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Determination of corticosterone concentrations in egg albumen: A non-invasive indicator of stress in laying hens

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ABSTRACT

Measurement of plasma corticosterone is difficult because the handling associated with blood sampling from birds is stressful. The use of non-invasive means of measuring stress could help to alleviate this problem. It was considered that the accumulation of plasma corticosterone into the egg albumen could provide a non-invasive indicator of stress in laying hens. The present study examined the relationship between plasma and egg albumen corticosterone concentrations and then determined what affect exposing hens to known stressors had on egg albumen corticosterone concentrations. Laying hens were given subcutaneous injections of either 0, 5, or 10 mg of corticosterone suspended in peanut oil and then the concentrations of corticosterone in the plasma and egg albumen determined. Also, groups of hens were handled, exposed to high ambient temperature and moved to new cages, all events known to be stress provoking, and then the concentrations of corticosterone in albumen determined. The injections increased plasma corticosterone concentrations substantially and these were directly related to the concentrations measured in the egg albumen. When hens were exposed to the various stressors, the level of corticosterone in the egg albumen increased. The corticosterone concentrations found in the egg albumen can provide a convenient non-invasive means of measuring stress in laying hens and other birds.

1. Introduction

Stress is a condition that places an animal in a state where its biological response mechanisms attempt to re-establish homeostasis. Stress is a syndrome that probably has no consistent biological response and the consequences vary extensively. However, most definitions of stress deal with the responses to adverse stimuli and therefore, have normally been defined according to changes in physiology and behavior.

In any habitat, birds need to cope with acute stressors such as poor weather, drought, predation, competition and disturbance [60,71,72]. These are unpredictable events that have important physiological consequences for individuals and their progeny. In the wild, chronic stress is unlikely as birds can avoid prolonged adverse conditions by using flight as means of escape [63]. Unlike wild birds, domestic poultry are often constrained by the behavioral repertoire available to them when attempting to cope with stressful events. This limitation could expose domestic poultry to situations resulting in chronic stress.

Adverse challenges frequently result in activation of the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic-adrenal-medullary system (SAMS) with consequent changes in plasma glucocorticoids and catecholamine concentrations, respectively [6]. In birds, the main glucocorticoid released from the adrenal gland is corticosterone [12]. In domestic and wild birds, corticosterone acts to modify behavior and physiology as individuals attempt to cope with unpredictable events in their environment [63,68,69]. While an increase for a short period, in plasma glucocorticoids, may make a positive contribution to an animal's ability to cope with a change in its circumstances, a chronic elevation is thought to be associated with a failure to adjust and this may have consequences that are detrimental to the animal [63]. There are difficulties with the interpretation of circulating plasma glucocorticoid concentrations because of diurnal variations and rapid changes in response to handling and blood sampling [3,13,71]. Some of these difficulties can be overcome by using non-invasive procedures such as measuring hormone concentrations in milk, saliva and faeces. Milk contains a variety of hormones and metabolites and the concentrations vary according to the amount circulating in the blood [54]. Saliva contains cortisol and the concentration is closely correlated with the degree of stress [20,35,53,66]. The measurement of glucocorticoid metabolites in faeces has been developed as a non-invasive measure of stress in ruminants [48,49,52], and birds [11,21,55].

In domestic hens, the deposition of albumen over 2–4 h during egg formation [17] could potentially provide an indication of the blood constituent concentrations during the period of accumulation or synthesis. The accumulation of steroids into the egg yolk has been

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given some consideration [14,22,23,26,27,40,59]. However, the accumulation of plasma constituents into the egg albumen has received limited attention. Offspring phenotype appears to be influenced by maternal contributions to the egg [16,22–24,43]. There is recent evidence that the concentration of corticosterone in the egg can affect the phenotypic development of the offspring [27,28,30,56,57]. Stress induced elevations in plasma corticosterone could result in greater accumulation of corticosterone in eggs and in turn influence embryonic development and post-hatch chick phenotype [16,27,30,58].

The objectives of the present studies were to develop an assay procedure for measuring corticosterone in egg albumen, to evaluate the relationship between egg albumen and plasma corticosterone concentrations and then determine the effects of known stressors on egg albumen corticosterone concentrations in laying hens. If such a relationship exists between egg albumen and plasma corticosterone concentrations then this could provide a non-invasive measure of stress in domestic hens and other birds.

2. Materials and methods

2.1. Birds and diets

In all studies Isa Brown hens were used. They were purchased from commercial sources at 16 weeks of age and fed *ad libitum* a commercial layer crumble diet containing 12.3 MJ/kg metabolisable energy and 160 g/kg protein. During the experimental studies hens were maintained on 16 h of light.

2.2. Corticosterone assay

2.2.1. Reagents

Phosphate buffered saline (PBS) was prepared by dissolving 4.33 g di-sodium hydrogen phosphate, 3.04 g sodium di-hydrogen orthophosphate, 9.0 g sodium chloride, 1 g sodium azide and 1.0 g gelatin in 1 L of distilled water and then the pH adjusted to 7.0 with 4N sodium hydroxide solution. Dextran-coated charcoal solution was prepared by dissolving 1.0 g of Dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 1 L of PBS and then adding 4.0 g of wood charcoal (Charcoal-Norit A; Fisher Scientific, New Jersey, USA). The 1,2,6,7-^{3}H Corticosterone (activity: 2.48 Bq/mmol) was obtained from Amersham Biosciences (United Kingdom). The radio-labelled corticosterone was diluted in ethanol and when needed, sufficient of this was dried down under nitrogen and then reconstituted in PBS to give approximately 12,000 CPM in 0.1 mL using a LKB-1215 Beta counter (Wallace Oy, Turku, Findland).

A standard stock solution of corticosterone was prepared by dissolving a weighed amount of steroid in ethanol. A working standard solution was prepared by taking a known volume of the standard stock solution and drying off the ethanol and then dissolving the precipitate in PBS and storing 1 mL aliquots at − 20 °C until needed. The assay standards, ranging in concentration from 0.1 to 25 ng/mL were prepared by serial dilution of the working standard in PBS. Internal quality controls for the albumen assay were prepared by injecting hens with corticosterone and collecting the albumen from eggs. After complete mixing these were stored as aliquots at − 20 °C. Internal quality controls for the plasma assays were prepared by adding known amounts of corticosterone to steroid free charcoal stripped hen plasma. A set of the appropriate quality controls was run in each of the albumen and plasma assays. Antiserum to corticosterone was purchased from Sigma chemicals (Sigma, St Louis, USA). Each vial of antiserum was reconstituted in 27.5 mL of PBS. For this antiserum the stated cross reactivities (%) with other steroids are Progesterone 15.7, 11-Deoxycorticosterone 20, 20α-Hydroxyprogesterone 8.8, Cortisol 4.5, Testosterone 7.9, 20ß-Hydroxyprogesterone 5.2, Cortisone, 3.2 and Oestradiol <0.1.

2.2.2. Albumen extraction procedure

After collection, the eggs were weighed, broken open and then the albumen was separated from the yolk, weighed and stored at − 20 °C until assayed. At a later stage, the albumen samples were thawed and shaken vigorously until completely mixed. A 5–6 g sub-sample of albumen was transferred to a 20 mL glass vial and to this was added 5 mL of distilled water and then the contents shaken. After complete emulsification, two aliquots (approximately 0.5 g) were weighed into glass culture tubes (12×75 mm). Diethyl ether (4 mL) was added and then the contents were shaken for 10 min. The tube and contents were then place in a − 80 °C freezer and when the aqueous phase was frozen, the solvent fraction was poured into a second culture tube. The diethyl ether was removed by heating under a constant flow of nitrogen gas. The precipitate was dissolved in 0.1 mL PBS. Plasma samples (0.1 mL) were dispensed in to glass culture tubes and 3 mL of diethyl ether added. The remainder of the procedure was the same as for the albumen samples. All samples were assayed in duplicate.

2.2.3. Corticosterone assay

To the albumen and plasma extracts (in 0.1 mL PBS) and standards (0.1 mL/tube), 0.1 mL of the antiserum solution was added and then after 30 min, 0.1 mL of the radio-labelled corticosterone was added and the contents mixed before being incubated at 4 °C overnight. The following day, 0.25 mL of the dextran-coated charcoal was added and the contents mixed and incubated for 15 min before being centrifuged at 2500 rpm for 15 min. The supernatant was poured into a 5 mL polyethylene scintillation vial (Packard Bioscience, Groningen, The Netherlands). To this was added 3 mL of counting scintillant (Opitphase Hisafe 3, Fisher Chemicals, Loughborough, UK) and then the level of radioactivity determined using a LKB-1215 Beta counter (Wallace Oy, Turku, Findland). The concentration of corticosterone was determined by comparing the bound radioactivity in the extracted samples with that in known assay standards. The recovery rate was determined by adding known amounts of labelled corticosterone to aliquots of both plasma and egg albumen and processing them as for the samples. The recovery rate was determined to be 92%. The assay sensitivity (defined as the concentration of hormone that produces binding that is 2 SD less than the zero binding) was 0.03 ng/tube. The intra-assay and inter-assay coefficient of variation for the albumen assay, estimated using 3 quality controls containing low, medium and high concentrations of corticosterone were: low (1.38 ng/g): 6.8% and 8.8%; medium (2.76 ng/g): 8.4% and 9.6%, high (4.25 ng/g): 12.4% and 9.7%, respectively. The intra-assay and inter-assay coefficient of variation for the plasma assay, estimated using 3 quality controls containing low, medium and high concentrations of corticosterone were: low (1.44 ng/mL): 5.2% and 7.5%; medium (4.90 ng/mL): 9.8% and 11.9%, high (6.47 ng/mL): 8.2% and 13.3%, respectively.

2.3. Experimental studies

All the experimental procedures involving hens were approved by the University of Sydney Animal Care and Ethics Committee and complied with the Australian Code of Practice for the use of Animals for Scientific Purposes.

In these studies, unless otherwise stated, hens were housed individually in conventional layer cages (25×55×45 cm). Following collection, eggs were weighed, broken open and the albumen separated from the yolk. The albumen was weighed and then stored at − 20 °C until assayed. When blood samples were collected they were maintained on ice until centrifugation, after which the plasma was collected and stored at − 20 °C until assayed.

2.3.1. Relationship between plasma and egg albumen corticosterone concentrations

A total of 60 hens, 56 weeks of age were used in the study. Hens were given a subcutaneous injection of either 0, 5 or 10 mg of