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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 ± 22.4 > SD-NR: 133.1 ± 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