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# Chronic Stress in Battery Hens: Measuring Corticosterone in Laying Hen Eggs

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**Abstract:** There has been growing social and scientific interest in recent years in laying hen welfare. Stress is an animal welfare parameter that has been found to alter laying hens' physiology and social behaviour. Our study aims to test the effects of the different housing systems (laying cage, barn and free range) on the laying hens' welfare. In this study we have taken a new approach to determine stress levels by measuring corticosterone and androgen concentrations in the eggs laid by hens housed in different farming systems. We found no relationship between commercial egg production type and androgen (testosterone and androstenedione) levels. Contrary to our expectations, we observed an almost significant positive trend between corticosterone levels and welfare quality: free range eggs contained the highest corticosterone concentrations. This would initially suggest that free range hens are more stressed than battery or barn hens. However, considering that chronic stressors can reduce an organism's hormonal output, our results could suggest that the low levels of corticosterone in battery hens are an indication of a chronic stress situation. Our data suggest that measuring corticosterone in eggs is a novel non-invasive method to determine stress levels in laying hens. This study would however need to be combined with a number of parameters (health, production and behaviour) to get an encompassing measure of laying hen welfare.

**Key words:** Corticosterone, laying hen, laying hen welfare, maternal effect, stress, testosterone

## Introduction

There has been growing interest in laying hen welfare in recent years, both in the scientific community and among the general public. Much of the present European legislation concerning laying hen welfare has been influenced more by public than by scientific evidence (Savory, 2004). Public opinion considers that the conventional laying cage system provides poor welfare for laying hens, preventing them from carrying out several of their natural behaviours, such as dust-bathing or nest building (Weeks and Nicol, 2006).

Public interest has grown in the last few years to improve laying hen welfare and ameliorate their housing conditions. Present day commercial production in the United Kingdom and Europe encompasses three main production system types; laying cage, barn and free range.

**The laying cage (battery system):** In this system hens are unable to carry out a wide range of natural behaviours due to space (550 $cm^2$ per individual) and equipment limitations. This housing method has received considerable social criticism and is to be abolished by EU legislation by 2012 in favour of enriched battery cages (750 $cm^2$, provided with perches and nesting material) (Council Directive 1999/74/EC).

**The barn system:** Better equipped than conventional laying cages, (i.e. with perches, feeders at different levels, floor areas covered with straw and wood carvings), these cages enable hens to carry out some of their natural behaviours.

**The free-range system:** Hens in this system are able to roam around freely in open spaces (2.5-4 $m^2$ per hen) and exhibit a wide range of natural behaviours. However, hens in this system are more likely to encounter cannibalistic events (Weeks and Nicol 2006).

Prolonged stress responses have been observed to alter an animal's immune functions and behaviour (McEwen and Wingfield, 2003) and are assumed to have a negative effect on animal welfare (Broom and Johnson, 1993). Measuring stress levels in laying hens could consequently be a useful way to control their welfare status. In birds, as in a lot of species Corticosterone is predominating (Nelson, 2000) and has been utilized to diagnose stress levels in different species (Beuving, 1983; von Holst, 1998). Short-term physical stress is a type of stressor that most organisms are faced with most of the time. However problems arise when a stress response is activated for too long or is too frequent, what is referred to as a chronic stress response. In some situations of chronic stress, the hormonal output has been observed to decrease over time (Sapolsky, 1992).

In birds, several studies have noted that social conditions experienced by the mother influence egg corticosterone (CORT) levels (Rettenbacher *et al*., 2004;

Saino *et al.*, 2005; Hayward and Wingfield, 2004). Handling methods, elevated temperatures and the presence of predators have been shown to increase CORT levels in laying hens' eggs (Saino *et al.*, 2005). This suggests that measuring egg CORT levels can be a useful, non-invasive method for determining stress levels in laying hens (Downing and Bryden, 2002; Hayward and Wingfield, 2004; Saino *et al.*, 2005).

It has been demonstrated that yolk androgens are deposited in the eggs as is corticosterone in a context dependent manner by the mother (Gil *et al.*, 1999; Müller *et al.*, 2002; Gilbert *et al.*, 2005). More elevated yolk androgen concentrations have been reported in situations where the mother has experienced some form of stress or aggression (Pilz *et al.*, 2003; Wittingham and Schwabl, 2002).

If these egg steroid hormone concentrations do indeed reflect stress levels experienced by laying hens, measuring these values as a proxy of welfare would have several advantages, a non-invasive sampling procedure, not involving handling or manipulative stress for the animals and integrative measures that would not vary markedly through time as do for instance punctual samples of plasma CORT (Downing and Bryden, 2002). In this study, we aim to determine; firstly whether egg steroid levels are reliable measures of stress in laying hens and secondly through egg steroid analyses which of the different egg production systems is more stressful to the hens. Since, we expect the free range system to be less stressful and more suitable for the hens (Beuving 1983; Broom and Johnson, 1993; von Holst, 1998), we predict eggs hatched in this system to contain lower steroid levels than eggs from more restrictive housing conditions.

## Materials and Methods

Eggs from eleven different brands that varied in egg production systems (laying cage, barn or free range) were bought at several nationally renown supermarkets in St Andrews (Scotland, UK) in November 2004 (total sample size = 57 eggs). Eggs were cracked open, the yolk was separated from the albumen and about 1 mL of each was transferred into separate 1.5 mL Eppendorf tubes and frozen at -20°C.

The hormone extraction and assay methods used in this study are described in detail in Rutstein *et al.* (2005) and Gilbert *et al.* (2005). About 300 mg of yolk were weighed out and the dried extracts from the extraction were resuspended in 300 µL of buffer. Albumin corticosterone was extracted from 500 µL samples using the same method except that three times more ether was used and the ethanol extraction was not necessary. Testosterone (T) and androstenedione (A4) assays were carried out using RIA kits (DSL-3800 and DSL-4000). CORT analyses were carried out with an EIA kit (OCTEIA AC-14F1). Sample sizes were not even because of extraction losses and final figures were: for A4 N = 55, for T N = 57, for CORT N = 42.

We used mixed models to analyze steroid egg concentration, declaring farm nested within farming system as a random factor. Since we predicted a priori an ordered difference between the three farming systems (barn conditions expected to be intermediate between cage and free range), we corrected p-values using a two-tailed ordered heterogeneity test ($r_S P_C$: Rice and Gaines, 1994).

## Results and Discussion

No relationship was observed between the different housing systems and any of the measured androgen concentrations (T: $F_{2,37} = 1.19$, $p = 0.36$; A4: $F_{2,35} = 0.15$, $p = 0.86$). This was unexpected as a female's T deposition in the egg has been shown to be context dependent and to increase with high breeding densities (Müller *et al.*, 2002; Rutstein *et al.*, 2005; Gilbert *et al.*, 2005; Pilz *et al.*, 2003). Increased aggression in females can result in higher T concentrations being deposited in the eggs she lays (Wittingham and Schwabl, 2002).

Contrary to our initial expectations, we found an almost significant trend for egg CORT levels to increase with improved welfare conditions of laying hens ($F_{2,24} = 2.05$, $p = 0.15$; $r_S P_C = 0.05$). Free-range eggs contained the highest CORT levels (Fig. 1), which might initially suggest to us that free-range hens are far more stressed than barn or battery hens. This concurs with the fact that free-range hens experience higher social stress levels compared to hens in isolation because of the pressure of having to feed communally and establish hierarchies. The low CORT levels of battery eggs might also indicate that the hens that laid the eggs were chronically stressed. Chronic stress has been shown to decrease faecal and circulating CORT levels if organisms have experience prolonged or frequent stress. However this is the first study in which chronic stress has been noted in yolk CORT measurements.

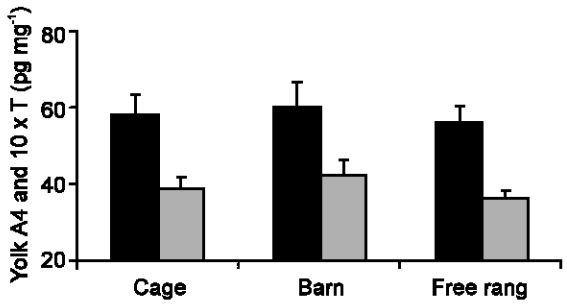


Fig. 1: Relationship between androstenedione (black bars) and testosterone (10x, shaded bars) concentrations and commercial housing types

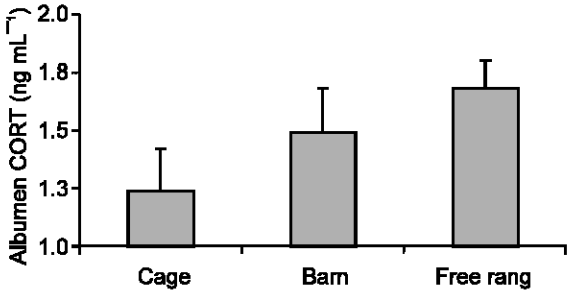


Fig. 2: Relationship between corticosterone concentrations and commercial housing system types

Egg steroid concentrations have been shown to reflect a laying hen's stress levels (Downing and Bryden, 2002), our study's findings are consistent with behavioural observations that agree that battery hens are subjected to unusually high levels of physical stress and are unable to fully express their natural behaviours (Appleby *et al*., 1993; Taylor and Hurnik, 1994).

**Conclusion:** Although, suggestive of chronic stress in battery hens, this study would need to be combined with a number of parameters (health, production, behaviour) to get an encompassing measure of laying hen welfare (Downing and Bryden, 2000) and to confirm the chronic stress condition of battery hens. Although, our study has not assessed the physiological status of the laying hens or the behaviour of the latter, we consider that our results are relevant if we take into account the extensive bibliography that shows context-dependent deposition of steroids in avian eggs (Rettenbacher *et al*., 2004; Saino *et al*., 2005; Hayward and Wingfield, 2004; Gil *et al*., 1999; Müller *et al*., 2002, Gilbert *et al*., 2005). Unlike this pilot study, future studies should be more extensive and make use of a combination of welfare parameters to determine which housing system is more suitable for the welfare of the laying hens (Rushen, 1991; Savory, 2004).

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