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# Temporal dynamic of adrenocortical and gonadal photo-responsiveness in male Japanese quail exposed to short days

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## ABSTRACT

The study evaluated whether different short-term endocrine testicular and adrenocortical responses to short photoperiod exposure can persist over time and particularly when birds exhibit spontaneous cloacal gland recovery. At 11 wk of age, 33 male Japanese quail exposed to long photoperiod were switched to short photoperiod (8L:16D). Another group of males was kept under long photoperiod ($n = 11$; LD quail). After 5 wk of short photoperiod exposure, quail were classified as nonresponsive or responsive to short photoperiod, depending on whether the cloacal gland volume was above or below 1,000 $mm^3$ and with or without foam production, respectively. Since 11 wk of age and during a 20-wk period, droppings of all quail were collected to determine corticosterone and androgen metabolites (AM) by enzyme immunoassays. Cloacal gland volume was also determined weekly. Both short photoperiod nonresponsive (SD-NR) and responsive quail showed overall significantly lower ($P < 0.01$) AM values (518.8 ± 11.9 and 248.6 ± 17.1 ng/g, respectively) than quail that remained under long photoperiod (814.3 ± 24.1 ng/g). However, nonresponsive quail showed a significantly smaller reduction in their AM levels than their responsive counterparts. During the first 6 wk of short photoperiod exposure, SD-NR quail showed similar corticosterone metabolites values than LD quail. Corticosterone metabolite profiles changed from 7 wk of short photoperiod exposure onward, with photoperiodic differences ($P < 0.01$) persisting up to the end of study (LD: $228.9 \pm 22.4 >$ SD-NR: $133.1 \pm 15.5 >$ short photoperiod responsive: 61.6 ± 17.9 ng/g, respectively). Testicular and adrenocortical glands showed different degrees of activity associated with cloacal gland photoresponsiveness to short photoperiod manipulation. Our findings suggest long-term effects of short photoperiod, both in the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenocortical axis activity of quail, including males that exhibited spontaneous cloacal gland recovery.

## 1. Introduction

The hypothalamic-pituitary-adrenocortical (HPA) axis plays an essential role in supporting mechanisms through which birds adjust their physiological stages in response to environmental cues [1]. Free-living bird species can seasonally modulate glucocorticoid release, which is commonly elevated during the breeding season [2], as in wild quail (*Perdicula* sp.) [3]. Under laboratory conditions, several groups have demonstrated that photoperiod affects HPA responses [4–6]. Indeed, male Japanese quail under long photoperiod

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showed significantly higher corticosterone and androgen metabolite concentrations than birds kept under short photoperiod [7]. Interestingly, a snapshot study showed that under short photoperiod some Japanese quail, whose hypothalamic-pituitary-gonadal (HPG) axis failed to respond to photoperiod, had intermediate corticosterone metabolite values between males that were kept under long photoperiod and males that did show a clear HPG inhibitory response to short photoperiod [7]. However, it remains unclear whether these photoperiodic endocrine testicular and adrenocortical responses persist over time, and particularly under short photoperiod, when Japanese quail exhibit spontaneous cloacal gland recovery. Development of the cloacal gland in quail, an androgen-dependent phenomenon, is a reliable indicator of testicular development and sexual activity [8–11].

Breeders are usually reared under a highly stimulatory light regime in poultry industry. Some reports about physiology and behavior indicate that reducing photoperiod could improve welfare issues [12,13]. However, because reproduction is strongly controlled by photoperiod length, shorter light exposure can alter breeding physiology and affect performance. Interestingly, in Japanese quail (*Coturnix* sp.) not all birds reduce their cloacal gland when exposed to short photoperiod [10,11].Therefore, quail can be classified as either nonresponsive or responsive to light manipulation, offering an interesting tool to assess reproductive physiology [7].In this species, we previously studied endocrine testicular and adrenocortical photoresponsiveness and observed that nonresponsive quail had high androgen and corticosterone metabolite concentrations [7], probably with similar general and metabolic activity to that of quail exposed to long photoperiod. The aim of the present study was to determine the dynamics of temporal variation of testicular and adrenocortical activity in male Japanese quail that are nonresponsive and responsive to short photoperiod manipulation. We determined whether observed differential photoperiodic endocrine testicular and adrenocortical responses to short photoperiod can persist over time, particularly when birds exhibit spontaneous cloacal gland and/or gonadal recovery.

## 2. Materials and methods

### 2.1. Animals and husbandry

The study animals were male Japanese quails (*Coturnix coturnix japonica*). Egg incubation, chick brooding, and lighting procedures were similar to those described elsewhere [14,15], with the exception that chicks were brooded in wood cages measuring 85 × 45 × 50 cm (length × width × height) in mixed-sex groups from 1 d to 4 wk of age. Briefly, birds were fed a starter ration (28% CP; 2,800 kcal of ME/kg) and water ad libitum. They were kept under long photoperiod (14L:10D; lights on at 6:00 AM) and controlled temperature (brooding temperature was 37.5°C during the first week of life, with a weekly decline of 3°C until room temperature, 24–27°C, was achieved). From 4 wk onward, birds were switched to a breeder ration (21% CP; 2,750 kcal of ME/kg). At that moment, Japanese quail were sexed by plumage coloration, and only males ($n = 44$) were randomly and individually housed in cages of two 5-tier cage batteries, each battery comprising 30 cages. Each cage measured 45 × 20 × 25 cm (length × width × height). The same experimenter measured cloacal gland size, foam production, and body weight weekly until the end of study (see details in the following).

At 11 wk of age, once all measurements were taken, 33 male Japanese quail exposed to long photoperiod were switched to short photoperiod (8L:16D; lights on at 10:00 AM). Another group of males was maintained under long photoperiod ($n = 11$). Following the procedure of Busso et al [7], after 5 wk of exposure to short photoperiod [10,16], quail that showed a reduction in the cloacal gland volume below 1,000 mm$^3$ and that did not exhibit any cloacal foam were classified as responsive to short days (SD-R; $n = 13$). The remaining males under short photoperiod were classified as nonresponsive (cloacal gland volume >1,000 mm$^3$; still exhibiting some cloacal foam production; SD-NR; $n = 20$). The classification criteria mentioned previously were based on the procedure of Oishi and Konishi [10] used to classify different types of photoperiodic responses and other studies informing that cloacal gland development, foam production, or a combination of both measurements are considered effective tools to predict the fertilizing ability of a male Japanese quail [17–20]. In addition, Biswas et al [21] showed that fertility was reduced in Japanese quail males that exhibited a cloacal gland volume below 1000 mm$^3$, showing also low foam weight and frequency of foam discharge, and low testosterone concentration [17].

### 2.2. Cloacal gland volume

Beginning at 11 wk of age, cloacal gland volume (Cvol) was determined in all males weekly and over a 20-wk period. Cloacal gland size length (mm) and width (mm) were measured using a digital caliper, and Cvol was calculated from these measurements according to Chaturvedi et al [16].

### 2.3. Molting

Beginning at 11 wk of age, all males were observed for shedding of feathers by weekly recording the presence or the absence of feather in the cage trays over a 20-wk period.

### 2.4. Sampling and steroid measurements

#### 2.4.1. Collection of droppings

From 11 wk onward, droppings were individually collected once a week over a 20-wk period. Briefly, cage trays were cleaned immediately after lights automatically turned on at 6:00 AM or 10:00 AM (quail maintained under long or short photoperiod, respectively) and 1 h later, fecal samples were collected. All samples were stored immediately at −20°C until hormonal analysis.

#### 2.4.2. Steroid extraction and immunoassays

A total of 0.5 g of each homogenized sample was extracted with 5 mL of 60% aqueous methanol by shaking for 30 min [22]. Following centrifugation (2500 × g, 15 min), aliquots of the supernatant (after a 1:10 dilution with assay buffer) were measured with a cortisone enzyme immunoassay (EIA) and an epiandrosterone EIA. Details of the EIAs (including immunogens, biotinylated steroid labels, and cross-reactions of the antibodies) are given elsewhere [23,24]. Both are group-specific EIAs, the cortisone EIA measuring immunoreactive corticosterone metabolites (CM) with a 3,11-dione structure and the epiandrosterone EIA measuring immunoreactive androgen metabolites (AM) with a 17-oxo structure. Sensitivity was 10 or 25 ng/g, respectively. In addition, interassay CVs of high and low controls were 6.5% and 6.2% for the cortisone EIA and 3.7% and 5.0% for the epiandrosterone EIA. The EIA for androgen metabolites in male Japanese quail was previously validated by Hirschenhauser et al [25]. The cortisone EIA has been successfully validated for Japanese quail droppings and used to measure adrenocortical activity in a previous study [7]. Considering that androgens and glucocorticoids are heavily metabolized, it is an advantage to work with group-specific antibodies, which recognize a group of metabolites rather than a specific steroid [26,27].

### *2.5. Statistical analyses*

All variables were subjected to repeated measures analysis of variance (ANOVA). A mixed linear model was performed to evaluate the effects of photoperiodic responses (SD-R, SD-NR, and long photoperiod [LD]), time (wk), and their interaction on CM and AM concentrations and Cvol measurements, taking into account photoperiodic responses as a fixed factor. Across analyses, "male quail" was incorporated as a random effect. Because a repetition factor model was applied, in the case that a bird did not naturally defecate in a particular time interval, concentrations between the previous and following collection time interval were interpolated to obtain an estimate value and avoid losing the whole hormonal profile for that bird. Hormonal data were rank-transformed to meet the ANOVA assumptions. Whenever repeated measures ANOVA reached significance ($P < 0.05$), Tukey post hoc tests were performed. Values are expressed as means ± SEM. All statistical analyses were performed using Infostat [28].

## 3. Results

Differences in mean Cvol because of photoperiodic response ($P < 0.001$, $F_{2,42} = 460.15$) and time ($P < 0.001$, $F_{19,798} = 9.48$) were evident. A significant interaction between these factors was also detected ($P < 0.001$, $F_{38,798} = 4.46$; Fig. 1). Post hoc test showed similar cloacal gland volume profiles for LD and SD-NR quail throughout the whole study. However, both groups clearly exhibited higher Cvol values than their SD-R counterparts ($P < 0.05$). The latter group exhibited the maximum inhibition of Cvol at 16 wk of age (5 wk after short photoperiod exposure). Immediately after that low milestone was reached, numerical Cvol increases were weekly observed (cloacal gland spontaneous recovery). At the end of the study, SD-R quail still did not recover the cloacal gland values observed at the beginning of the study (11 wk of age). Cloacal foam production profiles followed the pattern shown for Cvol ($P < 0.001$, $F_{40,924} = 2.9$), except that at 30 wk of age foam production in all groups recovered similar values to those detected at 11 wk of age, before the application of the photoperiodic treatment (data not shown).

Under short photoperiod, as expected, all birds exhibited feather loss. In SD-R quail feather loss (molting) finished as early as 2 wk after short photoperiod exposure, whereas for SD-NR males it was completed 4 wk later (17 wk of age).

Differences in mean CM concentrations because of photoperiodic response ($P < 0.001$, $F_{2,42} = 18.4$) and time ($P < 0.001$, $F_{19,798} = 15.39$) were evident. An interaction between these factors was detected as well ($P < 0.001$, $F_{38,798} = 2.92$; Fig. 2). Post hoc test showed that CM concentrations were similar in SD-NR and LD quail during the first 6 wk of the experiment, and also at the last week of the study, when spontaneous cloacal gland recovery was evident in these groups. Starting as early as 2 wk after short day exposure, SD-R quail showed reductions in CM values compared with SD-NR and LD quail ($P < 0.05$). After spontaneous cloacal gland recovery was initiated (21 wk of age), and until the end of the study, SD-R quail continued

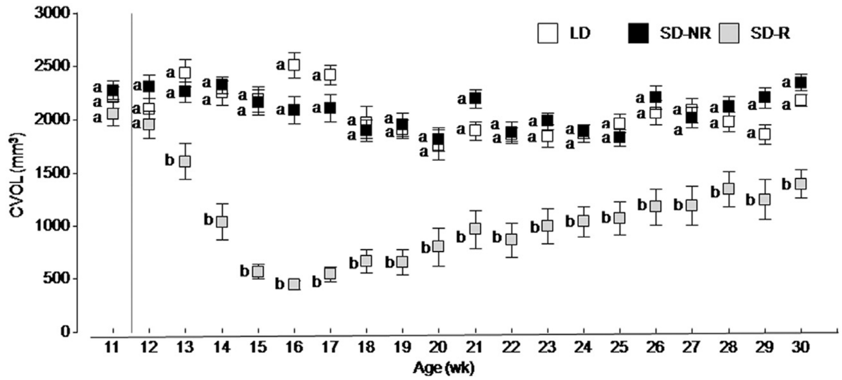

**Fig. 1.** Cloacal gland volume (Cvol) in male Japanese quail was registered weekly. After 11 wk of age (indicated by vertical line), birds were assigned to photoperiodic treatment (long or short photoperiod). At 16 wk of age, quail under short photoperiod were classified according to their cloacal gland photoresponsiveness as nonresponsive (SD-NR, n = 20) or responsive (SD-R, n = 13) to short photoperiod. Long photoperiod quail (LD) were kept on long days (n = 11). All values are expressed as mean ± SEM. Within each week, values with no common letter differ significantly ($P < 0.05$). To facilitate figure visualization, differences within treatments through time are mentioned in the text. SEM, standard error of the mean.

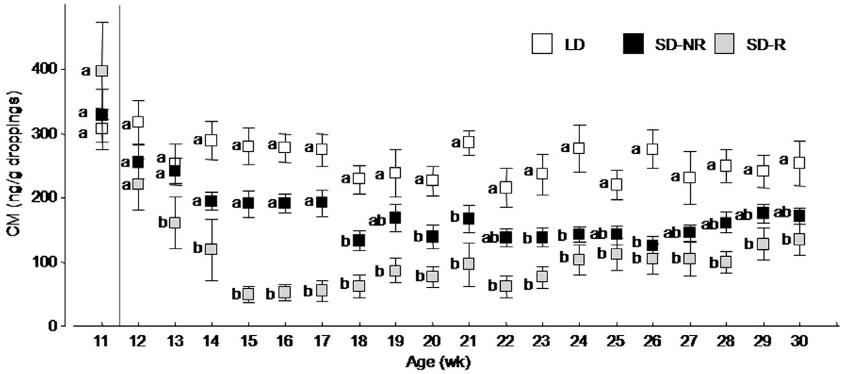

**Fig. 2.** Corticosterone metabolites (CM) in droppings of male Japanese quail collected individually over 1 h once a week. After 11 wk of age (indicated by vertical line), birds were assigned to photoperiodic treatment (long or short photoperiod). At 16 wk of age, quail under short photoperiod were classified according to their cloacal gland photoresponsiveness as nonresponsive (SD-NR, n = 20) or responsive (SD-R, n = 13) to short photoperiod. Long photoperiod quail (LD) were kept on long days (n = 11). All values are expressed as mean ± SEM. Within each week, values with no common letter differ significantly ($P < 0.05$). To facilitate figure visualization, differences within treatments through time are mentioned in the text. SEM, standard error of the mean.

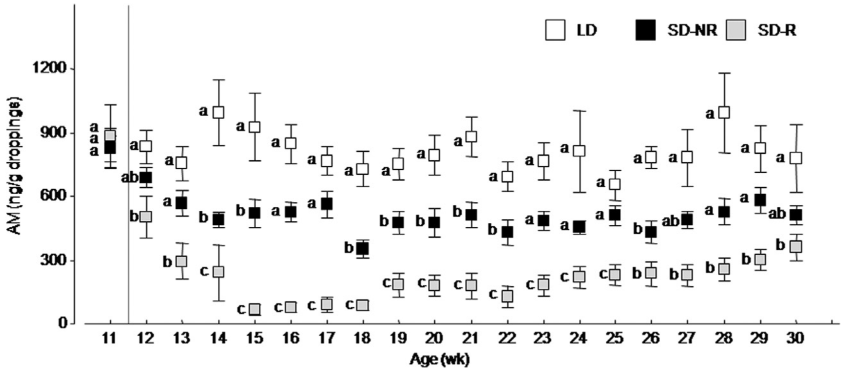

**Fig. 3.** Androgen metabolites (AM: 17-oxoandrogen metabolites) in droppings of male Japanese quail collected individually over 1 h once a week. For further details see legend of Figure 2.

showing lower CM values than LD males ($P < 0.05$) but not than their SD-NR quail counterparts.

Differences in mean AM concentrations because of photoperiodic response ($P < 0.001$, $F_{2,42} = 26.41$) and time ($P < 0.001$, $F_{19,798} = 7.99$) were found, as well as an interaction between these factors ($P < 0.001$, $F_{38,798} = 2.85$; Fig. 3). Post hoc test showed that after 3 wk of short photoperiod exposure, both SD-NR and SD-R quail had lower AM values than males kept under long photoperiod (LD quail; $P < 0.05$). Moreover, SD-R males showed AM concentrations even significantly lower than their SD-NR counterparts. Two wk after spontaneous cloacal gland recovery was initiated (23 wk of age) and until the end of the study, previously observed differences between SD-NR and LD quail were no longer present. Only in the last week of the study, no differences between SD-NR and SD-R males were detected.

## 4. Discussion

Japanese quail exposed to short photoperiod in our laboratory exhibited significant differences in the development of the cloacal gland among individuals, a phenomenon that has already been reported [10,11]. This divergent reproductive response is still not fully understood, and the neuroendocrine messengers involved in the degradation or “loss” of the inhibitory photoperiod signal in birds is unknown. The present study evaluates temporal endocrine testicular and adrenal activity in adult Japanese quail subjected to long (stimulatory) and short (inhibitory) photoperiods. Our findings contribute to the understanding of the HPA-HPG interaction by analyzing the endocrine responses at testicular and adrenocortical levels in birds differing in photosensitivity. Our findings suggest that short photoperiod can induce long-term effects on the HPA activity, even when birds clearly exhibited a spontaneous cloacal gland recovery. We also observed that high endocrine testicular activity and cloacal gland development coincided with high adrenocortical activity and vice versa. Similarly, in the peak reproductive phase, adrenal activity was enhanced in adult quail *Perdicula asiatica*, whereas in the reproductively inactive phase, adrenal activity was minimum as shown by decreased plasma corticosterone [3].

A reduction in cloacal gland and androgen hormones, with consequences on the reproductive capacity of birds, is expected when they are exposed to short days [11]. We observed that adrenocortical activity was also affected by short photoperiod exposure. Evidence of adrenocortical activity and seasonal variation in glucocorticoids in birds and other vertebrates indicate that concentrations of these steroids usually increase in the breeding season [2]. Photoperiod is a primary environmental component of seasonal variation and is apparently involved in the control of corticosterone secretion in birds [1], as detected for other neuroendocrine signals such as melatonin and thyroxin [29–32]. Previous data on corticosterone response to photoperiod are contradictory about the influence of natural seasonal variations in photoperiod on this hormone [33–35]. Seasonal variation in baseline glucocorticoid concentrations was reported in 72% of the studies in bird species [2]. However, photoperiod was found to have no significant effect on baseline concentrations of corticosterone neither in Mountain chickadees (*Poecile gambeli*) [35] nor in house sparrows (*Passer domesticus*) [34]. In the present study, we demonstrated that the temporal dynamic of adrenocortical activity in male Japanese quail was indeed affected by photoperiod. As found earlier in birds at different reproductive status, birds kept under long days had higher corticosterone concentrations than birds exposed to short days [7].

Our experiment showed a similar adrenocortical activity in LD and SD-NR Japanese quail during the first 6 wk of study, when both groups exhibited similar cloacal gland development and foam production. From 7 wk of photoperiod change, the SD-NR group showed a decrease in both CM and cloacal gland volume. Some small fluctuations were observed in the LD group for cloacal gland volume, which can be attributed to regular fluctuations during growth and aging processes. In those males, concentrations of corticosterone metabolites were found to remain high and without significant variations throughout the experiment. By contrast, after only 3 wk of exposure to short photoperiod, CM values in SD-R males were significantly reduced compared with those of LD and SD-NR quail. Therefore, our results demonstrate not only that CM are reduced in response to a change in photoperiod but also that the differences observed ($LD = SD\text{-}NR > SD\text{-}R$) persist over several weeks under constant photoperiod conditions. Because corticosterone secretion is influenced by the pituitary and hypothalamus, it could be considered that the source of variation may be induced at the upper levels of the nervous system. However, we cannot exclude additional direct effects of neurotransmitters on adrenocortical activity, also produced by the effects of photoperiod on neural pathways [6,36–38].

Because molting has been related to changes in adrenocortical activity [1], it may have affected adrenocortical activity in SD-NR quail, because from week 7, CM concentrations were reduced compared with those found for LD males. Indeed, there are extreme adjustments in energy partitioning for feathering [1]. The effect of molting on adrenal activity in SD-R quail was probably less evident because of the higher sensitivity of the HPA axis, which showed a sharp drop of adrenocortical activity from the earliest weeks of short photoperiod, even before the molting process was observed. Molting and reproductive processes are not simultaneous because they require high energy investment. Thus, molting usually occurs immediately after the breeding season [30]. However, in our study, during molting SD-NR exhibited similar values of AM and cloacal gland development as LD males. These results suggest that under suitable energy availability, males would be able to undergo both physiological processes simultaneously, which would be consistent with the hypothesis that both SD-NR and LD males have a similar reproductive potential.

From a practical perspective, classifying Japanese quail (and probably other galliform species) based on their reproductive response to short photoperiod could be considered a useful management tool to identify breeders in terms of resistance to abiotic factors, such as natural photoperiodic changes. This strategy would allow us to use more sustainable breeding environmental conditions than the current ones, for example, using fewer artificial light hours that would also most probably contribute to reduced aggressiveness between males while maintaining their reproductive capacity (ie, SD-NR males) [13]. However, the perspectives of applying this tool in the poultry industry still requires further research, using physiological and behavioral analyses especially focused on assessing whether SD-NR male Japanese quail can actually reproduce. Studies covering such aspects are currently ongoing in our laboratory. Basic studies such as those conducted in males should also be undertaken with females. On the other hand, reducing photoperiod might contribute to an improvement in animal welfare issues related to long photoperiod exposure [12,13,39]. Improved welfare would be associated with lower baseline CM values of SD-NR Japanese quail, probably less aggressive behaviors, increased cellular and humoral immune responses, and a potentially reduced stress responsiveness [12,13,39]. However, caution is advised because higher fecal CM do not necessarily reflect distress and may not necessarily be a welfare issue, unless other factors, such as behavior or health problems change accordingly.

## 5. Conclusions

Testicular and adrenocortical glands showed different degrees of activity associated with cloacal gland photoresponsiveness to short photoperiod manipulation. Our findings suggest long-term effects of a shift to short photoperiod, both in HPG and HPA axis activity of quail, including males that exhibited spontaneous cloacal gland recovery.

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