Yolk and albumen corticosterone concentrations in eggs laid by white versus brown caged laying hens

K. J. Navara1 and S. E. Pinson

Department of Poultry Science, University of Georgia, Athens 30602

ABSTRACT Maternal stress in birds can have permanent transgenerational effects through the transmission of stress hormones to offspring via the egg yolk. Previous studies have shown that White Leghorn hens show a heightened response to stress compared with Hy-Line Brown hens, producing significantly more corticosterone and displaying longer bouts of tonic immobility after handling, whereas baseline levels of corticosterone are similar between the strains. We tested the hypothesis that higher stress responsiveness would correspond to chronic accumulation and thus higher concentrations of corticosterone in egg yolks after exposure to stressors associated with routine maintenance. Eggs were collected from white and brown hens that were undisturbed except for daily feeding and routine egg collections. Corticosterone was quantified in plasma, egg yolks, and albumen and compared between strains. We predicted that corticosterone concentrations in yolk would be higher in eggs from white versus brown hens but that albumen corticosterone would not differ between strains due to the short term of albumen deposition. As predicted, yolk corticosterone concentrations were significantly higher in eggs produced by white hens, approximately twice those found in eggs laid by brown hens. Plasma and albumen concentrations of corticosterone were similar between groups. These results suggest that offspring hatching from eggs laid by White Leghorn hens are exposed to significantly more corticosterone through concentration in the egg yolk, which could permanently imprint offspring physiology and behavior.

INTRODUCTION

Exposure to stress can have serious effects on poultry from both welfare and production standpoints because hens exposed to stress not only exhibit fearful behaviors and physiological impairments, but stress also stimulates decreases in egg productivity (Mashaly et al., 2004) and can have long-term effects on offspring health as well (Hayward and Wingfield, 2004; Hayward et al., 2005; Janczak et al., 2007; Satterlee et al., 2007). When environmental changes are perceived as a threat, birds generate a stress response characterized by a neuroendocrine cascade through the hypothalamic-pituitary-adrenal (HPA) axis that ultimately results in the production of the stress hormone corticosterone (CORT; reviewed in Siegel, 1995). Corticosterone exerts many physiological effects on the female and also accumulates in eggs in both a chronic manner through incorporation into the yolk during the rapid yolk deposition phase of follicular development (Rettenbacher et al., 2005) and in an acute manner through passage into the albumen in the magnum just after ovulation (Downing and Bryden, 2008). Previous studies have shown spikes in both yolk and albumen CORT in response to stressful conditions (Bulmer and Gil, 2008; Downing and Bryden, 2008). Hormones in egg yolks and albumen can permanently affect the developmental trajectories of offspring. Yolk CORT, in particular, exerts mainly detrimental effects on chicks, decreasing growth, impairing immunity, and decreasing overall survival (Eriksen et al., 2003; Love et al., 2005; Rubolini et al., 2005; Saino et al., 2005). As a result, environmental conditions that modulate CORT production in females can have substantial transgenerational effects as well.

The magnitude of the hormonal response to a stressor as well as the behavioral response to that stressor in poultry appears to be strain-specific. Fraisse and Cockrem (2006) showed that White Leghorn hens produce significantly higher concentrations of CORT and exhibit longer durations of tonic immobility in response to a handling stressor compared with Hy-Line Brown hens. In general, white strains are more flighty and fearful than brown strains (Murphy, 1977). Inter-
Corticosterone content in egg yolk can be used as a long-term measure of CORT production in a female because yolk accumulates over a period of 7 to 12 d (Johnson, 1986). We predicted that eggs produced by white hens would contain greater concentrations of CORT in the egg yolk compared with eggs from brown hens due to overall differences in responsiveness to routine stressors during yolk deposition. Albumen, on the other hand, represents a short-term measure of CORT production because albumen deposition takes place over approximately 6 h (Downing and Bryden, 2008). We predicted that albumen CORT and baseline measures of CORT in plasma collected from unstressed birds would not differ between strains because these are short-term measures and would not likely reflect differences in overall responsiveness over time. Through these experiments, we aimed to determine whether concentrations of this key stress hormone, which is known to be deposited into eggs and to influence offspring quality, differ between brown and white hens.

MATERIALS AND METHODS

Bird Husbandry

Hy-Line W-36 and Hy-Line Brown layer hens were housed individually in layer cages. All birds had ad libitum access to food and water and were maintained on a light cycle of 14L:10D. Birds were monitored throughout the day for egg laying and eggs were collected manually. All birds were housed in 1 room and treated similarly, ensuring that interruptions caused by feeding and egg collection were similar for all birds. All eggs were collected on the same day. All animal handling and care was in compliance with the University of Georgia Institutional Animal Care and Use Committee (AUP#A2008-10079).

Yolk, Albumen, and Blood Sample Collection

Egg yolks were collected from eggs of 13 White Leghorn hens and 12 Hy-Line Brown hens and were homogenized and frozen at −20°C until hormone assays. Egg albumen was collected on a separate occasion from 15 white and 11 brown hens and thin and thick albumen were separated manually. Thin albumen was frozen at −20°C until hormone assays. Blood samples were obtained from 7 brown hens and 14 white hens via the brachial vein between 1800 and 2200 h via venipuncture of the brachial vein using a 1-mL syringe and a 27-gauge needle. Sampling occurred during the dark phase using red headlamps for visibility to avoid circadian disruption of the birds. Sampling was done at this time because the beginning of the dark phase is the time when both activity and CORT concentrations are at their lowest (Beuving and Vonder, 1977). Thus, samples collected here represent true baseline concentrations of CORT that are uncomplicated by exposure to daytime stressors. Blood samples were collected within 3 min of initial handling to avoid variation due to handling stress (Romero and Reed, 2004).

Hormone Quantification

Yolk samples were hand-homogenized by mixing and CORT was extracted through a double extraction followed by liquid column chromatography according to methods described by Schwabl (1993). Briefly, 50 mg of yolk was weighed and vortexed with 1,000 µL of deionized water. A small amount of tritiated CORT (1,000 cpm) was added to each sample for later calculation of recovery efficiency. Next, 3 mL of petroleum:dichloromethane (30:70 vol/vol: petroleum ether, BDH1145-4LG; dichloromethane, EX0190-8) was added, and the mixture was vortexed for 30 s and was allowed to settle for 20 min. Samples were then snap-frozen and the supernatant was poured off and dried using a N2 stream. Yolk samples were reconstituted in 1.0 mL of 10% ethyl acetate (JT9280-20) in isooctane (JT9479-3) and individual steroids were separated using Celite column chromatography (Celite Corporation, Lompoc, CA). Steroids were eluted in the following fractions: androstenedione, 2%; testosterone, 10%; and CORT, 50% ethyl acetate in isooctane. Only CORT was quantified for the current study. Extraction of CORT from thin albumen and plasma was done using a single extraction with 3 mL of pure diethyl ether. Corticosterone was quantified in yolk, albumen, and plasma using standard competitive binding RIA as described in Wingfield and Farner (1975). Briefly, samples were resuspended in 300 µL of PBS containing 0.02% sodium azide (EM-SX0199-1) and 0.1% gelatin (200004-458) (PBS gel) and duplicate aliquots of 100 µL each were added to assay tubes. An additional 50 µL was counted in a tube containing 4 mL of scintillation fluid by a scintillation counter to determine extraction recoveries. To each sample tube and to additional tubes containing a graduated curve of CORT, 50 µL of tritiated CORT (approximately 10,000 counts) as well as 50 µL of anti-rabbit anti-CORT antibody (07-120016, MP Biomedicals, Salon OH) were added. All of the tubes were incubated for 18 h, after which 500 µL of charcoal solution (0.25% charcoal, EM-CX0645-1; 0.025% dextran, 40627-1000) was added to each tube. Tubes were incubated for 10 min and centrifuged at 4,060 × g for 10 min. Supernatant was poured off into a scintillation tube, 4 mL of scintillation fluid
was added, and radioactivity was counted in a scintillation counter. Hormone quantities in each tube were analyzed according to the standard curve using a log-logit function. The final concentration for each sample was corrected for its individual extraction recovery percentage. Interassay variation across yolk, albumin, and plasma assays was 16%. Intrassay variation was 9.5 for yolk, 2.8 for albumen, and 3.5 for plasma. Average recoveries were 86% ± 0.03 for albumen, 65% ± 0.01 for yolk, and 90% ± 0.01 for plasma. These methods of quantifying CORT in chicken yolk, albumen, and plasma have each been validated previously (Downing and Bryden, 2008 for albumen and plasma and Royo et al., 2008 for yolk). All reagents were obtained from VWR (Atlanta, GA) unless otherwise noted.

**Statistical Analyses**

Concentrations of CORT in albumen, yolk, and plasma were compared among eggs collected from white versus brown hens using an ANOVA. All hormone data were normalized using log transformation because they were nonnormally distributed according to a Levene’s test for heterogeneity of variance. Data sets were analyzed for the presence of outliers using the Grubbs’ test for outliers. Two outliers were found for measures of yolk CORT. These data points were removed from the analyses. All α values less than 0.05 were considered significant. Statistical analyses were carried out using Statview software (SAS Institute, Cary, NC).

**RESULTS AND DISCUSSION**

Yolk CORT concentrations differed significantly between strains ($F_{1,23} = 10.82, P < 0.01$); concentrations of CORT in egg yolks produced by white hens were twice that in egg yolks produced by brown hens (1.61 ± 0.30 ng/g compared with 0.77 ± 0.176 ng/g; Figure 1A). Corticosterone concentration in albumen samples did not differ between eggs from white versus brown hens ($F_{1,24} = 0.14, P = 0.71$, Figure 1B; 0.44 ± 0.09 ng/mL compared with 0.52 ± 0.13 ng/mL; Figure 1B) nor did baseline concentrations of plasma CORT ($F_{1,19} = 0.03, P = 0.87$; 4.40 ± 1.99 ng/mL compared with 2.8 ± 0.44 ng/mL). Baseline levels of CORT were similar to those shown in other studies of laying hens (Davis et al., 2000; Dehnhard et al., 2003; Fraisse and Cockrem, 2006).

Our data show that, despite similar baseline plasma CORT concentrations in white and brown hens, white hens still accumulate higher levels of CORT in egg yolk over time. This is perhaps due to higher levels of CORT produced in response to routine stressors throughout the time of rapid yolk deposition, which occurs over a period of 7 to 12 d (Johnson, 1986). Prior studies have shown that white hens produce significantly more CORT in response to a handling stress compared with brown hens and show a greater fear response as well (Fraisse and Cockrem, 2006). Birds in the current study were routinely exposed to humans during feeding and egg collections; however, the extent to which the stress response to these routine interactions differs between brown and white hens remains to be tested. Our results differ from a previous study showing no differences in yolk or albumen CORT between white and brown hens (Singh et al., 2009). This disparity could result from specific strain differences among studies or could also result from differences in levels of stress experienced by birds between the 2 studies. For example, if birds in Singh et al. (2009) experienced less routine interruption or less exposure to other stressors, then birds in that study would show less variation and may not exhibit differences between strains. This is because baseline levels of CORT do not differ in these birds, but stress-induced CORT concentrations do (Fraisse and Cockrem, 2006).

Despite differences in yolk CORT, the lack of differences between baseline concentrations of CORT between white and brown hens suggests that neither group is experiencing a chronic elevation of CORT compared with the other. It is possible, however, that CORT concentrations do differ between strains at a different
time of day than when blood samples were collected in the current study. Here, samples were collected during the dark phase, the time of day when CORT concentrations are their lowest (Beuving and Vonder, 1977). However, albumen CORT, which is deposited over a much shorter period of time during the light period, did not differ between eggs produced by white and brown hens, and prior studies suggest that albumen CORT concentrations represent good measures of circulating plasma CORT in hens (Downing and Bryden, 2008). As a result, it is not likely that baseline levels of CORT differ during the light phase either.

Our findings lead us to question whether the differences in stress responsiveness and fear behavior observed between white and brown hens result from genetic differences between strains or whether these differences are driven by early exposure to yolk hormones. Indeed, exposure to elevated levels of yolk hormones can permanently imprint several body systems (Love et al., 2005; Rubolini et al., 2005; Satterlee et al., 2007). For example, implantation of female quail with CORT capsules increased yolk CORT concentrations significantly above controls and also permanently upregulated the HPA axis within offspring (Hayward et al., 2005). Perhaps if the linkage between higher stress responses and higher yolk CORT concentrations was broken for white hens, the stress responses of offspring would resemble the responses of brown hens. This idea remains to be tested.

High levels of CORT in egg yolks stimulates detrimental effects in chickens and other avian species, including reduced developmental stability of offspring (Eriksen et al., 2003), reduced growth (Hayward et al., 2005), reduced reproductive function (Satterlee et al., 2007), and impaired immune responsiveness (Love et al., 2005; Rubolini et al., 2005). However, to our knowledge, no significant differences in performance or survival between offspring of white and brown hens have been observed. Here, white hens deposited more than twice the amount of yolk CORT compared with brown hens, but the amount of CORT in these egg yolks and the range in concentrations was still small (0.8 to 1.6 ng/g). Indeed, many studies directly testing the effects of yolk CORT by experimentally manipulating CORT concentrations in eggs have raised CORT to concentrations above those found in the current study. However, it should be noted that yolk hormone concentrations that resulted from the implants used in Hayward and Wingfield (2004) were between 1 and 2.5 ng/g; these concentrations were in the same range of the levels we detected in our White Leghorn eggs. Thus, the range of variation in yolk CORT found in the current study may be sufficient to exert influence on development of offspring. Perhaps subtle variation in offspring performance that could be influenced by the variation in yolk CORT shown here has gone unnoticed or untested in these strains. Alternatively, perhaps White Leghorns are well-adapted to the ingrained level of stress responsiveness, thus reducing effects experienced by offspring.

Future studies should examine influences of this range of yolk CORT on offspring of white and brown hens as well as whether offspring from each strain have different abilities to respond to similar levels of yolk CORT.

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REFERENCES


