ABSTRACT Photorefractoriness (PR) in the turkey breeder hen is characterized by a lack of responsiveness to photoperiods that previously induced or maintained egg production. The consequence of PR is spontaneous regression of ovarian function and cessation of lay. Photosensitivity (PS) may be regained by giving at least 8 wk of short photoperiod (8L:16D) (light restriction). Following the transition from PR to PS, the birds may be photostimulated with long photoperiods, which allows for the recrudescence of ovarian function and normal egg production. Although the return of reproductive viability is the parameter for determining the successful recycle of ovarian function, there are no known reports of the physiological costs of this transition on immune function in the turkey breeder hen. We conducted an experiment to determine the immune responsiveness at various stages of recycle in the turkey breeder hen. Fifty photorefractory birds were selected and distributed equally among five treatment groups (time points). All birds were given an 8-wk period of light restriction (8L:16D) followed by a 12-wk period of photostimulation (16L:8D). The cellular (cutaneous basophil hypersensitivity CBH) and humoral (antibody titer) immune responses were determined in each treatment group (sequential time points): prelight restriction, 2-wk light restriction, 7-wk light restriction, 2-wk photostimulation, and 12-wk photostimulation. After 2-wk light restriction, there was a reduction in the cellular (64.1%) and humoral (59.5%) immune responses from that of the PR hens at the start. After 7-wk light restriction, the humoral responses increased (33.5%) as compared to the 2-wk light restriction time point. Upon photostimulation, both the cellular (23.3%) and humoral (52.4%) immune responses were reduced at 2 wk of photostimulation as compared to the prior 7-wk light restriction time point. Finally, there was a rise in cellular (45.7%) and humoral (72.3%) immune responses after 12 wk of photostimulation as compared to the prior 2-wk photostimulation time point. We concluded that recycling of PR turkey hens was associated with altered cellular and humoral immune responses characterized by initial decline then recovery in both the light restriction and the postphotostimulation periods.

(Key words: turkey, immune, recycle, photoperiod, photorefractory)

INTRODUCTION

As with most temporal zone birds, reproduction in the domestic turkey breeder hen is controlled by daylength. Exposure to a sufficiently long daylength allows for reproductive responsiveness to ambient light, therefore, producing hens with fully mature ovaries capable of maximal egg production (Siopes, 2000). However, this photosensitivity to long daylengths does not continue indefinitely in the turkey hen. If exposure to long photoperiod continues, the turkey will eventually lose its ability to respond to these long photoperiods and thus cease egg production. Turkeys reproductively unresponsive to long photoperiod are said to be photorefractory. Therefore, as has been described for the chicken and other birds, light control of reproductive activity in the turkey hen is a balance between two physiological states, photosensitivity (PS) and photorefractoriness (PR) (Sharp, 1993, 1996; Siopes, 2000). Reproductive function in PR turkeys will eventually spontaneously recrudesce (Siopes, 2001, 2002). However, spontaneously terminated PR in the turkey typically requires 20 to 21 wk of an unchanged long photoperiod (Siopes, 2001, 2002). This is certainly impractical when egg production is of commercial value in the turkey breeder hen. Therefore, the typical lighting management for turkey breeder hens includes a light restriction treat-

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Abbreviation Key: CBH = cutaneous basophil hypersensitivity; CRBC = Chukar red blood cells; PHA-P = phytohemagglutinin; PR = photorefractory; PS = photosensitive.
Husbandry

All turkey breeder hens were maintained in the same light-controlled facility. All management and husbandry practices were identical between treatments. The building was not temperature controlled, but was insulated and the rooms mechanically ventilated. Feed and fresh water were provided ad libitum throughout the study. During the prelay-light restriction period (light restriction), feed was calculated to contain 12% CP, 0.85% calcium, and 3,084 kcal ME/kg of feed. Starting at photostimulation and continuing for the remainder of the study, a pelleted breeder feed was provided that was calculated to contain 16% CP, 3.5% calcium, and 2,970 kcal ME/kg of feed. All photoperiods were provided using incandescent light with a mean intensity level of 54 lx at turkey head height.

Experimental Design

This experiment was conducted to evaluate the effect of prelay-light restriction and the renewal of photosensitivity on cellular and humoral immune responses in PR turkey breeder hens. At the end of their first year of egg laying, 50 PR hens were selected according to Siopes (2001) and distributed among five floor pens (treatment groups, n = 10 per treatment). All birds had been exposed to long photoperiods (16L:8D) during their first egg-laying season and spontaneously ceased lay with subsequent molt. A period of light restriction (8L:16D) was provided for 8 wk, followed by 12 wk of photostimulation (16L:8D) to stimulate egg production in the PS turkeys. This recycling of reproductive function is in accordance with the accepted light management practices for PR turkeys (Harper and Parker, 1957; Siopes, 1984, 1989, 1991, 1994).

The cellular and humoral immune responses were determined at selected, sequential time points during the recycle. The five time points selected for evaluation of immunity were prelight restriction, 2-wk light restriction, 7-wk light restriction, 2-wk photostimulation, and 12-wk photostimulation. Five treatment groups consisting of 10 birds per treatment were each assigned one of these time points and were subsequently measured for immune responses. That is, the in vivo immune responses were only measured once per treatment group and at one of these time points. In order to evaluate the cellular immune activity, the cutaneous basophil hypersensitivity (CBH) reaction to phytohemagglutinin (PHA-P), a lectin from Phaseolus vulgaris,3 was measured in the wing web of each bird (Stadecker et al., 1977). An 0.5 mL intradermal injection of PHA-P (100 µg/mL of sterile saline) was given in the wing web of each bird and the dermal swelling response was measured as the percent increase in wing-web thickness at the injection site 24 h post-PHA-P injection. The humoral immune response was evaluated, within the same PHA-P injected birds, by measurement of antibody titers following administration of a 0.5 mL intravenous injection of a 10% Chukar red blood cell (CRBC) suspension into the jugular vein. This injection was given at the same time as the PHA-P injection. The primary antibody response was measured 7 d following the CRBC injections using a microagglutination assay (Sever, 1962). The lymphoproliferative and primary antibody responses were reported as the level of immunity at the time of initial injection with PHA-P or CRBC, respectively. In addition, egg production data were recorded to ensure that recycling of ovarian function was consistent with previous reports in the turkey.

Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the General Linear Model procedure of the SAS institute (SAS Institute, 1990). Each treatment group was independent from the others and separated in time. Therefore, the mean value of each time point (treatment) was only compared to the mean value ob-

3Sigma Chemical Co., St. Louis, MO.
RESULTS

The egg production response of the recycled hens was consistent with previous reports for turkey hens. Egg laying resumed by 3 wk following relighting and peak hen-day egg production (68%) occurred at about 8 wk following relighting. The CBH response of turkey breeder hens at various stages of recycle is shown in Figure 1. All of the PR birds measured for CBH response prior to light restriction (prelight restriction) had greater dermal swelling responses to PHA-P as compared to these during the light restriction period and were comparable to previously reported levels for nonlaying hens (Scott and Siopes, 1994). However, light restriction caused suppression by 2 wk into the light restriction period. That is, CBH responses were decreased 64.1% in the 2-wk light restriction group as compared to values obtained for prelight restriction group as compared to values obtained for prelight restriction. The CBH response relative to reproductive state is qualitatively similar to the dynamics of the CBH responses during this time. Again, prelight restriction birds have greater antibody responses as compared to values in the light restriction period. Furthermore, there was a reduction in anti-CRBC antibody responses following light restriction. The primary antibody response was decreased by 59.5% by 2-wk light restriction. Contrary to the cellular immune activity data, the humoral immune responses were increased at the 7-wk light restriction time point 33.5% as compared to the 2-wk light restriction value. Consistent with the CBH data, the replacement of the prelay-light restriction with long photoperiods proved to be immunosuppressive to primary antibody responses. Measurements of antibody responses at the 2-wk photostimulation time point indicated a reduction in antibody responses by 52.4% relative to the values obtained for the 7-wk light restriction group. Finally, the measured antibody responses at 12-wk photostimulation indicated that the antibody responses were elevated by 72.3% relative to the 2-wk relighting time point. In fact, the values obtained for the 12-wk photostimulation time point returned to levels similar to hens at the start (prelight restriction period).

DISCUSSION

The results from this study confirmed that the cellular (CBH) and humoral immune responses were modulated in PR turkey breeder hens during recycling and renewal of photosensitivity for egg production. Dynamics of both immune responses during the recycling period were qualitatively similar. In short, there was immunosuppression at each daylength transition point followed by spontaneous immunoenhancement (Figures 1 and 2).

It was not surprising that CBH and humoral immune responses were at high levels in PR hens measured prior to light restriction. These PR birds are nonlaying and therefore should have no confounding effects of steroids on immune responses. In fact, earlier reports in the turkey hen have indicated that nonlaying hens have slightly increased dermal swelling responses to PHA-P as compared to laying hens (Scott and Siopes, 1994). However, it was surprising that the administration of short days to these PR hens was immunosuppressive. Most reports have indicated that shorter photoperiods are immunoenhancing when compared to birds placed in longer photoperiods or constant light (Kirby and Froman, 1991; Moore and Siopes, 1994). However, light restriction caused suppression by 2 wk into the light restriction period. That is, CBH responses were decreased 64.1% in the 2-wk light restriction group as compared to values obtained for prelight restriction. The CBH response relative to reproductive state is qualitatively similar to the dynamics of the CBH responses during this time. Again, prelight restriction birds have greater antibody responses as compared to values in the light restriction period. Furthermore, there was a reduction in anti-CRBC antibody responses following light restriction. The primary antibody response was decreased by 59.5% by 2-wk light restriction. Contrary to the cellular immune activity data, the humoral immune responses were increased at the 7-wk light restriction time point 33.5% as compared to the 2-wk light restriction value. Consistent with the CBH data, the replacement of the prelay-light restriction with long photoperiods proved to be immunosuppressive to primary antibody responses. Measurements of antibody responses at the 2-wk photostimulation time point indicated a reduction in antibody responses by 52.4% relative to the values obtained for the 7-wk light restriction group. Finally, the measured antibody responses at 12-wk photostimulation indicated that the antibody responses were elevated by 72.3% relative to the 2-wk relighting time point. In fact, the values obtained for the 12-wk photostimulation time point returned to levels similar to hens at the start (prelight restriction period).
in turn would increase corticosterone output (Esquifino et al., 1999). Corticosterone has known immunosuppression properties in the chicken (Gross and Siegel, 1983). Therefore, the initial immunosuppression observed in the present study could have been due to increased plasma corticosterone caused by an elevated melatonin output following light restriction. However, this does not explain the partial recovery of immune function during light restriction or the immunosuppression following reexposure to long photoperiods, where melatonin output is reduced.

All birds in the current study were initially PR, therefore, gonadotropin levels were also low and unchanging at the start and following the administration of light restriction. Therefore, it is apparent that the mechanisms underlying immunosuppression of CBH and humoral immune responses following light restriction are likely independent of gonadal steroid influence. Likewise is true for melatonin, if it is immunoenhancing, as most reports indicate. This leaves us with physiological state as an explanation of why immunosuppression occurred on short photoperiods and why our results appear to conflict with the existing literature noted above. That is, the change in photoperiod from long to short days has altered physiological conditions independent of the factors mentioned above. This would certainly include factors, such as initiation of the transition from PR to PS and changes in various hormone levels. This is consistent with reports in the European starling, in which a direct effect of reproductive state on immunity was observed that was independent of photoperiod, melatonin, and gonadal steroids (Bentley et al., 1998).

During the light restriction period, the humoral but not cell-mediated (CBH) immune responses were partially and spontaneously reconstituted. That is, continued exposure to short days allowed antibody responses to partially recover from the dramatic immunosuppression caused by light restriction. The current study did not provide evidence of the mechanisms allowing for this partial restoration of humoral immune responses. However, coincident with the spontaneous improvement in humoral immunity is a distinct change in physiological state, that is, the transition from PR to PS. We speculate that regaining of PS in the presence of elevated melatonin levels on short photoperiod may be involved in the spontaneous improvement in humoral immunity.

Although only the humoral immune response showed any recovery in hens during the light restriction period, both the CBH and humoral immune responses were significantly, but temporarily, suppressed following a return of long photoperiods. It is known that photosensitivity is restored by light restriction and that subsequent exposure to long photoperiod causes a rise in gonadal steroids within approximately 2 wk. In addition, endogenous melatonin is suppressed by this increase in daylength. If melatonin is immunoenhancing, both of these factors could be responsible for the immunosuppression observed following the short day (light restriction) to long day transition in the current study. This secondary immunosuppression was subsequently reconstituted in hens mea-
pressed after 12 wk of long photoperiod. This was particularly prominent in the humoral response, which returned to levels similar to the prelight restriction period (Figure 2). This was surprising considering that circulating gonadal steroids would be high and melatonin low, neither of which would be expected to promote immuno-suppression. However, by 12 wk, these hens have fully mature ovaries allowing for maximal egg production. It may be that development and maturation of ovarian tissue for egg production and reproductive traits following initial exposure to long photoperiods was responsible for the deleterious actions on immunity. The energy demands of developing the reproductive system are considerable, and once the energy requirements shift away from reproductive renewal, immune responses may be more able to be restored to normal levels.

Although the exact mechanisms of immunomodulation were not determined in the present study, we believe these results suggest that different mechanisms may be responsible for the immunosuppression observed during different stages of reproductive recycling in turkey breeder hens. Along with a difference in photoperiod, the endocrine profiles are different at each point of immunosuppression. At any rate, successful recycle of reproduction in PR hens comes at a significant biological cost to cellular and humoral immune responses in the turkey breeder hen. We believe this to be the first report of the cost of reproductive state change on immune function in the turkey breeder hen.

REFERENCES


