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CORPORATION LIMITED

Non-invasive assessment of stress in commercial housing systems

A report for the Australian Egg Corporation Limited

by Jeff Downing

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Non-invasive assessment of stress in commercial housing systems

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Foreword

This project determined the corticosterone concentrations in albumen of eggs collected from the three main production systems used in the Australian egg industry; conventional cages, free range and barn. Egg albumen corticosterone concentrations are used as a noninvasive measure of stress in laying hens because of their correlation with plasma corticosterone concentrations.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Abbreviations

ACTH	Adrenocorticotropic hormone
AGP	Alpha-1 acid glycoprotein
APP	Acute phase protein
Bn	Barn
CC	Conventional cage
CGB	Corticosteroid- binding globulin
CPM	Counts per minute
CNS	Central Nervous System
CRF	Corticotropin releasing factor
FC	Furnished cage
FR	Free Range
HHP	Hen housed production
H/L	Heterophil: Lymphocyte ratio
HPA	Hypothalamic-adrenal-axis
GR	Glucocorticoid receptor
MR	Mineralocorticoid receptor
PBS	Phosphate buffered saline
PVN	Paraventricular nucleus
RIA	Radioimmunoassay
SEM	Standard error of the mean
TI	Tonic Immobility

Executive Summary

The basis of the current study was to evaluate corticosterone concentrations in albumen of eggs collected from hens maintained in the three main production systems used in the Australian egg layer industry (Conventional cages (CC), Free range (FR) and Barn (Bn)) at 24, 32, 42, 52, 62 and 72 weeks of age. Commercial producers were initially contacted by the Australian Egg Corporation Limited detailing the project to be undertaken and requesting their involvement. Following a positive response from individual producers, meetings were arranged and details of how the egg collections were to be made was discussed. In the study, five free range (FR 1-5), four conventional cage (CC 1-4) and three barn (Bn 1-3) flocks were sampled. From each of the participating farms, ninety eggs were collected at random when the hens were at the appropriate ages. For all except farm CC4, eggs were supplied by the producers who randomly selected the eggs from those laid on one day in the week of the specified flock age. Eggs were collected at the same ages for all systems but because flocks were placed at different times, the collections started in different months, ranging from July 2009 to March 2010.

Egg and albumen weights, egg albumen corticosterone concentration and total corticosterone in albumen were determined for all eggs collected. The month of collection had no significant effects on egg weight ($P= 0.57$), albumen weight ($P=0.72$), egg albumen corticosterone concentration ($P=0.28$) or total albumen corticosterone in albumen ($P=0.48$). The collection age had a significant effect ($P<0.001$) on egg weight with it increasing from 24 to 42 weeks and then remaining relatively similar until the end of production. The age of collection had a significant effect on the egg albumen corticosterone concentration ($P= 0.02$) but no effect on the total amount of corticosterone in the albumen ($P=0.13$). This is difficult to understand because of the strong positive relationship between concentration and total amount of corticosterone.

When the farms are grouped into production (housing) systems it was found that the type of system had no significant effect on egg albumen corticosterone concentration ($P=0.78$) or on the total amount of egg albumen corticosterone ($P=0.48$). There was large variation in these measures for individual farms even in the same production system.

Grouping individual farms into the relevant production systems acted to mask some of the individual farm effects. The general pattern for many of the commercial farms is for the corticosterone concentrations to be high early in the production cycle and then for this to decrease as the hens age. The different patterns of albumen corticosterone concentrations observed on individual farms in the early part of the production provide evidence that:

- Farm management in the early phases of the production cycle could be relevant to the extent of challenges hens need to deal with in their environment.
- Early rearing management could be designed to familiarise hens to the production housing and accommodate their adaptation to the housing transfer.
- There is evidence that the egg albumen corticosterone concentrations were related to total mortality and egg production in the early phases of the production cycle. These observations warranted further investigation.

Overall Conclusions

When farms are grouped into production systems there were no differences in mean corticosterone concentrations. For each production system the variation between farms was large and this limited the value of such a comparison.

For most farms, the albumen corticosterone concentrations were high at the start of the production cycle and then decreased and remained stable during the later stages of the production cycle.

This pattern of albumen corticosterone concentrations suggests that farm management in the early phases of the production cycle could be relevant to the extent of challenges hens need to deal with in their environment. Also, early rearing management could be designed to familiarise hens to the production housing and accommodate their adaptation to the housing transfer.

The persistently low albumen corticosterone concentrations, low mortality, high egg production and large egg size recorded for farm CC4 where hens were housed individually, serves to illustrate the importance of group dynamics and social adaptation in laying hens.

A couple of large flocks in floor systems were capable of overcoming the issues of social stress and adaptation and were able to maintain low albumen corticosterone concentrations in the early phases of the production cycle. Again, it could reflect the role management of early rearing has in limiting the challenges faced by laying hens.

Four of the five farms that had more persistent elevated albumen corticosterone concentrations in the early production period were large free range or barn flocks.

The measurement of albumen corticosterone concentrations was sensitive enough to be correlated with a lice infestation (Farm FR1) and an outbreak of cannibalism (Farm Bn3).

In any flock there are some hens that perceive the challenges they face as more severe than others and have high albumen corticosterone concentrations.

The mean albumen corticosterone concentrations over the entire production cycle tended to be lower in flocks with lower mortality. However, further data is needed to establish a definitive relationship.

The data suggest that the elevated albumen corticosterone concentrations in the early stages of the production cycle are likely correlated with reductions in performance. Again, further data collection is needed to verify this relationship.

1 General Introduction

1.1 The role of measuring stress hormones in welfare evaluation

Historically, the use of stress hormones in welfare evaluation has probably received more attention than any other measure based on the number of research publications mentioning its involvement.

Stress is a condition that places an animal in a state where its biological response mechanisms attempt to re-establish homeostasis and if these systems are inadequate, pre-disposes the animal to pre-pathological and pathological states that impinge upon its well-being. It will include combinations of all conditions that the animal needs to deal with and can be external or internal factors. Any major perturbation to homeostasis requires adjustments by the hen to re-establish normality. If the changes are temporary and reversible the process is acclimation and if they are long-term and permanent the process is acclimatisation, when irreversible changes adaptation occurs.

Stress is an imprecise term but continues to be used because for many it is a simple way of explaining something that would otherwise require a much longer description (Ganong, 1963). Stress is essentially a syndrome and really has no consistent biological response and the consequences will vary extensively for individuals. Most definitions of stress deal with the consequences and are normally defined according to changes in physiology or behaviour. The absence or presence of stress provides a measure of animal wellbeing and responses to stress will remain as criteria for assessing an animal's wellbeing. The degree of change in biological systems is often used to evaluate the level of stress experienced by hens.

When challenged, the most energy conserving defence is for an animal to move away from the stressor influence. In intensive animal production systems avoidance is often limited, and this is where neuroendocrine responses are initiated that affect many biological systems, but this requires more energy to re-establish homeostasis. Responses to stress can be specific or nonspecific. The release of corticosterone has been identified as an essential feature of the non-specific response to stress (Hester *et al*., 1996a, b, and c). Mason (1971) provided evidence that many other parts of the neuroendocrine system, and not just the hypothalamic-adrenal-axis (HPA), were involved in the response to stress and there are specific responses depending on the type of stressor. Individuals differ to the extent of their physiological response initiated by any particular stressor with the final consequences depending on how the individual perceives the threat. Therefore, different biological mechanisms are available to individuals and the response to a stressor can vary between individuals.

Moberg's (1985) model of stress separates the animal's response into three phases, recognition of a threat to homeostasis, the stress response and the biological consequences of stress. Moberg's model recognises the importance of the central nervous system (CNS) in recognition of potential stressors and the influence it exerts over the responses necessary to cope with the disruption to homeostasis. Various modulators (experience, genetics, sex, age, psychological or physiological state) can influence the way the CNS responds to the stressor. In the second phase, the stress response is the change in biological function that enables the animal to cope with stress.

While normally these changes are sufficient for the animal to cope, cumulative effects of numerous stressors can be over bearing. The biological response diverts resources from

biological systems functioning before the perturbation to systems involved in re-establishing homeostasis. The degree to which this can be achieved is a measure of the animal's ability to cope. If the stressor(s) is major and persists, the biological cost may be great and lead to a pre-pathological state and then pathology. These final states are the consequences of failing to cope. The longer the animal exists in the pre-pathological state the greater the probability the animal will develop a pathological condition. The real welfare essence of Morberg's (1985) model is that the pre-pathological state represents a real threat to an animal's wellbeing. Identifying stress at this point is not an adequate measure for assessing welfare because the damage has already been done. Researchers and producers need to identify conditions that are stress provoking well before the pathological state is reached.

1.2 Physiological responses to stress

In essence, there are specific responses depending on the stressor and non-specific responses that are independent of the stressor. Non-specific responses include increased blood flow and production of glucose from glycogen, which prepares the animal for 'fright-flight' reaction, and these effects occur within minutes and are largely catecholamine-dependent. A consistent non-specific response is an increase in corticosterone secretion which is an acute response and responsible for glucose production from non-carbohydrate sources, principally protein. If the stress is continued for a chronic period, the corticosterone-induced changes are detrimental to the hen. Pathological consequences include; ulcers, hypertension and immunosuppression and effects can be permanent after removal of the stress and, if continued, can result in death. Short term stressors such as heat (Beuving, 1980), food and water deprivation (Beuving, 1980) transport (Broom and Knowles, 1989) and fear (Beuving *et al*., 1989) give rise to elevated corticosterone levels.

The endocrine, immune and central nervous systems interact and respond to stressors (physical, social or disease) in a coordinated manner. While the interactions between the brain and endocrine system have been long recognised, the participation of the nervous and endocrine systems in the regulation of immune-related responses is now appreciated. The bi-directional communication between the neuroendocrine and immune systems appears to be mediated by neurotransmitters, hormones, cytokines and receptors common to the three systems (Kelley, 1988; Blalock, 1989; Arkins *et al.*, 1993; Johnson, 1997; Johnson *et al.*, 1997). The coordinated response of these three systems during stress provides a primary example of how the brain-pituitary-immune axis serves to integrate the homeostatic responses of the animal (Husband, 1995). The effect of stress includes an increase in endocrine-immune output resulting in the release of hormones, particularly those of the hypothalamic-pituitary-adrenal axis (HPA), and cytokines and a change in the so-called "endocrine-immune gradient" (Elsasser, 1993). Metabolic changes following alterations in the gradient represent a homeorhetic response, which alters nutrient partitioning away from growth and skeletal muscle accretion to metabolic processes, which during immunological stress; support the immune response and disease resistance. Many cytokines, such as interleukin-1, tumour necrosis factor-□ (TFN-□) and interleukin-6, act directly on target tissues such as skeletal muscle, adipose, liver and bone, and indirectly alter the circulating levels of hormones such as growth hormone, insulin, glucagon and cortisol. In so doing, they orchestrate glucose homeostasis, increase net protein oxidation, muscle proteolysis, nitrogen excretion and net hepatic anabolism (Klasing, 1988; Johnson *et al.*, 1997). The net result is reduced growth rate and increased fat deposition.

1.3 The hypothalamic-pituitary-adrenal axis (HPA)

The HPA is a neuroendocrine system with classical activation by the nervous system, release of hormones and regulation by negative feedback control.

1.4 The adrenal gland

In birds, the adrenal glands consist of chromaffin and cortical (interrenal) tissues. Chromaffin tissue accounts for about 15-25% of the adrenal gland and is intermingled throughout the cortical tissue, being concentrated around blood spaces and is more abundant towards the centre of the gland. Chromaffin tissue is of two distinct cell types, those releasing adrenalin and those releasing noradrenaline (Ghosh, 1980). The cortical tissue is divided into the subcapsular zone, 20-40 cells thick and secretes aldosterone, and an inner zone produces glucocortcoids principally corticosterone (Holmes and Cronshaw, 1980).

1.5 The cortical hormones

The steroids of the adrenal are segregated into the glucocorticoids and the mineralocorticoids. In hens, the principle glucocorticoid is corticosterone and aldosterone is the principle mineralocorticoid (Holmes and Phillips, 1976).

1.6 Regulation of corticosterone secretion

Releasing factors, corticotropin releasing hormone (CRH) and vasopressin (AVP) are synthesised in the paraventricular nucleus (PVN) of the hypothalamus which receives inputs from other hypothalamic regions, the limbic systems (signals related to emotional state), the subfornical organ (monitors blood corticoids concentrations) and the brain stem (review in Mormede *et al*., 2007). The hypothalamic releasing factors stimulate ACTH secretion from the pituitary which acts on the adrenals to both stimulate the synthesis and release of corticosterone in birds (Etches, 1976; Freeman and Flack, 1981, Harbuz and Lightman, 1992: Minton, 1994; Carsia and Harvey, 2000). A negative feedback loop exists where corticoids act at the hypothalamus and pituitary to help bring the HPA back to basal activity.

The regulation of ACTH secretion remains less well defined in birds than mammals but seems to have many similarities. Synthesis and release of corticosterone is stimulated by ACTH. Corticosterone concentrations in adrenal effluent blood increase following an intravenous ACTH injection. The response to a challenge is realised in 2-3 min but is not maximal until 15-30 min and then lasts for around an hour after its termination (Harvey and Phillips, 1980). As with mammalian ACTH, the avian equivalent is a simple polypeptide of 39 amino acids (Li *et al*., 1978; Berghman *et al*., 1998).

1.7 Glucocorticoid receptors

Much more is now known about the role of glucocortcoid receptors and their involvement in regulating basal and stress induced responses to their hormone ligands. The effects of glucocorticoids are modulated by two receptors types, the high affinity mineralocorticoid (MR) and low affinity glucocorticoid (GR) receptors (Ruel *et al*., 1985; Sapolosky, *et al*.,

2000; Landys, *et al*., 2006). The differentiating roles of the glucocortcoids in peripheral and brain tissue are mediated by the differences in affinity and the presence of 11b-hydroxysteroid dehydrogenase (11b HSD) in some tissues. Aldostreone released from the adrenal is the principle ligand for the MR in tissues involved in water and electrolyte balance (review in Funder, 2006). In these tissues the MR is protected from corticoid activation because cells contain 11b- hydroxysteroid dehydrogenase which metabolises corticoids to inactive cortisone (Stewart and Krozowski *et al*., 1999). In the brain, cells don't posses this deactivating mechanism. This allows corticoids to interact with the high affinity MR receptor (review in Mormede *et al*., 2007). The difference in affinity helps differentiated glucocorticoid actions at low and moderated concentrations compared to those at high concentrations (Wingfield, *et al*., 1997).

Corticoids bind with intercellular MR and GR receptors, and then translocate to the nucleus to regulate gene transcription (Evans, 1988; Beato, 1989; Necela and Cidlowski, 2004; Pascual-Le Tallec *et al*., 2005; De Kloet, 2005). The GR has a role in regulating several hundred genes (Rogatsky, *et al*., 2003). Activation of the GR results in recruitment of cofactors that act in coordination to regulate gene transcription rates, and influence tissue development, immune function, homeostatic regulation and stress responses (review in Biddle and Hager, 2009). The authors of this review discuss some of the receptor-promoter interactions that occur following GR activation. The authors also point out that the rapid dynamic receptor/genomic associations must have implications for the physiological response of cells. The generally accepted model of GR function has the GR-ligand complex interacting at gene promoter regions where it recruits other protein complexes to convert chromatin from closed to open conformation which then allows transcription factors to bind (review in Biddle and Hager, 2009).

Young *et al*., (2004) reported that basal cortisol concentrations are maintained by pulsatile release from the adrenal. Stressors act on the HPA and cause the release of 'bursts' of cortisol. The highly conserved nature of glucocorticoid actions in animals (Busch and Hayward, 2009), would suggest that the corticosterone secretion in birds is likely to be similar in pattern. The basal pattern of corticosterone concentrations can be interrupted by surges of corticosterone in response to adverse events. Corticosteroids readily cross the blood brain barrier and basal concentrations act to coordinate daily activities (Joels *et al*., 2007). Joels and colleagues (2007) give a good overview of the roles that the specific mineralocorticoid (MR) and glucocorticoid (GR) receptors play in corticosteroid activated responses. At basal concentrations the corticosterone sensitive MR's in the brain are extensively occupied and activated most of the time (Joels *et al*., 2007). Evidence indicated that MR activation is needed to maintain neuronal integrity and excitatory tone in at least the hippocampus (Joels *et al*., 2007). The hippocampal MR is also involved in cognitive processes underlying appraisal of novel situations and selection of appropriate behavioural responses (De Kloet, 2005). The low affinity brain GR is only activated following stress induced surges of corticosterone or by the concentrations seen at the peak of the circadian rhythm (Conway-Campbell *et al*., 2007). One important action of GR is to normalise brain function some time after a stressful event (De Kloet, 2005). Stress induced increases in corticosteroids effect emotions, cognitive processes and motivation (McEwen, 2007). In rodents, changes to glucocorticoid receptor occupancy leads to altered interpretation of the environment and could determine an animal's emotional state and its adoption of a coping strategy to stress (Korte *et al*., 1995; 1996). So changes in receptor occupancy may be related to how animals cope with changes in their environment (Korte *et al*., 1995). This seems very important, because it has relevance in animal welfare, making a connection between behaviour, feelings and stress physiology.

In essence, activation of the brain MR receptor drives processes at the beginning of the stress response while GR activated processes dampen the initial reactions facilitating

recovery and adaptation. This has lead to the MR/GR balance hypothesis which is well explained in the review by Joels *et al*., (2007). If the balance between the beginning and end of the stress response to various stressors is upset, the individual loses the ability to maintain homeostasis which leads to physiological dysfunction and impaired behavioural adaptation, which, if a set point is surpassed, results in disease (Joels *et al*., 2007). Behavioural effects mediated by the brain MR occur rapidly, at a rate greater than would be possible via the nuclear activated pathway. These actions are initiated by presynaptic membrane localised MR which are connected to a rapid intercellular pathway in the hippocampus and this accounts for the rapid corticosterone effects. The affinity of the membrane MR for corticosterone is about 10 fold lower than MR mediating genomic effects and accounts for the activation at concentrations seen at the of peak of circadian rhythm or after exposure to stress provoking stimuli. These processes are dealt with in the comprehensive review by Joels *et al*., (2007).

The concept that the membrane MR's in the hippocampus control the initial stress response is important for appraisal and coping processes, whereas, GR activation is essential for management of the later adaptive phase (De Kloet *et al*., 1998; Joels *et al*. 2007).

1.8 Corticosteroid transport

Around 90% of circulating glucocoritcoids are bound to proteins, corticosteroid-binding globulin (CBG) and albumin which act to regulate their bioavailability (Breuner and Orchnik, 2002). Corticosterone-binding globulin has high-affinity low binding capacity whereas the non-specific-binding albumin protein has high capacity but low binding affinity (Wingfield *et al.*, 1984). Circulating concentrations of CBG are determined by endocrine status, most likely through an influence on liver synthesis (Kovacs and Peczely, 1983). Normally CBG is around 80-90% saturated and only requires small amounts of corticosterone to become fully saturated. A small increase in blood steroid concentration will increase the free circulating concentration greatly. Binding to the protein is probably important in controlling availability and in stabilisation of the free blood concentration. There are procedures for measuring the free and bound components (Barnett *et al*., 1996).

1.9 Clearance and metabolism

Corticosteroids are cleared from the circulation by simple exponential decay. Estimates of the half-life of corticosterone range from 10-22 min for different species of domesticated birds (Birrenkott and Wiggins, 1984; Kovacs and Peczely, 1983: Thomas and Phillips, 1975). The liver is the main site of degradation.

1.10 Diurnal rhythm

Plasma corticosterone and CBG levels show a distinct diurnal rhythm (Beuving and Vonder, 1977; Kovacs and Peczley, 1983: Wilson *et al.*, 1982) with CBG concentrations lagging approximately 4 hours behind (Siegel *et al*., 1976) those of corticosterone. Maximum concentration is observed at the end of the dark, start of the light periods with a trough in the concentration seen at night. The rhythm relates to changes in CRF, ACTH and hypothalamic activity. Shifts in the diurnal pattern occur in response to reproductive and nutritional status and in hens, also to the ovulatory cycle (Wilson and Cunningham, 1981 Wilson *et al*., 1982). There are also seasonal variations in the pattern of

corticosterone secretion. In birds, generally this pattern is influenced by such factors as feed supply (Ruis *et al*., 1997), light (Marple *et al*., 1972), weather patterns, migration and territorial behaviour. During the production cycle the corticosterone concentration is highest at peak production (Davis *et al*., 2000).

1.11 Factors stimulating corticosterone release

In general, any stress imposed on an animal and perceived to be a threat will stimulate corticosterone release, with this often thought to be a non-specific stress response. In laying hens, basal corticosterone concentrations are low, usually < 2 ng/mL (Craig and Craig, 1985; Lagadic *et al*., 1990; Littin and Cochrem, 2001). This compares to values which can be > 70 ng/mL in free living birds (Wada *et al*., 1999). The variation in corticosterone responses to stressors is probably an inherent characteristic (Littin and Cockrem, 2001). To adequately assess the corticosterone response to any stressor it would seem important not just to consider the peak response but to integrate the area under the response curve.

Genetic influences have marked effects on corticosterone responses to stress (Satterlee and Johnson, 1988). Factors reported to cause corticosterone release are feed and water deprivation (Imms, 1967; Nir *et al*., 1975; Scanes *et al*., 1980: Freeman *et al*., 1981; Scott *et al*., 1983: Beuving *et al*., 1989), heat or cold (Siegel and Latimer, 1970: Freeman and Manning, 1982; Edens and Siegel, 1975; Siegel and Latimer, 1984), infection (Curtis *et al*., 1980), restraint (Beuving and Vonder, 1978; 1986; Freeman and Flack, 1980; Scott *et al*., 1983: Downing and Bryden, 2002), social stress (Gross and Colmano, 1971; Gross and Siegel, 1973; Satterlee *et al*., 1983), transport (Freeman *et al*., 1984; Gross and Siegel, 1993) and fear (Katz *et al*., 1981; Harvey *et al*., 1984; Jones, 1996: Jones *et al*., 1988).

1.12 Feedback regulation and habituation

Plasma corticosterone regulates brain corticosterone receptor numbers (Sapolsky *et al*., 1984) and acts in a feedback loop to regulate synthesis and release of CRF and ACTH (Kamstra *et al*., 1983: Vale *et al*., 1983; Sapolsky *et al*., 1984). Some evidence suggests that corticosterone also acts on the adrenal to decrease its responsiveness to ACTH and on the liver to depress CBG synthesis (Malek, 1981). This information was used to support the reported habituation of the corticosterone response to prolonged heat (Siegel and Latimer, 1984), cold (Siegel and Latimer, 1970), under-feeding (Freeman *et al*., 1981) and exercise (Rees *et al*. 1983).

Rees and colleagues (1983) reported that the avian corticosterone response did not habituate to exogenous ACTH challenges. So adaptation to prolonged stress may result from a decrease in ACTH release, a consequence of habituation of the central nervous system and the control it has over CRF release. Adaptation probably increases the threshold required before a particular stimulus to elicit ACTH release. There are probably situations where adrenal compensation and stress-induced ACTH release result in an exaggerated adrenal response to a persistent stress or another unfamiliar stressor (Vernikos-Danellis, 1965).

The response from the adrenal can be modified by experience (Mason, 1971; Dantzer and Mormede, 1983). It is possible that animals can develop an expectancy and cognitive appraisal of various situations and failure of the expectancy may stimulate the HPA. For hens, feed deprivation results in elevated plasma corticosterone, however, the response is greater in hens previously fed *ad libitum* than in hens fed intermittently (Rees *et al*., 1984).

The involvement of the nervous system is critical to the intensity of the adrenal response. Physical stressors are unable to activate the HPA if emotional stimulation is absent. Since responses to stress are related to behavioural changes, the habituation of the nervous input stimulus could be responsible in part, for suppressed adrenal function.

There is some evidence that habituation can affect growth and egg production. Egg production is depressed by handling hens but only for those hens not accustomed to the procedure (Hughes and Black, 1976). Twice a day handling during the three weeks of brooding, increased growth rate in broilers and in female layer chicks but not male layer chicks (Jones and Hughes, 1981). Similar effects were reported by Thompson (1976) but not McPherson *et al*., (1961) and Reichman *et al*., (1978). However, in the later two studies, chicks were only handled once weekly. Irregular handling could be detrimental to growth whereas regular handling may enhance a chick's ability to cope with novel stressors.

Adaptation to the caretaker increases antibody response in hens (Gross and Siegel, 1979). The authors attributed this effect to a decrease in corticosterone. Heat exposure for 1 hour increases corticosterone levels in lymphatic tissue (Siegel and Gould, 1982); however, the levels decreased over seven subsequent exposures to heat. A suggested reason for this could be that prior stress increases the corticosterone binding to hypothalamic receptors and that this acts as a negative feedback to suppress CRF secretion (Davidson *et al*., 1968).

1.13 Measuring Corticosterone

Corticosterone can be measured in plasma, saliva, milk, urine, faeces and eggs. The main excretion route for glucocorticoids in the urine and the concentration in urine has a close linear relationship with plasma free cortisiol (Lindholm and Schultz-Moller, 1973).

1.13.1 Plasma corticosterone

Because of the highly conserved nature of the glucocorticoid function (Busch and Hayward, 2009), the relationship between glucocorticoids actions and fitness would have commonality across species. The response to stress will depend on the individuals' experience, perception and preferences (McEwen and Wingfield, 2003). So the perception by the animal will determine if a stimulus is stressful. Also, the degree of unpredictability of any challenge will influence the glucocorticoid response needed to handle the challenge (Wingfied, 1994). In birds, the effects of corticosterone are dose dependent (Hayashi *et al*., 1994; Hull *et al*., 2007; Wall and Cockrem, 2010). Therefore, the role that glucocorticoids play in physiology will depend on the circulating concentrations with the actions of low or moderated concentrations differing to those of 'stress' induced concentrations (Busch and Hayward, 2009).

Sustained elevations in corticosterone concentrations have deleterious effects (Bodnoff et.al., 1995; Rich and Romero, 2005); however, basal glucocorticoids concentrations need to be elevated for long periods for these to be pathological (Wingfield *et al*., 1998). In commercial production systems where the behavioural and even physiological options hens have might be limited, persistent exposure to stressors could alternate the individual animal from a state where it experiences the positive and negative effects of glucocorticoids at a frequent rate. Moderate to high glucocorticoids concentrations might be indicative of a response to a prolonged stressor or exposure to frequent stressors of short to moderate duration. The glucocorticoid response to one challenge is likely to

influence the animals' response to further challenges (Wingfied, 1994). Together, these connections would indicate that the change in circulating glucocorticoid concentration is a measure of the degree of challenge to the animal. With this in mind, limiting the number of potential challenges in a production system is going to decrease any likelihood of poor welfare.

This relationship between emotional state and the event supports the use of plasma ACTH and corticosterone as measures of adverse events experienced by animals (Mormede *et al*., 2007). However, care needs to be exercised in interpreting endocrine data as there is no simple relationship between plasma corticosterone concentrations and perception of stress (Mormede *et al*., 2007). Also, single-point blood samples are likely to be difficult to interpret.

1.13.2 Egg Corticosterone

Previous reports to the AECL (Downing and Bryden, 2002; 2005) identified the relationship between stressful events, plasma and egg albumen corticosterone concentrations. In these reports, examples are provided of how albumen corticosterone concentrations increase when hens are challenged with events that are known to be stressful.

Steroid hormones accumulate during egg formation (Rettenbacher *et al*., 2009). Royo *et al*., (2008) found that around about 80% of egg corticosterone was found in the yolk and 20% in albumen. During egg formation, yolk accumulation occurs over 7-12 days before ovulation (Johnson 1986) whereas the albumen is accumulated over 4-6 hours the day before the egg is laid. Accumulation of corticosterone in yolk and albumen could provide measures of long-term and short-tem stress, respectively.

Eggs collected from white and brown egg laying strains were found to contain corticosterone in the yolk at 0.5 – 1.6 ng/g and albumen at 0.4-0.5 ng/g (Navara and Pinson, 2010). The yolk concentration was higher in the white strain but no differences in the albumen concentrations were detected. Mean plasma corticosterone concentration (4.40 ± 1.99 Vs 2.8 ± 0.44 ng/mL) was higher in the white breed but the differences were not significant because of the very large individual variation observed in the white breed.

Singh *et al*., (2009) found no difference in yolk corticosterone concentrations for white and brown strains of laying hens. In their study, four strains (2 white and 2 brown) of laying hens were kept in CC (two tiers of three/birds/cage at 688/cm^2/bird), floor pens (21-24 birds/pen at 6000-7000 cm2 /bird) with nest boxes and perches. Egg samples were collected at 22 and 45 weeks of age and the H/L ratio determined at 19, 35 and 45 weeks. There were significant strain x age and strain x system interactions for H/L ratio. The H/L ratio was not different between cages and floor hens for three of the strains but one white strain had a higher ratio when housed in the cages than when housed in the floor pens. For hens in cages, the H/L ratio was highest at week 22 and then decreased with age. For hens housed in the floor pens it was higher at 45 weeks than 35 weeks. There was significant interaction between housing system and age for yolk and albumen corticosterone concentrations. In floor pen hens, the yolk corticosterone concentrations did not differ with age but in caged hens it was higher at 22 than at 45 weeks. The housing system has no effect on yolk corticosterone concentrations at 22 or 45 weeks. At week 22 albumen corticosterone concentrations were higher in floor birds and at 45weeks they were higher in caged birds. The range in values were, 25-28 ng/g for albumen and 20-30 ng/g for yolk on a dry matter basis, as these samples were freeze-dried. This is interesting because when intact, untreated samples are analysed, the concentrations in albumen are lower because of the short accumulation time. However, part of the difference in

concentration could be due to the higher water content of albumen compared to yolk. The age effects on H/L and egg corticosterone concentrations suggested that there was the possibility that hens had adapted to their environment. The authors suggest that there was general agreement in the pattern of H/L and yolk corticosterone concentrations and so yolk might be used a measure of long term stress in hens. The problem with using yolk as a measure of chronic stress is that there is no clear indication when the corticosterone was deposited. It is possible that a majority of the corticosterone could be deposited in a limited number of days and not really reflective of chronic stress. Measurement of the H/L ratio and the corticosterone concentrations in albumen were not aligned. This is probably because albumen corticosterone reflects plasma corticosterone concentrations in the short term and H/L is measuring longer term effects. A better alignment might be seen if more frequent albumen samples are collected in line with the physiological period required to develop the differences in heterophils and lymphocytes.

Cook *et al*., (2009) in the same 50 week old hens fitted with jugular catheters, but on different days, collected blood samples (8 x 1h) during a control period, following an ACTH injection and after a one minute handling episode. Eggs were collected for 5 days before treatments and for the 11 days following the treatments. Corticosterone concentrations were determined in freeze-dried albumen and yolk. Plasma corticosterone concentrations were elevated for around an hour following the ACTH injection but no increase was seen in egg albumen or yolk. Similarly, following the single handling episode there was no evidence of increased egg concentrations of corticosterone. There were some limitations to the study when trying to establish a relationship between plasma and egg corticosterone concentrations. First, the number of eggs evaluated was small. Second, the treatments are ones which elevate plasma corticosterone for short periods. Third, treatment effects were compared to control eggs collected on different days, and fourth, the assay extraction efficiencies were low for yolk (67%). Only small amounts of the serum corticosterone are sequestered into the egg and so short periods of elevated corticosterone are not going to be detected in the egg, especially for the albumen if the elevation occurs at a time outside it's deposition during egg formation. What is of significance here was that for individual hens, there was a strong relationship between egg albumen and yolk corticosterone concentrations and the mean serum corticosterone concentration measured on the day before the egg was laid. This relationship also held for total corticosterone output over the 8 hour sampling period as measured by the integrated area under the serum corticosterone curve. The authors estimated that about 1% of the serum corticosterone was sequestered into the egg. Their conclusion was that egg corticosterone concentrations are a biomarker of chronic stress but not acute stress where there is a short transient increase in corticosterone.

Using a very simplistic approach, Bulmer and Gil (2008) measured steroid hormones in eggs sourced from a supermarket and labelled as being produced in FR, Bn or CC systems. No relationship between egg androstenedione and testosterone concentrations and the type of production system were detected. There was a positive trend between increased egg corticosterone concentrations and the supposed improvements in housing welfare (Free range < Barn < CC). While the authors indicate that at first this might suggest higher stress in the FR systems they argue that under chronic stress down regulation of the HPA could account for lower corticosterone concentrations in birds from the CC system. Because of the egg collection method (off the supermarket shelf) any reason could be suggested but there is no evidence to support the author's arguments. However, on balance, their explanation seems less probable than the higher corticosterone concentrations in the floor based system being the result of social stress.

Sas *et al*., (2006) measured corticosterone in whole homogenised eggs collected on 2, 7 and 16 days after a one week adaptation period for hens moved into CC and FR housing.

For FR hens the corticosterone increased from day 2 to day 16 (0.37 ± 0.22 to 0.91 ± 0.75 ng/g) while for CC hens the concentration remained fairly constant ranging from 0.18 ± 0.11 to 0.22 ± 0.17. The FR birds had access to an outside run and on day 16 low temperature and winds were considered responsible for the higher corticosterone in these hens compared to those maintained in a stable temperature environment of the CC system. Adverse climatic conditions appear sufficient to elevate egg corticosterone concentrations.

1.13.3 Faecal Corticosteroid metabolites

Determination of faecal corticosteroid metabolites has become a routine non-invasive measure of stress in animals. The effects of rearing system (floor or cage), cage laying system (CC or FC) or genotype (White or brown strains) on faecal corticosteroids were reported by Buil *et al*., (2006). Corticosteroid concentrations were higher for hens housed in CC than the FC hens and there were differences between the two FC systems used. Floor reared pullets had higher faecal corticosteroid when moved to cages than cage reared hens. This suggested that there is an advantage to having the same rearing and laying systems. Genotype had significant effect on faecal corticosteroids with it being higher in a white strain of hens compared to a brown strain. Interactions between breed, cage system and age were also noted. Buil *et al*., (2006) indicated that the significant interactions between genotype and cage system suggests that there is a difference in the way genotypes respond to their environment. This is supportive of the concept, that selection of specific strains for different environmental conditions would be beneficial to improving welfare.

1.14 Chronic stress

Even when an adverse event is prolonged, the plasma corticoid concentrations decline after a time because of the negative feedback regulation and possible depletion of adrenal reserves following repeated exposure to the adverse stimuli. While chronic stress might act to elevate basal glucocorticoid concentrations these will not be detected using single point blood samples as is common practice (Barnett *et al*., 1988). While basal corticosterone and ACTH concentrations might not be elevated under chronic stress, evidence indicates that the activity of the system will change (review in Mormede *et al*., 2007). As Mormede *et al*., (2007) highlight, the resetting of this system is what Selye (1956) described as the ‘resistance phase’ in the concept of ‘general adaptation syndrome’. Measurement of concentrations in eggs where accumulation is over some time could provide a better integrate measure of small changes in basal corticosterone concentrations.

1.14.1 Heterophil to Lymphocyte ratio (H/L)

The H/L ratio is a haematological measure of stress (Gross and Siegel, 1983). Researchers have used this ratio as a selection criterion to establish lines susceptible or resistant to different stressors (Kassab *et al*., 2000). There are strain influences in the H/L ratio (Zekarias *et al*., 2000). Al Murrani *et al*., (1997) used H/L ratio to select a strain that had higher productivity and lower mortality when heat stressed.

Al-Murrani *et al*., (2006) produced two lines of hens based on selection for high and low H/L ratio. The R group had an average H/L ratio of 0.56 ± 0.01 while the S group has an average H/L ratio of 1.11 ± 0.02. Egg production, egg weight and body weight were

superior in the R group. Egg hatchability was also higher and there was less embryonic death. The effects were carried over into the hatched offspring with these being heavier and having lower mortality and better FCR, although there were some sex interactions at various ages. These data provide a link between susceptibility to stress and productivity. However, the study used local Iraqi strains of hens which have low egg production (50-60% hens housed). The relationship might not be as strong in commercial strains of laying hens where selection has been for high egg production and this probably means that selection has indirectly been against susceptibility to stress.

Moe *et al*., (2010) used the H/L ratio and response to immunisation to evaluate the effects of early rearing environment (floor or cage) on long-term stress when hens were transferred to three-bird CC or 8-bird FC for the laying period. Hens housed in FC had a higher H/L ratio (0.35 Vs 0.20) at 70 weeks of age and when adjusted for housing, the floor reared birds had higher H/L. As suggested by the authors, the different group sizes could have accounted for these cage effects. Floor reared hens with beak trimmed or intact beaks were placed in two CC (5 or 6 hens) or two FC (15 hens) systems at 18 weeks of age. Cage type has significant effect of H/L ratio with it being higher in FC caged hens (Guemene, *et al*., 2006).

At various sampling times throughout a full production cycle, cage density (361 and 482 cm^2/bird) had no effect on H/L ratio (Davis *et al*., 2000). Onbasilar and Aksoy (2005) recorded higher H/L in hens maintained in cages at 394 cm^2 compared to those housed at 656 or 1968 cm^2/bird. For a single episode of noise stress it took 18 hours for the H/L ratio to increase and 30 hours for it to return to control levels (Gross, 1990). In broilers the H/L ratio increased 3 hours after transport (Mitchell *et al*., 1992) and 1-3 hours after isolation stress (Jones *et al*., 1991). Two lines selected for corticosterone response to social stress had similar H/L ratios when exposed to a non-social stressor (Gross and Siegel, 1985). Maxwell (1993) suggested that the stress-immunity response has two phases. Mild or moderate stress results in heterophilia and an increase in H/L ratio while extreme stress results in basophilia that can become life threatening.

1.14.2 ACTH/ CRF stimulation test

The principle for this test is that under chronic stress, the adrenal gland becomes depleted and so the response to a maximal exogenous dose of ACTH or CRF will be less. It is considered a test for chronic stress (Koelbeck *et al*., 1986; Mormede *et al*., 2007). The test normally uses synthetic ACTH (1-24) administered intravenously at doses ranging from 0.5-2 $IU/kg^{0.75}$ or for CRF at 0.03-1 ug/kg (Review in Mormede, *et al*., 2007). The integrated responses in plasma are determined as the areas under the corticosterone (birds and rodents) or cortisol (mammals) curves. If using the CRF test, sensitivity of the adrenal gland is determined by the ratio of the integrated response of plasma ACTH to plasma corticosterone (Review in Mormede, *et al*., 2007).

Koelbeck *et al*., (1986) used two different doses of ACTH (0.33 and 1.0 IU/kg) in hens maintained in cages (3, 4 or 5 hens /cage at the same density of 0.035 m^2/hen) and floor pens (density of 0.094 and 0.373m^2/bird). The basal corticosterone concentrations were not different and ranged from 0.4 to 0.72 ng/mL. The corticosterone response to the ACTH injection peaked at 30 minutes, but no differences were observed at a dose of 1 IU/kg. At 0.33 IU/kg birds housed at 3 and 4 per cage had lower responses than those housed 5 hens per cage and the hens housed in floor pens. The authors suggested that having hens in different housing conditions might result in subtle differences in adrenal responses which are not apparent with maximal ACTH injection. This may bring into question the relevance of such a test, because it would seem impossible to determine what ACTH dose is needed

to measures just subtle differences. There also seems to be an issue with the large variation in response that can be seen when a set dose of ACTH 1-24 is used for a group of individual hens.

Colson *et al*., (2006) used the ACTH stimulation test as a measure of stress in hens housed in FC and aviary systems. The hens were reared in floor pens and moved to CC or an aviary, or reared in an aviary and then transferred to a laying aviary. Around the time of transfer (18 weeks) the H/L ratio was lower in aviary reared birds, but there was no difference at 66 weeks of age in the layer facilities. At week 66 of age, the basal corticosterone was higher in CC hens than in the aviary hens. The response to high dose (10µg/kg) ACTH challenge in week 20 was lower for CC birds and also birds reared in the aviary and moved to laying aviary compared to birds reared on the floor and moved to an aviary. No differences were seen at week 15 or 66 of age. The authors suggest that this indicated more stress in CC birds at week 20 because the response to ACTH was lower. This might be the case but the authors were reluctant to say that this was the case in hens reared in an aviary and moved to a laying aviary. In week 20, these particular birds had a similar response to the ACTH challenge as the CC housed laying hens. What seemed to be obvious is that there is large variation (large SD) in the individual responses to the ACTH challenge and this leaves questions as to its relevance as a suitable test.

The response to ACTH was determined in hens maintained in CC and FC systems housing different group sizes (Guesdon *et al*., 2006). The evaluation was done to an intramuscular injection of ACTH at 72 weeks of age. Cage system or group size had no effects on basal corticosterone concentration or the response to ACTH.

1.15 Acute phase proteins

Some recent work provides early evidence that acute phase proteins (APP's) might be a useful indication of physiological conditions. During pathophysiological conditions serum acute phase proteins (APP's) are involved in attempts to restore homeostasis (Salamano *et al*., 2010). One of these is alpha-1 acid glycoprotein (AGP) which is reported to be a positive contributor to this process while albumin is another and has a negative influence (Murata, 2007). APP's can be used to evaluate both inflammatory and non-inflammatory conditions (Murata, 2007).

Very recent work has evaluated the plasma concentrations of APG and the H/L ratio in hens maintained in different housing systems (Salamano *et al*., 2010). Hens were housed in CC (at four hens/cage and floor space of 550 cm^2), FC (at eight hens/cage and floor space of 750 cm^2), or one FR pen (of 24 hens and floor space of 1.2 m^2). The hens were moved to the facilities at 18 weeks and then bleed at 15 days, two months and four months after being relocated. The AGP values were higher in CC and FC than FR hens at 15 days but this had decreased in caged hens by two months and by four months the concentration was higher in FR birds. The H/L ratio was not different between housings systems ranging from 0.74 and 0.84. The authors noted that there was no evidence of disease, leaving them to suggest that the higher AGP at 15 days in caged hens could be due to social stress associated with establishment of the pen hierarchy, citing Grigor *et al*., (1995) to support this point. They go on to suggest that as the degree of aggression in the cages subsided, so did the concentration of AGP. The increase in AGP of FR hens at four months could indicate increased aggression in these birds. Further work is warranted to determine if measurements of AGP is worthy of consideration as a welfare indicator. A possible deterrent to their use might be the complex analytical procedures needed, although assay kits are now available.

1.16 The concept of allostatic load

McEwen and Wingfield (2003) described allostatic load as the difference between the metabolic energy required and that available. Glucocorticoid concentrations are linked to the allostatic load. If the allostatic load continues to increase, the animal enters a state of stress. With the allostatic load and glucocorticoid concentrations being linked, then it is reasonable to view high glucocortcoid concentrations as being related to conditions identified as stress. As the glucocorticoid concentrations rise in response to a challenge (stressor), the high affinity brain MR's are saturated (Wingfield and Romero, 2001; McEwen and Wingfield, 2003, Busch, *et al*., 2008) and binding to the low affinity GR receptors occurs and this initiates behavioural and physiological changes in an attempt to re-establish homeostasis (Wingfield, *et al*., 1998; Wingfield and Romero, 2001). When the high glucocorticoid concentrations are present for minutes or even hours, the physiological responses are varied and act to help the animal cope with the challenge. This is all positive for the animals but if these actions are not sufficient to correct the allostatic load and the elevated glucocorticoid concentrations continue, and the stress state becomes 'chronic' then glucocorticoid effects can have detrimental effects (McEwen and Wingfield, 2003). The prolonged elevation of glucocorticoids results in suppressed growth and immune function, hypertension, protein loss, increased fat deposition and neural loss (Reviews in Wingfield and Romero, 2001; Busch and Hayward, 2009). Glucocorticoids also influence behaviour, promoting actions which assist survival and essentially act to conserve energy by limiting reproductive functions.

For glucocorticoids to be useful measures of wellbeing, they need to be connected with fitness (Bush and Hayward, 2009). Baseline glucocorticoids are related to survival as they reflect allostatic load of the individual (Bush and Hayward, 2009). Bush and Hayward, (2009) provide a very useful conceptual model for the role of baseline plasma or faecal glucocorticoids and glucocorticoid responsiveness with survival or reproductive success. While these authors concentrate on application to wild birds, the model would seem to be relevant to commercial poultry. Their models also reflect the role that baseline and stress-level glucocorticoids have on coping and fitness. This model accounts for the positive, null and negative effects of glucocorticoids on survival and reproductive success. Their arguments are that glucocorticoid concentrations responsible for interacting with the mineralocorticoid receptor have positive or null effects (plateau) on the survival and reproductive success. The positive relationship implies that the physiological and behavioural consequences of the elevated basal glucocorticoids help the animal survive in the short-term. They go on to suggest that the inflection point where basal or stress-level glucocorticoids concentrations have a negative effect is where the mineralocorticoid receptor is saturated and binding to the glucocorticoid receptor occurs. For Bush and Hayward, (2009), the negative relationship between plasma glucocorticoids, glucocorticoid responsiveness and survival is indicative of chronic stress, initiated by frequent or prolonged elevated basal glucocorticoid concentrations. The same model is also used to link reproductive success with basal and glucocorticoid responsiveness. Basal line glucocorticoids need to be high enough to support the physiological and behavioural needs of reproduction but not high enough to disrupt the same systems. Bush and Hayward, (2009) also provide a model for the relationship between glucocorticoids, the duration and intensity of a challenge and fitness/coping. If the challenge is short in duration the relationship is positive. When the challenge is low grade or of short duration the effect on fitness/coping can be neutral. There is a negative relationship between fitness/coping and challenges which are of moderate grade and duration. However, when the glucocorticoids concentrations reach a peak, further increase in the duration of the challenge, will result in loss of protection from glucocorticoids as their concentration decreases due to feedback regulation. At this point the animals' fitness/coping declines rapidly.

1.17 Summary of earlier project data

From a previous AECL project (US-107A, 2005), the relationship between plasma and egg albumen corticosterone concentrations was confirmed and the details published by Downing and Bryden (2008). In the report referred to above, there were a number of experimental and field situations which supported the concept, that egg albumen corticosterone concentrations were reflective of stressful challenges to laying hens. Since the report, other researchers have identified that albumen corticosterone concentrations can be used as a measure of acute stress in birds. While it is clear that corticosterone accumulates in yolk, the concentrations are could be a measure of chronic stress because yolk is accumulated over a much longer period (10-12 days). An advantage of using the measures in egg albumen is that it is laid down over a short period and is reflective of blood corticosterone concentrations during this period.

As part of the studies detailed in project US-107A, 2005, the albumen corticosterone concentrations of hens maintained in different housing systems was assessed. In that study, Isa Brown hens were maintained in four different production systems, these being free range (FR), barn (Bn), conventional cages (CC) either 6 and 3 birds per cage in a naturally ventilated shed, and conventional cages maintained in an environmentally controlled shed (6 birds/cage). On one day during weeks 32 (peak production) and 70 (end of production) of age, eggs were collected from each housing facility and the corticosterone concentration in albumen determined by radioimmunoassay (RIA) (Downing and Bryden, 2008). At peak production, the egg albumen corticosterone concentrations were similar for the different housing systems. At the end of the production cycle the concentration of corticosterone in albumen was higher in the free range hens compared to the hens in the environmentally controlled shed. While there were little differences between production systems, within each, there were individual hens that had high egg albumen corticosterone concentrations (>2.0 ng/ml). In the study, samples of eggs were only collected at two ages and so there is no real indication of what happens over the full production period. It's possible that at different times during a full production cycle, the degree of stress hens experience in any housing systems could vary. To help understand what happens over the full production cycle, eggs need to be collected at regular intervals.

1.18 Project objectives

Corticosterone concentrations have consistently been used as a measure of stress in laying hens. The egg albumen corticosterone concentrations can be used as a non-invasive indicator of plasma corticosterone concentrations in hens. Therefore, the albumen corticosterone concentrations can be used in the same manner as plasma corticosterone concentrations are used as a measure of stress without the hindrances related to invasive blood sampling. The procedure also allows for large number of samples to be collected and for frequent sampling protocols. An increased sampling frequency overcomes the reliance on single time point samples and supports investigations looking at the pattern of change overtime. As a non-invasive procedure it can increase the sensitivity of welfare assessment beyond measurements of mortality, egg production, egg weight and body weight.

The objective of the current study was to evaluated corticosterone concentrations in albumen from eggs collected from hens maintained in the three main production systems used in Australia conventional cages (CC), free range (FR) and barn (Bn) at 24, 32, 42, 52, 62 and 72 weeks of age. While a main aim was to compare the differences between systems, the pattern on individual farms would help to identify potential periods when hens where experiencing conditions which result in stress.

2 General Materials and Methods

2.1 General Methodology

2.1.1 Determination of corticosterone concentrations in albumen

The corticosterone concentrations in egg albumen were determined by radioimmunoassay (RIA). Details of the assay were described previously in RIRDC project No.US-107A, 'non-invasive stress assessment of commercial egg industry practices'. Since this report there have been some slight modifications to the assay protocol.

2.1.1.1 Reagents

(i) Phosphate buffered saline (PBS): This 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 1L of distilled water and the pH adjusted to 7.0 with 4N sodium hydroxide solution.

(ii) Dextran coated charcoal solution: This was prepared by dissolving 1.0 g of dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 1 L of PBS and adding 4.0 g of wood charcoal (Charcoal-Norit A; Fisher Scientific, New Jersey, USA).

(iii) Corticosterone label: The 1,2,6,7-^{3}H corticosterone (approximate activity: 2.3-2.5 Bq/mmol) was obtained from Amersham Biosciences (United Kingdom) and later Perkin and Elmer (Glen Waverely, Victoria, Australia) . The labelled corticosterone was diluted in ethanol and when needed, dried down under nitrogen and then reconstituted in PBS to give approximately 13,000 CPM in 0.1 ml using a liquid scintillation analyser (Perkin Elmer, Tri–Carb 2800 TR).

(iv) Standards: A stock standard 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 stock standard solution and evaporating off the ethanol then dissolving the precipitate in PBS and storing 1 ml aliquots at -200C until needed. The assay standards, ranging in concentration from 0.1 to 51.8 ng/mL were prepared by serial dilution of the working standard in PBS.

(v) Quality controls: Internal quality controls for the albumen assay were prepared by collecting a number of egg and removing the albumen which was bulked together and homogenised (Ultra-Turaax, Janke and Kunker, IKA-WERCH).The albumen was dilute 1:1 with MQ water. To subsamples of the diluted albumen was added known amounts of corticosterone dissolved in PBS to give four internal quality controls plus the unsupplemented diluted albumen (five in total). A set of the quality controls was run in each of the albumen assays.

(vi) Antiserum: Corticosterone antiserum 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 manufactures stated cross reactivities (%) with other steroids are progesterone 15.7, 11-deoxycorticosterone 20, 20β-hydroxyprogesterone 8.8, cortisol 4.5, testosterone 7.9, 20β- hydroxyprogesterone 5.2, cortisone, 3.2 and oestradiol <0.1.

(vii) Albumen extraction procedure: After collection, the eggs were weighed, the shell broken open and the albumen separated from the yolk, weighed and stored at –20^{0}C until assayed. At a later stage, the albumen samples were thawed and homogenised (Ultra-Turaax, Janke and Kunker, IKA-WERCH), 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 the contents shaken. After complete emulsification, two aliquots (approximately 0.5-0.6 g) were weighed into glass culture tubes (12 x 75 mm), to which diethyl ether (4 ml) was added and the contents shaken for 10 minutes. The tube and contents were then placed in a –80^{0}C freezer and when the aqueous phase was frozen, the solvent fraction poured into a second culture tube. The diethyl ether was removed by heating under a constant flow of nitrogen gas. The remaining precipitate was dissolved in 0.1mL PBS.

(viii) Assay protocol: To the albumen extracts (in 0.1 mL PBS) and standards (0.1mL/tube), 0.1 mL of the antiserum solution was added and then after 30 min, 0.1 mL of radio-labelled corticosterone was added and the contents mixed before being incubated at room temperature (approximately 23^{0}C) for 2 days. On the third day, 0.25 mL of dextran-coated charcoal was added and the contents mixed and incubated for 20 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 H-safe 3, Fisher Chemicals, Loughborough, UK) and the level of radioactivity determined using a liquid scintillation analyser (Perkin Elmer, Tri–Carb 2800 TR).The concentration of corticosterone was determined by comparing the level of bound radioactivity in the extracted samples with that in known assay standards.

(x) Assay sensitivity: The recovery rate was determined by adding known amounts of labelled corticosterone to aliquots of 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 (0.98 ng/g); 6.2% and 8.8%; medium (1.63 ng/g); 7.8% and 9.0%, high (2.47 ng/g); 7.6% and 8.4%, respectively.

2.2 Animal Ethics

Hens maintained at the Poultry Research Unit, University of Sydney, Camden, NSW, Australia, were part of a flock maintained for student teaching and research and 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. All other flocks were commercial flocks.

2.3 Egg collections

From each of the participating farms, ninety eggs were collected at random when the hens were 24, 32, 42, 52, 62 and 72 weeks of age. For all commercial farms, eggs were supplied by the producer. Producers were instructed to randomly sample the eggs from the egg collection belt on one day in the relative collection week. For all except two farms, eggs were collected from the farms on the day they were sampled and transported to the laboratory where the albumen was collected and then stored frozen. For one farm on the north coast of NSW and the one at Tamworth, the eggs after collection were transported overnight to the laboratory and processed on arrival the next morning.

2.4 Descriptions of participating farms

Individual producers were initially contacted by the Australian Egg Corporation Limited detailing the project to be undertaken and requesting their involvement. Following a positive response from producers, a meeting was arranged and details of how the collections would be made were discussed. At the end of the production cycle each producer was asked to fill in a questionnaire giving details about the facilities and flocks involved in the study.

2.4.1 Free Range Farms

Farm 1-FR1

Location: North Western Sydney Basin, NSW, Australia
Strain of hen: Isa Brown
Shed capacity: 15,000 at placement at ten hens/m^2. The shed was divided into two roughly equal sections with a wire partition to prevent hens from 'swarming'
Shed Floor: Fully slatted
Feeding: Pan feeders (30 hens/feeder)
Watering: Nipple drinkers (6 birds/drinker)
Manure removal: Collection under the slats and removal at the end of the production cycle.
Environmental control: Tunnel ventilation with evaporative cooling
Lighting: 16 hours until last 8 weeks of the production cycle when it's increased to 17h. Low light intensity (6 lux)
Nests: Venamatic with side collection belts
Production: For the collection periods is given in table 3.13
Mortality: 5.01%
Floor eggs: Not available

Farm 2-FR2

Location: Southern Tablelands of New South Wales, Australia
Strain of hen: Isa Brown reared on the floor with perches and transferred at 15 weeks and trained to use the slatted area and perches. Hens have access to the free range area from 20 weeks
Shed capacity: 13,000 at 10 birds/m^2
Shed Floor: 2/3 raised slatted area and 1/3 concrete without litter. Ramps are provided between the concreted and slatted areas
Feeding: Automatic with pan feeders placed on the slats
Watering: Nipple drinkers on the slatted area
Manure removal: Collection under raised slatted area and removal at the end of the production cycle
Environmental control: Natural ventilation with installed extraction fans and foggers.
Lighting: 16 hours of light with the minimum set by the period of natural light
Nests: Three tiers of single bird nests
Production: For the collection periods in given in table 3.13
Mortality: 7.22%
Floor eggs: 6.5%

Farm 3-FR3

Location: Tamworth, NSW, Australia
Strain of hen: Isa brown
Shed capacity: 7,500 at ten birds/m^2
Shed Floor: Slatted area and litter area
Feeding: Hopper and pans
Watering: Nipple drinkers
Manure removal: Collection under raised slatted area and removal at the end of the production cycle
Environmental control: Natural ventilation with fans and foggers
Lighting: Low light intensity
Production: Average production 86%
Mortality: 4%
Floor eggs: After establishment 1%

Farm 4-FR4

Location: North Western Sydney Basin, NSW, Australia.
Strain of hen: Isa Brown
Shed capacity: 10,000 at placement at ten hens/m^2
Shed Floor: Fully Slatted
Feeding: Chain feeders with 6.25 cm/bird
Watering: Nipple drinkers (6 birds/nipple)
Manure removal: Collection under slatted floor and removal at the end of the production cycle
Environmental control: Tunnel ventilation with evaporative cooling
Lighting: 16 hours until last 8 weeks of the production cycle when it's increased to 17h. Low light intensity (6 lux).
Nests: Two tiers with centre belt collection
Production: For the collection periods is given in table 3.13
Mortality: 3.5%
Floor eggs: Not available

Farm 5-FR5

Location: North Western Sydney Basin, NSW, Australia.
Strain of hen: Isa Brown
Shed capacity: 15,000 at placement at ten hens/m^2
Shed Floor: Fully slatted
Feeding: Chain feeders with 10.7 cm/bird
Watering: Nipple drinkers (4 birds/nipple)
Manure removal: Collection under slatted floor and removal at end of production cycle
Environmental control: Tunnel ventilation with evaporative cooling
Lighting: 16 hours with low light intensity
Nests: Two tiers with centre belt collection
Production: For the collection periods is given in table 3.13
Mortality: 3.1%
Floor eggs: Not available

2.4.2 Barn Farms

Farm 6-Bn1

Location: Western Sydney Basin, NSW, Australia
Strain of hen: Isa Brown
Shed capacity: 2,500 at 10 birds/m^2
Shed Floor: Completely slatted
Feeding: Chain feeders
Watering: Nipple drinkers
Manure removal: Collection under slatted floor and removal at the end of the production cycle.
Environmental control: Natural ventilation with fans and foggers
Lighting: Additional lighting to match the maximum of the natural light period
Nests: Venamatic nests with belt collection
Production: Not provided as egg collected from different units on an egg common belt
Mortality: Not available
Floor eggs: Not available

Farm 7-Bn2

Location: North Western Sydney Basin, NSW, Australia.
Strain of hen: Isa Brown
Shed capacity: 9,500 at placement at seven hens/m^2
Shed Floor: Full litter flooring
Feeding: Automatic pan feeders
Watering: Nipple drinkers with cups
Manure removal: Clean out at end of production cycle
Environmental control: Natural ventilation with fans and foggers
Lighting: 16 h minimum with natural lighting and low intensity during any extended lighting
Nests: Rangers harvester nests
Production: 83% HHP
Mortality: 4.0%
Floor eggs: 3-4%

Farm 8-Bn3

Location: Mid North Coast, NSW, Australia.
Strain of hen: Isa Brown
Shed capacity: 10,000 at 10 hens/m^2
Shed Floor: One half raised slats and one half litter (wood shavings)
Feeding: Chain feeders
Watering: Nipple drinkers
Manure removal: Collection under the slatted area and removal at the end of production.
Environmental control: Tunnel ventilation with maximum setting at 27^0C and evaporative cooling
Lighting: 16 hours of full light except for the period leading up to the 72 week collection when the light was of low intensity (5-10 Lux)
Production: Not provided for commercial in confidence reasons
Mortality: Not provided for commercial in confidence reasons
Floor eggs: Not provided for commercial in confidence reasons

2.4.3 Conventional Cage Farms

Farm 9-CC1

Location: Western Sydney basin, NSW, Australia
Strain of hen: Isa Brown
Shed capacity: 44,000 at placement. Six tiers of five birds/cage
Feeding: Automatic hopper feeders
Watering: Nipple drinkers
Manure removal: Manure belt
Environmental control: Tunnel ventilation with humidity control. Temperature set for 23^{0}C but can reach 28-29^{0}C in summer
Lighting: 16 hours of light
Production: For the collection periods is given in table 3.13.
Mortality: 1.75%

Farm 10-CC2

Location: Western Sydney Basin, NSW, Australia
Strain of hen: Isa Brown
Shed capacity: 22,500 at placement with five hens/ cage with five tiers.
Feeding: Automatic hopper feeders
Watering: Nipple drinkers
Manure removal: Manure belt
Environmental control: Evaporative cooling with cross ventilation
Lighting: 15h of light at low intensity
Production: For the collection periods is given in table 3.13
Mortality: 6.5%

Farm 11-CC3

Location: North Coast of NSW
Strain of hen: Isa Brown
Shed capacity: 3,300 and single tier five birds/cage
Feeding: Chain feeders
Watering: Nipple drinkers
Manure removal: High rise shed with manure collection below
Environmental control: Natural with fans and foggers set for 34^{o}C
Lighting: Low light intensity
Production: Average production 89%
Mortality: 3%

Farm 12-CC4

Location: University of Sydney, Camden, NSW, Australia
Strain of hen: Isa Brown
Shed capacity: 1200. Single bird cages – Eggs were collected from a sub sample of 100 birds maintained in the facility
Feeding: Trough (30cm/bird)
Watering: Nipple drinkers
Manure removal: Manure belt
Environmental control: Tunnel ventilation with evaporative cooling.
Lighting: 15 h of normal intensity
Production: For the collection periods is given in table 3.13
Mortality: 2.0%

2.5 Statistical analysis

Egg weight, albumen weight, albumen corticosterone concentrations and total amount of corticosterone in albumen were response variables tested. Data was stored in Microsoft Excel®, and statistical analysis was conducted using the REML linear mixed model function of Genstat® 13th edition. Data were first tested for equality of variance using residual plots. When the equality of variance could be improved using a $\log_e$ transformation, data was transformed.

The fixed model included the effects of month of the year, collection age, production system and collection age x production system and the random model included the effects of farm and farm x collection age. Significance testing of fixed effects was conducted using Wald tests with a significance threshold of $P < 0.05$. Any non-significant interactions were removed from the model. The predicted means for all significant effects were copied to Microsoft Excel® as well as standard errors which were used to calculate the standard error of the mean (SEM). The least significant difference (LSD), which is equal to two times the standard error of differences (SED), was used to make pair wise comparisons of means. Microsoft Excel® was used to create graphical summaries of the back transformed means.

For individual farms, the effect of collection age and by association month were analysed by AVOVA (Statview 5, SAS Institute, NC. USA). When differences were significant individual comparisons were made using the Tukey-Kramer test with significance set at $P < 0.05$. Similarly the effects of system and age on the percentage of hens with high corticosterone concentrations (< 1.5 ng/g) were analysed in a similar manner.

The relationships between albumen corticosterone concentrations and measures of mortality and egg production were analysed by linear regression using Statview version 5.0.1 (SAS Institute).

3 Results

Collections were made at the correct age for all farms except farm Bn1, where the 72 week collection was not made as the producer moulted the flock at 68 weeks. Collection of eggs was made after this moult, but the analysis was not considered to be part of the project and so have not been included.

3.1 Collection values for individual farms

3.1.1 Free Range farms

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Jul	52.8 ± 0.6^{e}	32.1 ± 0.3^{e}	0.52 ± 0.01^{c}	16.6 ± 0.3^{d}
32	Sep	60.3 ± 0.5^{d}	35.6 ± 0.4^{cd}	0.92 ± 0.02^{a}	32.9 ± 0.8^{bc}
42	Nov	63.7 ± 0.5^{bc}	36.8 ± 0.4^{bc}	0.79 ± 0.02^{b}	29.0 ± 0.8^{c}
52	Jan	63.3 ± 0.6^{bc}	37.0 ± 0.5^{b}	0.96 ± 0.03^{a}	35.6 ± 1.2^{ab}
62	Mar	65.6 ± 0.5^{ab}	37.9 ± 0.4^{ab}	1.00 ± 0.03^{a}	37.9 ± 1.2^{a}
72	Jun	66.6 ± 0.6^{a}	38.7 ± 0.5^{a}	0.91 ± 0.03^{a}	35.0 ± 1.1^{ab}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-1 Farm FR1: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months for these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm FR1, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentrations and total corticosterone in albumen (all P<0.001) (see table 3.1). In general, the egg and albumen weights increased with age with maximum weight at 72 weeks. The weights at 24 and 32 weeks were significantly lower than at other collection times (P<0.05). The corticosterone concentration was lowest at 24 weeks (P<0.05) increasing at 32 weeks and then decreasing at 42 weeks before increasing again at 52 weeks where it remained until the last collection. A similar pattern was observed for the total amount of corticosterone in albumen.

For farm FR2, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.2). Egg weight was significantly greater at the end of production (P<0.05) and lower at 32 weeks (P<0.05). Weights were similar between 42 and 62 weeks of age. The albumen weights followed a similar pattern with highest weight at 72 weeks (P<0.05) and lowest at 32 weeks (P<0.05). The albumen corticosterone concentration was significantly lower (P<0.05) at weeks 52, 62 and 72 than at the earlier collection weeks, which were all different from one another (P < 0.05). The highest concentration was seen at week 32

(P<0.05). Exactly the same pattern and differences were observed for the total amount of corticosterone in albumen.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Aug	56.9 ± 0.2^{bc}	34.2 ± 0.2^{ab}	1.30 ± 0.03^{b}	26.7 ± 0.7^{b}
32	Oct	55.7 ± 0.4^{c}	32.8 ± 0.3^{c}	1.50 ± 0.04^{a}	41.5± 1.1^{a}
42	Dec	57.7 ± 0.4^{b}	33.3 ± 0.3^{bc}	1.06 ± 0.03^{c}	33.3 ± 1.1^{c}
52	Feb	58.0 ± 0.5^{b}	33.2 ± 0.4^{bc}	0.87 ± 0.03^{d}	32.3 ± 0.9^{d}
62	May	58.2 ± 0.5^{b}	34.2 ± 0.4^{ab}	0.89 ± 0.03^{d}	34.2 ± 0.9^{d}
72	Jul	59.9 ± 0.4^{a}	34.7 ± 0.4^{a}	0.92 ± 0.03^{d}	31.1 ± 1.0^{cd}
P Value		<0.001	0.002	<0.001	<0.001

Table 3-2. Farm FR2: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm FR3, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen (all P<0.001) (see table 3.3). Egg weight was significantly lower at weeks 24 and 32 than at other times (P<0.05). Albumen weight was higher at 42 and 72 weeks than at 24 and 32 weeks (P<0.05). The albumen corticosterone concentration was significantly higher (P <0.05) at week 24 than at all other weeks. The lowest concentration was seen at week 72. The total amount of albumen corticosterone followed a similar pattern as the albumen concentration.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	51.6 ± 0.4^{d}	31.4 ± 0.3^{c}	1.38 ± 0.04^{a}	43.1 ± 1.2^{a}
32	Nov	58.9 ± 0.4^{c}	34.5 ± 0.3^{b}	1.10 ± 0.03^{b}	38.5 ± 1.0^{b}
42	Feb	66.0 ± 0.5^{a}	38.4 ± 0.4^{a}	1.21 ± 0.03^{b}	47.0 ± 1.3^{a}
52	Apr	64.1 ± 0.5^{ab}	34.9 ± 0.4^{b}	0.92 ± 0.02^{c}	31.9 ± 0.8^{c}
62	Jul	63.8 ± 0.5^{b}	37.3 ± 0.4^{a}	0.83 ± 0.02^{cd}	30.8 ± 0.9c^{d}
72	Sep	64.2 ± 0.5^{ab}	37.3 ± 0.4^{a}	0.73 ± 0.03^{d}	26.8 ± 0.9^{d}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-3. Farm FR3: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm FR4, age of collection had a significant effect on egg and albumen weight, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001)

(see table 3.4). Egg and albumen weights were significantly higher at 62 and 72 weeks (P <0.05) than at other times. Egg and albumen weights were lowest at 24 weeks (P<0.05). The egg albumen corticosterone concentration was significantly higher (P<0.05) at week 24 and lowest at 42 and 72 weeks (P<0.05). The total amount of albumen corticosterone was significantly higher (P<0.05) at week 24 and lowest at 42 and 72 weeks (P<0.05).

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	55.7 ± 0.5^{d}	32.7 ± 0.4^{d}	1.23 ± 0.03^{a}	40.5± 1.1^{a}
32	Nov	59.4 ± 0.5^{c}	36.0 ± 0.4^{c}	0.97 ± 0.02^{b}	35.2 ± 1.0^{b}
42	Feb	63.7 ± 0.5^{b}	38.3 ± 0.4^{b}	0.73 ± 0.03^{d}	27.9 ± 1.3^{c}
52	Apr	65.0 ± 0.5^{b}	39.0 ± 0.4^{b}	0.85 ± 0.02^{c}	33.1± 0.9^{b}
62	Jul	67.3 ± 0.5^{a}	40.2 ± 0.4^{a}	0.87 ± 0.03^{c}	34.8 ± 1.1^{b}
72	Sep	67.7 ± 0.5^{a}	40.8 ± 0.4^{a}	0.76 ± 0.02^{d}	24.5 ± 1.0^{c}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-4. Farm FR4: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm FR5, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.10). Egg weight was significantly higher at 52 weeks (P<0.05) than at other times and were significantly lower at 24 and 32 weeks (P<0.05) compared to other collection times. Albumen weight was highest at 52 weeks and lowest at 24 weeks (P<0.05). The albumen corticosterone concentration was significantly higher (P<0.05) at week 24 and lowest at 32-62 weeks (P<0.05). The concentration increased at 72 weeks and was different to other collection times (P< 0.05). The total amount of corticosterone in albumen was highest at 24 and 72 weeks (P<0.05), when the concentration was highest.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	54.0 ± 0.4^{e}	32.8 ± 0.4^{d}	1.31 ± 0.03^{a}	43.0 ± 1.2^{a}
32	Nov	60.7 ± 0.4^{d}	36.3 ± 0.3^{bc}	0.83 ± 0.02^{c}	29.9 ± 0.8^{b}
42	Feb	64.9 ± 0.6^{ab}	37.8 ± 0.5^{ab}	0.85 ± 0.03^{c}	32.0 ± 1.3^{b}
52	Apr	66.5 ± 0.5^{a}	39.2 ± 0.4^{a}	0.74 ± 0.03^{c}	29.1 ± 1.1^{bc}
62	Jul	62.9 ± 0.5^{c}	37.1 ± 0.4^{b}	0.74 ± 0.02^{c}	27.5 ± 0.7^{c}
72	Sep	63.6 ± 0.5^{bc}	38.1 ± 0.4^{ab}	1.09 ± 0.03^{b}	41.7 ± 1.2^{a}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-5. Farm FR5: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

3.1.2 Barn farms

For farm Bn1, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.6). Egg weight increased significantly (P<0.05) from 24 weeks until 52 weeks and was similar then at 62 weeks. Albumen weight was significantly greater from week 42 onwards (P<0.05). Significantly higher (P<0.05) albumen corticosterone concentrations were observed at 24 and 32 weeks (P<0.05) than at other weeks except where it was similar for weeks 32 and 42. The lowest concentrations were found at 52 and 62 weeks (P<0.05). The total amount of corticosterone in albumen were similar for weeks 24 to 42 but significantly higher than for weeks 52 and 62 (P <0.05).

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	54.7 ± 0.4^{cd}	32.9 ± 0.4^{b}	1.27 ± 0.04^{a}	41.8 ± 1.2^{a}
32	Oct	56.0 ± 0.3^{c}	32.6 ± 0.3^{b}	1.24 ± 0.03^{ab}	40.8± 1.2^{a}
42	Jan	59.6 ± 0.3^{b}	34.7 ± 0.3^{a}	1.15 ± 0.03^{b}	40.1 ± 1.0^{a}
52	Mar	61.4 ± 0.5^{a}	35.8 ± 0.4^{a}	0.75 ± 0.02^{c}	26.7 ± 0.7^{b}
62	May	60.2 ± 0.5^{ab}	35.1 ± 0.4^{a}	0.72 ± 0.02^{c}	25.1 ± 0.7^{b}
P value		<0.001	<0.001	<0.001	<0.001

Table 3-6. Farm Bn1: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	55.3 ± 0.5^{c}	31.5 ± 0.3^{d}	1.13 ± 0.03^{c}	35.5± 1.1^{c}
32	Oct	61.2 ± 0.3^{b}	33.7 ± 0.3^{c}	1.66 ± 0.06^{a}	56.3 ± 1.9^{a}
42	Jan	59.1 ± 0.5^{b}	34.7 ± 0.4^{bc}	1.29 ± 0.04^{b}	44.9 ± 1.7^{b}
52	Mar	61.1 ± 0.4^{b}	35.6 ± 0.3^{b}	0.77 ± 0.02^{d}	27.2 ± 0.7^{d}
62	May	60.7 ± 0.5^{b}	34.9 ± 0.4^{bc}	0.73 ± 0.02^{d}	26.4 ± 1.1^{d}
72	Aug	65.5 ± 0.5^{a}	39.1 ± 0.4^{a}	0.71 ± 0.02^{d}	28.1 ± 0.9^{d}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-7. Farm Bn2: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm Bn2, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.7). Egg and albumen weights were significantly higher at 72 weeks (P<0.05) than at other times and significantly lower at 24 weeks (P<0.05) and were similar in weeks

32, 42, 52 and 62. The albumen corticosterone concentration was significantly higher (P<0.05) at week 32 than at other collection ages. The concentrations were similar at weeks 52, 62 and 72 but significantly lower than at other times (P<0.05). The total amount of corticosterone in albumen followed the same pattern as albumen concentrations.

For farm Bn3, age of collection had a significant effect on egg and albumen weight, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.8). Egg weight was significantly higher at 52 to 72 weeks (P<0.05) compared to other times and at 24 weeks lower than all other weeks (P<0.05). Albumen weights were similar at 52 to 72 weeks and were higher than at other times (P<0.05). The albumen corticosterone concentration was significantly lower (P<0.05) at 24 and 52 weeks compared to all other weeks which were similar. The total amount of albumen corticosterone was lowest at 24 and 52 weeks (P<0.05) in line with the lowest corticosterone concentrations.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Mar	55.7 ± 0.4^{d}	34.0 ± 0.3^{b}	0.69 ± 0.03^{b}	24.4 ± 1.0^{c}
32	May	59.6 ± 0.5^{c}	34.7 ± 0.4^{b}	0.81 ± 0.03^{a}	28.2 ± 1.0^{bc}
42	Jul	61.3 ± 0.5^{bc}	36.2 ± 0.4^{b}	0.88 ± 0.02^{a}	31.8 ± 0.9^{ab}
52	Oct	63.7 ± 0.5^{a}	37.7 ± 0.4^{a}	0.67 ± 0.02^{b}	25.4 ± 0.8^{c}
62	Dec	63.8 ± 0.5^{a}	37.8 ± 0.3^{a}	0.81 ± 0.02^{a}	30.3 ± 0.8^{b}
72	Feb	65.2 ± 0.5^{a}	38.6 ± 0.4^{a}	0.90 ± 0.03^{a}	34.8 ± 1.3^{a}
P		<0.001	<0.001	<0.001	<0.001

Table 3-8. Farm Bn3: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

3.1.3 Conventional cage farms

For farm CC1, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.9). Egg and albumen weights increased significantly until week 42 (P<0.05) and then remained similar up to week 72. The corticosterone concentrations were significantly higher at 32 weeks (P<0.05) than at all other weeks. The corticosterone concentrations were similar for all other collections except for the significant difference between weeks 24 and 62 (P<0.05). Total corticosterone in albumen was higher at 32 weeks (P < 0.05) and lower at 24 weeks compared to other times (P<0.05).

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Jul	56.1 ± 0.4^{d}	33.2 ± 0.3^{b}	0.81 ± 0.02^{c}	26.7 ± 0.7^{c}
32	Oct	57.9 ± 0.4^{c}	33.6 ± 0.3^{b}	1.23 ± 0.03^{a}	41.5 ± 1.1^{a}
42	Dec	64.0 ± 0.4^{ab}	36.7 ± 0.3^{a}	0.90 ± 0.03^{bc}	33.3 ± 1.1^{b}
52	Feb	65.3 ± 0.4^{a}	37.9 ± 0.3^{a}	0.85 ± 0.02^{bc}	32.3 ± 0.9^{b}
62	May	63.6 ± 0.4^{b}	37.3 ± 0.3^{a}	0.92 ± 0.03^{b}	34.2 ± 0.9^{b}
72	Jul	64.6 ± 0.5^{ab}	37.7 ± 0.4^{a}	0.83 ± 0.02^{bc}	31.1 ± 1.0^{b}
P value		<0.001	<0.001	<0.001	<0.001

Table 3-9. Farm CC1: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm CC2, age of collection had a significant effect on egg and albumen weights, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.10). Egg and albumen weights were significantly lower at 24 weeks (P<0.05) and were highest at 42, 62 and 72 weeks (P<0.05). The albumen corticosterone concentration was significantly higher (P<0.05) at week 24 than at other collection ages. There was significant decrease with time, with the lowest concentrations seen the second half of the production cycle. The total corticosterone in albumen was significantly higher at the first three collection ages compared to the later collection ages (P<0.05).

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Sep	51.6 ± 0.4^{c}	31.5 ± 0.3^{c}	1.27 ± 0.05^{a}	40.2 ± 1.7^{a}
32	Oct	60.5 ± 0.5^{b}	34.9 ± 0.3^{b}	1.13 ± 0.04^{b}	39.3 ± 1.4^{a}
42	Jan	63.5 ± 0.5^{a}	37.4 ± 0.4^{a}	1.05 ± 0.03^{bc}	39.4 ± 1.2^{a}
52	Mar	60.5 ± 0.5^{b}	35.1 ± 0.4^{b}	0.94 ± 0.03^{cd}	32.9 ± 1.0^{b}
62	May	62.3 ± 0.6^{ab}	36.7 ± 0.5^{a}	0.70 ± 0.02^{d}	25.2 ± 0.8^{c}
72	Aug	63.7 ± 0.5^{a}	36.2 ± 0.4^{ab}	0.83 ± 0.02^{d}	30.0 ± 0.8^{b}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-10. Farm CC2: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm CC3, age of collection had a significant effect on egg and albumen weight, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.11). There were extensive variations in egg and albumen weights. However, they were significantly higher at week 72 (P<0.05) and lower at 24 weeks (P<0.05). The egg albumen corticosterone concentration were significantly higher at 24 and 32 weeks

compared to other times (P<0.05). Lowest concentration was seen at the end of production. The total corticosterone in albumen was highest in weeks 24 and 32 (P<0.05). The total amount of albumen corticosterone followed a similar pattern as the concentration.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Dec	55.0 ± 0.4^{e}	33.5 ± 0.2^{d}	1.38 ± 0.03^{a}	46.2± 0.9^{a}
32	Jan	56.9 ± 0.4^{d}	33.9 ± 0.3^{cd}	0.95 ± 0.03^{b}	32.2 ± 0.9^{b}
42	Apr	59.7 ± 0.5^{bc}	35.6 ± 0.3^{b}	0.70 ± 0.02^{cd}	25.0 ± 0.9^{c}
52	Jun	60.5 ± 0.4^{b}	35.0 ± 0.4^{bc}	0.75 ± 0.02^{c}	26.2 ± 0.7^{c}
62	Aug	58.5 ± 0.5^{cd}	34.5 ± 0.4^{bcd}	0.68 ± 0.02^{cd}	23.2 ± 0.8^{c}
72	Nov	64.6 ± 0.4^{a}	37.9 ± 0.3^{a}	0.64 ± 0.02^{d}	24.3 ± 0.8^{c}
P		<0.001	<0.001	<0.001	<0.001

Table 3-11. Farm CC3. The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

For farm CC4, age of collection had a significant effect on egg and albumen weight, albumen corticosterone concentration and total corticosterone in albumen (all P<0.001) (see table 3.12). Egg and albumen weights were highest at week 52 (P<0.05) compared to other weeks, except week 42. Egg weight was lowest at week 24 (P<0.05). The albumen corticosterone concentration was significantly higher (P<0.05) at 72 weeks and lowest at 52 weeks (P<0.05). The total amount of albumen corticosterone was high and low at the same time as the concentration was observed to be high and low.

Age at collection (Weeks)	Month of collection	Egg weight (g)	Albumen weight (g)	Albumen corticosterone concentration ng/g	Total corticosterone in albumen (ng)
24	Mar	56.0 ± 0.4^{d}	34.3 ± 0.3^{bc}	0.72 ± 0.03^{b}	24.7 ± 1.0^{bc}
32	May	62.4 ± 0.4^{ab}	37.3 ± 0.4^{a}	0.78 ± 0.02^{ab}	29.1 ± 0.9^{a}
42	Jul	63.7 ± 0.5^{a}	37.3 ± 0.3^{a}	0.73 ± 0.02^{b}	27.3 ± 0.7^{ab}
52	Sep	62.9 ± 0.5^{ab}	37.1 ± 0.4^{a}	0.61 ± 0.02^{c}	22.8 ± 0.6^{c}
62	Dec	61.4 ± 0.5^{b}	35.7 ± 0.3^{b}	0.74 ± 0.02^{b}	26.2 ± 0.7^{ab}
72	Feb	60.7 ± 0.5^{c}	34.6 ± 0.4^{b}	0.83 ± 0.02^{a}	28.8 ± 0.7^{a}
P Value		<0.001	<0.001	<0.001	<0.001

Table 3-12. Farm CC4: The mean (± SEM) egg and albumen weights, albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected at different ages and the corresponding months of these collections. Within columns, values with different superscripts are significantly different (P<0.05).

3.2 Egg and albumen weights for all farms

The range in egg weights for individual farms is shown in figure 3.1. The pattern is reasonably consistent for all farms with the egg weight lowest at the start of the production cycle and then increasing until at a maximum weight between 52 to 72 weeks of age.

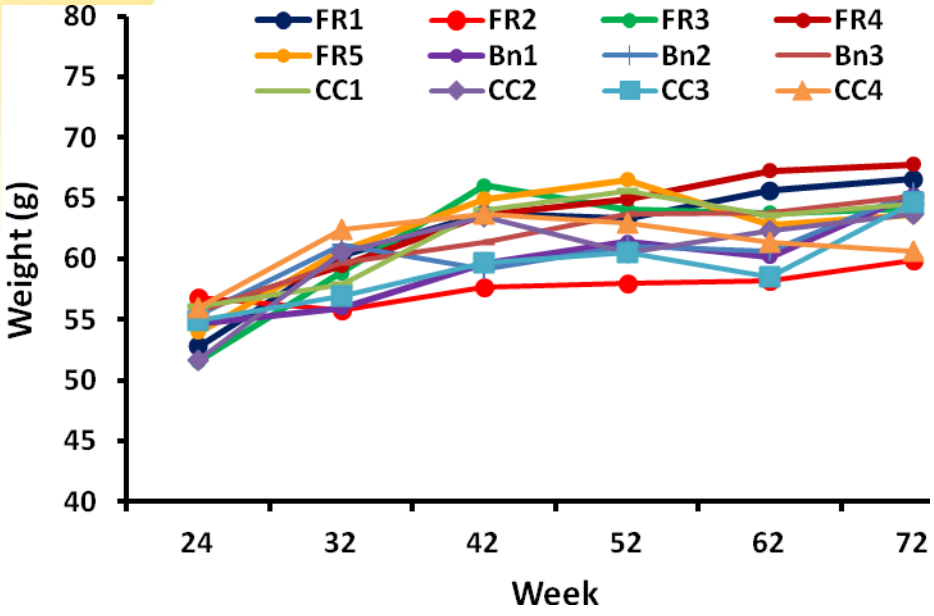


Figure 3-1. The egg weights for individual farms (FR- Free range farms 1-5; Bn – Barn farms 1-3; CC- Conventional cage farms 1-4).

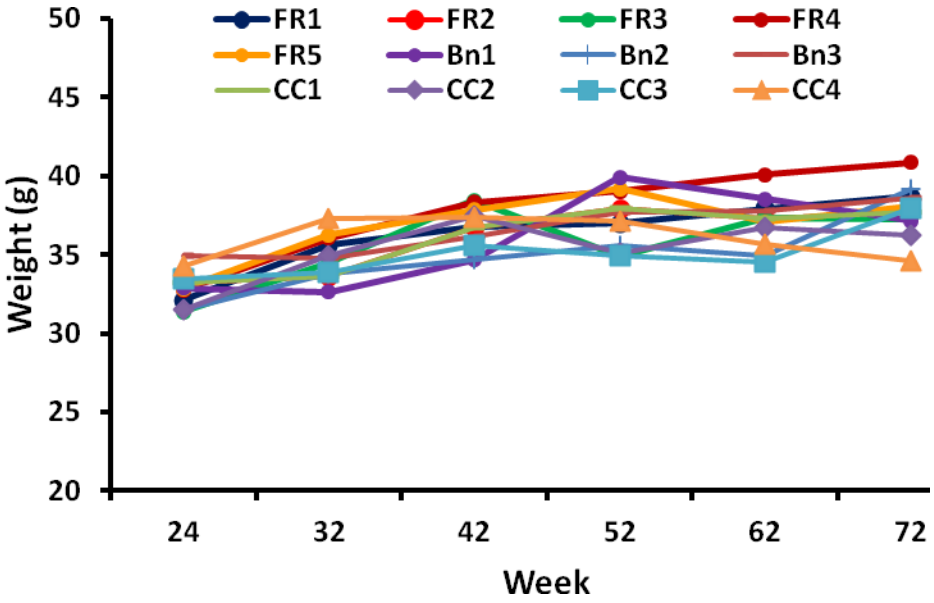


Figure 3-2. The albumen weights for individual farms (FR- Free range farms 1-5; Bn – Barn farms 1-3; CC- Conventional cage farms 1-4).

The range in albumen weights for individual farms is shown in figure 3.2. Because albumen weight is related to the egg weight (see Figure 3.13), the pattern is similar to that for the egg weights.

3.3 Albumen corticosterone concentrations and total corticosterone for all farms

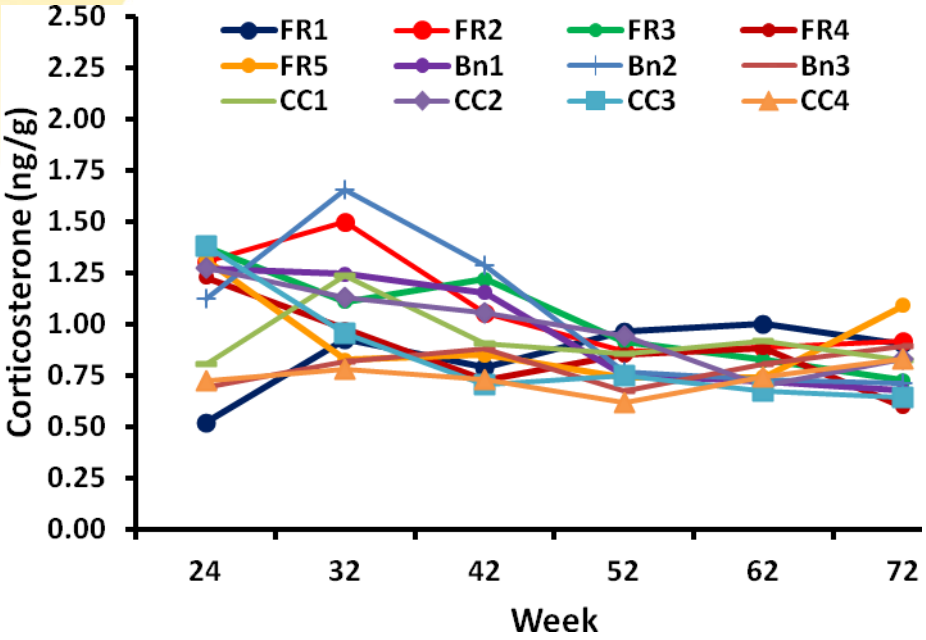


Figure 3-3. The egg albumen corticosterone concentrations for individual farms (FR- Free range farms 1-5; Bn – Barn farms 1-3; CC- Conventional cage farms 1-4).

The range in egg albumen corticosterone concentrations for individual farms is shown in figure 3.3. While the range varies for different farms, the general pattern for commercial farms is for the corticosterone concentrations to be higher in the early part of the production cycle and then for it to decrease and remain relatively stable over weeks 52 to 72. Exceptions to this general pattern are seen for farms CC4 and Bn3 where the range of concentrations was much smaller with the values early in the production cycle being similar to the concentrations seen for other farms in the later collections of the production cycle. A further point of interest is the relative low concentration seen at week 24 for farm FR1.

The range in total egg albumen corticosterone for individual farms is shown in figure 3.4. The range varies for different farms but the general pattern is similar to that seen for concentration because of the relationship between concentration and total albumen corticosterone (see Figure 3.14)

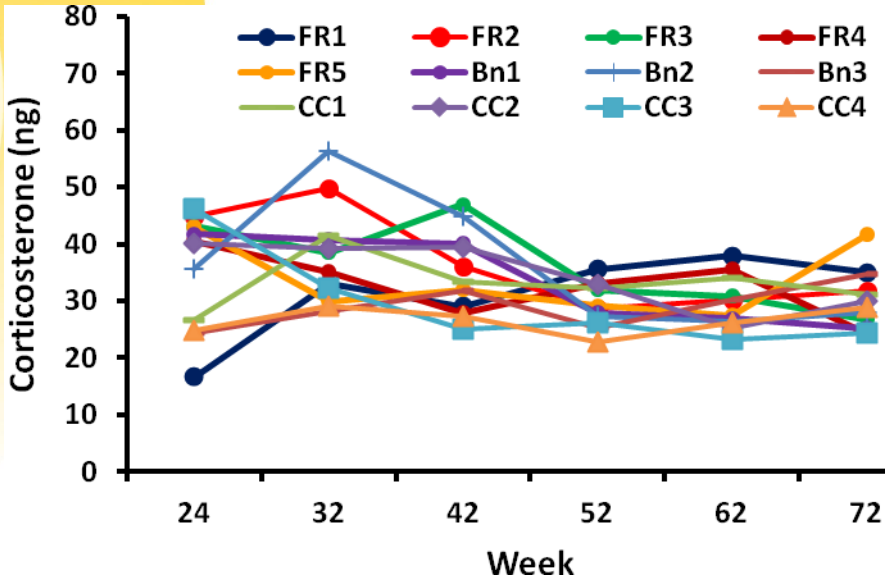


Figure 3-4. The range in total egg albumen corticosterone for the 12 individual farms (FR- Free range farms 1-5; Bn – Barn farms 1-3; CC- Conventional cage farms 1-4).

3.4 The comparisons between different production systems

3.4.1 Egg Weight

The effect of month of collection on egg weight is shown in figure 3.5. Month of collection had no significant effect on egg weight (P=0.57).

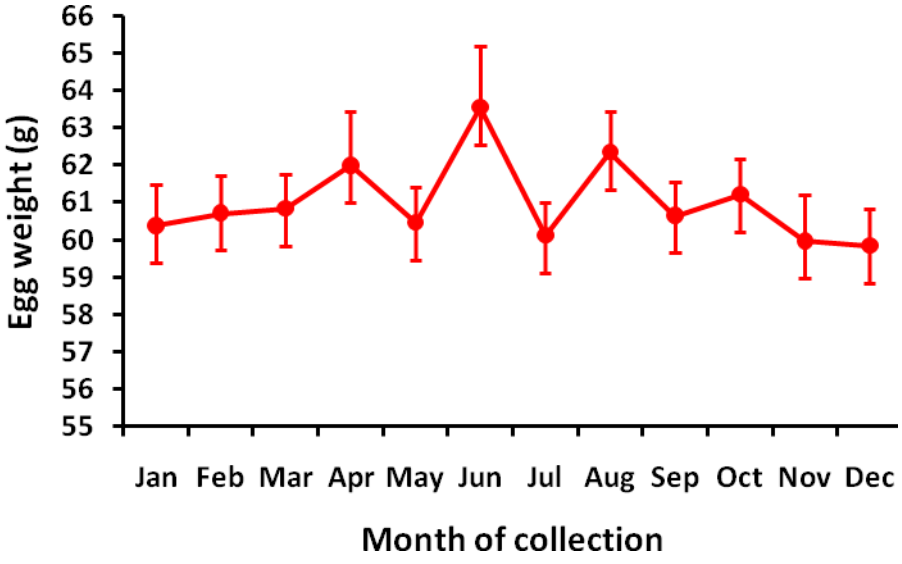


Figure 3-5. The mean (± SEM) egg weight for the different months were collections occurred in all three production systems. The effect of month was not significant (P=0.57).

The mean (± SEM) egg weight for the three production systems at the various collection ages is given in figure 3.6. The production system had no significant effect on egg weight (P=0.83). The collection age had a significant effect (P<0.001) with the collection age x system interaction not significant (P=0.68). The egg weight increased from 24 to 42 weeks and then remained relatively similar until the end of production. While it appears that at week 42, the egg weight was lower for the barn system, this was not detected in the analysis. This is possibly related to the lower egg weight seen for Bn2 at this time.

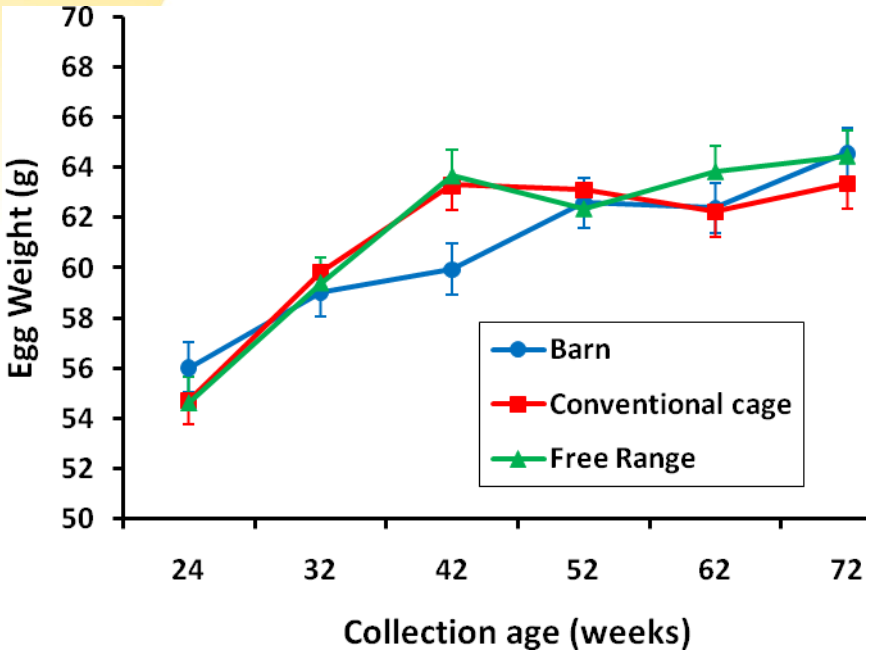


Figure 3-6. The mean (±SEM) egg weight for the three production systems, Conventional cages (n=4), Barn (n=3) and Free range (n=5). The effect of production system was not significant (P=0.83).

3.4.2 Albumen weight

The effect of month of collection on albumen weight is shown in figure 3.7. Month of collection had no significant effect on albumen weight (P=0.79).

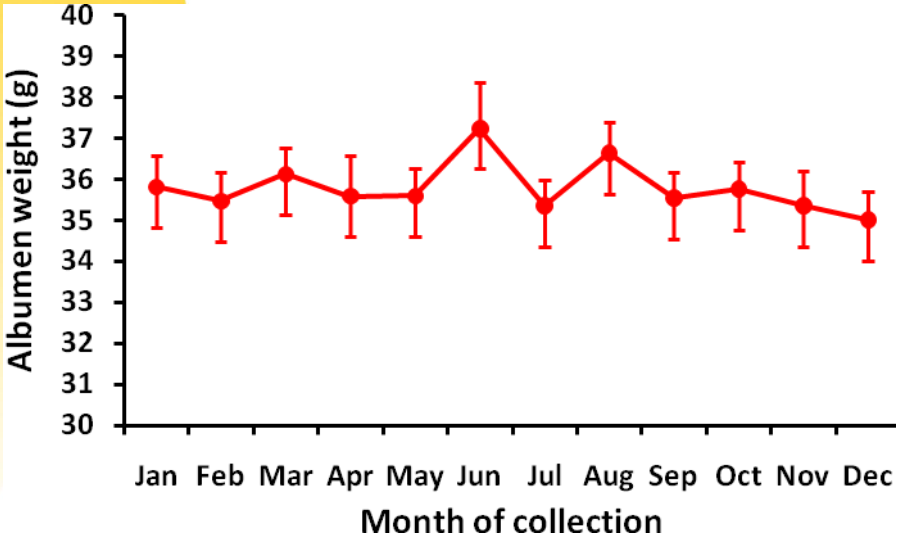


Figure 3-7. The mean (±SEM) egg albumen weight for the different months were collections occurred in all three production systems. The effect of month was not significant (P=0.79).

The mean (± SEM) egg albumen weight for the three production systems at the various collection ages is given in figure 3.8.

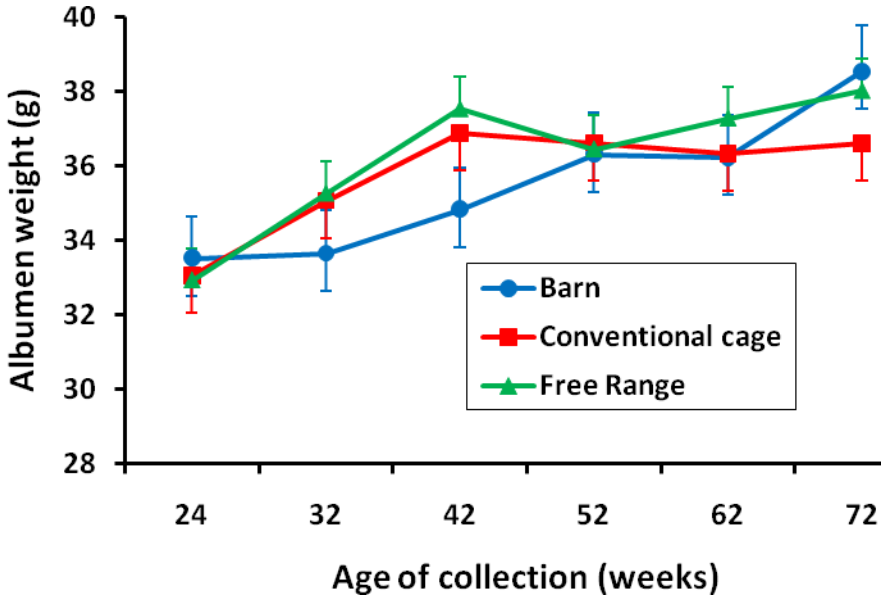


Figure 3-8. The mean (± SEM) egg albumen weight for the three production systems, Conventional cages (n=4), Barn (n=3) and Free Range (n=5). The effect of production system was not significant (P=0.72).

The production system had no significant effect on albumen weight (P=0.72). The collection age had a significant effect (P<0.001) with the collection age x system interaction not significant (P=0.44). The egg weight and albumen weight are related (see Figure 3.13) and for this reason the patterns are similar. The albumen weight increased from 24 to 42 weeks and then remained relatively similar until the end of production. While it does appear that at weeks 32 and 42 the albumen weight was lower for the barn system, this was not detected in the analysis. This again is possibly related to the lower albumen weight seen for farm 6 at these times.

3.4.3 Corticosterone concentrations

The effect of month of collection on albumen weight is shown in figure 3.9. Month of collection had no significant effect on corticosterone concentration (P=0.28).

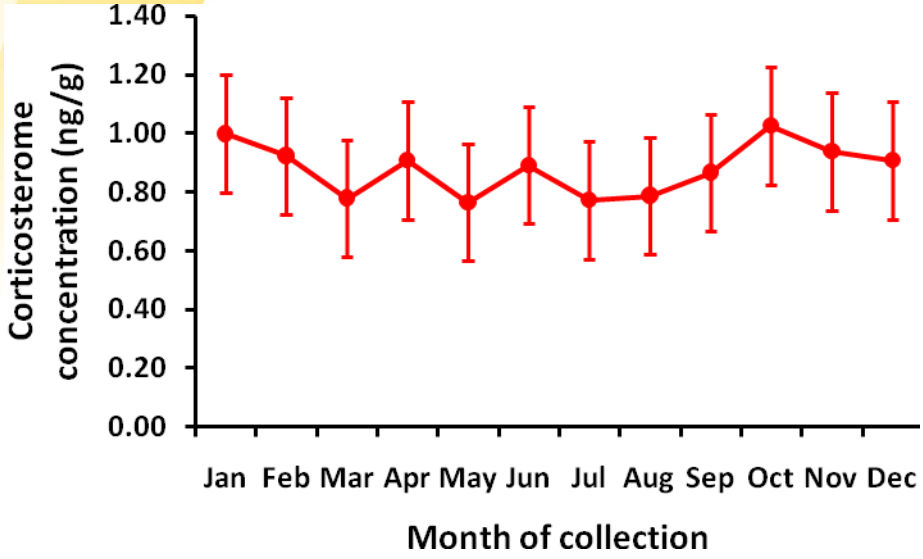


Figure 3-9. The mean egg albumen corticosterone concentrations for the different months of collections in all three production systems. The effect of month was not significant (P=0.28).

The mean egg albumen corticosterone concentration for the three production systems at the various collection ages is given in figure 3.10. The production system had no significant effect on egg albumen corticosterone concentration (P=0.78). The collection age had a significant effect (P=0.02) with the collection age x system interaction not significant (P=0.72). The albumen corticosterone concentrations were higher in the early stages of the production cycle. There seemed to be more variation in the mean values at different collection ages for the barn system.

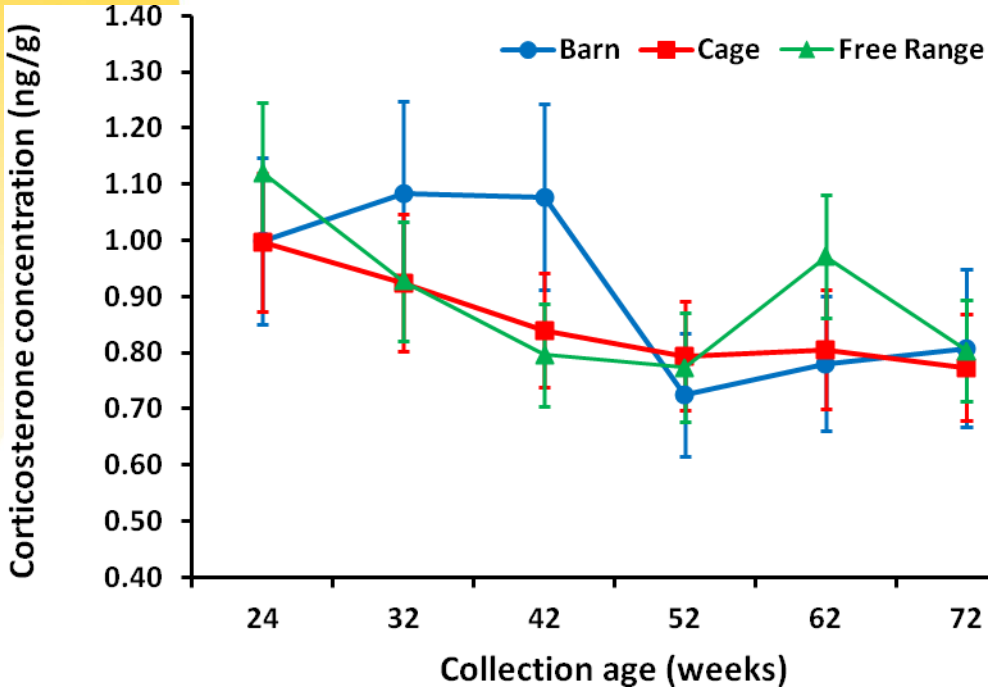


Figure 3-10. The mean egg albumen corticosterone concentrations for the three production systems, Conventional cages (n=4), Barn (n=3) and free range (n=5). The effect of production system was not significant (P=0.78).

3.4.4 Total egg albumen corticosterone

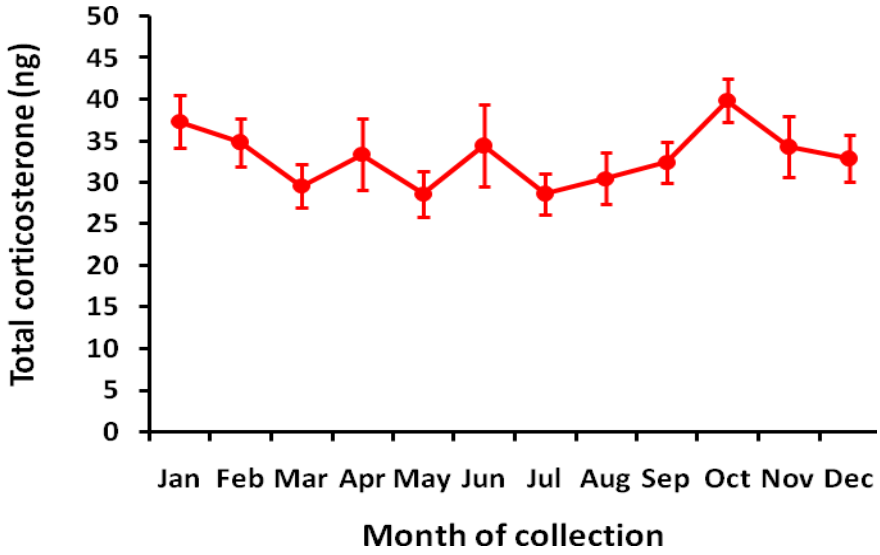


Figure 3-11. The mean total egg albumen corticosterone for the different months where collections occurred in all three production systems. The effect of month was not significant (P=0.16).

The effect of month of collection on total egg albumen corticosterone is shown in figure 3.11. Month of collection had no significant effect on total albumen in egg albumen (P=0.16).

The mean total egg albumen corticosterone concentrations for the three production systems at the various collection ages is given in figure 3.12. The production system had no significant effect on the total egg albumen corticosterone concentrations (P=0.48). The collection age had no significant effect (P=0.13) with the collection age x system interaction also not significant (P=0.79). There seemed to be more variation in the mean values at different collection ages for the barn system.

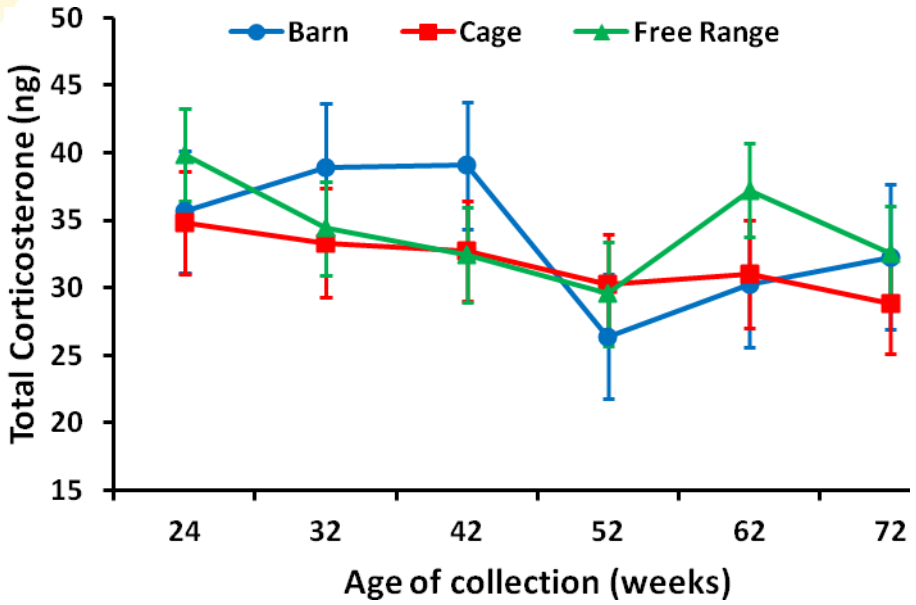


Figure 3-12. The mean total egg albumen corticosterone concentrations for the three production systems, Conventional cages (n=4), Barn (n=3) and Free Range (n=5). The effect of production system was not significant (P=0.48).

3.4.5 The patterns of corticosterone concentrations

Visual appraisal of the corticosterone concentrations for individual farms suggests that these may fit into three different patterns. These patterns are shown in Figure 3.13.

Pattern 1: Farms (Bn1, Bn2, FR2, FR3 and CC2) where the concentration starts high and remains high during the first half of the production cycle and then decreases and remains at a consistently lower concentration for the second half of the cycle.

Pattern 2: Farms (CC1, CC3, FR4 and FR5) where the concentration starts high and then declines from this point and quickly reaches a lower concentration that is maintained for the remainder of the production cycle.

Pattern 3: Farms (FR1, CC4 and Bn3) where the concentrations begin low and remain consistently low during the production entire cycle.

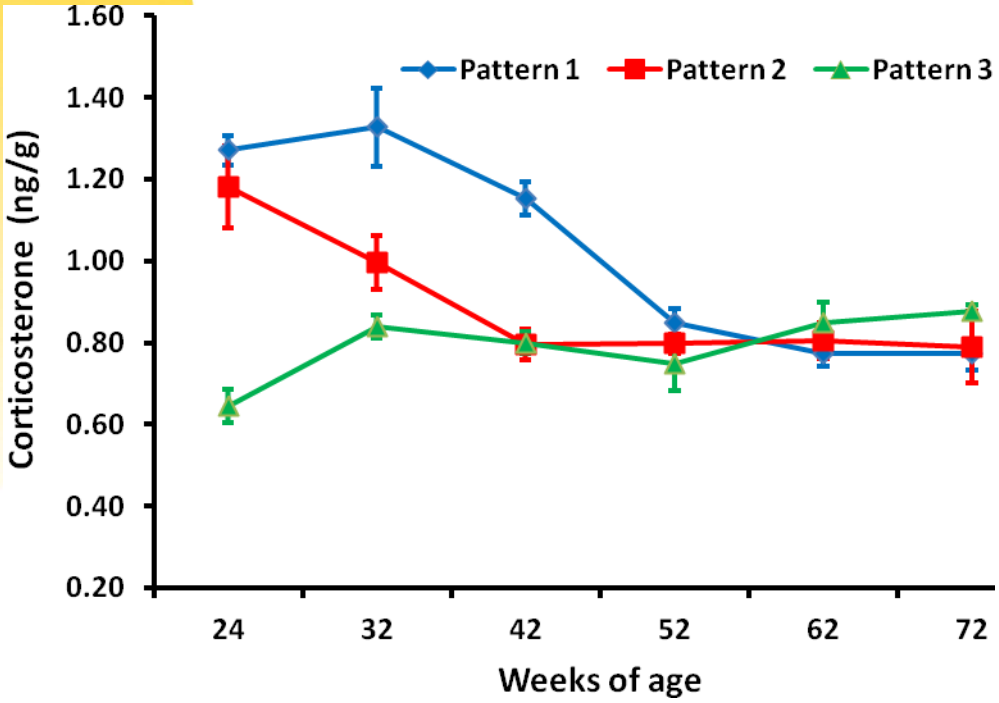


Figure 3-13. Different patterns of egg albumen corticosterone during the production cycle.

3.5 Relationship between egg and albumen weights

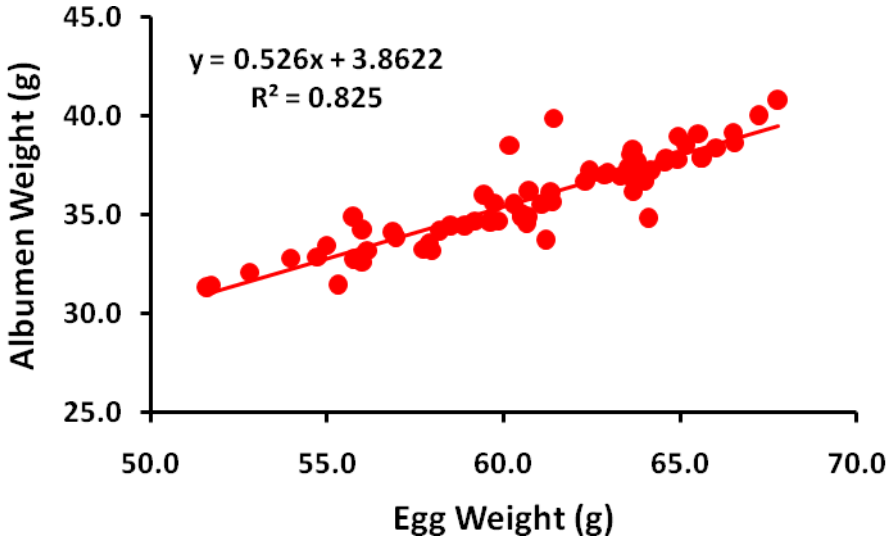


Figure 3-14. The relationship between the mean egg weight and albumen weight for the individual collection ages for all farms.

The relationship between the mean egg weight and albumen weight for the individual collection ages for all farms is shown in figure 3.14. As expected, there is a strong correlation between (r=0.91) mean egg and albumen weights.

3.6 Relationship between egg albumen corticosterone concentration and total albumen corticosterone

The relationship between the mean egg albumen corticosterone concentration and the total albumen corticosterone for the individual collection ages for all farms is shown in figure 3.15. As expected, there is a strong correlation between (r=0.97) mean egg albumen corticosterone concentration and the total albumen corticosterone.

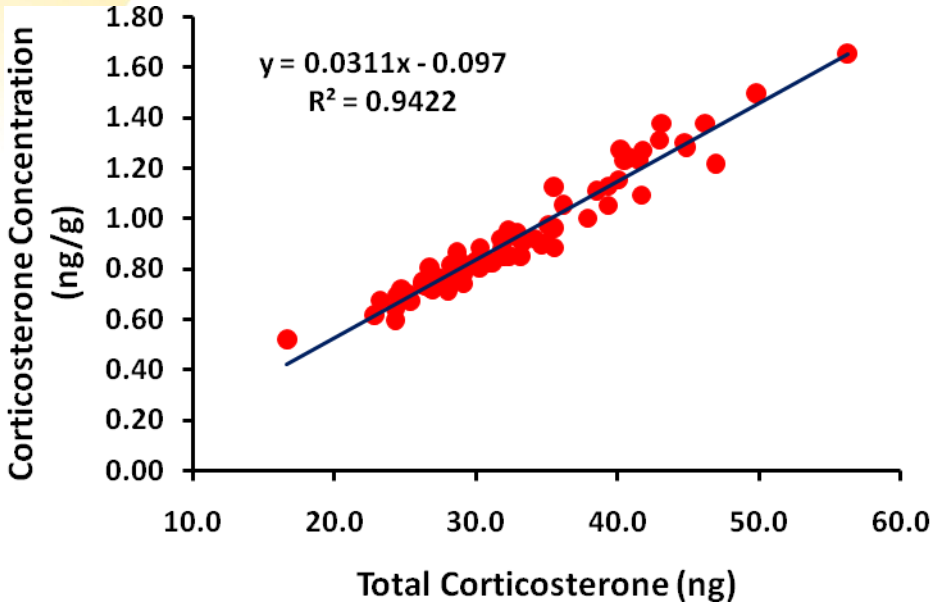


Figure 3-15. The relationship between the mean egg albumen corticosterone concentrations and the total albumen corticosterone for the individual collection ages for all farms.

3.7 The range in egg corticosterone concentrations for all farms

While the comparisons between production systems have been described (see section 3.3), there is another level where effects need to be considered. This is the level where the individual egg values for each of the collection times on each of the farms is evaluated. The plot of individual egg values for each farm highlights that there are some values which can be considered as high and indicate that there are probably some hens in these flocks with high plasma corticosterone concentrations. Figures 3.16 to 3.27 are plots of albumen corticosterone concentrations for all eggs collected from each of the 12 farms. An arbitrary value of 1.5 ng/g has been selected as the cut off point for the determination of the percentage of eggs with high corticosterone concentrations. This arbitrary value has been selected because for each housing system the farm with the lowest corticosterone concentrations are below this cut off value. The percentage of eggs with high corticosterone concentrations was calculated as the ratio of the number of eggs with values above 1.5 ng/g divided by the total number of eggs sampled. This percentage was calculated for each collection age for each of the 12 flocks involved in the study.

3.7.1 Free Range farms

The albumen corticosterone concentrations for all eggs collected on farm FR1 are shown in figure 3.16. The % of eggs with high values was minimal. A small percentage of high values were detected in the second half of the production cycle.

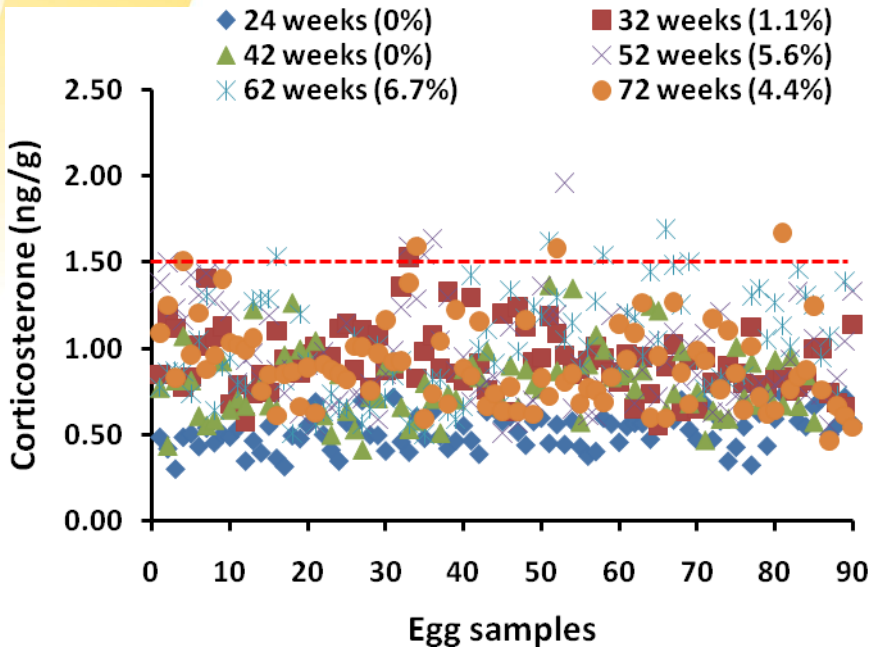


Figure 3-16. The albumen corticosterone concentrations for all eggs collected on farm FR1. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm FR2 are shown in figure 3.16. A high % of eggs collected at 24 and 32 weeks had high corticosterone concentrations. After this there were only a small number of eggs with values above 1.5 ng/g. In week 32 a small number of hens had values greater than 2.5 ng/g.

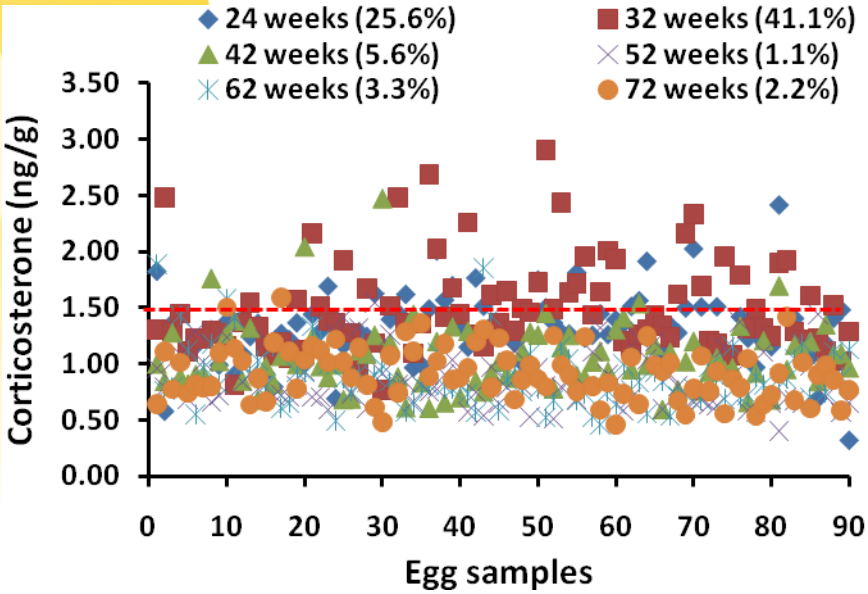


Figure 3-17. The albumen corticosterone concentrations for all eggs collected on farm FR2. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm FR3 are shown in figure 3.17. A very high % (41.1) of eggs collected at week 24 had high corticosterone concentrations. This % was lower at 32 and 42 weeks but still high compared to the collections after week 42 when the % was minimal.

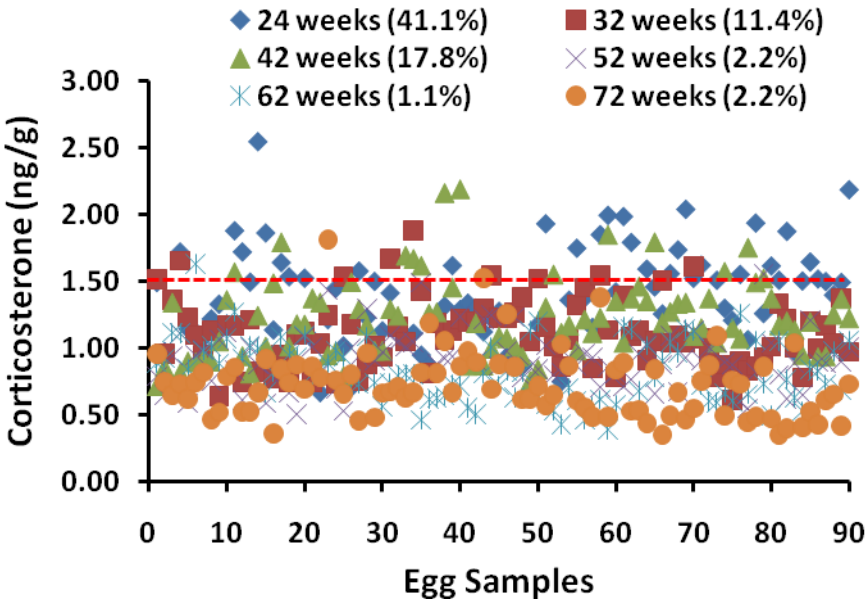


Figure 3-18. The albumen corticosterone concentrations for all eggs collected on farm FR3. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm FR4 are shown in figure 3.18. The % of eggs with values above 1.5 ng/g was moderate at week 24 (14.4) and after this very low for the later collections.

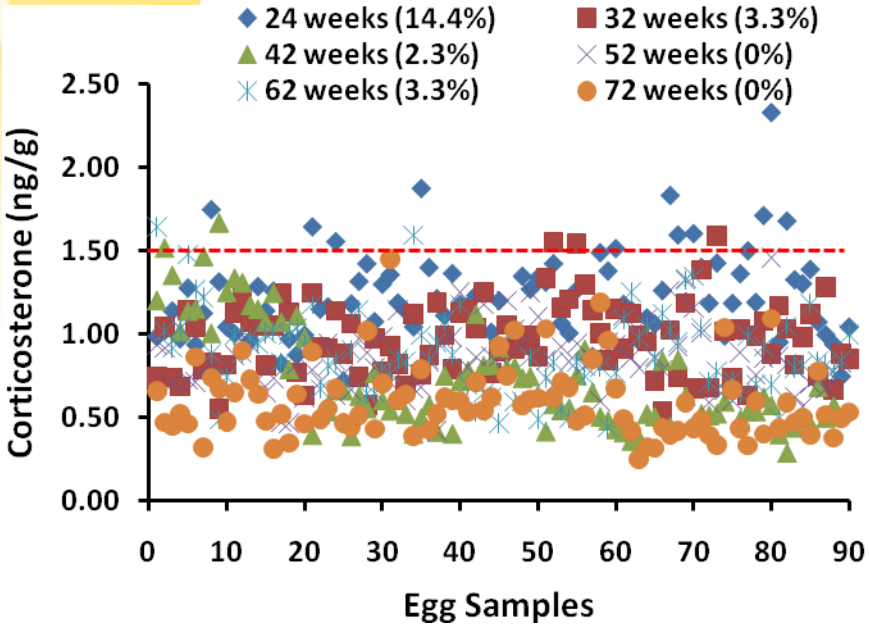


Figure 3-19. The albumen corticosterone concentrations for all eggs collected on farm FR4. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

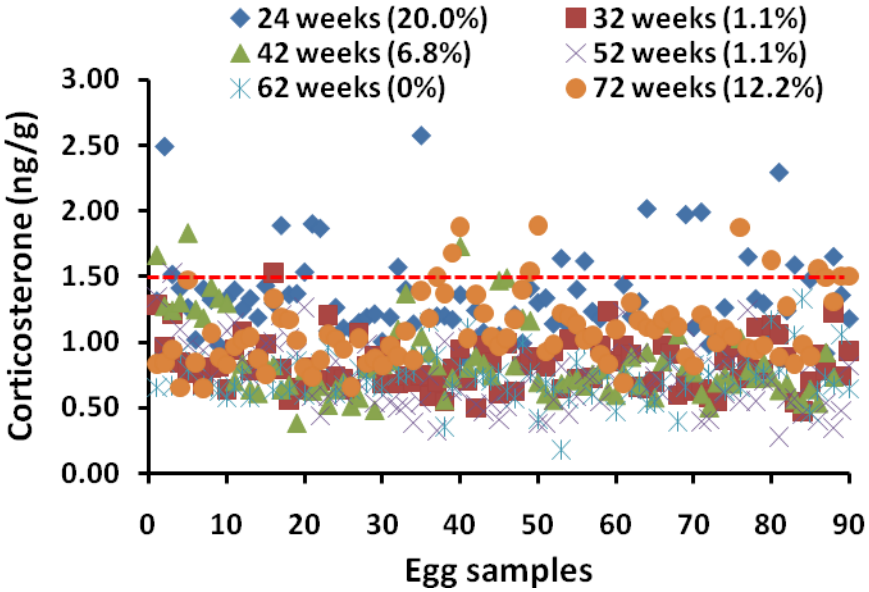


Figure 3-20. The albumen corticosterone concentrations for all eggs collected on farm FR5. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm FR5 are shown in figure 3.20. There were 20% of eggs with high values of corticosterone at week 24. The % was low thereafter except at the end of production where it was 12.2%.

3.7.2 Bran Farms

The albumen corticosterone concentrations for all eggs collected on farm Bn1 are shown in figure 3.21. A high % of eggs collected at 24 and 32 weeks had elevated corticosterone concentrations. This decreased somewhat at 42 weeks but was still moderately high at 14.4%. At 52 and 62 weeks no eggs were found to have values above 1.5 ng/g.

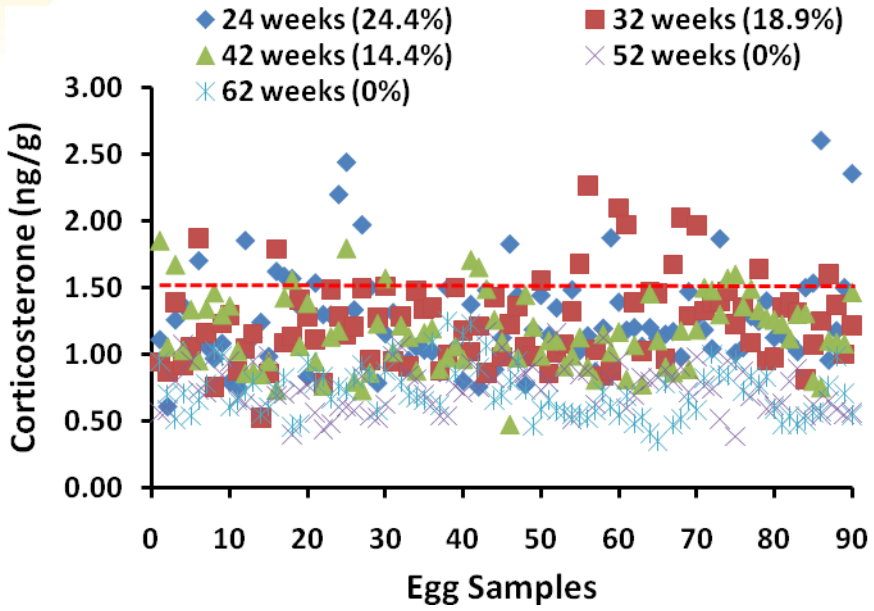


Figure 3-21. The albumen corticosterone concentrations for all eggs collected on farm Bn1. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

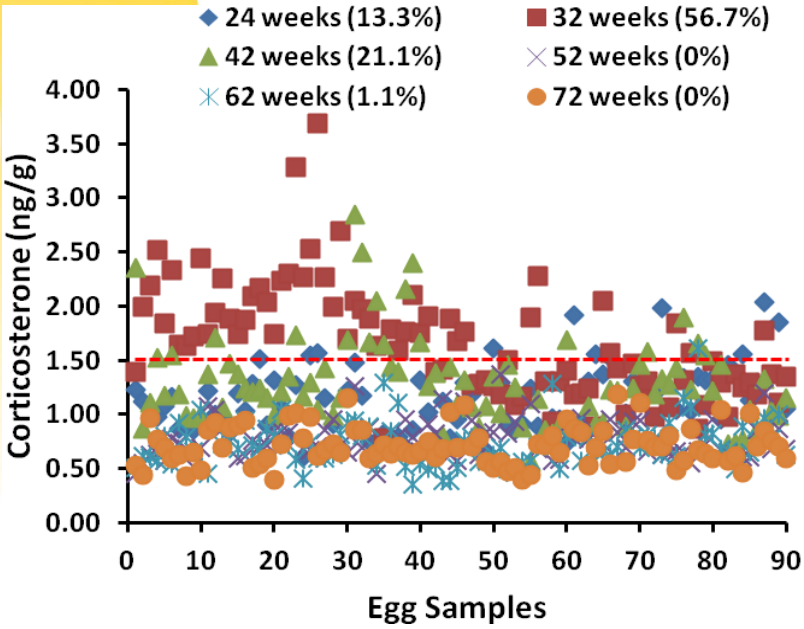


Figure 3-22. The albumen corticosterone concentrations for all eggs collected on farm Bn2. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm Bn2 are shown in figure 3.22. An exceptionally high % (56.7) of eggs collected at 32 weeks had high corticosterone concentrations. There were some eggs with very high values (> 2.5 ng/g) at this collection time. The % was lower at week 24 and 42 but still relatively high compared to later collection times when the % was minimal.

The albumen corticosterone concentrations for all eggs collected on farm Bn3 are shown in figure 3.23. The % of eggs with high values of corticosterone was exceptionally low at all collection times.

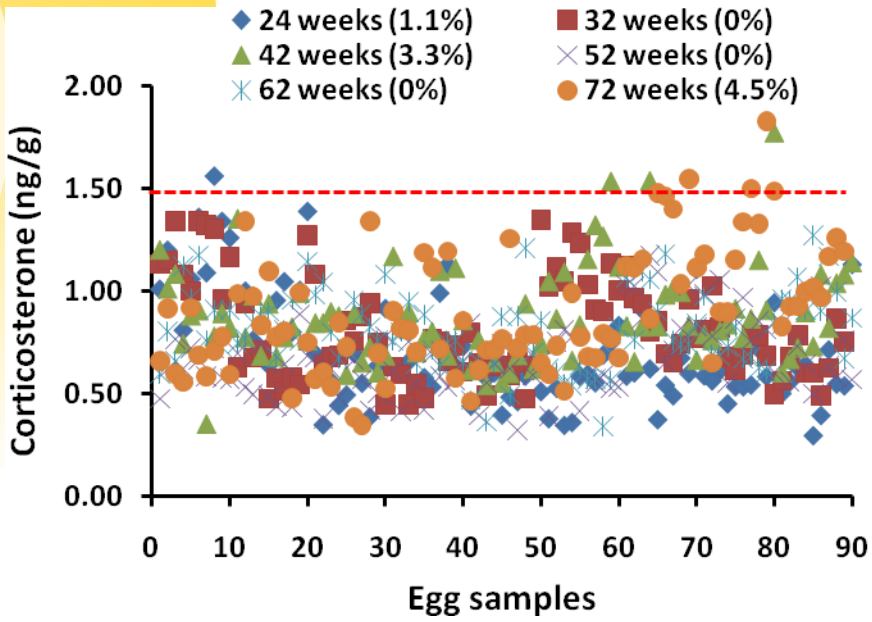


Figure 3-23. The albumen corticosterone concentrations for all eggs collected on farm Bn3. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

3.7.3 Conventional Cage farms

The albumen corticosterone concentrations for all eggs collected on farm CC1 are shown in figure 3.24. At the 32 week collection, 20% of the eggs had high corticosterone concentrations. At other times there were no eggs with values above 1.5 ng/g, or at best, only a small percentage.

The albumen corticosterone concentrations for all eggs collected on farm CC2 are shown in figure 3.25. A high % of eggs collected at 24 weeks had high corticosterone concentrations. This decreased somewhat at 32 weeks but remained at 14.4%. Only a low % of eggs with values above 1.5 ng/g were recorded after week 32.

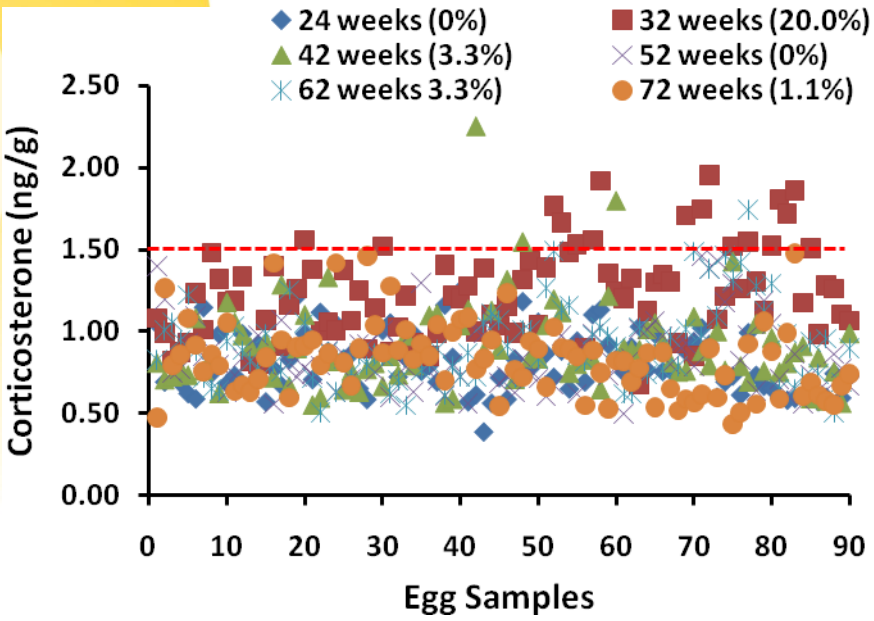


Figure 3-24. The albumen corticosterone concentrations for all eggs collected on farm CC1. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

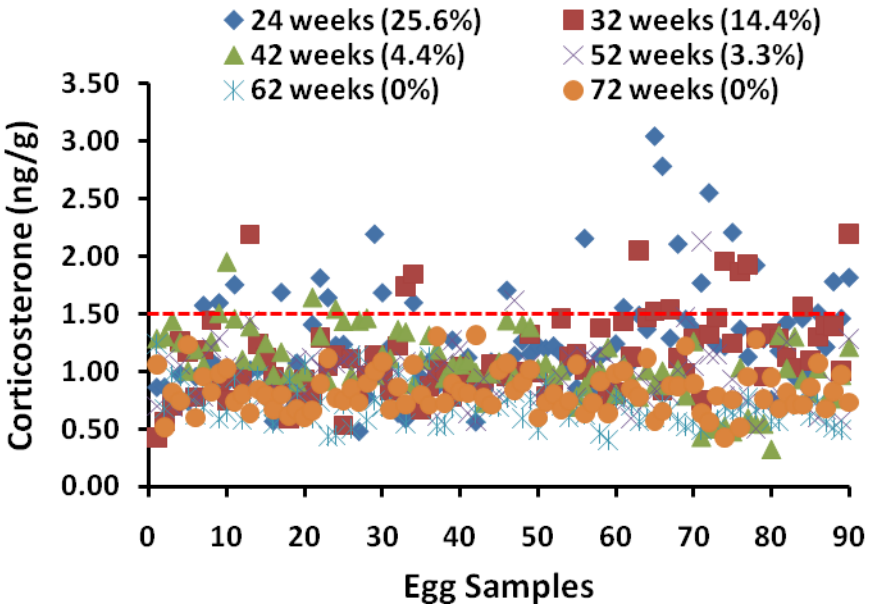


Figure 3-25. The albumen corticosterone concentrations for all eggs collected on CC2. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages

The albumen corticosterone concentrations for all eggs collected on farm CC3 are shown in figure 3.26. The only time that there was a high % of eggs with values above 1.5 ng/g was at week 24 (33.3) and after this the % was very low or zero.

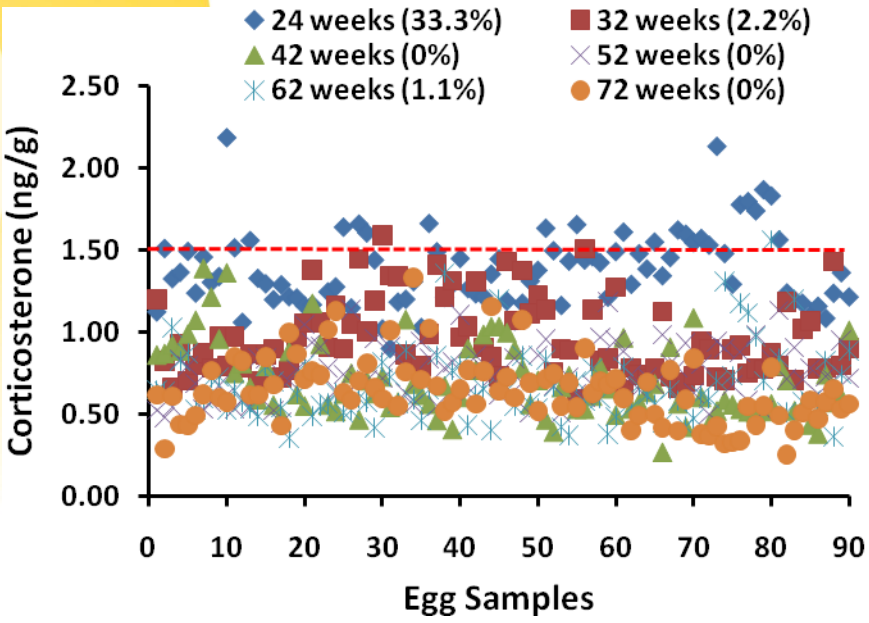


Figure 3-26. The albumen corticosterone concentrations for all eggs collected on farm CC3. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

The albumen corticosterone concentrations for all eggs collected on farm CC4 are shown in figure 3.27. The % of eggs with high values of corticosterone was exceptionally low at all collection times.

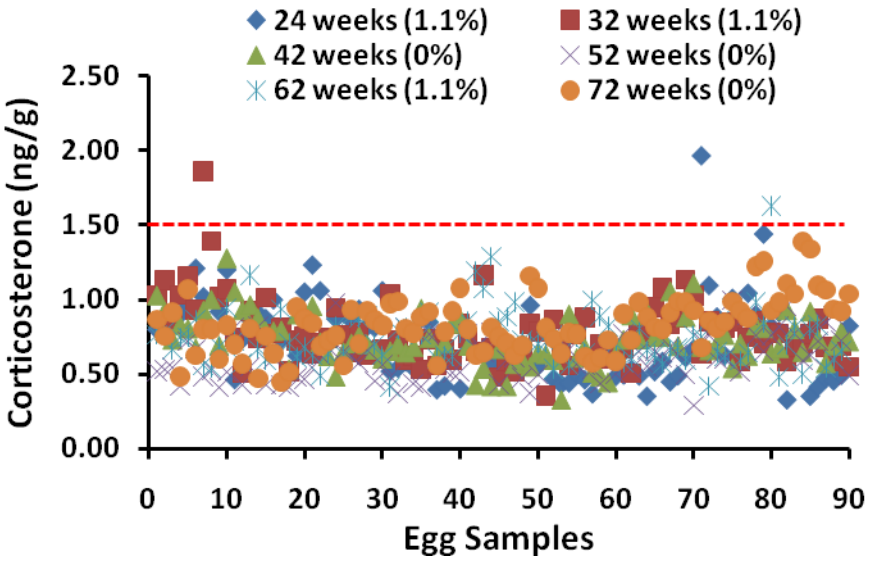


Figure 3-27. The albumen corticosterone concentrations for all eggs collected on farm CC4. The percentage of eggs with values above 1.5 ng/g is shown in the legend for all collection ages.

3.7.4 The mean percentage of egg with high corticosterone concentrations for the three production systems.

The mean (± SEM) percentage of hens with albumen corticosterone concentrations above 1.5 ng/g for the different production systems is shown in figure 3.28.

The effect of production system was not significant (P=0.39) while the effect of collection age was (P=0.001) but the interaction of system x age was not significant (P=0.77). The percentage of high values was significantly higher at weeks 24 and 32 compared to weeks 52 to 72 (P<0.05).

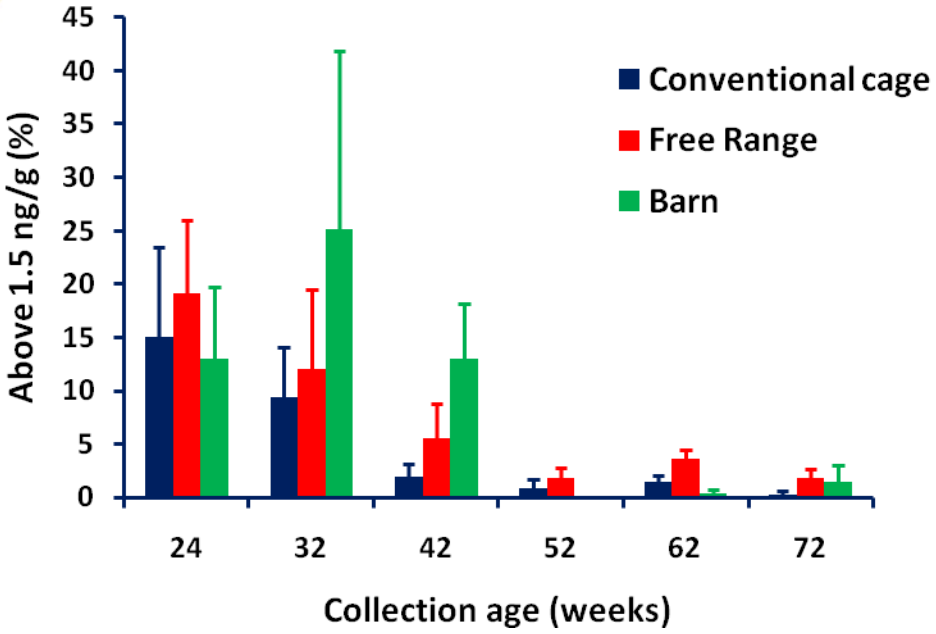


Figure 3-28. The mean (± SEM) percentage of hens with albumen corticosterone concentrations above 1.5 ng/g for the different production systems.

3.8 Egg production and mortality

Weekly egg production data were available for seven of the 12 farms. The hen housed egg production for the week in which eggs were collected for the corticosterone analysis is shown in table 3.13. Highest egg production was recorded for farm CC4 where hens were maintained in single cages. Low production was recorded in week 52 and 62 for farm FR1. At these times the flock was being treated for lice infestation.

The total mortality for these seven farms is reported in table 3.13. Highest mortality was recorded for farms FR2 and CC2 while the lowest was for farms CC1 and CC4.

Farm	24 Weeks (%)	32 Weeks (%)	42 Weeks (%)	52 Weeks (%)	62 Weeks (%)	72 Weeks (%)	Total Mortality (%)
FR1	70	91*	90	54	60	75	5.01
FR2	70.6	78.9	81.5*	82.4	80.4	70.1	7.22
FR4	66	83	90*	85	84.5	77.6	3.15
FR5	87.3	90.1*	87.6	84.1	84	76.1	3.08
CC1	77.4	91.6	98.2*	93.7	90	86.9	1.75
CC2	68.8	75.5	80.8*	80.4	78.4	67.3	6.50
CC4	94.7	97.1	97.5*	96.5	96.1	87.7	2.00

Table 3-13. The weekly hen housed egg production and total mortality for seven farms where data were available. * Highest production rate for each farm.

3.8.1 The relationship between egg albumen corticosterone concentration and the mean egg production

The relationship between egg albumen corticosterone concentration and the mean egg production for the week when eggs were collected is shown in figure 3.29. The correlation coefficient was 0.28 and the relationship was not significant at the P<0.05 level. Only around 8% of the variation in corticosterone production is accounted for by the rate of hen housed egg production. The higher corticosterone concentrations seen in the earlier weeks of the production cycle are unlikely to be due to the higher egg production seen at this time.

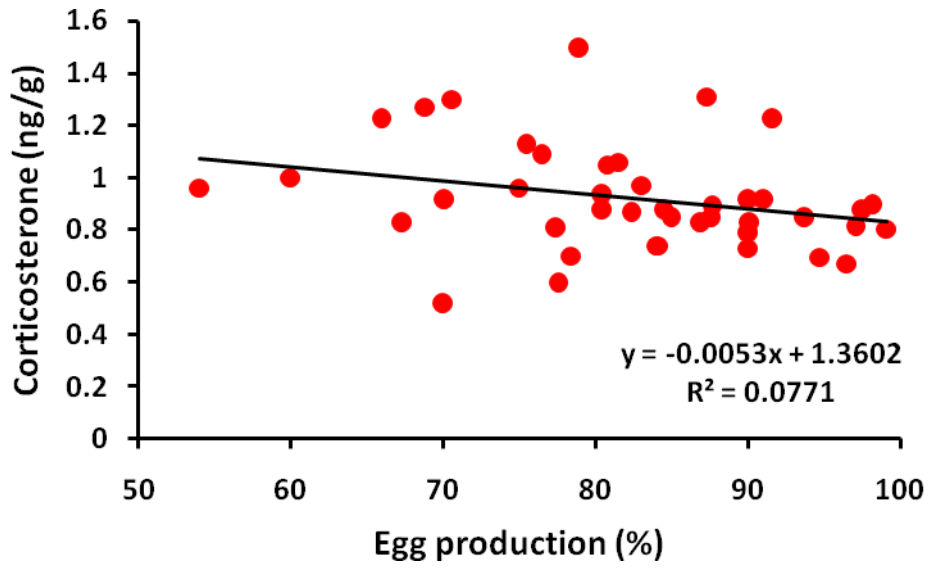


Figure 3-29. The relationship between egg albumen corticosterone concentration and the mean egg production for the week when eggs were collected.

3.8.2 The relationship between the mean albumen corticosterone concentration over weeks 24 to 42 and the average hen housed egg production.

Because the data indicate that there is the potential for the albumen corticosterone concentrations to be higher in the early half of the production cycle it would seem relevant to assess whether there is a relationship between egg production and albumen corticosterone during this phase of the production cycle. The relationship between mean egg albumen corticosterone concentration over weeks 24-42 and the average hen housed egg production over this same period is given in figure 3.30. The correlation coefficient was 0.79 and was significant at $P<0.05$. The relationship is negative with the albumen corticosterone concentrations being higher for flocks with the lower hen housed egg production. While the data available for this analysis is limiting, it does suggest that the conditions resulting in higher corticosterone concentrations also influence the rate of egg production during this early production stage.

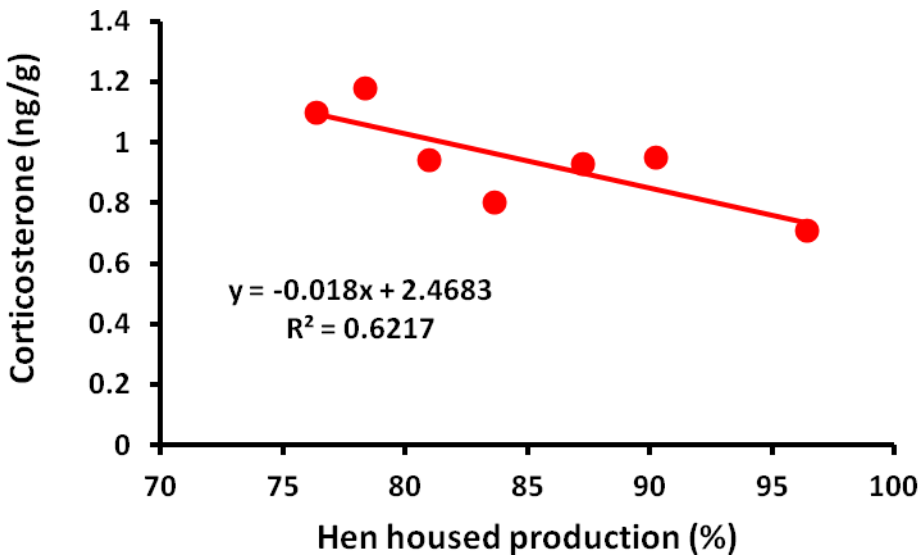


Figure 3-30. The relationship between mean egg albumen corticosterone concentration over weeks 24-42 and the average hen housed egg production over the same period

3.8.3 The relationship between the mean albumen corticosterone concentration and total mortality.

The relationship between mean egg albumen corticosterone concentration for the entire production period on individual farms and total mortality is shown in figure 3.31. The correlation coefficient was 0.73 and the relationship was marginally non-significant at $P<0.05$. There tended to be a positive relationship between total mortality and mean egg albumen corticosterone concentration with 53% of the variation in mortality accounted for by the differences in albumen corticosterone concentration.

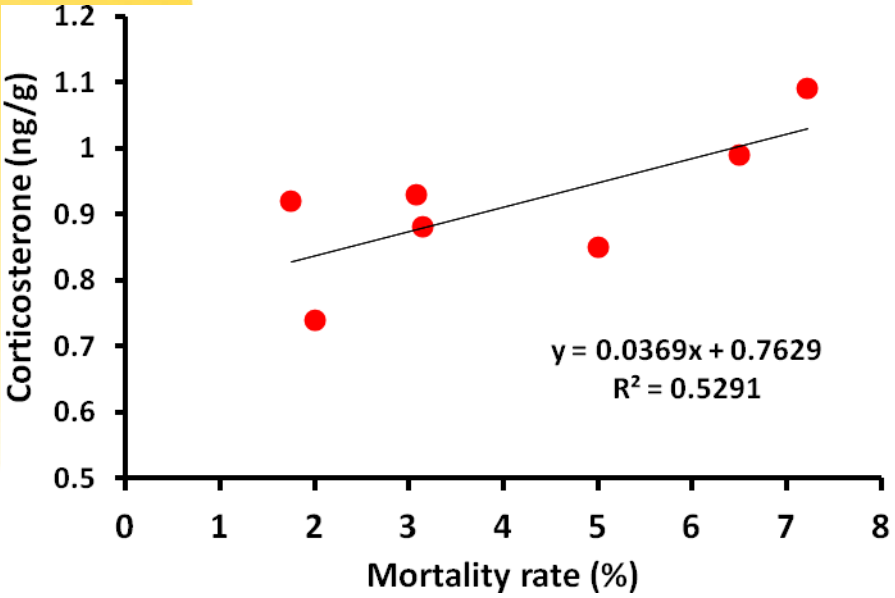


Figure 3-31. The relationship between mean egg albumen corticosterone concentration and the total mortality.

4 Discussion

Stress is relevant to welfare as it indicates a condition requiring an adjustment by the animal to adverse stimuli. If the adaptation is quick and successful then the consequences to the animal are minimal. If the stress is persistent, even if the adverse stimuli doesn't remain the same but varies, then the consequences for the animal can be poorer welfare. While plasma corticosterone concentrations are used as a measure of stress in poultry, blood sampling has significant limitations in that it is invasive, stressful in its own manner, really only gives a measure of corticosterone at a single time point and has limitations to the number of birds that can be sampled.

A major problem with using physiological measures as indicators of welfare is that often only single point samples are used in the assessment. Such sampling procedures are often difficult to interpret. Determining the change over time in physiological measures would provide researchers with better and more relevant information as to the status of the animals in their environment.

The use of egg albumen corticosterone concentrations as a measure of stress in poultry avoids many of the problems associated with blood collection and allows for regular sampling over a period of time. For this reason, the pattern of albumen corticosterone concentrations observed over the production cycle for individual farms can provide more detailed information as to the stress status of the flock during the production cycle.

Eggs were collected at the same ages for all farms but because flocks were placed at different times, the collections started in different months of the year. This highlights some of the problems encountered when moving from a controlled research environment to a commercial one. There are two levels to evaluate the results from the current study, collectively as part of a prescribed housing system and on an individual farm basis. The changes occurring within a farm are relevant to physiological status of the hens on that farm, irrespective of the housing type employed.

The month of collection had no significant effects on egg weight, albumen weight, corticosterone concentrations or total amount of corticosterone in albumen. As to be expected, the egg and albumen weights were significantly ($r=0.91$) correlated (see Figure 3.14). The egg albumen corticosterone concentrations and total corticosterone concentration are highly correlated ($r=0.97$) (see Figure 3.15). All the flocks which were part of the study were housed in facilities with some degree of environmental control and so are less likely to be affected by seasonal changes in temperature.

The collection age had a significant effect ($P<0.001$) on egg weight with it increasing from 24 to 42 weeks of age and then remaining relatively similar until the end of production. While it appears that at week 42 the egg weight was lower for the barn system this was not detected in the analysis. This is possibly related to the lower egg weight seen for farm Bn2 at this time. The production system had no significant effect on egg weight ($P=0.83$).

The age of collection had a significant effect on the egg albumen corticosterone concentration ($P= 0.02$) but no effect on the total amount of corticosterone in the albumen ($P=0.13$). This is difficult to understand because of the strong relationship between concentration and total amount of corticosterone (see Figure 3.15).

The type of production system had no significant effect on egg albumen corticosterone concentration ($P=0.78$) or on the total egg albumen corticosterone concentrations ($P=0.48$). With corticosterone concentrations used as a measure of stress in hens this

would suggest that the hens from any particular system were not any more stressed than those in another system. However, the grouping of farms into production systems masks some of the individual farm differences.

The albumen corticosterone concentrations for individual farms tended to fall into three patterns as shown in Figure 3.13. These patterns could reflect how the hens cope with the transition from the rearing environment to the layer housing. The decrease in albumen corticosterone concentration as hens age has been reported previously (Downing and Bryden, 2005). It seems to be an intrinsic feature as hens' age and may be related to decreased fearfulness and acclimatisation. Domestication of poultry has resulted in reduced fear, however, there are large variations in fearfulness due to strain and individual effects (Faure *et al*., 2003). Birds housed in different production systems have been shown to have different levels of fear, as determined by the TI test (Jones and Faure, 1981; Hansen *et al*., 1993). It may also represent habituation to the housing conditions so that some husbandry events are perceived as less stressful as hens age. A further consideration is the effect of culling and mortality. Those hens not coping in their environment are potentially ones that would have high corticosterone responses to the various challenges and are likely to be the ones removed from the flock either by culling or part of the flock mortality.

For some farms specific observations are worth noting. The corticosterone concentrations for farm FR1 increased at weeks 52, 62 and 72, and were as high as those seen at week 32. These elevated corticosterone concentrations corresponded to significant decreases in egg production in the week of the relevant egg collection (see table 3.13). On investigation, it was noted that this flock of hens were infected with lice at this time. Interestingly, this health issue was associated with a decrease in production but also an increase in egg albumen corticosterone concentrations. For farm Bn3 an increase in corticosterone concentrations was recorded in weeks 62 and 72. This corresponded to an outbreak of cannibalism which forced the producer to reduce light intensity in an effort to eliminate the problem. These stress provoking events were related to elevated albumen corticosterone concentrations. Farm CC4 had high egg production throughout the production cycle and relatively low egg albumen corticosterone concentrations throughout the cycle. Hens on this farm were maintained in individual cages and so would not have experienced the potential social stresses hens on other farms needed to cope with.

Social interactions can be stressful for laying hens (Craig and Guhl, 1969; Hughes *et al*., 1997; Bilick and Keeling, 2000; Keeling *et al*., 2003). Group effects on hen welfare had received considerable attention with small group sizes allowing for formation of fairly stable social hierarchies (Keeling, 1995). In group housing, there is competition for space and important social interactions take place which can be stressful (Keeling, 1995). Social structures, familiarity and competition are key issues responsible for the level of aggressive behaviour in group housed hens. The extensive interactions that occur during establishment of social orders in small groups of caged hens could result in stress that is seen as elevated plasma corticosterone and consequently egg albumen corticosterone (Downing and Bryden, 2009). In the floor based systems, hens are continually faced with changing social interactions. Therefore, important considerations in assessing welfare of hens are: the size of the enclosure, number of hens in the facility and the availability of resources (Mench and Keeling, 2001). In alternative production systems (barn and free range) hens experience a range of densities in the enclosure (Channing *et al*., 2001). It has been reported that hens are limited to identifying about 100 individuals in a group (Nicol *et al*., 1999) and that they prefer to be with familiar hens rather than unfamiliar hens (Bradshaw, 1992; Freire *et al*., 1997). Nicol and co-workers (1999) observed that aggressive behaviour was more prominent in small and medium sized flocks compared to larger flocks. In large flocks, hens probably adopt strategies that reduce social contact

(Oden *et al*., 2000; Mench and Keeling, 2001; Freire *et al*., 2003). In large flocks, victimisation of some hens could seriously compromise their welfare. Stressors are thought to have additive effects on plasma corticosterone concentration. The combination of changing environmental conditions and social interactions could have accounted for the higher albumen corticosterone concentration at the beginning of the production cycle. Such social interactions would not have taken place on farm CC4 (housed singly), where egg production was high and albumen corticosterone concentrations remained low.

There has been the suggestion that corticosterone concentrations are influenced by egg production level (Davis *et al*., 2000). In the current study, the relationship between egg production and albumen corticosterone was weak with only 8% of the variation in production being accounted for by the corticosterone concentration in the egg albumen. The general pattern for egg production is for it to increase from the start of the laying cycle and then peak at around 32-34 weeks (Marble, 1965).

For most farms, the albumen corticosterone concentration was highest at 24-32 weeks. Production data for the corresponding egg collection times was not available for all farms. For those seven farms where the production values were available (see table 3.13), peak production occurred around 42 weeks, a time when for most farms, the albumen corticosterone concentrations had begun to decrease. The higher corticosterone concentrations seen in the early part of the production cycle are unlikely to be related to egg production rate.

It has been suggested that the persistently low corticosterone concentrations observed in eggs from some farms could represent a state of chronic stress. There is a feedback regulatory mechanism controlling plasma corticosterone concentrations that acts to prevent the potential damage excessively high corticosterone concentrations could have on other physiological systems. However, the failure of the HPA axis to respond to further adverse stimuli would have dire consequences for the hen. When a hen is stressed, the biological response diverts resources from biological systems functioning before the perturbation to systems involved in re-establishing homeostasis. If the stress is major and persists for a long time, or there is a cumulative effect of numerous stressors, the biological cost can be great and lead to a pre-pathological state and then pathology (Moberg, 1985). The pre-pathological state represents a real threat to an animal's wellbeing because if it continues it can culminate in disease, although other effects include poor reproductive capabilities, failure to grow properly and development of abnormal behaviours. Chronic stress is associated with a high metabolic cost to the hen and not conducive to a high a rate of egg production. Ovarian regression is associated with increased plasma corticosterone (Etches and Williams, 1983; Etches *et al*., 1984a, b; Moudgal *et al*., 1991: Petitte and Etches, 1991), with the interruption to ovulation being dose-dependent (Moudgal *et al*., 1991). When a normal physiological range of around 3 ng/mL is maintained by subcutaneous infusion of corticosterone, egg production decreased by day 3 and completely ceased by day 8 (Etches *et al*., 1984a). When mini-osmotic pumps were implanted into hens to maintain a plasma corticosterone concentration of around 4 ng/mL, egg production ceased after 5-6 days of treatment (Downing and Bryden, 2002). Birds on farm FR1 were infested with mites in the later part of the production cycle. Before this infestation albumen concentration were low but increased after the infection at the same time as egg production decreased. Red mite infestation is considered a chronic stressor and results in elevated plasma corticosterone concentrations and reduced egg production in laying hens (Arkle *et al*., 2006; Kowalski and Sokol, 2009). So while the hens were at this low corticosterone concentration they were still able to respond to the parasite infestation. Similar arguments can be complied for the hens on farm Bn3 where at the end on the production cycle they had to deal with an outbreak of cannibalism.

Challenges with ACTH or CRF have been used to evaluate chronic stress. The principle being that under chronic stress the adrenal gland becomes depleted and so the response to a maximal exogenous dose of ACTH or CRF will be less (Koelbeck *et al*., 1986; Mormede *et al*., 2007). In the study reported by Rees *et al*., (1983), avian corticosterone response failed to habituate to exogenous ACTH administration (Rees *et al*., 1983). The test normally uses synthetic ACTH (1-24) administered intravenously at doses ranging from 0.5-2 IU/kg$^{0.75}$ or for CRF at 0.03-1 ug/kg (Review in Mormede, *et al*., 2007). There would appear to be some issues with this approach. Even Koelbeck and colleagues (1986) commented that having hens in different housing conditions might result in subtle differences in adrenal responses which are not apparent with following a maximal ACTH injection. As mentioned in section 1.13.2., this may bring into question the relevance of such a test, because it would seem impossible to determine what ACTH dose is needed to measure only subtle differences. There also seems to be an issue with the large variation in responses seen when a set dose of ACTH 1-24 is used for a group of individual hens. Using a fixed dose rate for all treated hens probably fails to account for individual sensitivity to ACTH. The test also relies on taking blood samples, which limits the sampling protocol to taking a fixed time sample after the initial challenge and comparing peak values at this time. The question remains as to whether this is the right time point to evaluate peak values for all hens.

The H/L ratio is a haematological measure of stress (Gross and Siegel, 1983: review: Maxwell, 1993). It is a procedure that could be used to help identify conditions resulting in chronic stress. Researchers have used this ratio as a selection criterion to establish lines susceptible or resistant to different stressors (Kassab *et al*., 2000). Maxwell (1993) suggested that mild or moderate stress results in heterophilia and an increase in H/L ratio while extreme stress results in basophilia that can become a life threatening. The difficulty is to determine what H/L ratio represents chronic stress. However, measuring the H/L ratio would be useful in helping to determine the degree of stress.

While consideration of mean corticosterone concentrations at a farm level can reveal evidence that is not seen when grouping these farms into their appropriate production systems, a further level of evaluation can be made by looking at the range of individual egg albumen corticosterones for a particular farm. For each collection age, the percentage of hens having corticosterone concentrations above 1.5ng/g are given in figures 3.16 to 3.27. For some hens, very high egg albumen corticosterone concentrations (> 2.5 ng/ml) were recorded. The relationship between plasma and egg albumen corticosterone indicates that these hens would have high plasma corticosterone concentrations (Downing and Bryden, 2005, 2008). Therefore, while group means provide a measure for assessing differences between farms and housing systems they provided little indication as to what is happening for a percentage of individual hens in any system that actually may be experiencing poor welfare. If these high values represent significant elevations from basal concentration they would represent efforts by hens to cope with challenging stimuli in the production environment that could adversely affect welfare. This ‘homeostasis approach’ to welfare is considered as valuable because it is strongly related to the ‘biological fitness of the individual’ (Broom, 1986; Broom and Johnson, 1993; Hemsworth and Coleman, 1998; Hemsworth and Barnett, 1991; Barnett and Hemsworth, 2003).

5 General Conclusions

When farms are grouped into production systems there were no differences in mean corticosterone concentrations. For each production system the variation between farms was large and limited the value of such a comparison. While the debate about the merits of different production systems will continue, what is happening on individual farms is very relevant to hen welfare.

For most commercial farms the albumen corticosterone concentrations were high at the start of the production cycle and this could reflect the extent of challenges that hens face early in the production cycle. The different patterns seen in albumen corticosterone concentrations thereafter could reflect the rate at which the hens are able to adapt to the challenges in their environment.

The different patterns of albumen corticosterone concentrations observed in the early part of the production provide evidence that:

- Farm management practices in the early phases of the production cycle could be relevant to the challenges hens need to deal with in their environment. Early rearing management could be designed to familiarise hens to the production housing and accommodate their adaptation to the housing transfer.

- The persistently low albumen corticosterone concentrations, low mortality, high egg production and large egg size recorded for farm CC4, where hens were housed individually, serves to illustrate the importance of group dynamics and social adaptation in laying hens.

- Commercial Farms FR1 and Bn3 were exceptions having persistently low albumen corticosterone concentrations through weeks 24-62. Clearly, large flocks in floor systems are capable of overcoming the issues of social stress and adaptation and can maintain low albumen corticosterone concentrations in the early phases of the production cycle. Again, it could reflect the role management or early rearing has in limiting the challenges faced by laying hens.

- Four of the five farms that had more persistent elevated albumen corticosterone concentrations (pattern 1) in the early production period were large free range or barn flocks.

- The measurement of albumen corticosterone concentrations could be correlated with a lice infestation (Farm FR1) and an outbreak of cannibalism (Farm Bn3).

- In any flock there are likely to be some hens that perceive the challenges as more severe than others and have high corticosterone concentrations.

- The mean albumen corticosterone concentrations over the entire production cycle tended to be lower in flocks with lower mortality. However, further data is needed to establish a definitive relationship.

- The data suggest that the elevated albumen corticosterone concentrations in the early stages of the production cycle are likely correlated with reductions in performance. Again, further data collection is needed to verify this relationship.

At this stage, the measurement of albumen corticosterone concentrations has highlighted the importance of early adaptation to housing system, which is likely to be improved with further knowledge and attention to management.

The high degree of variation between farms in a commercial setting necessitates that more farm collections are needed to further validate the sensitivity of albumen corticosterone concentrations as a technique for use in assessing welfare. Validation of the relationship with production and mortality need to be further explored.

6 Recommendations

Continued assessment of egg albumen corticosterone concentrations with particular interest to its correlation with production, body weight and mortality but also extend this to other measures of welfare such as plumage condition, health, feather pecking and cannibalism.

The comparison between production systems is not that useful because the variation between farms within a system can be large.

In a commercial context, the focus of welfare assessment should start at the flock level but eventually get to evaluating how individual hens cope within their environment.

More thorough practical benchmarking of body weights, plumage condition, body injury, other measures of stress (H/L) and mortality is required at the farm level.

The rearing management of pullets in assisting them to adapt to new housing; group dynamics and social interactions requires more attention.

The use of egg albumen corticosterone concentrations be promoted within the Poultry Science community as a technique that can further our understanding of bird welfare assessment.

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8 Plain English Compendium Summary

Project Title:	
• AECL Project No.:	US-108
• Researcher:	Jeff Downing
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• Phone:	02 903 67748
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• **Objectives**	To determine the corticosterone concentrations in albumen of eggs collected from the three main production systems used in the Australian egg industry, conventional cages, free range and barn.
• **Background**	The current study evaluated corticosterone concentrations in egg albumen from hens maintained in three main production systems of the Australian egg layer industry (Conventional cages (CC), Free range (FR) and Barn (Bn) at 24, 32, 42, 52, 62 and 72 weeks of age.
• **Research**	***In the study, five FR, four CC and three Bn flocks were sampled. Ninety eggs were collected at random when the hens were 24, 32, 42, 52, 62 and 72 weeks of age. Eggs were supplied by the producers who randomly selected them from those laid on one day in the week of the specified age. Eggs were collected at the same ages for all systems but because flocks were placed at different times, the collections started in different months (July 2009 to March 2010). Egg and albumen weights, egg albumen corticosterone concentration and total corticosterone in albumen were determined for all eggs collected.***
• **Outcomes**	The age of collection had significant effect on the egg albumen corticosterone concentration (P= 0.02) but no effect on the total amount of corticosterone in the albumen (P=0.13). When the farms are grouped into systems it was found that the production system had no significant effect on egg albumen corticosterone concentration (P=0.78) or on the total amount of egg albumen corticosterone (P=0.48). Grouping individual farms into the relevant production systems acts to mask some on the individual farm effects. The pattern of corticosterone concentrations for individual farms shows that there are some differences.
• **Implications**	When comparison is made on a production system basis, no differences in egg albumen corticosterone concentrations were found. This indicates that the degree of stress that hens need to cope within any system is similar although the stressors are likely to be different. It is suggested that social stress in the early part of the production system results in elevated corticosterone concentrations. Management and environment are likely to be important features influencing how hens cope in any system.
• **Publications**	US108A