Non-invasive Corticosterone Monitoring in Laying Hens with Different Egg Production Rate

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Introduction

Egg formation is a complex process taking about 24.5 h. Formation of egg yolk and ovulation are controlled by follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone, whereas, prostaglandins (PGF₂α, PGF₂) and arginine-vasotocin (AVT) are involved in the process of oviposition. Eggs are not produced continuously but in series. The preovulatory LH peak about 6 h before ovulation has to fall within the dark period to result in ovulation. As the average time between oviposition and ovulation of the next follicle is about 24.5 h the preovulatory LH peak is moving towards the light period. Failure of the preovulatory LH peak results in a break in egg formation. Egg series may comprise few eggs (bad layers) up to more than 100 eggs (good layers). One starting point for the differences between good and bad layers may be the capability of the hen to cope with stress. The process of egg production has to be regarded as severe metabolic stress to the hen indicated by elevated corticosterone concentrations preceding oviposition for one to two hours, which may be masked by the diurnal changes in levels of glucocorticosteroids (corticosterone, cortisol) (Johnson and Van Tienhoven, 1981). In laying hens secretion of corticosterone increases at the beginning of the dark period (Cunningham et al., 1994) and decreases during the light period (Beuving and Vonder, 1977). Quite high corticosterone levels in blood are found at oviposition time (Johnson and Van Tienhoven, 1981). As glucocorticoid levels in blood may be influenced by handling of hens determining glucocorticoid metabolites in feces may be an alternative.

The objective of the study was, therefore, to investigate whether concentrations of corticosterone and cortisol in feces correspond with the egg production rate of ‘good’ and ‘bad’ performing hens.
**Materials and Methods**

In a first step, the available method to determine glucocorticoids in pig feces (Claus and Weiler, 1996; Schmid, unpubl.) was adapted to feces of laying hens. The corticosterone and cortisol concentration was determined after extraction and solvent distribution by RIA technique using tritiated steroids as tracers. It was assured that feces contained a considerable amount of uric acid as 75 % of glucocorticoids are excreted by urine. In brief, samples of 0.5g only feces (without the white urine cap) were added to 4ml methanol and 500µl ultrapure water, and mixed for 30 minutes. Lipids were removed by solvent distribution against 3ml of petrolether. Corticosteroids were extracted from 100 µl of the methanol-water-phase using 5 ml tertiary butylmethylether. The recovery rate was determined by the addition of either $^{3}$H-cortisol or $^{3}$H-corticosterone to each sample and was 76.9% for cortisol and 90.5% for corticosterone, respectively. Thereafter, corticosterone and cortisol concentration was determined by RIA technique. The determination of cortisol was based on an antiserum raised in rabbits against cortisol-21-hemisucinate-BSA. It was used at a final dilution of 1:71.400. Cross reactivity was 18.8% for desoxycorticosterone, 15% for corticosterone and 7.5 % for progesterone, pregnenolon did not show any cross reactivity. In case of corticosterone, the antiserum (Dr. F. Schneider Dummerstdorf) was used at a final dilution of 1:36.400. The cross reactivity was 35.23% for desoxycorticosterone, whereas cortisol, progesterone, cortisone and pregnenolon showed no cross reactivity. The coefficients of the interassay variation were between 7% and 13% for corticosterone and cortisol, respectively. The intraassay variation was for both glucocorticoids below 2%.

To describe diurnal changes, feces were collected at 8.00, 13.00 and 18.00 h. For the main experiment fresh morning feces were collected daily from each hen separately between 7.30 and 10.00 h and stored deep frozen until analysis. Egg production rate, egg mass and yolk weight were recorded daily. The experiment was run over 35 days.

For the main experiment 16 laying hens of breed Bovans Goldline aged 50 weeks were used. Six hens did not lay eggs at the beginning of the experiment, whereas, the remaining hens showed variable egg production. Hens were kept in single cages (1,920 cm² floor space) in a climatic chamber providing normal environmental conditions. Feed and water were available ad libitum. Lighting was 14 h per day. Data were analysed by one factorial ANOVA and regression analysis using JMP programme (SAS Institute).
Results

Concentration of cortisol and corticosterone was higher in pure feces than in feces with visible uric acid cap (9.71 and 21.47, 6.56 and 9.99, ng/g fresh matter, respectively). As the visible proportion of uric acid was quite variable for further analyses pure feces were used. The corticosterone concentration was about twice as high than the cortisol concentration. In the same way both cortisol and corticosterone level was significantly higher in the morning than in the evening (10.06 and 19.98, 5.38 and 11.44, ng/g fresh matter, respectively).

Four hens showed egg production rate of 100 %, another four hens of >90 %, the remaining 8 hens of about 60 % with two hens laying not any eggs. Based on this observation two groups were built, 8 hens with medium to good production, 8 hens with bad production. Average egg mass and yolk mass was 68 g and 17 g in good and bad layers, respectively.

Cortisol and corticosterone concentration was lower with less variation during the experiment in ‘good’ performing hens, whereas, it was significantly higher and more variable in ‘bad’ layers. Correlation between cortisol and corticosterone was 0.75 to 0.96 (p≤0.05). No significant differences could be observed for cortisol content between ‘good’ and ‘bad’ layers (5.2 and 5.6 ng/g fresh matter, respectively), whereas, corticosterone concentration was significantly higher in ‘bad’ layers than in ‘good’ layers (16.3 and 12.7 ng/g fresh matter, respectively). Furthermore, corticosterone concentration in feces decreased in ‘good’ performing hens and increased in ‘bad’ performers during the experimental period. At the break day between two egg production series most hens showed higher corticosterone and cortisol levels in feces than on the days before and after the break.

Discussion

Beuving and Vonder (1977) reported a secretion peak of glucocorticoids in blood before start of the lighting period. Three to four hours later this increase is also measurable in feces (Dehnhard et al., 2003). As the daily light period started at 7.30 in the morning feces collection between 07.30 and 10.00 was sufficient to receive reliable results. It is well known that stress influences glucocorticoid secretion. Therefore, birds were acquainted to collection of feces before start of the experiment.

According to Bamberg (1987) 75 % of glucocorticoids are excreted with urine. In chickens corticosterone is the predominant glucocorticoid of the adrenal gland and is highly concentrated in urine (Wittmann, 1994). This indicates that concentration of glucocorticoids
should rather be determined in urine than in feces. As the proportion of urine is quite low and variable in deposited feces in chicken and as the concentration of glucocorticoids was higher in the present experiment in feces it seems appropriate to use only feces for analyses. Maybe, uric acid is more evenly secreted to and mixed with feces in chicken. The visible white uric acid cap on feces seems to contain mainly uric acid. This aspect has to be investigated in more detail.

Good performing hens had a lower corticosterone concentration in feces than bad performing ones. This indicates that hens are reacting differently to stress situations. This is also visible in the decreasing corticosterone concentration in feces in ‘good’ layers and in the increasing corticosterone concentration in ‘bad’ layers over the experimental period. Good layers are capable to cope better with stressfull situations (e.g. unfamiliar visitors in the experimental unit, noise during collecting feces) than bad layers. It has been reported that the ovulation (Johnson and Van Tienhoven, 1981) and the egg formation process (Gerken et al., 1994) may be stressful to laying hens. Therefore, it might be expected that these processes should result in higher corticosterone levels in good performing layers. As this was not the case it may be concluded that good stress compensation, visible in low levels of glucocorticoids, is necessary for a high egg production rate. Thus, the capacity of hens to buffer stress seems to be the key to a high laying rate.

Abstract

The process of egg production has to be regarded as severe metabolic stress to the hen. Elevated corticosterone concentrations have been described to precede oviposition for one to two hours, but may be masked by the diurnal changes in levels of glucocorticosteroids (corticosterone, cortisol). Increased corticoid levels were discussed either to be involved in the local induction of the process of ovulation and/or oviposition, or as result of those processes. The objective of the study was to investigate the relation between egg production rate and overall level of secretion of glucocorticosteroids determined as contents in feces. First, a method for determining glucocorticoids in feces was developed. Then, the diurnal rhythm of secretion of corticosterone and cortisol in feces was determined. In the third step, feces were collected and egg production was recorded daily in 16 Bovans Goldline laying hens during a 35 days period. Eight hens showed low to moderate and 8 hens high laying performance. Corticosterone concentrations in feces were about twofold higher than those of cortisol (20.0 and 10.1 ng/g fresh matter for corticosterone and cortisol respectively, p ≤ 0.01)
and reached a diurnal maximum in the morning samples. Corticosterone concentration was lower with less variation during the experiment in ‘good’ performing hens, whereas, it was significantly higher and more variable in ‘bad’ layers. Furthermore, corticosterone content in feces decreased in ‘good’ performing hens and increased in ‘bad’ performers during the experimental period. It was concluded that the capacity of hens to buffer stress is the key to a high laying rate.

Keywords: Laying hen, feces, stress, egg production, cortisol, corticosterone

References


Figure 1. Diurnal rhythm of corticosterone and cortisol secretion in feces of laying hens (FS = fresh matter)

<table>
<thead>
<tr>
<th>Corticosterone</th>
<th>Morning</th>
<th>Noon</th>
<th>Evening</th>
</tr>
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<tbody>
<tr>
<td>19.98</td>
<td>13.97</td>
<td>11.43</td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>10.06</td>
<td>7.32</td>
<td>5.38</td>
</tr>
</tbody>
</table>

Figure 2. Survey of corticosterone and cortisol secretion in feces of ‘good’ (left) and ‘bad’ (right) performing hens during the experimental period (FS = fresh matter)

Figure 3. Regression of corticosterone (left) and cortisol (right) secretion in feces of ‘good’ and ‘bad’ performing hens for days of measurement (FS = fresh matter)

y = 13.90 -0.147x (r² = 0.043)

y = 6.43 -0.193x (r² = 0.043)