Nutritional stress affects corticosterone deposition in feathers of Caspian tern chicks

Allison G. L. Patterson, Alexander S. Kitaysky, Donald E. Lyons and Daniel D. Roby

Stressful environmental conditions affect the adrenocortical function of developing animals, which can have consequences for their fitness. Discovery of the avian stress hormone corticosterone (CORT) in feathers has the potential to broaden the application of endocrine research in ecological and evolutionary studies of wild birds by providing a long-term measure of CORT secretion. Mechanisms of CORT deposition in feathers are not well known and few studies have related feather CORT to circulating plasma CORT during feather growth. Our objective was to experimentally test the validity of using feather CORT as a measure of CORT secretion in developing birds experiencing nutritional stress. Caspian tern Hydroprogne caspia chicks were fed ad libitum or restricted (35% less than ad libitum) diets for four weeks. We measured CORT in feathers from these chicks to examine the relationship between feather CORT concentrations and nutritional limitation, circulating plasma CORT, and feather development. We found that feather CORT was higher in controls fed ad libitum than in restricted individuals, despite higher levels of plasma CORT in restricted chicks compared to controls. Feather mass and growth rates were strongly and positively related to feather CORT concentrations in both treatments. This is the first experimental study to show that feather CORT concentrations can be lower in response to nutritional stress, even when plasma CORT concentrations are elevated. Our results indicate that CORT deposition in feathers may be confounded when feather mass and growth rates are compromised by nutritional stress. We conclude that feather CORT can be used for assessing nutritional stress in growing birds, but the direction of response depends on how strongly stress affects feather development.
between plasma CORT and feather CORT; however, only one was conducted under conditions of substantial nutritional stress (Will et al. 2014).

Understanding mechanisms of CORT deposition in the feathers during chick growth and development under conditions of nutritional stress is of particular physiological and ecological interest. Compared to molting adults, chicks face additional physiological demands during the time of feather growth, including concurrent requirements to grow alternative tissue and increase overall body mass. Consequently, the potential for nutritional stress to impact feather development is likely higher for chicks than for adults. During the period of feather growth most chicks are nest-bound and dependent on the prey availability their parents encounter at the foraging grounds, making chicks a good indicator of localized environmental conditions (Cairns 1987). Stress experienced during development has been related to survival and future fitness, linking environmental conditions during chick growth to overall population dynamics (Kitaysky et al. 2006, Monaghan et al. 2012). Other studies have related feather CORT to survival (Koren et al. 2012), reproductive effort (Bortolotti et al. 2008), and parental investment (Fairhurst et al. 2012); understanding the effects of nutritional stress on feather CORT could link population dynamics to environmental conditions at specific life history stages and nesting areas.

We tested the relationship between feather CORT, nutritional stress, and circulating plasma CORT using feathers from Caspian tern Hydroprogne caspia chicks exposed to experimentally-induced nutritional stress during feather development. Our objectives were 1) to examine the validity of using feather CORT as a measure of nutritional stress incurred by developing birds, and 2) to test relative contributions of CORT secretion vs feather development rates to variation in feather CORT concentrations. A previous study demonstrated that Caspian tern chicks raised on restricted diets had slower growth rates, smaller overall size, reduced feather growth, and higher levels of baseline and stress-induced plasma CORT than chicks fed ad libitum (ad lib) diets (Lyons and Roby 2011). If feather CORT is primarily related to circulating plasma CORT, we expected to find support for a positive relationship with nutritional stress and circulating CORT levels. If feather CORT is influenced by feather development, we expected to find support for a negative relationship with nutritional stress and feather mass and growth rate.

**Methods**

Feather samples were taken from Caspian tern chicks raised in captivity as part of an earlier study examining the effects of food availability on chick growth and development (Lyons and Roby 2011). Full details of the experimental protocols and results are reported by Lyons and Roby (2011). Ten chicks were raised on an ad lib diet and 10 were raised on a restricted diet. Each chick came from a different brood and was randomly assigned to a treatment. Diet treatments were applied from 11 d post-hatch until 39 d post-hatch. During this period chicks were fed by hand six times per day, chicks in the ad lib treatment also had access to food on trays throughout the day. Chicks in the restricted treatment were fed approximately two-thirds of the food consumed by ad lib chicks on the previous day. From day 11 to day 39 post-hatch chicks in the restricted diet treatment consumed on average 65% of the total biomass consumed by chicks in the ad lib diet treatment during the experiment. Measurements of chick mass and feather length (10th primary) were taken every one to two days. Baseline plasma CORT samples were collected at average ages of 19, 25, 31, and 39 d post-hatch. Blood samples were taken in the morning after an overnight fast, approximately one hour before first feeding, and within 3 min of removing a chick from its enclosure. Baseline CORT values did not differ between experimental groups prior to dietary treatments. At 39 d post-hatch, the temporal response to sustained stress (initial handling followed by restraint in a cotton bag) was measured by drawing blood within 3 min of capture (baseline level), and after 10, 30, and 50 min of sustained handling and restraint. At the end of the experiment, day 39, chicks fed ad lib had 23.5% greater body mass, 10.7% longer 10th primaries, and 13% greater total feather mass than chicks fed restricted diets. Chicks on the restricted diets had higher baseline plasma CORT concentrations and greater CORT response to sustained stress (Lyons and Roby 2011).

We used feathers from 14 chicks (seven from each diet treatment) that were euthanized at the end of the experiment and stored frozen at −20°C until feather CORT analysis. Because our study was conducted after the completion of the experiment by Lyons and Roby (2011), it was only possible to obtain feathers from these 14 chicks. The remaining six chicks were donated to an aviary following the initial study. We collected a 10th primary and a primary covert from each chick. Within feather differences in CORT could potentially reflect changes in individual CORT secretion through time (Bortolotti et al. 2009), however, other studies have found differences in CORT among feather section do not necessarily correspond to changes in plasma CORT during feather growth (Lattin et al. 2011). We were interested in examining differences in CORT among sections of the same feather, so each primary was divided into three sections – 20–40 mm (tip), 50–70 mm (middle), and 80–100 mm (base) measuring from the feather’s distal tip – and each section was assayed separately. These sections correspond to ages of 14–18, 20–23, and 25–28 d post-hatch for ad lib chicks and 14–19, 21–25, and 27–30 d for restricted chicks, thus allowing us to examine the temporal dynamics of feather CORT deposition. All 10th primaries were shorter than 14 mm on the first day of the diet treatment and longer than 100 mm (excluding the calamus) at the end of the experiment, therefore, all feather sections were grown during the time chicks were exposed to diet treatments.

Each sample was measured, weighed, and minced into <0.5 mm² pieces; then feathers were prepared and assayed following Bortolotti et al. (2008). The entire primary covert feather was used after removing the calamus. We used Sigma-Aldrich CORT antibody (C 8784, St Louis, MO, USA) for radio-immunoassay following Bortolotti et al. (2008) and Lattin et al. (2011). Validation assays were conducted on pooled samples containing two body feathers from each individual. Serial dilution of feather CORT extracts yielded slopes parallel to the serial dilution.
of standard CORT (Sigma-Aldrich). Hydrolysis of feather extracts did not increase specific binding, confirming earlier findings (Bortolotti et al. 2009) that CORT is likely deposited in feathers as a parental molecule. All samples had CORT levels above the assay detection limit (15.6 pg per sample). Intra-assay coefficient of variation was less than 2% and all samples were processed in a single assay. Mean recovery values for methanol extraction were 88.9% (SD ± 20); final feather CORT values were adjusted for individual recoveries. Feather CORT levels were normalized by feather length (pg mm$^{-1}$), which is thought to represent the temporal deposition patterns of CORT in feathers better than normalizing by sample mass (Bortolotti et al. 2008, 2009).

First, we examined trends in feather CORT values of 10th primary feather sections related to diet treatment and sample mass. We used mixed effects models to test for differences in feather CORT values of primary feather sections as a function of diet treatment, feather section, and feather sample mass; chick identity was included as a random effect. We compared 14 candidate models including all possible combinations of the three main effects, models including the main effects plus one of the interaction terms, and the null model.

Second, we examined how plasma CORT values, feather mass, and feather growth rate were related to feather CORT concentrations for the mean of all 10th primary sections and for primary coverts. One chick was excluded from this analysis because there were no stress-induced plasma CORT measurements taken from this individual. Linear models were used to examine the relationship between the mean of feather CORT from all 10th primary sections and baseline plasma CORT (average of five draws taken between day 10 and day 39 post-hatch), maximum stress-induced plasma CORT (individual peak in stress series CORT measurements minus baseline measurement on day 39), feather sample mass (mg), 10th primary growth rate (day 11–35 post-hatch), and diet treatment. We considered 10 models: each main effect, diet treatment paired with each of the four other main effects, and the null model. We did not consider models with more parameters in this analysis because of the small sample size ($n = 13$).

Akaike’s information criterion, corrected for small sample sizes (AICc), was used to compare models. The four most supported models and the null model are reported in AICc tables. Feather CORT values were log-transformed prior to analysis to achieve a normal distribution and equal variance. All analyses were conducted in R ver. 2.13.2; mixed effects models were performed using the ‘nlme’ package (Pinheiro et al. 2011).

Table 1. AICc model comparisons examining the effects of diet treatment, feather section, and feather mass on feather CORT (fCORT) concentrations (pg mm$^{-1}$) in 10th primary sections. Table includes the number of parameters in each model ($K$), the difference between each model and the top model ($\Delta$AICc), and model weights ($w_i$).

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>K</th>
<th>$\Delta$AICc</th>
<th>$w_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(fCORT) – Treatment + Section + Feather mass</td>
<td>-34.11</td>
<td>7</td>
<td>0.00</td>
<td>0.69</td>
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<td>log(fCORT) – Section + Treatment $\times$ Feather mass</td>
<td>-31.15</td>
<td>8</td>
<td>2.96</td>
<td>0.16</td>
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<tr>
<td>log(fCORT) – Treatment + Section $\times$ Feather mass</td>
<td>-30.43</td>
<td>9</td>
<td>3.67</td>
<td>0.11</td>
</tr>
<tr>
<td>log(fCORT) – Treatment $\times$ Section + Feather mass</td>
<td>-28.22</td>
<td>9</td>
<td>5.88</td>
<td>0.04</td>
</tr>
<tr>
<td>log(fCORT) – Null</td>
<td>2.12</td>
<td>3</td>
<td>36.22</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Results

Feather CORT for one of the 20–40 mm feather sections in the ad lib diet group was an outlier (49.43 pg mm$^{-1}$ with other values ranging from 8.86–24.75 pg mm$^{-1}$; Grubb’s test: $G = 5.1942$, $U = 0.3259$, p-value $< 0.001$). The other feather sections and primary covert from this chick had similar feather CORT values for their groups, suggesting that there was not a biological explanation for this extreme value. We report the feather section analysis excluding this outlier, but retaining other observations from this individual.

There was strong support for the 10th primary feather section model including diet treatment, feather section, and sample mass main effects (Table 1). All four top models included each of the main effects and there were only small differences in the parameter estimates among these models. In the best supported model, feather CORT from chicks on the restricted diet was lower on average than feather CORT for ad lib chicks by a factor of 0.81 (95% CI = 0.73–0.91, Fig. 1). After accounting for treatment and mass, feather CORT was highest in the tip sections (20–40 mm), and declined in the middle sections by a factor of 0.51 (95% CI = 0.38–0.70) and the base sections by a factor of 0.25 (95% CI = 0.14–0.43). Within feather sections and
treatments, there was evidence of a positive relationship between sample mass and feather CORT; for every 1 mg increase in mass, feather CORT increased by a factor of 1.05 (95% CI = 1.03–1.08).

The best supported model explaining mean feather CORT in all 10th primary sections included feather mass and treatment (Table 2, Fig. 2a). In this model average feather CORT increased by a factor of 1.03 (95% CI = 1.02–1.04) for every mg increase in feather mass and was lower in chicks on the restricted diet by a factor of 0.82 (95% CI = 0.71–0.94). Other models were not competitive with the treatment and feather mass model; however, all parameter coefficients in the top four models were significant (p < 0.05) indicating that maximum plasma CORT and feather growth rate were also related to feather CORT. After accounting for diet treatment, mean feather CORT in 10th primary sections increased by an average factor of 1.01 (95% CI = 1.00–1.02) for every ng ml\(^{-1}\) increase in maximum plasma CORT (Fig. 2b). Mean feather CORT in primary sections was positively related to feather growth rate, increasing by an average factor of 1.78 (95% CI = 1.35–2.37) for every mm d\(^{-1}\) increase in growth rate (Fig. 2c). There was no relationship between average baseline plasma CORT and mean primary CORT either

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Table 2. AIC\(_c\) model comparisons examining the relative effects of feather mass, feather growth rate, maximum plasma CORT, and baseline plasma CORT on mean feather CORT (fCORT) concentrations in all primary feather sections (pg mm\(^{-1}\)). Table includes the number of parameters in each model (K), the difference between each model and the top model (\(\Delta\text{AIC}_c\)), and model weights (\(w_i\)).

<table>
<thead>
<tr>
<th>Model</th>
<th>(\text{AIC}_c)</th>
<th>K</th>
<th>(\Delta\text{AIC}_c)</th>
<th>(w_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(fCORT) - Feather mass + Treatment</td>
<td>-17.50</td>
<td>4</td>
<td>0.00</td>
<td>0.93</td>
</tr>
<tr>
<td>log(fCORT) - Feather mass</td>
<td>-12.36</td>
<td>3</td>
<td>5.14</td>
<td>0.07</td>
</tr>
<tr>
<td>log(fCORT) - Max CORT + Treatment</td>
<td>-2.93</td>
<td>4</td>
<td>14.57</td>
<td>0.00</td>
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<tr>
<td>log(fCORT) - Feather growth</td>
<td>-2.08</td>
<td>3</td>
<td>15.42</td>
<td>0.00</td>
</tr>
<tr>
<td>log(fCORT) - Null</td>
<td>8.14</td>
<td>2</td>
<td>25.64</td>
<td>0.00</td>
</tr>
</tbody>
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Figure 2. Mean CORT in 10th primary sections (pg mm\(^{-1}\)) was positively related to (a) feather mass, (b) maximum-stress induced plasma CORT, and (c) feather growth rate; however there was no relationship with (d) average baseline plasma CORT. Feather CORT was consistently higher for chicks on the ad lib diet than for chicks on the restricted. Each graph depicts the model with the lowest AIC\(_c\) including one of the four parameters considered. Lines indicate means (solid lines) and 95% CI (dashed lines).
There was no support for an effect of plasma CORT on feather CORT in our AICc model comparisons. We observed a weak positive relationship between maximum stress-induced plasma CORT and feather CORT in 10th primaries (Fig. 2b). This relationship only occurred within diet treatments and this model had no support compared to the models that included feather mass – an indication that circulating plasma CORT had an effect on feather CORT, but was not the primary driver of feather CORT concentrations.

A recent study by Will and colleagues (Will et al. 2014) examined the relationship between feather CORT and nutritional stress in rhinocerous auklet *Cerorhinca monocerata* chicks raised under similar experimental conditions (∼50% food restriction between treatment and control groups; Sears and Hatch 2008) to those of Lyons and Roby (2011) for Caspian terns. Rhinocerous auklet chicks fed a restricted diet had elevated plasma CORT and elevated feather CORT (Will et al. 2014), this is in contrast to our results on its own (estimated change 0.96; 95% CI = 0.87–1.05) or after accounting for diet treatment (estimated change 0.97; 95% CI = 0.91–1.04; Fig. 2d).

Three models for CORT in primary coverts accounted for 76% of the model weights and were within 0.20 AICc (Table 3). The model with the lowest AICc included an effect of sample mass, indicating that average feather CORT increased by a factor of 1.02 (95% CI = 1.01–1.04) for every 1 mg increase in sample mass (Fig. 3a). In the second best model, feather CORT was lower on average in chicks on the restricted diet by a factor of 0.76 (95% CI = 0.60–0.91; Fig. 3b). The third competing model was a combination of these two main effects. The model including a main effect for feather growth rate was only 2.41 AICc higher than the best model, indicating weak support for a positive relationship between feather growth rate and feather CORT. Models including an effect of baseline plasma CORT or maximum plasma CORT received no support. The results for primary coverts were comparable to what was observed in 10th primaries.

Discussion

We found that the concentration of CORT in feathers of Caspian tern chicks was negatively related to nutritional restriction, even though baseline and stress-induced plasma CORT concentrations were elevated. This relationship was consistent across two feather tracts. Our result could reflect higher deposition rates of all feather materials in chicks fed ad lib: feathers of the ad lib chicks were heavier and grew faster than feathers of chicks on the restricted diet. There was evidence of a positive relationship between feather CORT and mass of the feather sample in 10th primaries and primary coverts, supporting the hypothesis that feather CORT deposition is influenced by the amount of material incorporated into growing feathers. Food limitation or reduced body condition during molt can reduce feather growth rates and result in feathers that are shorter and lighter (Strochlic and Romero 2008, DesRochers et al. 2009, Vágási et al. 2012). If CORT is passively deposited during feather formation, as has been proposed (Bortolotti et al. 2008, 2009), then less dense feathers would be expected to have lower feather CORT concentrations regardless of circulating CORT levels. This was the most likely explanation of lower feather CORT concentrations in feathers from chicks on restricted diets, despite elevated levels of circulating CORT for this group.

Table 3. AICc model comparisons examining the relative effects of feather mass, feather growth rate, maximum plasma CORT, and baseline plasma CORT on feather CORT (fCORT) concentrations in primary coverts (pg mm⁻¹). Table includes the number of parameters in each model (K), the difference between each model and the top model (ΔAICc), and model weights (w_i).

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>K</th>
<th>ΔAICc</th>
<th>w_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(fCORT) – Feather mass</td>
<td>−3.23</td>
<td>3</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td>log(fCORT) – Treatment</td>
<td>−3.20</td>
<td>3</td>
<td>0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>log(fCORT) – Feather mass + Treatment</td>
<td>−3.03</td>
<td>4</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>log(fCORT) – Feather growth</td>
<td>−0.82</td>
<td>3</td>
<td>2.41</td>
<td>0.08</td>
</tr>
<tr>
<td>log(fCORT) – 1</td>
<td>2.07</td>
<td>2</td>
<td>5.30</td>
<td>0.02</td>
</tr>
</tbody>
</table>

There was no support for an effect of plasma CORT on feather CORT in our AICc model comparisons. We observed a weak positive relationship between maximum stress-induced plasma CORT and feather CORT in 10th primaries (Fig. 2b). This relationship only occurred within diet treatments and this model had no support compared to the models that included feather mass – an indication that circulating plasma CORT had an effect on feather CORT, but was not the primary driver of feather CORT concentrations.

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for Caspian tern chicks. Unlike the response of Caspian terns, food limited rhinoceros auklet chicks preferentially allocated resources to feather growth at the expense of other tissues. Restricted auklet chicks grew feathers with similar mass, length, and growth rate as the unrestricted chicks (Sears and Hatch 2008). Variation in feather development in response to nutritional stress is the main difference between these two studies, providing support for our hypothesis that lower feather density was associated with decreased CORT deposition in feathers of Caspian tern chicks raised on restricted diets.

Nestlings of different species respond to nutritional limitation in different ways (Schew and Ricklefs 1998). As discussed above, rhinoceros auklet chicks on restricted diets preserved feather growth at the expense of mass gain and skeletal growth (Sears and Hatch 2008). In common murres Uria aalge primary growth was more sensitive to change in diet quantity and quality than all other morphological measurements (Benowitz-Fredericks et al. 2006). The effect of nutritional limitation on feather growth of Caspian tern chicks was intermediate to these two examples; feather mass and growth rate were reduced, but feathers were not affected as strongly as skeletal growth or mass gain (Lyons and Roby 2011). Additional studies of species with different feather growth responses to nutritional stress would enhance our understanding of the link between feather quality and feather CORT deposition.

Our experiment involved a substantial nutritional restriction (a 35% decrease from an ad lib diet), at lower levels of nutritional restriction circulating CORT might increase without any effect on feather quality and feather CORT might more closely track plasma CORT concentrations. Within species comparisons of the feather CORT/plasma CORT relationship for different levels of dietary restriction would also improve interpretation of feather CORT from free-living chicks.

Three other studies that have related feather CORT to circulating plasma CORT found higher feather CORT was related to higher plasma CORT concentrations (Bortolotti et al. 2008, Lattin et al. 2011, Fairhurst et al. 2013). Two of these studies involved adults induced to molt flight feathers; the process of CORT deposition in adults replacing feathers could conceivably be different than for chicks growing new feathers. Free living adults undergoing molt would presumably have fewer energetic demands than a developing chick and more flexibility to adapt to limited food availability (e.g. by delaying the timing of molt, re-growing feathers more slowly, or migrating to a different area), therefore, there may be few circumstances where adult feather density would be reduced enough to effect CORT deposition.

We observed a decreasing temporal trend in feather CORT within the same feather, which was consistent between diet treatments and did not reflect changes in concentrations of circulating plasma CORT. Methanol extractions may be less effective with greater sample mass (Millsbaugh and Washburn 2004, Wasser et al. 2010, Lattin et al. 2011), which could explain the differences we observed among feather sections and supports a previous conclusion that samples from different sections of a feather or different feather tracts are not directly comparable (Lattin et al. 2011). Extraction efficiency does not explain our overall result that nutritional stress had a negative effect on feather CORT; this would have resulted in higher feather CORT levels in the restricted group, which had lighter feathers. Instead, there was a positive relationship between sample mass and feather CORT within sections taken from the same feather region. The question of how CORT deposition varies along the shaft of a feather deserves more experimental investigation and highlights the importance of carefully selecting which feather groups and sections to compare.

Our study shows that the interpretation of feather CORT may be equivocal unless patterns of feather growth and material deposition are known. We demonstrated that feather density can have a stronger effect on feather CORT than plasma CORT, which resulted in a negative relationship between nutritional stress and feather CORT. Results of this study indicate that feather CORT can be used for detecting nutritional stress in growing birds; however, it would be inappropriate to interpret lower feather CORT measurements as an indication of lower CORT secretion when there are systematic differences in feather density. Feather CORT has the potential to be an informative tool for measuring long-term hormonal activity in birds. Without experimental validation, researchers should refrain from making direct inference between levels of CORT in feathers and CORT secretion. The relationship we observed between feather density and feather CORT supports the hypothesis that feather CORT is passively deposited during feather growth (Bortolotti et al. 2008, 2009). Resolving the question of how CORT is deposited in feathers should be a priority for future research.

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References


