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Corticosterone metabolites in blue tit and pied flycatcher droppings: Effects of brood size, ectoparasites and temperature

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Abstract

The hypothalamic-pituitary-adrenal (HPA) axis of birds induces the secretion of corticosterone (CORT) as a response to different ecological variables. In this study we tested experimentally if manipulations of brood size or ectoparasitism led to subsequent differences in the concentration of excreted CORT metabolites of adult and nestling blue tits (*Cyanistes caeruleus*). No significant effect of the manipulation of brood size was detected in adults or nestlings. No significant effect of ectoparasitism was detected in males or nestlings, although females from uninfested nests showed lower concentrations of excreted CORT metabolites. In addition, we analysed if weather conditions had an influence on the concentration of excreted CORT metabolites of blue tits and pied flycatchers (*Ficedula hypoleuca*) breeding in the same forest. We detected no effect of weather conditions on adults, but nestlings of both species showed a negative correlation between their excreted CORT metabolites and the average mean temperatures they were subjected to during their growth. This effect was not found in blue tits in a colder year, suggesting that the sensitivity of the HPA axis to ambient temperature may be subjected to interannual variation. Moreover, we found a positive effect of the maximum temperature on the day of sampling on the concentration of CORT metabolites of blue tit nestlings in one of the years. These results suggest that weather conditions may act as environmental stressors to which the HPA axis of blue tit and pied flycatcher nestlings may be sensitive.

Keywords: Corticosterone; Droppings; Ectoparasites; Brood size; Weather conditions; Cyanistes caeruleus; Parus caeruleus; Ficedula hypoleuca

Introduction

The endocrine response to stress includes the secretion of glucocorticoids as a modulator mechanism of physiological processes directed to overcome the stressful events. The hypothalamic-pituitary-adrenal (HPA) axis is known to respond to different ecological variables which can generate stressful situations. Thus, under stress, the corticotropin-releasing hormone (CRH) and vasopresin (AVP) are secreted by the hypothalamus and stimulate the secretion of adrenocorticotropic hormone (ACTH), which regulates the synthesis of glucocorticoids. In birds, the main steroid secreted under this situation is corticosterone (CORT). Among the actions modulated by glucocorticoids we can mention their cardiovascular effects, their suppressive actions on immune and inflammatory reactions, their effect on mobilization of lipids and proteins or their action stimulating appetite or their inhibitory effect on reproduction (see Sapolsky et al., 2000 for a review). Basal concentrations of glucocorticoids, secreted constitutively, can also exert some of these actions and are involved in the maintenance of basic life processes (Landys et al., 2006).

Feeding behavior constitutes a daily activity necessary for survival that seems to be affected by glucocorticoids. Food availability and internal energy stores are factors that can influence feeding behavior and may determine the level to

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† José Luis Osorno died on 2 April 2004. We mourn the loss of a bright behavioural ecologist and excellent colleague.

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which glucocorticoids vary (Landys et al., 2006). In adult birds, it has been reported that corticosterone facilitates foraging behavior under nutritional stress (Astheimer et al., 1992) and increases under uncertain food availability (Reneerkens et al., 2002). For nestling birds, food availability may depend on food shortages and on competition between nest mates for the food brought by the parents. The size of the brood may be determinant in the availability of food as is demonstrated by a number of studies in which the body mass of the nestlings is negatively associated to the brood size (Martin, 1987; Merilä and Wiggins, 1995; Moreno et al., 1997; Sanz and Tinbergen, 1999; Saino et al., 2001). Thus, it could be expected that the HPA axis may be responsive to manipulations of brood size, given that the secretion of glucocorticoids may help to mobilize energy to overcome food shortages (Kitaysky et al., 1999; Sapolsky et al., 2000) and also increase begging frequencies and parental provisioning rates (Kitaysky et al., 2001a). Accordingly, corticosterone may be secreted as a response to the stress imposed by enlarged broods, as it is shown in the study of Saino et al. (2003) in nestlings of barn swallows (*Hirundo rustica*). Enlarged broods may also result in an increase on corticosterone levels on parents as a reflect of increased parental effort (see Ilmonen et al., 2003). Thus, a first objective of the present study was to evaluate whether a brood size manipulation experiment performed in a population of blue tits (*Cyanistes caeruleus*) in 2003 (see Merino et al., 2006) had an effect on the corticosterone levels of adults and nestlings.

Parasitism constitutes a stressor that may have consequences for life-history traits in wild birds (Loye and Zuk, 1991). The susceptibility to parasite infections may be associated to the immunosupression ocurred during chronic stress. The glucocorticoids secreted under prolonged stressful situations may exert immunosupressive and anti-inflammatory actions that may result in impaired disease resistance (Sapolsky et al., 2000; Sapolsky, 2002). As a consequence, under non-manipulated conditions, a positive correlation between parasitism and glucocorticoid levels might be reflecting the higher susceptibility to infection induced by potential chronic stress. On the other hand, release of glucocorticoids under parasitism may help the organism to face this pressure by increasing energy uptake through increased feeding behavior or mobilization of lipids (Raouf et al., 2006). In fact, a recent experiment by Raouf et al. (2006) has shown that free living cliff swallows *Petrochelidon pyrrhonota* are responsive to ectoparasite loads and colony size, with higher levels of corticosterone in individuals from parasitized nests and large colonies. Also, a positive correlation between the degree of infestation with ticks and baseline CORT levels of red-legged kittiwakes was detected by Kitaysky et al. (2001b). Other consequences of parasites have been evaluated in relation to modifications of behavior in order to compensate the deleterious effect of parasitism (Loye and Zuk, 1991; Simon et al., 2005). Increased parental provisioning seems to be a compensatory strategy in parasitized bird nests (Hurtrez-Boussès et al., 1998; Bouslama et al., 2002; Merino et al., 1998a,b), although evidences exist for variation in this behavior (Møller, 1994; Tripet et al., 2002). Thus, a second objective of the present study was to evaluate the effects of ectoparasitism on corticosterone levels and compensatory behavioral responses. Thus, we manipulated ectoparasitic load in nests of a free-living population of blue tits and measured if parasitism affected corticosterone levels of birds and parental provisioning behavior.

As a third objective, we evaluated if weather conditions function as ecological variables to which the HPA axis of birds may respond. Physiological changes orchestrated by glucocorticosteroids may be a useful mechanism to cope with perturbations of the environment, such as climate changes (Wingfield, 2003). As corticosterone may favour energy mobilization, via its stimulatory effects on glycogenolysis, lipolysis and proteolysis (Sapolsky et al., 2000) an increased adrenal activity under cold weather may have a role in the thermoregulation of birds. This possibility is suggested by the negative covariation of corticosterone levels and ambient temperature found by Frigerio et al. (2004) in Greylag geese (*Anser anser*). Moreover, several studies on birds have detected an increase in corticosterone levels as a response to severe weather episodes (Wingfield, 1984; Astheimer et al., 1995; Romero et al., 2000). Therefore, climatic variables (the maximum temperature registered on the day of sampling, the average mean temperature of the nestling period and the number of days of precipitation) were evaluated in this study as possible predictors of corticosterone levels of adult and nestling blue tits and pied flycatchers (*Ficedula hypoleuca*).

Methods

All procedures conform to the requirements of animal welfare and conservation of Spanish laws.

Species and study area

The blue tit (*Cyanistes caeruleus*) is a small (10–11 g) hole-nesting passerine of European woodlands. It is a resident bird, which adapts readily to breeding in nest boxes. Egg laying in central Spain typically begins in late April, clutch sizes range from 4 to 14 eggs with a mean of 9 eggs and the number of fledglings averages 7. Females incubate and brood the chicks alone and both sexes feed the young (Potti et al., 1988; Moreno et al., 1996; Fargallo and Johnston, 1997). Similarly, the pied flycatcher (*Ficedula hypoleuca*) is a small (12–13 g) hole-nesting passerine of European woodlands. It is a summer visitor, which adapts readily to breeding in artificial nest-boxes. Egg laying in the study population typically begins in late May and clutch sizes range from 2 to 7 eggs with a mode of 6 eggs (mean 5.73) (Sanz and Moreno, 1995). Nest-boxes are periodically inspected and the dates of clutch initiation, clutch sizes and hatching dates (1 = 1 April) are determined.

The study was conducted in 2003 and 2005 in a deciduous forest of Pyrenean oak *Quercus pyrenaica* at 1200 m a.s.l. in the vicinity of La Granja, Segovia province, central Spain (40° 53′ N, 4° 01′ W.). Nest-boxes (125 × 17 mm bottom area) are cleaned every year after the breeding season.

In 2005 we visited nests on two consecutive days and we collected fresh droppings of nestlings in both visits. An effect of the visit of the previous day could be expected in the concentration of excreted CORT metabolites of nestlings the following day, thus, age of nestlings was considered in the analyses. In addition, previous studies have detected age-related changes in baseline CORT in nestlings of altricial and semi-altricial species (Schwabl, 1999; Kern et al., 2001; Love et al., 2003). Also, adrenocortical responses to stress have been shown to increase with age in nestlings of white storks (*Ciconia ciconia*) (Blas et al., 2006) and American kestrels (*Falco sparverius*) (Love et al., 2003). Adults of blue tits and pied flycatchers were captured at the nest boxes using traps activated when parents got inside the nest-box to feed the nestlings. They were held in cotton bags before ringing and measurement. Some birds defecated during handling. The whole manipulation did not take longer