Acta Agriculturae Scand Section A, 2009; 59: 80–84

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Environmental effects on steroid hormone concentrations in laying hens' eggs

A. M. JANCZAK[1], P. TORJESEN[2] & S. RETTENBACHER[3]

[1]*Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Sciences, N-0033 Oslo, Norway,* [2]*Hormone Laboratory, Oslo University Hospital, Aker, N-0514 Oslo, Norway, and* [3]*Institute of Biochemistry, Department of Biomedical Sciences, University of Veterinary Medicine, A-1210 Vienna, Austria*

Abstract
The hormone content of a birds' egg may reflect the environmental conditions of the bird. In this study we measured hormone concentrations of eggs from hens living under different housing conditions. Eggs from 16 floor-housed and 16 singly caged hens were analysed for androstenedione and estradiol. The concentrations of these hormones were highest in the yolk of eggs from floor-housed hens ($P \leq 0.05$). The concentration of estradiol in the albumen of eggs was highest for caged birds ($P \leq 0.0001$). In caged hens, the concentrations of both hormones varied significantly over days in the egg yolk, but not in the albumen. As the concentration of androstenedione and estradiol in the yolk of chicken eggs is environmentally dependent, these hormones may provide a mechanism by which the hen signals the state of the environment to her progeny.

Keywords: *Chicken, egg, gonadal steroids, maternal effects, poultry.*

Introduction

Apart from genetic inheritance, parents, especially the mother, can influence offspring development by the transfer of non-genetic material. Research on hormone-mediated maternal effects in birds has highlighted the influence of variable yolk steroid hormone concentrations on offspring phenotype (morphology, physiology and behaviour). Pioneered by Schwabl (1993), research on yolk steroid hormones, mostly androgens and their possible implications for the offspring evolved rapidly (for reviews see Gil, 2003, 2008; Groothuis et al., 2005). Interestingly, environmental conditions experienced by the laying female can result in modulated yolk androgen concentrations, suggesting an influence of the environment on the physiology of the mother and consequently on the development of her offspring (Schwabl, 1996, 1997; Whittingham & Schwabl, 2002). However, as most research focuses on non-domestic species, there is little information on how environmental cues translate into modified steroid hormone profiles in the domestic chicken egg. Housing conditions of chickens have been demonstrated to be a potential factor to influence the endocrine status of the animals (Hester, 2005). Therefore, also in conventional laying hen and broiler breeds, maternal effects may contribute to phenotypic variation in their progeny. It has been clearly demonstrated that administration of steroid hormones, such as corticosterone into chicken eggs has the potential to alter a suite of behavioural as well as physiological traits of the developing embryo (Eriksen et al., 2003; Janczak et al., 2006). Unlike in other bird species, where research mainly focuses on maternal androgens, little is known about steroid hormones, such as androgens and estrogens and their modulation by environmental conditions in chickens. This information is important for the development of valid experimental models of maternal effects in chickens (see Lindqvist et al., 2007), and could also have potential implications for recommendations regarding housing of layer or broiler breeders pending further studies on the effects of varying egg steroid hormone content.

Correspondence: Andrew M. Janczak, Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Sciences, N-0033 Oslo, Norway. Tel: +47 22 59 74 76. E-mail: andrew.janczak@veths.no

(Received 24 December 2008; revised 27 April 2009; accepted 6 May 2009)

ISSN 0906-4702 print/ISSN 1651-1972 online
DOI: 10.1080/09064700903023348

The egg yolk is formed in the ovary from proteins and lipids produced in the liver. Thus, the hormone levels of the yolk should represent the hormone levels in the ovary during the phase of continuous yolk formation from 5 to 6 days before ovulation (Moran, 1987). The albumen proteins are formed by cells located at the oviduct, mainly in the magnum mucosa. As these cells do not produce hormones, the general serum levels of hormones during the gradual accumulation of albumen over 2–4 hours during egg formation, should be reflected in the albumen fluid. A positive correlation between plasma and albumen corticosterone concentrations was found (Downing & Bryden, 2008).

The present experiment aimed at describing the influence of housing conditions on androstenedione and estradiol concentrations in chicken eggs collected from hens living under loose-housing conditions at a low density and from hens living in single cages at a higher density. Eggs were collected at day 14, 18 and 21 after the caged hens were moved from the floor system to single cages and hormone levels in yolk and albumen were determined.

Material and methods

Birds

Thirty-two female and five male White Lohmann-selected Leghorns were loose-housed on the floor from the time of hatching in a single solid walled room (540 × 250 cm) on pine sawdust at a density of 2.74 birds m^{-2}. Birds were fitted with numbered leg bands at five weeks of age to allow individual identification. The temperature of the room in which chicks were housed was held at 32°C in the first two days, and gradually reduced to 20°C by six weeks of age. The animals had daily one hour of darkness in the first week (24:00–01:00 h), and 12 hours of darkness thereafter (18:00–06:00 h). Birds had ad libitum access to water (four troughs per pen) and conventional starter feed for laying hens in round feeders (four feeders per pen) placed at ground level the first two days of life and at head height from the third day of age. Birds were fed and water troughs were cleaned every day between 9:00 and 10:00 h. Birds had access to perches from hatching and elevated nest boxes were provided from 14 weeks of age.

Treatments

At the age of 20 weeks, 16 of the hens were moved to another room where they were housed individually in cages measuring 24 × 45 cm (9.25 birds m^{-2}) for the remainder of the experiment under the same lighting and temperature conditions as described previously. This comprised the cage treatment condition. The 16 birds in the floor-treatment were kept in the original unaltered pen together with the original five cocks at a density of 1.55 birds m^{-2}.

Norwegian legislation limits the maximum density of laying hens in traditional pens to 14 birds m^{-2} and for loose-housed hens to 9.5 birds m^{-2}. Experimental birds were housed at 2.74 birds m^{-2} before treatment. This was changed to 1.55 birds m^{-2} for birds on the floor system and 9.25 birds m^{-2} for birds in cages during the treatment. The floor-housed hens were thus housed at very low densities, whereas the caged hens were housed at a density similar to that used for conventional loose-housed hens. The main argument for using the present design was not to mimic conventional bird densities but to suddenly change the housing environment and severely limit the movement of birds in pens (as a putative stressor) and compare them to birds that could move freely and interact with cockerels at a low density.

Egg sampling

Eggs produced by hens in both treatment conditions were collected on three different days (14, 18 and 21), starting 14 days after the treatment was applied in order to measure concentrations of androstenedione and estradiol in egg yolk and egg albumen. After an egg was opened onto a plate, a pipette was applied to the outermost layer of the albumen and then the yolk in order to collect a 5 ml sample of each. Neither the yolk nor the albumen was homogenised prior to sampling. After collection, samples were stored at −20°C for 1–2 months prior to analysis.

Hormone measurement

In the lab, the thawed chicken egg yolk and albumen samples were extracted with diethyl ether. Recovery rates were not determined in this analysis but are about 75% for similar protocols with bird yolk (Rutstein et al., 2004) and about 85–95% if the very same extraction method is applied to other biological material (Torjesen, personal observation). Androstenedione was extracted from the yolk as described by Schwabl (1993), with slight modifications, using 10 ml of diethyl ether for initial extraction. The final extracts were dissolved in 2 ml 2,2,4-trimethylpentane of which 0.2 ml was used for the androstenedione assay. A separate extract was made for the assay of estradiol in the yolk. In this case the final extract was dried in nitrogen and then dissolved in the appropriate assay buffer for the estradiol assay.

Extractions from the albumen were made by adding 0.5 ml distilled water to 0.5 ml albumen, and extracting with 7 ml of diethyl ether. After freezing, the ether phase was dried under nitrogen and the residue was dissolved in the appropriate assay buffer.

Estradiol was determined using a fluoroimmunoassay according to the instructions of the kit (DELFIA, Perkin Elmer, Turku, Finland). The analytical sensitivity of the assays is 13.6 pg/ml. The intra-assay coefficient of variation was 5% and the inter-assay coefficient of variation was 10%. Androstenedione was determined using an in house radioimmunoassay (RIA) employing a specific antiserum (Cat. No A0795, Sigma-Aldrich, St. Louis, MO, USA). Cross-reactivity of the androstenedione antibody was 67% for 5α-androstane-3,17-dione and 6% for dehydroepiandrosterone. The intra-assay coefficient of variation for androstenedione was 5% and the inter-assay coefficient of variation was 10%. The sensitivity of the androstenedione assay was 10 pg/ml.

Statistics

The data conformed to the assumptions of normality. Data collected from caged birds were therefore analysed using a repeated measures analysis of variance testing for the effect of the day on which eggs were collected (day 14, 18 and 21) on the concentrations of androstenedione and estradiol in egg yolk and egg albumen. Egg samples from floor-housed birds were excluded from this analysis because they were not identified individually. A separate analysis of variance was used to test for treatment effects (floor or cage housing) on egg hormone content. For models that were significant when testing for treatment effects on hormone concentrations, the treatments were subjected to a post-hoc comparison of means within sample collection day using a two-tailed *t*-test. Inter-individual and intra-individual coefficients of variation (CV) were calculated to facilitate description of variability of the steroid hormones in egg yolk and egg albumen. The intra-individual CV's were calculated for each bird over the three days on which eggs were collected from cage-housed birds. Inter-individual CV's were calculated as the mean of the CV's for different days.

Results

Caged birds had lower egg yolk concentrations of androstenedione ($F_{(1,91)} = 31.71$; $P \leq 0.0001$) and estradiol ($F_{(1,91)} = 5.50$; $P \leq 0.02$) than egg yolks from floor-housed birds. The egg albumen concentration of androstenedione ($F_{(1,91)} = 0.02$; $P \leq 0.90$) was not effected by housing conditions, but the concentration of estradiol in the egg albumen was highest for hens in cages ($F_{(1,91)} = 27.11$; $P \leq 0.0001$; Figure 1). In caged birds, a significant variation between days was found for egg yolk concentrations of androstenedione ($F_{(2,45)} = 19.88$; $P \leq 0.0001$) and estradiol ($F_{(2,45)} = 6.47$; $P \leq 0.004$). In the egg albumen, no significant variation between days was observed: androstenedione ($F_{(2,45)} = 0.77$; $P \leq 0.47$) and estradiol ($F_{(2,45)} = 1.80$; $P \leq 0.18$). The intra and inter-individual CV's for androstenedione levels in the yolk were 48 and 34%, respectively, and 17 and 21% in the albumen. Both intra and inter-individual CV's were 25% for estradiol in the yolk and 18 and 15% in the albumen.

Discussion

The estradiol and androstendione concentrations were about five to ten times greater in the yolk than in the albumen. Thus, the yolk levels of these hormones appear to represent local ovarian hormone production during the phase of yolk formation, while the albumen concentrations most likely represent hormone levels in the general circulation during the phase of albumen deposition.

Variation between groups

To the knowledge of the authors, this is the first experiment establishing environmental influences on the concentrations of androstenedione and estradiol in chicken's eggs. The concentrations of androstenedione and estradiol were higher in the yolk of eggs from hens housed on the floor, whereas caged birds had higher albumen concentrations of estradiol.

For wild bird species such as sparrows (Schwabl, 1997) and tree swallows (Whittingham & Schwabl, 2002), where higher yolk androgen levels were found in colonies with higher breeding density and therefore more female–female interactions, it has been suggested that these elevated hormonal levels are a result of aggressive encounters with conspecifics (Gil, 2008). Living on the floor enables all kinds of social interactions between individual chickens (Hester, 2005). Therefore, the different levels of androstenedione and estradiol in our experiment could also be an effect of the two housing environments which differed mainly in terms of available space and social conditions. The results could also indicate that differences in activity may be important for the yolk steroid content of hen's eggs. Another explanation could be that females in the cages had lower levels of steroids due to lack of the stimulating presence of the males (Gelez & Fabre-Nys, 2004) or that gonadal activity was downregulated because of stress (Gibson et al., 1986).

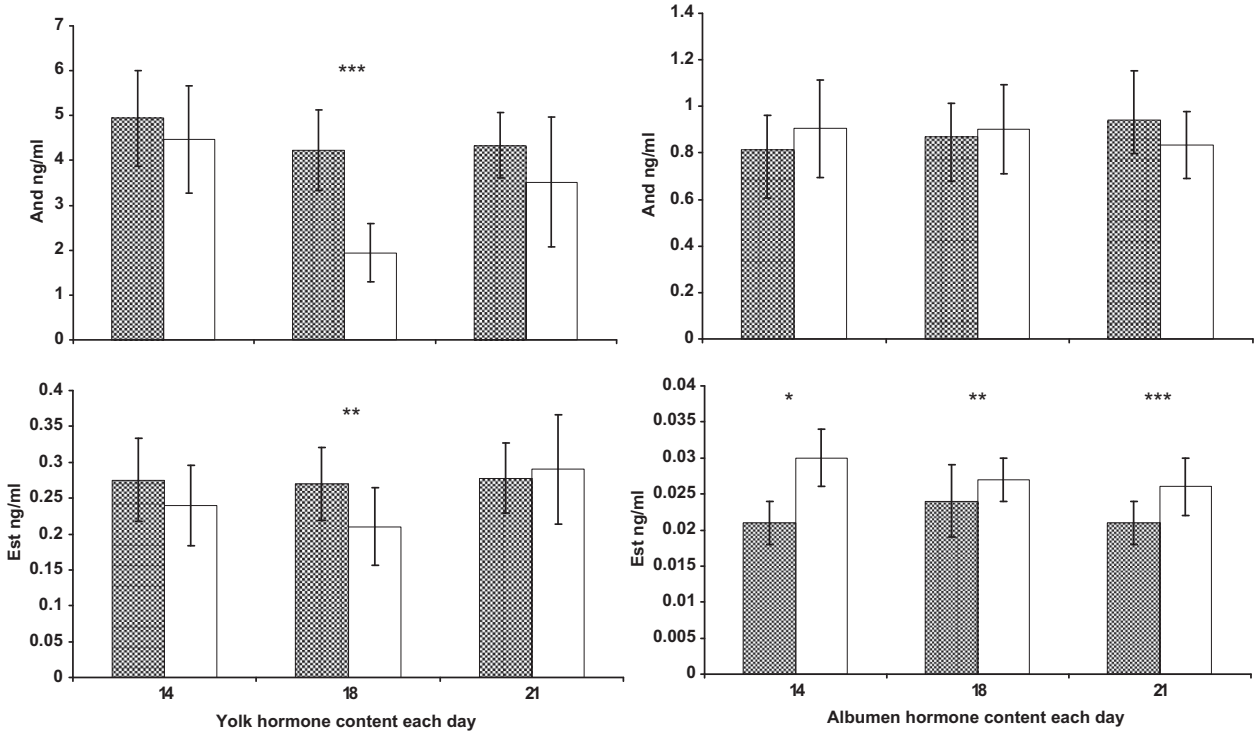

Figure 1. Mean and standard deviation (SD) for hormones in the yolk and albumen of eggs from hens living on the floor (grey bars) or in cages (white bars). Significant differences are presented as: *($P \leq 0.05$), **($P \leq 0.01$) and ***($P \leq 0.001$), based on a two-tailed *t*-test.

Treatment effects on the albumen steroid content of eggs were found only for estradiol concentrations, which were highest for eggs from hens housed in cages. Estradiol is a rather polar substance and is therefore more likely than androstenedione to diffuse from the more lipophilic yolk into the albumen. The low overall concentrations of estradiol are in accordance with others' findings (e.g. Elf & Fivizzani, 2002; Williams et al., 2005), however up to now it is not known if such low levels are of biological relevance. The different timing of albumen and yolk production (Sharp, 1999) might explain why treatment effects on albumen hormone concentrations were not observed in the present experiment. Eggs were collected 14 days after the treatment was started and therefore it is possible that changes in albumen levels may have occurred prior to sampling and then stabilised as reported by Downing and Bryden (2008).

Variation between days

The concentrations of androstenedione and estradiol varied significantly between the days on which egg samples were collected. In numerous bird species, hormone concentrations change within clutches (Groothuis et al., 2005). Both increasing as well as decreasing patterns have been described (Groothuis et al., 2005). The changing concentrations found in the present study could be the result of a clutch-like increase or decrease of hormone levels over the laying period. In the domestic chicken, Elf and Fivizzani (2002) found no changes in hormone concentrations of six consecutively laid eggs but found significant differences between individual females. However in our study, the variation of steroid hormones between days within the same individual and between individuals on the same day appeared to be very similar. A larger difference was found between the CV for steroid hormone measured in the yolk and albumen, as there tended to be a larger variation in hormones measured in the yolk than for hormones measured in the albumen. This was especially pronounced for androstenedione, which had an intra-individual CV of 48 in the yolk but only 17 in the albumen. This finding might be partly explained by the fact that the yolk was not homogenised prior to analysis, which probably contributed additional variation.

Our findings establish that the concentrations of steroid hormones in yolk and albumen of laying hens are environmentally dependent. This suggests that the environmental conditions of layer hens may have consequences for the development of their progenies' phenotype via steroid-mediated maternal effects. Further research should thus focus on the effect of different housing systems on offspring development and performance in both layer and

broiler breeders, which would be relevant for basic as well as for applied research and might even contribute to ameliorating housing conditions for parent birds.

Acknowledgements

This work was funded by the Norwegian Research Council and the Norwegian Centre for Poultry Science. Morten Bakken, Janicke Nordgreen and three anonymous reviewers gave helpful comments to this article.

References

Downing, J. A., & Bryden, W. L. (2008). Determination of corticosterone concentrations in egg albumen: A non-invasive indicator of stress in laying hens. *Physiology of Behavior*, *95*, 381–387.

Elf, P. K., & Fivizzani, A. J. (2002). Changes in sex steroid levels in yolks of the leghorn chicken, *Gallus domesticus*, during embryonic development. *Journal of Experimental Zoology*, *293*, 594–600.

Eriksen, M. S., Haug, A., Torjesen, P. A., & Bakken, M. (2003). Prenatal exposure to corticosterone impairs embryonic development and increases fluctuating asymmetry in chickens. *British Poultry Science*, *44*, 690–697.

Gelez, H., & Fabre-Nys, C. (2004). The "male effect" in sheep and goats: A review of the respective roles of the two olfactory systems. *Hormones and Behavior*, *46*, 257–271.

Gibson, S. W., Hughes, B. O., Harvey, S., & Dun, P. (1986). Plasma concentrations of corticosterone and thyroid hormones in laying fowls from different housing systems. *British Poultry Science*, *27*, 621–628.

Gil, D. (2003). Golden eggs: Maternal manipulation of offspring phenotype by egg androgen in birds. *Ardeola*, *50*, 281–294.

Gil, D. (2008). Hormones in avian eggs: Physiology, ecology and behaviour. *Advances in the Study of Behavior*, *38*, 337–396.

Groothuis, T. G. G., Müller, W., von Engelhardt, N., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews*, *29*, 329–352.

Hester, P. Y. (2005). Impact of science and management on the welfare of egg laying strains of hens. *Poultry Science*, *84*, 687–696.

Janczak, A. M., Braastad, B. O., & Bakken, M. (2006). Behavioural effects of embryonic exposure to corticosterone in chickens. *Applied Animal Behaviour Science*, *96*, 69–82.

Lindqvist, C., Janczak, A. M., Nätt, D., Baranowska, I., Lindqvist, N., Wichman, A., Lundeberg, J., Lindberg, J., Torjesen, P. A., & Jensen, P. (2007). Transmission of stress-induced learning impairment and associated. Brain gene expression from parents to offspring in chickens. *PLoS ONE*, *2*, e364 (doi:10.1371/journal.pone.0000364).

Moran, E. T. (1987). Protein requirement, egg formation and the hens ovulatory cycle. *Journal of Nutrition*, *117*, 612–618.

Rutstein, A. N., Gilbert, L., Slater, P. J. B., & Graves, J. A. (2004). Mate attractiveness and primary resource allocation in the zebra finch. *Animal Behavior*, *68*, 1087–1094.

Schwabl, H. (1993). Yolk is a source of maternal testosterone for developing birds. *Proceedings of the National Academy of Sciences USA*, *90*, 11446–11450.

Schwabl, H. (1996). Environment modifies the testosterone levels of a female bird and its eggs. *Journal of Experimental Zoology*, *276*, 157–163.

Schwabl, H. (1997). The contents of maternal testosterone in house sparrow *Passer domesticus* eggs vary with breeding conditions. *Naturwissenschaften*, *84*, 406–408.

Sharp, P. J. (1999). Chickens, control of reproduction in. In E. Knobil and J. D. Neill (Eds.), *Encyclopedia of Reproduction* (pp. 572–580). London: Academic press.

Whittingham, L. A., & Schwabl, H. (2002). Maternal testosterone in tree swallow eggs varies with female aggression. *Animal Behavior*, *63*, 63–67.

Williams, T. D., Ames, C. E., Kiparissis, Y., & Wynne-Edwards, K. E. (2005). Laying-sequence-specific variation in yolk oestrogen levels, and relationship to plasma oestrogen in female zebra finches (*Taeniopygia guttata*). *Proceedings: Biological Sciences*, *272*, 173–177.