<td>0.09 ± 0.005 lx</td>
<td>0.38 ± 0.01 lx</td>
</tr>
<tr>
<td>8L:8dim:8D</td>
<td>6.9 ± 0.45 lx</td>
<td>6.4 ± 0.42 lx</td>
<td>0.09 ± 0.005 lx</td>
</tr>
<tr>
<td>8dim:8L:8D</td>
<td>0.09 ± 0.005 lx</td>
<td>7.9 ± 0.51 lx</td>
<td>0.12 ± 0.006 lx</td>
</tr>
<tr>
<td>16L:8D</td>
<td>7.9 ± 0.51 lx</td>
<td>0.12 ± 0.006 lx</td>
<td>0.09 ± 0.005 lx</td>
</tr>
</tbody>
</table>

1L = photoperiod; D = darkness.
Plasma melatonin concentration data are given in Table 3. In the samples taken at 0700 h (1 h before the lights came on), concentrations were similar for the 8- and 16-h groups and for the birds given the dim light after the main photoperiod; however, all three groups had significantly lower concentrations than the dim/bright birds. In samples taken at 0900 h (1 h after the lights came on), there were no significant differences in melatonin concentration among any groups, although the dim/bright group had the highest level. At 1200 h (4 h after lights-on), the dim/bright group had significantly higher plasma melatonin than any of the other three groups, which were not significantly different from each other. At 1500 h (7 h after lights-on) and at 1700 h (1 h after lights out in the 8-h controls and 1 h after transfer from dim to bright or from bright to dim), melatonin concentrations were similar in all groups. At 2000 h, when the 16-h group and dim/bright birds were brightly illuminated, but the bright/dim group was half way through its dim period, concentrations for the three groups were not significantly different. At 2300 h, concentrations for the bright/dim group were significantly higher than the dim/bright group. Birds were similar, but both were significantly higher than the dim/bright group.

**DISCUSSION**

The 1-wk advance in mean AFE for the mixed-intensity groups compared with the 8-h controls in this trial discredits the hypothesis that birds respond to a period of very dim light (below the threshold for complete photoinduction; Lewis et al., 1999) that precedes a bright photoperiod but ignores one that follows it. However, the 5-wk-later maturities recorded for the dim/bright and bright/dim treatments, compared with birds transferred to 16-h photoperiods, agrees favorably with the 3-wk-later maturity reported by Lewis et al. (1999) for a photoperiod of 3 h dim light:8 h bright light:3 h dim light relative to 14-h controls.

Both sets of data show that, although not ignoring it, pullets do not link a very dim light period to the main photoperiod to form a subjective long-day that is equal to the sum of the two phases. Notwithstanding that the mean AFE for the two mixed groups were remarkably similar, it is possible that they were the result of two different processes coincidentally producing the same magnitude of advance in maturity. In the case of the

### TABLE 2. Sexual maturity, oviposition time, and the proportion of eggs laid before the bright photoperiod in hens given an 8L:16D, 16L:8D, 8L:8dim:8D, or 8dim:8L:8D regime

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Age at first egg (days)</th>
<th>Mean oviposition time (h)</th>
<th>Proportion of eggs laid before bright photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td>8L:16D</td>
<td>141.4 ± 2.40</td>
<td>07.18 ± 0.41</td>
<td>0.74b</td>
</tr>
<tr>
<td>8L:8dim:8D</td>
<td>135.5 ± 2.56</td>
<td>07.00 ± 0.37</td>
<td>0.71b</td>
</tr>
<tr>
<td>8dim:8L:8D</td>
<td>134.2 ± 1.91</td>
<td>12.83 ± 0.39</td>
<td>0.89b</td>
</tr>
<tr>
<td>16L:8D</td>
<td>101.8 ± 1.53</td>
<td>10.16 ± 0.21</td>
<td>0.10b</td>
</tr>
</tbody>
</table>

a,b,cColumn means with different superscripts are significantly different at P < 0.05.  
L = light at 7.0 ± 0.46 lx, dim = light at 0.09 ± 0.005 lx, D = darkness.

### TABLE 3. Effect of 8L:16D, 8L:8dim:8D, 8dim:8L:8D and 16L:8D lighting regimes and sampling time on plasma melatonin concentration (pg/mL) in brown-egg hybrid pullets

<table>
<thead>
<tr>
<th>Time</th>
<th>8L:16D Pooled SED (Residual df)</th>
<th>8L:8dim:8D Pooled SED (Residual df)</th>
<th>8dim:8L:8D Pooled SED (Residual df)</th>
<th>16L:8D Pooled SED (Residual df)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0700</td>
<td>24.2± ± 4.7</td>
<td>24.8± ± 9.8</td>
<td>27.3± ± 5.1</td>
<td>25.0± ± 7.3</td>
<td>0.0016</td>
</tr>
<tr>
<td>0900</td>
<td>18.7± ± 5.2</td>
<td>9.8± ± 4.7</td>
<td>26.6± ± 7.6</td>
<td>15.3± ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>1200</td>
<td>6.3± ± 2.4</td>
<td>8.7± ± 3.9</td>
<td>21.9± ± 5.6</td>
<td>2.5± ± 0.0</td>
<td>0.0056</td>
</tr>
<tr>
<td>1500</td>
<td>2.5± ± 0.0</td>
<td>2.5± ± 0.0</td>
<td>2.5± ± 0.0</td>
<td>5.6± ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>1700</td>
<td>4.6± ± 2.1</td>
<td>4.3± ± 1.8</td>
<td>3.4± ± 0.9</td>
<td>2.5± ± 0.0</td>
<td>NS</td>
</tr>
<tr>
<td>2000</td>
<td>4.1± ± 1.6</td>
<td>2.5± ± 0.0</td>
<td>2.5± ± 0.0</td>
<td>2.5± ± 0.0</td>
<td>NS</td>
</tr>
<tr>
<td>2300</td>
<td>30.8± ± 8.1</td>
<td>25.0± ± 0.0</td>
<td>25.0± ± 0.0</td>
<td>4.3± ± 5.5</td>
<td>0.0008</td>
</tr>
<tr>
<td>0100</td>
<td>55.8± ± 9.6</td>
<td>6.9± ± 4.4</td>
<td>4.3± ± 5.5</td>
<td>4.3± ± 5.5</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Within a row, means with different superscripts (a,b,c) are significantly different at P < 0.05. Within a column, means with different superscripts (x,y,z) are significantly different at P < 0.05.

L = light at 7.0 ± 0.46 lx, dim = light at 0.09 ± 0.005 lx, D = darkness.
birds given dim light after the main photoperiod, sexual maturity might have been advanced by the dim light fully illuminating a nonphase-shifted photoinducible phase, thus mimicking a mildly stimulatory bright photoperiod (Figure 2b). In contrast, sexual maturity of the birds given the dim light before the main photoperiod, the photoinducible phase, which would normally occur early in the scotoperiod of an 8L:16D schedule, might have been phase-advanced so that it was partially, although not fully, illuminated by the main photoperiod and similarly mimicked a mildly stimulatory photoperiod (Figure 2c).

Evidence from trials involving quail exposed to different light/dark cycles shows that changes in lighting conditions can advance the photoinducible phase so that a normally nonstimulatory photoperiod becomes photoinductive (Juss et al., 1995; Follett et al., 1998). The response to dim light given after a bright photoperiod, but falling within the photoinducible phase, is likely to be proportional to illuminance, at least up to the 1.7 lx threshold necessary for full stimulation of domestic pullets (Lewis et al., 1999). Such graded responses of sexual development have been reported by Bentley et al. (1998) for male European starlings (*Sturnus vulgaris*) exposed to various intensities of light for the complete photoperiod and for Japanese quail transferred from a nonstimulatory bright:dim regimen to continuous illumination at 1, 5, or 10 lx (Meyer et al., 1988). Bentley et al. (1998) concluded that decreased light intensity for a complete 18-h photoperiod could alter a bird’s perception of day length (slower testicular growth and no demonstration of photorefractoriness), even though the 3-lx intensity was above that which would be regarded as fully stimulatory for domestic pullets. Follett and Millette (1982) also concluded that variations in the light intensity of a 1-h night-interruption pulse given to quail were able to induce different rates of sexual maturation.

If the hypotheses to explain the similar AFE in this trial for both mixed intensity groups are correct, it must be concluded that the photoinducible phase is only phase-shifted by dim light given before, and not after, a main photoperiod. However, an alternative explanation might be that dim light that precedes a main photoperiod pulls the photoinducible phase forward, but that dim light following it pushes the photoinducible phase forward, and that both occurrences are able to phase-shift the photoinducible phase and produce similar degrees of photostimulation.

Although light is the strongest cue for determining oviposition time in laying hens, other zeitgebers, such as temperature, noise cycles, and feeding times, have been shown to exert a modifying influence. However, where the ovulatory cycle has been affected by these cues, the laying hens were exposed to continuous illumination (Wilson and Abplanalp, 1956; Cain and Wilson, 1974; Bhatti and Morris, 1977) or to continuous darkness (Abplanalp, 1966). When a group of hens were given a temperature cycle of 12 h at 30°C and 12 h at 20°C, but 6 h out of phase with a 12L:12D lighting schedule, mean oviposition time was modified by about 45 min only (Bhatti and Morris, 1977).

The daily temperature fluctuation in this trial was only 2°C and was temporally similar in all rooms, as the birds were not serviced during the dim phase. There was no delay in oviposition time for the bright/dim treatment in which birds would also have heard noise from adjoining rooms during their dim phase. Thus, it is unlikely that the 5.6-h-later oviposition time for the dim/bright treatment (Table 2) could be attributed to anything other than the influence of the dim light. Had the dim/bright hens ignored the dim phase (for setting the ovulatory cycle), as apparently the bright/dim hens did, mean oviposition time would have been delayed by 8 h. Accordingly, the
5.6-h-later oviposition time for the dim/bright birds indicates a 2.4-h advance in the phase setting of the clock that controls the pre-ovulatory release of luteinizing hormone (LH).

The 3-h delay in oviposition time and tighter distribution of individual laying times for the 16-h birds in this trial, compared with the 8-h controls, was similar to that reported by Bhatti et al. (1988) for the same treatments. However, the data for bright/dim hens contrast with their findings that the mean time of lay for birds given 8 h of bright light (20 lx) followed by 8 h of dim light (1.25 lx) is similar to birds given a conventional 16-h photoperiod. As the threshold intensity for stimulation of the photoperiodic mechanism, at least for that involved in sexual maturation, appears to lie somewhere between 0.9 and 1.7 lx (Lewis et al., 1999), it is not surprising that 8 h at 1.25 lx in this earlier trial was linked to the brighter phase to create a 16-h day length. Additionally, the 0.09-lx mean illuminance used in this trial was less than the 0.3 lx reported by Bhatti et al. (1988) to have been treated as darkness when given to domestic fowl alone or in association with bright light.

Although dusk is a stronger signal than dawn, both interfaces normally influence the phase setting of pre-ovulatory LH release (Bhatti and Morris, 1978). It is possible, therefore, that the transition from bright to dim light, for the bright/dim birds, was strong enough to act as a dusk signal and for the birds to respond as if given an 8-h d and 16-h night and lay at a similar time to the 8-h controls (Table 2). In contrast, the transition from dark to dim for the dim/bright birds, although strong enough to advance the biological clock for phase setting pre-ovulatory LH release by 2.4 h, was not potent enough to be regarded as a normal dawn.

Estimates of plasma melatonin concentrations (≈20 pg/mL) for the 8L, 16L, and bright/dim birds at 0800 h (dawn), interpolated from the 0700 and 0900 h data, suggest that dawn for the dim/bright birds was likely to have occurred at about 1100 h. This finding represents a 5-h advance in the circadian melatonin rhythm and indicates a 13-h subjective day (1100 to 2400 h). A similar exercise, using the bright/dark interface of the 8L, 16L and dim/bright groups (≈10 pg/mL) to estimate the dusk of the bright/dim birds, suggests that dusk occurred at about 2100 h, thus producing another 13-h subjective day (0800 to 2100 h).

Whereas the similarity of the 2 subjective d, as indicated by the melatonin rhythms, might appear to offer another explanation for the similarity of the sexual maturities for the two mixed-intensity groups, earlier reports of sexual maturities for domestic pullets that had been transferred from 8- to 13-h photoperiods at around 10 wk of age do not support this hypothesis. Whereas AFE for the mixed-intensity groups in this trial was advanced by 7 d, Lewis et al. (1997) showed that a transfer from 8- to 13-h photoperiods advances mean AFE by 26 d, compared with constant 8-h controls. These findings suggest that the clock-slave that controls melatonin secretion is different from that which controls sexual maturation and support the conclusion of Juss et al. (1995) that the rhythm of the photoinducible phase is not driven by the rhythm of melatonin secretion. A similar uncoupling of the melatonin clock and that controlling the occurrence of the photoinducible phase has been demonstrated in quail (Follett
and Pearce-Kelly, 1991; Juss et al. 1993, 1995; Kumar et al., 1993), in which 27-h ahemeral days resulted in a phase advance in the melatonin rhythm but not in the photoinducible phase.

The sexual maturity, oviposition time, and plasma melatonin concentration data from this trial show that occurrence of very dim light relative to a normal photoperiod does not equally influence all physiological mechanisms. Whereas the location of a dim phase seems to be important for the clock setting of pre-ovulatory LH release, as illustrated by the differences in mean oviposition time, it is minimally important for the control of sexual maturation (by setting the photoinducible phase) and for the setting of the clock for melatonin secretion. These data also suggest that, whereas dusk is more important than dawn for setting the ovulatory cycle (Bhatti and Morris, 1978), dawn and dusk have equal potency when providing the bird with information on day length changes for controlling sexual development. It is, therefore, important that poultry houses are truly light tight if complete control of sexual maturity is required. However, provided light leakage is less than 0.9 lx, sexual maturity is unlikely to be advanced by more than a week. The dissimilar effects of the dim/bright and bright/dim schedules for time of lay, despite the apparently similar amplitude of phase shift of dawn for the dim/bright and of dusk for the bright/dim birds (as indicated by plasma melatonin concentrations), suggest that melatonin is also not linked to the phase setting of pre-ovulatory LH release nor to the control of gonadal development. Ahemeral days have been used to uncouple the circadian oscillators that control melatonin secretion, locomotor activity, photoinductibility, and ovipositional circadian rhythms in quail (Simpson and Follett, 1982; Follett and Pearce-Kelly, 1991; Juss et al., 1993, 1995; Kumar et al., 1993; Follett et al., 1998), and light intensity has been shown to modify sexual maturation in quail (Follett and Milette, 1982; Meyer et al., 1988) and starlings (Bentley et al., 1998), but this seems to be the first evidence that these circadian oscillators can be uncoupled using a mixture of bright light, dim light, and darkness within a 24-h cycle.

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