

Time course of changes in egg-shell quality, faecal corticosteroids and behaviour as welfare measures in laying hens

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Abstract

The aim of this study was to assess the extent to which three non-invasive measures of welfare in laying hens (egg-shell quality, corticosteroid levels as measured from the birds' faeces and behavioural preferences) were correlated over a period of five days in two groups of birds. One group had access to an enriched test area (bark chips on the floor and a tray of sprouted wheat); the other group had access to a comparably sized barren area (bare wire mesh floor). The measure of preference used was the amount of time hens spent in the test area as measured each day. It was predicted that birds with access to the less preferred environment would show higher levels of faecal corticosteroids and egg-shell anomalies. However, although the birds showed a preference for the enriched environment from Day 1, the other two measures did not follow the same pattern. Faecal corticosteroid metabolites showed an initial increase in both groups, which declined significantly by Day 4, with the 'enriched' birds in fact showing a trend for higher levels than the 'barren' birds. Shell thickness also showed a change over the five days, but with a different time course: declining to a minimal level on Day 3 and then rising again by Day 5. No measure of shell quality was significantly different between the two environments, but there was a trend for changes in shell thickness to be more pronounced in eggs from enriched birds. The results indicate the caution that needs to be exercised in using shell quality or corticosteroid measurements in isolation from assessments of what the animals themselves prefer.

Keywords: animal welfare, behaviour, corticosteroid, hens, preference, shell quality

Introduction

Single measures of animal welfare can be very misleading (Dawkins 1980; Broom 1988; Mason & Mendl 1993), yet it remains unclear how different measures — physiological, biochemical or behavioural — should be put together to give an overall picture of an animal's welfare. Physiological measures such as hormonal levels may give very variable results (Rushen 1986, 1991), and behavioural measures such as preference may be misleading because animals do not always choose what is good for their health in the long term (Dawkins 1990; Fraser & Matthews 1997). One of the obstacles to an agreed way of integrating different welfare measures is a lack of information on how the different components are, or are not, correlated with each other, and therefore how increases or decreases over time should be interpreted.

The aim of this study was to take three different measures of welfare in the laying hen — shell quality, levels of corticosteroid as measured in the faeces, and behavioural preference — and to see how they correlated over a period of five days. All three measures are non-invasive and are thus potentially of value as measures that cause minimal interference with the animal, but egg-shell changes are potentially

the most valuable of all for use on commercial poultry farms since they provide a 'window' into a hen's physiological state without disturbing it. Any disruption to a hen's usual environment can induce the formation of abnormal egg-shells (Hughes *et al* 1986; Solomon *et al* 1987). These shell abnormalities are associated with disturbances of the oviduct (Watt 1989), and consequently analysis of egg-shell appearance and structure potentially provides a non-invasive way of assessing bird welfare (Mills *et al* 1987, 1991). However, egg-shell changes cannot yet be confidently interpreted in welfare terms because it is not clear whether the observed shell changes are specific to conditions that indicate reduced welfare (as inferred by other measures) or are a more generalised response to any environmental change, including those that the birds find pleasurable. Until this basic information is available, it is difficult to interpret egg-shell quality in welfare terms.

In this study, the hypothesis that egg-shell changes are indicative of reduced welfare was tested by comparing changes in egg-shell quality and in faecal corticosteroids in two groups of hens: one given access to a preferred environment and the other to a less preferred environment. It was predicted that the hens in the less preferred environment

would show higher levels of both measures. However, since egg-shell changes take some hours to appear, we felt it was important to conduct the experiment over more than one day. This was also important in view of possible changes both in behaviour and faecal corticosteroids as the experiment progressed. Plasma corticosteroid is metabolised by the liver with a half-life of 10-15 mins in chickens, and its metabolites can be picked up from faeces approximately 4 h after an induced change in blood levels (Cockrem & Rounce 1994; Lord 2001; Dehnhard *et al* 2003). We therefore conducted the experiment over a period of five days and looked for correlations between our three measures over this period.

Methods

Animals

The subjects were 32 ISA Brown hens. From the age of 15 weeks, the hens were housed in pens (3.5 × 1 m and approximately 4 m high) in groups of six or eight. The pens had wood-shavings on the floor and each contained a wooden nest box and two perches, so that both the wire mesh floor and the bark chips subsequently presented to them in the experiment were novel. The birds were kept on a 14h light : 10h dark cycle at 18–19°C and were fed a mixture of commercial layer mash, corn and grit on an *ad libitum* basis. [Au: OK?]

For the tests, pairs rather than single birds were used to minimise distress from social isolation. Birds were paired at random with another bird from the same social group and were therefore familiar to one another before the test. Since it was possible to house only a limited number of birds at one time, the birds were tested in two batches (Jan/Feb 1999 and July/August 1999), but the age of the birds at testing was similar (29–34 weeks) for both batches.

Apparatus

Two pairs of birds were tested at any one time in the same room. Each pair was tested in a pen that was divided into two solid-sided compartments connected by a doorway through which the hens could move freely. On one side of the doorway (60 × 30 cm) was a 'facilities' compartment (86 × 86 cm), which contained a feeder, drinking dish and nest box with a floor of wood shavings. On the other side of the doorway was a 'test' compartment (79 × 95 cm). For one group of birds the test compartment was 'barren' and had a fine (3 × 3.5 cm) wire mesh floor, and for the other it was 'enriched' and had a thick layer of bark chips and a tray (23 × 37 cm) of sprouted wheat. The two enclosures were situated next to each other in a controlled temperature room (18°C with a 14 h light period and a light intensity of approximately 200 lux). Two pairs of birds (one in the barren and one in the enriched enclosure) were observed at the same time and their behaviour simultaneously recorded on video. The position of the barren and enriched treatments was alternated each week.

Procedure

Each week for eight weeks, two pairs of birds were removed from their home cages at 0900h on a Monday. One pair was

placed in the facilities compartment of the barren enclosure and the other in the facilities compartment of the enriched enclosure. They remained in the apparatus for five days continuously and their behaviour was recorded on two separate video cameras.

Measures

Shell quality

Eggs were collected from the home pens during the Tuesday, Wednesday and Thursday of the week prior to the first experimental period and used as controls (Day 0). All eggs laid were collected from the experimental pens daily throughout each experimental period. Each egg was given a code number and further assessment of egg-shell quality was carried out 'blind' in terms of treatment type.

The eggs were initially examined to look for any superficial egg-shell abnormalities that would result in them being classed as seconds within the commercial environment. The shells were classified either as normal or abnormal in appearance according to the criteria described by Hughes *et al* (1986). The intact eggs were then weighed to an accuracy of 10 mg. The total thickness, mammillary thickness ('T. Mamm') and effective thickness ('T. Effective') of each egg-shell were subsequently derived by measuring transverse sections of shell with a scanning electron microscope following the methodology of Bain (1990).

The incidence of 12 ultrastructural faults at the level of the mammillary layer was assessed according to the methodology developed by Bain (1990) and later modified by Fraser (1996). As outlined by these authors, these scores were summarised by means of a total score for each egg.

Faecal corticosteroid

At 1200h each day the enclosures were cleared of faeces. All faeces subsequently produced were collected at 1700h for measurement of faecal corticosteroids. The afternoon period was chosen to avoid the morning laying period and the intrinsic glucocorticoid increase that occurs with oviposition and ovulation (Beuving & Vonder 1977, 1981). Samples consisted of faeces combined from both birds in each enclosure: faeces from birds in the enriched enclosure were combined at the point of collection, and faeces from birds in the barren enclosure were collected separately for the purposes of a pilot experiment, but a composite sample was created by mixing equal aliquots of individual samples before hormone extraction. After collection, faeces were either freeze-dried or frozen directly and then freeze-dried. Samples were processed using the solvent extraction and I¹²⁵ radioimmunoassay analysis methods described in Wasser *et al* (2000).

To provide a baseline comparison, faeces were collected from the birds in their home pens during one day of the week before transfer to the experimental enclosures. Each bird was independently observed so that faeces could be attributed to individuals, but because of the labour-intensive nature of this procedure, these 'pre-transfer' faeces were only collected from birds that were to be placed in the barren experimental enclosure. For the first four pairs of

Table 1 Daily means (and SE) for fresh weight and thickness profiles. For each measurement, data sharing superscript symbols († or ‡) are not significantly different ($P > 0.05$).

	Day					
	0 (pre-test)	1	2	3	4	5
Fresh weight (g)	54.5 (0.08)	55.7 (1.23)	56.0 (0.96)	57.3 (1.16)	55.6 (1.04)	56.0 (1.04)
Total thickness (mm)	0.299 (0.002) [†]	0.296 (0.004) [†]	0.288 (0.003) [‡]	0.274 (0.008) [†]	0.293 (0.005) [†]	0.296 (0.004) [†]
T. Mamm (mm)	0.038 (0.0003) [‡]	0.041 (0.0017) [†]	0.034 (0.002) [‡]	0.032 (0.001) [‡]	0.036 (0.002) [‡]	0.037 (0.002) [‡]
T. Effective (mm)	0.26 (0.001) [†]	0.255 (0.004) [‡]	0.255 (0.003) [‡]	0.242 (0.008) [†]	0.257 (0.004) [‡]	0.259 (0.004) [†]

birds, each bird of the pair was observed for alternating hours between 1300–1700h, and all faeces seen to be produced were collected immediately. For the second four pairs of birds, each bird was observed constantly from 1300–1600h.

Environmental preference

The hens' responses to the two environments were assessed by measuring the % of time the birds spent in the test compartment relative to the facilities compartment. If birds with access to the enriched test compartment spent more time in their test compartment compared to birds with access to the barren test compartment, this would be taken as a preference for the enriched environment. Time spent in each compartment was measured from the videotapes by pausing the video every 5 mins and noting whether none, one or both birds were present in the test compartment.

Statistical analysis

A pair of birds was treated as an independent unit for statistical purposes for all response variables. To compare faecal corticosterone levels, behaviour, egg weight and shell thickness profiles between days and treatments, data from enriched and barren-housed birds were analysed with the following General Linear Model (GLM): = Pair (Environment) + Day + Environment + Environment*Day; random: Pair.

To compare superficial egg-shell abnormalities and total ultrastructural score between days, a non-parametric test was needed and Friedman's test was used on the enriched and barren data separately using the model: = Pair + Day. To compare these measures between environments, a Mann-Whitney test was used on data from each day separately.

The following GLM was used to compare faecal corticosterone in barren-housed birds between pre- and post-transfer days: Corticosterone = Pair + Day; random: Pair.

Results

Shell quality

Superficial abnormalities

Abnormal-shelled eggs accounted for only a relatively small percentage (7.7%) of all eggs laid during the trial, so it was not particularly surprising that no significant effects of day or environment were found for this measure.

Egg weight and thickness profiles

No significant differences were found for egg weight with regard to day ($F = 1.61$; $df = 5$; $P = 0.167$). A significant effect of day, however, was found with regard to total shell thickness ($F = 6.31$; $df = 5$; $P < 0.001$), T. Mamm ($F = 3.62$; $df = 5$; $P = 0.006$) and T. Effective ($F = 3.14$; $df = 5$; $P = 0.013$). Further analysis revealed significant differences between the total thickness of shells from Day 3 and all other days apart from Day 2 ($P = 0.015$; Table 1). T. Mamm decreased significantly between Day 1 and Day 3 ($P = 0.031$; Table 1). The T. Effective measure followed a similar pattern to total shell thickness with a significant decrease between the control (Day 0) and Day 3 ($P = 0.014$; Table 1) and a significant increase between Day 3 and Day 5 ($P = 0.044$).

No significant effect of environment (enriched versus barren) was found for fresh weight ($F = 0.37$; $df = 1$; $P = 0.550$) or for any measure of shell thickness (total thickness: $F = 1.98$; $df = 1$; $P = 0.182$, T. Mamm: $F = 0.82$; $df = 1$; $P = 0.380$, T. Effective: $F = 1.62$; $df = 1$; $P = 0.224$). It did, however, appear from Figures 1 and 2 that there was a trend for changes in thickness measurements to be more pronounced in eggs from the enriched pen.

Ultrastructural quality

No significant effect of day was found for total ultrastructural score in eggs from the barren pen ($S = 1.85$; $df = 4$; $P = 0.764$), while significant differences were found in the data from the enriched pen ($S = 10.74$; $df = 4$; $P = 0.030$). The total score significantly decreased between the control (Day 0) and Day 2 ($P = 0.014$; Table 2), indicating improvements in ultrastructure, before displaying a significant increase between Day 2 and Day 5 ($P = 0.042$).

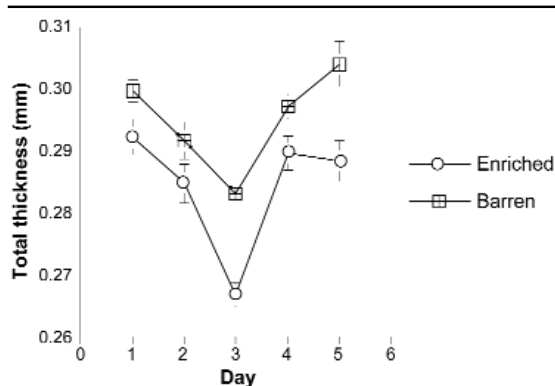
No significant environment effect was found for total ultrastructural score on any of the experimental days (Table 2).

Faecal corticosteroid

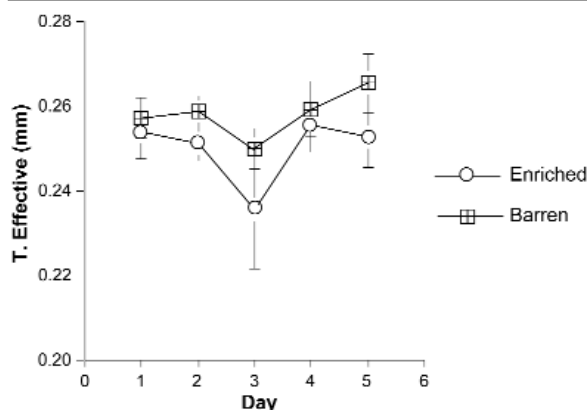
The faecal corticosteroid of birds from both treatments differed significantly between days ($F_{1,13.08} = 7.98$; $P = 0.014$; Table 3), with levels being higher on Day 1 of the experiment than on Day 4. The influence of environment did not quite reach statistical significance ($F_{1,15.20} = 3.52$; $P = 0.080$), although Figure 3 displays a trend towards higher levels of faecal corticosteroid in the enriched environment. There was no interaction between day and environment (Table 3).

Table 2 Pen means (and SE) for ultrastructure total score for each day of the experiment. For each treatment, data sharing superscript symbols are not significantly different ($P > 0.05$).

	Day					
	0 (pre-test)	1	2	3	4	5
Enriched	28.7 (0.41) [†]	27.0 (1.77) ^{‡‡}	25.0 (1.83) [‡]	26.3 (1.00) ^{‡‡}	29.3 (1.27) ^{‡‡}	30.9 (1.76) [†]
Barren	28.7 (0.41)	28.7 (0.81)	29.8 (2.20)	28.7 (1.63)	26.6 (1.36)	28.1 (1.51)

Figure 1

Mean total shell thickness of eggs from enriched and barren pens on different days of the experiment. Vertical bars represent standard errors.

Figure 2

Mean T. Effective values of eggs from barren and enriched pens on different days of the experiment. Vertical bars represent standard errors.

Comparison of pre- versus post-transfer corticosteroid levels for birds transferred to the barren enclosure showed a significant difference between days ($F_{2,14} = 6.28$; $P = 0.011$; Table 4; Figure 4). On Day 1, between 1200–1700h (following transfer at 0900h), corticosteroid levels were significantly higher than on the pre-transfer day ($T = 3.14$; $P = 0.019$). By Day 4, corticosteroid levels had declined so that they were not significantly different to the pre-transfer day ($T = 0.19$; $P = 0.988$), and were significantly lower than they had been on Day 1 ($T = -2.99$; $P = 0.025$).

Table 3 ANOVA table of faecal corticosterone comparison between days and environments (* $P < 0.05$).

Variable	F (df)	P
Pair	0.80 (14)	0.660
Day	7.98 (1)	0.014*
Environment	3.52 (1)	0.080
Day \times Environment	0.07 (1)	0.790

Preference tests

Birds whose test compartment was enriched spent significantly more time in it than did birds whose test compartment was barren ($F_{1,59.75} = 10.68$; $P = 0.002$; Figure 5). The amount of time spent in the test compartment increased significantly over the five experimental days ($Y = 20.005 + 2.6039$ [day]; $P = 0.001$), and this did not vary significantly between environments ($F_{1,62} = 0.94$; $P = 0.335$).

Discussion

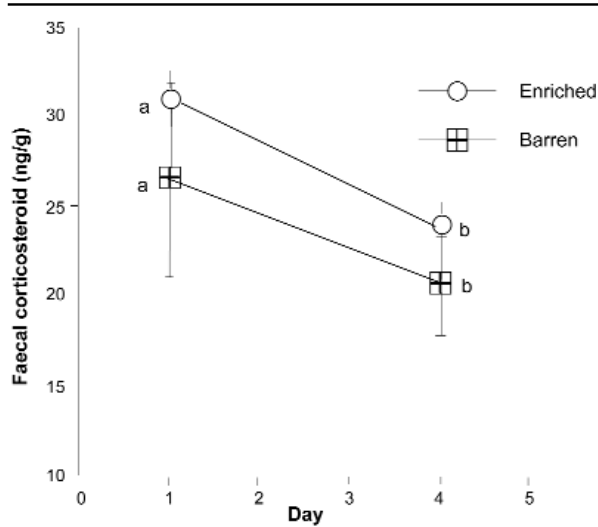
Hens given access to the enriched environment spent more time there than birds given access to the barren environment spent in the comparable part of their pen. In this sense, the enriched environment was the preferred environment. This preference was apparent at the very beginning of the experiment and persisted throughout the five days. However, neither egg-shell quality nor faecal corticosteroid levels followed this pattern.

In both environments, faecal corticosteroid metabolites increased at the beginning of the experiment and decreased again within four days. Contrary to our prediction, the group with access to the preferred environment did not show lower levels of faecal corticosteroids. There was no significant difference between the two groups and, in fact, there was a trend in the opposite direction, with higher corticosteroid levels being recorded in birds with access to the enriched environment.

Similarly, no measure of shell quality differed significantly between the two environments but, again contrary to prediction, changes in shell thickness were more pronounced in the eggs of birds given access to the enriched environment. Shell thickness also showed a change over the five days of the experiment but with a different time course to that of corticosteroids. Shell thickness declined to a minimum on Day 3 and then rose again on Day 5.

Thus, the time from the beginning of the experiment was a better predictor both of faecal corticosteroid levels and egg-shell quality measures than whether birds had access to a more preferred or a less preferred environment. This strongly suggests that for both groups of birds, the effect of

Figure 3



Mean levels of faecal corticosteroid for birds from the two pen types on Days 1 and 4 of the experiment. Vertical bars represent standard errors. Levels sharing letters are not significantly different at $P < 0.05$.

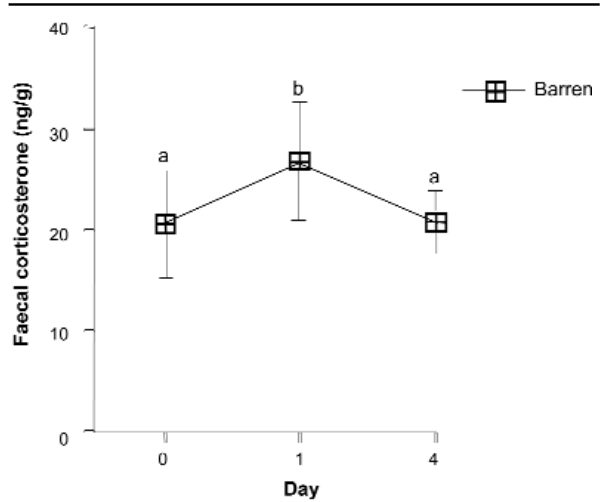
Table 4 ANOVA table of pre- and post-transfer faecal corticosterone comparison for birds transferred to the barren environment (* $P < 0.05$).

Variable	F (df)	P
Day	6.28 (2)	0.011*
Pair	2.70 (7)	0.054

starting the experiment (which included being transferred to the pens from their home cage) and then settling down, was more important than which experimental group they were in. It may have been that the difference between the enriched and barren environments used here was not sufficiently great for faecal corticosteroids and shell quality to be used as comparative welfare measures, but it was sufficient to pick up a behavioural preference. This result therefore sounds a cautionary note for how welfare measures should be assessed; particularly physiological measures that may be more of a response to environmental change (such as being moved to a new environment) than an indication of reduced welfare *per se*.

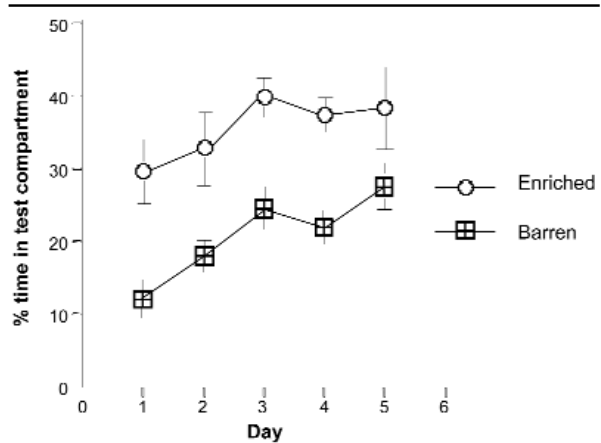
Higher plasma corticosterone levels in an enriched environment have been shown in pigs (de Jong *et al* 1998). This supports the contention (Rushen 1986; Broom 1988; Dawkins 1998) and the implication of the present study that corticosteroid elevation is not exclusive to aversive or less preferred situations. One possible reason for this is that enriched environments make animals more active and thus lead to higher corticosterone in birds; although in humans and primates this has been shown to occur only during very intense exercise (Mason 1971; Luger *et al* 1987; Nelson 2000), and pre-trained ducks showed no increase during

Figure 4



Mean levels of faecal corticosterone for birds placed in the barren pen, as measured before the experiment started (Day 0) and on Days 1 and 4 of the experiment. Vertical bars represent standard errors. Levels sharing letter are not significantly different at $P < 0.05$.

Figure 5



Preference for the enriched or barren test compartment measured as the % of total time spent in the test compartment by birds from barren and enriched pens. Vertical bars represent standard errors.

exercise (Harvey & Phillips 1982). Nevertheless, the potential for activity to increase corticosterone emphasises the importance of combining corticosterone measurements with behavioural measurements (particularly of movement and feeding) when using corticosterone level as a welfare measure, since corticosterone may increase independently of any psychological distress due to its role in inducing gluconeogenesis (Orth *et al* 1992; Causey Whittow 2000). Future studies should record time spent feeding in order to exclude a purely metabolic function of any corticosterone elevation.

Shell quality changes are also to be expected in the face of a change in the environment, which may indicate nest disturbance and favour a delay in egg-laying, which in turn causes egg-shell changes. In addition, elevated faecal glucocorticoid levels may influence the shell formation process itself, and may do so via a mechanism involving the mobilisation of glycogen stores at the expense of lipid and protein production. Shell matrix proteins are produced in a variety of tissues including the liver and the oviduct itself (Hincke *et al* 1992). These proteins have a fundamental role to play in the shell formation process in so far as they appear to direct crystal growth. Any impairment in their production and/or release will ultimately manifest itself in terms of a change in a range of shell parameters such as thickness. Sahin and Forbes (1998) reported that the feeding of birds with corticosterone over a four week period resulted in reduced egg production and reduced protein efficiency for egg production accompanied by a higher rate of fat deposition. These authors hypothesised that this may be due to the increased demand for protein used for fat anabolism. Obviously these authors were demonstrating a chronic effect of increased corticosterone levels whereas the current study only elicited a short-term effect and as such was unlikely to have caused such severe results. Nevertheless, a slight change in the protein balance may be sufficient to alter the shell formation process.

Many authors have reported changes in the ultrastructure of the egg-shell following a presumed stress event (Solomon *et al* 1987; Watt 1989; Brackpool 1995). Thus, shells tend to display a variety of adverse intra-shell defects immediately following a stress experience such as vaccination, transfer to a strange group, etc. In the current study, ultrastructural analyses revealed a significant effect of day in eggs from the enriched pen, with a reduction in total score values — indicating improved egg-shell quality — 24 h after the birds were moved into the experimental pen. In the absence of supporting evidence relating to the % of cracks and to breaking strength, the effect of these ultrastructural changes on shell performance must remain unqualified. Nevertheless it would appear that there has been a compensatory mechanism, at the ultrastructural level, in response to the decline in shell thickness that became significant on Day 3. Brackpool *et al* (1993) reported a similar compensation effect, at the level of the mammillary layer, during a time of reduced shell thickness following heat stress. The present results indicate that the welfare implications of changes to egg-shell quality still need to be investigated.

Animal welfare implications

Our results raise two important points with regard to assessing welfare when different ‘indicators’ are combined. The first is that the three different measures used here — shell quality, faecal corticosteroid levels and behavioural preferences — have very different time courses. The preference of the hens for the enriched environment was apparent on Day 1, but the maximum effects on shell quality were seen on Day 3. Faecal corticosteroid levels initially rose but had fallen again by Day 4. This means that

in choosing an ‘indicator’ of welfare (for example, to monitor the effects of an environmental change), it is important not to rely on just one moment in time, but to take into account how that indicator changes with time.

The second important point to emerge from this study is that transfer to a new environment, even an enriched one, can produce symptoms (changes in shell thickness and elevated corticosteroid levels) that would be called “stress” if the animals’ own preferences were not independently known. In a commercial environment, neither behavioural preferences nor corticosteroid levels would be routinely measured, whereas shell quality is of primary economic importance. However, it is clear from this study that there can be a 48 h time delay before the shell quality changes are most apparent and also that the shell changes *per se* have to be carefully interpreted as they are not related to behavioural preferences or corticosteroid levels in any simple way.

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