AUSTRALIAN EGG CORPORATION LIMITED

Non-Invasive Stress Assessment of Commercial Egg Industry Practices

A report for the Australian Egg Corporation Limited

by Jeff Downing

Co researchers:
Wayne Bryden, John Barnett and
Geoff Stewart

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Researcher Contact Details

Name:	Jeff Downing
Address:	Faculty of Veterinary Science, University of Sydney, 425 Werombi Road, Camden, NSW 2570
Phone:	02 4650 0600
Fax:	02 4655 0693
Email:	jeffd@camden.usyd.edu.au

In submitting this report, the researcher has agreed to AECL publishing this material in its edited form.

AECLContact Details:
Australian Egg Corporation Limited
A.B.N: 6610 2859 585
Suite 4.02, Level 4
107 Mount Street
North Sydney NSW 2060

Phone:	02 9409 6999
Fax:	02 9954 3133
Email:	enquiries@aecl.org
Website:	http://www.aecl.org

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Foreword

The welfare of hens in modern production systems has been of concern to various interest groups for sometime and this has certainly been the case in more recent times. To adequately assess welfare there is a need to determine how hens cope with the environment created for them. Those conditions that result in biological dysfunction are the ones most likely to adversely affect a hen's welfare. A group of criteria based on measures of physiology, behaviour, immunology and production are needed to give a comprehensive measure of a hen's welfare. Understanding the relationship between animal production and stress is important in issues of animal welfare. Chronic stress is associated with poor welfare. Therefore measures of stress are one of the physiological measures of hen welfare.

As part of a previous AECL project (US-71A), a procedure for measuring corticosterone in egg albumen was developed as a non-invasive measure of stress in hens. This procedure has been used in the present project to measure the level of stress in hens maintained under various experimental and commercial production conditions. Parts of the present project were conducted in collaboration with other researchers working on AECL funded projects. The effects that temperature, floor space, cage density, age, type of housing and the provision of furnished cage features have on stress are investigated as part of this project.

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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David Witcombe
Program Manager R&D
Australian Egg Corporation Limited

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About the Author

Dr Jeff Downing completed his undergraduate degree at Macquarie University in 1987 and then a PhD in 1995. During this time he was employed at the CSIRO, Division of Animal Production. During his 25 years at CSIRO he was involved in research pertaining to the regulation of food intake and then later the interactions between nutrition and ovarian follicle function in ruminants. During his final years at CSIRO his work on ovarian function shifted to broiler breeders. In the late 1990's he moved to the University of Sydney. During this period his work has concentrated on the non-invasive measurement of stress in laying hens, omega-3 enrichment of poultry meat and fatty acid metabolism in broilers.

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Abbreviations

Abbreviation	Explanation
ACTH	Adrenocorticotrophic hormone
AVP	Arginine vasopressin
C	Centigrade
CBG	Corticosterone binding globulin
CPM	Counts per minute
cm	Centimetre
CNS	Central nervous system
CRF	Corticotrophin releasing factor
d	Day
FFA	Free fatty acids
g	gram
h	hour
H/L	Heterophil to lymphocyte ratio
HPA	Hypothalamic pituitary adrenal axis
Kg	Kilogram
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
min	Minute
MJ	Mega Joule
mg	Milligrams
ml	Millilitre
mRNA	Messenger Ribonucleic acid
NaOH	Sodium hydroxide
ng	Nanogram
nmol	Nanomolar
NO	Novel object
PBS	Phosphate buffered saline
PNMT	Phenylethanolamine-N-methyltransferase
REML	Restricted maximum likelihood
RIA	Radioimmunoassay
RIRDC	Rural Industries Research and Development Corporation
rpm	Revolutions per minute
sec	Second
SEM	Standard error of the mean
SRBC	Sheep red blood cells
TI	Tonic immobility
TNF-α	Tumour necrosis factor alpha

Executive Summary

The present project is concerned with stress and welfare in laying hens. It follows on from a previously funded RIRDC, Egg Industry Project; No US-71A, 'Development of a non-invasive test of stress in laying hens', which resulted in the development of a procedure for determining corticosterone concentrations in egg albumen. As part of the earlier study, it was found that corticosterone concentrations in albumen increase when hens are stressed. This procedure formed the basis for determining the corticosterone concentrations in hens when subjected to commercial husbandry practices.

Chapter 1. Literature Review

A comprehensive literature review of the relevant research areas is provided in chapter 1. Earlier sections of the review deal with the role that the glucocorticoids have in physiological function. The relationship between stress and welfare is given detailed consideration. This is important because chronic stress is associated with poor welfare and measures of stress should continue to be one of the criteria used in assessing hen welfare. A large component of the review concentrates on the connection between environmental and husbandry influences and stress and how this affects hen welfare. The role of space allocation and group size in conventional cages is dealt with, as is stress and welfare in alternative production systems. In later parts of the review consideration is given the role of fear in the stress response of individual hens and the possibility of using measures of fear as indices to predict stress responses in hens.

Chapter 2. The relationship between plasma corticosterone concentration and the corticosterone concentration in egg albumen of laying hens

In a previous RIRDC project, No US-71A, 'Development of a non-invasive test of stress in laying hens', the relationship between plasma and albumen corticosterone concentrations was not clearly established although it was obvious that when hens were exposed to known stressors the level of corticosterone in albumen increased. From these earlier studies, it was concluded that the subcutaneous administration of corticosterone would be the best approach to developing an experimental model to achieve the initial project objective. Therefore, the objectives of the studies described in this chapter were to develop an experimental model that adequately evaluated the relationship between plasma and albumen corticosterone concentrations and to use this to test for the relationship between plasma and egg albumen corticosterone concentrations. Five studies are detailed in this chapter, and after the first, each progressed from the observations made in the previous experiment. The critical developments were to determine the dose of corticosterone that was needed as a subcutaneous injection, and the timing of this so that eggs could be collected at an appropriate time.

There is a clear relationship between plasma and albumen corticosterone concentrations (Figures 2.10 and 2.11). The correlation coefficient of 0.87 was high for a biological relationship and this was consistent in two similar experiments. From the present studies, around 76% of the variation in egg albumen corticosterone concentration could be accounted for by variations in plasma corticosterone. High corticosterone concentrations remain one of the physiological measures used to determine if hens are stressed. Using corticosterone concentration in egg albumen avoids many of the problems associated with taking blood samples.

Chapter 3. The effects of housing laying hens in groups and different cage densities on the plasma and egg albumen corticosterone concentrations

The objective of the study presented here was to evaluate what effects group housing in conventional cages at different cage densities had on plasma and egg albumen corticosterone concentrations. While the effect of group number and cage space allowance were not segregated in the experimental protocol, a purpose of the study was to simulate conditions in commercial practice. Hens that had previously been housed individually in conventional layer cages (47 cm x 28 cm x 48 cm: 1175 cm^2/bird) were transferred to group cages (46 cm x 50 cm x 62 cm). Hens were housed 5, 4, 3 or 2 hens per cage or individually. The floor space allocated to each of the treatments was 460, 575, 767, 1150 and 2300 cm^2/bird respectively. There were ten replicate cages for each of the cage treatments. On days 2, 4, 8, 11, 16, 26, 43, 54, 83, and 110 of the study a blood sample was taken from one hen in each pen. Daily egg production was recorded for the entire study. On the day following each of the blood sampling periods all eggs were collected and oviposition times recorded. Corticosterone concentrations were determined in plasma and egg albumen samples.

On any individual week there were no significant differences in mean egg production, although the egg production tended to be lower for hens housed 5/cage during some weeks. There were no significant effects of hen number/pen on egg or albumen weights. On any individual collection day, the plasma corticosterone concentrations were not different when hens were housed 5, 4, 3 or 2 per cage or singularly, although this just failed to reach significance (P=0.075). On any individual collection day, the albumen corticosterone concentrations were not different when hens were housed 5, 4, 3 or 2 per cage or singularly and again this just failed to reach significance (P=0.06). On any individual collection day, the total amount of corticosterone in albumen was not different when hens were housed 5, 4, 3 or 2 per cage or singularly. Over the period of the study the amount of corticosterone in albumen was significantly lower in hens housed 2 or as singles in a cage.

The space available to hens in conventional cages remains the reason for most complaints about the use of conventional cages. In this study, at the highest cage density (5/bird cages) the space allocation was 460cm^2/bird. From a review of the literature (see section 1.6.2.) it was not unexpected to find no differences in egg production at this space allocation and group size. There were no differences in plasma and egg albumen corticosterone concentrations, delays in oviposition time or any obvious increase in the number of abnormal eggs. It would appear that hens are not chronically stressed by reducing the space allocation to 460 cm^2/bird. While the effects of group size and space are compounded, in this study it probable that group size is of more concern for hen welfare so long as the space available is above a minimum value.

Chapter 4. The effects of high ambient temperature on the plasma and egg albumen corticosterone concentrations in laying hens

Temperature is probably the most important environmental factor that influences egg production and egg quality. However, increases in ambient temperature have only small effects on egg production until a critical temperature is reached and after this, temperature can have a large effect. While the tendency to house caged hens in controlled environment sheds will continue; the proportion of hens kept in alternative housing systems will also increase. Hens kept in free range and barn systems can be exposed to a wide range in ambient temperature and heat stress remains an important consideration for the welfare of these hens. Changes in egg production, food intake and bodyweight are all end point measures of changes in physiological function when hens are stressed. In the study presented here the effects of long-term exposure to high ambient temperature is evaluated. End point measures of physiological function (egg production and food intake) are used as part of the

assessment. Also, plasma and egg albumen corticosterone concentration and the total amount of corticosterone in albumen are used as indicators of acute and chronic stress.

At 36 weeks of age, hens were allocated to individual pens (45 x 30 x 40 cm) and housed in one of three environmentally controlled rooms (36 hens/room). To acclimatise hens to climate room surroundings, all rooms were set to maintain a temperature of 20^0C for the first two weeks. After this time, and designated as day one of the study, one room was maintained at 20^0C (moderate temperature) for the duration of the study, while another room was increased and maintained at 32^0C (high temperature). In the third room the temperature was maintained at 20^0C and then at 0900h, two days before each egg collection the temperature was increased to 32^0C (alternate temperature regime), it remained at 32^0C until the completion of blood sampling when it was returned to 20^0C. Temperature treatments were maintained for a period of 12 weeks. Egg production was recorded daily and feed intakes were determined in the last two weeks of the study. On days 3, 8, 15, 22, 29, 39, 58, 70 and 82 after the start of the temperature treatments, all eggs laid by hens in all rooms were collected. The day before egg collection, blood samples were taken from each group. Corticosterone concentrations were determined in plasma and egg albumen samples.

The bodyweight of the hens housed at the high temperature was significantly different to the other treatments. Egg production of the hens housed at 20^0C or the alternate temperature regime was not different. Production was significantly lower for hens at 32^0C. The mean egg weight was significantly higher ($P<0.05$) for the hens housed at 20^0C compared to the other treatments. Early in the study, oviposition times were similar but towards the end of the study the mean oviposition time of hens held at 32^0C was significantly longer than for hens held at 20^0C. Hens housed at 20^0C had significantly higher ($P<0.05$) feed intakes than hens housed at 32^0C. The mean (±SEM) plasma corticosterone concentrations were not different. Over the period of the study, albumen corticosterone concentration was higher in the hens at 32^0C however; on any individual collection day there were no significant treatment effects. This difference was not observed for the total amount of corticosterone in albumen.

Results from the present study indicate that hens held at a temperature of 32^0C make physiological adjustments as determined by changes in egg production, body weight and food intake in an effort to function in high temperature. However, these adjustments do not seem to involve chronic elevation in corticosterone secretion during a moderate period of high temperature exposure.

Chapter 5. The effects of short-term exposure to a range of environmental temperatures on plasma and egg albumen corticosterone concentrations and the total amount of corticosterone in albumen

As discussed in chapter 4, high ambient temperature will depress egg production, however so will low ambient temperature. There are a number of reports detailing various aspects of high ambient temperature on stress in layers; however, there is limited information in the literature as to the effects of cold exposure on egg production and corticosterone concentrations in laying hens. In the study presented here, the effects of short-term exposure to various ambient temperatures ($10\text{-}33^0$C) is evaluated. End point measures of physiological function such as food intake and egg production are used as part of the assessment. Also, both plasma and egg albumen corticosterone concentration and the total amount of corticosterone in albumen are also used as indicators of stress.

Hens at 42 weeks of age were allocated to individual pens and housed in an environmentally controlled room. The study was run over a period of 12 weeks with the temperature alternating, on a weekly basis, between the treatment temperatures and the thermoneutral temperature (20^0C). The treatment temperatures were 10, 15, 20, 25 30 and 33^0C. Feed intakes were determined on a weekly basis and water intake over 24h period. On the last day of each treatment period all eggs laid were

collected and a blood sample was taken at the end of collection period. Corticosterone was determined in plasma and egg albumen samples.

Body weight was similar at the start and conclusion of the study. When hens were housed at a temperature of 33^0C the egg production tended to be lower than at other times. There were no differences in egg weights. Oviposition time tended to be longer at high temperature compared to low temperature. High or low temperature had no effect on plasma or egg albumen corticosterone concentrations.

Temperature is a stressor of hens, however the disturbance to homeostasis will depend on the absolute temperature and period of exposure. When the temperature ranged from 10-30^0C for a period of 7 days hens appeared to have little problem coping as determined by measures of feed intake, egg production and corticosterone concentrations in both plasma and egg albumen. At 33^0C hens needed to make more demanding adjustments to the temperature conditions. It would seem that a temperature of around 30^0C is the upper limit to the ambient temperature before hens are required to make major physiological adjustments if exposed for a moderate period of time.

Chapter 6. The effects of providing a nest box, dustbath and perch in furnished cages on egg albumen corticosterone concentration and total amount of corticosterone in albumen as non-invasive measures of stress

In developed countries over 90% of egg production is from hens maintained in conventional cages. However, conventional cages fail to provide a nest box, dust bath and perch and this is considered to be a welfare concern by some. The continued pressure to ban conventional cages in some parts of the world has increased the enthusiasm for alternative housing systems. The furnished cage is considered an alternative especially in the EU. The work reported in the present study is one component of a large study conducted at the Animal Welfare Science Centre, Werribee, Victoria, and Australia, with Associate Professor John Barnett as the chief investigator. Full details of the project have been provided to the Australian Egg Corporation Limited (Project Number: Dav-197A). This larger study examined the welfare of hens maintained in cages provided with individual items of furniture or combinations of these items. In two further treatments the effect of space and stocking density were examined. In the main study, various aspects of egg production, morphology, immunology, behaviour and physiology were used to assess hen welfare in cages supplied with the different pieces of furniture. Details presented in chapter 6, relate to the measurement of corticosterone in egg albumen as a non-invasive measure of stress.

The experiment used 66 Victorsson Trivelburen cages imported from Sweden and modified during installation to meet the requirements of the experimental design. The final experimental design with the number of factors and treatments was (3 nest box* 3 dust bath* 2 perch) + 1 space control + 1group size control) with three replicate for each treatment. A total of 60 pens were used with each housing 8 hens except for the group size treatment which housed 16 hens per cage. In the larger experiment, a number of production and welfare measures were made. There were two sampling periods each conducted over 8 weeks. The first started at 29 weeks of age and the second at 59 weeks of age. Five sets of measurements were made in both sampling periods. On two consecutive days during weeks 33 (period 1) and 63 (period 2) of age, all eggs laid were collected. Corticosterone in egg albumen was determined by RIA.

There was no significant effect of treatment on egg or albumen weight although eggs were heavier at 63 weeks. Albumen corticosterone concentration and total amount of corticosterone in albumen were lower at week 63 compared to week 33. Within the standard cages there were no significant effects of providing individual pieces of furniture (perch, nest box and dust bath) on albumen corticosterone concentrations or total amount of corticosterone in albumen. At 33 weeks of age, both the egg

albumen corticosterone concentration and total amount of corticosterone in albumen was significantly higher when the number of hens housed together was increased (16 birds in double with cages). This difference was not evident at 63 weeks of age.

As a general conclusion from the comprehensive study and results detailed here, any effects of having a dustbath, nest box or perch in the cage is minimal. Some behavioural changes occurred but like many behavioural studies it is difficult to determine how important these are to hen welfare. Of the physiological criteria used to assess welfare in this study, except the increase in bone strength when a perch was present, none seemed to indicate that hens were adversely stressed by not having a dustbath, nest box or perch present.

Chapter 7. The effects of alternative housing systems on egg albumen corticosterone concentrations and total amount of corticosterone in albumen as a non-invasive measure of stress

The continued pressure to ban conventional cages in some parts of the world has increased the enthusiasm for alternative housing systems. In Australia, alternative production systems are mostly of the barn or free range type. Like all systems each has advantages and disadvantages. Much welfare research comparing cages and floor systems have used small pens in the experimental design. These studies provide valuable information, however they often fail to be representative of the large flock sizes and enclosures used in commercial practice. In an effort to more fully evaluate the welfare of hens in alternative housing systems under commercial conditions, the AECL funded a study at the University of Queensland, Gatton, Queensland, Australia.

The ‘Isa 2000’ strain laying hens were maintained in four different production systems. The housing systems were free range, barn, conventional cages (6 and 3 birds per cage) in a naturally ventilated shed, and conventional cages maintained in an environmentally controlled shed. In the main study, various egg production, morphological, immunological, behavioural and physiological criteria were used to assess hen welfare in these systems. The present report gives details of one component of the larger study looking at the effects of housing system on egg albumen corticosterone concentration as a non-invasive measure of stress. 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 RIA.

There were some minor differences in egg and albumen weights. 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). The percentage of hens with values above 2.0 ng/ml was higher at peak production than at the end of production. The relationship between plasma and egg albumen corticosterone established in chapter 2 indicates that these hens have high plasma corticosterone concentrations. An important consideration from a welfare perspective would be to know if individual hens have persistently high concentrations.

Chapter 8. The effect of hen age on the response to stress

During the production cycle, the behaviour patterns of the hen relate to adjustments being made for the environment, social interactions and age. In turn, these changes in behaviour are related to the physiological needs of the hen at the different stages of the cycle with age seemingly to have a major influence on behaviour and levels of fear. In chapters 6 and 7, evidence was provided suggesting that the corticosterone concentrations in hens diminish with age. This could indicate that hens are less responsive to stress as they age. The higher corticosterone concentrations at a younger age could predispose hens to greater risk from disease because of the association between glucocorticoids and immunosuppression. In the present study, the effect of age on the hens corticosterone response to stress was investigated.

At 24, 34, 54 and 70 weeks of age (treatment periods 1-4) hens were subject to a procedure, known to be stressful. Fifty hens were removed from their single cages and transferred to group cages (46 cm x 50 cm x 62 cm) and housed 5 birds per cage (total of 10 cages). At each treatment period, the same fifteen hens remained in their single pens and acted as a control group. The day hens were moved was designated as day 0 of each treatment period. On day 0 starting at 0500h, the hens were moved to group pens and remain there for 10 days. On days –2, 0, 1, 5 and 9, between 1600-1700h, a 1.5ml blood sample was taken from 2 hens in each of the group cages and also from the fifteen hens that remained in the single pens as the control treatment. On days 1, 2, 6 and 10 of each period, all eggs laid were collected. Corticosterone in plasma and albumen was determined by RIA.

Hens moved to group cages tended to have lower egg production in all periods compared to hens not moved. Oviposition time was longer in group-housed hens. The plasma corticosterone concentration decreased as the hens' aged. During the course of the study plasma corticosterone concentration was higher in the group-housed hens compared to hens remaining in their individual cages. When hens were moved, plasma corticosterone increased compared to the control hens in periods 1, 2 and 3 but not period 4. The failure to observe an increase in period 4 (70 weeks) may be related to a diminished responsiveness to this stressor as the hens aged.

As a general conclusion, the patterns in plasma and egg albumen corticosterone concentrations suggests that as hens age their basal plasma corticosterone concentrations decrease and their corticosterone responsiveness to a novel stressor is also diminished. This could be related to observations indicating that fearfulness decreases with age as higher levels of fear are associated with increased corticosterone concentration.

Chapter 9. The relationship between fearfulness and the response to stress in laying hens

In any production system, a hen is exposed to many stressors, factors that influence its behaviour, physiology and emotions. Individuals having enhanced fear responses could potentially have their welfare compromised. Behavioural and adrenocortical responses tend to be tightly linked with metabolic and environmental stressors. Chronic elevation of plasma corticosterone increases fearfulness. Chronically elevated corticosterone concentrations are believed to be associated with poor welfare and failure of hens to cope with their environments. The objective of the present study was to determine if there is a relationship between behavioural measures of fearfulness and a hen's adrenal response to stress. If there is an association, then simple behavioural measures of fear could be of value in identifying hens whose welfare could be potentially compromised in various production systems. It could also be possible to use such tests as selection criteria when attempting to establish strains of hens more suited to the extensive production systems gaining popularity.

One hundred and fifty, Isa-Brown laying hens were used. All hens were subjected to the tonic immobility test on three occasions with a mean time calculated for each bird. The hens were ranked according to this mean time. Two groups were selected according to the ranking. Group 1, short immobility times, consisted of hens with a tonic immobility time of less than 70 seconds. Group 2, slow tonic immobility times, consisted of hens with a tonic immobility time of greater than 300 sec. The tonic immobility test was conducted on all hens between 24 and 26 weeks of age. During week 28 of age, the selected hens (total of 49) were exposed to a situation know to be stressful and levels of corticosterone in egg albumen and plasma determined. This part of the study was conducted over 7 days. At 0500h on day 1, the selected hens were removed from their individual cages and placed in a transport crate, and after 4 hours, transferred to a new individual cage located on the other side of the layer shed. A blood sample (1ml), was taken from all selected hens starting at 1600h on day 1. Eggs were collected on the day before hens were moved (day-1) and then days 2, 3, 5 and 7. Corticosterone in plasma and albumen was determined by RIA.

For the two groups, there were no differences in plasma and egg albumen corticosterone concentrations or the total amount of corticosterone in albumen. For individual hens there were no significant correlation between TI and any measure of corticosterone. The relocation stress failed to elicit a large elevation in corticosterone concentration, however, the presence of a large number of abnormal eggs indicate that there was a major disturbance to egg formation on day 1.

In the present study, the two treatment groups were selected to have very different mean tonic immobility duration. This was done to ensure that there were two distinct populations so that any difference in corticosterone would have been maximal. Both the plasma corticosterone concentration and the corticosterone in egg albumen were similar in both groups. The failure to find a relationship between the corticosterone response to stress and the behavioural tests of fear could be related to the layer strain used. A probable consequence of selection for production in cages has also been the selection for decreased fearfulness in the strain of layer used. While fear is reported to be associated with physiological and morphological measures often related to stress, the tests used here do not seem to be related to corticosterone response. These tests may be related to adrenomedullary activation although this was not determined in the present study. The large number of abnormal eggs on the first collection day suggests that catecholamine release could be high during relocation.

General Conclusions

Present layer strains have been selected for production in conventional cages. This has produced lines of hens that in general, are capable of adapting to many stress-provoking stimuli experienced in this type of housing system. Hens cope well with temperature ranging from 10-30^{0}C. Hens need to make some major physiological adjustments when coping and small temperature increases above 30^{0}C. After about two weeks at 32^{0}C, production decreases but there was no major shift in corticosterone concentrations. After 1 week at 33^{0}C production declines but again minimal effects are observed on corticosterone concentrations.

In both conventional and furnished cages, space availability had little effect on corticosterone concentrations. This is probably true so long as the space is above some minium, and this area seems to be well below the space required by legislation. However, evidence from the present studies semm to indicate that group size may have an influence on stress levels in caged hens. There is need for further research to evaluate what effects group size has on stress and welfare and to establish what is an appropriate group size for both conventional and furnished cages.

Evidence, using a range of criteria for determining welfare, indicates that the provision of a dustbath or nest box has minimal effect on welfare and no effect on corticosterone concentrations. While the provision of a perch increased bone strength it has no effects on corticosterone concentrations. In this study, there was a range of individual corticosterone concentrations with some hens having high

levels. This could represent intrinsic genetic differences within the population or possibly a transient increase in these hens. It would be difficult to determine the reason for these elevated levels as it would require the determination of an, 'unstressed baseline' measure for individual hens. The aim in commercial production should be to minimise the amount of stress.

Alternative production systems provide a range of stimuli that hens need to cope with. However a comparison of different systems indicate that all have similar effects on corticosterone concentration. Comparisons based on group mean levels suggest that hens are not unduly stressed. However, when the range in individual corticosterone concentrations is viewed there are some hens with high levels. Again these high levels could be the result of genetic differences in the population, the result of transient increases or due to persistently elevated corticosterone levels. As stated above, this could only be determined if it was possible to have an, 'unstressed baseline' measures for individual hens. This could be possible in a research environment but not feasible in commercial production. The goal in commercial production should be to minimise the amount of stress and the range of stress levels experienced by the flock. The non-invasive measurement of stress provides the egg industry with a technique that can help in the valuation of procedures and their effect on stress in hens.

Age modifies a hen's response to stress. Basal and stress-induced corticosterone concentrations are greater at younger ages. This suggests that greater husbandry care should be exerted to prevent stress early in the production cycle as this will improve welfare. Because of the association between glucocorticoid levels and immune function it suggests that young hens are more likely to be prone to pathological states. In highly selected lines of laying hens used in commercial production, fear does not seem to be associated with the corticosterone response to stress. Therefore, in these lines, behavioural measures of fear are not a good indication of how hens react to stress.

Selection for egg production in cages has produced strains of hens capable of coping with many of the stressors associated with this production system. Egg production remains a good end point measure of welfare. If a hen's welfare is not good two of the functions first affected are growth and reproduction. Therefore, if hens are not coping with their environment decreased egg production will be a consequence. This type of selection has indirect effects because it also selects for livability, resistance to disease, reduced fearfulness and ability to cope with the stress inherent with the housing system. Therefore, selection for egg production in alternative systems will result in strains of hens best able to cope in these systems. Hens better able to cope will also experience better welfare. The problem with this approach remains, the difficulty of identifying production from individual hens. Research is needed to determine adequate direct or indirect measures of individual production in alternative housing systems. While selection for production will most likely improve welfare in any system, there still remains some hens that will experience difficulty in coping and it is these hens whose welfare will be compromised. Selection for production is an end point measure of welfare. Selection for production will progressively select for strains of hens less venerable to stress in a particular production system.

Chapter 1

Literature Review

1.1. Animal Welfare

1.1.1. Definitions of Welfare

Since the publication of 'Animal Machines' by Harrison (1964) there has been increased emphasis on critically evaluating welfare in animal production systems (Craig and Swanson, 1994). An interest in welfare can stem from numerous factors and these include economics, culture, philosophical attitudes, science, aesthetics, knowledge and religion (Craig and Swanson, 1994). With this range of interests it becomes obvious that attitudes to welfare can vary greatly. Kellert (1988) indicated that the attitude had a lot to do with the relationship that different professions had with animals. Farmers were utilitarian (interested in animals as resources), white collar professionals were humanistic (animals as companions), naturalistic (concerned with wildlife) and ecological (concerned with wildlife and environment). Organizations related to animal matters have been categorised as concerned with exploitation, use, welfare, protection, rights and liberation (Morgan, 1986). The extent of concern an individual has for animal welfare depends largely on which perspective predominates and the intensity of the emotions involved.

Because individual perspectives can vary greatly, there are many definitions of welfare. For this reason a strict definition is difficult because it can't encompass all perspectives. Broom (1991) indicated that the definition must refer to criteria of significance to the animal and not some aspect given to the animal by man. Perhaps an appropriate general definition that is suitable to many interests is: 'Animal wellbeing (welfare) is a state or condition of physical and psychological harmony between the organism and its surroundings characterized by the absence of deprivation, aversive stimulation, overstimulation or any other imposed condition which adversely affects health and productivity of the organism' (Hurnik, 1988). Broom (1986) describes welfare of an animal as it's state with regard to its attempts in coping with its environment. This seems to be a valuable definition, as it emphasis the condition or state of the animal at any particular time. It essentially deals with how the individual is coping with it's situation at any time (Broom, 1996). A critical component of this concept is that it makes reference to how much a hen has to do in coping (Broom, 2001). Not all situations can be controlled and so at any time hens are exposed to events that alter the environment and in turn this requires some modification by the individual to cope with the change. Through evolution hens have been provided with various adaptive mechanisms whereby adjustments can be made to cope with change. Therefore, inherent in this concept, the welfare of the hen is closely linked with the extent that the individual is successful in coping with the changes. Also, explicit in this concept is the appreciation that when assessing welfare there is a need to understand the biology of the animal (Broom, 2001) so that welfare can be measured in a scientific manner (Broom, 2001).

Therefore in assessing welfare, scientists need to generate the knowledge needed to understand the biology of the hen and how this is modified in response to disturbances to its environment. To properly assess welfare in modern production systems there is a need to determine how hens cope with the environment created. Those conditions that result in biological dysfunction are the ones most likely to adversely impinge on a hen's welfare.

1.1.2. Approaches to assessing welfare

There are various scientific approaches taken to determine welfare in farm animals (Duncan and Fraser, 1997: Barnett and Hemsworth 2003). In their assessment, Barnett and Hemsworth (2003) give a good overview of the various approaches that have been taken and these are considered below.

1. Hen welfare - 'the feelings approach': With this approach the state of an animal is measured in terms of it's emotions, how it feels. Feelings are part of the hen's coping responses to any challenge placed on its biological fitness. Some researchers consider that this should be the main approach to assessing welfare (Dawkins, 1990; Duncan, 1993). In general, negative feelings are associated with poor welfare and positive feelings with good welfare (Dawkins, 1980; Broom, 1991). Pain and fear that cause suffering are negative emotions and should be minimised to enhance welfare. Positive feelings such as comfort and pleasure are associated with good welfare (Duncan and Fraser, 1997). There are major difficulties associated with studying emotions and so this approach is a real challenge for scientists (Barnett and Hemsworth 2003).

2. Hen welfare – 'the needs approach': Animals have needs, which are strongly associated with a motivational desire to meet these needs (Duncan, 1998). Therefore using this approach, the environment is appropriate if the animal's needs are meet (Appleby, 1997). A need can be defined as a 'requirement, which is part of the basic biology of an animal to obtain a particular resource or respond to a particular environmental or bodily stimulus' (Broom, 2001). Needs are often determined by preferences tests (Broom and Johnson, 1993; Barnett and Hemsworth, 2003). Unsatisfied needs can be associated with negative feelings that will in turn be associated with poorer welfare.

3. Hen welfare – 'the nature of the species approach': Animals display behaviours that are considered as natural when maintained in the 'wild'. Welfare is considered to be poorer if hens are maintained in an environment where any of these behaviours are restricted or absent. Barnett and Hemsworth, (2003) suggested that there are major concerns with this approach because

- it assumes that the full range of behaviours is required in all environments
- the difficulty in determining what is natural behaviour
- domestication and selective breeding have produced breeds and strains far removed from their wild ancestors.

4. Hen welfare – 'the five freedoms approach': In the UK, a report by the Brambell committee (Brambell *et al*., 1965) indicated that for welfare to be good, intensively housed animals needed to be provided with certain minimal requirements. In 1992, the UK Animal Welfare Council adopted the five freedoms as criteria to ensure good welfare for intensively housed animals (FAWC, 1992). The five freedoms are:

- Freedom from hunger and thirst
- Freedom from discomfort
- Freedom from disease
- Freedom to express normal behaviour
- Freedom from fear and distress

In essence, under this approach a hen's welfare is compromised if any of these 'freedoms' are not meet. However, one problem with this approach is that there is not an adequate definition for some of the terminology, which is required for proper scientific evaluation using these criteria (Barnett and Hemsworth, 2003).

5. Hen welfare – 'the homeostasis approach': This approach concentrates on the changes made by the hen's biological systems as it attempts to adjust and maintain homeostasis. It is how much has to be done by the hen to cope with its environment which is related it's welfare (Broom, 1986). This approach is considered by some 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). At any time, the extent to which an

individual hen is coping will be a measure of its wellbeing. If coping is not successful then the hen will develop pathological states that result in poor welfare or death. In this approach the measures of welfare are based on an understanding of the biology of the hen and particularly what the hen does in it's attempts to cope with change in the environment (Broom, 2001). Therefore, using this approach hen welfare can be assessed by the magnitude of changes in behavioural, morphological or physiological responses or the cost to 'biological fitness'. In essence the measures of biological success are determined by the ability to survive, grow and reproduce and ensure perpetuation of the species. Therefore, measures of growth, reproduction and mortality are indicators of the cost to 'biological fitness' and in effect of welfare.

In measuring welfare the multidisciplinary approach is often considered the best approach (Broom, 1991). Some measures are related more to short-term disturbances and others to long-term problems (Broom, 1991). Criteria that can be considered included;

- Level of productivity
- Behaviour
- Physiology
- Disease and immunity
- Anatomy
- Survivability

1.2. Stress

1.2.1. Definition of stress

In attempting to relate stress and welfare the following definition may be useful. Stress is a condition that places an animal in a state where it's biological response mechanisms attempt to re-establish homeostasis and if these systems are inadequate, pre-disposes the animal to pathological states that impinge upon it's well-being. This includes combinations of all conditions that the animal needs to deal with and these can be external or internal factors. Any major perturbation in 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, with irreversible changes adaptation occurs.

1.2.2. Stress and welfare

Understanding the relationship between animal production characteristics and stress is important in issues of animal welfare. The underlying concept is that if an animal is stressed it's well-being is compromised. It remains abundantly clear that stress means different things to different individuals. It is an imprecise term, however it continues to be used because for many it is a simple way of explaining something that would otherwise require many words to describe (Ganong, 1963). Stress is not a single entity that results in a particular measurable response. It is a syndrome that probably has no consistent biological response and the consequences vary extensively for individuals. However, most definitions of stress have dealt with the consequences and therefore, have normally been defined according to changes in physiology or behaviour. The absence or presence of stress provides a measure of animal wellbeing.

The responses to stress are important criteria used to assess an individual's state of wellbeing. For a hen to survive it must maintain it's biological systems within limits, that is maintain homeostasis. Anything that disrupts these systems can be considered a stressor and any efforts to re-establish homeostasis can be considered part of the hen's stress response. In any production system hens have to deal with changes in their environment which challenge homeostasis. An appropriate set of

responses need to be instigated that act to negate the disruption to homeostasis. It is the level of changes in the biological systems that are often used to evaluate the level of stress experienced by hens.

1.2.3. Stress models

In the wild there are a range of responses when animals are threatened (Engel and Schmale, 1972). When challenged, the defence which is the most energy conserving is for animals to alter their behaviour. Often this is easily achieved by simply moving away from the stressor. Intensive farming practices often deprive animals of this option. When avoidance is limited, neuroendocrine responses are initiated that affect many biological systems. This strategy is much more costly, with energy reserves being diverted away from growth and reproduction to re-establishing homeostasis.

The responses to stress maybe specific or nonspecific. Specific responses depend on the stressor. Selye (1950) identified many stimuli that were potential stressors and considered that the response to stress was non-specific. Selye developed the 'general adaptation syndrome' consisting of three components, an initial emergency reaction, the resistance phase and eventually exhaustion. It's during the resistance phase that the animal is capable of coping however, when this fails the animal enters the exhaustive phase. A genetic basis for the behavioural and physiological responses to stressors is found in lines of hens selected for either high (HGPS) or low (LGPS) productivity and survivability in group cages (Craig and Muir, 1996a; Cheng *et al*., 2001a, b). The HGPS line showed better adaptation to a variety of stressors including heat and cold, handling and social effects (Hester *et al*., 1996a, b, c). The release of corticosterone was identified as an essential feature of the non-specific response to stress.

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 were specific response depending on the type of stressor. At this time, it was recognised that the lack of a glucocorticoid response was not a definitive indication that an animal was not being stressed. 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 treat. Therefore, different biological responses are available to individuals and the response to a stressor can vary between individuals.

There is probably no unifying model of stress that will predict the biological consequences of all stessors. Moberg (1985) developed a model that separated the animal's response into three phases, recognition of a threat to homeostasis, the stress response and the biological consequences of stress. In essence, different stressors will initiate different combinations of responses and these vary between individuals. In this model, Moberg emphasised the importance of the central nervous system in recognition of potential stressors and the influence exerted 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 central nervous system (CNS) responds to the stressor. In the second phase of Moberg's model, the stress response (biological response) is not a single non-specific response but differs for individuals. It is the change in biological function that enables the animal to cope with stress. These changes are normally sufficient to eliminate or modify the effects of the stressor. However, it is the cumulative effects of numerous stressors that can be over bearing for the animal. 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. In most cases the biological cost is small. However, if the stressor is major and persists for a long time, or there is a cumulative effect of numerous stressors, 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 higher the probability the animal will develop a pathology.

Morbeg (1985) argues that it is the pre-pathological state that represents a real threat to an animal's wellbeing. Disease is a serious consequence, although other effects include poor reproductive capabilities, failure to grow properly and development of abnormal behaviours. Therefore, pathology is an endpoint that represents stress in animals. 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 before they result in pathology.

1.3. Physiological responses to stress

As mentioned previously, responses to stress can be specific or nonspecific. Specific responses depend on the stressor. For example the response to cold involves huddling, increased metabolic rate and changed blood flow patterns. Nonspecific responses are independent of the stressor. There is a stereotypic pattern of physiological responses to stress involving many organ systems. 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 in minutes and are catecholamine-dependent. A consistent non-specific response is an increase in corticosterone which is an acute response and responsible for glucose production from non-carbohydrate sources, principally protein. These changes are beneficial to the hen and result in some level of energy expenditure. 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.

Recent evidence suggests that 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 has recently been appreciated. In turn, the immune system influences neural activity and endocrine secretions. 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 (see Kelley, 1988; Blalock, 1989; Arkins *et al.*, 1993; Johnson, 1997; Johnson *et al.*, 1997). Furthermore, leukocytes not only synthesize and secrete cytokines but also growth hormone, prolactin and insulin-like growth factors. In addition to the 'communication' between the endocrine and immune systems, there is also a dense innervation of lymphoid tissues allowing changes in central nervous system output, following environmental change, to directly impact on immune function (review: Marsh, 1992; Ottway & Husband, 1994). 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).

A key question for those concerned with poultry production is how well do bird's cope with modern systems of animal management. Within its genetic capacity, an animal adjusts continually to changes in environmental conditions or stress, but at a metabolic cost. The effect of stress is 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-α (TNF-α) 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.

Amino acid redistribution occurs during immunological stress, with muscle being the major source of nitrogen. Some of the amino acids are used for B-cell proliferation and synthesis of immunoglobulins others provide the carbon skeleton for increased gluconeogenesis by the liver, where large quantities are incorporated into acute-phase proteins. These aspects of protein turnover are accompanied by a significant nitrogen loss. Calculations by Reeds *et al.* (1994) show that the amount of muscle protein mobilized is considerably in excess of the quantity of acute-phase proteins synthesized due to demands for phenylalanine, tryptophan and tyrosine. The net loss of body nitrogen occurs following the oxidation of amino acids remaining after completion of acute-phase protein synthesis (Reeds *et al.*, 1994). Research conducted with broiler chickens (Klasing & Barnes, 1988) and pigs (Stahly, 1996) demonstrate that animals subjected to greater immunological stress require less essential amino acids to achieve their decreased protein accretion rates than animals not stressed. Similar studies have not been conducted with laying hens.

1.4. The hypothalamic-pituitary-adrenal axis (HPA)

1.4.1. The adrenal gland

The adrenal gland provides an example of the co-operation existing between the endocrine, nervous and immune systems in the control of a variety of functions (Ganong, 1963). In birds, the adrenal glands consist of chromaffin and cortical (interrenal) tissue that is not divided into a distinct outer cortex and inner medulla. Chromaffin tissue accounts for about 15-25% of the adrenal tissue and is intermingled throughout the cortical tissue. However, it is concentrated around blood spaces and is more abundant towards the centre of the gland. Chromaffin tissue is of two distinct types, that releasing adrenalin and that releasing noradrenaline (Ghosh, 1980). The cortical cells are arranged in cords that radiate out from the centre of the gland. The arrangement of specific cell types along the cords does give rise to a level of structural zonation and function. The cortical tissue is divided into the subcapsular zone, 20-40 cells thick and producing aldosterone, and an inner zone that produces corticosterone (Holmes and Cronshaw, 1980).

1.4.2. The adrenal hormones

1.4.3. The cortical hormones

While there is no division of the avian adrenal into distinct regions as in mammals, it is still appropriate to consider the hormones synthesised and secreted from the adrenal as two groups. The cortical hormones, those secreted by the cortical tissue and the medullary hormones, those secreted by the chromaffin cells. The steroids of the adrenal are divided into the glucocorticoids and the mineralocorticoids. For mammals corticosterone, cortisol, cortisone and 11-dehydrocorticosterone are the principle glucocorticoids while 11-deoxycorticosterone, 17-hydroxy-11-deoxycorticosterone and aldosterone are the principle mineralocorticoids. In hens the main corticosteroids are corticosterone, cortisol, cortisone and aldosterone with the principle glucocorticoid being corticosterone and aldosterone the principle mineralocorticoid (Holmes and Phillips, 1976). In avian species corticosteroids have overlapping activities. Other steroids are found in varying amounts including oestradiol, progesterone and androgens.

1.4.4. Regulation of corticosterone secretion

Activation of the hypothalamic-pituitary-adrenal axis (HPA) results in secretion of glucocorticoids from the adrenal cortical tissue (Harbuz and Lightman, 1992: Minton, 1994). The regulation of corticosteroid release involves a sequence of events starting with the release of hypothalamic factors, then ACTH (adrenocorticotrophic hormone) from the pituitary and eventually corticosteroid from the adrenal (Etches, 1976; Freeman and Flack, 1981). The regulation of ACTH secretion remains less well defined in birds than mammals but seems to have many similarities. In mammals, ACTH release is regulated by corticotropin releasing factor (CRF) and arginine vasopressin (AVP). Both CRF and AVP are synthesized and secreted by the paravocellular cells of the paraventricullar nucleus (Whitnall, 1993) and thus a mixture of CRF and VP is presented to the pituitary and they act to augment one another (Caraty *et al*., 1990; Alexander *et al*., 1991; Jacob and Minton, 1993). Vasopressin augmentation could be a strategy to elevate ACTH action during stress. *In vitro* studies in rats have shown that oxytocin, angiotensin II, adrenalin and noradrenaline have limited capacity to stimulate ACTH release (Watanabe and Orth, 1988). It is also known that cytokines are involved in regulating the HPA (Whitnall, 1993). Interleukin-1, interleukin-6 and TNF have been implicated and probably increase ACTH release during infection and inflammation.

Stress-induced responses of the HPA can be considered in two domains, the effects of acute stress and the effects of chronic or repeated stress. A variety of acute stressors have been shown to elicit an increase in plasma ACTH and corticosterone. Physical stress increases CRF mRNA and proenkephalin mRNA while psychological stress stimulates CRF mRNA only (Lightman and Young, 1987, 1988; Harbuz *et al*., 1991). Hypertonic-saline stress increases CRF mRNA and AVP mRNA (Lightman and Young, 1988). The level of CRF mRNA remains unchanged after cold stress (Harbuz and Lightman, 1989) or ether stress (Watts, 1991). Studies into chronic stress are difficult because of the absence of a good experimental model. Many investigators have attempted to emulate chronic stress by using an acute stress applied over several days. Plasma ACTH levels don't remain elevated in chronic stress (Hashimoto *et al*., 1988). Chronic stress is associated with near normal circulating ACTH and corticosterone levels. There has been the suggestion that AVP plays an important role in chronic stress. The proposal is that endogenous AVP is essential for the pituitary to remain responsive to stress during times it has become refractory to CRF stimulation (Scaccianoce *et al*., 1991).

Glucocorticoid secretion from the adrenal is under the primary influence of adrenocorticotrophic hormone (ACTH), synthesized and released from the pituitary. Synthesis and release of corticosterone is stimulated by ACTH. Corticosterone levels in adrenal effluent blood increase 250% following an intravenous ACTH injection. The response is realised in 2-3 min but is not maximal until 15-30 min (Harvey and Phillips, 1980). As with mammalian ACTH, the avian equivalent is a simple polypeptide of 39 amino acids (Li *et al*., 1978).

Corticosterone action probably involves the hippocampus, with high to moderate level of receptors found in this area of the brain (De Kloet, 1991). Changes in receptor occupancy may be related to how animals cope with changes in their environment (Korte *et al*., 1995). Individual stressors may activate specific components of the HPA and sympathetic-adrenomedullary system (De Boer *et al*., 1990; Korte, *et al*., 1997). This selective function may result in altered adrenal function with behaviour and physiological modifications being related to the relative changes in corticosteroids and catecholamines. In quail selected for increased struggling activity, noradrenaline response is higher than in line that struggles less (Satterlee and Edens (1987). Hens selected for high or low levels of feather pecking have different adrenal hormone response to manual restraint. The high feather pecking line had the greatest noradrenaline response but lowest corticosterone response (Korte *et al*., 1997).

1.4.5. Corticosteroid transport

Glucocorticoids are transported in the blood bound to protein carriers in a reversible association. Corticosterone is bound to a specific binding protein, corticosterone-binding globulin (CBG) or to

non-specific-binding protein (probably albumen). Corticosterone-binding globulin has high-affinity low binding capacity whereas the non-specific-binding 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 50-80% 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 stabilization of the free blood concentration.

Corticosterone receptors are of the steroid/thyroid hormone nuclear receptor type that control differential gene expression by enhancing specific gene expression (Evans, 1988; Beato, 1989). There are two types of receptors in the CNS. Type-I receptors are thought to regulate basal CRF gene expression at the nadir of diurnal ACTH secretion (Dallman *et al*., 1987) and regulate peak ACTH secretion (Dallman *et al*., 1989). Type-II receptors are involved in stress-induced ACTH release (Ruel and de Kloet, 1985). Selectivity of gene expression is achieved by restricting the expression of different receptors in specific cells. Different sets of genes in different cells will be accessible to the hormone receptor complex (Evans, 1988). Transcription of specific mRNAs result in proteins that elicit the specific cellular response attributed to corticosterone. The duration of the corticosterone response is determined in part by the rate of degradation of the receptor /hormone complex. The hippocampus contains moderate to high glucocorticoid receptor numbers (De Kloet and Ruel, 1987). 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).

1.4.6. Clearance and metabolism

Basal concentrations of plasma corticosterone for chickens range from 0.4-12 ng/ml using radioimmunoassay (Radke *et al*., 1984: Satterlee *et al.*, 1980). Corticosteroids are cleared from the circulation with a 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.

1.4.7. 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 levels lagging approximately 4 h behind (Siegel *et al*., 1976) those of corticosterone. Maximum concentration is observed at the end of the dark, start of the light, periods. 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, weather patterns, migration and territorial behaviour. During the production cycle the corticosterone concentration is highest at peak production (Davis *et al*., 2000). In the same study it was high at the time of a forced moult initiated by feed deprivation at the end of the production cycle. The increase in corticosterone at these times is probably related to the high demand for nutrients.

1.4.8. Factors stimulating corticosterone release

Any stress imposed on animals and perceived to be a threat will stimulate corticosterone release. Corticosterone release is thought to be a non-specific stress response. In laying hens, basal corticosterone levels are low, usually less than 2 ng/ml (Craig and Craig, 1985; Lagadic *et al*., 1990; Littin and Cochrem, 2001) and this compares to values of greater than 70 ng/ml in free living birds

(Wada *et al*., 1999). The variation in corticosterone responses to stressors is probably an inherent characteristic (Litten and Cockrem, 2001). To adequately assess the corticosterone response to any stressor it would seem important not just to consider the peak response but also the rate of increase, rate of decrease and area under the response curve.

Genetic influences have marked effects on corticosterone responses to stress. After 12 generations of selection, two quail lines evolved that had showed large differences in their corticosterone response to immobilisation stress. At the end of the 12 generations the mean (± SEM) plasma corticosterone concentration in the highly responsive line was 12.84 ± 0.82 ng/ml and in the low responsive line 4.47 ± 0.53 ng/ml (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 Seigel, 1973; Satterlee *et al*., 1983), transport (Freeman *et al*., 1984; Gross and Seigel, 1993) and fear (Katz *et al*., 1981; Harvey *et al*., 1984; Jones, 1996: Jones *et al*., 1988).

1.4.9. Feedback regulation and habituation

Plasma corticosterone regulates brain corticosterone receptor numbers (Sapolsky *et al*., 1984) and feedback to regulate synthesis and release of CRF and ACTH (Kamstra *et al*., 1983: Vale *et al*., 1983; Sapolsky *et al*., 1984). Corticosterone also acts on the adrenal to decrease its responsiveness to ACTH and on the liver to depress CBG synthesis (Malek, 1981). The habituation of the corticosterone response that occurs in response to prolonged stress, heat (Siegel and Latimer, 1984), cold (Siegel and Latimer, 1970), underfeeding (Freeman *et al*., 1981) and exercise (Rees *et al*. 1983), could be accounted for by this feedback mechanism. Avian corticosterone response does not show habituation to exogenous ACTH (Rees *et al*., 1983). 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 elicits 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). This could be the situation where there is continued elevation in plasma corticosterone (Beuving and Vonder, 1978) or adrenalin (Freeman and Manning, 1979) following handling stress.

The response from the adrenal can be modified by experience (Mason, 1971; Dantzer and Mormede, 1983). It is possible that an animal can develop an expectancy and cognitive appraisal. Failure of 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, then habituation of nervous input stimulus could be responsible in part, for suppressed adrenal function.

Neonatal exposure to thermal stressors appears to improve tolerance to heat in later life (Reece *et al*., 1972; Arjona *et al*., 1988). Acclimatising chickens to high temperatures later in life can also be achieved by exposing them to feed restriction during neonatal growth (Zulkifli *et al*., 1994a). Does this habituation occur without the presence of the glucocorticoid during the initial stress? This is a question that Zulkifli and colleagues (1994b) have attempted to answer. In their work, neonatal chickens were feed 60% *ad libitum* and were then treated with or without metyrapone. Metyrapone inhibits the conversion of deoxycorticosterone to corticosterone (Dominguez and Samuels, 1963). Fasting increased corticosterone concentrations in hens (Zulkifli *et al*., 1993). For those chickens

treated with metyraprone later growth under heat stress was compromised. Also, those not treated with metyraprone had superior disease resistance. The authors concluded that short-term disruption to homeostasis during the neonatal stage without any associated change in corticosterone levels inhibits the ability of the animal to cope in subsequent responses to stressors. Their suggestion is that corticosterone plays a role in habituation and prepares an animal for later disruptions to homeostasis.

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 stresses.

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 1h 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).

1.5. Hormones of the sympathetic-adrenal-medullary (chromaffin cells) axis

1.5.1. Catecholamines

The amino acid L-tyrosine which is derived from the diet or from the essential amino acid L-phenylalanine, is the precursor of the catecholamines. The oxidation of tyrosine to dopa (3,4-dihydroxyphenylalanine) occurs in the adrenals and is catalysed by tyrosine hydroxylase. The next step is the decarboxylation of dopa to hydroxtryramine (3,4-dihydroxphenylethylamine) under the catalytic influence of aromatic L-amino acid decarboxylase. Hydroxtryramine is converted to noradrenaline under the influence of the enzyme Dopamine β-hydroxylase. Methylation of noradrenaline to adrenalin is catalyzed by phenylethanolamine-N-methyl transferase. In the synthesis of the adrenal medullary hormones, hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine is the rate-limiting step.

1.5.2. Control of catecholamine release

The chromaffin cells, homologous to neural tissue, contain adrenalin and noradrenaline in granules or membrane-bound vesicles, each is synthesized in distinct cell types (Ghosh, 1980). Stimulation of the sympathetic nervous system results in release of catecholamines from the chromaffin cells. The response is apparently unrelated to the severity of the stress imposed (Jurani *et al*., 1980: Lahiri, 1982). The chromaffin cells are also influenced by blood-borne factors and hormones. The catecholamines can be released relative to their individual adrenal concentrations or adrenalin can be released preferentially because the conversion of noradrenaline to adrenalin can take place by the induction of phenylethanolamine-N-methyltransferase (PNMT). Adrenalin and noradrenaline interact with specific cell surface receptors (Lands *et al*., 1967; Caron and Lefkowitz, 1993). The response in the tissue will depend on the relative numbers of each receptor type and the ability of the agonists to interact with these receptors.

Factors stimulating catecholamine release are varied. Environmental stressors such as heat and cold (Lin and Sturkie, 1968), restraint (Zachariasen and Newcomer, 1975) and forced exercise (Rees *et al*., 1984) can be potent stimulators of release. There are also many chemical and hormonal stimuli including insulin (Pittman and Hazelwood, 1973), ACTH (Zachariasen and Newcomer, 1974), corticosterone (Zachariasen and Newcomer, 1975) and anaesthesia (Sturkie *et al*., 1970), which influence release.

1.6. The effects of corticosterone

1.6.1. Effects on energy and protein metabolism

Environmental stressors exert their effects on corticosterone through psychological rather than physical qualities (Dantzer and Mormede, 1983). Therefore, corticosterone could provide a measure of an animal's mood and well being. Corticosterone administration increases food intake, suppresses growth rate and increases carcass and liver fat levels (Nagra and Meyer, 1963; Freeman and Manning, 1975; Bartov *et al*., 1980; Bartov, 1982, 1985). Some of these effects are seen after as little as 4 days of treatment (Bartov, 1982). Corticosterone is involved in glucose homeostasis and carbohydrate metabolism with a prominent action being to promote glucogenesis from non-carbohydrate sources such as amino acids and fatty acids (de La Cruz *et al*., 1981; Saadoun *et al*., 1987; Simon, 1984; Joseph and Ramachandran, 1992). Corticosterone increases plasma glucose and liver glyconeogenesis in birds (Snedecor *et al*., 1963: Stamler *et al*., 1954). The glucose is largely derived from protein catabolism (Nagra and Meyer, 1963) and the shift in metabolism favours fat deposition as there is an increase in plasma free fatty acids (FFA) and an increase in the saturated fat to unsaturated fat ratio (Nagra and Meyer, 1963).

Growth of broilers is impaired in a dose-dependent manner by corticosterone administration (Saadoun *et al*., 1987; Tur *et al*., 1989; Siegel *et al*., 1989). The depressed growth rate in birds (Davison *et al*., 1983: Siegel and Van Kampen, 1984) is often in spite of an increase in feed intake (Bartov *et al*., 1980: Siegel and Van Kampen, 1984). There are at least three factors contributing to the decreased growth rate (Siegel and Van Kampen, 1984). Firstly, there is an increase in protein catabolism as indicated by increased nitrogen excretion, uric acid excretion and water intake. Secondly, a reduction in absorptive efficiency as indicated by similar energy retention but increased food intake. Thirdly, there is an increase in energy retention in the form of fat. For chickens, corticosterone administration results in hyperglycaemia and glycogenolysis in liver and muscle (Joseph and Ramachandran, 1992). There is evidence that corticosterone influences glucagon release (Marco *et al*., 1972) and action (O'Neil and Langstow, 1978) as well as antagonizing insulin action

(Natarajan *et al*., 1987). So the effects on carbohydrate metabolism could be related to changes in the insulin/glucagon ratio.

Corticosterone is a potent lipogenic agent in chickens (Nagra and Meyer, 1963; Bartov, 1982: Saadoun *et al*., 1986). Hens treated chronically with corticosterone show a dose-dependent decrease in body weight and increase in abdominal and liver fat (Bartov, 1982; Simon 1984; Williams *et al.*, 1985; Saadoun *et al*., 1987). The increase in fattening is attributable, in the main, to an increase in protein catabolism as is suggested by elevated plasma uric acid levels in corticosterone-treated hens. Abdominal fat pad weight is increased following corticosterone treatment (Siegel *et al*., 1989; Akiba *et al*., 1992; Hayashi *et al.*, 1994). The reported decrease in thyroxine (T4) following feeding of corticosterone (Hayashi *et al*., 1994) could be related to the fat changes, as there is a relationship between metabolism, fat deposition and T4.

When glucocorticoids are administered to animals the effects on protein metabolism are similar to those seen for stress atrophy of skeletal muscle and lymphoid tissue (Rousseau and Baxter, 1979). Changes in protein metabolism reflect changes in the relative rates of protein synthesis and degradation (Tomas *et al*., 1979; Hayashi *et al*., 1992). Skeletal muscle protein degradation is increased in a dose-dependent manner by feeding corticosterone but there is no effect on protein synthesis (Hayashi *et al*., 1994). Corticosterone acts at the liver to increase glucose production and induce synthesis of enzymes involved in amino acid catabolism (Kenney, 1969). In addition, corticosterone acts to amplify the actions of other hormones involved in protein metabolism. Psychological stress increases catecholamine and glucagon release (Bloom *et al*., 1972; Freeman and Manning, 1976).

1.6.2. The effects on reproduction

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). Corticosterone injections will delay oviposition with high doses needed towards the period of peak egg production. There are reports showing that corticosterone treatment induces ovulation (Etches and Cunningham, 1976: Etches and Croze 1983). Injections of ACTH will induce ovulation (Etches and Cunningham, 1976) and LH release (Wilson and Sharp 1976a; Etches and Croze, 1983), however, the levels needed are outside the normal physiological range. A normal physiological range (around 3 ng/ml) can be maintained by subcutaneous infusion of 30 ug/h of corticosterone (Etches *et al*., 1984a). When this level of corticosterone was infused into hens, egg production dropped by day 3 and completely ceased by day 8 (Etches *et al*., 1984a). Similar effects can be achieved by dietary restriction or feeding diets deficient in calcium or sodium (Douglas *et al*., 1972; Summers and Leeson, 1977; Whitehead and Sharp, 1976; Williams *et al*., 1985). These dietary effects may be mediated by changes in corticosterone as levels increase with a reduction in food intake (Etches *et al*., 1984a).

Reduction in food intake causes an immediate lowering of plasma LH, progesterone and oestradiol (Tanabe *et al*., 1981). Nutritionally-induced ovarian regression is also associated with a greatly reduced pituitary responsiveness to LHRH which is not the case in corticosterone-induced ovarian regression (Etches *et al*., 1984a). Both oestradiol and progesterone (Booney and Cunningham, 1976; Wilson and Sharp, 1976b) are required to maintain LH release. After the infusion of corticosterone, ovarian regression is associated with a decrease in progesterone, oestradiol and LH (Etches *et al*., 1984a) with the sequence of hormonal changes suggesting that corticosterone acts directly at the ovarian level. Puberty is advanced in quail selected for a low corticosterone response to restraint (Marin *et al*., 2002). Corticosterone levels are elevated during a period of moult initiated by feed deprivation (Hoshino *et al*., 1998a, b; Davis *et al*., 2000). The high corticosterone is probably related to the demand for nutrients at this time. When hens were denied access to feed, corticosterone concentration was significantly higher by 20h and remained high for the 68 h duration of feed deprivation (Beuving *et al*., 1989).

Adrenalin induces follicle atresia *in vitro* (Moudgal *et al*., 1985) and *in vivo* (Moudgal *et al*., 1990a) and affects ovulation rate (Moudgal and Razdan, 1981). Adrenalin also delays oviposition (Sturkie, 1976), however this effect is dependent on the dose and stage of the laying cycle (Moudgal *et al*., 1990a). Any effects corticosterone has on reproduction could be being mediated through its effects on catecholamine synthesis. Corticosterone stimulates the conversion of noradrenaline to adrenalin by inducing PNMT. When alpha-methyl-p-tyrosine, a blocker of catecholamine synthesis, is administered with corticosterone any detrimental effects corticosterone has on ovulatory events are prevented (Moudgal *et al*., 1991).

1.6.3. The effects on immune function

It is a widely held view that the immunosuppressive effects of stress are mediated by glucocorticoids. Chronically high corticosterone concentrations decrease disease resistance (Gross 1992). Adrenal activation precedes lymphocytosis (Siegel, 1985: Beuving *et al*., 1989). Glucocorticoid receptors are located on lymphocytes and monocytes (Comsa *et al*., 1982). Stress increases the incorporation of corticosterone into lymphoid cells (Gould and Siegel, 1981), inhibiting glucose uptake and protein synthesis and causing lymphocytosis, resulting in decreased cell number and antibody production. Immunosuppression has been reported after stress or ACTH injections (Thaxton and Siegel, 1970; 1972: Siegel 1987). Pharmacological doses of corticosterone can suppress cell-mediated immunity (Gross *et al*., 1980; Edens *et al*., 1983; Gross and Seigel, 1983). Regression of thymus, spleen and bursa occurs following ACTH or corticosterone injections (Siegel and Beane, 1961; Siegel, 1962). Corticosterone causes a reduction in lymphocyte number (Davison and Flack, 1981) and an increased susceptibility to disease (Gross *et al*., 1980).

The heterophil to lymphocyte (H/L) ratio is considered to be a good indicator of stress (review: Maxwell, 1993). Corticosterone infusion increases the H/L ratio (Jones, 1987b; Maxwell *et al*., 1992; Alodan and Mashaly, 1999) as will ACTH infusion (Wolford and Ringer, 1962). Factors known to be stressful that increase the H/L ratio include fasting and feeding frustration (Gross and Seigel, 1983; Jones, 1989; Beuving *et al*., 1989; Zulkifli *et al*., 1995**;** Davis *et al*., 2000), exposure to heat and cold (Campo and Davila, 2002), lack of foraging material (El-Lethey *et al*., 2000), noise (Gross, 1990), transport (Mitchell *et al*., 1992), social stress (Anthony *et al*., 1988), isolation stress (Jones *et al*., 1991b) and increasing cage density (Hester *et al*., 1996a).

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). For a single episode of noise stress it took 18h for the H/L ratio to increase and 30h to return to control levels (Gross, 1990). In broilers the H/L ratio increased 3h after transport (Mitchell *et al*., 1992) and 1-3h after isolation stress (Jones *et al*., 1991b). 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.

Hens in stressful environments produce lower antibody response to a variety of antigens (Gross, 1972; Gross and Siegel 1975; Thompson *et al*., 1980; Edens *et al*., 1983). Corticosteroids or ACTH infusions decrease antibody titers in birds (Thaxton *et al*., 1968; Thaxton and Seigel, 1970). After challenging hens with Marek's disease the severity is increased by continuous corticosterone infusion (Powell and Davison, 1986). Within 12h of heat treatment or ACTH injection antibodies to three specific antigens decreased (Thaxton and Siegel, 1972). Pretreatment with suppressor corticosterone compounds modified this effect (Siegel and Latimer, 1974). Exposure to heat decreases cell-mediated immunity in hens (Regnier and Kelley, 1981), as will a decrease in actual body temperature (Siegel, 1971). Any reduction in antibody production could increase the susceptibility to disease.

1.7. The effects of catecholamines

1.7.1. The effects on metabolism

Plasma catecholamine levels are closely associated with glucose (Cramb *et al*., 1982) and lipid (Campbell and Scanes, 1985) metabolism. Noradrenaline and adrenaline promote hyperglycaemia and this is associated with increased glycogenolysis, increased gluconeogenesis and decreased glucose utilization by peripheral tissues, with adrenalin being more potent than noradrenaline. Adrenalin stimulates hepatic gluconeogenesis by increasing the availability of substrates, lactate and glycerol, these being generated during lipolysis. Catecholamines are also responsible for a decrease in the metabolic clearance rate of glucose (Himms-Hagen, 1967).

Catecholamines act to inhibit insulin release (Clutter *et al*., 1980), independently of blood glucose levels. Both adrenalin and noradrenaline stimulate glucagon release (Steffens and Strubble, 1983) which acts to stimulate hepatic glycogenolysis and gluconeogenesis. Adrenaline and noradrenaline are released in response to the flight or fright syndrome, whereas glucagon is released in response to nutritional stress. A decrease in the insulin/glucagon ratio results in mobilization of metabolic fuels such as glucose and free fatty acids (FFA's). In domestic fowl, lipogenesis occurs in the liver with the adipose tissue functioning primarily for lipid storage and mobilization (Goodridge and Ball, 1967; O'Hea and Leveille, 1968; 1969). Mobilization of fats stores involves lipolysis and changes in blood supply. In isolated chicken hepatocytes adrenalin inhibits lipogenesis in a dose-dependent manner and noradrenaline has a similar effect but with a lower potency (Campbell and Scanes, 1985; Capuzzi *et al*., 1975; Cramb, *et al*., 1982). The antilipogenic effects of adrenalin can be reversed using both α- and ß-adrenergic antagonists (Campbell and Scanes, 1985).

Adrenalin infusion has been reported to decrease proteolysis but not to stimulate protein synthesis (Castellino *et al*., 1990; Mathews *et al*., 1990). Clenbuterol, a $ß_2$-adrenergic agonist, increases carcass protein content in chickens (Takahashi *et al*., 1993). The clenbuterol effect on protein accretion in chickens is due to a decrease in the rate of protein degradation as this ß-agonist is reported to have no effect on rates of protein synthesis in chickens (Muramatsu *et al*., 1991). Feeding corticosterone to chickens (10 mg/kg of diet) increased carcass fat and abdominal fat and decreased carcass protein (Takahashi *et al*., 1993). When clenbuterol was also added to the diet (0.33 mg/kg) there was a significant effect on the corticosterone reduction in carcass protein but no effect on the carcass fat levels. In the chicken, ß2-agonists could act predominantly on protein deposition.

1.7.2. The effects on reproduction

The ovarian follicle is intensively innervated and contains adrenergic fibres (Gilbert 1969). A role for catecholamines in ovulation is suggested by the report that anti-adrenergic drugs inhibit and agonists induce ovulation in birds (Kao and Nalbandov, 1972; Moudgal and Razdan, 1981; 1985). As the largest ovarian follicle advances towards ovulation the levels of adrenalin and noradrenaline increase and the dopamine levels decrease (Moudgal and Razdan. 1983). These changes are not seen in the second largest follicle where levels remain low. Adrenalin *in vitro* (Moudgal *et al*., 1985) and *in vivo* (Moudgal *et al*., 1990a) causes atresia of ovarian follicles and *in vivo*, reduces egg production (Sykes, 1955). Adrenalin levels in the egg are a good measure of stress in hens (Moudgal *et al*., 1990b) and poor egg shell quality (Moudgal *et al*., 1990c). These deleterious effects can be prevented if an inhibitor of catecholamine synthesis is administered (Moudgal *et al*., 1991). In groups of hens laying at either 40% or 70% egg production, the high layers had significantly higher noradrenaline and lower adrenalin levels in the eggs compared to the low layers (Moudgal *et al*., 1992). The values suggest that there was greater conversion of noradrenaline to adrenalin, that is greater PNMT activity, in the poor layers. There is an increase in PNMT activity during stress or after corticosterone treatment (Zachariasen and Nemcomer, 1975). Dopamine levels in eggs do not appear to be an indicator of stress (Moudgal *et al*., 1992).

1.8. Ambient Temperature

Poultry are homeotherms and need to maintain their core body temperature within narrow limits. This is done through an integration of the nervous and endocrine systems resulting in a combination of behavioural and physiological responses. A rectal temperature of 45^0C seems to be the upper limit of safety for hen survival (Wilson, 1948). Ambient temperatures above 40.5^0C will result in mortality if sustained for a short period of time (Wilson, 1948). Hens are not able to cope with an ambient temperature of 38^0C for more than an hour if the exposure is sudden (Sykes and Fataftah, 1986). There is a general model of thermogenesis for farm animals. As part of this, the thermoneutral zone is the range of ambient temperature were metabolic rate is not changed and thermoregulation is achieved by both physiological and behavioural means (Van Kampen, 1981). The limits to this range are referred to as the upper and lower critical temperatures and these vary according to the humidity (Van Kampen, 1981) and probably other factors.

Heat dissipation via the combs and wattles is important when the ambient temperature reaches 30^0C (Van Kampen, 1974), as is heat loss from the skin area under the wings (Kettlewell, 1989). More extensive efforts to decrease heat load involve panting (Hales, 1983). When heat stress is extreme cardiovascular failure will occur. For hens the optimum ambient temperature is 21^0C (Charles, 1985). High producing commercial laying hens, need to maintain a high metabolic rate and this increases their susceptibility to heat stress (Blem, 2000). The insulative effect of the plumage, lack of sweat glands and relatively low respiratory water evaporative rate increases the propensity of hens to heat stress (Lee *et al*., 1983; Etches *et al*., 1995). Both growth rate and body weight can be predisposing factors influencing the birds response to heat stress (Reece *et al*., 1972; Wilson *et al*., 1975). Activity levels decrease in an effort to decrease metabolic heat production (Hutchinson and Sykes, 1953). Water intake increases with high ambient temperature and this acts as a heat sink for birds (Teeter *et al*., 1987a).

While the tendency to house caged birds in controlled environment sheds will continue, the proportion of hens kept in alternative housing systems will also increase. Hens kept in barn and free range systems can be exposed to a wide range of ambient temperature and heat stress remains an important consideration in the welfare of hens maintained in these types of housing systems. Various methods have been employed to reduce the incidence of exposure to high temperatures. Building design and insulation have been used to decrease shed temperatures (Carr and Carter, 1985), drinking water can be cooled (Beker and Teeter, 1994) and water cooling of the shed and birds all help to alleviate heat stress (Reilly *et al*., 1991; Wolfenson *et al*., 2001).

1.8.1. Effects of ambient temperature

1.8.2. Food intake, feed utilization and body weight

Layer performance is depressed during heat stress. Effects associated with heat stress are decreased body weight and food intake, altered nutrient absorption, respiratory alkalosis, decreased blood flow to some organs and endocrine changes (Sykes and Fataftah, 1986; Scott and Balnave, 1988; Etches, *et al*., 1995; Samara *et al*., 1996; McKee *et al*., 1997; Balnave and Muheereza, 1998; Wolfenson *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004). In chickens, some consequences of high ambient temperature are decreases in feed intake, growth rate, feed conversion efficiency and increased fat deposition (Geraert *et al*., 1993; 1996). A reduction in feed intake decreased metabolic activity and therefore whole body heat production (MacLeod and Jewitt, 1984).

The decrease in feed conversion efficiency and increase in fatness observed under high ambient temperature suggest that there are important changes in nutrient use. The increase in fatness observed

in broilers suggests that there is a diversion of nutrients towards lipogenesis although it could also result from inhibition of lipolysis. The increased fatness is not associated with increased circulating triglyceride levels (Geraert *et al*., 1996). Energy digestibility has been reported to increase (Keshavarg and Fuller, 1980) or be unchanged (Geraert, *et al*., 1992) under conditions of chronic heat stress. Protein retention is lower in heat stressed broilers (Geraert *et al*., 1996). Amino acid digestibility decreases (Zuprizal *et al*., 1993) although jejunal amino acid and hexose uptake increase in broilers during heat exposure (Mitchell and Charlisle, 1992). Broilers housed at 32^0C had lower blood glucose and amino acid levels than broilers at 22^0C. In the same study, the broilers at 32^0C had depressed growth rates even in comparison to pair-fed broilers held at 22^0C (Geraert *et al*., 1996b). Plasma protein concentration is lower under heat stress (Zhou *et al*., 1998) as is blood calcium (Abel-Raham, 2000).

In laying hens, cyclic periods of high temperature (15.6-35^0C) had no effect on feed intake (Deaton *et al*., 1981). In more recent study, hens were maintained at constant temperatures of 23.9^0C and 35^0C or subjected to a daily cyclic temperature regime (23.9^0C to 35^0C) (Mashaly *et al*., 2004). The feed intake was decreased as the severity of the heat stress increased. Feed intake is higher and feed conversion efficiency better in cooler temperatures (Payne, 1966; Siegel, 1971; Stockland and Blaylock, 1974). High ambient temperature decreases body weight (Scott and Balnave, 1988; Samara *et al*., 1996; Abel-Raman, 2000; Garaces *et al*., 2001; Mashaly *et al*., 2004). Some of the effects on body weight can be alleviated by imposing a dark period during normal light periods (Sahin and Kucuk, 2001) although there is a limit to how short the period can be for this effect (Schilder *et al*., 2001). The changes in body weight are most probably associated with the changes in feed intake.

1.8.3. Egg production

Temperature is probably the most important environmental factor that influences egg production and egg quality. Prolonged exposure to high temperatures is associated with decreased egg production (Hester *et al*., 1996b; Muiruri and Harrison, 1991; Samara *et al*., 1996; Whitehead *et al*., 1998; Mashaly *et al*., 2004) as is cold stress (Davis and Stopes, 1989; Spinu and Degen, 1993). Egg production is better in cool temperatures (de Andrade *et al*., 1977; Stockland and Blaylock, 1974). Increases in ambient temperature have only small effects on egg production until a critical temperature is reached and after this temperature has a large effect. Egg production is relatively constant across a range of temperature. This range has been reported to be 10-27^0 C by Van Kampen, (1981) and 15-27^0 C by Marsden *et al*., (1987). There is a rapid fall in egg production, egg quality and feed intake when the temperature is greater than 28-30^0C (Al-Saffar and Rose, 2002). This is the temperature where panting and acute effects of heat stress begin.

High ambient temperature results in decreased egg weight (de-Andrade *et al*., 1977; Emery *et al*., 1984; Balnave and Muheereza, 1997; Samara *et al*., 1996; Scheideler *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004) inferior shell and albumen quality (Emery *et al*., 1984; Odom *et al*., 1985; Sauver and Picard, 1987; Mahmoud *et al*., 1996; Mashaly *et al*., 2004) and increased egg breakage (Tanor *et al*, 1984; Roland, 1988).

When hens of a line selected for survivability in multi-bird cages were compared to an unselected line, egg production was depressed when the housing temperature was lowered to 0^0C from 18-24^0C, but the decrease was not as great in the selected line (Hester *et al*., 1996b). When housed individually or in 12-bird cages the selected line had a higher production rate at the low temperature. Using the same lines of hens and increasing the temperature from 30^0C to 38^0C for 3h decreased egg production, however again it was better in the selected compared to the unselected line (Hester *et al*., 1996b). At the high temperature, mortality was lower in the selected line when kept in 12-bird cages but similar when housed singularly. At a constant temperature of 35^0C the mortality rate in hens was 31.7% compared to 5% when the temperature was 23.9^0C over the same production period (Mashaly *et al*., 2004).

1.8.4. Immune function

Both high and low temperature are reported to cause immunosuppression (Heller *et al*., 1979; Davis and Stopes, 1989; Spinu and Degen, 1993; Hester *et al.,* 1996a; Dabbert *et al*., 1997; Zulkifi *et al*., 2001) and lymphocyte proliferation (Puthpongsiriporn *et al*., 2001). Climatic stress can alter B-cell function resulting in reduced antibody production (Thaxton and Siegel, 1970; Spinu and Degen, 1993). High temperature can decrease (Thaxton and Sigel, 1970; Gross, 1992; Zulkifi *et al*., 2001; Mashaly *et al*., 2004), have no effect (Donker *et al*., 1990) or increase (Henken *et al*., 1983) antibody response to specific antigens. Cold stress is reported to have no immunosuppressive effect (Dabbert *et al*., 1997; Hangalapura *et al*., 2003), to be immunosuppressive (Svensson *et al*., 1998) or enhance immunity (van Loon *et al*., 2004). There is little doubt that genotype and severity of the heat stress influence the degree of immunological perturbation. The B- and T-cell proliferation rate was not adversely affected by maintaining hens at a constant temperature of 23.9^0C and 35^0C or on a daily cyclic temperature regime ranging from 23.9^0C to 35^0C. The white blood cell levels were lower after 4 weeks at 35^0C (Mashaly *et al*., 2004).

A short period (1-2h) at 42^0C will decrease leukocyte number (Ben-Nathan *et al*., 1976). The heterophil to lymphocyte (H/L) ratio is an indicator of stress (Gross and Siegel, 1983; Gross, 1989; van Niekerk *et al*., 1989; Maxwell, 1993). In fact some researchers consider corticosteroid levels not a good indicator of stress (Beuving *et al*., 1989) and that the H/L ratio is a more sensitive measure (Siegel, 1985; Gray *et al*., 1989 Maxwell *et al*., 1992). High temperatures increase the H/L ratio (McFarlane and Curtis, 1989; McFarlane *et al*., 1989; Mogenet and Youbicier-Simo, 1998; Mashaly *et al*., 2004). The increase in the H/L ratio was dependent on the severity of the heat stress (Mashaly *et al*., 2004). Cold temperatures will also increase the H/L ratio (Wolford and Ringer, 1962).

1.8.5. Endocrine changes

1.8.6. Metabolic hormones and steroids

Most studies investigating hormonal changes to temperature have concentrated on the regulation of thermogenesis. The T3 levels appear to be lower and T4 levels are unchanged (Rudas and Peters, 1984; Geraert *et al*., 1996) or increase (Moss and Balnave, 1978) during heat stress. While Geraert *et al*., (1996) found this to be the case when broilers were heat stressed at 2-4 weeks of age, the T3 levels were unchanged when the birds were heat stressed at 4-6 weeks of age, however the T4 levels were lower. The changes in thyroid hormones seem to be independent of feed intake (Leclercq and Rudeaux, 1988). Administration of either T3 or T4 to chickens does not prevent the growth retardation associated with high temperature (May, 1989). Growth hormone levels are increased during heat stress (Mitchell and Goddard, 1990) and this could be associated with thyroid hormone regulation or a response to the decrease in food intake seen with high ambient temperature.

Basal progesterone levels are lower in heat stressed hens (Wolfenson *et al*., 2001) and it has been proposed that this is due to reduced steroid secretion from granulosa cells during heat stress (Novero *et al*., 1991).

1.8.7. Corticosterone

Increased corticosterone concentration seems to be a critical requirement for survival during severe disruption to homeostasis however, chronic exposure is seemingly damaging to health and welfare (Sapolsky, 1992). Temperature extremes increase plasma corticosterone concentration in chickens, laying hens and turkeys (El-Halawani *et al*., 1973; Brown and Nester, 1973; Buckland *et al*., 1974; Edens and Seigel, 1975; Edens, 1978; Beuving and Vonder, 1978; Geraert *et al*., 1996; Etches *et al*.,

1995; Siegel, 1995; Hester, 1996c; Wolfenson *et al*., 2001; Downing and Bryden, 2002). Corticosterone has been measured in studies of high and low ambient temperature but few if any have investigated the corticosterone levels in chronically heat-stressed birds. Broilers exposed to 32^0C from 4 to 6 weeks of age had increased circulating corticosterone concentrations and decreased basal insulin sensitivity (Geraert *et al*., 1996). Corticosterone administered to chickens decreases body weight gain, increases protein catabolism and lipid deposition (Decuypere and Buyse, 1988) as well as inhibiting insulin sensitivity (Taouis *et al*., 1993). Cold stress reduces egg production and immune function and the changes were not necessarily the result of changes in corticosterone (Davis and Stopes, 1989). Turkeys selected for either high or low corticosterone response to cold temperatures had improved egg production in the low corticosterone line (Brown and Nester, 1973, 1974).

Exposure to a temperature of 42^0C for 1-2h increased plasma corticosterone concentration (Ben-Nathan, *et al*., 1976). When laying hens were exposed to 37^0C for 10 h/d over 10 consecutive days there was a transient increase in plasma corticosterone concentration (Wolfenson *et al*., 2001). The concentration was elevated on day 2 (1.38 v 2.19 ng/ml), however by day 10 these had returned to normal. Transient increases in corticosterone concentrations have been reported by others following acute episodes of heat (Etches *et al*., 1995; Siegel, 1995; Hester, 1996: Downing and Bryden, 2002). A major problem with many studies is that samples are taken infrequently and therefore give little indication of changes over time. Repeated exposure to heat stress resulted in a diminished corticosterone response over time (Siegel and Gould, 1982).

In turkey hens, corticosterone levels increased substantially in the two weeks prior to lay in both pen and caged birds (Zadworny *et al*., 1986). Food intake is insufficient after the onset of lay to maintain high egg production so there is a need to mobilise body reserves. Corticosterone may stimulate utilization of body reserves and therefore the basal level of corticosterone seen in laying hens may actually be elevated corticosterone levels when compared to non-laying hens and increases above basal levels are the result of metabolic efforts to meet nutrient demands during stress. This is supported by the study of Zadworny *et al*., (1986) where corticosterone levels were measured over 16 weeks. In caged turkey hens the corticosterone concentrations were higher than for hens maintained in pens after 4-6 weeks, however the level of egg production is much lower in the penned hens because a higher percentage become broody. Therefore, here the higher egg production was related to higher plasma corticosterone concentrations. Male turkeys not heat stressed, showed a rhythm in plasma corticosterone concentration which peaked at 1000h, then fell to a constant level between 1300 and 2200h and then declined to the lowest levels at 0600h (El-Halawani *et al*., 1973). Both, heat- (32^0C) or cold-stressed (7^0C) birds showed no rhythm, maintaining high levels over the 24h treatment period. However, the maximum levels were never greater than the diurnal peak concentration seen in non-stressed birds. In the same study, turkeys were exposed to the high and low temperatures for 5 weeks. Plasma corticosterone concentrations increased after initial exposure and then returned to control levels by 2 weeks for birds at 32^0C and by 3 weeks for birds at 7^0C. In the cold hens housed at high density had lower corticosterone concentrations than hens at low density and this situation was reversed in a hot humid environment (Edens *et al.*, 1982).

In chickens selected for their low corticosterone response to ACTH, survivability at 45^0C was greater than those selected with high corticosterone responses (Edens and Seigel, 1975). When hens were exposed to a cyclic temperature (4.4^0C to 37.8^0C) plasma corticosterone concentration was higher compared to hens at 21^0C on the first day of heat stress (Siegel, 1971). After one week the concentration had returned to the values seen in the hens at 21^0C.

1.8.8. Habituation and temperature

Early stressful events experienced by animals can influence responses to subsequent stressors (Sapolsky, 1992). The individual responses to heat stress are very variable (Arad and Mardar, 1984). Many strains of laying hen have been developed in climates were they have had little opportunity to

develop tolerance to heat. For these strains, acclimatisation and acclimation provide some protection against heat stress. After several days exposure to intermittently high temperature hens are able to survive conditions that would initially be lethal.

When hens were exposed to an ambient temperature of 38^0C, the initial period of exposure before the rectal temperature reached 45^0C was only 1h (Sykes and Fataftah, 1986). After 7 days of intermittent exposure to high temperature the period for the rectal temperature to reach 45^0C increased to 4h. This is typical of what is considered to be the process of acclimatisation. At high temperatures the capacity for hens to increase heat loss is limited and so acclimatisation probably involves physiological adjustments that decrease heat production (Sykes and Fataftah, 1986). Following acclimatisation of hens exposed to 38^0C intermittently, oxygen consumption fell by 19-29% and continuous exposure to 35^0C resulted in a 14% decrease in heat production at the end of 7 days (Sykes and Fataftah, 1986). This is consistent with the observation that heart rate is lower in hens acclimatised to high temperature (Hutchinson and Sykes, 1953). Exposure to progressively higher temperatures, up to 44^0C for 2h/d had an accumulated effect with regard to acclimatisation (Arad *et al*. 1981). Acute exposure to a daily cyclic temperature ranging from 24^0C to 35^0C for 2-4 days can acclimatise birds to high temperature subsequently (Davis *et al.,* 1991). Physiological changes in basal metabolic rate are involved in the process of acclimatisation. Fasting either days or hours before acute heat periods can increase heat tolerance (McDonald *et al*., 1990; Francis *et al*., 1991; Teeter *et al*., 1992). The fasting needs to be before the period of heat stress, however this only needs to be 3-6h to achieve an effect (Teeter *et al*, 1987b).

An important question remains; can hens acquire and then retain the ability to acclimatise and help protect them at some later period of high ambient temperature? The temperature to which hens are acclimatised is important. If acclimatised to a low temperature then heat stress will begin at 25^0C. For this reason heat stress is more pronounced in hens maintained in temperate climate (Balnave, 2004). Rearing hens at high or moderate temperatures had no effect on acclimatisation at a later time (Njoya and Picard, 1994). Payne (1966) also found minimal effect of rearing temperature (22^0 C or 33^0 C) on layer performance at different temperatures (17-30^0 C). A high rearing temperature tended to give lower production in other studies (Stockland and Blaylock, 1974; Cowan and Micie, 1983). Kyarisiima and Balnave (1996) reared pullets in cool or hot temperatures and found that layer performance in hot temperatures was improved if the pullets were reared in the cool environment and this effect was related to greater food intake during the layer period.

It's possible that habituation requires some exposure to glucocorticoids during the initial stressful event. High circulating corticosterone concentrations may be important in preparing the body to respond to subsequent stressors. Neonatal exposure to acute heat stress appears to improve heat tolerance in chickens (May *et al*., 1987). Reports suggest that chickens can be acclimatised to high temperature by restricting fed availability during the neo-natal period (Zulkifli *et al*., 1994a). Acclimatisation to heat is also associated with metabolic changes and this could be where corticosterone influences acclimatisation. Fasting has stimulatory effects on the HPA axis (Zulkifli *et al.*, 1993). While early feed restriction improved heat tolerance in chickens; blocking corticosterone synthesis during the period of feed restriction depressed the acclimatisation effects (Zulkifli *et al*., 1994b). These authors suggest that transient perturbations in homeostasis during the neonatal stage without an increase in corticosteroid may prevent acclimatisation of birds. Increased corticosterone concentrations in the neonatal stage could alter corticosterone receptor number in the brain so that at later stages animals are more responsive to raised corticosterone levels, corticosterone action being to prepare the body for the disruption to homeostasis**.** By being more responsive later in life, less corticosterone is actually released due to enhanced feedback regulation (Sapolsky *et al*., 1984). Other researchers reject this as a prerequisite for acclimatisation (Munck *et al*., 1984; Munck and Guyre, 1986). Responses to exogenous CRF are influenced by age, dose rate and frequency of injections. In early life responses to CRF are either absent or equivocal (Freeman and Manning, 1977). After a 7 day pre-treatment of chickens with a daily injection of CRF, the peak corticosterone response to an ACTH injection given on day 8 was similar to controls (Freeman *et al.*, 1979), however the total

corticosterone response was lower in the CRF pre-treated birds. Therefore, habituation may involve a decrease in the total amount of corticosterone released in response to a stressful stimulus.

Water depletion is reported to reduced heat tolerance (Arad, 1983). This was not found to be the case by Sykes and Fataftah (1986). In heat, water needs increase to meet an increase in respiratory evaporative cooling (Belay and Teeter, 1993). Water intake needs to be sufficient to meet specific needs and maintain homeostasis.

1.9. Space, group size and cage density

It needs to be noted that in many cage studies the effects of space and density are compounded because often the change in space allowance is achieved by changing the group size. In production systems both the space per bird and the size of the group influence aspects of hen behaviour, physiology and wellbeing. There are various procedures for determining the space needs of hens (Cooper and Albentosa, 2004). One way is to make direct measures of the space needed by hens for them to perform particular activities. Dawkins and Hardie (1989) estimated that hens needed 475 cm^2 for standing, 850 cm^2 for scratching, 1150 cm^2 for preening and 1876 cm^2 to partake in wing flapping. Another way is to evaluate the hen's response to altering the space available to perform various activities (Nicol 1987a). Reducing space available to hens will decrease the incidence of comfort activities (Nicol, 1987b).

The limited space available to hens maintained in conventional cages and the behavioural limiting constraints it causes have been of concern to various interest groups. There remains contention over how important specific activities are to the wellbeing of hens. The activities requiring extensive space are not often seen in caged hens (Albentosa and Cooper, 2003; Albentosa, *et al*., 2003). Providing more space to hens increases foraging, locomotion, preening and dustbathing (Albentosa and Cooper, 2003; Albentosa, *et al*., 2003).

There is one question that continually perplexes researchers working in the area of hen welfare. If an activity is absent or prevented, does this mean that the hen is going to be stressed or frustrated because it can't perform the activity? The intensity that hens exert in an effort to maintain a larger space is not great (Linberg and Nicol, 1996). Although, when provided with alternatives they appear to prefer increased space (Dawkins, 1985; Nicol, 1986). This would suggest that some comfort activities are not a prerequisite when a large effort is required to obtain the space needed to perform them. The effort exerted can also be influenced by previous rearing conditions (Faure, 1991).

There is competition for space and, importantly, social interactions take place when hens are housed in groups (Keeling, 1995). The space available for a hen will be limited by other hens in the group as well as any limits imposed by the size of the enclosure. In alternative systems, the floor space per hen may be the same as in cages, however the usable space available will be greater as hens have greater total space available to them (Keeling, 1995). Restriction of personal space can be an important psychological disturbance to hens. Cooper and Albentosa (2004) report that for hens maintained as groups, the social interactions can be catergorised as gregarious, facilitative, competitive and aggressive. In general hens prefer to spend time with other hens (gregariousness) and in the presence of other hens will perform activities instigated by flock mates (facilitation). Especially if resources are limiting, competition can be intense for these and will include space (Dawkins, 1985: Nicol, 1986). Social structures, familiarity and competition are key issues responsible for the level of aggressive behaviour in group housed hens. 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). Groups of familiar hens maintained in cages tend to distribute themselves evenly over the available space while unfamiliar hens tend to distribute themselves

unevenly and engage in aggressive behaviour (Linberg and Nicol, 1996). In alternative production systems (barn and free range) hens show a range of densities in the enclosure (Channing *et al*., 2001).

In barn and free range systems hens need to deal with problems associated with identifying other individuals, establishing their position in the hierarchy and dealing with aggressive interactions. In large enclosures group size is probably important. 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). For example hens in a free range systems may do this by not leaving the shed (Grigor *et al*., 1995). In cage systems hens readily establish stable social hierarchies and this is probably responsible for the much reduced level of aggressive interactions (Keeling, 1995). Linberg and Nicol (1996) reported that hens tend to prefer a small group size than a large group size, although this may depend on the hens' position in the hierarchy. The primary welfare concern of conventional cages is the lack of resources to meet the hen's behavioural repertoire (Appleby and Hughes, 1991). The purpose of furnished cages is to alleviate the restriction on some behaviours (Appleby *et al*., 2002). The question still remains; what is an acceptable amount of space for caged hens?

1.9.1. Adaptive mechanisms to avoid social stress

In large flocks, victimisation of some hens could seriously compromise their welfare. Severe victimisation can result in extensive feather loss (McAdie and Keeling, 2000; Freire *et al*., 2003). The extent of feather coverage on certain areas of the body is closely related to the level of pecking experienced by individuals (Freire *et al*., 1999; 2003). In large flocks victimised hens appear to adapt by escaping to areas of relative safety. In a perchery, hens that spent more time on the slats under the perches, were found to have the worst feather condition on the back, tail and head regions, all areas associated with increased incidence of feather pecking from higher ranking hens (Freire *et al*., 2003). These hens had lower body weights but no other anatomical indicators of stress, there being no changes in liver, spleen and bursa of Fabricius weights. Changes in these organs have been reported when broilers are infused with ACTH (Puvadolpirod and Thaxton, 2000). The time spent on the slats had no relationship with the time hens took to move away from other hens (avoidance test). Freire *et al*., (2003) suggested that escaping to the slats may be sufficient for hens to avoid the stress associated with victimisation and for there to be no physiological and anatomical changes normally evident when hens are stressed. The lower body weights might be evidence that the avoidance is an adaptation, which does not completely alleviate the consequences of stress associated with victimisation.

1.9.2. Space, group size, cage density and production

The aggressive and competitive behaviour of hens housed as groups is reflected by the production level and efficiency. In laying hens, egg production has been reported to decrease with an increase in cage density (Dorminey *et al*., 1972; Lei *et al*., 1972; Feldkamp and Adams, 1973; Martin *et al*., 1980; Cunnignham and Ostrander, 1981; Quart and Adams, 1982; Mashaly *et al*., 1984; Adams and Craig, 1985; Patterson and Muir, 1986; Anderson and Adams, 1992; Lee and Craig, 1991; Brake and Peebles, 1992; Anderson, 1995; Bell *et al*., 1998). Adams and Craig (1985) undertook a statistical analysis of published data looking at the effects of crowding on production performance. Three density categories were examined, low (432-561 cm^2/hen), medium (355-426 cm^2/hen) and high (271-348 cm^2/hen) and only studies using 2-6 hens per cage were used in the evaluation. The data indicated that there was a curvilinear relationship between density and production with performance decreasing more rapidly at higher densities. Reducing average space per hen from 387 to 310 cm^2/hen decreased egg production by approximately twice the level as decreasing the average space from 516 to 386 cm^2/hen. Decreasing the space per hen below 380 cm^2 seems to have a large influence on egg production.

Group size and available floor space have separate and additive effects on egg production (Adams and Jackson, 1970). Increasing group size (4 to 6 hens) and decreasing cage space per hen (407 to 316 cm^2) decreased egg production and, like the previous report, the effects were separate and additive (Cunningham *et al*., 1988). Robinson (1979) found that space and group size effects on production only became significant when the group size was small (2 hens/pen) and floor space was minimal (350 cm^2). In 6 bird cages increasing the space available from 361 to 482 cm^2/bird increased production from 77.4% to 82.3% (Anderson *et al*., 2004).

For hens housed in cages individually (1394 cm^2) or at low density (2 hens at 1394 cm^2/bird) or high density (2 hens at 679 cm^2/bird), both increasing the number of hens per pen and decreasing the space available, reduced egg production without affecting feed conversion efficiency or body weight (Mench *et al*., 1986). The production effects of housing 1, 2 or 3 hens in small cages at 1160 (S1), 580 (S2) or 390 (S3) cm^2/bird respectively or 3 (L3), 4 (L4) , 5 (L5) or 6 (L6) hens in large cages at 770, 580, 460 and 390 cm^2/bird were studied by Koelkebeck and Cain (1984). In large cages, increasing the number of hens in the same space (L3, L4, L5 and L6) had no effect on egg production. In large cages the area per hen may decrease as the group size increases, however the increased cage size may be important in alleviating the negative effects seen with the same space allocation in small cages. When the group size is held constant (3 hens) and the space per hen is halved (L3; 770 cm^2/bird vs S3; 390 cm^2/bird) egg production was significantly lower. Overall, in this particular study egg production was favoured by having one hen per cage.

Carey and Kuo, (1995) recorded egg production for two strains of hens over a full production cycle. Hens were housed as groups of 6, 8, 12 or 24 and the floor space maintained at 364 cm^2/bird, and feeder space at 10 cm/bird. There were no effects of group size on egg production and egg mass. When hens were maintained in groups of 4 or 6 and given either 312 or 406 cm^2/bird floor space, the area and group size decreased egg production by 2.2 and 2.6%, respectively (Cunningham, 1988). Over a 10 month period egg production was lower from hens housed 7/cage (75.3%) compared to 5/cage (77.6%) with a 6% increase in feed intake and 9% increase in body weight for hens in the smaller group (Cunningham and Gvaryahu, 1987).

Age can be a modifying influence on the effects of cage density. Increasing the number of hens per cage from 1 to either 3, or 4 had no effect on production at 24-28 weeks of age (Lei *et al*., 1972). In the same study but a different experiment, increasing the number per cage from 1 to either 3 or 5, reduced egg production and feed efficiency at 52-56 weeks of age from hens housed 5 per cage (Lei *et al*., 1972). These changes were not associated with the ability of hens to utilise energy, with lipid metabolism being unaffected by cage density. When two strains of hens selected for differences in

avoidance behaviour (Craig *et al*., 1983) were housed 4 or 8 hens per cage at 348, 464 and 580 cm^2/bird, production over one full cycle was lower at 348 cm^2/birds. There were no differences between hens at 464 and 580 cm^2/bird (Okpokho *et al*., 1987). Also, there was a higher percentage of second quality eggs at the highest density. There were no effects on production or body weight although degree of nervousness was higher in the 8 bird cages even though the strains differed in level of nervousness because of initial selection (Okpokho *et al*., 1987). This study suggests group size (<8) has very limited effect on production so long as the space available is somewhere between 348 and 464 cm^2/bird. Craig *et al*., (1986b) found no association between fearfulness of hens at high cage density and other indicators of wellbeing and production. Using a number of measures of stress, the level of stress was reported to be higher in 2 compared to 1-bird cages (Barnett *et al*., 1994; Barnett *et al*., 1997a).

Taken together these studies suggest that space only becomes a factor in limiting egg production if it is less than some critical value. As a prediction this value seems to be somewhere around 350-380 cm^2/bird. This critical space allocation would be influenced by hen size and so varies for individuals or strains of laying hens.

1.9.3. Space, group size, cage density and feed intake

As density increased by changing the number of hens maintained in a fixed cage size, feed intake and body weight decreased and feed conversion efficiency was poorer in pullets (Patterson and Siegel, 1998). These observations were compounded by differences in feeder and waterer space availability as density increased. In the study, body weight increased as the space available increased from 196 cm^2 to 372 cm^2/bird. Increasing the space available for pullets from 221 to 304 cm^2/bird had no effect on feed intake or body weight when the feeder and watering space available per hen was held constant (Anderson and Adams, 1992). Increasing the space available to pullets to 582 cm^2 increased feed intake but not body weight because as suggested by the authors, the increased energy intake was used to support the increased general activity as a result of the additional space available to the pullets (Leeson and Summers, 1984). Increasing the cage density decreased food intake (Lee and Craig, 1991) and feed conversion efficiency (Roush *et al*., 1984) while no effects on either measure were recorded by Iscan *et al*., (1998).

As group size was increased, feed intake decreased (Quart and Adams, 1982; Cunningham and Gvaryahu, 1987). However like many studies the effects are confounded by space being modified as the group size increased as well as feeder space being compromised. For two strains of hens, housed in groups of 6, 8, 12 or 24 with the floor space maintained at 364 cm^2/bird and feeder space at 10 cm/bird, there were no effects on feed conversion efficiency. However, the smaller groups consumed less feed that again could be the result of a lower activity level in the smaller groups (Carey and Kuo, 1995).

1.9.4. Space, group size, cage density and corticosterone

Siegel (1959; 1960) reported increased adrenal weight for hens when the population density increased. Adrenal weight increased and bursa weight decreased in birds housed at 360 cm^2 compared to 920 cm^2 (Siegel, 1960).

A number of reports indicate that there is a positive relationship between cage density and plasma corticosterone concentrations (Davami *et al*., 1984; Craig *et al*., 1986). Cheng *et al*., (2003) used two lines of hens selected for high (HGPS) or low (LGPS) group productivity and survivability in multi-bird cages, to investigated the adrenal and plasma dopamine responses to a change in group size and space allowance. Hens from the HGPS and LGPS lines were housed singularly at 1084 cm^2/bird or as groups of 10 at 542 cm^2/bird. Plasma corticosterone was not different between lines, however the levels were significantly lower for hens housed singularly compared to hens housed in groups of 10. Adrenal weights were higher in the HGPS line without group size having any effect. While the

adrenal weight was lower in the LGPS lines there was a significant increase in the weight when birds were housed as groups of 10. Adrenal hypertrophy has been used as an indicator of stress (Siegel, 1960). The heavier adrenal weight of the HGPS hens is probably due to hereditary hypertrophy and thought to be associated with their increased productivity and survivability (Hester *et al*., 1996b; Cheng *et al*., 2003). It is also suggested that this adaptation allowed these hens to respond more readily to stressors (Hester *et al*., 1996 b, c; Cheng *et al.*, 2001b). Adrenal hypertrophy in the LGPS hens housed as groups is a stress-induced response and this kind of change has been used to indicate animals that are stressed. The dopamine levels were higher in the LGPS line, however for both lines it was higher in birds housed individually than as groups. This is probably associated with isolation stress or increased activity in single pens (Kehoe *et al*., 1996). It is clear that in any population of hens there is a range in the responses to various stressors.

Decreasing the area per bird increases corticosterone (Lei *et al*., 1972; Mashaly *et al*., 1984) as does a decrease in personal space (Compton *et al*., 1981). Mashaly and colleagues (1984) reported that the compounding effects of group size and cage density resulted in corticosterone increasing from 1.38 ng/ml (3 hens at 516 cm^2) to 1.65 cm^2 (4 hens at 387 cm^2) and 2.32 ng/ml (5 hens at 317 cm^2). The difference for hens at 3 and 4 per cage compared to 5 hens per cage was significant. Increasing the number of hens per cage from 1 (1394 cm^2/bird) to either 2 (696 cm^2/bird), 3 (465 cm^2/bird) or 4 (349 cm^2/bird) in one experiment and from 1 to either 3 or 5 (279 cm^2/bird) in a second experiment, resulted in elevated plasma corticosterone concentrations with large differences seen when hens were housed 5 per pen (Lei *et al*., 1972). The difference in plasma corticosterone concentration when the space per hen went from 696 cm^2/bird to 465 cm^2/bird was 0.5 ng/ml, from 465 cm^2/bird to 347 cm^2/bird was 4 ng/ml and from 465 cm^2 to 279 cm^2/bird was 6 ng/ml.

Hens maintained in groups of 6 and provided with a floor space of 361 or 482 cm^2/bird showed no differences in plasma corticosterone at various sampling times during a full production cycle or during a moult induced at the end of the production cycle (Davis *et al*., 2000). Moving hens and housing them individually in pens with a space of 2900 cm^2/bird or in cages at 949 cm^2/bird or as groups of 4 (464 cm^2/bird) or 6 (310 cm^2/bird) in cages had various effects on plasma corticosterone (Craig *et al*., 1986). In all treatments the plasma corticosterone levels were elevated after moving especially for the first 5 days but had declined by 2-3 weeks. Hens housed in floor pens and six bird cages had plasma corticosterone concentrations 12% higher than did hens housed in cages individually or 4 birds per cage. Although not significant, these differences tended to persist throughout the 22 weeks of the study. Housing hens in cages, singularly at 1394 cm^2/bird, at low density 2 hens at 1394 cm^2/bird or high density 2 hens at 679 cm^2/bird had no effect on plasma corticosterone or H/L ratio (Mench *et al*., 1986). Housing 1, 2 or 3 hens in small cages at 1160 (S1), 580 (S2) or 390 (S3) cm^2/bird respectively or 3 (L3), 4 (L4), 5 (L5) or 6 (L6) hens in large cages at 770, 580, 460 and 390 cm^2/bird respectively resulted in no differences in plasma corticosterone (Koelkebeck and Cain, 1984). In this study, there are direct comparisons (S2 Vs L4 and S3 Vs L6), which indicate that changing the group size has no effect on plasma corticosterone concentration. No effects of cage density on T3 and T4 concentration were found by Davis *et al*., (2000).

As an observation, it would seem from the available data an area somewhere between 317 and 287 cm^2/bird might be the lower limit before available space effects on plasma corticosterone concentrations are observed. Small and moderate group sizes do not seem to affect plasma corticosterone concentration so long as the area per hen is adequate. This might not be the case when the group size is large, where social stress might be influential.

1.9.5. Space, group size, cage density and immunological function

Cage density had no effect on the antibody response to sheep red blood cells (SRBC) (Patterson and Siegel, 1998; Hester *et al*., 1996a). As pullets age the response to SRBC immunisation increased (Patterson and Siegel, 1998). In this same study, cage density had no effect on heterophil and lymphocytes numbers and consequently the H/L ratio, even though there were changes in feed intake and body weight. This is supported by Davis *et al*., (2000) but not be Hester *et al*., (1996a) who found that as density increased, the H/L ratio was higher.

1.9.6. Space, group size, cage density, morphology and mortality

One advantage of maintaining a small group size in conventional cages is the low level of cannibalism experienced (Abrahamasson and Tauson, 1995). As the group size increases the incidence of cannibalism also increases (Bell, 1995). In some studies mortality increased as the cage density increased (Adams and Craig, 1985; Moinard *et al*., 1998), in others no effect is recorded (Muir, 1996; Abrahamsson and Tauson, 1997, 1998). There were no effects on mortality when two strains of hens were housed in groups of 6, 8, 12 or 24 with the space maintained at 364 cm^2/bird and feeder space at 10 cm/bird (Carey and Kuo, 1995). When two strains of hens selected for differences in avoidance behaviour (Craig *et al*., 1983) were housed 4 or 8 hens per cage, at 348, 464 and 580 cm^2/bird, mortality over one full cycle was higher at 348 cm^2/bird with no differences between hens at 464 and 580 cm^2/bird (Okpokho *et al*., 1987).

When group sizes were 4, 8, 14 and 28 and cage space was 2882, 1442, 824 and 412 cm^2/bird respectively, aggressive acts tended to increased in a curvilinear manner (Al-Rawi and Craig, 1975). There were more aggressive acts when the space was 824 cm^2 than 412 cm^2 and 2882 cm^2. Possible reasons for these observations are that at 412 cm^2/bird space may limit the incidence of attacks while at 2882 cm^2/bird there may be sufficient space so that personal space is not invaded as frequently or there is less competition for resources. The compounding effects of space and group size make a definitive explanation difficult. In a further study, when the group size was 4, 8 or 14 hens all maintained at a fixed area of 412 cm^2/bird, there were more aggressive acts in the 8 and 14 bird cages (Al-Rawi *et al*., 1976).

1.10. Furnished cages

In developed countries over 90% of egg production is obtained from hens maintained in conventional cages (Bell, 1995). Interest in furnished cages followed from concerns that conventional cages failed to provide hens with the opportunity to perform certain behaviours. The support for furnished cages largely derives from the failure of conventional cages to meet aspects of the 'Five Freedoms' (Appleby, 1993b; Baxter 1994; Tauson 1995). In the EU, conventional cages are to be replaced with furnished cages by 2013. In the UK, it has been estimated that it will cost around 400 million pounds to comply with the EU directive to phase out the conventional cages presently used. With the industry yearly profit being about 10 million pounds realising this type of investment may be difficult (Hutton, 2004).

In an effort to improve welfare, conventional cages have been modified in various ways (Tauson, 1986; Appleby and Hughes, 1990; Appelby and Smith, 1991; Duncan *et al*., 1992; Nicol, 1992; Sherwin and Nicol, 1992; Hughes *et al*., 1993; Sherwin, 1993; Appleby, 1993a, b; Flemming *et al*., 1994; Abrahamssom *et al*., 1995; Appleby and Hughes, 1995). Limiting the floor slope has greatly improved foot condition, while changing the cage front design has limited trapping accidents and skeletal damage during depopulation. Adding solid sides to conventional cages decreased mortality

(Tauson, 1984b) or increased mortality (Ramos *et al*., 1986; Glatz and Barnett, 1996) especially in hot weather (Glatz and Barnett, 1996). Providing solid partitions between cages had no effect on body weight, feed conversion efficiency or egg production (Cunningham and Gvaryahu, 1987). While Ramos *et al*., (1986) found no effects on egg quality, food conversion efficiency or feather condition there was an increase in body weight. There is a significant level of aggression between hens in adjacent cages with wire partitions (Hughes, 1980; Cunningham and Gvaryahu, 1987). Solid sides in cages had no effect on the number of aggressive interactions between neighbours as these occurred at the feed trough (Cunningham and Gvaryahu, 1987). Barnett *et al*. (1997a) investigated different aspects of hen welfare in conventional cages with solid or wire sides and compared these with hens in a floor pen under Australian conditions. Plasma corticosterone concentrations and H/L ratio were lower for hens housed in cages with solid sides compared to wire sides or the floor pen. Feather condition was better in cages with solid sides and was similar to that seen in the floor pen. Body weight was lower for hens from cages with solid sides compared to wire sides.

Conventional cages fail to provide a nest box, dust bath and perch. Nesting, dust bathing and perching evolved as components of a hen's survival mechanisms in the wild. An essential feature of some research has been efforts to identify how important the maintenance of these behaviours are to hens following their domestication and while being maintained in an environment that modifies the factors influencing survival. Much research is based on the principle of determining preference levels of hens for various cage modifications. Operant and thwarting tests, where a hen is prevented from performing certain behaviours have also been used (Duncan and Wood-Gush, 1972; Keeling, 2004). Other approaches to assessing the impact of these features on hen welfare are limiting. Freedom of movement does not seem to be improved by cage design with group size and probably most importantly space/bird being the more important considerations (Appleby *et al*., 2002).

1.10.1. Nest Boxes

Nesting is thought to be controlled essentially by internal cues (Wood-Gush, 1975). In the wild hens display nesting behaviour and this trait seems to be present even in highly selected hybrid lines, however the level of motivation for nesting seems to vary widely between lines (Cooper and Appleby, 1996; Freire *et al*., 1996; Zimmerman *et al*., 2000). Duncan (1992) has ranked the absence of a nest box in conventional cages as the largest welfare issue for hens in this production system. Hens will work hard to facilitate an environment that allows nesting behaviour (Follensbee *et al*., 1992). Failure to provide a nest has been considered to cause frustration in hens (Duncan, 1970; Brantas, 1980; Kite, 1985; Mills and Wood-Gush, 1982; 1985; review: Appleby *et al*., 1992b; Yue and Duncan, 2003). In a study by Yue and Duncan (2003), hens were housed 4 per cage with a floor space of 625 cm^2/bird. Cages were provide with a nest or no nest and others with a nest which birds had access to for 3 weeks and then denied access during the fourth week of each month of a full production cycle. Hens provided with a nest showed significantly less pre-laying pacing than the other treatments. Restricting the availability of the nest increased pre-laying pacing to the same level as seen in hens without a nest. In this study around 30% of the hens provided with a nest did not use them. In this study it would have been of interest to know if these hens had the same pre-laying behaviour as the hens which actually used the nests.

As well as a genetic component, early experience probably influences the extent to which nests will be used (Faure and Jones, 2004). When provided, most of the eggs are laid in the nest box (Abrahamsson and Tauson, 1997). Although there are some hens that fail to use nests for laying when available (Sherwin and Nicol, 1993; Abrahamsson and Tauson, 1998; Barnett and Cronin, 2004). However, this may depend on the nest box being suitable. Hollow nests were reported to be suitable by Sherwin and Nicol, (1993) but unsuitable by Applelby *et al*., (2002) who found that only 42% of hens used them and the eggs were paler which suggested that oviposition may have been delayed. Some form of enclosure seems to be an important component of nest use (Appleby and McRae, 1986**;** Appleby and Smith, 1991**;** Walker and Hughes, 1998).

Major problems associated with nests are the laying of eggs outside the nest, higher incidence of cracked eggs and dirty eggs, roosting in nest boxes and dust bathing if nest material is available. In furnished cages some hens spend a lot of time in the nests (Appleby *et al*., 2002). This is possibly an avoidance strategy to being victimised. For hens this may alleviate an otherwise stressful environment and allow the hen to maintain near normal plasma corticosterone concentrations.

1.10.2. Dustbathing

Selection programs in quail show that dustbathing is under low genetic control (Gerken, 1991). Hens do not seem to be highly motivated to use a dust bath (Petherick *et al*.., 1993; 1995). Neither is there a strong motivation to access litter (Faure and Lagadic, 1994). The effect of providing litter on feather pecking is unclear with some research suggesting it decreased the incidence (Norgaard-Nielsen *et al*., 1993; Rudkin, 1996; Sanotra *et al*., 1995) and another, that it increased the incidence (Sanotra *et al*., 1995).

Vacuum dustbathing could indicate a high degree of motivational frustration (Appleby *et al*., 1992b) to the absence of a dustbath. Bathing behavior can take place on cage floors (Lingberg and Nicol, 1997; Appleby et al., 2002). In fact a high percentage of hens may prefer this (Abrahamsson *et al*., 1996; Abrahamsson and Tauson, 1997) and therefore cage space might be more important than the presence of a dust bath**.** A recent AECL funded project found that dustathing did not take place if a perch was not present (Barnett and Cronin, 2004). Dust baths are difficult to manage (Appleby *et al*., 1998; Appleby *et al*., 2002). A litter area consisting of artificial turf with a sprinkling of sand is used as much as a dust bath with sand (Appleby *et al*., 2002). When provided, hens will use a dust bath, however this does not seem to be an essential behaviour in light of recent work. Differences between individuals in dustbathing activity could simply be variations in the response to novelty (Jones and Hocking, 1999).

1.10.3. Perches

Strain differences in the use of a perch, are reported by Faure and Jones (1982). Also, individual variations in the extent of perch use have been reported (Lambi and Scott, 1998). Hens are motivated to roost as daylight decreases (Kent *et al*., 1997; Olson and Keeling 2002). A high percentage (91 %) of hens will use a perch at night (Abrahasson and Tauson, 1997). When placed in conventional cages, hens spent 46.5% of the time they were being observed on the perch (Barnett *et al*., 1997b). Presence of a perch increases the hens behavioural repertoire (Hughes and Appleby, 1989; Duncan *et al*., 1992; Abrahamsson and Tauson, 1993; Abrahamsson and Tauson, 1997) and reduces feed intake (Braastad, 1990; Tauson and Abrahamsson, 1994).

It is clear that the presence of a perch increases bone strength (Hughes and Appleby, 1989; Knowles and Broom, 1990; Gregory *et al*., 1991; Duncan *et al*., 1992; Appleby *et al*., 1992a; Hughes *et al*., 1993; Wilson *et al*., 1993; Alvery and Tucker, 1994; Flemming, *et al*., 1994; Abrahamssom, 1997; Barnett *et al*., 1997b; Barnett and Cronin, 2004) however, alone this will not prevent bone breakages during depopulation if hens are not handled correctly (Gregory and Wilkins, 1989; Gregory *et al*., 1992).

When provided with perches, hens prefer the highest perches (Newberry *et al*., 2001). Oval or rectangular perches are preferred to round perches (Robertson *et al*., 1989; Lambie and Scott, 1998) and oval perches decrease the incidence of bumble-foot (Oester, 1994). Tauson, (1984a) reported a 2% increase in production when a perch was provided, however others found no improvement (Robertson *et al*., 1989; Duncan *et al*., 1992). Disadvantages of having perches are increased incidence of dirty and cracked eggs (Tauson, 1984a; Appleby, *et al*., 1992a; Glatz and Barnett, 1996). Some of these problems are created by hens laying while standing on a perch (Duncan *et al*., 1992; Appleby *et al*., 1998; Barnett and Cronin, 2004). There is evidence that perches increase deformation of the sternum (Appleby *et al*., 1993). In 1 or 2 bird cages providing a perch had no effect on plasma corticosterone, H/L ration or body weight (Barnett *et al*., 1997b).

1.10.4. Welfare of hens in furnished cages

Production, morphology and behaviour are criteria that are often used to assess welfare of laying hens in furnished cages. In a 3 year study, the effects of cage furniture on production criteria was investigated by Appleby *et al*., (2002). Hens were maintained in conventional cages at 4 (625 cm^2/bird) or 5 (500 cm^2/bird) hens per cage. These were compared with other treatments consisting of having 5 (750 cm^2/bird), 6 (715 cm^2/bird) and 8 (625 cm^2/bird) hens in cages having a front rollaway nest, dust bath and perch or the same group sizes in cages having only a nest and perch. A further treatment had 4 (875 cm2), 5 (700 cm2) and 7 (500 cm2) hens per cage with a nest having two hollow nests. In years one and three of the study, cage design had no effect on total egg production, mortality or body weight. In year two, there were some differences, with nest type (front rollaway 85% and hollow nest 88%) and perch number (one perch 89%, two perches 85%) having an influence on production. In general, for all years egg quality (dirty, cracked) was inferior from furnished compared to conventional cages. Hollow nests were inferior to rollaway nest with a lower percentage (42% vs 82% in year 1) of hens using them and the incidence of cracked and dirty eggs greater. Even when present about 20% of hens did not use the nest and this suggests that either some hens find no need for a nest or that the design is not suitable to meet the hens needs. Group size had an effect on production with levels being higher in pens having fewer birds. This ranged from 84.9% (5 birds) to 81.0% (7 birds) in year 1 and 87% (4 birds) to 84% (8 birds) in year 2. In year 2 mortality was higher as hen number per cage increased. Generally, in the study, as the hen number per pen increased, feed intake per bird tended to decrease. While these effects are reported according to group size they are results which are compounded by the fact that as group sized increased the space available to hens also decreased. Because of these compounding effects it remains unclear as to how significant group size and space allowance are in relation to welfare. Feather and foot damage were less in the furnished cages.

Comparisons between conventional and furnished cages when the group size is increased were reported by Abrahamsson and Tauson (1997). The group size in the conventional cage was set at 4 hens with a space of 600 cm^2/bird and in the furnished cages varied from 4, 5, 6, or 8 hens per cage with a fixed space allowance of 600 cm^2/bird. Egg production ranged from 82.7-84.8% without cage type or group size in furnished cages having any effect. Similarly, there were no differences in egg mass/hen. There was a significantly lower percentage of cracked eggs in the conventional cages, however there were more dirty eggs from these cages. There were no differences in feed consumption, feed conversion efficiency, body weight or tibia bone strength. In furnished cages group size had no effects on mortality, percentage of hens using the nests and perches and level of feather pecking. This suggests that a moderate increase in group size in furnished cages will not adversely affect hen welfare.

Egg production has been reported to be similar (Abrahamsson *et al*., 1995; Abrahamsson and Tauson, 1997, 1998; Appleby *et al*., 2002) or slightly better (Appleby and Hughes, 1995) from furnished cages compared to conventional cages. There is an increased level of cracked eggs (Abrahamsson and Tauson, 1997, 1998) and less (Abrahamsson and Tauson, 1997, 1998) or similar (Tauson, 2002) level of dirty eggs. The number of cracked eggs largely depends on how far the eggs need to roll out of the cage (Tauson, 2002). Production levels were recorded to be higher in furnished cages than a floor based system (Tauson and Holm, 2001).

The presence of furnished features in a cage may result in synchronization of behaviours. The level of a particular behaviour being performed by a hen can be facilitated by others undertaking the behaviour. This social facilitation and synchronization could cause problems if the cage features are inadequate to meet the demand. Olsson *et al* (2002) reported that dust bathing does not seem to be socially facilitated. In furnished cages some hens spend a lot of time in the nests (Appleby *et al*., 2002). This is possibly an avoidance strategy to being victimised and may alleviate an otherwise stressful situation.

Costs of production are 10-20% higher in furnished cages than conventional cages (Elson, 1994). Mortality is comparable to conventional cages (Tauson, 2000). Higher amounts of feed use are reported for hens in furnished cages compared to conventional cages (Appleby *et al*., 2002) but no difference in feed conversion efficiency (Tauson and Abrahamsson, 1996). Keel bone deformation and incidence of bumble foot can be higher in furnished cages (Tauson and Abrahamsson, 1994; Abrahamsson *et al*., 1996), however feather condition and body condition can be better (Maria *et al*., 2001). A number of workers have reported that in general they consider that hen welfare is improved in furnished cages compared to conventional cages (Baxter, 1994; Tauson and Abrahamsson, 1996; Duncan, 2001; Appleby *et al*., 2002).

1.11. Alternative Housing Systems

In developed countries over 90% of egg prediction is obtained from hens maintained in conventional cages (Bell, 1995). This has resulted from the advantages in production cost ease of management, improved health (parasites and coccidosis) and improved air quality. Some simple changes in conventional cage design have gone some way to improving hen welfare. However, the continued pressure to ban conventional cages in some parts of the world has increased the enthusiasm for alternative housing systems. The furnished cage is considered an alternative in the EU although there is also increased interest in alternative systems to using cages. In some EU countries percheries are commonly used as a housing system and there are reports identifying positive and negative aspects on hen welfare. In Australia alternative production systems are mostly barn or free range systems.

Problems identified with aviary systems included increased labour costs, incidence of floor eggs, management difficulties, high ammonia and dust levels and increased disease risk (Van Emos and Fiks-van Niekerk, 2004). As a general comment higher levels of ammonia and dust make air quality inferior in floor based systems compared to cage systems (Tauson and Holm, 2001). In a survey of Dutch aviary systems with a free range component it was found that there is a substantial possibility of disease problems and increased mortality with producers finding these difficult to correct (Van Emos and Fiks-van Niekerk, 2004).

1.11.1. Alternative systems and egg production

Koelkebeck and Cain (1984) compared production from small cages (1-3 birds), large cages (3-6 birds) cages, pens and a free range system over a 10 month period. Egg production was higher from cages than pens or free range. The low production from floor penned hens could have been related to an outbreak of coccidiosis although production data for all systems over a two month period when the outbreak occurred were deleted from the analysis. This highlights one of the welfare concerns associated with floor based systems, the need to prevent this type of disease outbreak. In floor pens reducing the space allocation from 940 cm^2/bird to 373 cm^2/bird had no effect on egg production. This may lend further support to the concept that if the space allocation is greater than 350-380 cm^2/bird there is no effect of space on egg production.

During weeks 19-25 of age, egg production was lower for hens maintained in floor pens with no foraging straw available compared to pens with straw (El-Lethey *et al*., 2000). In a survey of Dutch aviary systems approximately 25% of farms identified having a production problem with the main complaint being a failure of flocks to reach peak production levels (Van Emos and Fiks-van Niekerk, 2004). Production rate in floor based systems is often a reflection of mortality rates (Tauson, 2002).

Feed intake is normally around 1-4% higher in floor systems compared to cages and egg weights often slightly lower (Elwinger and Tauson, 1999). The increased feed intake could be related to an increase in activity and in some cases the incidence of feather pecking (Peguri and Coon, 1993). In the EU there can be large variation in egg quality with different floor based systems (Abrahamsson

and Tauson, 1995). There can be a large percentage of floor eggs because of variation in the percentage of hens that will use the nests (Tauson and Holm, 1998).

1.11.2. Alternative systems and morphology

Increased activity is associated with greater bone strength in poultry (Knowles and Broom, 1990). The lack of activity in cages is often viewed as the reason for the high incidence of brittle bones in hens (Moinard *et al*., 1998). Similarly, the increased bone strength of hens housed in floor systems (Gregory and Wilkins, 1989; Gregory *et al*., 1990; Newman and Leeson, 1998) or furnished cages (Abrahamsson and Tauson, 1997; 1998) is because activity is greater. There are strain differences in the level of activity (Savory and Mann, 1997), however the effect of genetics is minor compared to the influence of the housing system (Faure and Jones, 2004).

Asymmetry of the leg bones is greatest in birds that spend large amounts of time on the slats in perchery systems (Moller *et al*., 1995: Yngvesson and Keeling, 2001). Birds spending less time on the slats and therefore more time in the perched areas were found to have a high incidence of keel bone breakage (Freire *et al*., 2003). Therefore, increased freedom to move around the perchery in itself might present a problem for hen welfare. Hens have a problem with jumping downwards and this can cause bone breakage in poorly designed aviary or perchery systems (Moinard *et al*., 2004). Appleby *et al*., (1993) found more keel bone damage for hens in cages than in a floor system.

Failure to beak trim is reported to decrease performance and welfare of hens in non-cage systems (Hughes and Gentle, 1995; Abrahamsson and Tauson, 1995). Feather pecking is a routine behaviour and therefore some level of feather pecking can be expected in all production systems (Appleby and Hughes, 1991). In general, floor systems have more instability as regards to feather pecking (Tauson and Holm, 2001). If excessive, feather pecking can cause welfare problems and even death (Savory, 1995; Huber-Eicher and Wechsler, 1997; 1998). Increased levels of nervousness are associated with increased incidence of feather pecking (Craig *et al*., 1983). Hens with better plumage have been considered to be less stressed (Abrahamsson, *et al*., 1996; El-Lethy *et al*., 2000). A number of husbandry considerations can influence the level of feather pecking and damage associated with it. Increasing cage density increases the degree of feather pecking (Craig *et al*., 1986; Appleby *et al*., 1993; 2002; Barnett *et al*., 1997a). Plumage damaged was significantly greater in pens housing 7 hens compared to those housing 5 hens (Cunningham and Gvaryahu, 1987). When hens from two strains were housed in 6-bird cages at 361 or 482 cm^2/bird, there were no differences in extent of feather pecking (Anderson *et al*., 2004).

In conventional cages the level of aggression towards pen neighbours is comparable to that of cage mates (Cunningham and Tienhoven, 1983). The level of feather pecking decreased in conventional cages fitted with solid sides (Tauson, 1977; 1984b; Barnett *et al*., 1997a). *Ad libitum* feeding decreases the incidence of pecking at inedible objects (Savory and Mann, 1999). The opposite of this, feed deprivation increases the incidence of pecking at inedible objects (Anderson *et al*., 2004). In floor pens feather pecking is lower when hens are provided with forage material (Huber-Eichler and Wechsler, 1997; 1998; El-Lethey *et al*., 2000). In the study by El-Lethey *et al*., (2000), the absence of foraging straw also increased H/L ratio, tonic immobility duration and decreased the humoral response to SRBC and tetanus toxin, all measures considered to be indicators of stress. The authors suggested that the level of feather pecking is associated with the level of stress. In this same study feeding the diet in mash or pelleted form had no effect on the degree of feather pecking.

In perchery systems, feather condition is often poor with the birds spending increased time on the slatted areas having the poorest feather condition, especially on areas associated with aggressive feather pecking such as the back, tail and head (Freire *et al*., 2003). In a line of hens selected for a low incidence of feather pecking, the plasma corticosterone concentration was lower than in a line selected for a high incidence of feather pecking (Korte *et al*., 1997). The high line showed greater behavioural inhibition in an open field test (Jones *et al*., 1995). Some recent studies report that

feather condition is better in floor pens than cages (Taylor and Hurnick, 1994; McAdie, and Keeling, 2000). Under controlled experimental conditions, often the comparisons are made between cages and small flock floor pens. In commercial environments the floor systems are very large and the incidence of feather damage will certainly be influenced by the flock size.

1.11.3. Alternative systems and mortality

Mortality in cage systems has been reported to be lower (Koelkebeck and Cain, 1984), higher (Craig and Craig, 1985; Mench *et al*., 1986) or similar (Mou and Katle, 1990) compared to floor pens. In cage systems the mortality is lowest when the cage density is kept low and group size is small (Koelkebeck and Cain, 1984; Adams and Craig, 1985). Housing hens in pens with a space of 2900 cm^2/bird or singularly in cages at 949 cm^2/bird or groups of 4 (464 cm^2/bird) or 6 (310 cm^2/bird) hens had various effects on mortality (Craig *et al*., 1986). In 6-bird cages mortality was approximately 15% and this was about 5 times greater than for the other treatments. Mortality was 14.3% for hens maintained in cages in groups of 14 at 361 cm^2/bird and 3.5% for hens maintained in groups of 14 in floor pens at 2940 cm^2/bird (Craig and Craig, 1985). In one study, mortality levels were 16% for a free range system and 5.5% in a perchery (Hafez *et al*., 2001). Beak trimming can decrease mortality in floor based systems (Craig and Muir, 1996; Bell *et al*., 1998).

In the survey of Dutch aviary systems with an added outer free range area, mortality levels ranged from 8-28% with the main causes being E. coli infection, metabolic exhaustion, feather pecking and cannibalism (Van Emos and Fiks-van Niekerk, 2004). In an effort to alleviate the problem many producers vaccinate against E.coli and Salmonella. In aviary systems the incidence of bumble-foot can be greater and hyperkeratosis lower than in cage systems (Tauson and Abrahamsson, 1994). In some aviaries there can be a high percentage of birds found emaciated (Engstom *et al*., 1993).

1.11.4. Alternative systems and immunology

In floor pens, hens provided with straw forage material had higher humoral responses to SRBC and tetanus toxin (El-Lethey *et al*., 2000). In the same study feeding the diet as a pellet or mash had no effect on the immunological response. In a comparison of hens in floor pens and cages the antibody responses were reduced for hens in the floor pen (Erhard *et al*., 2000). Feeding the diet in pellet or mash for had no effect on the H/L ratio (El-Lethey *et al*., 2000). Different light regimes had an influence on the H/L ratio (Garcia-Davila and Campo, 2001).

1.11.5. Alternative Systems and Behaviour

During the production cycle the behaviour patterns of the hen relate to adjustments being made for the environment, social interactions and age. These changes in behaviour are related to the physiological needs of the hen at the different stages of the production cycle with age seemingly having a major influence on behaviour and levels of fear (Anderson *et al*., 2004).

Caged birds show less locomotor activity (Black and Hughes, 1974; Koelkebeck and Cain, 1984; Mench *et al*., 1986) than penned hens. Mench *et al*., (1986) found no difference in the level of maintenance behaviour between hens housed in cages or pens. In this study, the minimum space allowance was 697 cm^2 and so it would appear that this is sufficient to ensure maintenance behaviour in low density cages (2 hens per cage). This may change as the number of hens in the cage increases.

There is a strong correlation between the mean number of aggressive acts per pen and egg production (Al-Rawi *et al*., 1976; Cunningham, 1988). Chickens and hens seem to be able to establish hierarchies after no more than three interactions (Chase, 1985; Litten and Cockrem, 2001), however this would seem to depend on the size of the group. In another study, based on the level of interaction, Cunningham (1988) considered that the hierarchy had been completely established after

6 weeks of hens being placed into group cages. In this study hens were housed in groups of 4 or 6 and allocated spaces of 316 and 406 cm^2/bird. Hens were observed between 23-26 and 37-40 weeks of age. Aggressive activity decreased with age. At the younger age both a decrease in cage space/bird and an increase in the group size increased significantly the level of aggressive activity whereas at the older age cage space had no effect. The effect of age was also recorded by others (Choundary *et al*., 1972; Choundary and Craig, 1972). The dominant hens were found to be more active in 6 bird-cages than in 4-bird cages (Cunningham 1988). This could indicate that any problem with aggression could be magnified in large group sizes. There is a strong stability in the linear social order in pens and cages (Rushen, 1982; Chase, 1985, Cunningham, 1988). In cage studies the dominant hen initiated 40-50% of all aggressive acts (Cunningham, 1981; Craig and Ramos, 1986). The lowest ranked hen was found to have initiated some events especially at feeding (Cunningham, 1988) although feeding was not found to be a contributing factor in an earlier study (Cunningham, 1981).

Cunningham (1988) commented that in studies of social order it might be important to consider using a social tension index that looked at the level of submission in hens. This is because the number of acts on an individual is the important welfare consideration. Naturally it's not the dominant hens that have a problem. In 6-bird cages the lowest ranked hen is subjected to more attacks than the lowest hen in 4-bird cages (Cunningham, 1988). A White Leghorn line selected for low agonistic behaviour was found to have a lower mortality rate and better production than hens with high agonistic behaviour (Choundary *et al*., 1972), however in a similar type of study no relationship was found (Al-Rawi *et al*., 1976). In a White Leghorn line selected for increased survivability and decreased feather loss in multi-bird cages (Muir, 1996) hens tended to react less intensely to a range of stresses (Hester *et al*., 1996 a, b, c).

At 23-26 weeks of age, cage space and group size had an influence on social stress and egg production (Cunningham, 1988). Social order affects plasma corticosterone in free ranging wild birds (Schwabl *et al*., 1988). In poultry, plasma corticosterone was not related to social rank (Cunningham *et al*., 1987; Mench and Ottinger, 1991; Litten and Cockrem, 2001). This may simply relate to the fact that hens accept their position in the hierarchy once the order is established. When individually housed hens were moved to group pens for 4h/day there was a negative correlation between social rank and adrenal weight but no such relationship was found in hens maintained in groups continuously (Siegel, 1971). In the study of Litten and Cockrem (2001), position in the social rank was established by pairwise comparisons of hens and the response to handling determined. While the response between birds varied significantly (0.42- 6.0 ng/ml before handling and 1.12-19.6 ng/ml 25 min after handling) there was no relationship to position in the social ranking. These kinds of studies indicate that the lowest hens in the order are not more stressed by adverse stimuli but they don't really measure whether the lowest ranked hens are subjected to more or less stress when maintained in group environments.

1.11.6. Alternative housing and corticosterone

Of three different housing systems, cages, free-range and strawyards; hens in strawyards had significantly lower circulating plasma corticosterone concentrations than hens in cages or free range (1.43 ± 0.37, 0.93 ± 0.24 and 0.32 ± 0.09 ng/ml respectively). The caged hens tended to have higher corticosterone concentrations than free range hens (Gibson *et al*., 1986). In the same study, levels of T4 were not affected by the housing system but T3 levels were significantly lower for the free-range hens compared to the caged hens. The T3 levels may be a response to temperature differences in this study (range 3^0C in strawyards and free range to 20.7^0C in cages) with T3 being a regulator of metabolic activity in hens (Klandorf *et al*., 1981). Contrary to this, Koelkebeck and Cain (1984) found that corticosterone levels were lower in hens maintained in cages (0.79 ng/ml) compared to floor pens (1.72 ng/ml) and a free range system (0.95 ng/ml). Other studies, support there being lower plasma corticosterone concentrations for hens housed in cages compared to floor pens (Edens *et al*., 1982; Craig *et al*., 1985: Barnett *et al*., 1997a). Others report no difference (Barnett and Bartlett 1981; Craig and Craig, 1985) and one a higher concentration (Gibson *et al*., 1986).

Koelkebeck and Cain (1984) compared production from small (1 to 3 birds) cages, large (3 to 6 birds) cages, pens and a free range system over a 10 month period. Plasma corticosterone concentration was significantly higher in floor pens than in cages or free range. Also, the concentration was higher as the density decreased from 3725 (approximately 1.2 ng/ml) to 1870 (approximately 1.7 ng/ml) and 940 cm^2 per hen (approximately 2.2 ng/ml), with the difference between 3725 and 940 cm^2 per hen being significant.

Lines of hens selected for either a high level or low level of feather pecking (Blokhuis and Beutler, 1992). At 52 weeks of age individually caged hens from both lines were subjected to manual restraint for 8 min and the catecholamine and corticosterone responses determined (Korte *et al*., 1997). The high feather pecking line had higher noradrenaline concentrations but adrenaline concentrations were similar. Both lines had elevated corticosterone concentrations following restraint with the concentration being significantly greater in the low feather pecking line (approximately: 4 vs 7 ng/ml at 8 min).

During a full production cycle and subsequent moult, cage density (361 and 482 cm^2/bird) had no effect on plasma corticosterone concentration, H/L ratio, T3 or T4 concentrations (Davis *et al*., 2000). However, the hormone concentrations varied through the production cycle and the changes were related to the metabolic demands associated with physiological stress. For hens maintained in cages as groups of 14 at 360 cm^2/bird or floor pens of 14 hens at 2490 cm^2/bird there were no differences in basal corticosterone or ACTH initiated corticosterone response (Craig and Craig. 1985). Barnett and colleagues (1997a) found that the corticosterone response to ACTH was larger for hens from floor pens compared to hens from cages.

1.12. Human interactions

In modern cage production systems, hens are housed as groups, in multi-tiered multi-rowed cage designs. The position of the cage in the poultry shed can influence the level of fearfulness displayed by hens. Hens housed in the top tier show greater avoidance of approaching humans compared to hens in the bottom tier (Hemsworth and Barnett, 1989). Jones and colleagues (1985a) reported that hens in the top rows of cages were more fearful of a novel object. This was also observed by Hemsworth and Barnett (1989), however this only occurred in the early stages of their study and unlike Jones (1985a, b) they found being in the top tier had no effect on tonic immobility duration. Hens that withdrew and failed to look forward when an observer stood in front of the cage had the highest plasma corticosterone concentrations when handled during blood sampling. There was also a correlation between both egg production and avoidance of humans with hens less fearful of humans having the best production (Hemsworth and Barnett, 1989). These workers also found a significant negative correlation between production and the level of corticosterone response to handling. Handling has been shown to reduce egg production (Hughes and Black, 1976). Hens housed in the pens located in the areas most frequented by stockpersons are least fearful, suggesting that familiarity with humans decreased fearfulness (Hemsworth and Barnett, 1989).

1.13. Abnormal egg formation

Stressors imposed on hens can result in delayed oviposition (Carter, 1977: Hughes and Gilbert, 1984; Hughes *et al*., 1986; Mills *et al*., 1991; Reynard and Savory, 1999). These delays often result in eggs with abnormal shells. The type of abnormality seems to depend on the time of the delay with the minimum oviposition delay required to produce an abnormality seeming to be around 1.35h (Hughes *et al*., 1986). The abnormally can be alterations to the shell, absence of a shell, decreased shell thickness, modification of the normal egg shape (misshapen, slabsided and equatorial bulges) and various levels of calcium deposited on the outer cuticle (Watt and Solomon, 1988). Pale eggs are

often related to delayed oviposition (Walker and Hughes, 1998). The effects on shell whitening depend on when the stress is imposed (Reynard and Savory, 1999). The frequency of eggs with extra calcium on the outer surface is greatest at peak production and this was found to diminish with age (Mills *et al*., 1991; Yue and Duncan, 2003). The amount of extra calcium deposited during the reproductive life of a hen has negligible effects on skeletal integrity (Yue and Duncan, 2003).

Moving hens housed as groups of 4, 3 or 2 in pens to cages caused a delay in oviposition with some hens retaining the egg overnight (Hughes *et al*., 1986). Over the first 6-8 days after moving, egg production decreased from around 90% to 50% and then gradually increased after this. A higher percentage of the eggs laid were abnormal, especially during the first 6 days after the move. Crating and wing banding of hens increased the number of abnormal eggs laid and interestingly, the number of abnormal eggs laid by hens in a pen adjacent to the wing banded hens increased, even though they themselves were not handled (Hughes *et al.*, 1986). This is of interest because it could suggest that a disturbance in any part of a production shed could influence hens in another part of the shed. When hens, which are trained to lay in nest boxes, are prevented from doing so, the percentage of normal eggs laid decreased from 90% to 42 % in the first 24h (Hughes *et al.*, 1986).

It was predicted that adrenalin affected uterine motility and shell gland contraction resulting in poor shell quality and other egg deformities (Hughes and Black, 1976). Administration of exogenous adrenalin increases the incidence of abnormal eggs laid. Adrenalin (0.1-0.25 mg) given as s/c injection produced a dose-dependent increase in the number of abnormal eggs laid (Hughes *et al.*, 1986). Similarly the injection of ACTH causes a dose-dependent increase in number of abnormal eggs laid (Flickinger, 1966). Corticosterone plays a role in the ovulatory cycle of the hen with the plasma concentration peaking around 44 min before oviposition (Beuving and Vonder, 1977). There is evidence indicating that corticosterone can also affect oviposition time and the incidence of abnormal eggs. In hens 66-74 weeks of age, plasma corticosterone concentration was higher (approximately 1.0 ng/ml) in hens laying soft-shelled or membranous eggs in the afternoon compared to hens laying normal hard shelled eggs (Klingensmith *et al*., 1984). However, the changes in plasma corticosterone seem to depend on when oviposition occurred, with hens laying in the morning showing no difference in plasma corticosterone. The blood samples were taken around the time of oviposition and the result is interesting, because it indicates that differences in plasma corticosterone can be observed at the time of the oviposition peak in corticosterone. It has been considered that measuring stress-induced changes in plasma corticosterone is difficult at this time because of the diurnal peak in corticosterone related to oviposition. In the study by Klingensmith and colleagues (1984) no difference in adrenal cholesterol or corticosterone concentrations were found for hens laying soft-shelled or membranous eggs and this is similar to results of Wilson *et al*., (1981).

For egg abnormalities to occur, it appears that the stress needs to be imposed during the time of oviposition (Reynard and Savory, 1999). In this study, hens were subjected to a 6h period of relocation stress and the effects on oviposition time and egg quality determined. For those hens failing to lay during the period of stress oviposition occurred soon after the stress ceased, if the delay from their normal oviposition time was less than 2.4h. Generally if the delay was > 2.4 h at the time the stress was removed then oviposition time was much longer and these eggs showed the most extensive abnormalities. These authors proposed that there was a critical period of oviposition delay after which the egg will be retained for a long period and at the subsequent oviposition an abnormal egg is laid. The critical period of delay after which an egg is retained for a lengthy period ranged from 1.5-4.3h for individual hens. The authors make a very feasible argument that once the delay is greater than the critical time, oviposition delay is prolonged because the increase in uterine contractions needed for explosion of the egg is disrupted. During stress, elevated catecholamines or corticosterone at a critical period may interfere with the normal uterine function delaying oviposition and the eventual laying of abnormal eggs.

Walker and Hughes (1998) reported various effects on egg quality, when they compared conventional cages and furnished cages. The furnished cages were fitted with either an enclosed nest

box, enclosed nest box and dustbath or a cage with two hollow nests in the floor and hens were maintained at different densities (5 hens at 1000 cm^2/bird and 7 hens at 745 cm^2/bird). In cages with a hollow nest, only 40% of eggs were laid in the nests and the egg shells were paler. Cage density, provision of enclosed nests or dustbath had no effect on shell colour. Hens housed 3 per cage at 600 cm^2/bird or 4 hens at 450 cm^2/bird had no effect on egg shell colour, however the colour was darker for eggs from hens housed individually at 1040 cm^2/bird (Mills *et al*., 1987). In the report from Roland (1978) the percentage of misshapen eggs was related to group size and increased or decreased by changing the number of hens in the cage.

1.14. Genetic Selection

The best welfare for laying hens is most likely to be achieved if the environmental and genetic approaches are integrated (Jones and Hocking, 1999). Selection criteria such as behavioural traits, susceptibility to different stressors, needs and the motivation behind these along with productivity can all influence welfare (Faure and Jones, 2004). Selection in general doesn't act to eliminate a trait but rather modify the threshold before an effect is observed (Faure *et al*., 2003). Criteria that are phenotypically and genetically associated could be useful in breeding programs aimed at improving welfare in production systems. A link between behavioural traits and physiological measures of welfare could be the basis of valuable selection criteria especially in large group production systems. Such an association would need to be predictable and universal to be of any use.

After 6 generations of selection for group production in multi-bird cages the mortality decreased to 8.8% and this compared to 68% in an unselected lines. The mortality rate in the selected line was similar to hens housed in individual cages (9.1%) (Muir, 1996). The selection for group production alleviated the need to beak trim hens. When hens were housed 12 birds/cage, the group selected line had a mortality rate of 20% and this compared to a rate of 89% in a commercial line selected for individual cage production (Craig and Muir 1996). The selected line also had better feather condition and fewer agonistic interactions (Craig and Muir 1996). In a review of recent work, Muir and Cheng (2004) considered that selection for group productivity and survivability altered the neuroendocrine and immunological systems in the selected hens so that they were more capable of maintaining behaviour and physiological homeostasis in cage environments. Lines of quail selected for long (LT) or short tonic (ST) immobility duration or high (HS) and low corticosterone (LS) response to immobilisation, influenced the way the lines responded to a variety of stressors (Satterlee and Johnson, 1988; Mills and Faure, 1991; Jones *et. al*., 1991a, b). The corticosterone response in the LS line was lower than the HS line when subjected to various stressors (Jones *et. al*., 1991a, b; 2000).

Selection for traits that increase adaptive capability will be of no value unless they are associated with economic traits such as egg production. Selection for a characteristic in one type of housing system may not be of use in another system. For traits associated with improved welfare the need will be to select for these in the particular housing system of interest. Selection for adaptation to multi-bird cage systems clearly indicates that this approach can be successful, however in alternative systems, identification of production from individual hens will be the compelling limitation.

1.15. Fearfulness

Fear is a primary emotion and one of the innate feelings experienced by humans and non-human animals (Leventhal, 2000; Dantzer, 2002). Fearfulness was recognised as an animal state in the Brambell Committee investigation on animal welfare (Brambell *et al.*, 1965). Fear acts to protect animals from danger (Salzen, 1979). It has been described appropriately as the 'adaptive psycho-physiological (emotional) response to real or perceived danger' (Jones, 1996). Responses to fear have been described for poultry (reviews: Duncan, 1985; Jones, 1987c, d). Fear can inhibit all other emotional states (Jones, 1996) and can be expressed as an acute or chronic state and like some other emotions it is inevitable in an animal's life (Fraser, 1993). While fear can't be completely eliminated from a production system, excessive or prolonged states of fear are considered to represent a state of

poor welfare (Jones, 1985c; Jones, 1996). Ways of reducing fearfulness and increasing a hen's adaptability to its environment should be considered as improving welfare.

1.15.1. Fear provoking stimuli

Within in any housing system many factors can interfere with how hens respond to fear, with inappropriate fear responses adversely affecting welfare (Jones, 1996). Two of the most potentially fear-provoking stimuli are sudden changes in the environment (novelty) and exposure to humans (Murphy, 1978; Duncan, 1989; Jones, 1987b; Wiepkema and Koolhaas, 1993; Jones, 1996). Potentially frightening events include; unfamiliar inanimate objects (novel objects) and food, loud noises, visual and physical contact with humans, sudden appearance and movement of animate and inanimate objects, disruption of social environment, predators and transportation (Wood-Gush, 1971; Schiff, 1965; Duncan, 1985; Duncan *et al*., 1986; Cunningham 1988; Duncan, 1989; Mills and Nicol, 1990; Grigor, 1993; Scott *et al*., 1998). Handling is frightening for hens (Scott and Moran. 1992, 1993). The extent and intensity of these events are influenced by the production system with previous experiences and genetic makeup having major influence.

1.15.2. Measuring fearfulness

Several measures may be used to assess fear (Wood-Gush, 1983; Jones, 1996). Tests can be based on, level of nervousness (Anderson and Admas, 1994), latency of feeding (Ramos *et al*., 1986), tonic immobility (Gallup, 1979), measures of avoidance or escape (Craig *et al*., 1986; Griogor, 1993), feather damage (Craig *et al*., 1984) and reactions to a moving object (Cunningham and Ostrander, 1981).

Tonic immobility (TI) is a commonly used measure of fearfulness (Gallup, 1979; Jones, 1996). It is believed to have a genetic basis because selection for TI produces divergent lines differing in TI duration (Mills and Faure, 1991). It is an unlearned response of birds when they are restrained for a short period. It is a state of immobilisation induced by mild restraint in which the bird has reduced responsiveness to external stimuli (Jones, 1996). It is normally induced by placing the bird on it's back in a U- or V-shaped cradle and restraining it for a short period (15 sec) by placing a hand on the breast area. The time taken for the bird to right itself from this state is the duration of tonic immobility. The more frightened a bird is when the state of tonic immobility is induced, the longer will be the time the bird takes to right itself. Catching and holding time before measuring the TI response has no effect on the TI duration (Craig *et al*., 1984). The tonic immobility varies greatly between individuals resulting in very large measures of SD (Beuving *et al*., 1989; Cashman *et al*., 1989). This presents a major problem in experimental studies where group numbers are small and hens are allocated randomly to treatments. When designing experiments it might be more appropriate to measure TI before allocating hens to experimental groups or to select groups of hens with known TI values and then determine what changes occur after various treatments.

Corticosterone infusion increases the TI duration (Jones *et al*., 1988). The TI duration is prolonged in broilers following transportation with the journey length being important (Cashman *et al*., 1989). Manual harvesting increases TI duration compared to mechanical harvesting in broilers (Duncan *et al*., 1986). Feed withdrawal decreases TI duration (Campo and Alvarez, 1991). Handling is frightening for hens (Scott and Moran, 1992; 1993) and the rougher the handling the longer TI duration becomes (Jones, 1992). In floor pens, hens provided with straw foraging material had shorter TI duration than hens without straw (El-Lethey *et al*., 2000). These authors suggest that TI could be a measure of stress because other measures indicated that the hens without straw were stressed. In a commercial broiler line the best and worst producers had similar TI (Skinner and Nobel, 2003). In quail selected for egg production no association was found for this trait and TI (Minvielle *et al*., 2002). In this study there were only small variations in individual TI duration, therefore selection for production may have also selected for a particular range of TI values. In the F2 generation from parent lines of quail selected, one for egg production (Minvielle *et al*., 2000) and the

other for long TI duration (Mills and Faure, 1991), no relationship between TI and egg production was found (Mignon-Grasteau and Minvielle, 2003). Using various tests for fear including TI, there were no significant effects of rearing environment, age or habituation on fear levels (Okpokho and Craig, 1986).

The novel object test measures the degree to which a bird will avoid a novel object placed in the hen's environment. Often a multi-coloured rod is used and placed in the feed trough of the bird's home cage (Craig, *et al*., 1983; Jones, 1996). The amount of avoidance reflects the bird's fearfulness. Tests based on a birds avoidance of humans, activity in an open field or novel environment and its emergence from hole-in-the wall have all been used to assess fearfulness and details of their implementation are described by Jones (1996).

Feather pecking can be genetically manipulated (Kjaer *et al*., 2001). Associations between feather pecking and fear have been examined by a number of workers (Okpokho *et al*., 1987; Jones *et al*., 1995; Keeling and Jensen, 1995; Albentosa *et al*., 2003b). Better feathered hens are considered to be less fearful (Hughes and Duncan, 1972; Quart and Admas, 1982; Okpokho *et al*., 1987; Na-Lampang and Craig, 1990). The TI duration is reported to be related to the extent of feather pecking (Jones *et al*., 1995). In five strains of hens, those with the poorest feather condition were found to have the shortest TI (Campo *et al*., 2001). This suggests that the hens with the worst feather condition were less fearful and this observation disagrees with the previous studies. In the study of Campo and colleagues (2001), hens with the poorest feather condition had a significantly higher H/L ratio even though the increase in heterophil number and decrease in lymphocyte number were separately, not significantly different. When force moulted, hens having the greatest feather loss also have the greatest H/L ratio (Alodan and Mashaly, 1999). It is possible that feather condition could provide a measure of fearfulness and wellbeing.

Tests for fear may measure different aspects of behaviour (Rushen, 2000). Tests used across different genetic lines have produced inconsistent results (Craig *et al*., 1984; Albentosa *et al*., 2003). Strong strain and line differences in TI have been reported (Craig *et al*., 1984; Campo and Alvarez, 1991; Albentosa *et al*., 2003). For individual birds, good correlation between different measures of fear have been reported in some studies (Jones and Mills, 1983**;** Mills and Faure, 1986; Jones *et al*., 1987, 1988, 1991; Gerken and Petersen, 1992). However, a recent report indicates that the relationship between different measures is not strong (Albentosa *et al*., 2003). The tests may not be measuring the same trait with fear being a very situation-dependent variable that is modified by factors such as age. There are reports which indicate that within tests responses are consistent with age (Hocking *et al*., 2001: Mills and Faure, 2001) while others indicate that age influences how hens respond to various tests of fearfulness (Albentosa *et al*., 2003). The TI duration has been shown to increase (Campo and Carnicer, 1993) with age. Using the Hansen test (Hansen, 1976) as the fear measure, level of fear increased (Anderson *et al*., 2004) or decreased (Anderson and Adams, 1992) with age. The level of fear increased during a forced moult (Anderson *et al*., 2004).

1.15.3. Housing systems and fearfulness

Domestication of poultry has resulted in reduced fear, however there are large variations because of strain and individual effects (Faure *et al*., 2003). Birds housed in different production systems have been shown to have different levels of fear (Jones and Faure, 1981; Hansen *et al*., 1993). Hen behaviour changed as the environment was changed, with no adverse effects on production or fear levels (Anderson and Adams, 1991, 1992). Significantly higher levels of fear were recorded at peak production (Anderson and Adams, 1992) than other stages of the production cycle. Increased fearfulness has been associated with decreased production (Craig *et al*., 1983; Bessi, 1984). The TI duration is reported to be shorter (Jones and Faure, 1981; Kujiyat *et al*., 1983) or longer (Anderson and Adams, 1994) in pens than cages. In a cage system no differences in TI was observed for hens in different tiers (Scott *et al*., 1998). At the end of lay, free range hens had a shorter TI time than hens housed in conventional cages (Scott *et al*., 1998).

1.15.4. Space, group size, cage density and fearfulness

Group size, space allowance or density had no effects on fearfulness in a study using floor pens (Lee, 1989). Hens housed 1, (1548 cm^2/bird), 2 (774 cm^2/bird), 3 (516 cm^2/bird), or 4 (387 cm^2/bird) hens per cage showed no differences in fearfulness when tested for TI at 29, 36 and 52 weeks of age (Lee and Moss, 1995). Removing or adding a hen to some groups similarly had no effect on TI times. Using the Hansen test for fear (Hansen, 1976), the level of fearfulness was not affected by the cage density (361 or 482 cm^2/bird) (Anderson *et al*., 2004).

1.15.5. Fear, corticosterone and catecholamines

Frightening stimuli result in the release of catecholamines (Dantzer and Mormede, 1983; Harvey *et al*., 1984) and corticosterone (Beuving, 1980; Harvey *et al*., 1984). Fear is associated with high adrenalin response (Henry, 1986). In a White Leghorn strain fitted with an indwelling catheter, an experimenter in novel clothing elicited an increase in adrenalin and noradrenalin within 1 min (Beuving and Blokhius, 1997). In the same study manual restraint had a similar effect although the responses were greater. These workers also found a negative correlation between adrenalin response and the TI duration.

High corticosterone release is associated with behaviours that indicate fear in some species (Dantzer *et al*., 1980; Katz *et al*., 1981; Harvey *et al*., 1984: Jones *et al*., 1988; Jones, 1990). As measures of fear increase so do plasma corticosterone concentration and H/L ratio (Davis *et al*., 2000). Chronic elevation of corticosterone increases fearfulness that is likely then to increase corticosterone release, initiating a cycle which can have adverse effects on welfare (Jones *et al*., 1988). Chronically elevated corticosterone is associated with long TI (Jones *et al*., 1988). Lines of quail selected for long (LT) or short tonic (ST) immobility duration or high (HS) and low corticosterone (LS) response to immobilisation had modified responses to a variety of stressors (Mills and Faure, 1991). Using mini-osmotic pumps implanted subcutaneously to deliver corticosterone at 15 ug/h significantly increased plasma corticosterone concentration in hens from 0.15-0.48 ng/ml to 1.1-2.9 ng/ml. The increase in plasma corticosterone significantly increased the TI duration and H/L ratio (Jones *et al*., 1988). This gives strong support to the proposal that chronic HPA activity and fear are related in laying hens. Chickens selected for high activity in a novel environment were found to have lower basal and stress-induced corticosterone concentrations (Faure, 1980). Highly fearful birds have increased H/L ratio and increased corticosterone concentrations when subjected to feed deprivation (Beuving *et al*., 1989).

Broilers that re-establish contact with others after being separated in a maze have lower corticosterone responses to an acute stressor (Marin and Jones, 1999). This suggests that broilers that are less fearful have diminished corticosterone responses. This has practical implications because in a further study it was found that the broilers which re-established contact quicker also had better growth rates (Marin *et al*., 2003).

Quail selected for high (HSR) social reinstatement (desire to return to flock mates) showed more activity, higher corticosterone response and H/L ratios when isolated compared to those selected for low (LSR) social reinstatement (Mills *et al*., 1993). The HSR line is probably less fearful but the higher corticosterone and H/L ratios when these birds are isolated is worthy of comment. The selection has been for one behavioural trait, isolation response, and while this may indicated less fearful birds they are actually probably more fearful when isolated. If birds can only identify a limited number of conspecifics in large housing systems, then HSR birds may actually be exposed to situations that adversely affect their welfare. While this selection procedure is no doubt for experimental purposes it does provide reason for caution when identifying selection criteria to be used to improve welfare in alternative egg production systems.

When groups of White Leghorn hens with either long or short TI duration were injected with ACTH they had similar corticosterone responses of 12.99 ± 1.4 and 12.5 ± 0.8 ng/ml, respectively (Beuving *et al*., 1989). In the same study, deprivation from visible feed for 68h increased corticosterone concentration without significant differences between the groups, although they were consistently higher in hens with long TI duration during the period of feed unavailability. During the period of feed deprivation, the H/L ratio increased in both groups of hens, however it was significantly higher in the hens with long TI duration. The prolonged changes in corticosterone and H/L ratio indicate that the hens did not readily adapt to the frustration caused by feed deprivation (Beuving *et al*., 1989).

1.16. Handling

In 8 and 14-week old laying strain cockerels, prolonged immobilisation (5h) increased plasma corticosterone (Wodzicka-Tomaszewska *et al*., 1982). Levels rose within ten minutes and continued to increase and were at their maximum values at 5h. The concentration of T3 and T4 decreased significantly over the same period. During immobilisation a TRH injection increased T3 and T4 concentrations in cockerels. The authors suggested that this indicated that the lower T3 and T4 concentrations following restraint were not the result of hormone depletion but probably rather inhibitory effects of the increased corticosterone. In mammals, elevated glucocorticoid levels are associated with inhibition of thyroid hormone release essentially by suppressing TSH secretion (Reichlin, 1966).

Holding hens in crates increases plasma corticosterone concentrations (Beuving and Vonder, 1978; Scott *et al*., 1983). Regular handling in early life has been reported to diminish the response to fear in later life (Jones *et al*., 1991a). Chickens handled 10 times/week for 3 weeks had higher corticosterone responses on the earlier handling and bleeding episodes than at later handling and bleeding episodes (Freeman and Manning, 1979). Adapting hens to handling over a 7-day period reduced the corticosterone response to handling and bleeding at a later time (Webb and Mashaly, 1984). In the same study, the corticosterone responses to bleeding hens by brachial venipuncture was greater than bleeding through an indwelling brachial vein catheter. There is greater variation in the individual corticosterone responses to handling than there is between different sampling days when samples were collected over 7 days (Litten and Cockrem, 2001). There was also large variation in the area under the corticosterone response curve for individual hens. This probably means that it is important to measure individual responses to stress provoking stimuli rather than rely on group averages when evaluating stress responses and using these to indicate welfare status.

Manual restraint was reported to increase plasma corticosterone within 45 sec (Beuving, 1980). Plasma corticosterone increases if the sampling time is long, however no effects are observed if the sampling time is less than 150 sec (Beuving and Vonder, 1978; Craig and Craig, 1985; Lagadic *et al*., 1990). Others found no effect if the sample was taken within 60 sec (Etches, 1976) or 120 sec (Craig and Craig, 1985). For all studies forming part of this report a maximum limit of 2 min was placed on the taking of blood samples. Following restraint plasma corticosterone peaks at about 10 min (Beuving and Vonder, 1986). Peak levels of corticosterone after handling stress are 4-8 ng /ml (Litten and Cockrem, 2001).

One problem with blood sampling is what effect the disturbance of removing one hen from the enclosure for sampling has on the corticosterone concentrations of other hens in the enclosure. When hens housed 4 per cage were bled in sequence (within group) or taken alternatively from different cages (between group) there were no effects on corticosterone concentration. This is supported by the work of others (Harvey *et al*., 1980; Jones and Harvey, 1987). In 6-bird cages the order of bleeding had an effect on the plasma corticosterone concentration. There was no effect for the first two hens bleed but concentration increased for the third and subsequent hens being bled (Davis *et al*., 2000).

For studies detailed in this report no more than two hens from the same group cage were bled at any sampling time.

Chapter 2

The relationship between plasma corticosterone concentration and the corticosterone concentration in egg albumen in laying hens.

2.1 Introduction

In a previous RIRDC project, No US-71A, 'Development of a non-invasive test of stress in laying hens', the relationship between plasma and albumen corticosterone concentrations was not clearly established although it was obvious that when hens were exposed to known stressors the level of corticosterone in albumen increased. The experimental model used in the previous studies involved infusion of corticosterone using mini-osmotic pumps. This resulted in a constant rate of corticosterone administration over 14 days and subsequent cessation of laying after 3-5 days. This caused problems associated with the timing of the egg sampling relative to the start of the infusion. From these earlier studies it was concluded that the subcutaneous administration of corticosterone would be the best approach to developing an experimental model to best achieve the initial project objective.

2.2. Objective

The objective of the studies described in this chapter was to develop an experimental model that adequately evaluated the relationship between plasma and egg albumen corticosterone concentrations and test for the relationship between plasma and egg albumen corticosterone concentrations. To this end, five experiments were completed and these are described here.

2.3. Experiment 1

2.3.1. Objective

To determine, the dose-response relationship between a subcutaneous injection of corticosterone and plasma corticosterone concentration in laying hens. This study determined the dose that could be used in evaluating the relationship between plasma and egg albumen corticosterone levels.

2.3.2. Materials and Methods

Birds and treatments: Hens (n=8) were given subcutaneous injections of either 0, 1.25, 2.5, 5 or 10 mg of corticosterone suspended in 1 ml of peanut oil. A vegetable oil was used as the carrier due to the poor solubility of corticosterone in saline. Hens receiving no corticosterone were injected with

peanut oil alone. Injections were given between 1600-1700h, and this was designated as day 1 of the study.

Egg collection: The eggs collected the following morning (day-2) were discarded because the albumen present would have been deposited prior to the injection. Eggs collected on days 3 and 4 were weighed the albumen removed and weighed then stored at -20^0C until assayed.

Blood sampling: Starting at 1600h on days 2 and 3, blood samples (1-1.5 ml) were taken from all hens. The blood samples were centrifuged within 40 minutes of being collected, the plasma harvested and stored at -20^0C until assayed. The plasma levels of corticosterone at this time would be representative of those existing during the accumulation of albumen during day 2 and 3 and be the source of corticosterone present in eggs laid on days 3 and 4.

Corticosterone assay: The corticosterone levels in plasma and egg albumen were determined by radioimmunoassay (RIA). Details of the assay were described previously in RIRDC project No.US-71A, 'Development of a non-invasive test of stress in laying hens'. Since this report there have been some modifications to the assay protocol.

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 (activity: 2.48 Bq/mmol) was obtained from Amersham Biosciences (United Kingdom). The labelled corticosterone was diluted in ethanol and when needed, dried down under nitrogen and then reconstituted in PBS to give approximately 12,000 CPM in 0.1 ml using a LKB-1215 beta counter (Wallace Oy, Turku, Finland).

(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 -20^0C until needed. The assay standards, ranging in concentration from 0.1 to 25 ng/ml were prepared by serial dilution of the working standard in PBS.

(v) Quality controls: Internal quality controls for the albumen assay were prepared by injecting hens with corticosterone and collecting the albumen from their eggs. After complete mixing, albumen was stored as aliquots at -20^0C. Internal quality controls for the plasma assays were prepared by adding known amounts of corticosterone to steroid free charcoal stripped hen plasma. A set of the appropriate quality controls was run in each of the albumen and plasma assays.

(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 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, broken open and the albumen separated from the yolk, weighed and stored at -20^0C until assayed. At a later stage, the albumen samples were thawed and shaken vigorously until completely mixed. A 5-6 g sub-sample of

albumen was transferred to a 20 ml glass vial and to this was added 5 ml of distilled water and the contents shaken. After complete emulsification, two aliquots (approximately 0.5g) 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 place in a -80^0C 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) Plasma extraction procedure: Corticosterone in plasma was extracted by dispensing 0.1 ml into glass culture tubes and then adding 3 ml of diethyl. The remainder of the procedure was the same as for the albumen samples. All samples were assayed in duplicate.

(ix) Assay protocol: To the albumen and plasma 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 4^0C overnight. The following day, 0.25 ml of dextran-coated charcoal was added and the contents mixed and incubated for 15 min before being centrifuged at 2500 rpm for 15 min. The supernatant was poured into a 5 ml polyethylene scintillation vial (Packard Bioscience, Groningen, The Netherlands). To this was added 3 ml of counting scintillant (Opitphase H-safe 3, Fisher Chemicals, Loughborough, UK) and the level of radioactivity determined using a LKB-1215 Beta counter (Wallace Oy, Turku, Finland). 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 both plasma and egg albumen and processing them as for the samples. The recovery rate was determined to be 92%. The assay sensitivity (defined as the concentration of hormone that produces binding that is 2 SD less than the zero binding) was 0.03 ng/tube. The intra-assay and inter-assay coefficient of variation for the albumen assay, estimated using 3 quality controls containing low, medium and high concentrations of corticosterone were: low (1.38 ng/g); 6.8% and 8.8%; medium (2.76 ng/g); 8.4% and 9.6%, high (4.25 ng/g): 12.4% and 9.7%, respectively. The intra-assay and inter-assay coefficient of variation for the plasma assay, estimated using 3 quality controls containing low, medium and high concentrations of corticosterone were: low (1.44 ng/ml); 5.2% and 7.5%; medium (4.90 ng/ml); 9.8% and 11.9%, high (6.47 ng/ml); 8.2% and 13.3%, respectively.

Statistics: The relationship between the plasma and the egg albumen corticosterone concentrations were subjected to simple regression analysis. Differences between treatments were analysed by ANOVA.

2.3.3. Results

The corticosterone levels in plasma for samples collected on day 2 and in the albumen taken from eggs collected on day 3 are shown in Figure 2.1. An injection of 2.5 mg or greater of corticosterone significantly increased plasma corticosterone concentration. For eggs collected on day 3, no differences in albumen corticosterone concentration were observed except for hens injected with 10 mg of corticosterone levels were higher. No differences were detected in total corticosterone amount in albumen. Not all hens laid eggs on day three. Some hens in the groups injected with 5 and 10 mg injection failed to lay. Also many hens which did lay had a delayed oviposition compared to the control hens. The corticosterone levels in plasma for samples collected on day 3 and in the albumen taken from eggs collected on day 4 are shown in Figure 2.2. The mean plasma corticosterone remained higher for hens injected with 10 mg of corticosterone as where the concentrations in the albumen. For injections of corticosterone less than 10 mg the plasma concentrations of corticosterone were no higher than the controls. The relationship between plasma (day 2) and albumen (day 3)

corticosterone concentrations is given in Figure 2.3. There was no evidence of there being any relationship.

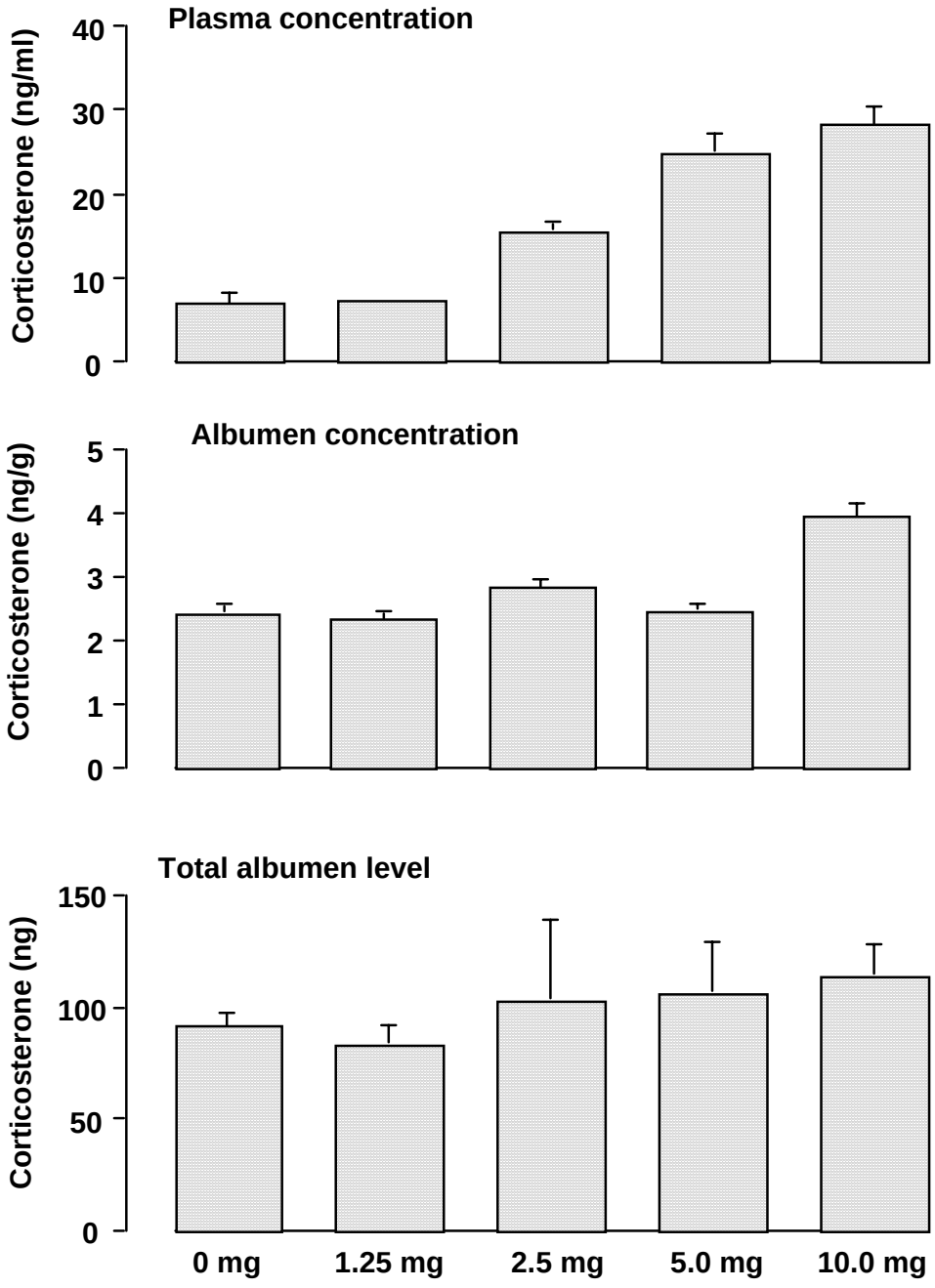

Figure 2.1: Plasma and egg corticosterone at 2 and 3 days, respectively, after injection of corticosterone. The mean (± SEM) plasma corticosterone (upper panel) for hens treated with 0, 1.25, 2.5, 5.0 and 10.0 mg of corticosterone given as a single subcutaneous injection. The mean (± SEM) concentration of corticosterone in albumen (middle panel) and the total amount of corticosterone in albumen (lower panel) of eggs collected following the injection of corticosterone. The injection was given on day 1 at 1600h, the plasma sample taken on day 2 at 1600h and the eggs collected on day 3.

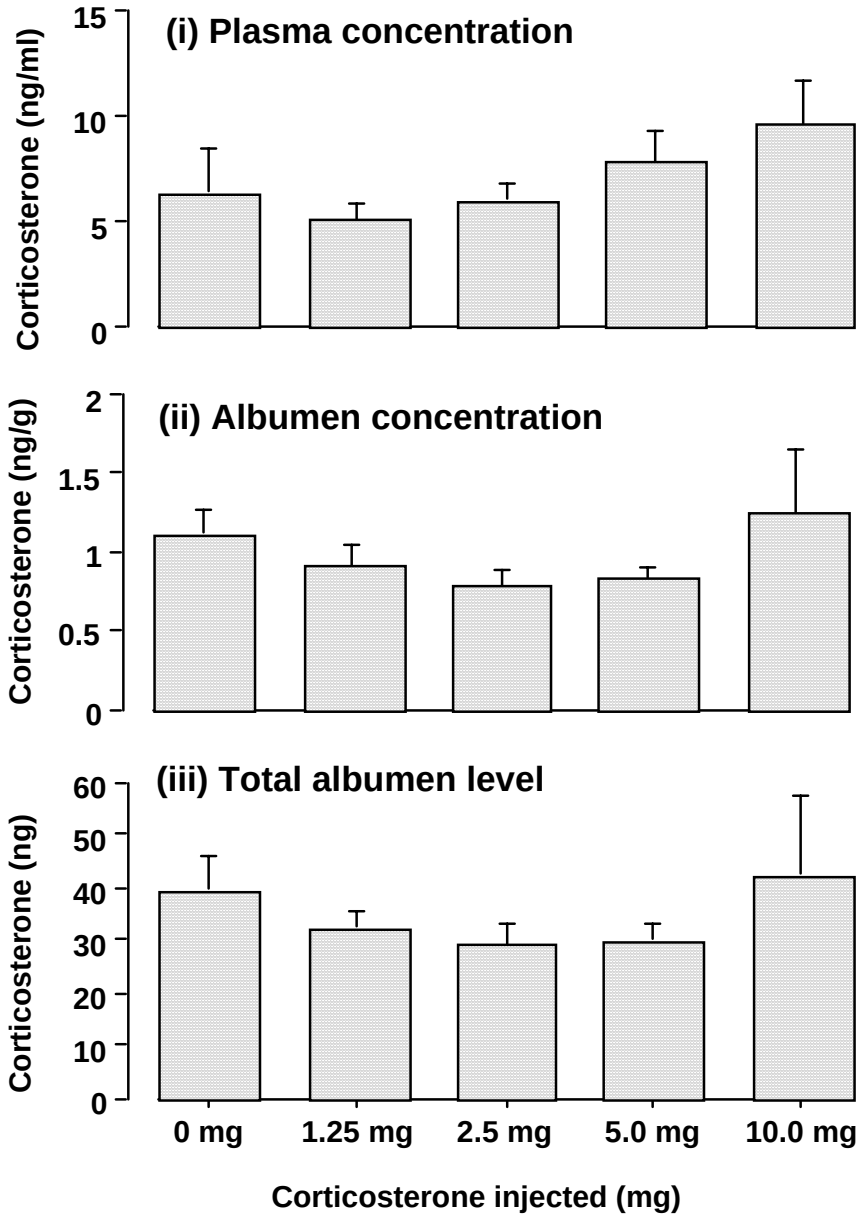

Figure 2.2: Plasma and egg corticosterone at 3 and 4 days, respectively, after injection of corticosterone. The mean (± SEM) plasma corticosterone (i) for hens treated with 0, 1.25, 2.5, 5.0 and 10.0 mg of corticosterone, given as a single subcutaneous injection. The mean (± SEM) concentration of corticosterone in albumen (ii) and the total amount of corticosterone (iii) in albumen of eggs collected following the injections of corticosterone. The injection was given on day 1 at 1600h, the plasma sample taken on day 3 at 1600h and the eggs collected on day 4.

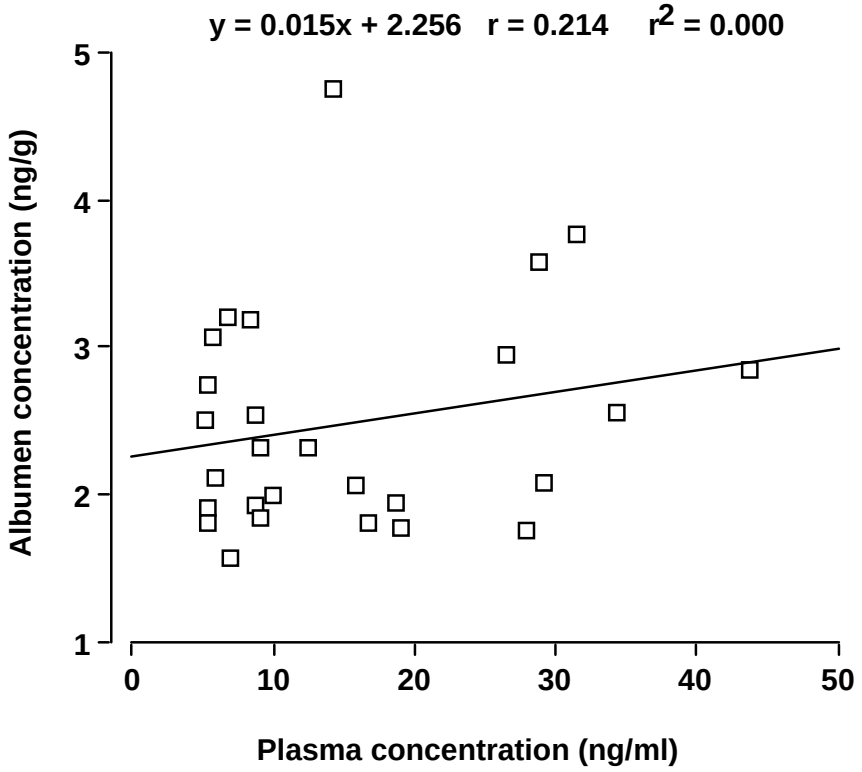

Figure 2.3: The relationship between the plasma and albumen corticosterone concentrations for hens injected with different doses of corticosterone. The injection was given on day 1 at 1600h, the plasma sample taken on day 2 at 1600h and the eggs collected on day 3.

2.3.4. Discussion

Injections of corticosterone less than 10 mg failed to maintain elevated plasma corticosterone above control values after 1-2 days. Therefore the range of doses used was not sufficient to adequately determine the relationship between plasma and albumen corticosterone concentrations. Also, because some hens did not lay following the injections of corticosterone, the size of the treatment groups was inadequate to collect sufficient eggs to appropriately determine the relationship.

2.4. Experiment 2

2.4.1. Introduction

In this study the experimental design gave consideration to the key observations made in experiment 1. Firstly, higher doses of corticosterone were used and group size was increased. The period of egg collection was extended to 7 days after the injection. Because there was a delay in oviposition time for hens injected with corticosterone in experiment 1, the oviposition time for individual hens was recorded in this study.

2.4.2. Objective

To determine the dose-response relationship between corticosterone injected subcutaneously and plasma corticosterone concentration.

2.4.3. Materials and methods

Birds and treatments: Hens (n=10) were given subcutaneous injections of either 0, 7.5 or 15 mg of corticosterone suspended in 1 ml of peanut oil. Hens receiving no corticosterone were injected with peanut oil alone. Injections were given between 1600-1700h, and this was designated as day 1 of the study.

Sampling: For the same reasons given in experiment 1 the eggs collected the following morning (day 2) were discarded. All eggs laid were collected on days 3 to 8. Eggs were weighed, broken open and the albumen removed, weighed and stored at -20^0C until assayed. Starting at 1600h on days 2, 4 and 7 blood samples were taken from all hens. It was considered that the plasma levels at this time would be representative of those existing during the accumulation of albumen during day 2, 4 and 7 and be the source of corticosterone present in eggs laid on days 3, 5 and 8. The oviposition time of individual hens was recorded between 0800h and 1600h on each of the egg collection days. Corticosterone concentrations in plasma and egg albumen were determined by RIA.

Statistics: The relationship between the plasma and the egg albumen corticosterone concentrations were subjected to simple regression analysis. Differences between treatments were analysed by ANOVA.

2.4.4. Results

The plasma corticosterone concentrations are given in Figure 2.4. On day 2 there was a clear dose response with both the 7.5 and 15 mg dose significantly increasing the plasma corticosterone concentration compared to control hens. The differences had greatly diminished by day 4 and by day 7 all treatment groups had similar mean plasma corticosterone concentrations. The albumen corticosterone concentration and total amount of corticosterone in albumen for eggs collected on day 3 are shown in Figure 2.5. The corticosterone concentration in albumen was higher in hens treated with both the 7.5 and 15 mg doses of corticosterone and the total amount of corticosterone in albumen also increased in line with the concentration changes. For the control hens the albumen corticosterone concentration were similar on all days.

By day 4, the levels of albumen corticosterone in eggs from hens treated with the 7.5 mg dose had returned to the levels seen in control hens and remained similar on day 7. For this treatment group, only 6 of the 10 treated hens continued to lay eggs beyond day 3. For the hens treated with 15 mg of corticosterone only one hen continued to lay after day 3. The relationship between plasma corticosterone concentration (day 2) and albumen concentration (day 3) is given in Figure 2.6.

Oviposition time for the control hens was relatively constant over the seven days. For the hens treated with either 7.5 or 15 mg of corticosterone the time of oviposition on day 2 was delayed until after 1600h but occurred sometime before 0800h on day 3. Therefore eggs collected on the morning of day 3 were actually eggs which would have normally been laid on day 2.

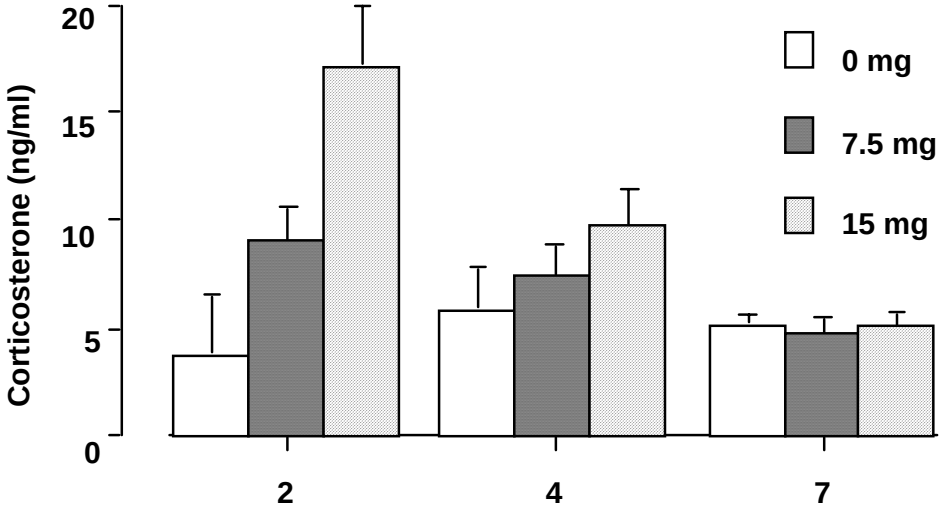

Figure 2.4: The mean (± SEM) plasma corticosterone concentration for hens treated with 0, 5 or 15 mg of corticosterone, given as a single subcutaneous injection. The injection was given on day 1 at 1600h, the plasma samples were taken on days 2, 4 and 7 at 1600h.

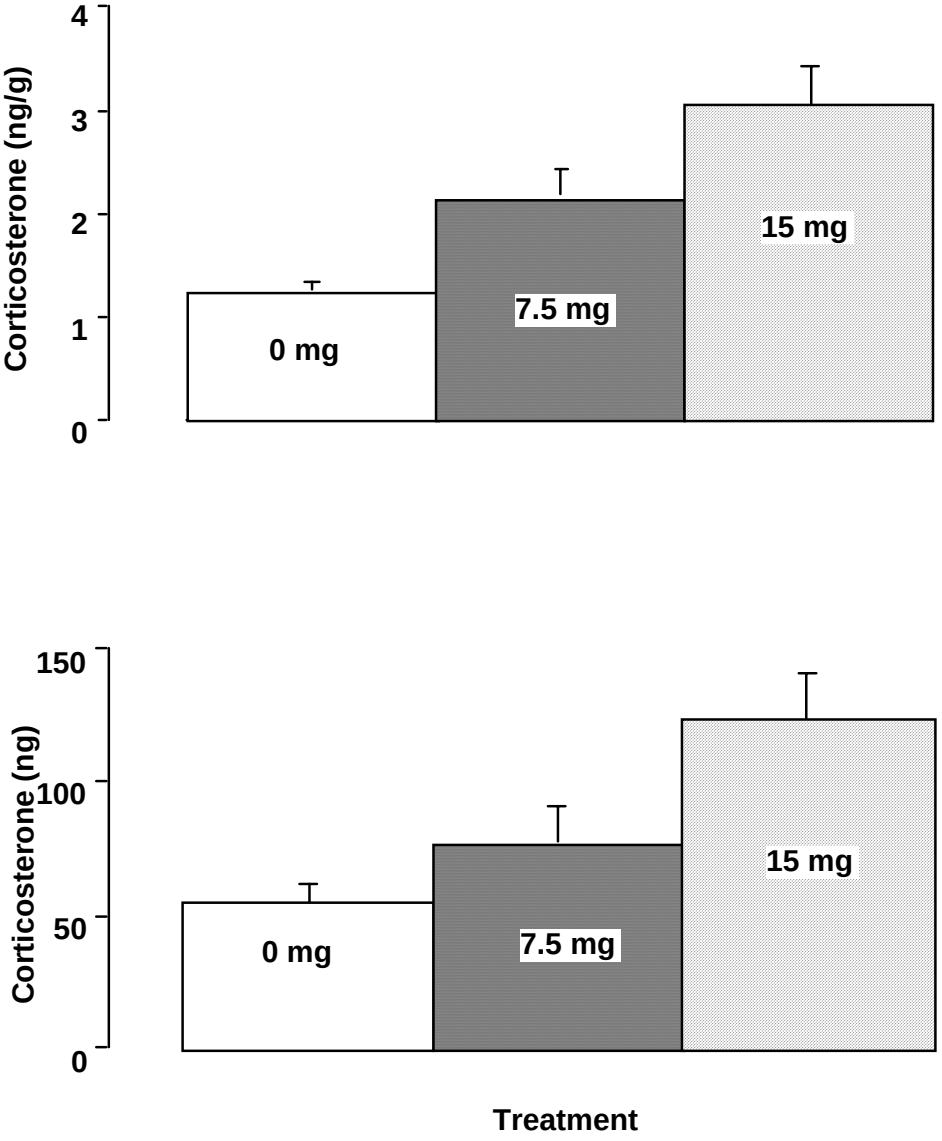

Figure 2.5: The mean (± SEM) concentration of corticosterone in albumen (upper panel) and the total amount of corticosterone (lower panel) in the albumen of eggs collected following the subcutaneous injection of corticosterone. The injection was given on day 1 at 1600h, the plasma sample taken at 1600h on day 2 and the eggs collected on day 3.

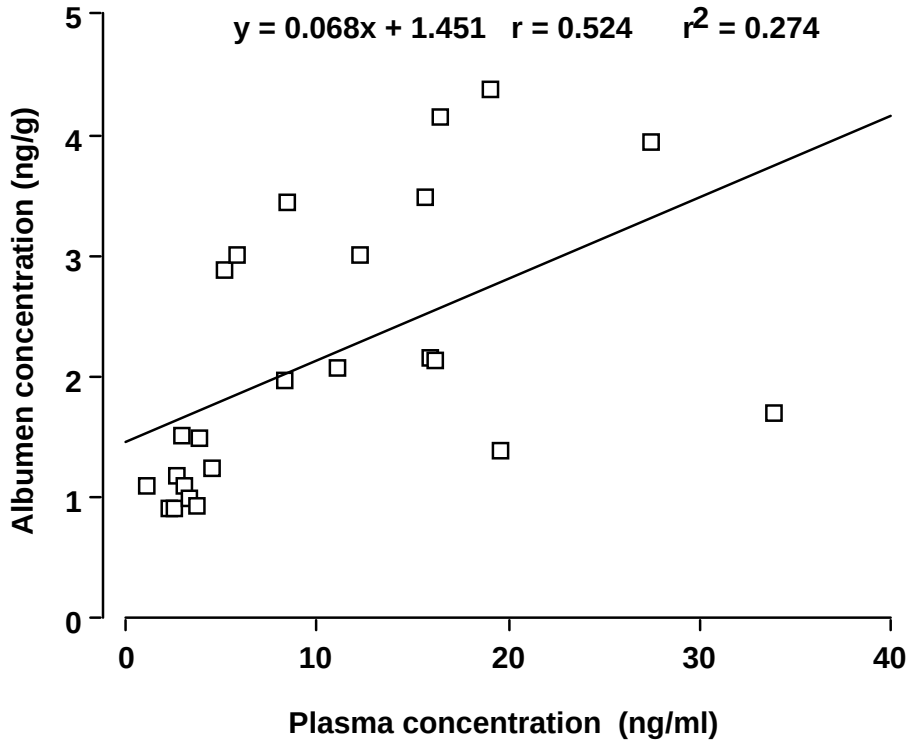

Figure 2.6: The relationship between the plasma and egg albumen corticosterone concentrations following a single subcutaneous injection of 0, 7.5 or 15 mg of corticosterone. The injection was given on day 1 at 1600h, the plasma sample taken at 1600h on day 2 and the eggs collected on day 3.

2.4.5. Discussion

The doses of corticosterone used had a significant physiological effect on both oviposition and ovulation. Injecting hens with 15 mg of corticosterone completely disrupted egg laying with only one hen laying after day 3 and this hen had a plasma level of corticosterone similar to the mean of the control group. Therefore, in any further studies doses of less than 15 mg need to be used. The relationship shown in Figure 2.6 indicated that the experimental model would be appropriate for determining the relationship between plasma and albumen corticosterone concentrations however, further refinement would be required to determine the correct doses and timing of the injection.

2. 5. Experiment 3

2.5.1. Introduction

Following from experiment 2 further refinement of the experimental protocol was required.

2.5.2. Objective

To further evaluate the dose-response relationship between corticosterone injected subcutaneously and plasma corticosterone concentration.

2.5.3. Materials and methods

Birds and treatments: Hens were given a subcutaneous injection of 0 mg (n=15), 5 mg (n=15) or 10 mg of corticosterone (n=20) suspended in 1 ml of peanut oil. Hens receiving no corticosterone were injected with peanut oil alone. Injections were given between 1600-1700h, and this was designated as day 1 of the study.

Sampling: After the injections were given all eggs laid were collected, weighed and then broken open and the albumen harvested and weighed before being stored at -20^0C until assayed. Starting at 1600 h on days 2 and 3, blood samples were taken from all hens, the plasma harvested after centrifugation and stored at -20^0C until assayed. On days 1 to 4 of the study the oviposition times for individual hens were recorded. Corticosterone concentrations in plasma and the egg albumen were determined by RIA.

Statistics: The relationship between the plasma and the egg albumen corticosterone concentrations were subjected to simple regression analysis. Differences between treatments were analysed by ANOVA.

2.5.4. Results

The plasma corticosterone levels are given in Figure 2.7. On day 2, 24 h after the injection, the mean plasma concentration was 4 fold higher in hens treated with 10 mg of corticosterone and 3 fold higher for hens injected with 5 mg corticosterone compared to the concentration for control hens. For all hens treated with either 5 or 10 mg of corticosterone, oviposition was delayed and for 21 of the 35 hens treated with these doses it was delayed beyond 1600 h and for 7 hens it didn't occur at all. Therefore many of the eggs collected on day 3 were those that would have normally been laid on day 2. Only 9 of the 20 hens treated with 10 mg corticosterone laid an egg on day 4 and these were the result of a delayed oviposition, however the delay was not as long as seen on day 3. Eleven of the 15 hens treated with 5 mg laid on day 4 but again oviposition time was delayed. The albumen concentration and total amount of corticosterone in albumen for eggs collected on day 3 are given in Figure 2.7. Again it needs to be stressed that for the corticosterone treated hens; the albumen is probably collected from eggs that would have normally been laid on day 2. Similar levels were also seen in the albumen from eggs laid on day 4 (not shown). The relationship between plasma and albumen corticosterone concentrations is given in Figure 2.8. The values include all eggs laid on days 3 and 4 for which there are corresponding blood samples taken on days 2 and 3.

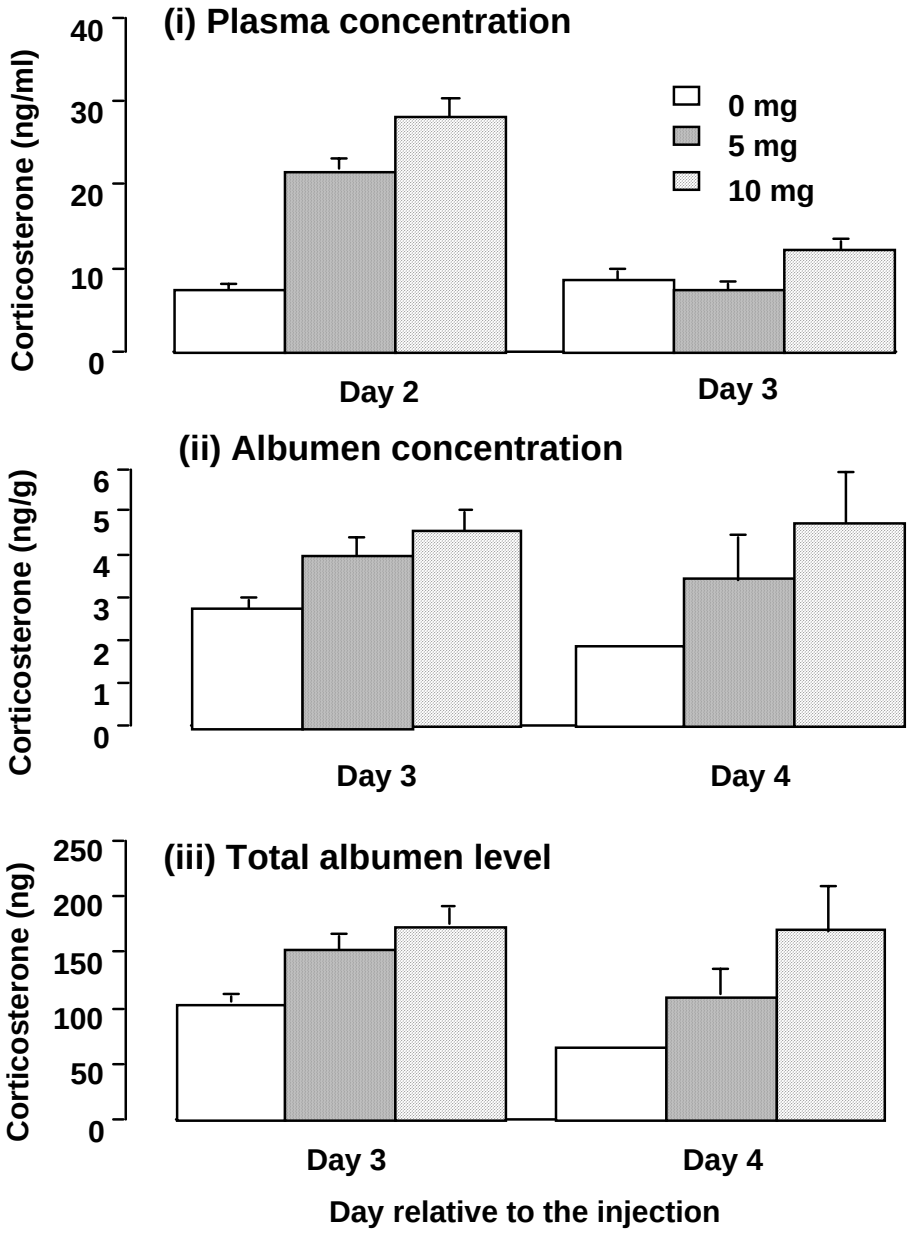

Figure 2.7: The effect of treating hens with 0, 5 or 10 mg of corticosterone given as a single subcutaneous injection at 1600h on plasma and egg albumen corticosterone – sampling on days 2-4. The mean (± SEM) plasma corticosterone (upper panel), the mean (± SEM) concentration of corticosterone in albumen (middle panel) and the total amount of corticosterone (lower panel) in albumen of eggs collected following the injection of corticosterone. The injection was given on day 1 at 1600h; the plasma sample taken on day 2 and 3 at 1600h and the eggs collected on days 3 and 4.

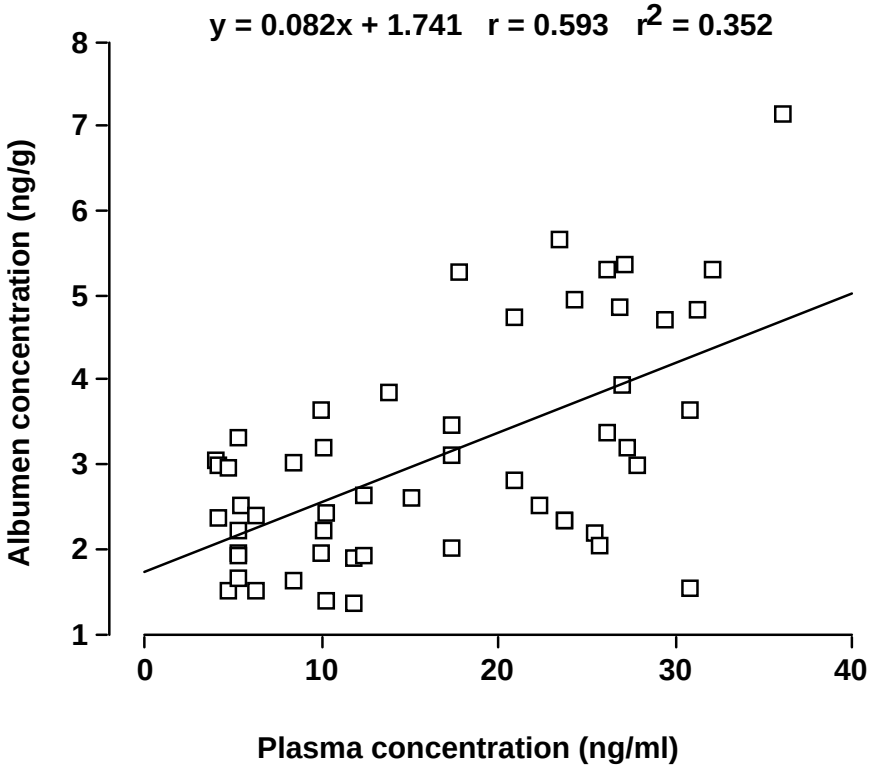

Figure 2.8: The relationship between the plasma corticosterone concentration and the concentration found in albumen for hens given a subcutaneous injection of 0, 5 or 10 mg of corticosterone at 1600h. The injection was given at 1600h on day 1; the blood sample was taken on day 2 at 1600h and eggs collected on day 3.

2.5.5. Discussion

The results from this experiment highlighted the need for a major modification to the experimental model. For the hens used in this study, oviposition does not normally occur before 0600 h and so ovulation would occur normally around this time. If, as has been reported, corticosterone can interfere with ovulation then an injection given at 1600h insures that high plasma corticosterone levels are present at the net expected ovulation. This is the probable cause for hens failing to lay after the initial oviposition which in fact is the result of an egg being formed in the oviduct before the injection was given. The effects of corticosterone on ovulation and delayed oviposition indicated that the timing of the corticosterone injection needed to be altered. If the injection was given at 0600h the time effects on ovulation are likely to be negligible on the day of injection. Although oviposition would probably be delayed, the eggs formed on the day of the injection would be exposed to elevated corticosterone for the entire period of albumen accumulation. It is also likely that an egg would be collected from almost all the treated hens on the day after the injection even though ovulation would probably be blocked after this and hens injected with high doses of corticosterone would cease to lay.

2.6. Experiment 4

2.6.1. Introduction

Following on from the observations made in experiment 3, a further study was initiated where the corticosterone injection was given to hens at 0600h and the relationship between the plasma and albumen corticosterone concentrations determined.

2.6.2. Objective

To evaluate the dose-response relationship between plasma corticosterone concentration and the corticosterone concentration in albumen when hens are given a subcutaneous injection at 0600h.

2.6.3. Materials and methods

Birds and treatments: Isa Brown hens, 56 weeks of age were housed individually, in conventional cages (30 x 40 x 45 cm) and fed a commercial layer diet. The hens were given a subcutaneous injected of 0 mg (n=20), 5 mg (n=20) or 10 mg (n=20) of corticosterone suspended in 1 ml of peanut oil. Hens receiving no corticosterone were injected with peanut oil alone. Injections were given at 0600 h on day 1 of the study.

Sampling: A 1-2 ml blood sample, was collected from all hens between 1600-1700 h on the day 1 and 2. The blood was centrifuged within 40 min of being collected, the plasma harvested and then stored at -20^0C until assayed. Oviposition times on day 2 and 3 were recorded, the eggs collected and weighed; the albumen was then collected, weighed and then stored at -20^0C until assayed. Corticosterone levels in albumen and plasma were determined by RIA.

Statistics: The relationship between the plasma and the egg albumen corticosterone concentrations were subjected to simple regression analysis. Differences between treatments were analysed by ANOVA.

2.6.4. Results

The plasma and albumen corticosterone concentrations and total amount of corticosterone in albumen are shown in Figure 9. The subcutaneous injections of corticosterone significantly elevated plasma concentrations. On day 1 the concentrations were 1.77 ± 0.13, 14.94 ± 1.65 and 23.95 ± 2.28 ng/ml and day 2, 1.99 ± 0.20, 12.08 ± 1.34 and 14.50 ± 2.33 ng/ml for the hens injected with 0, 5 and 10 mg corticosterone, respectively. The injections of corticosterone significantly increased both the concentration and total amount of corticosterone in albumen on day 2. However, the concentration and total amount of corticosterone in the albumen from eggs collected on day 3 were not different from the controls. There were no albumen determinations for the 10 mg corticosterone injected group on day 3 because only one hen laid. The relationship between plasma and albumen corticosterone concentration is shown in Figure 10. There was a significant positive correlation between plasma and egg albumen corticosterone concentrations. As with previous studies oviposition time was delayed.

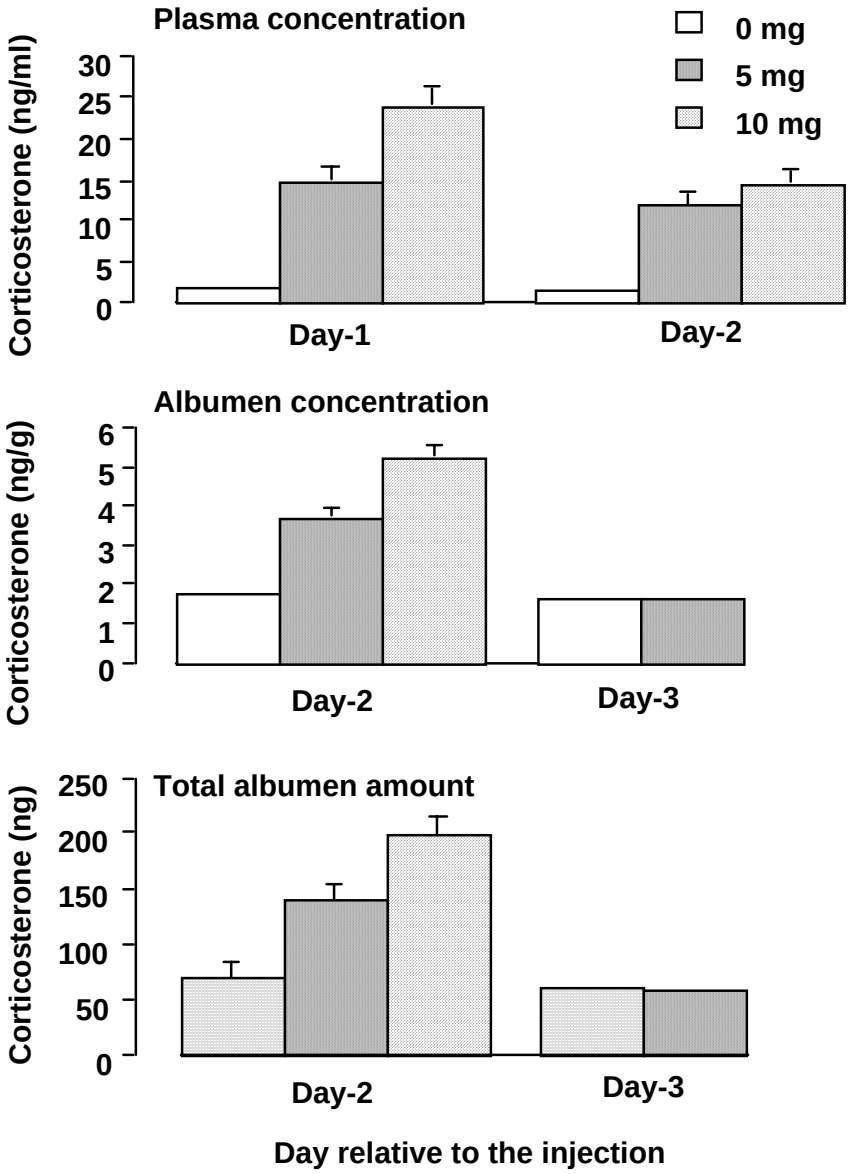

Figure 2.9: The effect of treating hens with 0, 5 or 10 mg of corticosterone given as a single subcutaneous injection at 0600h on plasma and egg albumen corticosterone – sampling on days 1-3. The mean (± SEM) plasma corticosterone (upper panel) for hens treated with 0, 5.0 or 10.0 mg of corticosterone given as a single subcutaneous injection. The mean (± SEM) concentration of corticosterone in albumen (middle panel) and the total amount of corticosterone (lower panel) in albumen of eggs collected following the injection of corticosterone. The injection was given on day 1 at 0600h; the plasma samples taken on day 1 and 2 at 1600h and the eggs collected on days 2 and 3.

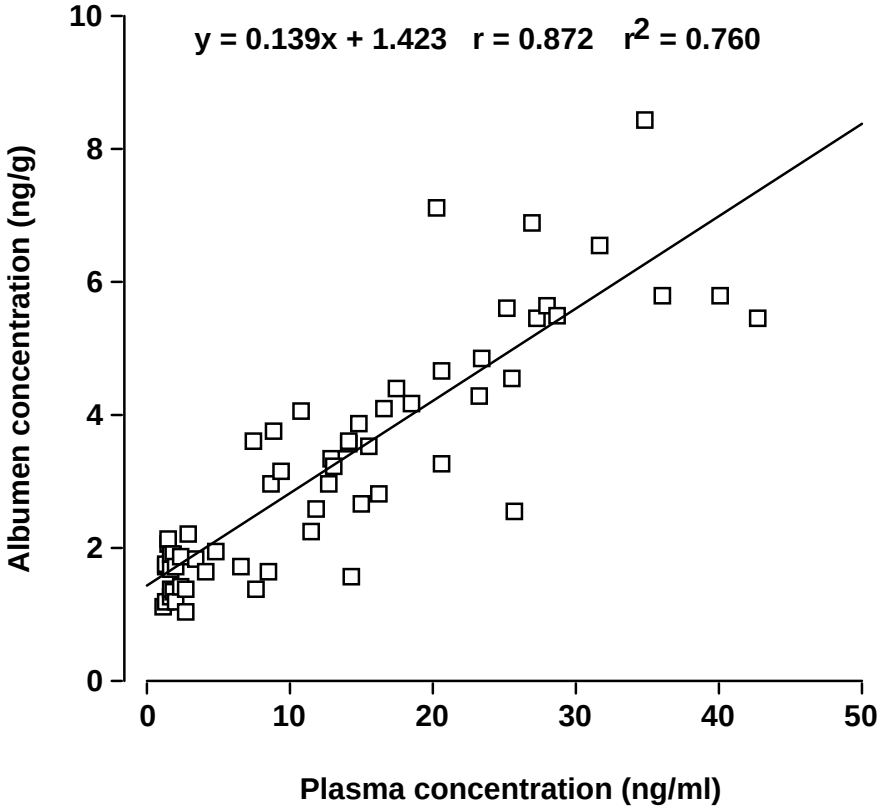

Figure 2.10: The relationship between the plasma corticosterone concentration and the concentration found in albumen for hens given a subcutaneous injection of 0, 5 or 10 mg of corticosterone at 0600h. The injection was given at 0600 h on day 1, the blood sample taken at 1600 h on day 1 and eggs collected on day 2.

2.6.5. Discussion

Thirty-eight of the 40 hens injected with corticosterone laid an egg on day 2. Therefore, injecting the corticosterone at 0600h allowed at least one egg to be collected from most hens; containing albumen that would have been laid down during the period when the plasma corticosterone concentration was elevated. The likely effects of elevated corticosterone concentrations on ovulation considered to be operating in previous studies appeared evident in this study. Only one hen from the group injected with 10 mg of corticosterone and 7 from the group injected with 5 mg laid on day 3. While the mean plasma levels of corticosterone of hens given the 5 mg injection remained elevated on day 2 there was no corresponding increase in albumen concentration for eggs collected on day 3. The mean plasma concentration was derived from all hens sampled while the albumen concentration was derived from the 7 hens that laid on day 3. For these 7 hens the plasma concentrations were not different from the mean value for control hens. Therefore only hens with plasma levels similar to control hens laid on day 3. There is a clear relationship between plasma and albumen corticosterone concentrations (Figure 10). The correlation coefficient of 0.87 is high for a biological relationship. From the present study, 76% of the variation in egg albumen corticosterone concentration can be accounted for by the variation in plasma corticosterone.

2.7. Experiment 5

2.7.1. Introduction

The experimental model used in experiment 4 is considered to be appropriate for determining the relationship between plasma and egg albumen corticosterone concentrations. Because this relationship is critical to using corticosterone levels in albumen as a non-invasive measure of stress in laying hens, the results from experiment 4 needed to be confirmed. For this reason the study described in experiment 4 was repeated.

2.7.2. Objective

To confirm the results of the previous study, that established the relationship between plasma and egg albumen corticosterone levels.

2.7.3. Materials and methods

Birds and treatments: Isa Brown hens, 62 weeks of age were housed individually, in conventional cages (30 x 40 x 45 cm) and fed a commercial layer diet. The hens were given a subcutaneous injected of 0 mg (n=20), 5 mg (n=20) or 10 mg (n=28) of corticosterone suspended in 1 ml of peanut oil. Hens receiving no corticosterone were injected with peanut oil alone. Injections were given at 0600 h, and this was designated as day 1 of the study.

Sampling: A 1-2 ml blood sample was collected from all hens between 1600-1700 h on day 1. The blood was centrifuged within 40 min of being collected, the plasma harvested and then stored at -20^0C until assayed. Oviposition times on day 2 were recorded, the eggs collected and weighed; the albumen was then collected, weighed and then stored at -20^0C until assayed. Corticosterone levels in albumen and plasma were determined by RIA.

Statistics: The relationship between the plasma and the egg albumen corticosterone concentrations were subjected to simple regression analysis. Differences between treatments were analysed by ANOVA.

2.7.4. Results

The mean (± SEM) plasma corticosterone concentrations were 0.68 ± 0.07 ng/ml, 5.44 ± 0.39 ng/ml and 10.6 ± 1.7 ng/ml for the hens treated with 0, 5 and 10 mg corticosterone, respectively. Both levels of injected corticosterone significantly ($P<0.05$) elevated plasma corticosterone concentrations on day 1. Individual hen plasma and albumen corticosterone concentrations are given in Figure 2.11. As in experiment 4, there was a significant linear relationship between the plasma and albumen corticosterone concentrations.

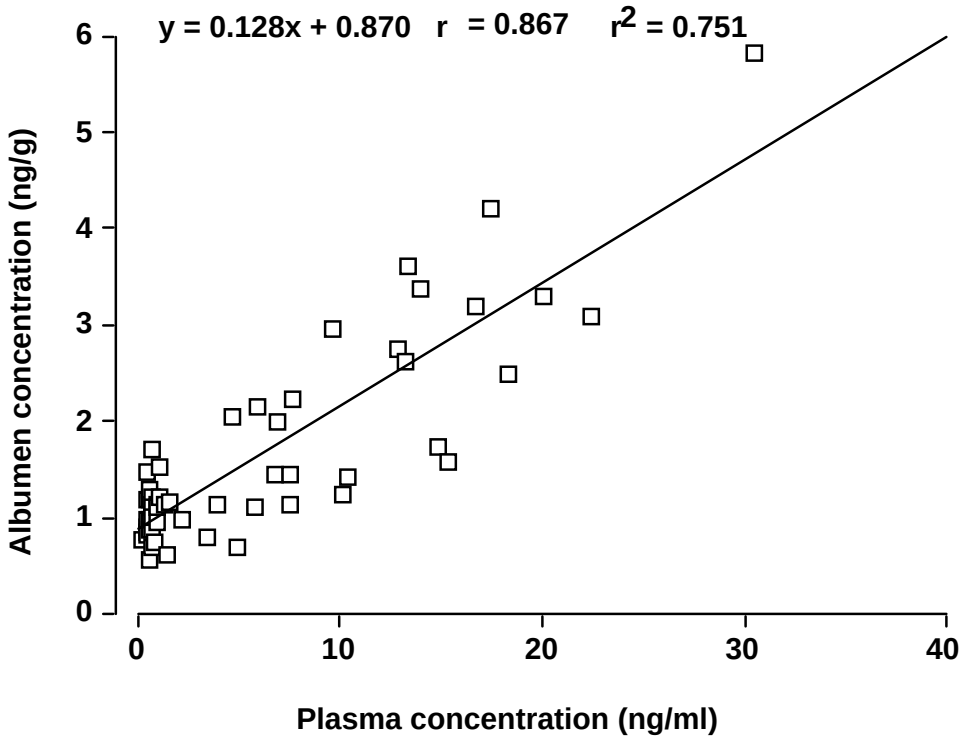

Figure 2.11: The relationship between the plasma corticosterone concentration and the concentration found in albumen for hens given a subcutaneous injection of 0, 5 or 10 mg of corticosterone at 0600h (repeat experiment). The injection was given at 0600h on day 1, the blood sample taken at 1600h on day 1 and eggs collected on day 2.

2.7.5. Discussion

The observations from the present study confirm those seen in experiment 4. Of the 48 hens injected with corticosterone, 42 laid an egg on day 2. This rate of lay is similar to that seen in experiment 4. The mean plasma corticosterone levels for all groups were lower than observed in experiment 4. However, the relationship between plasma and albumen corticosterone concentrations detailed in experiment 4 is evident in the present study. The correlation coefficient of 0.87 is similar to that seen in experiment 4 and again it is pertinent to emphasis that this is high for a biological relationship. It is estimated that 75% of the variation in egg albumen corticosterone concentration can be accounted for by the variation in plasma corticosterone and compares with the 76% obtained in experiment 4.

2.8. General discussion

As reported in RIRDC project, No US-71A, 'Development of a non-invasive test of stress in laying hens', the albumen corticosterone concentration and total amount of corticosterone in albumen are increased when hens are subjected to stressors known to increase plasma corticosterone concentrations. This suggested that corticosterone is sequested into albumen during egg formation. At the completion of project No US-71A, the relationship between plasma and egg albumen corticosterone had not been established because the experimental model used was inadequate. The first objective of the present project was to develop an experimental model, allowing the

determination of the relationship between these two physiological measures. From the previous project it was evident that a continuously high plasma corticosterone concentration caused ovarian follicle regression. At the final stages of the earlier project, it was concluded that giving the hens a single subcutaneous injection of corticosterone could effectively increase the plasma corticosterone concentration.

The first two experiments were dose response studies to determine what amount of corticosterone needed to be injected to increase plasma corticosterone concentrations sufficiently to test for the relationship of interest. From these studies it was determined that doses of 5 and 10 mg of corticosterone would be appropriate for this purpose. Another obstacle to using exogenous corticosterone to increase plasma corticosterone relates to the time when the injection had to be administered. When the injection was given late in the evening very few egg were laid on the second day which equated to albumen being sequested during the day following the injection.

Ovarian regression is associated with increased plasma corticosterone (Etches and Williams, 1983; Etches *et al*., 1984a: Moudgal *et al*., 1991: Petittee and Etches, 1991), with the rate of ovulation interrupted in a dose-dependent manner (Moudgal *et al*., 1991). A normal physiological range (around 3 ng/ml) can be maintained by subcutaneous infusion of 30 ug/h of corticosterone (Etches *et al*., 1984a). When this level of corticosterone was infused into hens, egg production dropped by day 3 and completely ceased by day 8 (Etches *et al*., 1984a). After the infusion of corticosterone, ovarian regression is associated with a decrease in progesterone, oestradiol and LH (Etches *et al*., 1984a) with the sequence of hormonal changes suggesting that corticosterone acts directly at the ovarian level.

By injecting hens in the evening, plasma corticosterone would be high during the expected time of the LH surge the next day. This would probably prevent ovulation and formation of an egg. The result would be failure of egg laying on the second day and this is what was observed in many of the corticosterone-treated hens. Injecting hens in the morning (0500-0600h) would elevate plasma corticosterone concentrations only after the LH surge had occurred in most hens. Therefore, ovulation would take place with an egg laid the following day. The albumen from this egg would have been sequestered during egg formation the previous day when plasma corticosterone was elevated by the morning injection. However, no further egg would be laid as the high circulating corticosterone would inhibit a further LH surge. This is what was observed for many hens after given the 10 mg dose of corticosterone and to a lesser extent the 5 mg dose.

Once an appropriate experimental protocol was established it was used to determine the relationship between plasma and egg albumen corticosterone concentrations. There is a clear relationship between plasma and albumen corticosterone concentrations (Figures 2.10 and 2.11). The correlation coefficient of 0.87 is high for a biological relationship. From the present studies, around 76% of the variation in egg albumen corticosterone concentration can be accounted for by the variation in plasma corticosterone. There was excellent agreement between experiments 4 and 5. There were some differences in the plasma concentrations in experiments 4 and 5 with the concentrations being higher in experiment 4. However, the relationship still remained with the albumen concentrations also being higher in experiment 4. Therefore, the amount of corticosterone deposited in the albumen seems to depend on the concentration in the plasma.

2.9. Implications

Excessive stress is associated with poor welfare. Many of the stresses to which hens are exposed result in activation of the HPA axis, with some consequences being changes in plasma and tissue levels of glucocorticoids and catecholamines secreted from the adrenal (Harbutz and Lightman, 1992). High corticosterone concentrations remain one of the physiological measures used to determine if hens are stressed. Using plasma corticosterone as a measure of adrenal activation has

problems associated with sampling procedure and with taking more than one point sample. Non-invasive techniques of measuring stress hormone concentrations would reduce these problems. The positive relationship between the plasma and egg albumen corticosterone concentrations allows for this measure to be used as a non-invasive determination of plasma corticosterone in hens. The gradual accumulation of albumen over 4-6 h during egg formation provides a reflection of the circulating corticosterone concentrations over this time. It might also magnify small differences in plasma corticosterone concentrations because of the period of accumulation.

Chapter 3

The effects of housing laying hens in groups and different cage densities on the plasma and egg albumen corticosterone concentrations

3.1. Introduction

The limited space available to hens maintained in conventional cages and the behavioural limiting constraints it causes have been of concern to various interest groups. There is continued contention over how important specific behavioural activities are to the wellbeing of hens. In any production system that houses hens as groups, the space available to an individual is limited by flock mates and the specifications of the enclosure. When resources are limiting, hens will compete for these and this includes space (Dawkins, 1985; Nicol, 1986). Therefore important considerations in assessing hen welfare are the size of the enclosure, number of hens and the availability of resources (Mench and Keeling, 2001). Groups of familiar hens maintained in cages tend to distribute themselves evenly over the available space while unfamiliar hens tend to distribute themselves unevenly with associated increased aggressive behaviour (Linberg and Nicol, 1996a). Therefore, in cages if the space is adequate and there is a stable hierarchy in place, much of what would potentially have a negative influence on welfare may be avoided. This again highlights the need for continued scientific evaluation of what is adequate space.

There are a number of reports indicating that egg production decreases as the cage density increases (Dorminey *et al*., 1972; Lei *et al*., 1972; Feldkamp and Adams, 1973; Martin *et al*., 1980; Cunnignham and Ostrander, 1981; Quart and Adams, 1982; Mashaly *et al*., 1984; Adams and Craig, 1985; Patterson and Muir, 1986; Anderson and Adams, 1991; Lee and Craig, 1991; Brake and Peebles, 1992; Bell *et al*., 1998). Egg production is regarded as a good measure of general welfare by some researchers. The effect of group size and cage space availability, are reported to have separate and additive effects on egg production (Adams and Jackson, 1970; Mench *et al*., 1986; Cunningham *et al*., 1988). Koelkebeck and Cain (1984) using different group sizes and space allowances found that in general, egg production was favoured by having one hen per cage. When two strains of hens were housed in groups of either 6, 8, 12 or 24 and the cage space maintained at 364 cm^2/bird and feeder space at 10 cm/bird, no effects on egg production and egg mass were recorded (Carey and Kuo, 1995). When group size was 4 or 8 hens each housed at different space allowances, there were no effects on egg production or body weight (Okpokho *et al*., 1987). Accepting egg production as an indicator of hen wellbeing, these and other data reviewed in section 1.6.2 suggest that space only becomes a factor if it is less than some critical value. As a prediction this value seems to be somewhere less than 350-380 cm^2/bird. Group size, if kept below 8, would seem to have minimal effect of production (see section 1.6.2).

While production may be an indicator of welfare, a more comprehensive evaluation requires assessment using a number of criteria. A number of reports indicate that there is a positive relationship between cage density and plasma corticosterone concentrations (Davami *et al*., 1984;

Craig *et al*., 1986a). Decreasing the area per bird increased corticosterone (Lei *et al*., 1972; Mashaly *et al*., 1984) as does a decrease in personal space (Compton *et al*., 1981). Mashaly and colleagues (1984) reported that the compounding effects of group size and cage density resulted in corticosterone increasing from 1.38 ng/ml (3 hens at 516 cm^2) to 1.65 ng/ml (4 hens at 387 cm^2) and 2.32 ng/ml (5 hens at 317 cm^2). The difference between the hens at 3 and 4 per cage compared to those at 5 hens per cage was significant. From these and other data reviewed in section 1.6.4 it would seem that a cage space somewhere between 317 and 287 cm^2/hen might be the lower limit before available space effects on plasma corticosterone concentrations are observed. Small and moderate group sizes do not seem to greatly affect plasma corticosterone concentration so long as the area per hen is adequate. This might not be the case when the group size is large, where social stress might be influential.

While plasma corticosterone is one physiological measure of stress, and chronic stress is associated with poor welfare, it is difficult to measure because continuous blood sampling is not an option. In most studies the assessment is made on samples taken at one time point and on a small sub-sample of hens. Measuring corticosterone in albumen can eliminate most of the problems associated with having to take blood samples.

3.2. Objective

The objective of the study presented here was to evaluate what effects group size and cage density of hens in conventional cages, have on the plasma and egg albumen corticosterone concentrations. While the effects of group number and cage space allowance were not segregated in the experimental protocol, the purpose of the study was to simulate conditions observed in commercial husbandry. In the study, cages similar to those available commercially were used with the objective being to evaluate what occurs when group size is modified in such cages.

3.3. Materials and methods

Birds and management: Isa Brown hens, 58 weeks of age were used in the study. They were fed *ad libitum,* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h light with light on at 0600h.

Cages: Hens previously housed individually in conventional layer cages (47 cm x 28 cm x 48 cm) (1175 cm^2/bird) were transferred to group cages (46 cm x 50 cm x 62 cm). Birds were housed 5, 4, 3 or 2 hens per cage or as a single hen per cage. The floor space allocated to each of the treatments was 460, 575, 767, 1150 and 2300 cm^2/bird, respectively. There were ten replicate cages for each of the housing treatments. The hens were moved between 0800h and 0930h on day 1 of the study.

Blood sampling: Two weeks and one week before the hens were transferred a 1 ml blood sample was taken by jugular venipuncture from 36 of the hens to be used in the study. These samples were collected starting at 1600h and taken within 2 min of the hen being first handled. These samples were taken to determine plasma corticosterone levels before the birds were moved. Blood was centrifuged and the plasma harvested and stored at -20^0C until assayed. On days 2, 4, 8, 11, 16, 26, 43, 54, 83, and 110 of the study a 1ml blood sample was taken from one hen in each pen by jugular venipuncture. The blood samples were collected between 1500 and 1630h and again taken within 2 min of the hen being first handled. The blood was centrifuged and the plasma harvested and stored at -20^0C until assayed.

Egg collection: Daily egg production was recorded for the entire period of the study. On the day following each of the blood sampling periods, all eggs were collected and oviposition times recorded. Egg collection started at 0800h, 2h after lights on, and this was designated as an oviposition time of

zero. Any egg laid before this time was regarded to have an oviposition time of zero. Eggs were collected on the hour up to and including 1600h. All eggs collected at any particular hour were considered to have the same oviposition time. Therefore, all eggs collected at 0900h were considered to have an oviposition time of +1 h and those at 1000h of +2 h etc.

Albumen collection: The first two eggs laid by hens in cages housing 5, 4 or 3 birds and all eggs laid by hens in cages housing 2 birds or a single bird were identified and weighed. These were then broken open and the albumen collected, weighed and then stored at -20^0C until assayed.

Corticosterone determinations: The corticosterone levels in plasma and egg albumen were determined by RIA as previously described in section 2.3.2.

Statistics: Values are given as means ± SEM. Differences between treatments were assessed by ANOVA and if significant ($p<0.05$) then multiple comparisons were made using the Tukey test. The means are for all eggs collected for individual treatments. Egg production was analysed on a weekly basis as the mean hen production per cage. All analysis was conducted using the 'Statview' computer program (SAS Institute Inc, NC, USA).

3.4. Results

Egg production: The mean weekly egg production is given in Table 3.1. On any individual week there were no significant differences in mean egg production, although the mean egg production tended to be lower for hens housed 5/cage during some weeks. The mean egg production for the 16 week treatment period were, 5.78 ± 0.09, 5.88 ± 0.09, 6.04 ± 0.08, 5.94 ± 0.11 and 6.30 ± 0.11 for hens housed 5, 4, 3, 2 or 1 bird(s) per cage, respectively. The overall mean production for the treatment period was higher in birds housed singly compared to those housed 5, 4 or 2 birds per cage.

Egg weight: The mean (±SEM) egg weights for those days when albumen samples were taken are given in Table 3.2. There were no significant effects of hen number/cage on egg weight.

Albumen weight: The mean (±SEM) egg albumen weights are given in Table 3.3. There were no significant effects of hen number/cage on egg albumen weight.

Oviposition times: The mean (±SEM) oviposition time are given in table 3.4. There were no significant effects of hen number/cage on oviposition time.

Plasma corticosterone: At two weeks and one week before being transferred to the group cages the mean (± SEM) plasma corticosterone concentrations were 0.55 ± 0.04 ng/ml and 0.82 ± 0.09 ng/ml, respectively. The mean (± SEM) plasma corticosterone concentrations during the experimental period are shown in Figure 3.1. The effect due to hen number per cage just failed to be significant ($P=0.075$). The effect of day was significant ($P<0.0001$) but there was no significant treatment x day interaction. Plasma concentration of corticosterone was higher on days 4 and 43 than all other days except 2 and 83 ($P<0.05$). The concentrations on days 2 and 4 were higher than on days 8 and 11 ($P<0.05$). On any individual collection day, the plasma corticosterone concentrations were not different for hens housed 5, 4, 3 or 2 per cage or singly.

Albumen corticosterone concentration: The mean (± SEM) albumen corticosterone concentrations are shown in Figure 3.2. The levels ranged from around 1 to 2.6 ng/g. The effect due to hen number per cage just failed to be significant ($P=0.06$), however the effect of day was significant ($P<0.0001$) but there was no significant treatment x day interaction. The albumen corticosterone concentrations were higher on days 2 and 4 than all other days except day 1 which in turn, was similarly higher than all other days except day 11 ($P< 0.05$). On days 8 and 11 the concentration was higher than later collection days ($P< 0.05$) while the concentration on day 16 was higher than days 43 and 54 ($P<$

0.05). On any individual collection day, the albumen corticosterone concentrations were not different when hens were housed 5, 4, 3 or 2 per cage or singly.

Total albumen corticosterone level: The mean (± SEM) total amount of corticosterone in albumen is shown in Figure 3.3. The effect due to hen number per cage was significant (P=0.011), as was the effect of day of collection (P<0.0001) but there was no significant treatment x day interaction. Over the course of the study the amount of corticosterone in albumen was lower in hens housed as 2 or as singles in a cage. On day 1, the amount of corticosterone in albumen was lower than on days 2 and 4 but higher than on other days (P< 0.05). The corticosterone amount in albumen was higher on days 2 and 4 than all other days (P< 0.05) and on day 11 they were higher than other days and this was similar for day 8 except when compared to days 11 and 26. The amount in albumen was also higher on day 26 than day 43. On any individual collection day, the amount of corticosterone in albumen was not different when hens were housed 5, 4, 3 or 2 per cage or singly.

Week	Number of hens per cage				
	5	4	3	2	1
1	5.28 ± 0.21	5.82 ± 0.29	6.07 ± 0.25	5.22 ± 0.43	6.20 ± 0.25
2	5.40 ± 0.27	5.46 ± 0.38	6.07 ± 0.22	5.15 ± 0.56	6.50 ± 0.22
3	5.44 ± 0.20	5.80 ± 0.37	6.06 ± 0.25	5.70 ± 0.50	6.60 ± 0.27
4	5.36 ± 0.24	5.84 ± 0.31	5.73 ± 0.38	5.85 ± 0.33	6.20 ± 0.20
5	6.02 ± 0.12	5.47 ± 0.38	5.89 ± 0.32	6.05 ± 0.19	6.30 ± 0.30
6	6.13 ± 0.14	5.98 ± 0.31	6.24 ± 0.28	6.35 ± 0.18	6.00 ± 0.39
7	5.08 ± 0.14	5.25 ± 0.21	5.43 ± 0.24	5.30 ± 0.15	5.60 ± 0.34
8	5.78 ± 0.17	6.03 ± 0.16	6.17 ± 0.39	6.40 ± 0.16	6.40 ± 0.37
9	6.15 ± 0.22	5.97 ± 0.23	6.02 ± 0.23	6.45 ± 0.39	6.40 ± 0.31
10	5.93 ± 0.19	6.13 ± 0.26	6.27 ± 0.31	6.05 ± 0.44	6.20 ± 0.55
11	5.83 ± 0.26	5.95 ± 0.26	5.83 ± 0.29	6.00 ± 0.45	6.10 ± 0.28
12	6.33 ± 0.30	6.43 ± 0.33	6.73 ± 0.26	6.33 ± 0.46	7.00 ± 0.27
13	5.65 ± 0.22	5.50 ± 0.32	5.62 ± 0.26	5.44 ± 0.40	5.73 ± 0.36
14	6.04 ± 0.29	6.02 ± 0.21	6.20 ± 0.20	6.03 ± 0.39	5.91 ± 0.31
15	6.20 ± 0.28	5.56 ± 0.21	6.36 ± 0.31	6.44 ± 0.29	7.27 ± 0.24
16	5.79 ± 0.31	5.92 ± 0.30	5.88 ± 0.24	6.22 ± 0.22	6.36 ± 0.28

Table 3.1: The mean (± SEM) weekly egg production for hens maintained in conventional cages at 5, 4, 3, or 2 hens per cage or a single hen per cage.

Day after being moved into cages	Number of hens per cage				
	5	4	3	2	1
1	65.0 ± 0.9	65.0± 0.9	67.2 ± 1.2	62.6 ± 1.4	64.1 ± 2.2
2	65.0 ± 1.1	65.8 ± 1.0	65.5 ± 1.0	63.4 ± 1.3	63.4 ± 2.6
4	65.4 ± 0.8	65.3 ± 1.2	63.8 ± 0.9	63.0 ± 1.6	62.4 ± 1.1
8	63.3 ± 1.0	65.4 ± 1.0	65.3 ± 0.9	61.9 ± 1.1	64.2 ± 2.1
11	64.9 ± 1.2	64.5 ± 0.9	64.3 ± 1.7	63.2 ± 1.4	62.5 ± 2.8
16	63.6 ± 0.8	63.9 ± 1.1	64.4 ± 0.8	64.8 ± 1.0	64.5 ± 1.5
26	66.2 ± 1.1	66.0 ± 1.1	64.1 ± 1.0	64.8 ± 1.1	65.3 ± 1.5
43	66.0 ± 1.0	67.7 ± 0.9	66.6 ± 1.0	64.4 ± 1.4	63.9 ± 2.3
54	68.3 ± 1.1	66.9 ± 1.3	66.8 ± 0.8	64.9 ± 1.2	66.5 ± 1.7
83	65.1 ± 1.2	67.4 ± 1.2	67.8 ± 1.7	65.1 ± 1.6	69.8 ± 1.8
110	67.0 ± 1.1	68.1 ± 1.1	66.5 ± 1.1	65.9 ± 1.5	66.9 ± 2.1

Table 3.2: The mean (± SEM) egg weight (g) for hens maintained at 5, 4, 3, or 2 hens per cage or as a single hen per cage.

Day after being moved into cages	Number of hens per cage				
	5	4	3	2	1
1	37.8 ± 0.8	37.0 ± 0.7	39.3 ± 1.2	35.6 ± 1.1	36.1 ± 1.9
2	37.4 ± 1.0	36.9 ± 0.9	38.2 ± 0.7	35.1 ± 0.9	37.2 ± 1.8
4	37.6 ± 0.8	37.8 ± 1.1	36.6 ± 0.8	35.5 ± 1.1	34.0 ± 1.0
8	37.2 ± 0.6	37.5 ± 0.8	37.9 ± 0.9	34.9 ± 0.8	36.4 ± 2.0
11	37.2 ± 1.1	37.0 ± 0.6	37.4 ± 0.7	34.9 ± 0.9	34.4 ± 2.0
16	36.6 ± 0.7	35.3 ± 0.9	36.8 ± 0.8	35.3 ± 0.7	35.6 ± 1.0
26	37.6 ± 1.0	36.5 ± 0.9	36.5 ± 0.7	35.7 ± 0.7	35.8 ± 1.7
43	38.3 ± 0.8	38.4 ± 0.5	39.5 ± 0.9	36.4 ± 0.9	36.6 ± 1.7
54	39.2 ± 0.8	38.0 ± 1.0	37.3 ± 0.8	35.6 ± 0.8	37.2 ± 1.6
83	36.5 ± 0.9	37.4 ± 1.1	37.9 ± 1.3	35.9 ± 1.0	37.0 ± 1.6
110	38.3 ± 0.8	38.5 ± 0.8	37.5 ± 0.9	36.6 ± 0.9	38.1 ± 2.1

Table 3.3: The mean (± SEM) albumen weight (g) for hens maintained at 5, 4, 3, or 2 hens per cage or as a single hen per cage.

Day after being moved into cages	Number of hens per cage				
	5	4	3	2	1
1	2.13 ± 0.29	1.53 ± 0.39	2.40 ± 0.22	2.56 ± 0.54	2.14 ± 0.70
2	2.58 ± 0.34	2.23 ± 0.31	2.44 ± 0.31	2.67 ± 0.46	2.22 ± 0.72
4	2.46 ± 0.22	3.37 ± 0.32	2.63 ± 0.47	2.28 ± 0.56	2.70 ± 0.58
8	3.37 ± 0.20	3.03 ± 0.41	2.83 ± 0.44	2.45 ± 0.57	2.44 ± 1.09
11	2.06 ± 0.12	3.09 ± 0.37	1.76 ± 0.10	2.16 ± 0.63	1.50 ± 0.40
16	3.02 ± 0.34	2.09 ± 0.28	2.08 ± 0.39	1.80 ± 0.56	3.56 ± 0.73
26	2.41 ± 0.35	2.49 ± 0.41	3.03 ± 0.75	1.80 ± 0.30	1.90 ± 0.57
43	2.81 ± 0.22	2.71 ± 0.33	2.62 ± 0.48	2.15 ± 0.30	3.44 ± 0.75
54	2.93 ± 0.33	3.07 ± 0.28	2.10 ± 0.38	2.13 ± 0.41	2.80 ± 0.74
83	1.74 ± 0.29	2.06 ± 0.50	1.00 ± 0.29	1.17 ± 0.31	0.89 ± 0.42
110	1.52 ± 0.17	1.86 ± 0.30	1.32 ± 0.29	2.25 ± 0.63	1.44 ± 0.53

Table 3.4: Mean (± SEM) oviposition times (h) for hens maintained at 5, 4, 3, or 2 hens per cage or as a single hen per cage.

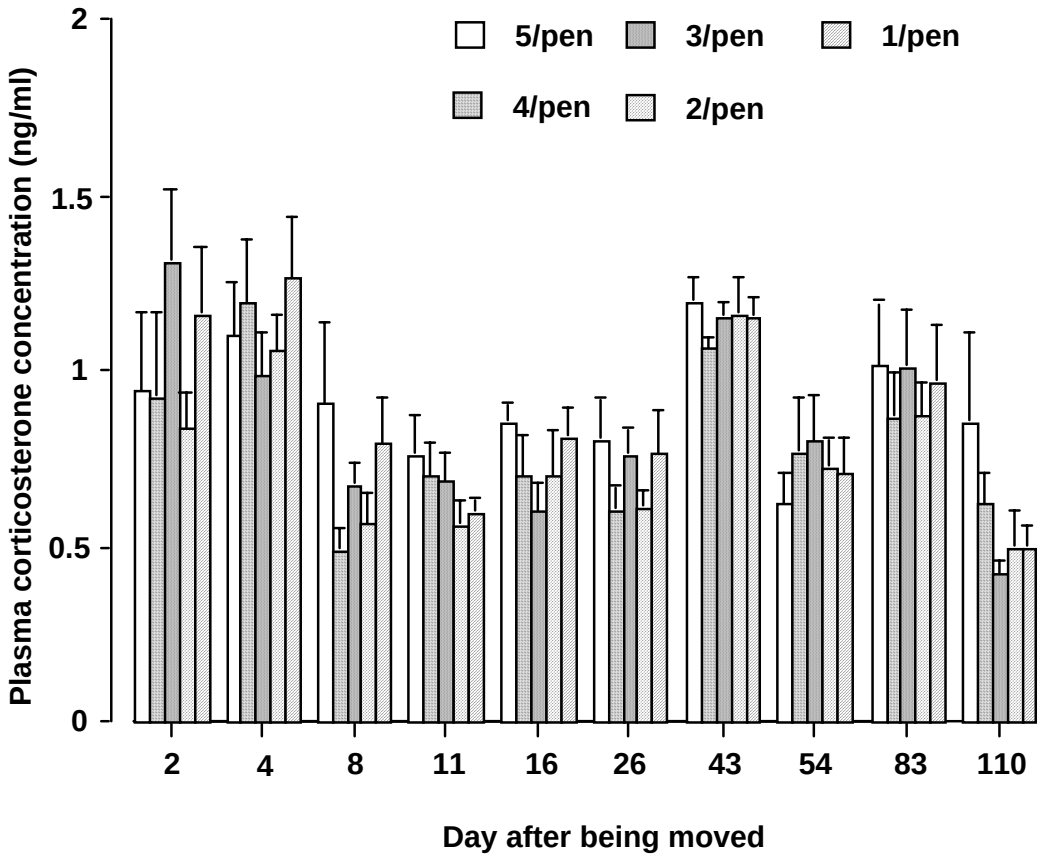

Figure 3.1: The mean (± SEM) plasma corticosterone concentration for hens maintained at 5, 4, 3 or 2 hens per cage or as a single hen per cage.

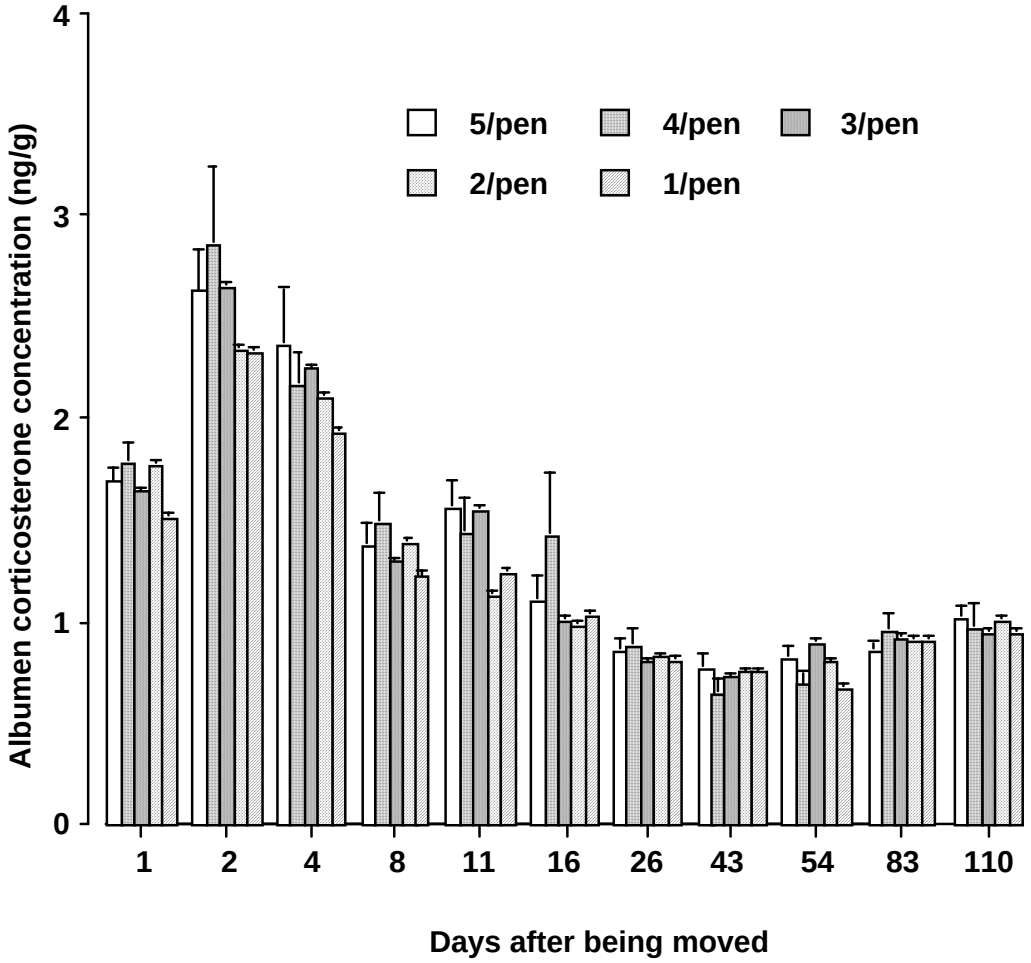

Figure 3.2: The mean (± SEM) egg albumen corticosterone concentration for hens maintained at 5, 4, 3 or 2 hens per cage or as a single hen per cage.

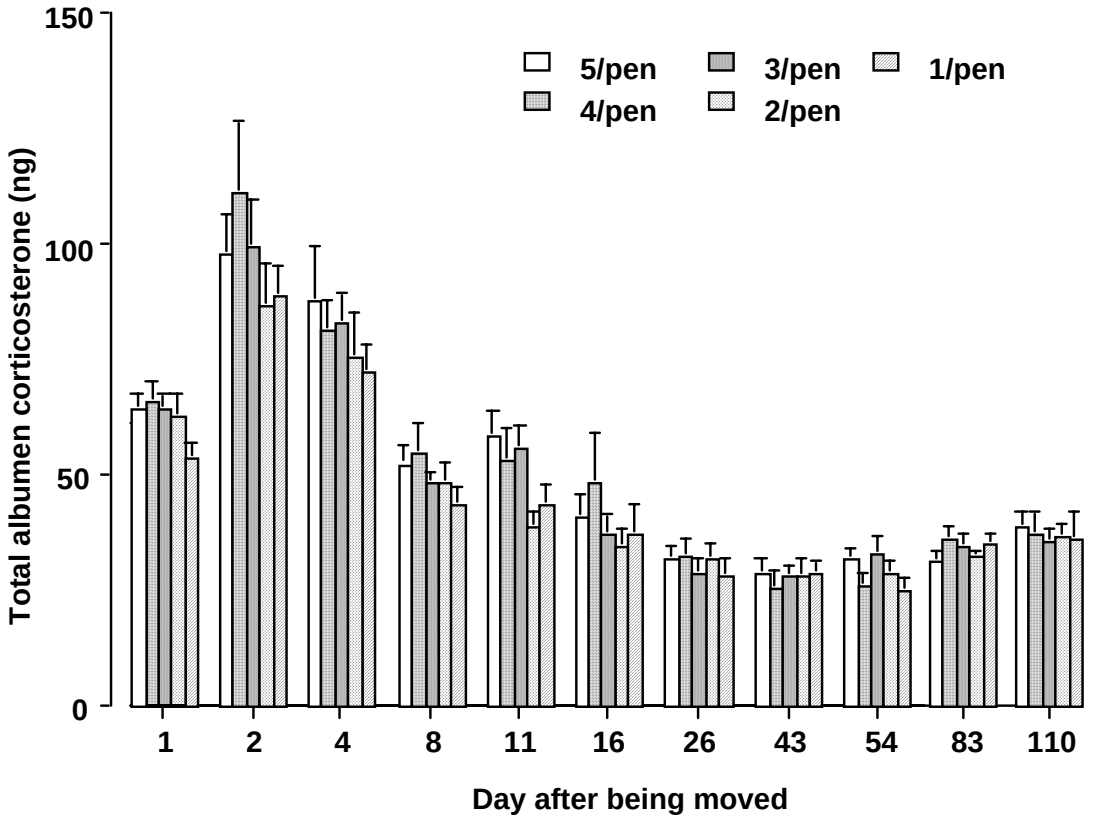

Figure 3.3: The mean (± SEM) total amount of corticosterone in albumen for hens maintained at 5, 4, 3 or 2 hens per cage or as a single hen per cage.

3.5. Discussion

Like many other studies, in the present study, no clear distinction can be made between any stress effects associated with either differences in cage density (cm^2/bird) or the number of hens in the group. Therefore, potentially any effects on stress could be a contribution from the effects due to available space and the influence of social interactions associated with group size.

As the group size increased, and consequently the space allowance decreased, no significant effects on plasma corticosterone concentrations were observed. On individual sampling days the number of hens in the cage had no effect on the egg albumen corticosterone concentration. The egg albumen concentrations were higher on days 2 and 4 after the hens were moved to the group cages. These gradually decreased by day 16 and then remained at a similar level for the remainder of the study. The increase in albumen concentrations observed on days 2 and 4 were most probably a result of stress associated with the moving of hens to a new novel environment. This effect has been observed and reported previously (Downing and Bryden, 2002).

Plasma corticosterone concentrations were higher on days 2 and 4 and on day 43. The higher concentrations on days 2 and 4 were consistent with the higher albumen concentrations on these days. Craig *et al,* (1986a) reported that moving hens and housing them individually in pens with a space of 2900 cm^2/hen or in cages at 949 cm^2/hen or groups of 4 (464 cm^2/hen) or 6 (310cm^2/hen) had various effects on plasma corticosterone. In all treatments the plasma corticosterone levels were elevated after moving especially over the first 5 days but had declined by 2 to 3 weeks (Craig *et al.*, 1986a). In the current study, the elevated plasma corticosterone concentration on day 43 did not coincide with higher egg albumen corticosterone concentrations on the same day. The blood samples

were taken on the day the albumen would have been deposited during egg formation but after it had been deposited, as the samples were collected after 1600h. Because of the difference in timing it can't be assumed that the concentrations are representative of the concentrations existing during albumen accumulation in egg formation. The plasma concentrations are those existing at a single time point. The hens used in this study were housed in a large facility with other hens and it is possible that some unforeseen event or activity caused the elevation in plasma corticosterone concentration on day 43. This is plausible because the plasma concentration was higher in all treatment groups on this day. The egg albumen corticosterone concentrations tended to be higher on days 8 and 11 compared to later days. This was not seen for the plasma samples, as concentrations were similar after day 4. As discussed earlier, plasma samples are taken at a single time point whereas egg albumen is accumulated over a period of 4-6 h. Small differences in plasma corticosterone during albumen accumulation might be magnified because of the time over which the albumen is accumulated.

Increasing the number of hens/cage from 1 (1394 cm^2/bird) to either 2 (696 cm^2/bird), 3 (465 cm^2/bird) or 4 (349 cm^2/bird) in one experiment and from 1 to either 3 or 5 (at 279 cm^2/bird) in a second experiment, affected plasma corticosterone concentrations (Lei *et al*., 1972). The difference in plasma corticosterone concentration when the space per hen went from 696 to 465 cm^2 was 0.5 ng/ml, from 465 to 347 cm^2 was 4 ng/ml and from 465 to 279 cm^2 was 6 ng/ml. Also, hens maintained in groups of 6 and provided with a floor space of 361 or 482 cm^2/bird showed no differences in plasma corticosterone at various sampling times during a full production cycle or a moult induced at the end of the production cycle (Davis *et al*., 2000). Hens housed in floor pens and six-bird cages had plasma corticosterone concentrations 12% higher than did hens housed in cages individually or 4 birds per cage. Although not significant, these differences tended to persist throughout the 22 weeks of the study (Craig *et al*., 1986). In the present study, at the highest density (5-bird cages) hens had a space allocation of 460cm^2/bird. From the observations made by Lei *et al*., 1972, Craig *et al*., (1986) and Davis *et al*., (2000) none, or at best, only a small effect on plasma corticosterone would be expected by maintaining hens at 460 cm^2/bird. During the current study there was a tendency for the egg albumen corticosterone concentrations to be higher at the larger group sizes and this resulted in a significantly lower total corticosterone in albumen for hens maintained singly or two per cage. The number of hens per cage had no effect on egg weight or albumen weight. There being no differences in egg albumen weight and egg albumen, the fact that total amount of corticosterone albumen was different suggests that the small differences in corticosterone concentration might be real.

The number of hens per cage had no significant effects on egg production although there was a tendency for the level to be lower for hens housed 5 per cage during some weeks, however, this was not a consistent pattern throughout the study. In laying hens, egg production has been shown to decrease with an increased in cage density (Dorminey *et al*., 1972; Lei *et al*., 1972; Feldkamp and Adams, 1973; Martin *et al*., 1980; Cunnignham and Ostrander, 1981; Quart and Adams, 1982; Mashaly *et al*., 1984; Adams and Craig, 1985; Patterson and Muir, 1986; Anderson and Adams, 1991; Lee and Craig, 1991; Brake and Peebles, 1992; Bell *et al*., 1998). Adams and Craig (1985) undertook a statistical analysis of published data looking at the effects of crowding on production performance. Three density categories were examined, low (432-561 cm^2/hen), medium (355-426 cm^2/hen) and high (271-348 cm^2/hen) and only studies using 2-6 hens per cage were used in their evaluation. The data indicated that there was a curvilinear relationship between density and production with performance decreasing more rapidly at higher densities. Reducing average space per hen from 387 to 310 cm^2/hen decreased egg production by approximately twice the level as decreasing the average space from 516 to 386 cm^2/hen. Decreasing the space per hen below 350-380 cm^2/bird seems to have a large influence on egg production (see section 1.6.2). In two strains of hens housed 4 or 8 hens per cage at 348, 464 and 580 cm^2/bird, production over one full cycle was lower at 348 cm^2/bird with no differences between hens at 464 and 580 cm^2/bird (Okpokho *et al*., 1987). When two strains of hens were housed in groups of 6, 8, 12 or 24 and the floor space maintained at 364 cm^2/bird and feeder space at 10 cm/bird, there were no effects on egg production and egg mass (Carey and Kuo, 1995). In the present study, at the highest cage density (5-bird cages) the space

allocation was 460cm^2/bird. From the above reports it was not unexpected to find no differences in egg production.

3.6. Implications

The space available to hens in conventional cages is a major objection to the use of this type of housing system. Both egg production and corticosterone concentrations are two of the criteria used when investigators are assessing welfare of laying hens. Using these criteria hens housed 5 per cage at 460cm^2/bird had similar egg production, plasma and egg albumen corticosterone concentrations as hens housed in smaller group sizes with more space per hen. The lower total corticosterone amount in albumen for hens housed singly or 2 per cage indicates some subtle changes in corticosterone secretion or accumulation although these are not totally obvious from the plasma or albumen corticosterone concentrations.

3.7. Recommendations

The space that is required by hens to ensure good welfare continues to be debated by different interest groups. Corticosterone is one measure of physiological function used to assess stress. The results of the present study suggest that if the space available is above some minimum than group size could have an important effect on stress in caged hens. There is a need for an extensive study evaluating what effects group size in conventional cages has on stress and wellbeing using a number of assessment criteria. A study of this type needs to avoid the compounding effects of group size and cage density, as well as ensuring things like feeder space are appropriate, so as to avoid stress resulting from competition for resources.

Chapter 4

The effects of high ambient temperature on the plasma and egg albumen corticosterone concentrations in laying hens

4.1. Introduction

Poultry are homeotherms with a need to maintain their core body temperature within narrow limits and to do this there is an integration of the nervous and endocrine systems resulting in a combination of behavioural and physiological responses during adjustments to ambient temperature. For hens the optimum ambient temperature is 21^0C (Charles, 1985). High producing commercial laying hens, need to maintain a high metabolic rate and this increases their susceptibility to heat stress (Blem, 2000). Also, the insulative effect of the plumage, lack of sweat glands and relatively low respiratory water evaporative rate increases the propensity of hens to heat stress (Etches *et al*., 1995).

Temperature is probably the most important environmental factor that influences egg production and egg quality. Prolonged exposure to high temperatures is associated with decreased egg production (Hester *et al*., 1996b; Muiruri and Harrison, 1991; Samara *et al*., 1996; Whitehead *et al*., 1998; Mashaly *et al*., 2004). However, increases in ambient temperature have only small effects on egg production until a critical temperature is reached and above this temperature can have a large effect. There is a rapid fall in egg production, egg quality and food intake if the temperature is greater than 28-30^0C (Al-Saffar and Rose, 2002). This is the temperature where panting and acute effects of heat stress begin.

Increased corticosterone concentration seems to be a critical requirement for survival during severe disruption to homeostasis, with chronic exposure seemingly damaging to health and welfare (Sapolsky, 1992). Temperature extremes increase plasma corticosterone concentration in chickens, laying hens and turkeys (El-Halawani *et al*., 1973; Brown and Nestor, 1973; Buckland *et al*., 1974; Edens and Seigel, 1975; Edens, 1978; Beuving and Vonder, 1978). Corticosterone has been measured in studies of high and low ambient temperature but few, if any, have investigated the plasma corticosterone concentrations in chronically stressed birds. Transient increases in corticosterone concentrations have been reported by others following acute episodes of heat (Etches *et al*., 1995; Siegel, 1995; Hester, 1996c). A major problem with many studies is that samples are taken infrequently and therefore give little indication of changes over time. Repeated exposure to heat stress can diminish the corticosterone response over time (Siegel and Gould, 1982).

Layer performance is depressed during heat stress with decreased food intake, altered nutrient absorption, respiratory alkalosis, decreased blood flow to some organs and endocrine changes (Sykes and Fataftah, 1986; Scott and Balnave, 1988; Etches, *et al*., 1995; Samara *et al*., 1996; McKee *et al*., 1997; Balnave and Muheereza, 1998; Wolfenson *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004). High ambient temperature decreases body weight (Scott and Balnave, 1988; Samara *et al*., 1996; Abel-Rahman, 2000; Garaces, 2001; Mashaly *et al.*, 2004). The changes in body weight are most probably associated with the changes in food intake.

Individual responses to heat stress are very variable (Arad and Mardar, 1984). Early stressful events experienced by animals can influence responses to subsequent stressors (Sapolsky, 1992). Many strains of laying hen have been developed in climates where they have had little opportunity to develop tolerance to heat. For these strains, acclimatisation and acclimation provide some protection against heat stress. At high temperatures the capacity for hens to increase heat loss is limited and so acclimatisation probably involves physiological adjustments that decrease heat production (Sykes and Fataftah, 1986).

While the tendency to house caged birds in controlled environment sheds will continue; the proportion of hens kept in alternative housing systems will also increase. Hens kept in free range and barn systems can be exposed to a wide range in ambient temperature and heat stress remains an important consideration in the welfare of these hens.

Changes in reproductive rate, morphology and physiology are criteria used in assessing hen welfare. High ambient temperature is a stressor of poultry. Changes in egg production, food intake and bodyweight are all end point measures of changes in physiological function when hens are stressed. A consistent non-specific response to stress is an increase in corticosterone 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.

4.2. Objective

In the study presented here the effects of long-term exposure to high ambient temperature is evaluated. End point measures of physiological function (egg production and food intake) are used as part of the assessment. Also both plasma and egg albumen corticosterone concentration and the total amount of corticosterone in albumen are used as indicators of acute and chronic stress.

4.3. Materials and methods

Birds and management: Isa Brown laying hens were used in the study. From 16 weeks of age the hens were housed individually in conventional cages (47 cm x 28 cm x 48 cm). Throughout the study hens were fed *ad libitum* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h of light with lights on at 0530h. At 36 weeks of age hens were allocated to individual pens (45 x 30 x 40 cm) and housed in one of three environmentally controlled rooms (36 hens/room).

Experimental design: To acclimatise the hens to the environment room and surroundings all rooms were set to maintain a temperature of 20^0C for the first two weeks. After this time, and designated as day one of the study, one room was maintained at 20^0C (moderate temperature) for the duration of the study, while another room was increased and maintained at 32^0C (high temperature). In the third room the temperature was maintained at 20^0C and then at 0900h, two days before each egg collection the temperature was increased to 32^0C (alternate temperature regime). The temperature was maintained at 32^0C until the completion of blood sampling when it was returned to 20^0C. The temperature treatments were maintained for a period of 12 weeks.

Egg collection: Individual egg production was recorded daily. On days 3, 8, 15, 22, 29, 39, 58, 70 and 82 after the start of the temperature treatments, all eggs laid by hens in all rooms were collected. Egg collection was started at 0830 h, 3 h after lights on. This was designated as zero oviposition time and so any egg laid before this time had an oviposition time of zero. Eggs were collected each hour after 0830 h until 1530 h. For eggs collected at 0930 h the oviposition time was recorded as +1 h, for those at 1030 h the oviposition time was +2 h and similarly the oviposition time increased 1 h for each additional hourly collection. The eggs were weighed then broken open and the albumen separated out and weighed and stored at -20^0C until assayed.

Blood sampling: Beginning at 1530h after the finish of the egg collection, a 1ml blood sample was taken from 15 hens in each room. In each room the hens were divided into two groups of 18 and the blood sampling was alternated between these two groups on consecutive weeks. After collection, the blood was centrifuged and the plasma harvested and stored at -20^{0}C until assayed.

Corticosterone determination: The corticosterone concentration in egg albumen and plasma were determined by RIA as described in section 2.3.2.

Feed Intakes and bodyweights: During the last two weeks of the study (weeks 11 and 12) feed intakes were determined for each treatment. The intakes were measured for groups of three birds (experimental unit) and therefore, there were 12 replicates for each room. Hens were weighed before and at the end of the study.

Statistical analysis: Values are given as means ± SEM. Differences between treatments were assessed by ANOVA and if significant ($p<0.05$) then multiple comparisons were made using the Tukey test. The analysis of egg production was done on the basis of individual weekly production. The analysis of feed intake was done on the mean weekly intake for the experimental unit. All analyses were conducted using the 'Statview' computer program (SAS Institute Inc, NC, USA).

4.4. Results

Body weight: When moved to the climate rooms at 36 weeks of age, the bodyweight of a random sample of 24 hens, was 2075 ± 35 g. The mean bodyweights at completion of the study were 2162 ± 38, 1798 ± 39 and 2057 ± 33 g for hens held at 20^{0}C, 32^{0}C or the alternate temperature, respectively. The bodyweight of the hens housed at the high temperature was significantly lower than the other treatments.

Egg production: Details of the mean egg production are given in Figure 4.1. While egg production was recorded daily for all hens, the data is given as the mean weekly hen production. There was a significant effect of temperature treatment on egg production ($P<0.0001$) but no significant effect due to week. For the two weeks before the start of the study, the egg production was similar for all treatments. Egg production of the hens housed at 20^{0}C or the alternate temperature regime was not different, although they tended to be lower in the early part of the study for the hens on the alternate temperature regime. For hens housed at 32^{0}C, egg production started to decline by week 3. From week 3 the production was significantly lower for hens at 32^{0}C compared to hens housed at 20^{0}C except for weeks 6 and 11. During weeks 8, 9, 10 and 12 egg production was significantly lower for hens at 32^{0}C than hens housed at the alternate temperature regime.

Egg weight: The mean (± SEM) egg weights are given in Table 4.1. At the first collection period (day 3), egg weight was lower for hens housed at 20^{0}C compared to the other treatments. For other collection days, the mean weight was significantly higher ($P<0.05$) for the hens housed at 20^{0}C compared to the other treatments. For hens housed at 32^{0}C and the alternate temperature regime, egg weight was similar on most collection days, however, they differed significantly ($P<0.05$) on collection days 29, 70 and 82.

Albumen weight: The mean (±SEM) albumen weights are given in Table 4.2. On initial sampling days the albumen weights were similar. On collection days 29 to 82, the albumen weights were significantly lower for the hens housed at 32^{0}C when compared to those housed at 20^{0}C. When hens were maintained under the alternate temperature regime, albumen weights were similar to other treatments, except on collection day 58 ($P<0.05$).

Oviposition times: The mean (± SEM) oviposition times are given in Table 4.3. In the early days of the study, oviposition times were similar although some differences were recorded on days 15 and 29 (see figure 3.3). Later in the study (days 58 to 82) consistent differences were observed. At this time the mean oviposition time of the hens held at 32^0C was significantly later ($P<0.05$) than for hens held at 20^0C. By day 70 hens experiencing the alternate temperature regime also tended to have delayed oviposition time compared to the hens held at 20^0C.

Feed intake: The mean feed intakes are given in Table 4.4. Hens housed at 20^0C had significantly higher ($P<0.05$) feed intakes than hens housed at 32^0C for both weeks 11 and 12 and the hens housed under the alternate temperature regime for week 12. Hens housed under the alternate temperature regime had higher intakes than those housed at 32^0C, however the difference was significant ($P<0.05$) only for week 11.

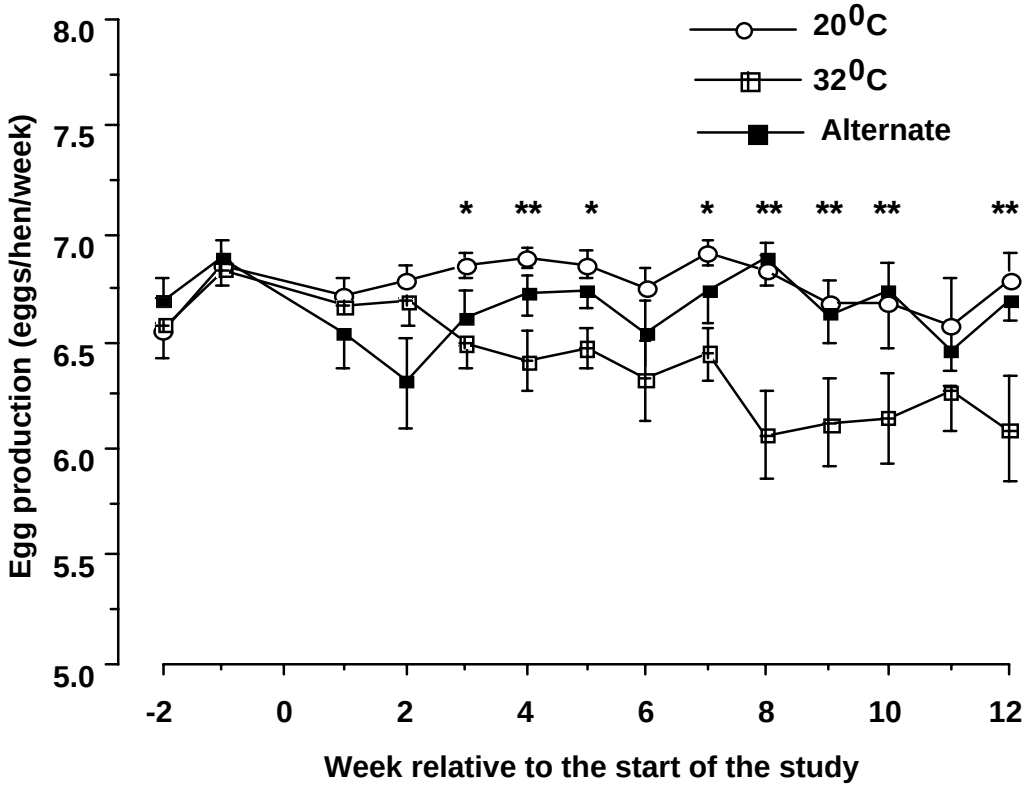

Figure 4.1: The mean (± SEM) egg production for hens housed continuously at 20^0C and 32^0C or the alternate temperature regime. For individual weeks, significant differences ($P<0.05$) between hens held at 32^0C compared to 20^0C are indicated by * and significant differences between hens held at 32^0C compared to those at 20^0C and the alternate temperature regime are indicated **.

Day after the start of the study	Treatment temperature		
	20^0C	32^0C	Alternate (20^0C and 32^0C)
3	52.4 ± 0.7^b	58.6 ± 0.8^a	58.4 ± 0.7^a
8	60.6 ± 0.5^a	58.0 ± 0.8^b	57.9 ± 0.7^b
15	61.2 ± 0.6^a	$57.7 \pm 0.1.3^b$	57.5 ± 0.7^b
22	61.6 ± 0.6^a	57.5 ± 0.7^b	56.9 ± 0.8^b
29	62.0 ± 0.7^a	57.1 ± 0.8^c	58.6 ± 0.9^b
39	61.9 ± 0.7^a	56.2 ± 0.8^b	57.9 ± 0.9^b
58	62.8 ± 0.7^a	55.5 ± 0.8^b	59.8 ± 0.7^b
70	62.9 ± 0.7^a	56.6 ± 1.0^c	59.7 ± 0.7^b
82	62.5 ± 0.5^a	56.4 ± 0.9^c	60.1 ± 0.8^b

Table 4.1: The mean (±SEM) egg weight (g) for hens housed continuously at 20^0C and 32^0C or at 20^0C and then 32^0C for two days before egg collection (alternate temperature regime). Within rows, values with different superscripts are significantly different ($p<0.05$).

Day after the start of the study	Treatment temperature		
	20^0C	32^0C	Alternate (20^0C and 32^0C)
3	34.3 ± 0.9	32.2 ± 0.9	33.0 ± 0.7
8	34.0 ± 0.4	32.6 ± 0.8	32.2 ± 0.6
15	34.5 ± 0.5^{a}	32.2 ± 0.9^{b}	32.7 ± 0.6^{ab}
22	33.9 ± 0.5	32.3 ± 0.7	32.5 ± 0.6
29	35.0 ± 0.5^{a}	32.4 ± 0.5^{b}	33.6 ± 0.7^{ab}
39	34.8 ± 0.5^{a}	32.2 ± 0.7^{b}	33.3 ± 0.7^{ab}
58	36.3 ± 0.8^{a}	31.6 ± 0.7^{c}	33.9 ± 0.5^{b}
70	35.7 ± 0.6^{a}	32.0 ± 1.0^{b}	33.3 ± 0.7^{ab}
82	35.5 ± 0.4^{a}	32.8 ± 0.7^{b}	33.8 ± 0.6^{ab}

Table 4.2: The mean (±SEM) albumen weight (g) for hens housed continuously at 20^0C and 32^0C or at 20^0C and then 32^0C for two days before egg collection (alternate temperature regime). Within rows, values with different superscripts are significantly different ($p<0.05$).

Day after the start of the study	Treatment temperature		
	20^0C	32^0C	Alternate (20^0C and 32^0C)
3	0.17 ± 0.10	0.24 ± 0.09	0.38 ± 0.13
8	0.39 ± 0.16	0.48 ± 0.16	1.39 ± 0.36
15	0.14 ± 0.07^b	0.97 ± 0.25^a	0.84 ± 0.25^a
22	0.28 ± 0.12	0.94 ± 0.27	0.77 ± 0.19
29	0.13 ± 0.08^b	0.57 ± 0.14^a	0.41 ± 0.15^{ab}
39	0.41 ± 0.21	0.53 ± 0.21	0.76 ± 0.26
58	0.14 ± 0.08^b	1.23 ± 0.38^a	0.38 ± 0.12^a
70	0.41 ± 0.21^b	1.38 ± 0.27^a	0.90 ± 0.25^{ab}
82	0.32 ± 0.11^b	1.57 ± 0.32^a	0.84 ± 0.25^{ab}

Table 4.3: The mean (±SEM) oviposition times (h) for hens housed continuously at 20^0C and 32^0C or at 20^0C and then 32^0C for two days before egg collection (alternate temperature regime). Within rows values with different superscripts are significantly different ($p<0.05$).

Week after the start of the temperature treatment	Temperature treatment		
	20^0C	32^0C	Alternate
Week 11	916 ± 17^{ab}	739 ± 32^{c}	863 ± 26^{b}
Week 12	1001 ± 25^{a}	760 ± 17^{b}	811 ± 28^{b}

Table 4.4: The mean (±SEM) feed intake (g) for hens housed at 20^0C, 32^0C or an alternate temperature regime. Within rows values with different superscripts are significantly different ($p<0.05$).

Plasma corticosterone concentration: The mean (±SEM) plasma corticosterone concentrations are shown in Figure 4.2. There was no significant effect due to the temperature treatment but the day of collection had a significant effect on plasma corticosterone. The concentration on day 3 was higher than all other collection days. The concentration on day 82 was higher than day 15 and all collection days after this time.

Albumen corticosterone concentration: The mean (± SEM) albumen corticosterone concentrations are shown in Figure 4.3. There was a significant effect of treatment and day of collection however, no significant interactions between treatment and day. Over the period of the study, albumen corticosterone concentration was higher in the hens at 32^0C (1.12 ± 0.02 ng/g) compared to those at 20^0C (1.04 ± 0.02 ng/g) but not the hens on the alternate regime (1.09 ± 0.02 ng/g). On any individual collection day there were no significant treatment effects.

Total albumen corticosterone: The mean (± SEM) total amount of corticosterone found in egg albumen is shown in Figure 4.4. There was no significant treatment effect. There was a significant day of collection effect. The amount of corticosterone in albumen was higher on day 15 than other days. For both days 3 and 82 the amount of corticosterone in albumen was higher than on days 8, 29, 39 and 70. The total amount of corticosterone found in albumen on days 22 and 58 was greater than on days 8 and 70 and the amount on day 39 was greater than on day 70. On any individual collection day there was no effect of temperature treatment on the total amount of corticosterone found in egg albumen.

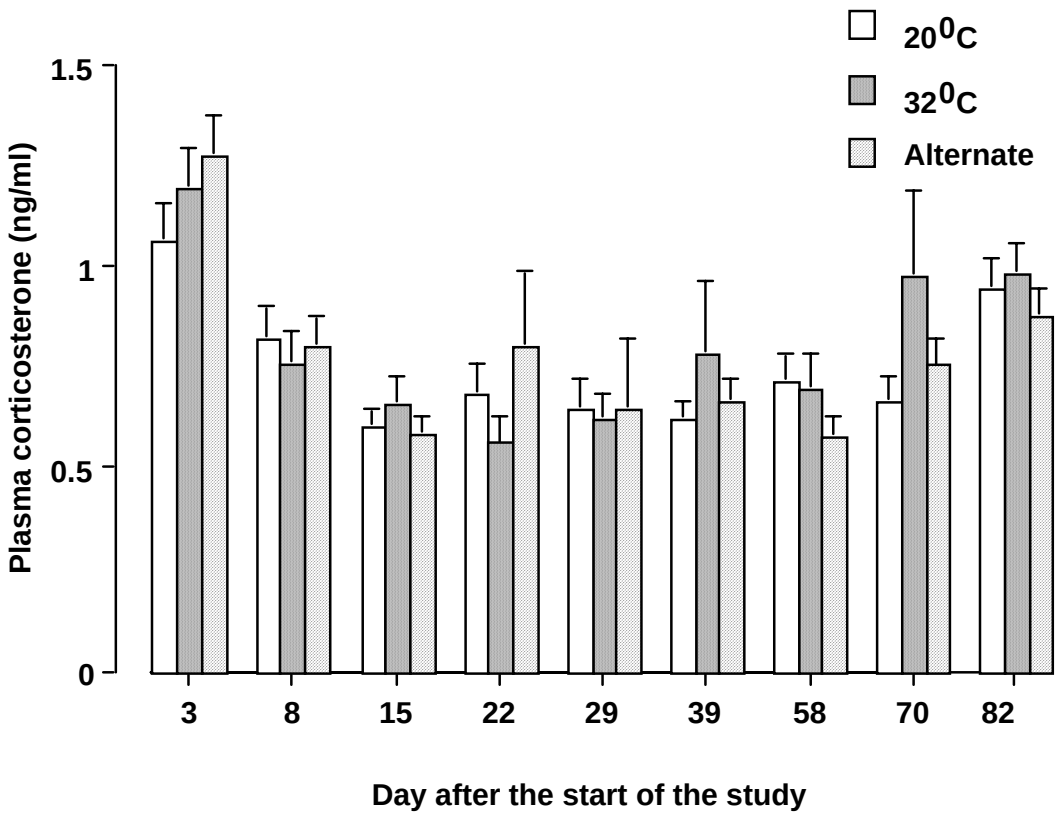

Figure 4.2: The mean (± SEM) plasma corticosterone concentration for hens housed continuously at 20^0C and 32^0C or at 20^0C and then 32^0C for two days before egg collection (alternate temperature regime).

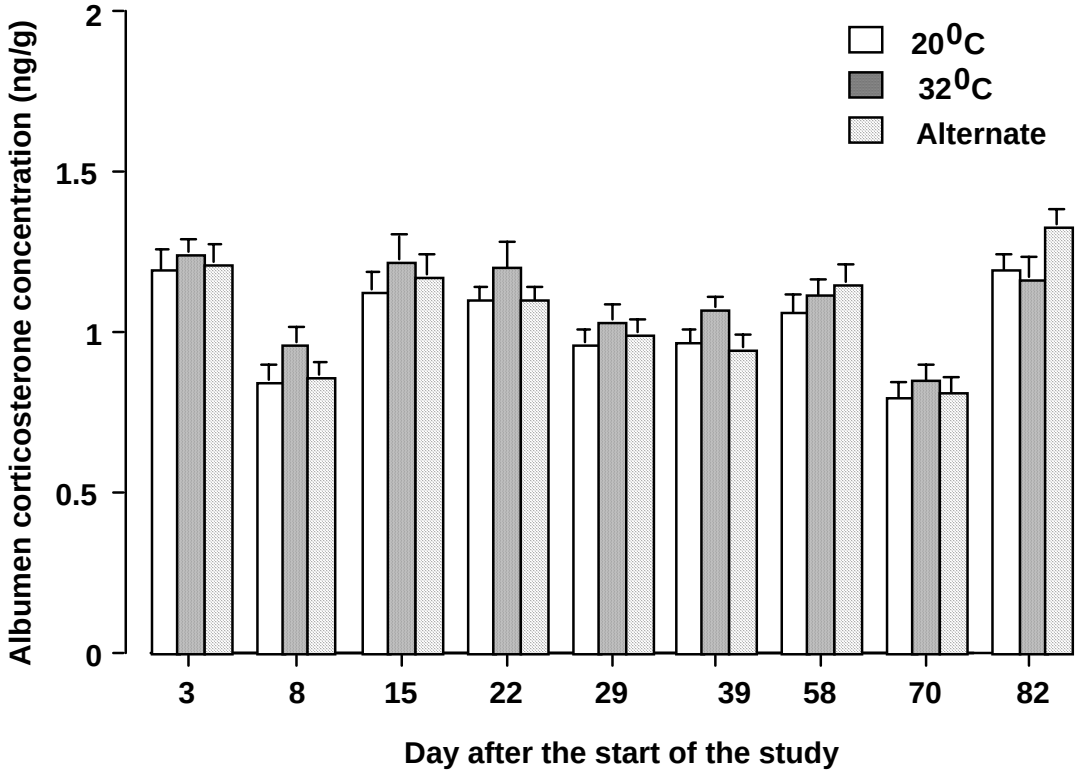

Figure 4.3: The mean (± SEM corticosterone concentration in albumen for hens housed at continuously at 20^0 and 32^0C or at 20^0 and then 32^0C for two days before egg collection (alternate temperature regime).

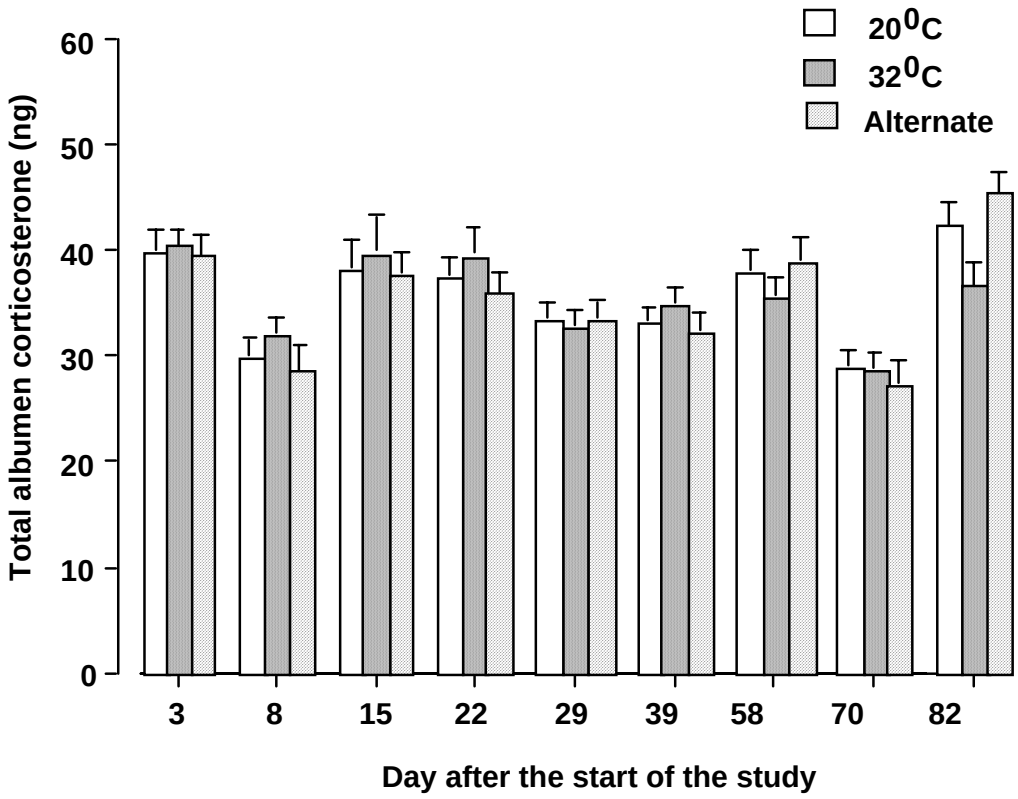

Figure 4.4: The mean (± SEM) total amount of corticosterone in egg albumen for hens housed at continuously at 20^0C and 32^0C and at 20^0C and then 32^0C for two days before egg collection (alternate temperature regime).

4.5. Discussion

Heat is a stressor of hens. Changes in egg production, food intake and bodyweight are all end point measures of changes in physiological function when hens are stressed. At the start of the study body weight was 2075 ± 35 g and for the hens maintained at 20^0C and the alternate temperature regime body weights were similar to this at the end of the study (2162 ± 38 and 2057 ± 33 g respectively). However, hens maintained at 32^0C had a significantly lower body weight at the end of the study (1798 ± 39 g). The changes in body weight are likely to be related to the differences in feed intake. At the end of the study feed intake was significantly higher for the hens housed at 20^0C and the alternative temperature compared to the hens at 32^0C.

Heat stress is reported to decrease body weight and feed intake. Also, to alter nutrient absorption, cause respiratory alkalosis, decrease blood flow to some organs and alter secretions from various endocrine glands (Sykes and Fataftah, 1986; Scott and Balnave, 1988; Etches, *et al*., 1995; Samara *et al*., 1996; McKee *et al*., 1997; Balnave and Muheereza, 1998; Wolfenson *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004). From these reports it is not unexpected to see the lower body weight and feed intake in the hens housed continuously at 32^0C. In laying hens, cyclic periods of high temperature (15.6-35^0C) had no effect on food intake (Deaton *et al*., 1981). In a more recent study, hens were maintained at constant temperatures of 23.9^0C and 35^0C or subjected to a daily cyclic temperature regime ranging from 23.9^0C to 35^0C (Mashaly *et al.,* 2004). Feed intake decreased as the severity of the heat stress increased. In the present study, hens subjected to the alternate temperature treatment were exposed to a heat stress for 2 days prior to each sampling period. While during this

period, feed intake was probably reduced the period of heat stress was not sufficiently severe enough to affect body weight.

High temperature decreased weekly egg production. Egg production for hens maintained at 20^0C and the alternate temperature were similar. At 32^0C egg production was lower and the decline became obvious after about 3 weeks at this temperature. The decline in egg production continued until about week 8 where it remained at this level for the remainder of the study. Temperature is probably the most important environmental factor that influences egg production and egg quality. Prolonged exposure to high temperatures is associated with decreased egg production (Hester *et al*., 1996b; Muiruri and Harrison, 1991; Samara *et al*., 1996; Whitehead *et al*., 1998; Mashaly *et al*., 2004). There is a rapid fall in egg production, egg quality and food intake when the temperature is greater than 28-30^0C (Al-Saffar and Rose, 2002). High ambient temperature decreases egg weight (de-Andrade, 1976; Emery *et al*., 1984; Balnave and Muheereza, 1997; Samara *et al*., 1996; Scheideler *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004). From the day 8 sampling, egg weight for hens at 20^0C was significantly higher than for the other treatments. Egg weight was determined on the day of egg collection at a time when hens from the alternate temperature regime had been maintained at 32^0C for two days. Food intake measures were made on a weekly basis and these indicated limited effects of the alternate treatment regime. The lower egg weight for this treatment would suggest that the effect of maintaining hens at 32^0C probably had immediate effects on feed intake. The changes in egg production and egg weight are most probably associated with the changes in feed intake. The differences in egg weight occur earlier than the observed differences in albumen weight. Albumen weight was lower for the hens housed at 32^0C compared to those at 20^0C from the 29 day sampling until the end of the study (day 82) although they tended to be lower from the very first sampling day. This may suggest that the earlier effects on egg weight might be due to differences in shell weight.

Delays in time of oviposition are considered by some to be associated with stress in laying hens (Carter, 1977: Hughes and Gilbert, 1984; Hughes *et al*., 1986; Mills *et al*., 1991; Reynard and Savory, 1999) and these delays often result in the laying of eggs with abnormal shells. Administration of exogenous adrenalin increases the incidence of abnormal eggs laid (Hughes *et al.*, 1986: Downing and Bryden, 2002). There is evidence indicating that corticosterone can also affect oviposition time and the incidence of abnormal eggs (Klingensmith *et al*., 1984; Downing and Bryden, 2002). It was predicted that adrenalin affected uterine motility and shell gland contraction (Hughes and Black, 1976). While adrenalin was not measured in the current study, the delay in oviposition could be the result of increased adreno-medullary function.

On individual collection days, plasma corticosterone concentration was not affected by the temperature treatment. Plasma concentrations were higher on day 3 compared to other days and on day 82 compared to collection days after day 8. There appears to be no apparent reason for the higher level on day 3 as the hens were acclimatised to the rooms for 2 weeks before the treatments were applied. Temperature extremes increase plasma corticosterone concentration in chickens, laying hens and turkeys (El-Halawani *et al*., 1973; Brown and Nester, 1973; Buckland *et al*., 1974; Edens and Seigel, 1975; Edens, 1978; Beuving and Vonder, 1978: Downing and Bryden, 2002). The egg albumen corticosterone concentration was not different between treatments on individual collection days, although over the period of the study the concentration was higher in the hens maintained at 32^0C. This difference was not observed for the total amount of corticosterone in albumen. The concentration effects are probably related to the differences in albumen weights discussed earlier.

In an earlier study, hens housed at 30^0C had higher plasma and egg albumen corticosterone concentrations than hens housed at 20^0C (Downing and Bryden, 2002). In that study, the sampling was done after 28-32 weeks of exposure to the elevated temperature and hens were housed two per cage. In the present study, hens were held in the elevated temperature for 12 weeks. Maintaining hens at the high temperature in the present study probably resulted in physiological responses that allowed hens to cope with the changed environment. These 'coping strategies' probably resulted in maintenance of homeostasis and normal corticosterone concentrations. However, there are signs, towards the end of the present study, that the coping strategies may have been under pressure to

accommodate the effects of the high temperature. At 32^0C hens had decreased feed intake, had lost significant bodyweight and egg weight decreased with time and the oviposition time was extended. An increased period of exposure at this temperature might initiate more extensive physiological adjustments in an effort to maintain homeostasis which could increase adrenal activity, this was observed when hens are held at a temperature of 30^0C for 28-32 weeks (Downing and Bryden, 2002). Also in this earlier study having two hens in the cage might be responsible for an additive effect of imposing a social stress in addition to the temperature stress. It is also possible, that by having the hens in close proximity, the effective ambient temperature was higher when the birds were housed 2 per cage.

4.6. Implications

Results from the present study indicate that hens held at a temperature of 32^0C make physiological adjustments as determined by changes in egg production, body weight and feed intake in an effort to function in high temperature. However, these adjustments do not seem to have involved chronic elevation in corticosterone secretion during a moderate period of high temperature.

4.7. Recommendations

The duration of the present study was for 12 weeks. Towards the end of this period there was evidence that hens were beginning to have some difficulty coping with high temperature. Under normal production systems hens are not usually required to cope with the high temperatures continually. The results indicate that hens are capable of coping adequately with a temperature of 32^0C for a period but this might be close to their limitations before they experience difficulty in coping. Being at their physiological limit, any stressor imposed in addition to heat could readily cause a severe stress response.

Chapter 5

The effects of short-term exposure to a range of environmental temperatures on plasma and egg albumen corticosterone concentrations and the total amount of corticosterone in albumen

5.1. Introduction

Poultry are homeotherms with a need to maintain their core body temperature within narrow limits and this is done by an integration of the nervous and endocrine systems. The effects result in a combination of behavioural and physiological responses that allow the hen to cope with the changes in ambient temperature. For hens the optimum ambient temperature is 21^0C (Charles, 1985). In chapter 4, the effects of exposing hens to high ambient temperature on corticosterone concentrations and egg production were examined. In naturally ventilated sheds and different climatic zones hens are exposed to a wide range of ambient temperature.

Changes in reproductive rate, morphology and physiology are criteria that are used when assessing hen welfare. Temperature is probably the most important environmental factor that influences egg production and egg quality. As discussed in chapter 4 high temperature will depress egg production, however so will low ambient temperature. Egg production is depressed when the housing temperature was lowered to 0^0C from 18-24^0C, and the temperature increased from 30^0C to 38^0C for 3 h (Hester *et al*., 1996b). The hens used in this study were lines selected for survivability and production in multi-bird cages and an unselected line. The selected line performed better under both temperature extremes and this may suggest that the ability of hens to cope with social stress may provide some measure that allows them to better cope with the climatic stress. Turkeys selected for either high or low corticosterone response to cold temperature had improved egg production in the low corticosterone line (Brown and Nester, 1973; 1974). Feed intake is higher and feed conversion efficiency better in cooler temperatures (Payne, 1966; Siegel, 1971; Stockland and Blaylock, 1974).

Male turkeys that are not heat stressed show a rhythm in plasma corticosterone concentration, peaking at 1000h, then falling to a constant level between 1400 and 2200h and then a decline to the lowest levels at 0600h (El-Halawani *et al*., 1973). However, both, heat- (32^0C) or cold-stressed (7^0C) birds show no rhythm and maintain high levels over the 24h-treatment period. Temperature extremes increase plasma corticosterone concentration in chickens, laying hens and turkeys (El-Halawani *et al*., 1973; Brown and Nester, 1973; Buckland *et al*., 1974; Edens and Seigel, 1975; Edens, 1978; Beuving and Vonder, 1978). In temperate climates laying hens can be exposed to short periods of low temperature. While the tendency to house caged hens in controlled environment sheds will continue; the proportion of hens kept in alternative housing systems will increase. Hens kept in free range systems can be exposed to a wide range in ambient temperature and heat stress remains an important consideration in the welfare of these hens. There are a large number of reports detailing

various aspects of high ambient temperature on stress in layers, however, there is limited information in the literature as to the effects of cold exposure on egg production and corticosterone concentrations in laying hens. In cold climates hens are maintained in insulated housing systems and are therefore protected from extreme cold. In temperate climates laying hens can be exposed to short periods of low temperature.

5.2. Objective

In the study presented here, the effects of short-term exposure to various ambient temperatures (10-33^{0}C) is evaluated. End point measures of physiological function such as feed intake and egg production are used as part of the assessment. Also, both plasma and egg albumen corticosterone concentration and the total amount of corticosterone in albumen are used as indicators of stress.

5.3. Materials and methods

Birds and management: Thirty six Isa Brown hens, 42 weeks of age, were allocated to individual cages and housed in an environmentally controlled room. Throughout the study hens were fed *ad libitum* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h of light with lights on at 0700 h. To help acclimitise hens to the environmentally controlled room, they were housed at the thermoneutral temperature of 20^{0}C, for four weeks before the study commenced.

Experimental protocol: The study was run over a period of 12 weeks with the temperature alternating, on a weekly basis, between the treatment temperatures and the basal temperature (20^{0}C). The pattern of ambient temperature was:

Week 1 - temperature maintained at 25^{0}C
Week 2 - temperature maintained at 20^{0}C
Week 3 - temperature maintained at 15^{0}C
Week 4 - temperature maintained at 20^{0}C
Week 5 - temperature maintained at 30^{0}C
Week 6 - temperature maintained at 20^{0}C
Week 7 - temperature maintained at 10^{0}C
Week 8 - temperature maintained at 20^{0}C
Week 9 - temperature maintained at 36^{0}C
Week 10 - temperature maintained at 20^{0}C
Week 11 - temperature maintained at 33^{0}C
Week 12 - temperature maintained at 20^{0}C

Feed Intakes and bodyweights: Hens were weighed at the beginning and end of the study. Feed intakes were determined on a weekly basis and water intakes were determined over a 24h period on the fifth day of each weekly treatment period. Both feed and water intakes were determined for groups of three hens (experimental unit) therefore there were 12 replicates of this experimental unit.

Egg collection: Individual egg production was recorded daily. On the seventh day of each of weeks 1, 2, 3, 5, 7, 11 and 12 all eggs laid were collected. Egg collection was started at 0800h, 1h after lights on. This was designated as zero oviposition time and so any egg laid before this time was considered to have an oviposition time of zero. Eggs were collected each hour after 0800h until 1600h. Therefore, those eggs collected at 0900h were assigned an oviposition time of +1h, and those at 1000h a time of +2h and similarly the oviposition time increased 1h for each additional hourly collection. The eggs were weighed then broken open and the albumen separated out and weighed and then stored at -20^{0}C until assayed.

Blood sampling: Beginning at 1600h after the finish of the egg collection, a 1 ml sample of blood was taken from 18 hens. The hens were divided into two groups of 18 hens and the blood sampling was alternated between these two groups on different weeks. After collection, the blood was centrifuged and the plasma harvested and stored at -20^{0}C until assayed.

Corticosterone measurement: The concentrations of corticosterone in egg albumen and the plasma samples were determined by RIA as described in section 2.3.2.

*Statistical analysis***:** Values are given as the mean ± SEM. Differences between treatments were assessed by ANOVA and if significant ($p<0.05$) then multiple comparisons were made using the Turkey/Kramer test (Statview', SAS Institute, Cary. NC, USA). The analysis of plasma and egg albumen corticosterone levels and oviposition time were conducted on individual hen responses whereas for feed and water intake the analysis was conducted on the individual mean for each experimental unit. Egg production was analysed using the Kruskal-Wallis Non-parametric ANOVA (Instat'program).

5.4. Results

At the start of week 9 the temperature was raised to 36^{0}C. During the first day of this treatment it became obvious that hens were going to have difficulty coping at this ambient temperature. When hens were exposed to an ambient temperature of 38^{0}C, the initial period of exposure before the rectal temperature reached 45^0 C was 1h (Sykes and Fataftah, 1986). A rectal temperature of 45^{0}C seems to be the upper limit of safety for hen survival (Wilson, 1948). In consideration of the hens welfare the treatment was terminated and the temperature of the room returned to 20^{0}C. The temperature remained at 20^{0}C for the remainder of week 9 and then week 10 as for the treatment protocol. The data from week 9 were not included in the statistical analysis.

Body weight: There was no significant difference in bodyweight. At the start of the study, the mean (± SEM) bodyweight was 1945 ± 30 g and at the end of the study it was 1962 ± 31 g.

Feed intake: The mean (± SEM) feed intakes are given in Figure 5.1. There was no consistent pattern in feed intake that seemed to be related to ambient temperature. However, there were some significant differences. When hens were housed at 33^{0}C and 25^{0}C the feed intake was significantly lower than at other temperatures except when at 15^{0}C and at 20^{0}C in week 6. Also, the difference in feed intake at 20^{0}C in week 12 and at 25^{0}C was significant. At 15^{0}C the intake was significantly lower than at 10^{0}C and 20^{0}C during weeks 4 and 8. The intake at 20^{0}C (week 6) was lower than at 10^{0}C and 20^{0}C (week 4).

Water Intake: The mean (± SEM) water intakes are given in Figure 5.1. The water intakes during the periods when the temperature was at 20^{0}C were similar except for week 12 when it was significantly higher than for week 4. The water intake was significantly higher at 33^{0}C than at other times. Also, the intakes were significantly higher at 30^{0}C compared to when the temperature was lower. At 25^{0}C the intake was significantly higher than at lower temperatures except when at 20^{0}C during weeks 2 and 12 of the study.

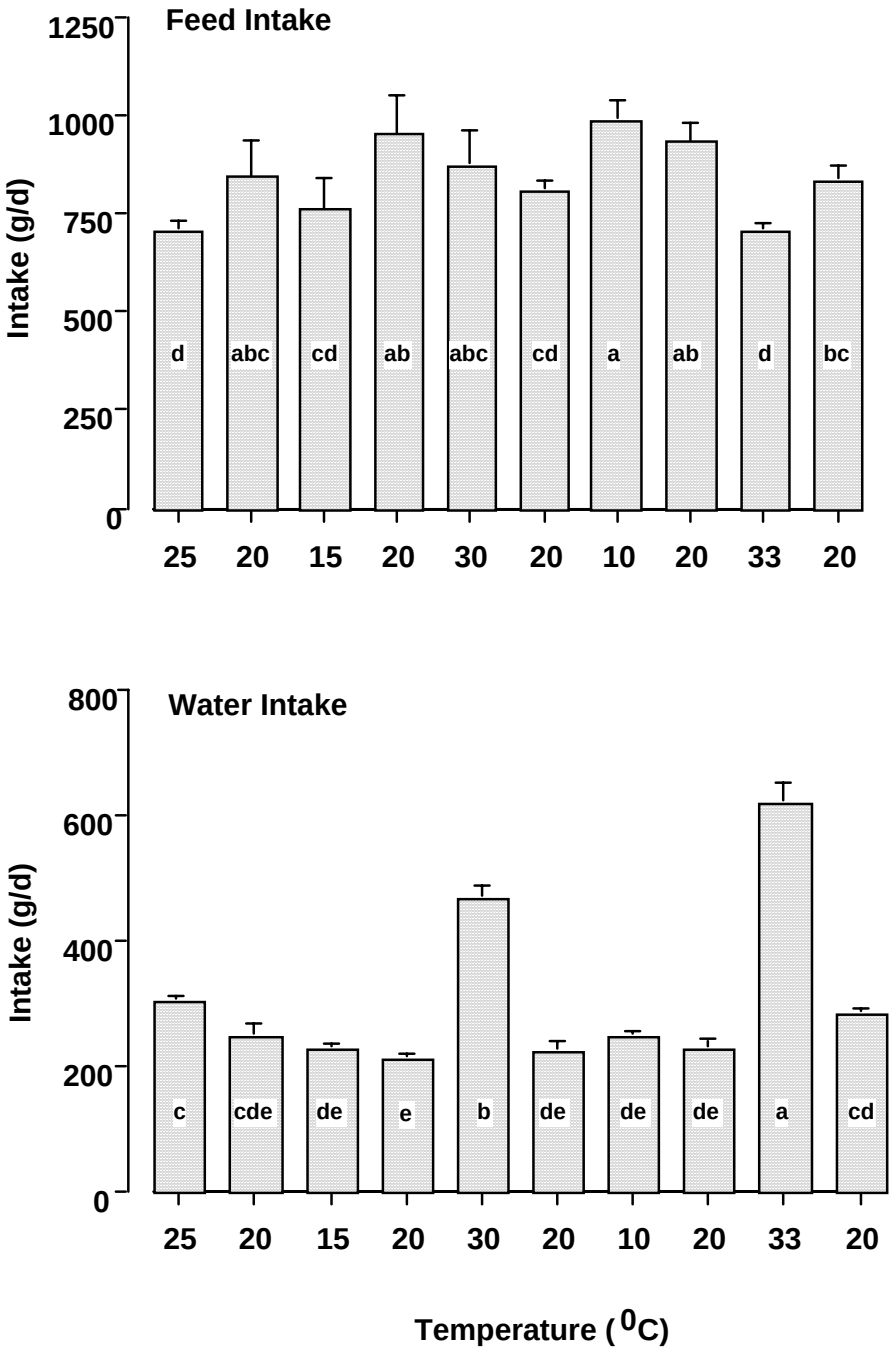

Figure 5.1: The mean (± SEM) feed intake (upper panel) and water intake (lower panel) for hens housed at different ambient temperatures. Columns with different letters are significantly different ($p<0.05$).

Egg production: Details of the mean egg production are shown in Figure 5.2. While egg production was recorded daily for all hens, the data are given as the mean weekly production for each temperature treatment. When hens were housed at a temperature of 33^0C the egg production tended to be lower and was significantly different compared to the hens housed at 25^0C or 20^0C in week 4.

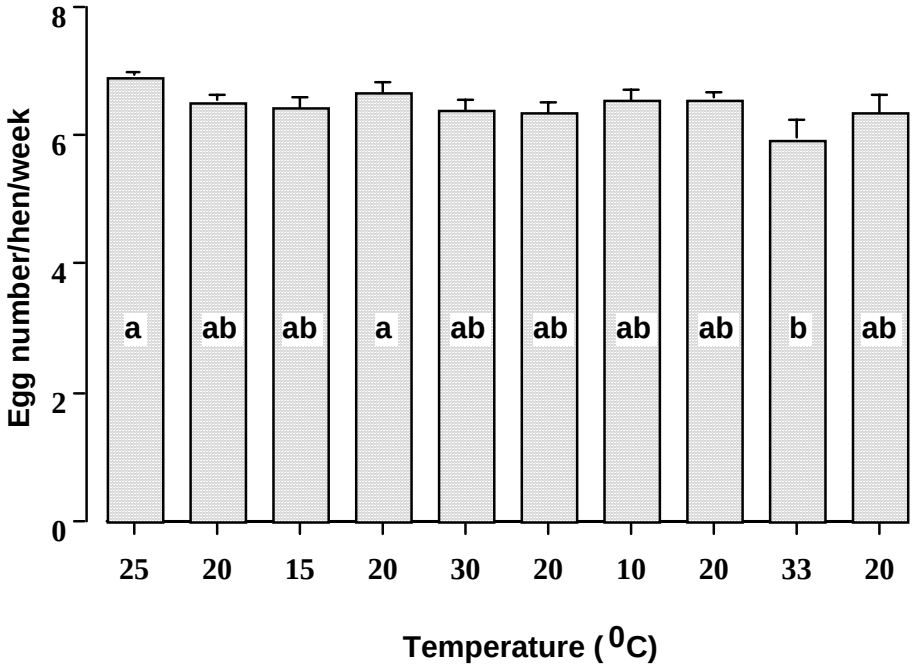

Figure 5.2: Mean (± SEM) weekly egg production for hens housed at different ambient temperatures for one week. Within treatments values with different letters are significantly different ($p<0.05$).

Egg and albumen weights: The mean (± SEM) egg and albumen weights are given in Table 5.1. There were no differences in egg weights. There was a significant difference in egg albumen weight for hens housed at 10^0C and 33^0C. There were no other significant differences in albumen weight.

Oviposition times: The mean (± SEM) oviposition times are given in Table 5.1. There were some differences in oviposition times. Oviposition tended to be longer at 25^0C, 33^0C and 20^0C (week-12). At 20^0C (week 12) and at 25^0C the oviposition time was significantly longer than at 15^0C, 30^0C and 20^0C week 2. At 33^0C the oviposition time was significantly longer compared to 15^0C and 30^0C ($P<0.05$).

Plasma corticosterone concentration: The plasma corticosterone concentrations are given in Figure 5.3. Plasma corticosterone was significantly higher when hens were housed at 20^0C in week 2 than at 20^0C in week 12. There were no other differences.

Albumen corticosterone concentration: The mean (± SEM) egg albumen corticosterone concentrations are shown in Figure 5.4. For individual sampling days mean values ranged from 1.05 ± 0.05 ng/g to 1.23 ± 0.08 ng/g. There were no significant differences between temperature treatments.

Total albumen corticosterone: The mean (± SEM) total amount of corticosterone in the albumen is shown in Figure 5.4. The levels ranged from 34.7 ± 2.0 to 42.6 ± 3.1 ng per egg. There were no significant differences between temperature treatments.

Week and temperature	Egg weight (g)	Albumen weight (g)	Oviposition time (h)
1 (25^0C)	61.8 ± 1.0	36.0 ± 0.8^{ab}	2.7 ± 0.3^{ab}
2 (20^0C)	61.3 ± 0.8	35.7 ± 0.7^{ab}	1.5 ± 0.3^{cd}
3 (15^0C)	61.8 ± 0.8	35.7 ± 0.7^{ab}	1.0 ± 0.2^{d}
5 (30^0C)	62.1 ± 0.8	34.7 ± 0.6^{ab}	1.4 ± 0.3^{cd}
7 (10^0C)	62.9 ± 0.9	36.9 ± 0.8^{a}	2.1 ± 0.4^{bc}
11 (33^0C)	59.5 ± 1.1	33.3 ± 0.8^{b}	2.9 ± 0.4^{ab}
12 (20^0C)	60.2 ± 1	34.6 ± 0.8^{ab}	3.1 ± 0.5^{a}

Table 5.1: The mean (± SEM) egg weight, albumen weight and oviposition time for hens housed at different ambient temperatures. Within columns values with different superscripts are significantly different ($P<0.05$).

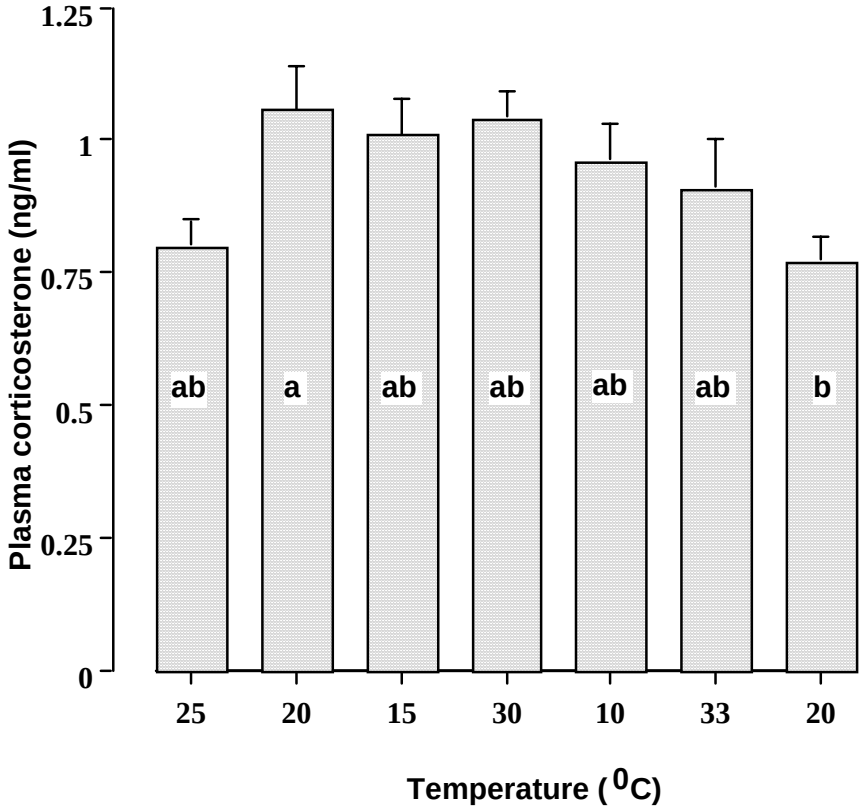

Figure 5.3: The mean (± SEM) plasma corticosterone concentrations for hens housed in conventional cages at different ambient temperatures. Treatments with different letters are significantly different ($P<0.05$).

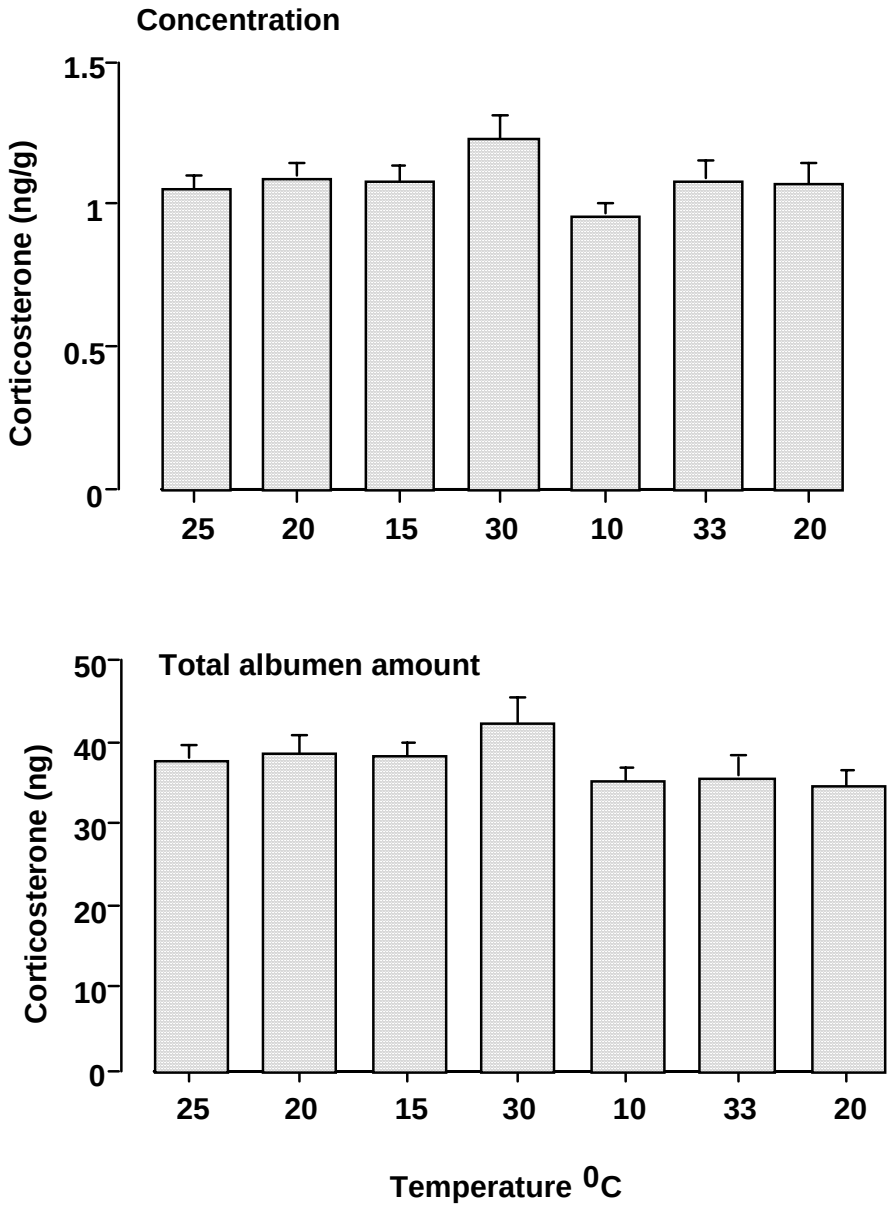

Figure 5.4: The mean (± SEM) egg albumen corticosterone concentrations (upper panel) and total amount of corticosterone in albumen for hens housed at different ambient temperatures. There were no significant differences between treatments.

5.5. Discussion

It is known that hens need to make various physiological adjustments to cope with changes in ambient temperature with many of these associated with modifications in energy utilisation and the need to conserve or dissipate body heat during thermoregulation. Because of the need to maintain body temperature within the thermal neutral zone, feed intake is influenced by ambient temperature. For this reason, some changes in feed consumption at the different temperatures were to be expected. There were differences in feed intake. However, there was no clear pattern, with significant differences observed at the same temperature (weeks 4 and 6). While feed intake changes with age and production level, neither of these would seem to be mitigating factors here because the feed intake measurements were only 2-3 weeks apart and there were no differences at this same

temperature during weeks 2 and 12. There were also no differences in egg production or egg weight for weeks 4 and 6. The lower feed intake in week 6 at 20^0C could be a carry over effect from the higher temperature (30^0C) during week 5. In the experimental design 20^0C was selected as the thermal neutral temperature and other 7-day temperature treatments were alternated with a 7-day period at 20^0C. Seven days may not have been sufficient time for hens to adjust to some changes especially after a period of high temperature.

At 25^0C and 33^0C feed intake was significantly lower than at other temperatures. The decrease seen when the temperature was at 33^0C is consistent with expectations but why it decreased at 25^0C is hard to explain especially when no decrease was observed when the temperature was at 30^0C. It is further perplexing because at 33^0C the decreased feed intake was associated with a significant decrease in egg production, however at 25^0C there was no corresponding decrease in egg production. A reduced feed intake decreases metabolic activity and therefore body-heat production (MacLeod and Jewitt, 1984) and this is important for thermoregulation. While some of the feed intake data is confusing it seems that for temperatures above 30^0C, ambient temperature has a significant negative effect on food intake and associated with this is a decrease in egg production.

Water intake increases with high ambient temperature and this acts as heat sink for birds (Teeter *et al*., 1987a) in their efforts to maintain body temperature. In general, the observations from the present study concur with this. At 30^0C and 33^0C water intake was significantly greater than at other temperatures. Even at 25^0C water intake was higher than at lower temperatures especially when at 10^0C and 15^0C. At moderate ambient temperature hens may use a combination of modifying feed and water intake to maintain body temperature. At high ambient temperature major changes in both feed and water intake are probably needed to maintain body temperature. At low ambient temperature feed intake is increased to maintain body temperature. In the present study the highest feed intake was recorded for the hens at 10^0C.

Egg production was significantly lower at 33^0C and similar at other temperatures. Temperature is probably the most important environmental factor that influences egg production and egg quality. Prolonged exposure to high temperatures is associated with decreased egg production (Hester *et al*., 1996b; Muiruri and Harrison, 1991; Samara *et al*., 1996; Whitehead *et al*., 1998; Mashaly *et al*., 2004) as is cold stress (Davis and Stopes, 1989; Spinu and Degen, 1993). Egg production is reported to be relatively constant across a specific range of temperatures. This range has been reported to be 10-27^0 C by Van Kampen, (1981) and 15-27^0 C by Marsden *et al*., (1987). The data from the present study would concur with these reports. High ambient temperature results in decreased egg weight (de-Andrade, 1976; Emery *et al*., 1984; Balnave and Muheereza, 1997; Samara *et al*., 1996; Scheideler *et al*., 2001; Kirunda *et al*., 2001; Mashaly *et al*., 2004). There were no significant effects of temperature on egg weight in the current study although it tended to be lower when birds were exposed to a temperature of 33^0C and higher when exposed to 10^0C. These trends resulted in albumen weight being significantly lower when the temperature was at 33^0C compared to 10^0C. As a general comment oviposition time tended to be shorter at moderate temperatures (15-30^0C). Stressors imposed on hens result in delayed oviposition (Carter, 1977: Hughes and Gilbert, 1984; Hughes *et al*., 1986; Mills *et al*., 1991; Reynard and Savory, 1999). The delay in oviposition at the temperature extremes could be indicative that there is some disturbance to physiology and homeostasis.

Increased corticosterone concentration seems to be a critical requirement for survival during severe disruption to homeostasis, with chronic exposure seemingly damaging to health and welfare (Sapolsky, 1992). The severity of the disturbance is probably important with regard to the involvement of corticosterone in homeostatic re-establishment. Temperature extremes increase plasma corticosterone concentration in chickens, laying hens and turkeys (El-Halawani *et al*., 1973; Brown and Nester, 1973; Buckland *et al*., 1974; Edens and Seigel, 1975; Edens, 1978; Beuving and Vonder, 1978; Garaert *et al*., 1996; Etches *et al*., 1995; Siegel, 1995; Hester *et al*, 1996c; Wolfenson *et al*., 2001; Downing and Bryden, 2002). In the present study, the only difference in plasma corticosterone concentration was seen when the temperature was at 20^0C in weeks 2 and 12. It is difficult to understand why there is this difference at the same temperature. There was no effect of

temperature on egg albumen corticosterone concentration or total amount of corticosterone in egg albumen. Therefore, the differences in plasma corticosterone were not evident in egg albumen corticosterone concentration. The egg albumen was laid down at a different time to when the plasma was taken. Blood samples were taken on the same day as the eggs were collected. For these eggs, the albumen would be deposited on the day before the blood samples were taken. In this study, it was considered the plasma corticosterone would be stable by day 7 at any particular treatment and for logistical reasons blood samples and egg collection was done on the same day. In retrospect it would have been more appropriate to take blood samples on the day the albumen was deposited although this would need to be after the albumen had been deposited (i.e. late evening). From this observation all future studies were designed to ensure blood samples were taken on the day of albumen formation and deposition.

5.6. Implications

Temperature is a stressor of hens, however the disturbance to homeostasis will depend on the absolute temperature and period of exposure. When the temperature ranged from 10-30^{0}C for a period of 7 days hens appeared to have little problem coping as determined by measures of feed intake, egg production and corticosterone concentrations in plasma and egg albumen. At 33^{0}C hens needed to make more demanding adjustments to the temperature conditions. It would seem that around 30^{0}C is the limit to the ambient temperature above which hens are required to make major physiological adjustments when exposed for moderate periods of time.

5.7. Recommendations

The range of temperatures where hens seem to have minimal effort in maintaining homeostasis is 15-30^{0}C. In this range there are limited effects on feed intake and egg production. This concurs with other studies, 10-27^0 C by Van Kampen, (1981) and 15-27^0 C by Marsden *et al*., (1987). The results presented here, and those in chapter 4 where egg production decreased by week 3 when the temperature was at 32^{0}C, indicate that 30^{0}C is probably the upper limit before significant effects on feed intake and egg production are observed.

Chapter 6

The effects of providing a nest box, dustbath and perch in furnished cages on egg albumen corticosterone concentration and total amount of corticosterone in albumen as non-invasive measures of stress.

6.1. Introduction

In developed countries over 90% of egg production is from hens maintained in conventional cages (Bell, 1995). Interest in modifying conventional cages stemmed from concerns that conventional cages failed to provide for some of the hen's behavioural needs. In an effort to improve the welfare of hens in conventional cages certain modifications were provided (Tauson, 1986; Duncan *et al*., 1992; Nicol, 1992**;** Sherwin and Nicol, 1992; Hughes *et al*., 1993; Sherwin, 1993; Appleby, 1993; Flemming *et al*., 1994; Abrahamssom and Tauson, 1995; Appleby and Hughes, 1995: Barnett *et al*., 1997a , b). Cage modifications can help to improve hen welfare.

However, conventional cages fail to provide a nest box, dust bath and perch. Nesting, dust bathing and perching evolved as components of hen's survival mechanisms in the wild. Duncan (1992) ranked the absence of a nest box, as the largest welfare issue for hens maintained in conventional cages. Nesting is thought to be controlled essentially by internal cues (Wood-Gush, 1975) and it has been reported, that hens will work hard to facilitate an environment that allows nesting behaviour (Follensbee *et al*., 1992). The nesting traits displayed by hens in the wild seem to be present even in highly selected hybrid lines, although the level of motivation for nesting seems to vary widely between lines of hens (Cooper and Appleby, 1996; Freire *et al*., 1996; Zimmerman *et al*., 2000).

Hens do not seem to be highly motivated to use a dust bath (Petherick *et al*., 1995) or access litter (Faure and Lagadic, 1994). Bathing behavior can take place on cage floors (Lingberg and Nicol, 1997; Appleby *et al*., 2002) and in fact a high percentage of hens may prefer this (Abrahamsson *et al*., 1996; Abrahamsson and Tauson, 1997). Therefore cage space may be more important than the presence of a dust bath.

Presence of a perch in cages increases the hen's behavioural repertoire (Hughes and Appleby, 1989; Duncan *et al*., 1992; Abrahamsson and Tauson, 1993, 1997). Individual variations in the extent of perch use have been reported (Lambi and Scott, 1998). When placed in a conventional cage, hens spent 46.5% of the time while being observed on the perch (Barnett *et al*., 1997b). There is clear evidence that having a perch in the housing enclosure increases bone strength (Hughes and Appleby, 1989; Knowles and Broom, 1990; Gregory *et al*., 1991; Duncan *et al*., 1992; Appleby *et al*., 1992a; Hughes *et al*., 1993; Wilson *et al*., 1993; Alvery and Tucker, 1994; Flemming, *et al*., 1994; Abrahamsson and Tauson, 1997; Barnett *et al*., 1997b; Barnett and Cronin, 2004) and this is viewed as improving welfare.

The continued pressure to ban conventional cages in some parts of the world has increased the enthusiasm for alternative housing systems. The furnished cage is considered an alternative in the EU and with conventional cages to be phased out by 2012 there has been increased research interest in evaluating their effects on hen welfare.

Production, morphology and behaviour are criteria used to assess welfare of laying hens in furnished cages. Mortality is comparable to conventional cages (Abrahamasson *et al*., 1995; Tauson, 2000) as is egg production (Abrahamsson *et al*., 1995; Abrahamsson and Tauson, 1997, 1998; Appleby *et al*., 2002). There is an increase in the level of cracked eggs (Abrahamsson and Tauson, 1997, 1998) with the percentage of dirty eggs less (Abrahamsson and Tauson, 1997, 1998) or similar compared with conventional cages (Tauson, 2002). Appleby and Hughes (1995) found slightly better production from furnished cages. A number of workers have reported that, generally, hen welfare is improved in furnished cages compared to conventional cages (Baxter, 1994; Tauson and Abrahamsson, 1996; Duncan, 2001; Appleby *et al*., 2002).

While there is pressure in the EU to find an alternative to the conventional cage, in Australia the need has not been as demanding and therefore there has been less research in evaluating the impact on welfare of the features incorporated into furnished cages. Even in the reported literature comprehensive welfare evaluation of specific pieces of cage furniture is limiting. The work reported in the present study is one component of a large study conducted at the Animal Welfare Science Centre, Werribee, Victoria, Australia, with Associate Professor John Barnett as the chief investigator. Full details of the project have been provided to the Australian Egg Corporation Limited (Project number DAV-197A).

In the large study at Werribee, welfare of hens maintained in furnished cages and the effects of individual items of furniture were investigated. This was done by housing hens in cages (8 hens per cage) containing various combinations of the main furnished features (i.e. perch, dust bath and nest box). Also the effects of stocking density and space per bird were examined. The welfare assessment was done by determining the effect of various cage features on a range of behavioural and physiological criteria that are considered important to hen welfare.

Five sets of measurements were made as part of this assessment. These were:

1. Behavioural measures: general activity, egg laying activity, feeding, head movements, perch use, dust bath use and nest box use were all determined following video surveillance.
2. Blood sampling for immunological measures: total white cell count, the number of heterophils, basophils, eosinophils and monocytes were also determined. The responsiveness of the immune system was assessed by measuring firstly, the ability of white blood cells to produce interleukin-6 when challenged and secondly, by the proliferation rate of lymphocytes.
3. Blood sampling for determination of plasma corticosterone concentrations and response to an ACTH injection.
4. Body condition: body weight, feather damage and cover, claw length and foot condition, injuries to the body areas, keel bone deformation and bone strength.
5. Corticosterone levels in egg albumen as a non-invasive measure of adrenal function

In addition, egg production was determined during parts of the study.

6.2. Objective

The present report gives details of one component of the larger study that determined the effects of cage furniture on egg albumen corticosterone concentration as a non-invasive measure of stress. As detailed in chapter 1, social interaction, fear, frustration, space and group size all have been reported to influence the HPA axis. Elevated corticosterone concentrations remain one of the measures of stress and indicate physiological adjustments by hens to perturbations of homeostasis. For this reason they are used as one criteria when assessing welfare.

Details of the results for measures 1 to 4 and production performance are given in the report for the AECL, project number DAV-197A. The remainder of the present chapter reports on the procedures and results relating to the measurement of corticosterone in egg albumen.

6.3. Materials and methods

Birds and husbandry: Hy-Line Brown hens were floor reared and vaccinated against infectious laryngotracheitis, infectious bronchitis, avian encephalomyelitis, *Mycoplasma gallisepticum*, egg drop syndrome and Marek's disease. The pullets were transferred to the Werribee research facility at 15-16 weeks of age and placed in furnished cages (9 hens per cage). At 21 weeks of age hens were identified using leg bands. At this time, cages were standardised for hen weight within a treatment, and the number per cage fixed at 8. The hens were fed commercial diets and during egg production this contained 17% crude protein. Water was provided *ad libitum* using three water nipples in each cage. The cages were in a climate-controlled shed maintained at 21^0C and 40% humidity. Lighting was computer controlled and was increased by 30 min each week from 11:13 h light/dark at placement to 16:8 light/dark at 26 weeks of age with lights on at 0500h. There was a daily 'sunrise' and 'sunset' period of 30 min at 0500h and 2030h respectively. Light intensity was 20 lux at placement and this was reduced to 5 lux at 28 weeks of age. Daily shed maintenance started at 0930h and generally was completed within 4h. Daily egg collection was carried out between 1100 and 1200h. Feed was added when necessary and residual feed was removed and weighed at monthly intervals.

Cages: The experiment used 66 Victorsson Trivelburen cages imported from Sweden and modified during installation to meet the requirements of the experimental design. Cages were 1206 mm wide, 498 mm deep and 455 mm high. The nest box was 241mm wide, 270 mm high and extended the full depth of the cage at one end. The floor of the nest box was covered with a layer of 'astro turf'. For the treatments with a dust bath, access was available for 6h daily after a wire grid opened mechanically at about midday. For the treatments that received litter, two cups of wood sawdust were added to the dust bath. For treatments that were provided with the 'dust bath space" no litter was provided. Perches were made from 'oval-shaped' wood with cross-sectional measurements 36mm x 30mm with the top and bottom surfaces being flat. The perch was placed 90 mm above the floor and 190 mm from the back of the cage. All cages were provided with a claw trimming plate, placed at the front of cage.

Treatments: In the study there were 20 treatments using 60 cages. Details of the individual treatments are given below and in Table 6.1.

The treatments

Main effect	Treatment effect
Dust bath	1. Dust bath provided 2. No dustbath, but with the equivalent space occupied by the dust bath available to the hens 3. No dustbath, with the extra cage space not available to the hens
Nest Box	1. Nest box provided 2. No nest box, but with the equivalent space occupied by the nest box available to the hens 3. No nest box, with the extra cage space not available to the hens
Perch	1. Perches provided 2. No perches provided

Additional treatments were imposed to investigate the effects of cage floor space and group size on bone strength. The space control treatment consisted of a cage without dust bath, nest box or perch but with double the floor area to enable hens to move around more freely. The number of hens were the same as for the other treatments. An additional treatment with double the number of hens (group size) was also included to examine the effects on bone strength. In these last two treatments the dividing wall between two adjacent cages was removed to give a pen with approximately twice the floor area.

Therefore, the final experimental design with the number of factors and treatments was (3 nest box* 3 dust bath* 2 perch)+ 1 space control + 1 group size control) with three replicates for each treatment. Details of the treatments are given in table 6.1. A total of 60 pens were used with each housing 8 hens except for the group size treatment which housed 16 hens per cage.

Experimental design: In the larger experiment, a large number of production and welfare measures were assessed. There were two sampling periods each conducted over 8 weeks. The first started at 29 weeks of age and the second at 59 weeks of age. Five sets of measurements were made in both sampling periods.

Egg collection for determination of corticosterone concentration: This report deals with the periods where eggs were collected for corticosterone analysis. On two consecutive days during weeks 33 (period 1) and 63 (period 2) of age, all eggs laid were collected. Egg collection was started at 0600h, 1h after lights on. This was designated as zero oviposition time and so any egg laid before this time had an oviposition time of zero. Eggs were collected each hour after 0600h until 1700h. For eggs collected at 0700h the oviposition time was recorded as +1 h, for those at 0800h the oviposition time was +2h and similarly the oviposition time increased by 1h for each additional hourly collection. The eggs were weighed then broken open and the albumen separated out, weighed and then stored at -20^0 until assayed.

Treatment	Dust Bath				Nest Box				Perch	
	Yes	No	Space		Yes	No	Space		Yes	No
1	X						X			X
2			X		X				X	
3			X			X			X	
4			X		X					X
5			X				X		X	
6	Space control				Space control				Space control	
7			X			X				X
8		X					X		X	
9	X				X				X	
10			X				X			X
11	X				X					X
12		X			X				X	
13		X					X			X
14	X						X		X	
15	Group size				Group size				Group size	
16		X				X			X	
17		X			X					X
18	X					X				X
19	X					X			X	
20		X				X				X

Table 6.1: Treatment design for the furnished cage study. The presence of individual cage furniture for any particular treatment is represented with an X.

Corticosterone determination: The corticosterone concentration in the egg albumen was determined by RIA as described in section 2.3.2.

Statistical analysis: Values are given as the mean ± SEM. For egg weight, albumen weight and oviposition times any differences between treatments were assessed by ANOVA with 20 treatments and 3 replicates per treatment on two consecutive days for each of the two collection periods. Level of significance was set at $P<0.05$.

Full details of the procedures used in the analysis of the egg albumen corticosterone concentration and total amount of corticosterone in albumen are provided in the report to the AECL Project number DAV-197A. The following description is taken from that report. Measurements were analysed as a 3 nest box treatment by 3 dust bath treatment by 2 perch treatment factorial plus 2 added control treatments, using a restricted maximum likelihood analysis (GenStat Committee, 2000) with random effects for the replicates and blocks within replicates. When the variance components for the blocking structure were estimated as being negative, this was allowed to stand. In some cases, when numerical convergence could not be obtained with these random effects, a simpler blocking structure, such as a block random effect not nested within replicates or only a random replicate effect, was used. In every case individual cages were used as the experimental unit. Where appropriate, the data for each cage were transformed so that the residuals from a saturated treatment model were homogeneous. Starting with a fully saturated model for each measurement, the factorial model was reduced, in a stepwise manner, to the simplest model that reasonably fitted the data using Wald tests. With most measurements it was reasonable to consider a model with no treatment effects of nest box, dust bath or perch. In these cases predicted means, and backtransformed means as appropriate, are presented for nest box, dust bath and perch treatments using models that only have a fixed effect of the treatment being examined and a fixed effect to account for the two larger cage size control treatments. Also, a treatment comparison was made of predicted means between the 16 hen large cage, the 8 hen large cage and the combined small cage pens using an analysis that only included these treatments. A likelihood ratio test for fixed effects in REML models (Welham and Thompson, 1977) was used to test for the effects of nest box, dust bath and perch main effects. These tests compared a model with only fixed effects for cage size and allocated hen number with models that also included fixed effects of nest box, dust bath and perch, respectively. A similar type of likelihood ratio test for fixed effects was carried out for testing cage size and allocated hen number effects against a model with no treatment effect at all. In some cases it was not possible to calculate the likelihood ratio test due to non-convergence, and in these cases the Wald test was used instead.

6.4. Results

Egg weight: The mean (± SEM) egg weights for period 1 (days 1 and 2) and period 2 (days 3 and 4) are given in Table 6.2. There was no significant effect of treatment on egg weight, however the day effect was significant as was the interaction between treatment and day. Egg weight in period 2 was significantly greater than in period 1 ($P<0.05$). There were no significant effects due to dust bath, nest box or perch and no interactions between these cage features.

During week 33 (61.7 ± 0.3 and 62.2 ± 0.2 g for day 1 and 2, respectively) there were no significant treatment, day or treatment x day affects on egg weight. In week 63 (63.8 ± 0.7 and 65.1 ± 0.3 g for days 3 and 4, respectively) there were significant treatment, day and treatment x day effects on egg weight ($P<0.05$). Egg weight for treatment 7 (no dust bath but space, no nest box and no perch; 58.2 ± 4.3 g) was significantly lower compared to egg weight for treatment 3 (No dust bath but space, nest and perch; 66.7 ± 2.7 g), treatment 11 (dust bath, nest box and no perch; 66.2 ± 0.9 g) and treatment 19 (dust bath, no nest box and no perch: 67.3 ± 0.5 g). These differences were due to the low egg

weight observed for treatment 7 on day 3. Increasing the space available to the hens or increasing the group size had no effect on egg weight.

Albumen weight: The mean (± SEM) albumen weights for period 1 (days 1 and 2) and period 2 (days 3 and 4) are given in Table 6.3. There was a significant effect of treatment, day and treatment x day interaction on egg albumen weight ($P<0.05$). Treatment 19 (dust bath, no nest box, no perch: 40.0 ± 0.6 g) had a significantly greater albumen weight than did treatment 7 (no dust bath but space, no nest and no perch: 36.9 ± 1.4 g), treatment 8 (no dust bath, no nest but space, perch: 37.1 ± 0.7 g) and treatment 9 (dust bath, nest and perch: 36.9 ± 0.2 g).

There was no dust bath, nest box or perch effect on albumen weight although there was a significant interaction between nest box and perch. Albumen weight was greater when there was no nest box but a perch present (39.2 + 0.3 g) compared to when the space for a nest box was available and a perch (37.4 ± 0.3 g). The albumen weight on day 4 (38.3 + 0.3 g) was greater than day 1 (37.6 ± 0.2 g) and day 3 (37.6 = 0.3 g) with no other day effects being significant. On day 3, albumen weight for treatments 7 (no dust bath but space, no nest box and no perch: 29.5 ± 1.4 g) and 8 (no dust bath, no nest box but space and perch: 33.3 ± 0.6 g) were lower than for treatments 3 (no dust bath but space, no nest and a perch; 41.5 ± 1.6 g) and 19 (dust bath, no nest and a perch: 39.9 ± 0.5 g).

Oviposition time: The mean (± SEM) oviposition time for period 1 (days 1 and 2) and period 2 (days 3 and 4) are given in Table 6.4. There was a significant treatment effect, however there were no day or treatment x day effects ($P<0.05$). Treatment 16 (no dust bath but space, no nest and a perch: 5.1 ± 0.3h) and treatment 18 (dust bath, no nest box but space and no perch: 4.9 ± 0.2h) had longer oviposition times than treatment 1 (dust bath, no nest but space and no perch: 3.1 ± 0.3h), treatment 7 (no dust bath but space, no nest box and no perch: 3.4 ± 0.2h), treatment 11 (dust bath but space, nest box and no perch: 3.4 ± 1.4h) and treatment 20 (no dust bath but space, no nest box but space and no perch: 3.4 ± 0.1h). Some features of the furnished cages had an effect on the oviposition time with some significant interactions. Nest box presence or absence had no effect on oviposition time. Oviposition was shorter if a dust bath was present compared to when not present (3.9 ± 0.1 vs 4.3 ± 0.1h). Oviposition was shorter if a perch was absent compared to when present (3.9 ± 0.1 vs 4.2 ± 0.1h). In the absence of a nest box, adding a dust bath increased oviposition time compared to when a nest box or the space taken up by the nest box was available (4.6 ± 0.1, 3.7 ± 0.2 and 3.3 ± 0.2h, respectively). Also, in the absence of a nest box, oviposition time is longer if a perch is available in the presence or absence of a dust bath.

Albumen corticosterone concentration: The egg albumen corticosterone concentration for all treatments, for period one (33 weeks of age: days 1 and 2) and period 2 (63 weeks of age: days 3 and 4) are shown in Figures 6.1 and 6.2, respectively. Albumen corticosterone concentration was lower at week 63 compared to week 33. Within the standard sized cages there were no significant effects of providing furnished features (perch, nest box and dust bath) on albumen corticosterone concentrations. The mean values for the effects of having a dust bath, nest box or perch in the cage are given in Table 6.5. At 33 weeks of age, there was no effect of providing hens with increased space, however there was a significant effect of increasing the number of hens housed together (16 birds in double with cages, see table 6.6). This difference was not evident at 63 weeks of age.

Total corticosterone in albumen: The total amount of corticosterone in egg albumen for all treatments, for period one (33 weeks of age: days 1 and 2) and period 2 (63 weeks of age: days 3 and 4) are shown in figures 6.3 and 6.4, respectively. The effects seen were similar to those seen for corticosterone concentration. The total amount of corticosterone in albumen was lower at week 63 compared to week 33. Within the standard cages there were no significant effects of providing furnished features (perch, nest box and dust bath) on albumen corticosterone concentration. The mean values are given in Table 6.7. At 33 weeks of age, there was no effect of providing hens with increased space (see Table 6.8), however there was a significant effect of increasing the number of

hens housed together (16 birds per pen, see Table 6.8). This difference was not evident at 63 weeks of age.

Treatment	**Egg weight (g)**			
	Day 1	**Day 2**	**Day 3**	**Day 4**
Treatment 1	60.6 ± 0.8	62.9 ± 2.4	64.4 ± 2.8	62.7 ± 1.2
Treatment 2	62.1 ± 1.0	62.5 ± 0.03	65.9 ± 0.7	65.1 ± 1.2
Treatment 3	63.2 ± 1.0	63.1 ± 0.1	67.4 ± 6.1	66.1 ± 0.9
Treatment 4	61.5 ± 0.7	63.0 ± 0.6	65.6 ± 1.7	64.7 ± 0.6
Treatment 5	61.4 ± 0.1	62.6 ± 0.5	63.8 ± 1.6	64.6 ± 1.9
Treatment 6	62.0 ± 1.1	61.1 ± 0.3	66.2 ± 1.8	65.6 ± 1.9
Treatment 7	62.6 ± 2.2	62.1 ± 0.9	48.5 ± 0.1	68.0 ± 1.1
Treatment 8	61.9 ± 0.9	62.6 ± 0.1	56.2 ± 0.2	65.4 ± 1.1
Treatment 9	58.4 ± 1.1	60.9 ± 0.4	63.9 ± 0.7	63.9 ± 0.4
Treatment 10	61.9 ± 1.0	62.2 ± 0.3	64.2 ± 0.9	65.1 ± 2.3
Treatment 11	63.2 ± 0.3	63.1 ± 2.0	67.3 ± 1.3	65.1 ± 1.3
Treatment 12	62.4 ± 0.4	63.7 ± 2.2	64.2 ± 0.7	63.8 ± 1.0
Treatment 13	61.3 ± 0.1	61.3 ± 0.3	65.7 ± 0.3	66.1 ± 0.8
Treatment 14	62.5 ± 0.8	61.1 ± 1.9	64.5 ± 1.4	64.9 ± 0.7
Treatment 15	62.6 ± 0.1	61.7 ± 0.3	66.0 ± 1.2	64.1 ± 0.6
Treatment 16	62.5 ± 2.7	61.7 ± 0.8	64.7 ± 1.3	65.3 ± 0.5
Treatment 17	62.3 ± 2.5	61.7 ± 1.3	65.6 ± 1.1	65.3 ± 1.9
Treatment 18	62.8 ± 0.9	61.1 ± 0.9	66.4 ± 1.7	64.6 ± 0.5
Treatment 19	60.8 ± 0.2	60.6 ± 0.4	66.8 ± 1.0	67.9 ± 0.4
Treatment 20	62.6 ± 0.7	60.6 ± 3.0	63.6 ± 0.6	63.9 ± 1.0

Table 6.2: The mean (± SEM) egg weight for hens at 33 weeks (days 1 and 2) and 63 weeks (days 3 and 4) of age. Hens were housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-).

Treatment	Albumen weight (g)			
	Day 1	Day 2	Day 3	Day 4
Treatment 1	36.8 ± 0.9	38.4 ± 1.2	37.5 ± 1.9	36.7 ± 1.2
Treatment 2	37.2 ± 0.5	37.1 ± 0.1	38.3 ± 0.8	38.2 ± 1.1
Treatment 3	37.8 ± 1.1	39.1 ± 0.1	41.5 ± 1.6	38.8 ± 0.7
Treatment 4	36.7± 0.6	37.8 ± 0.6	37.8 ± 0.9	37.4 ± 0.4
Treatment 5	36.7 ± 1.0	37.7 ± 1.0	37.4 ± 1.4	37.4 ± 1.2
Treatment 6	37.4 ± 0.9	35.9 ± 0.9	39.0 ± 1.7	37.7 ± 1.3
Treatment 7	38.3 ± 1.0	39.4 ± 0.8	29.5 ± 0.4	40.5 ± 1.3
Treatment 8	37.4 ± 0.5	38.7 ± 0.9	33.3 ± 0.6	38.8 ± 0.8
Treatment 9	36.3 ± 0.6	36.9 ± 0.4	37.1 ± 0.3	37.2 ± 0.1
Treatment 10	37.8 ± 0.3	37.5 ± 0.4	38.0 ± 0.8	38.1 ± 2.1
Treatment 11	38.9 ± 0.6	39.2 ± 0.8	40.3 ± 1.1	37.0 ± 2.2
Treatment 12	37.1 ± 0.5	37.2 ± 0.6	37.1 ± 0.1	37.8 ± 0.4
Treatment 13	37.6 ± 0.7	37.3 ± 0.3	38.5 ± 0.6	38.9 ± 0.8
Treatment 14	37.4 ± 0.1	37.6 ± 0.9	38.9 ± 0.9	38.4 ± 0.3
Treatment 15	37.6 ± 0.4	37.9 ± 0.5	39.0 ± 0.9	37.7 ± 0.5
Treatment 16	37.1 ± 1.0	38.0 ± 0.2	38.3 ± 0.5	39.4 ± 0.5
Treatment 17	38.2 ± 1.5	39.2 ± 0.9	38.7 ± 1.1	39.0 ± 1.6
Treatment 18	38.6 ±0.7	37.9 ± 0.2	38.2 ± 1.3	37.5 ± 0.7
Treatment 19	40.9± 2.1	38.5 ± 0.8	39.9 ± 0.5	40.7 ± 0.7
Treatment 20	36.7 ± 0.6	38.5 ± 0.9	37.3 ± 0.8	37.7 ± 1.0

Table 6.3: The mean (± SEM) albumen weight for hens at 33 weeks (days 1 and 2) and 63 weeks (days 3 and 4) of age. Hens were housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-).

Individual hen corticosterone responses: While, in the standard 8-bird cages, there were no effects of providing furnished features on corticosterone concentration there was a range of corticosterone responses from individual hens in cages. In figure 6.5 (33 weeks of age, day 1 sampling) and figure 6.6 (63 weeks of age, day 3 sampling) individual hen corticosterone concentrations are shown for each pen. In these figures a line has been drawn through each graph identifying values above and below 2.0 ng/g. This value has been chosen arbitrarily, and it is considered that values above this are high and probably indicate that these hens have elevated plasma corticosterone concentrations. In week 33 (day 1 collection) for the 8-bird standard cages 13.1% of

eggs collected had albumen corticosterone concentrations above 2.0 ng/ml. In week 63 (day 3 collection) this had reduced to 3.2%.

Week and temperature	Oviposition time (h)			
	Day 1	Day 2	Day 3	Day 4
Treatment 1	3.4 ± 0.4	3.0 ± 0.4	3.5 ± 0.3	4.1 ± 0.7
Treatment 2	3.9 ± 0.4	4.3 ± 0.8	3.3 ± 0.3	3.8 ± 0.9
Treatment 3	3.7 ± 0.1	3.8 ± 0.4	4.6 ± 0.7	4.9 ± 0.7
Treatment 4	4.6 ± 1.0	4.4 ± 0.9	4.4 ± 0.5	3.2 ± 0.3
Treatment 5	4.0 ± 0.6	4.1 ± 0.9	5.1 ± 0.6	5.0 ± 0.8
Treatment 6	4.1 ± 0.2	4.4 ± 1.0	3.9 ± 1.0	3.1 ± 0.4
Treatment 7	4.0 ± 0.2	3.1 ± 0.3	2.7 ± 0.3	3.8 ± 0.6
Treatment 8	4.7 ± 0.4	4.2 ±0.3	3.6 ± 0.1	5.1 ± 0.9
Treatment 9	4.4 ± 0.5	3.8 ± 0.6	3.5 ± 0.5	4.2 ± 0.4
Treatment 10	4.3 ± 0.4	4.4 ± 0.4	4.6 ± 1.0	4.6 ± 0.6
Treatment 11	3.2 ± 0.4	3.2 ± 2.0	3.1 ± 0.1	4.1 ± 0.1
Treatment 12	4.1 ± 0.8	3.3 ± 0.9	5.2 ± 0.3	4.0 ± 0.3
Treatment 13	4.5 ± 0.6	4.0 ± 0.9	4.1 ± 0.7	4.0 ± 0.3
Treatment 14	3.4 ± 0.4	3.7 ± 0.6	3.3 ± 1.0	3.2 ± 1.0
Treatment 15	4.0 ± 0.4	3.5 ± 0.4	4.7 ± 0.6	4.6 ± 0.4
Treatment 16	5.4 ± 0.3	5.4 ± 0.2	5.2 ± 1.1	4.7 ± 0.7
Treatment 17	4.2 ± 0.1	4.4 ± 0.5	4.1 ± 0.5	3.6 ± 0.4
Treatment 18	4.9 ± 0.4	5.1 ± 0.4	4.8 ± 0.7	5.1 ± 0.2
Treatment 19	4.5 ± 0.1	4.8 ± 0.2	3.4 ± 0.2	3.9 ± 0.4
Treatment 20	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.4	3.4 ± 0.2

Table 6.4: The mean (± SEM) oviposition time for hens at 33 weeks (days 1 and 2) and 63 weeks (days 3 and 4) of age. Hens were housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-).

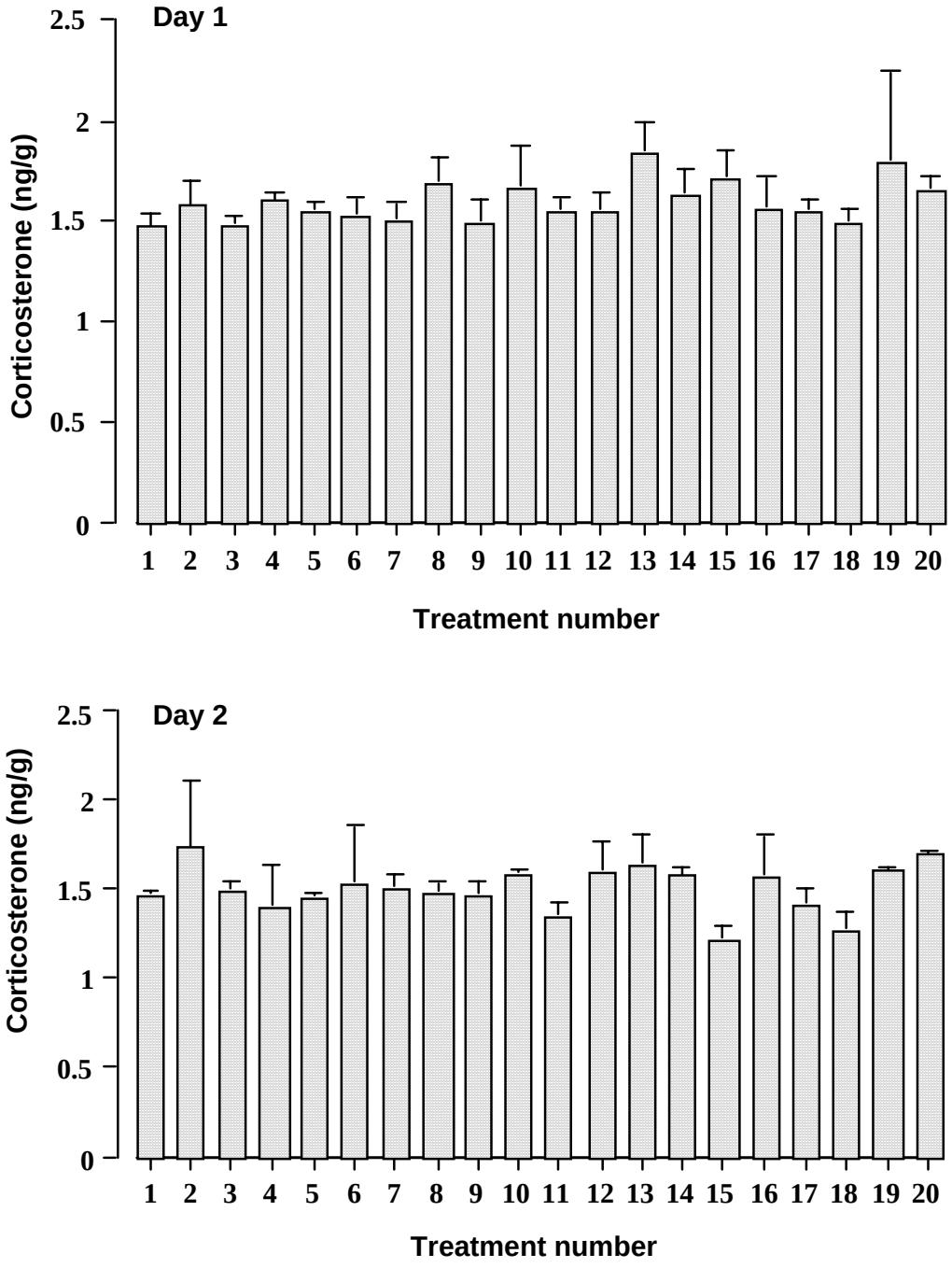

Figure 6.1: The mean (± SEM) egg albumen corticosterone concentration for hens at 33 weeks (days 1 and 2) and housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-). Two other treatments examined the effects of group size and cage space (treatments 6 and 15).

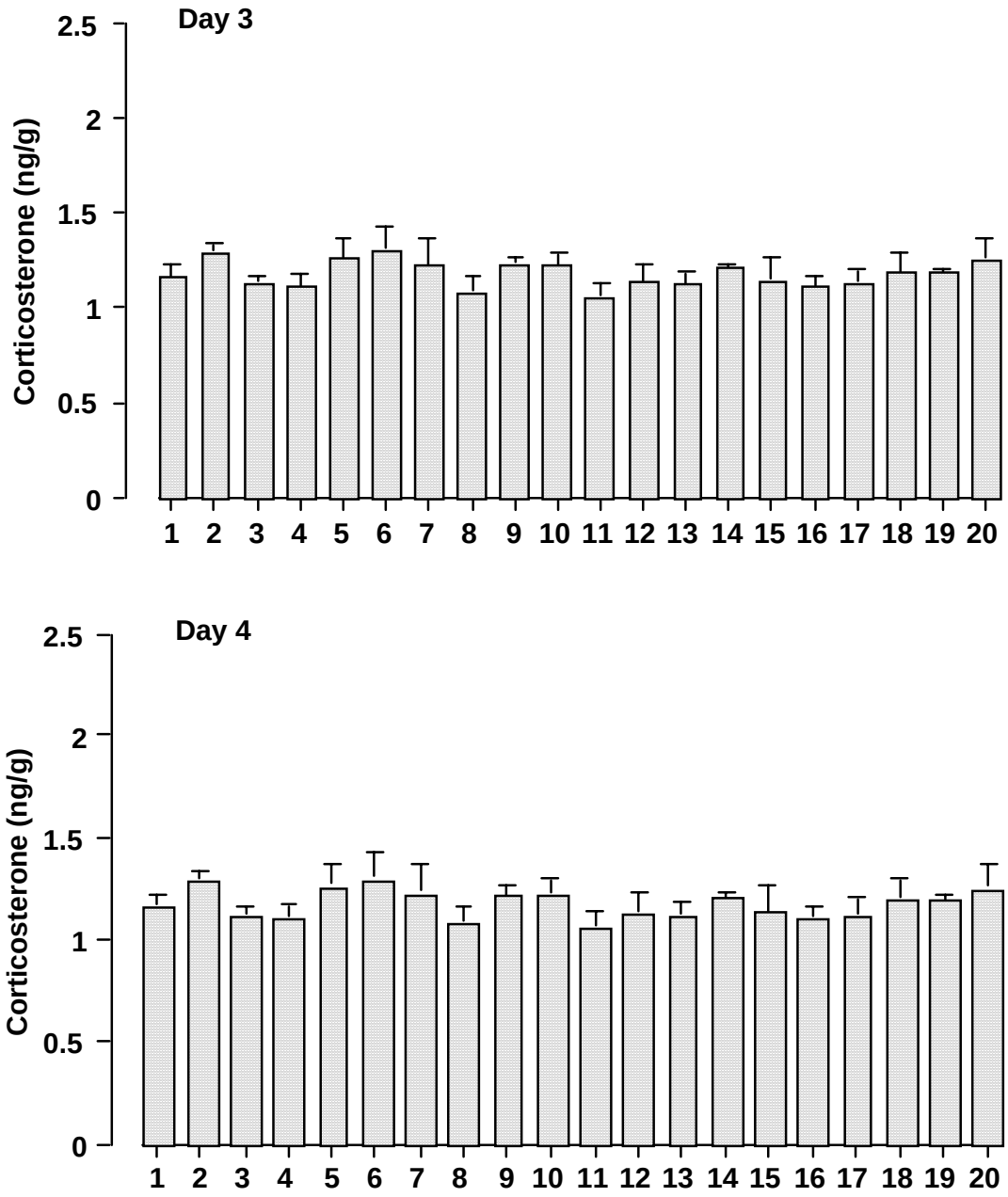

Figure 6.2: The mean (± SEM) egg albumen corticosterone concentration for hens at 63 weeks (days 3 and 4) of age and housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-). Two other treatments examined the effects of group size and cage space (treatments 6 and 15).

Age (weeks)	Albumen Corticosterone (ng/g)			*P value*
	No perch	**Perch**	**Std Error**	
33	1.54	1.52	0.04	0.704
63	1.10	1.14	0.02	0.84

Age (weeks)	Albumen Corticosterone (ng/g)				*P value*
	No dust bath	**Space**	**Dust bath**	**Std Error**	
33	1.54	1.55	1.49	0.049-0.050	0.471
63	1.10	1.12	1.10	0.030	0.772

Age (weeks)	Albumen Corticosterone (ng/g)				*P value*
	No nest box	**Space**	**Nest box**	**Std Error**	
33	1.50	1.56	1.53	0.049-0.050	0.471
63	1.10	1.15	1.10	0.029	0.772

Table 6.5: The mean egg albumen corticosterone concentration when hens were provided with a dust bath, nest box or perch in the cage.

Age (weeks)	Albumen Corticosterone (ng/g)				*P value*
	Large cage 16 hens	**Large cage 8 hens**	**Standard cage 8 hens**	**Std Error (large cages)**	
33	1.75	1.48	1.53	0.128	**0.021**
63	1.17	1.24	1.12	0.076	0.086

Table 6.6: The mean and SEM egg albumen corticosterone concentrations when group size and available space were increased. The control cage contained 8 hens (standard cage 8 hens). Group size was doubled to 16 and the cage space kept constant (large cage 16 hens). Cage space was increased two fold by maintaining the same group size and doubling the cage space (large cage 8 hens).

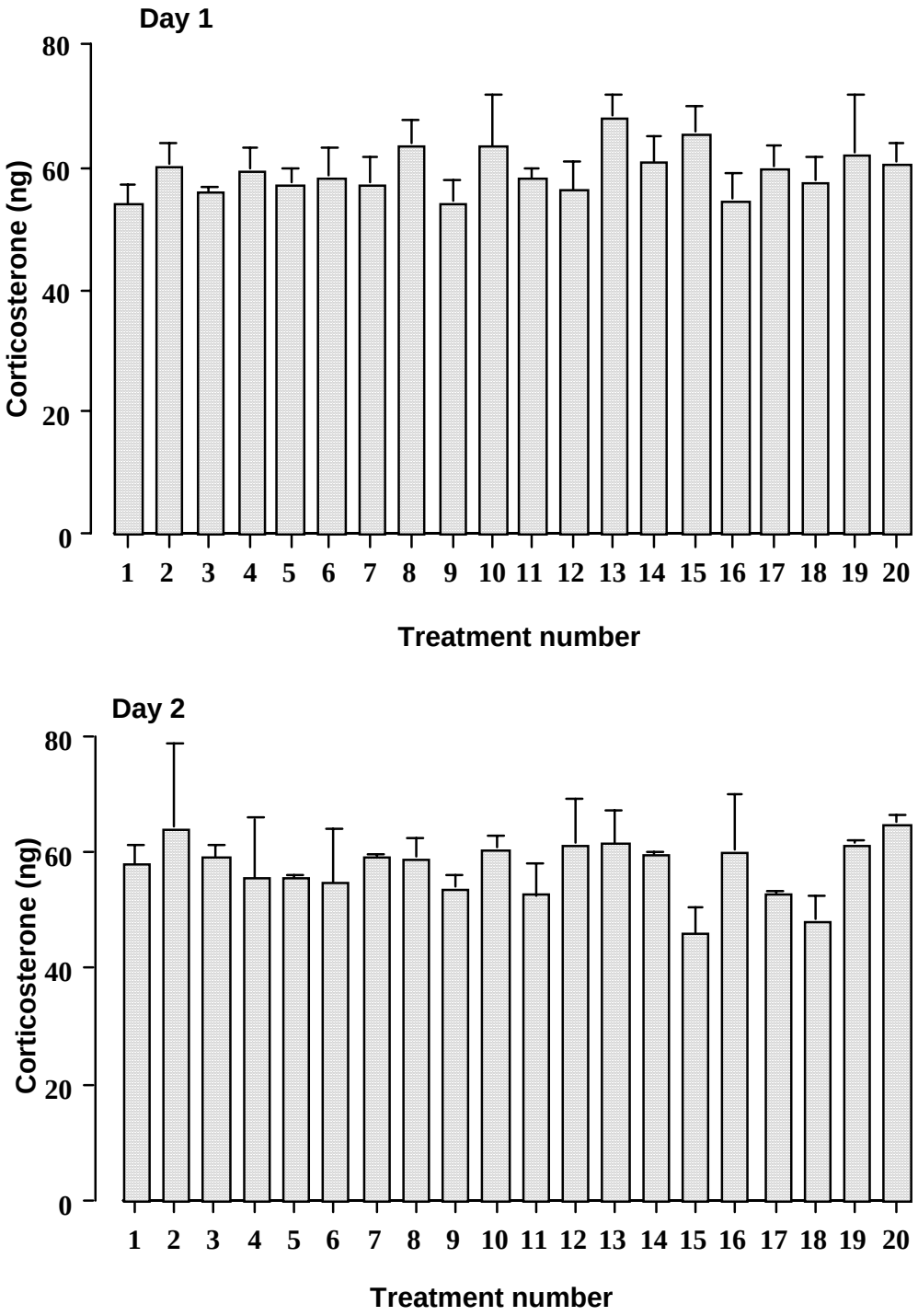

Figure 6.3: The mean (± SEM) total amount of corticosterone in egg albumen for hens at 33 weeks (days 1 and 2) of age and housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-). Two other treatments examined the effects of group size and cage space (treatments 6 and 15).

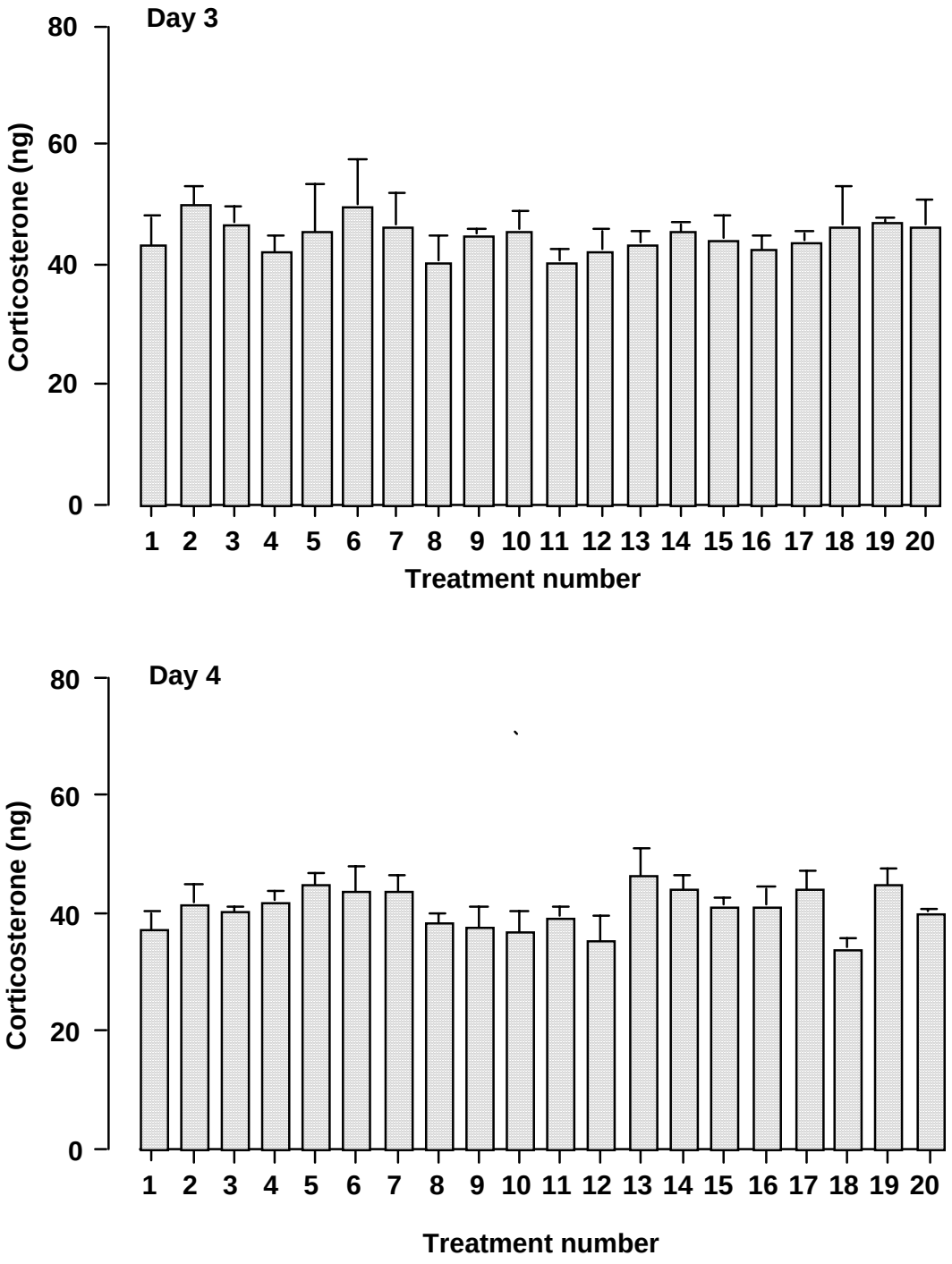

Figure 6.4: The mean (± SEM) total amount of corticosterone in egg albumen for hens at 63 weeks (days 3 and 4) of age and housed in cages with different combinations of dust bath (+/-), nest box (+/-) and perch (+/-). Two other treatments examined the effects of group size and cage space (treatments 6 and 15).

Age (weeks)	Albumen Corticosterone (ng)			*P value*
	No perch	**Perch**	**Std Error**	
33	58.2	56.4	1.61	0.275
63	41.9	43.3	1.21	0.265

Age (weeks)	Albumen Corticosterone (ng)				*P value*
	No dust bath	**Space**	**Dust bath**	**Std Error**	
33	57.3	58.4	56.2	1.98	0.561
63	42.6	43.1	42.0	1.49-1.50	0.790

Age (weeks)	Albumen Corticosterone (ng)				*P value*
	No nest box	**Space**	**Nest box**	**Std Error**	
33	56.3	58.3	57.3	1.98	0.608
63	43.4	43.1	41.3	1.42-1.45	0.772

Table 6.7: The mean total amount of corticosterone in albumen when hens were provided with a dust bath, nest box or perch in the cage.

Age (weeks)	Albumen Corticosterone (ng/g)				*P value*
	Large cage 16 hens	**Large cage 8 hens**	**Standard cage 8 hens**	**Std Error (large cages)**	
33	66.3	54.4	57.3	5.1	**0.019**
63	45.8	46.6	42.6	3.7	0.209

Table 6.8: The mean total amount of corticosterone in egg albumen when group size and available space were increased. The control cage contained 8 hens (standard cage 8 hens). Group size was doubled to 16 and the cage space kept constant (large cage 16 hens). Cage space was increased two fold by maintaining the same group size and doubling the cage space (large cage 8 hens).

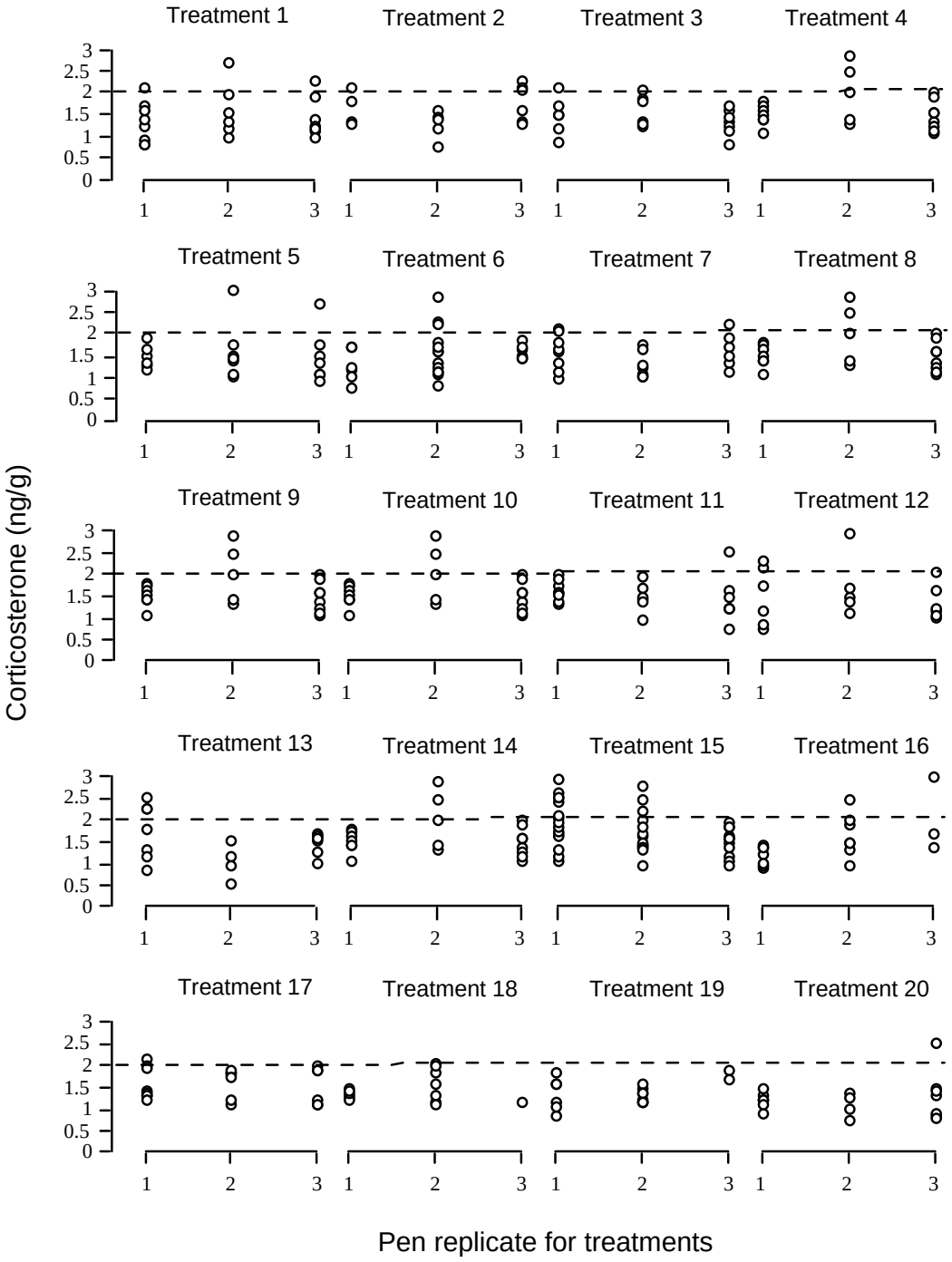

Figure 6.5: The egg albumen corticosterone concentration for individual hens at 33 weeks of age (day 1). There were 20 treatments with 3 replicate pens for each treatment. Two treatments examined the effects of cage space and group size (treatments 6 and 15, respectively).

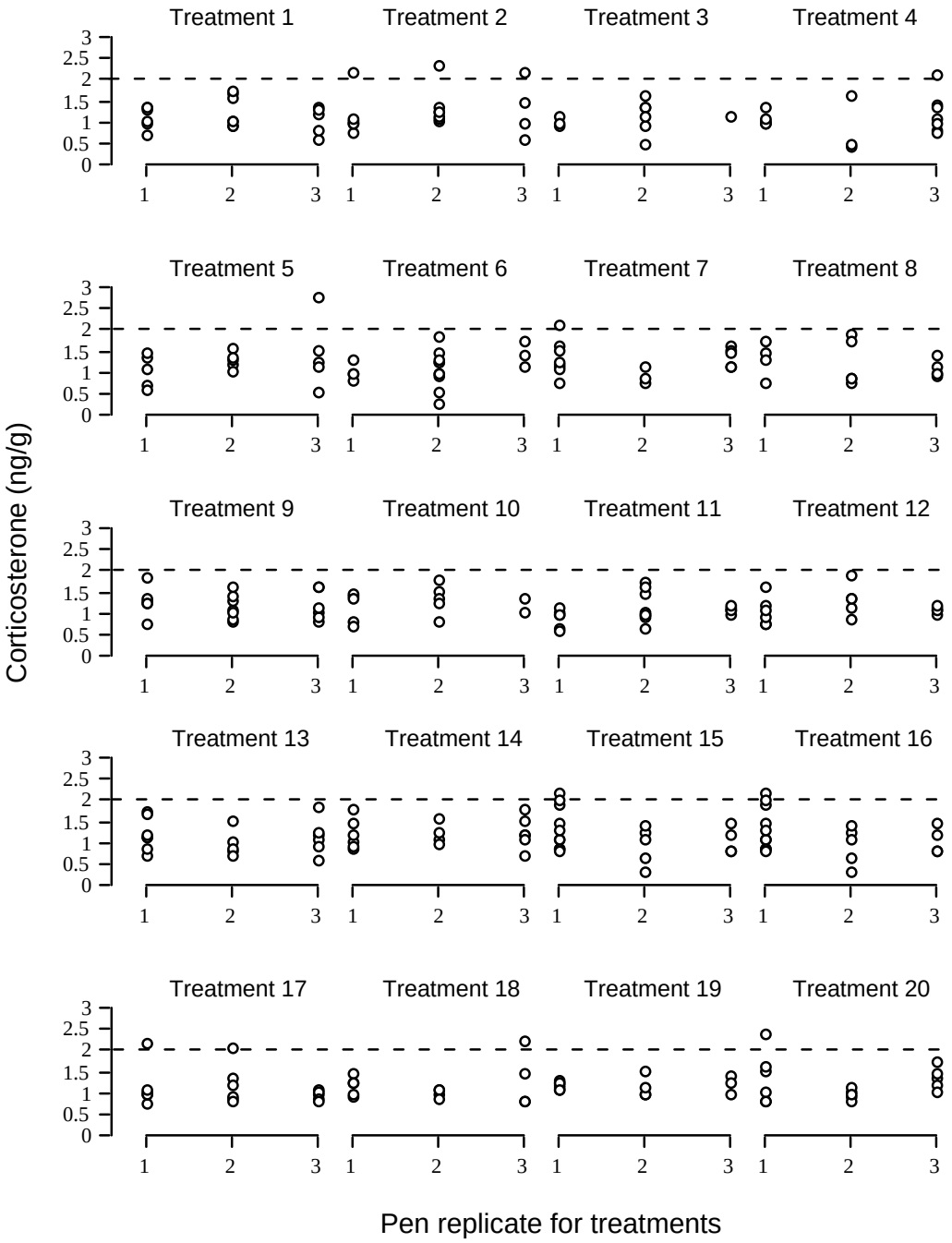

Figure 6.6: The egg albumen corticosterone concentration for individual hens at 63 weeks of age (day 3). There were 20 treatments with 3 replicate pens for each treatment. Two treatments examined the effects of cage space and group size (treatments 6 and 15, respectively).

6.5. Discussion

The results presented in this chapter relate to one component of a large study assessing the welfare of laying hens in furnished cages (AECL Project No: DAV-179A). In the larger project several criteria were used to determine what effects specific features of cage furniture had on hen welfare. The cage

features under investigation were dustbath, nest box and perch. The study employed a factorial design to evaluate the effects of each feature and combinations of these features. A further two treatments were added that looked at the effects of group size and space allocation.

The data presented in this chapter deals with the effects of cage furniture on egg albumen corticosterone concentration and the total amount of corticosterone in albumen. However, reference will be made to some of the results from other measures of welfare detailed in the report to the AECL for project DAV-179A. In the overall study measurements were made at two times, peak production (weeks 29-36 of age) and towards the end of production (weeks 59-66 of age). The egg samples for determination of corticosterone concentration were collected during both periods on two consecutive days during week 33 (period 1: days 1 and 2) and week 63 (period 2: days 3 and 4).

Egg production was measured for a limited time at the beginning of each period (22-29 weeks and 48-55 weeks) and therefore the data may be inadequate to make conclusive statements with regard to the production levels and welfare. It has been suggested that reproductive rate as determined by egg production is a good measure of stress and consequently overall welfare. The presence or absence of a dustbath, nest box or perch had no effect on egg production. Production was higher during the first collection period (93%) than the second collection period (80%). These production levels are normal for this strain of hen (AECL report: DAV-179A). Egg production increases to a peak and then gradually declines until the end of production (Etches, 1990) therefore the decline in production seen in the present study was expected.

Eggs collected for corticosterone analysis were significantly heavier in period 2 than in period 1 with no difference due to the day of collection in either period. Egg weight increases as hens age and is greatest at the end of lay (Etches, 1996). Therefore, the differences in egg weight between the two periods is as would be expected. There were no effects of cage furniture on egg weight in either period. For some unexplained reason on day 3, treatment 7 (no dustbath, no nest box but space, no perch) had eggs weights significantly lower than treatments 3, 11 and 19. This may simply be an aberration as there was no logical explanation for this result especially as there was no difference on day 4. Increasing the available cage space or group size had no effect on egg weight. During period 1 a doubling of the space in 8 bird cages increased egg production (100% vs 93%), however production was similar in period 2 (AECL report: DAV-179A).

There were no effects of dustbath, nest box or perch on egg albumen weight. The albumen weights were higher on day 4 than on days 1 and 3. However, the mean differences were small, being less than 0.9 g. On day 3 there were significantly lower weights in treatments 7 and 8 compared to some other treatments and this is probably related to the lower egg weights in these two treatments on this day. Other differences in albumen weight were small.

Delays in oviposition time have been associated with stress (Carter, 1977: Hughes and Gilbert, 1984; Hughes *et al*., 1986; Mills *et al*., 1991; Reynard and Savory, 1999) and the laying of abnormal eggs (Hughes *et al*., 1986; Walker and Hughes, 1998; Reynard and Savory, 1999). The presence or absence of a nest box had no effect on oviposition time. The presence of a dustbath shortened oviposition time while having a perch increased oviposition time. Large variations in individual oviposition times were observed. Differences associated with the presence or absence of a dustbath or perch were small, being only 20-24 min. Stressed-induced changes in oviposition time are generally of large magnitude. It has been reported that the delay needs to be at least 2.4h or longer than the normal oviposition time for there to be effects on levels of abnormal eggs laid (Reynard and Savory, 1999). The differences in oviposition time seen in the present study do not seem to be large enough to be indicative of stress.

In the comprehensive furnished cage study, immunological measures were one of the criteria used in the evaluation of welfare. There was no effect of furniture on white blood cell count, differential

white blood cell count, cell proliferation rates and immunological responses to antigens. With these results there were no differences in H/L ratio, a measure of stress in hens (review: Maxwell, 1993). Group size and increased space allowance had no effects on white blood cell counts and differential white cell counts but the ability of cells from hens in 16-bird cages to produce interleukin-6 was reduced, suggesting that there was some level of immunosuppression for this treatment (AECL Report: DAV-197A). Cage density had no effect on the antibody response to SRBC (Patterson and Siegel, 1998; Hester *et al*., 1996a). As pullets age the response to SRBC immunisation increased (Patterson and Siegel, 1998). In this same study, cage density had no effect on heterophil and lymphocyte numbers, and consequently the H/L ratio, even though there were changes in feed intake and body weight. This is supported by Davis *et al*., (2000), but not by Hester *et al*., (1996a) who found that as density increased the H/L ratios were higher.

Within the standard 8-bird cages the presence of individual pieces of cage furniture had no effects on egg albumen corticosterone concentration or the total amount of corticosterone in albumen. The albumen corticosterone concentration and the total amount of corticosterone in albumen were significantly higher at 33 weeks of age than at 63 weeks of age ($P<0.05$). The effects of age on both plasma and albumen corticosterone concentrations are examined and discussed in chapter 8. The plasma corticosterone concentrations at both periods were not affected by the presence of individual features of cage furniture (AECL Report: DAV-197A) with plasma corticosterone concentrations during both periods being similar (range: 3.04 -3.35 ng/ml in period 1 and 3.26-3.53 ng/ml in period 2). The percentage corticosterone change in response to an ACTH challenge was similar for all types of cage furniture (AECL Report: DAV-197A).

There were some significant effects of group size and space allowance on physiological and behavioural measures (AECL Report: DAV-179A). At 33 weeks of age hens in 16-bird cages had significantly higher egg albumen corticosterone concentration and total amount of corticosterone in albumen (see tables 6.6. and 6.8). This difference was not evident at 63 weeks of age. While not significant, during both periods the plasma corticosterone concentrations tended to be higher in the 16-bird cages and the standard 8-bird cages compared to the 8-bird cages with twice the space allocation (AECL Report: DAV-197A). During the first period the percentage change in ACTH-induced corticosterone response was significantly lower in the 16-bird cages compared to 8-bird cages with the same space allocation. This was not the case during period 2 (AECL Report: DAV-197A).

In any housing system both the space per bird and the size of the group influence aspects of hen behaviour, physiology and wellbeing. A number of reports indicate that there is a positive relationship between cage density and plasma corticosterone concentrations (Davami *et al*., 1984; Craig *et al*., 1986a). Decreasing the area per bird increases corticosterone (Lei *et al*., 1972; Mashaly *et al*., 1984) as does a decrease in personal space (Compton *et al*., 1981). While corticosterone concentrations will depend on the cage space a review of the literature (see section 1.6.4.) indicates that space effects seem to be small if the area per bird is greater than 350-380 cm^2/bird. If this suggestion holds, then in the present study where the area was 750 cm^2/bird, no effects of space on corticosterone concentration would be expected.

When the area per hen is adequate, then larger group sizes could increase the level of social stress and this could account for the increase in egg albumen corticosterone concentration seen in the 16-bird group. Using the immunological and corticosterone results for the basis, the present data suggested that the hens maintained in groups of 16 were more stressed than those in 8-bird cages with the equivalent cage space (750cm^2/bird). 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).

The absence of a nest has been suggested to be the major welfare concern associated with conventional cages (Duncan, 1992). Failure to provide a nest has been considered to cause frustration in hens (Duncan, 1970; Brantas, 1980; Kite, 1985; Mills and Wood-Gush, 1982; 1985; review:

Appleby *et al*., 1992b; Yue and Duncan, 2003). The egg laying performance and corticosterone measures observed in this study do not indicate hens are stressed when a nest is not present.

For hens in standard 8-bird cages there was a wide variation in egg albumen corticosterone concentration. At peak production there was a higher percentage (13.3%) of the hens that had concentrations above 2 ng/ml than at the end of production (3.2%). This is in agreement with the decrease in mean corticosterone concentration at the end of production. While there were no treatment differences the high values found in some hens could have relevance with regard to the welfare of these hens. Mean corticosterone values may suggest that welfare is adequate, however for those individuals with high corticosterone concentrations this might not be the situation. From the relationship described in chapter 2 hens with albumen corticosterone concentrations above 2 ng/ml have high plasma corticosterone concentrations. If the same hens persistently have high corticosterone concentrations and this represents a level much higher than the normal baseline 'unstressed' level, then it is possible that their welfare is being compromised. If this is the situation, then it indicates that when assessing welfare, experimental protocols should be designed to determine what happens to individual hens. This is difficult with group housing because identifying individual hen egg laying is a major obstacle and determining baseline 'unstressed' basal levels is probably not achievable.

6.6. Implications

As a general conclusion, from the comprehensive study and results detailed here, any effects of having a dustbath, nest box or perch in the cage is minimal. Some behavioural changes occurred but like many behavioural studies it is very difficult to determine how importantly these contribute to hen welfare. Of the physiological criteria used to assess welfare in this study, except the increase in bone strength when a perch was present, none seemed to indicate that hens were adversely stressed by not having a dustbath, nest box or perch.

6.7. Recommendations

The measures of welfare used in the comprehensive study suggest that the absence of furniture from the cage has only very limited influence on welfare. The measures of corticosterone used in this study suggest that hens were not unduly stressed. While group measures may indicate this, examination of individual corticosterone measures also provide evidence that there is the possibility that some hens may have problems. If high corticosterone values are truly elevated levels and not due to genetic differences the welfare of some hens may be compromised. Ideally, there appears to be a need to identify whether there are individual hens which have persistently high corticosterone levels, and if these are simply transient or inherent variations due to individual genetic differences. However, in practice this is difficult and not practical in commercial production systems. Therefore, assessment of stress in commercial situations needs to be based on the mean value for the group and husbandry practices should be directed at minimising the changes in corticosterone levels in hens. If the housing system causes an elevation in corticosterone levels, hens are more likely to develop pathological conditions.

Chapter 7

The effects of alternative housing systems on egg albumen corticosterone concentrations and total amount of corticosterone in albumen as a non-invasive measure of stress

7.1. Introduction

The continued pressure to ban conventional cages in some parts of the world has increased the enthusiasm for alternative housing systems. The furnished cage is considered an alternative in the EU although there is also increased interest in floor based systems. In Australia, alternative production systems are mostly of the barn or free range type. Like all systems, each has advantages and disadvantages. Problems identified with floor systems include increased labour costs, high incidence of floor eggs, management difficulties, high ammonia and dust levels and increased disease risk (Van Emos and Fiks-van Niekerk, 2004). In a survey of Dutch aviary systems approximately 25% of farms indicated having a production problem, with the main complaint being a failure to reach peak production levels (Van Emos and Fiks-van Niekerk, 2004).

Egg production in floor based systems is often a reflection of mortality rates (Tauson, 2002). Mortality in cage systems has been reported to be lower (Koelkebeck and Cain, 1984) or higher when compared to floor pens (Craig and Craig, 1985; Mench *et al*., 1986) or even similar (Mou and Katle, 1990). In a survey of Dutch aviary systems having a free range component, mortality levels ranged from 8-28%, with the main causes being E. coli infection, sudden deaths, feather pecking and cannibalism (Van Emos and Fiks-van Niekerk, 2004). Disease outbreaks are an important welfare concern in floor based systems. In general, floor systems have more instability as regards to feather pecking (Tauson and Holm, 2001) and if excessive can cause welfare problems and even death (Huber-Eicher and Wechser, 1997; 1998). Failure to beak trim is reported to decrease performance and welfare of hens in non-cage systems (Hughes and Gentle, 1995; Abrahamsson and Tauson, 1995). Beak trimming can decrease mortality in floor based systems (Craig and Muir, 1996; Bell *et al*., 1998). However, beak-trimming in its own right remains a welfare concern.

Of three different housing systems; cages, free-range and strawyards, hens in strawyards had significantly lower circulating plasma corticosterone concentrations than hens in cages or free range (1.43 ± 0.37, 0.93 ± 0.24 and 0.32 ± 0.09 ng/ml, respectively). The hens in the cages tended to have higher corticosterone concentrations than free range hens (Gibson *et al*., 1986). Koelkebeck and Cain (1984) found that corticosterone concentrations were lower for hens maintained in cages (0.79 ng/ml) compared to floor pens (1.72 ng/ml) and free range systems (0.95 ng/ml). Other studies support lower plasma corticosterone concentrations in hens maintained in cages compared to floor pens (Edens *et al*., 1982; Craig *et al*., 1985: Barnett *et al*., 1997a). Still others report no difference (Barnett and Bartlett 1981; Craig and Craig, 1985). Barnett and colleagues (1997a) found that the corticosterone response to ACTH was greater for hens from floor pens compared those from cages.

Birds housed in different production systems have been shown to have different levels of fear as determined by the tonic immobility test (TI) for fearfulness (Jones and Faure, 1981; Hansen *et al*., 1993). The TI duration is reported to be shorter (Jones and Faure, 1981; Kujiyat *et al*., 1983) or longer (Anderson and Adams, 1994) for hens in pens compared to cages. At the end of lay, free range hens had a shorter TI time than hens housed in conventional cages (Scott *et al*., 1998). Levels of fear have been associated with production, plasma corticosterone concentration, feather condition, mortality and behaviour, measures used to assess welfare.

Much welfare research comparing cages and floor systems have used small pens in the experimental design. These studies provide valuable information, however they often fail to be representative of large flock sizes and enclosures used in commercial practice. In an effort to more fully evaluate the welfare of hens in alternative housing systems under commercial conditions, the AECL funded a study at the University of Queensland, Gatton, Queensland, Australia. In this study the welfare of hens in four housing systems; conventional cages in a naturally ventilated shed, conventional cages in an environmentally controlled shed, barn and free range, have been evaluated. In the main study various egg production, morphological, immunological, behavioural and physiological criteria were used to assess hen welfare in the different production systems.

7.2. Objective

The present report gives details of one component of the larger study looking at the effects of housing system on egg albumen corticosterone concentration as a non-invasive measure of stress. As detailed in chapter 2, social interaction, fear, frustration, space and group size have been reported to have effects on various aspects of the HPA axis. Elevated corticosterone concentrations remain one of the measures of stress and indicate physiological adjustments by hens to perturbations of homeostasis.

7.3. Materials and methods

Birds and feeding: The 'Isa 2000' strain of laying hen was used in all flocks. The hens were purchased from a commercial source, delivered at 16 weeks of age and then maintained in the various housing systems for one full production cycle. All hens received the full range of vaccinations recommended by the commercial supplier.

Housing systems:
Conventional cages: There were four rows of wire-floored cages placed back to back and maintained in a naturally ventilated saw-tooth shed. In one row, hens were housed 3 birds per cage. In three double rows hens were housed 6 birds per cage. This was achieved by removing 2/3 of the wire partition between two adjacent cages.

Environmentally controlled shed: There was one row of conventional cages in three tiers. Each cage housed 6 hens.

Barn: There were three individual barn facilities constructed in the one shed. Each of these was built along guidelines provided by the RSPCA (2005). The barn system consisted of approximately 1/3 floor litter area and 2/3 raised slatted floor area, part of this housed central colony nests with automatic belt egg collection. Fresh feed was delivered six-times daily using a flat chain conveyor, with water being provided by a nipple line with underneath spillage cups. Both the feeding and water systems were located on the slatted area. The stocking density for each barn pen was approximately 7 hens/m^2. Initially the hens were not beak-trimmed in accordance with RSPCA guidelines.

Free Range: The free range sheds were built with design aid from the Queensland Free Range Egg Producers Association. There were three replicate free range facilities consisting of a shed containing feed and water, nests and perches and a free range area to house 600 hens (1500 hens/ hectare). Perches were supplied at 15cm/hen housed and one nest box per 5 hens. Nest boxes were constructed 4 tiers high with 8 nests per tier. After one month of lay, the nest boxes were removed from ground level and maintained at 30 cm above the floor for the remainder of the production cycle. Fresh feed was supplied 6 times daily using automatic flat chain feeders and water by two lines of nipple drinkers with underneath spillage cups.

Egg collection: Period 1: On one day during week 32 of age, 40 eggs were collected at random from each of the 3 replicates of the barn and free range systems. A similar number of eggs were collected from the top, middle and bottom rows of the environmentally controlled facility and from the three rows of conventional cages housing 6 hens per cage and from the single row of conventional cages housing 3 hens per cage. There were a total of 120 eggs collected from each of the production systems except the conventional cages housing 3 hens per cage were the total number of eggs collected was 40.

Period 2: On one day during week 70 of age, eggs were collected from each of the housing systems. In the free range systems all egg were collected and identified according to whether they were laid in the first, second, third or fourth tier of the nest boxes, with the top level being the first row. After collection, a sub-sample of eggs was taken and the proportion used determined by the number of eggs found in each of the tiers. This procedure resulted in 45, 40 and 44 eggs being collected from the three individual free range facilities. This sampling procedure was undertaken so as to avoid any possibility that the nest site might have been preferred by any particular group of hens. In the barn system, 40 eggs were collected at random from each of the three facilities (total 120 eggs). In the environmentally controlled facility all eggs laid from each of the tiers were collected. Each egg was identified according to the cage from which it had been collected. By doing this, it was possible to determine the range in egg albumen corticosterone concentrations for individual cages. From the top, middle and bottom tiers a total of 50, 49 and 46 eggs were collected, respectively. The number of hens in each cage was also recorded during the collection. A total of 60 eggs were collected from hens housed in 6-bird conventional cages. Eggs were identified according to the row and cage from which they were collected. As with the environmentally controlled facility, this allowed for determination of the range in egg albumen corticosterone concentrations for individual cages. Because of the mortality during the course of the larger study, the number of hens in each cage was not always 6 as had been the case at the start of the study. At the time of the egg collection the number of hens in each cage was recorded. A total of 60 eggs were collected from hens housed in 3-bird conventional cages. Eggs were identified according to the row and cage from which they were collected. As for the environmentally controlled facility, this allowed for determination of the range in egg albumen corticosterone concentrations for individual cages. After collection the eggs were weighed and then broken open and the albumen separated out, weighed and stored at -20^0 until assayed. The corticosterone concentrations in the egg albumen was determined by RIA as described in section 2.3.2.

Statistical analysis: Values are given as the mean ± SEM. There were three replicates for the free range facilities and three for the barn facilities, although these were constructed in the one shed. Any differences between replicates was analysed by ANOVA and where no differences were evident then the data was combined and compared to values for the conventional cage systems. In the environmentally controlled shed, differences between tiers were analysed by ANOVA and where no differences were found data was combined and used in comparison to other housing systems. Differences between housing systems were assessed by ANOVA and, if significant ($p<0.05$) differences were found, by multiple comparisons using the Tukey/Kramer test ('Statview', SAS Institute, Cary. NC, USA).

7.4. Results

Egg weights: The egg weights are given in Table 7.1. In periods 1 and 2, the egg weights were not different between replicate facilities for the free range and barn systems. Also, there were no differences in weight for eggs collected from the three tiers of conventional cages in the environmentally controlled shed. There was a significant effect of housing type and period of collection on egg weight ($p>0.05$) but no significant interaction between housing and period. The mean egg weight for period 1 was 62.8 ± 0.2 g and period 2 was 66.2 ± 0.22 g. During the course of the study the weight of those eggs collected from the barn system were significantly greater ($P<0.05$) than for the eggs collected from the environmentally controlled shed and the conventional cages housing 3 hens per cage (65.0 ± 0.3 g, 63.9 ± 0.3 g and 63.6 ± 0.5g, respectively). At 32 weeks, hens housed 6 birds per cage had significantly higher egg weight than hens maintained in cages in the environmentally controlled shed. In week 70 the differences in egg weight just failed to be significant ($p=0.064$).

Albumen weights: The egg and egg albumen weights are given in Table 7.1. In periods 1 and 2, the albumen weights were not different between replicate facilities for the barn and free range system. Also, there were no differences in weight for eggs collected from the three tiers of conventional cages in the environmentally controlled shed. There was no significant effect of housing type on the weight of egg albumen, however there was a significant difference ($P<0.001$) between the two collection periods with no interaction between housing and period. The mean egg weight for period 1 was 36.1 ± 0.2 g and for period 2 was 37.7 ± 0.2 g. Albumen weight tended to be lower for hens housed in the environmentally controlled shed and birds housed in conventional cages 3 hens per cage. This is in line with the lower egg weights seen in these treatments.

Albumen corticosterone concentration: The egg albumen corticosterone concentrations for periods 1 and 2 are given in Figure 7.1. In periods 1 and 2, egg albumen corticosterone concentrations were not different between replicate facilities for the free range and barn systems. Also, there were no differences in egg albumen corticosterone concentrations for eggs collected from the three tiers of conventional cages in the environmentally controlled shed. Over the period of the study, there was no significant effect of housing type on the egg albumen corticosterone concentration. There was a significant effect due to the period of collection ($p< 0.001$) and the interaction between period and housing type approached significance ($P=0.062$). The corticosterone concentration for period 1, was 1.54 + 0.02 ng/g and for period 2, was 1.27 ± 0.01 ng/g. When the analysis was conducted on the two periods independently, the egg albumen corticosterone concentrations were similar for all treatments in period 1 (range 1.52 to 1.57 ng/ml). However, in period 2 the difference in concentration between hens housed in the free range (1.36 + 0.3 ng/g) and conventional cages in the environmentally controlled shed (1.20 ± 0.03 ng/g) were significantly different ($p< 0.05$).

Total albumen corticosterone levels: The total amount of corticosterone in egg albumen for periods 1 and 2 are given in Figure 7.2. In periods 1 and 2, total corticosterone in albumen was not different between replicate facilities for the free range and barn systems. Also, there were no differences in total corticosterone in albumen for eggs collected from the three tiers of conventional cages in the environmentally controlled shed. There was no significant treatment effect on the total amount of corticosterone in albumen. There was a significant difference between the collection periods ($p<0.001$) with no significant interaction between housing type and period of collection. The mean (± SEM) total corticosterone in albumen for period 1 was 56.0 ± 0.7 ng and for period 2 was 48.1 ± 0.6 ng. If the analysis is done on the two periods independently, the total amount of corticosterone in albumen was similar for all treatments in period 1 (range 55.8 to 56.4 ng). In period 2, the difference in total corticosterone in albumen between hens housed in the free range (51.9 ± 1.4 ng) and conventional cages in the environmentally controlled shed (44.9 + 1.2 ng) were significantly different ($p< 0.05$).

Housing type	Egg weight (g)		Albumen weight (g)	
	32 weeks	70 weeks	32 weeks	70 weeks
Barn	63.0 ± 0.4[ab]	67.0 ± 0.4	36.5 ± 0.3	37.7 ± 0.4
Free Range	62.7 ± 0.5[ab]	66.9 ± 0.4	36.0 ± 0.3	37.8 ± 0.4
Conventional cages - environmentally controlled	61.9 ± 0.4[b]	65.5 ± 0.5	35.7 ± 0.3	37.4 ± 0.4
Conventional cages - 6 hens per cage	63.9 ± 0.4[a]	65.9 ± 0.4	36.4 ± 0.4	38.3 ± 0.4
Conventional cages – 3 birds per cage	62.4 ± 0.5[ab]	65.2 ± 0.8	36.1 ± 0.4	36.6 ± 0.6

Table 7.1: The mean (±SEM) egg and albumen weights (g) for hens maintained in conventional cages (naturally ventilated or environmentally controlled sheds) or in alternative barn and free range housing systems. Within columns values with different superscripts are significantly different ($p<0.05$).

The range in egg albumen corticosterone concentrations: For each of the production systems the range in egg albumen corticosterone concentrations for individual hens are given in figures 7.3 to 7.7. In period 1 the majority (approximately 80%) of the individual values lie in the range of 1- 2 ng/g. The percentage of values greater than 2.0 ng/g and below 1.0 ng/g are shown for each of the production systems. As will be discussed later, values above 2.0 ng/g will be considered as high and values below 1.0 ng/g as low. In period 1, the percentage of values above 2.0 ng/g ranged from 20.8 in the conventional cage system (3 birds/cage) to 11.7 in the conventional cage system (6 birds/cage). The percentage of values below 1.0 ng/g ranged from 1.7 in the conventional cage system (6 birds/cage) to 6.7 in the free range system. In period 2, there was much lower percentage of hens with corticosterone concentrations above 2.0 ng/g and higher percentage below 1.0 ng/g. For period 2, the percentage of values above 2.0 ng/g ranged from 11.4 in the conventional cage system (3 birds/cage) to 3.4 in both the conventional cage (6 birds/cage) and barn systems. The percentage of values below 1.0 ng/g ranged from 28.8 in the environmentally controlled conventional cage system to 15.6 in the barn system.

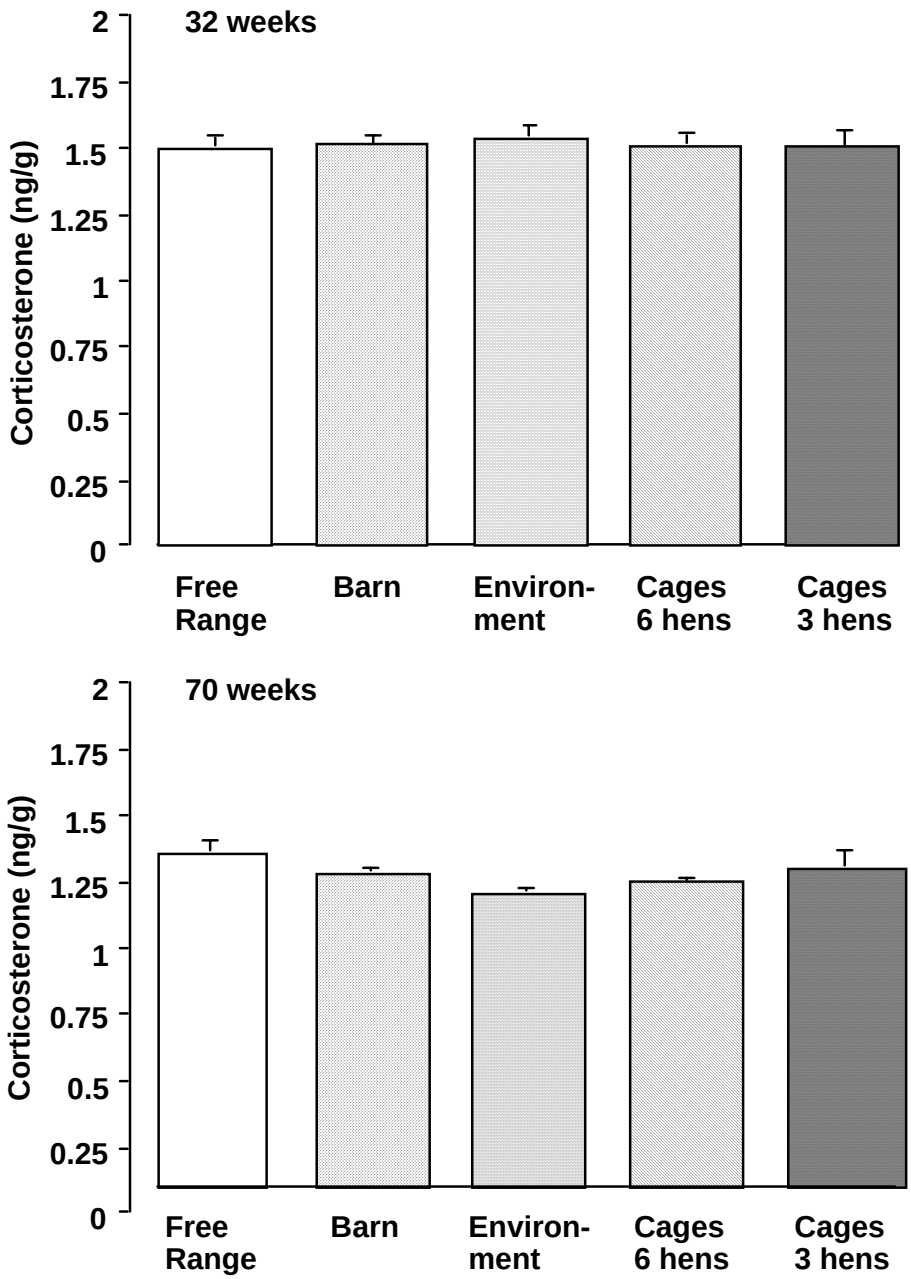

Figure 7.1: The mean (± SEM) egg albumen corticosterone concentration for hens housed in conventional cages (at either 6 or 3 birds per cage), conventional cages in an environmentally controlled shed, barn or free range housing facilities.

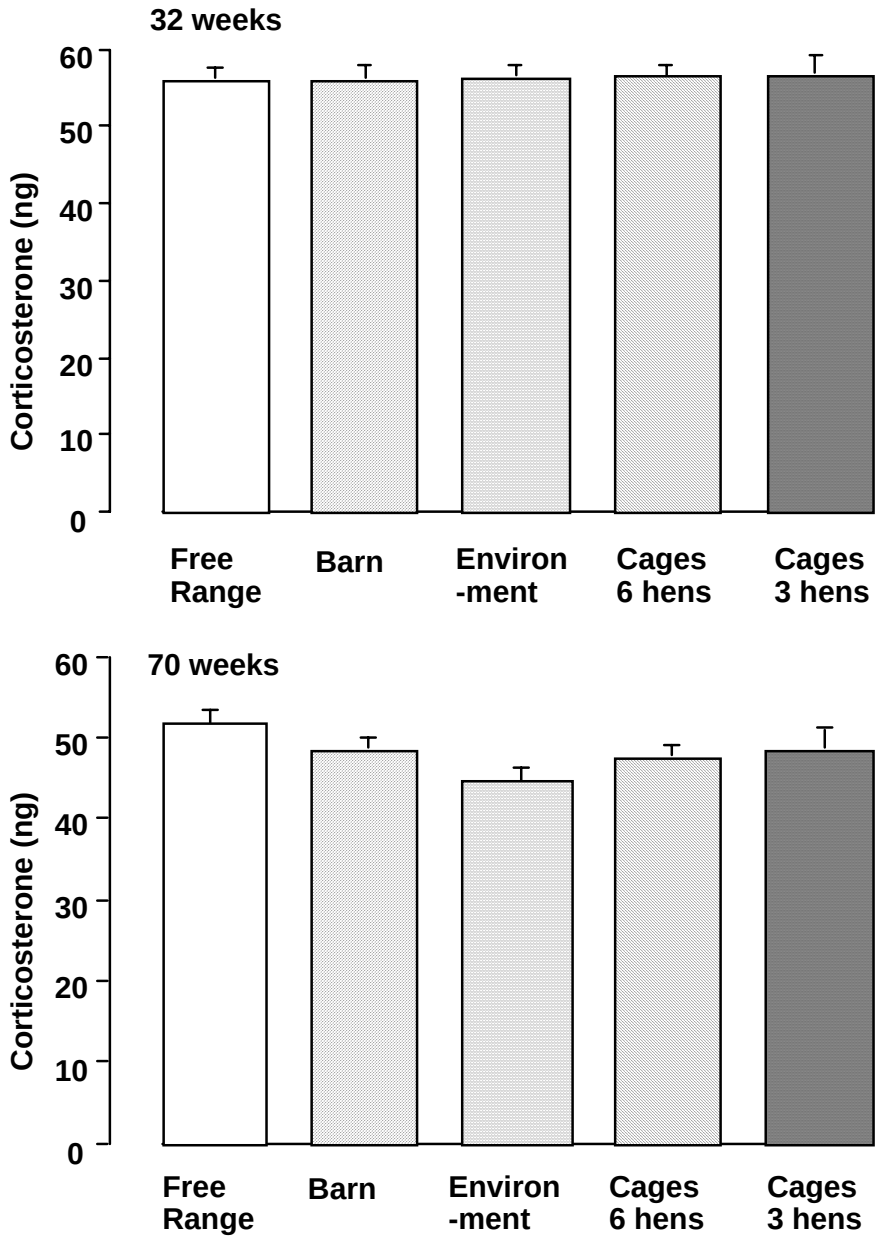

Figure 7.2: The mean (± SEM) total amount of corticosterone in egg albumen for hens housed in conventional cages (at either 6 or 3 birds per cage), conventional cages in an environmentally controlled shed, barn or free range housing facilities.

The effect of group size in conventional cage systems: In the conventional cages systems the number of hens per cage was set a 6 or 3 at the start of the study. By the time of egg collection in period 2 (70 weeks of age) the number of hens in individual cages varied because of mortality. In the conventional cages (6 birds/cage), maintained in the open-sided saw tooth shed, there was a large variation in the number of hens in each cage. For this system there were 5 cages containing 6 hens, 5 cages containing 5 hens, 16 cages containing 4 hens, 23 cages containing 3 hens and 11 cages containing 2 hens. This provided the opportunity to study what effects group size had on egg albumen corticosterone concentration. Any analysis would be compounded by the effect of also increasing the space available per hen as the group size decreased. The egg albumen corticosterone concentration

and the total amount of corticosterone in albumen are shown in Figure 7.8. There was no significant effect of group size on either the egg albumen corticosterone concentration or total amount of corticosterone.

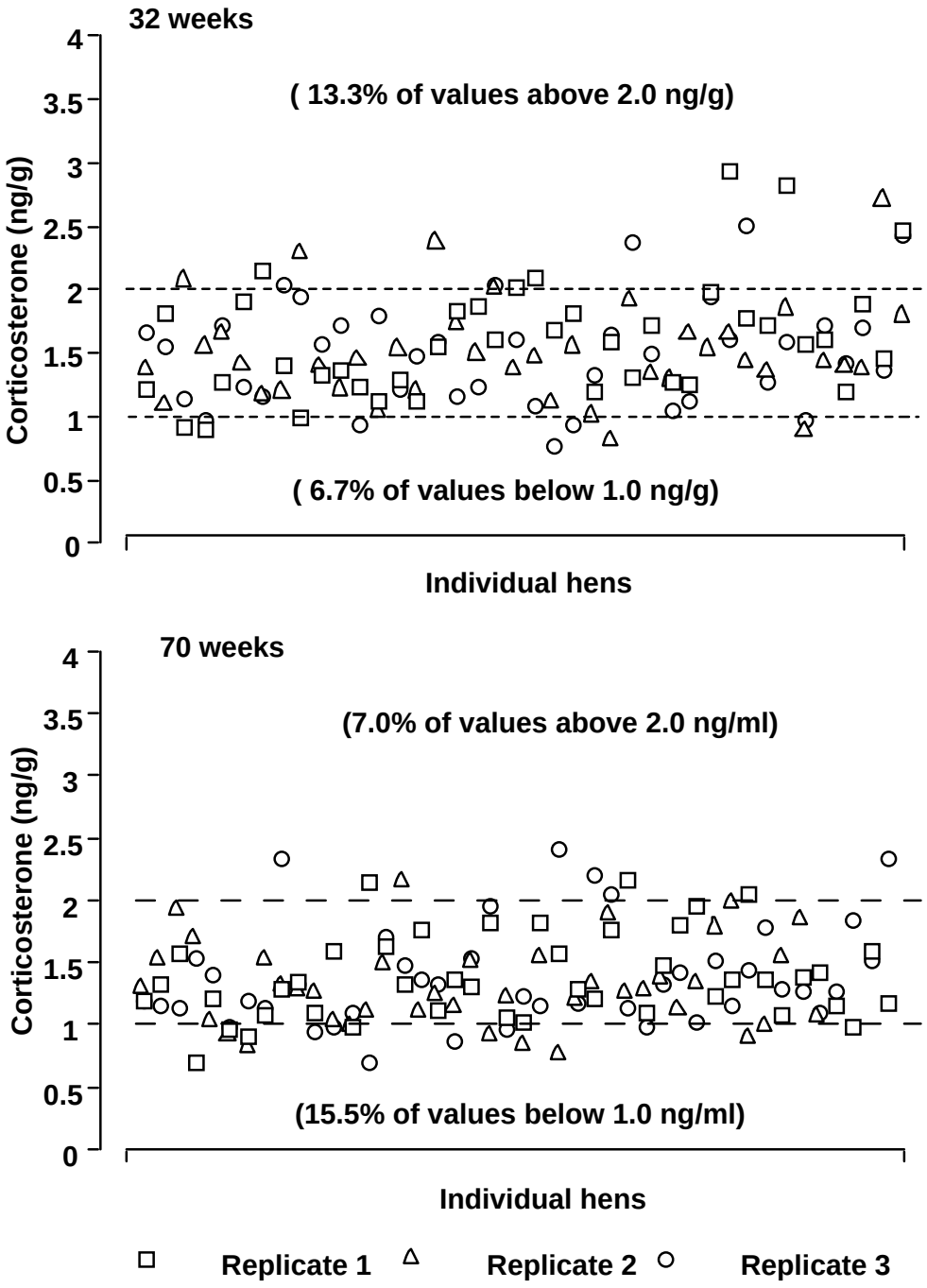

Figure 7.3: The range of albumen corticosterone concentrations for eggs collected from hens maintained in three replicates of a free range production system.

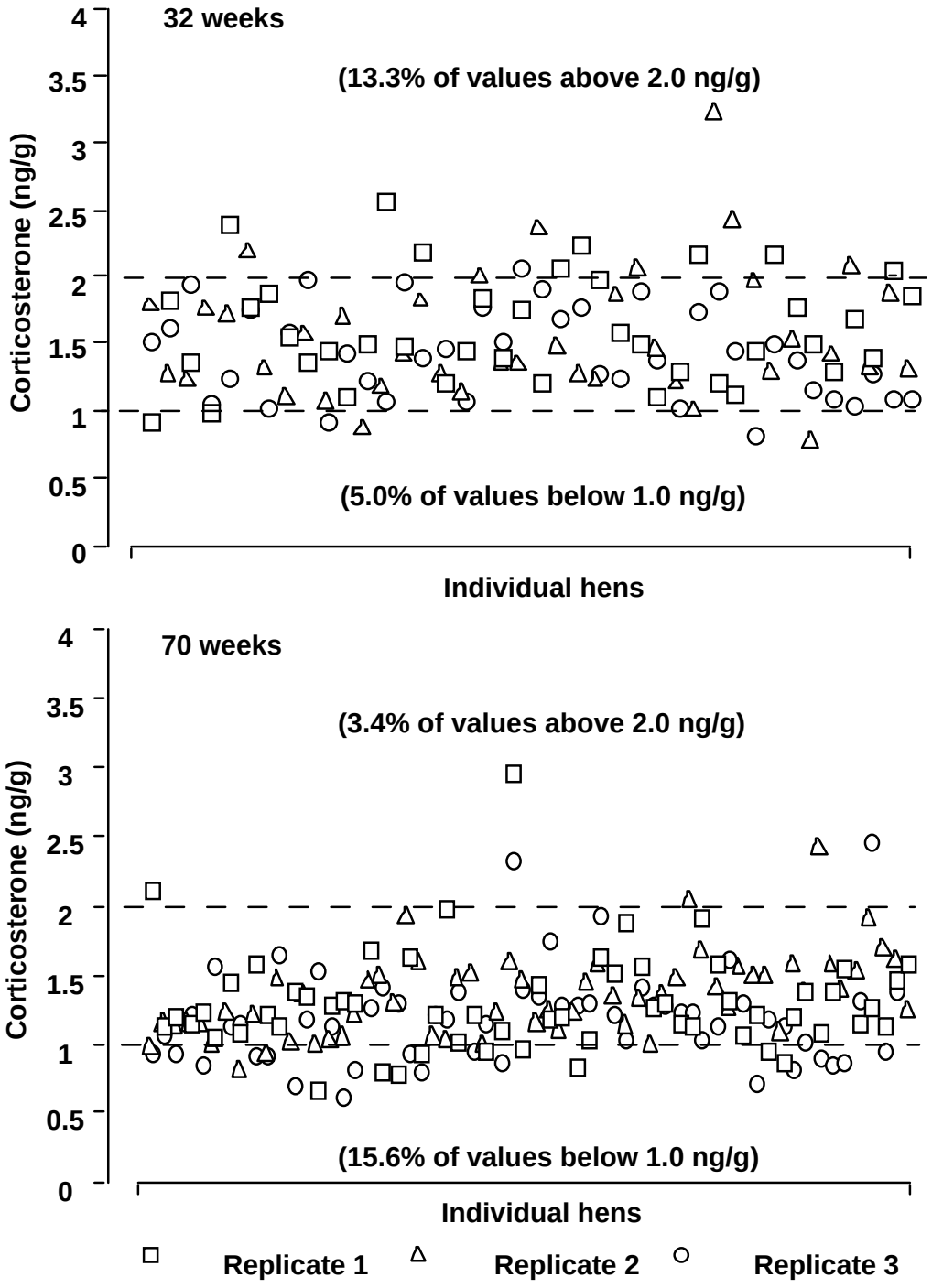

Figure 7.4: The range of albumen corticosterone concentrations for eggs collected from hens maintained in three replicates of a barn production system.

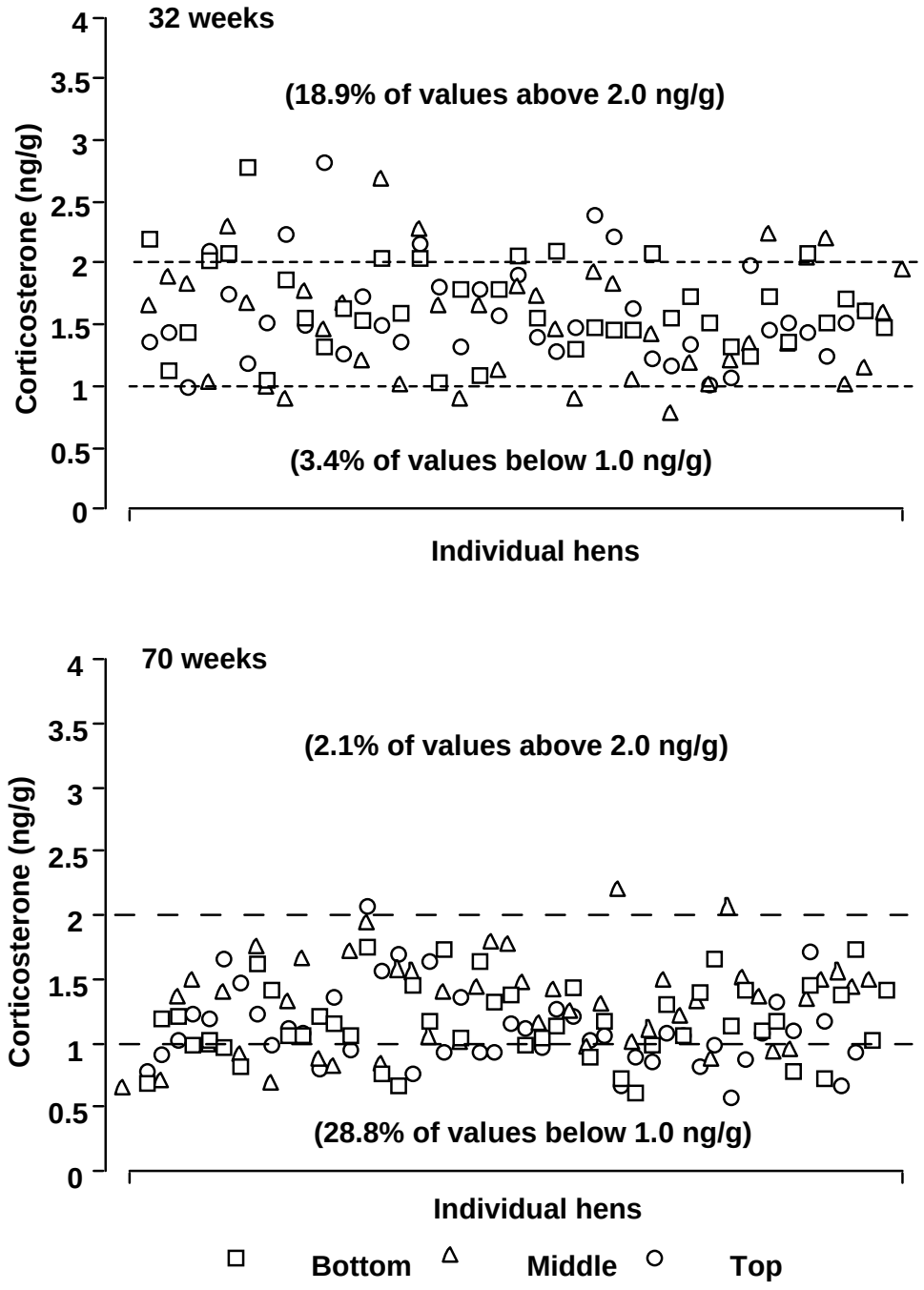

Figure 7.5: The range of albumen corticosterone concentrations for eggs collected from hens maintained in three tiers (bottom, middle and top) of conventional cages maintained in an environmentally controlled shed.

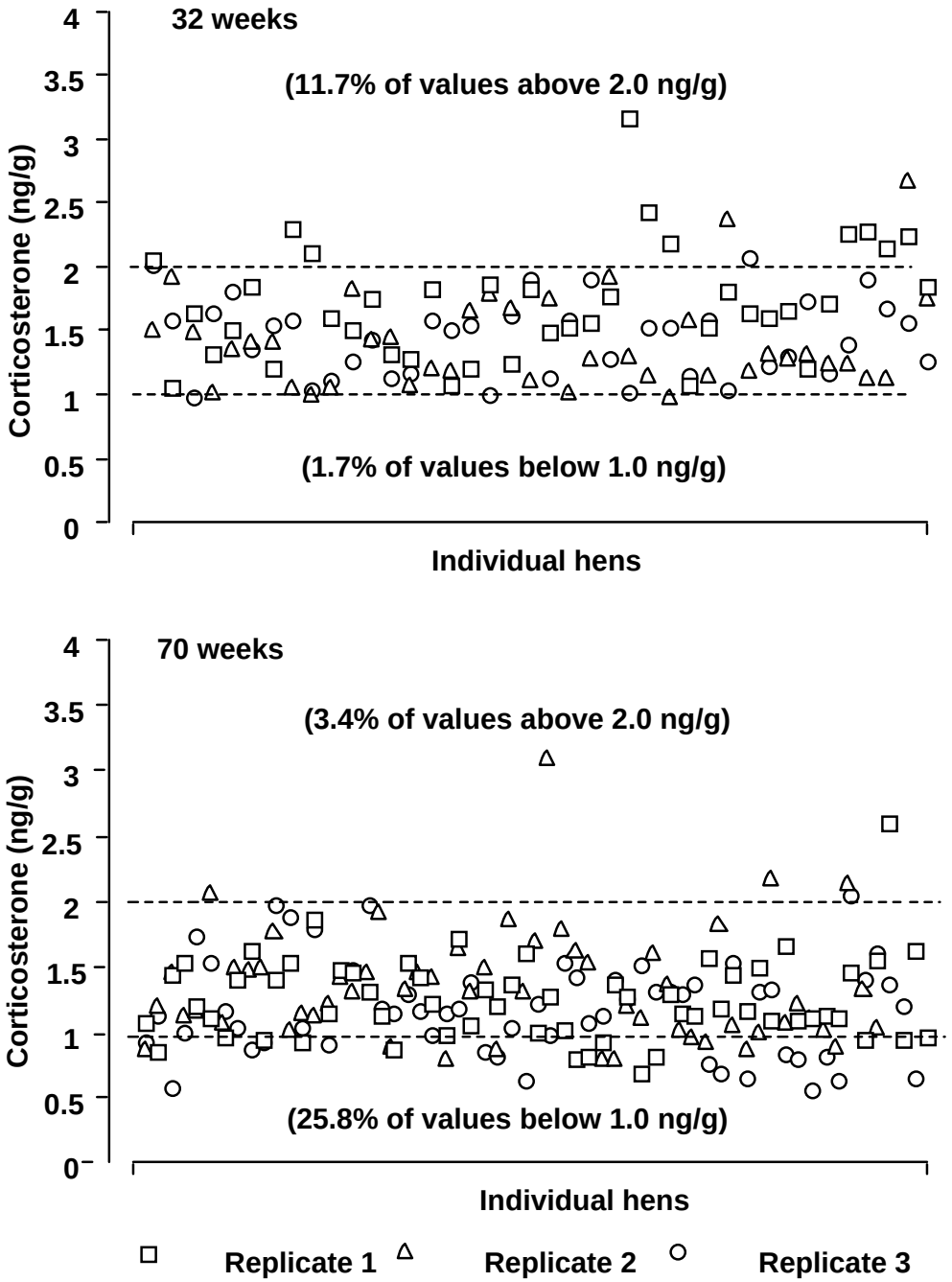

Figure 7.6: The range of albumen corticosterone concentrations for eggs collected from hens maintained in a single tier of conventional cages (initially 6 birds/cage) in an open sided saw-tooth shed.

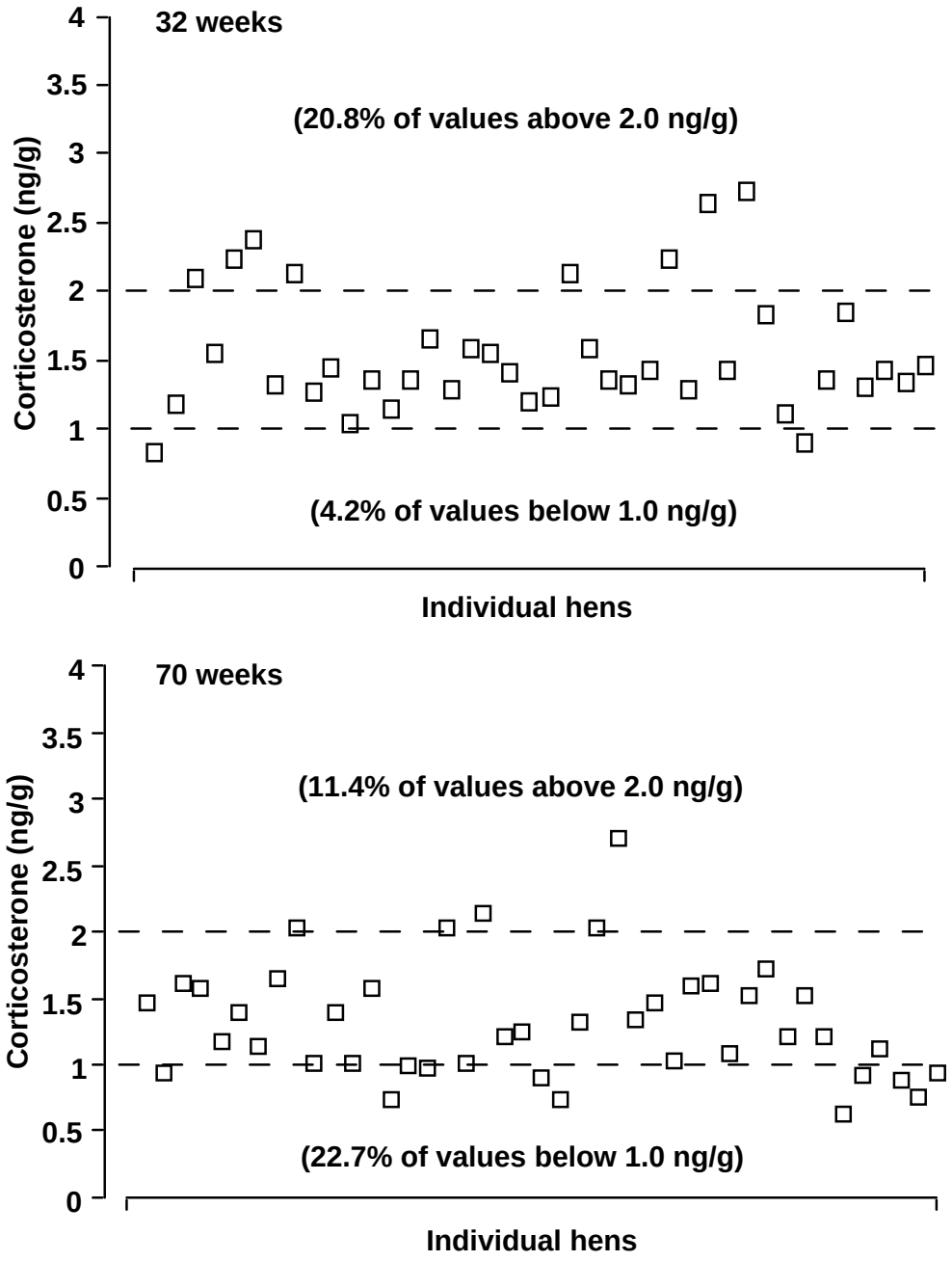

Figure 7.7: The range of albumen corticosterone concentrations for eggs collected from hens maintained in a single tier of conventional cages (initially 3 birds/cage) in a naturally ventilated saw-tooth shed.

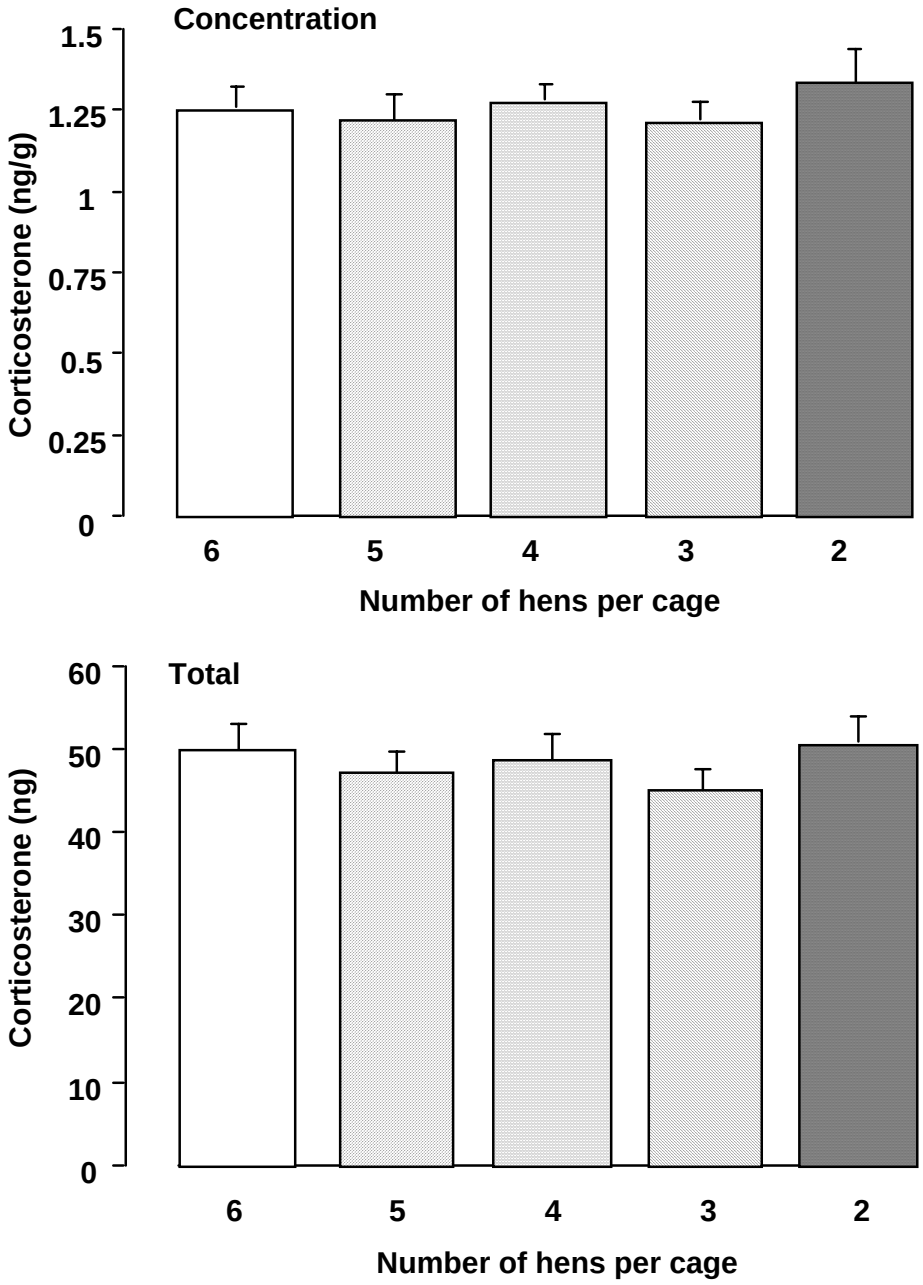

Figure 7.8: The mean (± SEM) egg albumen corticosterone concentration (upper panel) and total amount of corticosterone in albumen (lower panel) for hens maintained in conventional cages at 6, 5, 4, 3, or 2 birds/cage in a naturally ventilated shed.

7.5. Discussion

The discussion here relates to the measurement of corticosterone in albumen as a non-invasive measure of stress in a housing study conducted at the University of Queensland. In the larger study a number of criteria were used to assess welfare of hens in conventional cage, barn and free range production systems. Full details are given as part of the report to the AECL for project UQ-93A, conducted by Professor Wayne Bryden and Mr Geoff Stewart. There were three replicate treatments

for the barn and free range systems. No differences between replicates were found, so for each of these systems the data were combined and compared to the conventional cage systems. For the conventional cage systems, hens were housed 6 or 3 per cage in a naturally ventilated shed or 6 per cage in three tiers of conventional cages in an environmentally controlled shed. Measurements were made at around peak production (32 weeks) and then towards the end of production (70 weeks).

Egg weight was significantly higher towards the end of production compared to peak production. While production levels decrease towards the end of the production cycle egg weight increases (Etches, 1996). Therefore, this result is to be expected. Egg weight was higher in the barn system compared to conventional cages in the environmentally controlled shed and the conventional cages with 3 hens /cage. The difference in egg weight when the two collection periods were compared was also evident in albumen weight. While not different, the albumen weight for hens housed in conventional cages in the environmentally controlled shed and the conventional cages with 3 hens /cage tended to be lower and this again is in line with the tendency for egg weights to be lower for these treatments.

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, when the analysis was done on the period of collection. In the environmentally controlled shed environmental conditions would be more predictable than in other systems. Here the group size is small and the changes in environmental temperature minimal. The small group size allows for fairly stable social hierarchies to be formed. 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. In the floor based systems hens are continually faced with changing social interactions and environment. 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 corticosterone concentration at the end of production in free range hens compared to those housed in the environmentally controlled facility. In the free range system there was a higher percentage (7%) of hens with egg albumen corticosterone concentrations above 2.0 ng/ml and a lower percentage (15.5%) below 1.0 ng/ml at the end of production compared to other housing systems. The respective figures for the environmentally controlled shed were 2.1% and 28.8%. At the end of production there were no significant differences in corticosterone concentration between hens housed in the barn or conventional cages in the naturally ventilated shed and free range facilities although they tended to be higher in the free range system. These housing systems were exposed to similar environmental conditions and therefore exposed to similar temperature variations.

Within each production system there were individual hens that had high egg albumen corticosterone concentrations (>2.0 ng/ml). The percentage of hens with values above 2.0 ng/ml was higher at peak production than at the end of production. This is related to the lower mean levels seen later in the production cycle. This same phenomenon has been seen observed and described in experimental chapters 6 and 8 of this report. As discussed in chapter 8 this characteristic seems to be an intrinsic feature as hens' age and may be related to decreased fearfulness. 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. While decreased fearfulness could be a possible explanation for these differences, it is also plausible that the difference in corticosterone concentration is a result of there being naturally, higher physiological levels at peak production (Davis *et al*., 2000).

As a side issue, the effect of hen number per cage was investigated for those hens housed in the conventional cages in the naturally ventilated shed. At the start of the study hen number per cage was

fixed at 6, however by the end of production cycle the number per cage varied because of mortalities. Hen number per cage had no effect on the egg albumen corticosterone concentration at the end of production. These observation concur with studies detailed in chapter 3 where the number of hens in a fixed cage size had no effects on egg albumen corticosterone concentrations.

As mentioned previously, the number of hens having corticosterone concentrations above 2.0 ng/ml was higher at peak production. An important consideration from a welfare perspective would be to know if individual hens have persistently high concentrations. If this were the situation it could represent a potential welfare problem for these hens. However, it may simply represent genetic differences between hens. It would be necessary to have an 'unstressed' baseline value for individual hens and measures of the changes in individuals to clarify this point. For some hens, very high egg albumen corticosterone concentrations (>3 ng/ml) were recorded. The relationship between plasma and egg albumen corticosterone established in chapter 2 indicates that these hens have high plasma corticosterone concentrations. Therefore, while group means provide a measure for assessing differences between treatments or 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. It could be more appropriate when measuring welfare if experimental protocols considered what changes occur at the individual level. 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). While this may be ideal it is not practical for determining optimum conditions for commercial production. Under commercial conditions the objective is to optimise the group mean and to minimise the changes that occur because of the husbandry procedures.

7.6. Implications

In this study, egg albumen corticosterone concentrations were used as a non-invasive measure of stress in hens to evaluate the effects of different housing systems. Housing type had similar effects on the egg albumen corticosterone concentrations. While this suggests that the effects are similar for each housing systems there is evidence that in each system some hens have high corticosterone concentrations. This could simply be the result of genetic differences but could also indicate that some hens are stressed. If the same hens have persistently high corticosterone concentrations their welfare might be compromised. It is the changes in corticosterone concentration that remains the important consideration and under commercial conditions this is difficult to assess on an individual basis.

7.7. Recommendations

Understanding the relationship between animal production characteristics and stress is important in issues of animal welfare. The underlying concept is that if an animal is stressed it's well-being is compromised. Stress is not a single entity, however a non-specific response to many stressors is an increase in corticosterone release. The high corticosterone concentrations in egg albumen for some hens might represent individual variation in basal plasma concentration or responsiveness rather than actually represent hens under stress. If it is a response to stress, than it could be these hens are at greatest risk to immunological suppression and eventually to pathologies (Moberg, 1985) and reduced egg production (Etches and Williams, 1983; Etches *et al*., 1984a: Moudgal *et al*., 1991: Petittee and Etches, 1991). To further evaluate the risk to individuals by using egg albumen corticosterone concentration and production as criteria there is a need to identify egg production from individual hens. This would be a major challenge under commercial conditions and essentially not practical. To determine the optimum conditions in commercial production it is essential to make comparisons based on the group means and to take actions that minimise conditions that adversely affect the mean values.

Chapter 8

The effect of hen age on the response to stress

8.1. Introduction

During the production cycle the behaviour patterns of the hen are associated with adjustments being made for the environment, social interactions and age. These changes in behaviour are related to the physiological needs of the hen at different stages of the production cycle, with age seemingly to have a major influence on behaviour and levels of fear (Anderson *et al*., 2004).

Age appears to influence some measures used in the assessment of welfare. It can be a modifying influence on the effects of cage density. Increasing the number of hens per pen from 1 to either 3 or 4, had no effect on production at 24-28 weeks of age (Lei *et al*., 1972). In the same study, but a different experiment, increasing the number per cage from 1 to either 3 or 5, reduced egg production and feed efficiency at 52-56 weeks of age from hens housed 5 per cage (Lei *et al*., 1972). During a full production cycle and subsequent moult, cage density (361 and 482 cm^2/bird) had no effect on plasma corticosterone concentration, H/L ratio or T3 or T4 concentrations (Davis *et al*., 2000). However, the hormone concentrations varied through the production cycle and the changes were related to the metabolic demands associated with physiological stress. Significantly higher levels of fear were recorded at peak production (Anderson and Adams, 1992). As pullets age the response to SRBC immunisation increased (Patterson and Siegel, 1998). There is some evidence suggesting that the H/L ratio is lower in older birds (Mench *et al*., 1986).

Early stressful events experienced by animals can influence responses to subsequent stressors (Sapolsky, 1992). It is possible that habituation requires some exposure to glucocorticoids during the initial stressful event. High circulating corticosterone concentrations may be important in preparing the body to respond to subsequent stressors. Neonatal exposure to acute heat stress appears to improve heat tolerance in chickens (May *et al*., 1987). Increased corticosterone concentrations in the neonatal stage could alter corticosterone receptor number in the brain so that at later stages animals are more responsive to raised corticosterone levels. The corticosterone acts to prepare the body for the disruption to homeostasis. By being more responsive later in life, less corticosterone is released due to the enhanced feedback regulation (Sapolsky *et al*., 1984). Responses to exogenous CRF are influenced by age, dose rate and frequency of injections.

Chickens and hens seem to be able to establish hierarchies after no more than three interactions (Chase, 1982; Littin and Cockrem, 2001). However, this would seem to depend on the size of the group (Cunningham, 1988). In another study, based on the level of hen interactions, Cunningham (1988) considered that the hierarchy had been completely established after 6 weeks of hens being placed in group-cages. In this study hens were housed in groups of 4 or 6 and allocated spaces of 316 and 406 cm^2/bird. Hens were observed between 23-26 and 37-40 weeks of age. Aggressive activity decreased with age. At the younger age both a decrease in cage area/bird and an increase in the group size increased significantly the level of aggressive activity whereas at the older age cage space had no effect. The effect of age was also recorded by others (Choundary *et al*., 1972; Choundary and Craig, 1972).

In chapters 6 and 7, evidence was provided that suggests corticosterone concentrations in hens diminished with age. In those studies, the egg albumen corticosterone concentrations were lower at the end of production compared to peak production. This could indicate that hens are less responsive to stress as they age. The higher corticosterone concentrations at a younger age could predispose hens to greater risk from disease because of the association between glucocorticoids and immunosuppression (see Section 1.5.1.3).

8.2. Objective

In the present study the effect of hen age on plasma and egg albumen corticosterone concentrations and the response to stress was investigated.

8.3. Materials and methods

Birds and husbandry: Sixty five Isa Brown laying hens were obtained from a commercial source and delivered to the research facility at 16 weeks of age. Birds were floor reared and then acclimatised to cages and nipple drinkers for 4 weeks before delivery. Hens were vaccinated against Marek's disease, infectious bronchitis, fowl pox, avian encephalomyelitis, infectious laryngotracheitis and Newcastle disease. Birds were beak trimmed at 12 weeks of age. From the time of delivery, hens were housed individually in conventional cages (47 cm x 28 cm x 48 cm). Throughout the study hens were fed *ad libitum* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h of light with lights on at 0500h.

Experimental design: At 24, 34, 54 and 70 weeks of age (treatment periods 1-4) hens were subject to a procedure know to be stressful. Fifty hens were removed from their single cages and transferred to group cages (46 cm x 50 cm x 62 cm) and housed 5 birds per cage (total of 10 cages). To avoid any possibility of habituation to other members of the group, different hens were housed together at each of the treatment periods. At each treatment period the same fifteen hens remained in their single cage and acted as a control group for the duration of the study. The day hens were moved was designated as day 0 of each treatment period. On day 0 starting at 0500h, the hens were moved to group cages and remained there for 10 days.

Blood sampling: The blood sampling procedures were the same for all the treatment periods. On day -2, between 1600-1700h, a 1.5ml blood sample was taken from 30 of the hens in the group to be moved and also from the fifteen hens that remained as the control treatment. On days 0, 1, 5 and 9, again between 1600-1700h, a 1.5ml blood sample was taken from 2 hens in each of the group cages and also from the fifteen hens that remained in the single cages as the control treatment. Blood remained on ice and was centrifuged within 45 min of being collected. The plasma was collected and stored at -20^0C until assayed.

Egg collection: On day -1 of each treatment period, 30 eggs were collected from the hens to be moved the following day and all eggs laid by the 15 hens which were to remain as the controls. On days 1, 2, 6 and 10 of each period all eggs laid by hens in the group cages and those remaining in the single cages were collected. Egg collection was started at 0600h, 1h after lights on. This was designated as zero oviposition time and so any egg laid before this time had an oviposition time of zero. Eggs were collected each hour after 0600h until 1530h. For eggs collected at 0700h the oviposition time was recorded as +1 h, for those at 0800h the oviposition time was +2 h and similarly the oviposition time increased 1h for each additional hourly collection. The eggs were weighed then broken open and the albumen separated out and weighed and stored at -20^0C until assayed. Daily egg production was recorded for the treatment periods.

Measurement of corticosterone: The corticosterone concentration in egg albumen and plasma were determined by RIA as described in section 2.3.2.

*Statistical analysis***:** Values are given as means ± SEM. Differences between treatments, period and days of collection were assessed by ANOVA and if significant ($p<0.05$) then multiple comparisons were made using the Tukey test. In the analysis, the experimental unit was taken to be the cage. For further evaluation, effects within periods were analysed in a similar manner and when treatment effects were significant comparisons on individual days were made by unpaired Student's t-test. All analysis was conducted using the 'Statview' computer program (SAS Institute Inc, NC, USA). When individual comparisons are made the significance level was set at ($P<0.05$).

8.4. Results

Egg production: The mean daily egg production from the time the hens were moved until the end of measurements in each period is given in Table 8.1. The hens housed in the group cages tended to have lower egg production than hens housed continuously in single cages, however this just failed to reach significance ($P=0.056$). The period of collection had a significant effect ($p<0.001$) on daily egg production while there was no interaction between the period of collection and type of cage housing. The mean (± SEM) daily egg production for periods 1, 2, 3 and 4 were 0.93 ± 0.01, 0.95 ± 0.01, 0.93 ± 0.02 and 0.78 ± 0.3 respectively. The production level during period 4 was lower than for the other periods.

Egg weights: The mean egg weights are given in Table 8.2. No significant effects were observed for treatment (singles vs group housing) or for day of collection and no interactions between treatment, period and day. There was a significant effect of collection period on egg weight ($P<0.001$). The mean (± SEM) egg weights for periods 1, 2, 3 and 4 were 51.0 ± 0.6, 59.8 ± 0.4, 62.7 ± 0.4 and 65.1 ± 0.4 g, respectively, with the differences for all comparisons being significant.

Treatment	**Period**			
	1	**2**	**3**	**4**
Single	0.95 ± 0.02	0.98 ± 0.02	0.95 ± 0.02	0.79 ±0.03
Group	0.91 ± 0.01	0.93 ± 0.01	0.92 ± 0.05	0.76 ± 0.05

Table 8.1: The mean (± SEM) daily egg production for hens housed in individual cages (single) or in individual cages and then moved to group cages (5 birds/cage). The production levels are for the time from when hens were moved to group cages until the end of the measurements in each period.

Period	Treatment	Egg weight (g)				
		Day -1	Day 1	Day 2	Day 6	Day 10
1	Single	47.8 ± 2.4	51.3 ± 2.0	49.7 ± 1.2	53.0 ± 1.6	53.4 ± 2.2
	Group	51.4 ± 1.6	50.0 ± 1.0	49.3 ± 0.5	51.7 ± 1.5	52.1 ± 1.0
2	Single	60.7 ± 1.9	59.3 ± 0.9	58.6 ± 0.9	60.0 ± 1.0	60.1 ± 1.3
	Group	59.4 ± 0.9	59.3 ± 1.6	60.6 ± 0.6	60.5 ± 0.9	59.9 ± 0.4
3	Single	60.7 ± 1.9	63.9 ± 1.5	63.7 ± 1.3	64.4 ± 1.3	63.3 ± 1.4
	Group	59.0 ± 0.8	64.5 ± 0.6	63.7 ± 0.9	64.9 ± 0.7	64.6 ± 1.1
4	Single	64.6 ± 1.7	64.6 ± 1.4	64.1 ± 1.5	63.9 ± 1.7	64.7 ± 1.4
	Group	66.5 ± 1.3	65.7 ± 1.2	66.0 ± 0.9	66.2 ± 1.1	64.7 ± 1.1

Table 8.2: The mean (± SEM) egg weights for hens housed in individual cages (single) or in individual cages on day -1 and then moved to group cages (5 birds/cage) on day 0.

Albumen weights: The mean egg albumen weights are given in Table 8.3. No significant effects were observed for day of collection. There were significant effects due to treatment (single vs group; $P<0.05$) and collection period ($P<0.001$) on egg albumen weight and a significant interaction between treatment and collection ($P<0.05$). The mean (± SEM) albumen weight for the group hens was 35.4 ± 0.3 g and the single hens was 34.5 ± 0.3 g. The means (± SEM) for periods 1, 2, 3 and 4 were 31.8 ± 0.4, 34.9 ± 0.3, 35.8 ± 0.3 and 37.3 ± 0.5 g, respectively, with the differences for all comparisons being significant except when period 2 is compared to period 3. When the analysis is completed for individual periods, treatment differences were found to be significant in period 4 where for group hens, the albumen weight was 38.5 + 0.8 g and the single hens was 36.2 ± 0.6 g.

Period	Treatment	Egg albumen weight (g)				
		Day -1	Day 1	Day 2	Day 6	Day 10
1	Single	31.3 ± 1.6	32.5 ± 1.2	31.4 ± 1.1	32.5 ± 1.0	33.2 ± 1.3
	Group	30.6 ± 1.0	30.6 ± 0.6	30.7 ± 0.5	32.7 ± 0.9	32.6 ± 0.6
2	Single	34.3 ± 1.1	34.6 ± 0.8	34.1 ± 0.7	34.3 ± 0.8	34.8 ± 1.3
	Group	34.6 ± 0.6	35.7 ± 0.7	36.5 ± 0.6	35.3 ± 0.6	35.9 ± 0.3
3	Single	34.5 ± 1.1	35.4 ± 1.3	34.7 ± 1.2	37.3 ± 1.4	35.6 ± 1.2
	Group	35.2 ± 0.7	36.8 ± 0.6	35.9 ± 0.7	37.1 ± 0.6	36.5 ± 0.7
4	Single	35.7 ± 1.5	36.1 ± 1.2	35.7 ± 1.2	35.7 ± 1.5	38.1 ± 1.1
	Group	38.5 ± 0.9	36.9 ± 1.1	40.3 ± 1.1	40.3 ± 2.7	39.7 ± 3.3

Table 8.3: The mean (± SEM) egg albumen weights for hens housed in individual cages (single) or in individual cages on day –1 and then moved to group cages (5 birds/cage) on day 0.

Oviposition time: The mean (± SEM) oviposition times are given in Table 8.4. There were significant treatment ($P<0.001$) and period ($P<0.001$) effects with the interaction between period and day also significant ($P<0.01$). The oviposition time was longer in the group-caged hens (3.7 ± 0.1 vs 3.4 ± 0.1h). The oviposition times in periods 1 (4.4 ± 0.1h) and 2 (3.8 ± 0.1h) were significantly longer than periods 3 (2.9 ± 0.1 h) and 4 (3.0 ± 0.1h) ($p<0.05$). On days –1 and 6 the oviposition time decreased from period 1 to 3 and then was similar in period 4. On days 1 and 2 the oviposition was similar in period 1 and 2, decreased to period 3 and then was similar in period 4. On day 10 the oviposition time increased from period 1 to 2, decreased to period 3 and then remained similar in period 4.

Oviposition time: Effects within periods:
Period 1 (24 weeks): There was a significant treatment and day effect ($P<0.01$). The oviposition time was not different on days, -1 and 10, however on days 1 and 2 they were longer in the group caged hens compared to the single caged hens. The oviposition time on day –1 was longer than on day 3.

Period 2 (34 weeks): There was a significant treatment effect ($P<0.05$) and no day effect. On days –1, 1, 6 and 10 the oviposition times were not different between treatments. On day 2 the oviposition time was longer in the group-caged hens.

Period 3 (54 weeks): There were no significant effects during period 3.

Period 4 (70 weeks): There were no significant effects during period 4.

Period	Treatment	Oviposition time (h)				
		Day -1	Day 1	Day 2	Day 6	Day 10
1	Single	5.1 ± 0.4	3.6 ± 0.3	3.1 ± 0.3	4.1 ± 0.6	3.6 ± 0.4
	Group	4.9 ± 0.3	4.6 ± 0.2	4.5 ± 0.3	4.5 ± 0.4	4.6 ± 0.3
2	Single	3.6 ± 0.3	4.0 ± 0.3	3.9 ± 0.4	3.3 v 0.3	3.6 ± 0.6
	Group	3.4 ± 0.2	4.1 ± 0.2	5.0 ± 0.3	4.0 ± 0.3	4.3 ± 0.3
3	Single	2.9 ± 0.3	2.9 ± 0.4	2.2 ± 0.5	2.9 ± 0.4	2.5 ± 0.3
	Group	3.1 ± 0.3	2.8 ± 0.3	2.9 ± 0.2	2.9 ± 0.3	3.0 ± 0.3
4	Single	3.2 ± 0.3	2.2 ± 0.9	2.5 ± 0.3	3.1 ± 0.3	3.1 ± 0.4
	Group	3.6 ± 0.5	2.9 ± 0.2	2.7 ± 0.1	3.2 ± 0.3	2.9 ± 0.2

Table 8.4: The mean (± SEM) oviposition time for hens housed in individual cages (singles) or in individual cages on day -1 and then moved to group cages (5 birds/cage) on day 0.

Plasma corticosterone: The plasma corticosterone concentrations are shown in Figure 8.1. There are no values for day 1 of period 1 (24 weeks) because of a mishap that resulted in loss of the samples before analysis. There were significant differences due to treatment ($P < 0.001$), period of collection ($P < 0.0001$), day of collection ($P < 0.01$) and interactions between treatment and day ($P < 0.0001$). Other interactions were not significant. Over the course of the study, the corticosterone concentrations was significantly higher in the hens that were moved to group cages compared to the hens that remained in their individual cages (0.70 ± 0.02 vs 0.59 ± 0.02 ng/ml).

There was a decrease in corticosterone concentration over the course of the study. The overall means and treatment means for each period are given in Table 8.5. The overall concentration at 24 weeks (period 1) was significantly higher than for all other periods and at 34 and 54 weeks (periods 2 and 3) the concentration was higher than at 70 weeks (period 4). These differences were evident in the single treatment group whereas the difference between period 3 and 4 were not evident in the group treatment.

Because of the experimental protocol a significant effect of day of collection on corticosterone concentration was expected. The overall means and treatment means for each day are given in Table 8.6. The single and group treatments had similar concentrations on the day before the group hens were moved. The concentration increased significantly in the group treatment after the hens were moved and was higher than other days except day 9. In the single treatment there was a significant decrease on days 0, 1 and 5 compared to day –2. In both treatments plasma concentration increased on day 9 to levels similar to day -2.

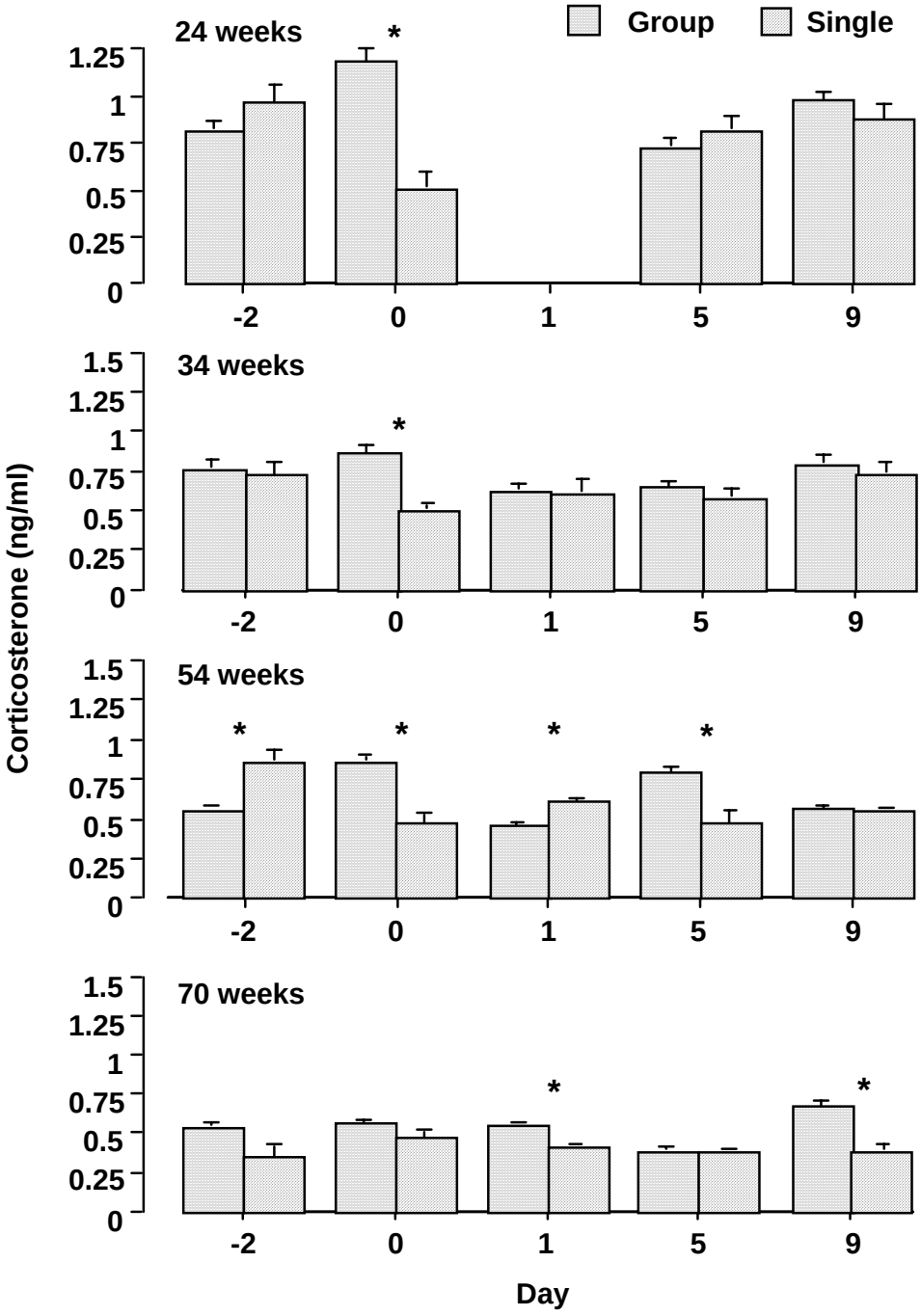

Figure 8.1: The mean (± SEM) plasma corticosterone concentrations for hens housed in individual cages (single) or in individual cages and then moved to group cages (5 hens/cage) for 10 days at 24, 34, 54 and 70 weeks of age. On individual days for columns marked with an asterisk treatments are significantly different ($p < 0.05$).

Plasma corticosterone: *Effects within periods*

Period 1 (24 weeks): There was a significant treatment effect but no significant effect of day or treatment and day interaction. The concentration of corticosterone was not different on days -2, 5 and 9, however on day 0 concentration was higher in the group caged hens compared to the single caged hens. For the group treatment, concentration was higher on days 0 and 9, however lower on days -2

and 5. The mean increase when the hens were moved (day –2 vs 0) was 0.38 ng/ml. In the single treatment, from day –2 to 0 there was a decrease of 0.45 ng/ml in mean plasma concentration.

Period 2 (34 weeks): There was a significant treatment effect and no day effect or interaction between treatment and day. The corticosterone concentrations were similar for treatments on all days except day 0 when it was significantly higher for the group treatment. The mean increase for hens that were moved (day –2 vs 0) was 0.10 ng/ml. In the single housed hens there was a decrease in corticosterone concentration from days –2 to 0 of 0.23 ng/ml.

Period 3 (54 weeks): There was no significant treatment effect but a significant ($P<0.05$) day effect and interaction between treatment and day ($P<0.001$). There were significantly higher corticosterone concentrations in the group treatment on days 0 and 5 whereas the single treatment hens had significantly higher concentrations on days –2 and 1. The mean increase for hens that were moved (day –2 vs 0) was 0.33 ng/ml. In the single housed hens there was a decrease in corticosterone concentration from days –2 to 0 of 0.38 ng/ml.

Period 4 (70 weeks): There was a significant ($P<0.001$) treatment effect with no other effects. The concentration of corticosterone was significantly higher in the group housed hens on days 1 and 9. The mean increase for hens that were moved (day –2 vs 0) was 0.04 ng/ml. In the single housed hens the increase in corticosterone concentration from days –2 to 0 was 0.13 ng/ml.

Period	**Corticosterone concentration (ng/ml)**		
	Overall	**Single**	**Group**
1 (24 weeks)	0.86 ± 0.04^{a}	0.79 ± 0.05^{a}	0.92 ± 0.06^{a}
2 (34 weeks)	0.68 ± 0.02^{b}	0.64 ± 0.03^{b}	0.74 ± 0.04^{b}
3 (54 weeks)	0.61 ± 0.02^{b}	0.59 ± 0.03^{b}	0.63 ± 0.03^{bc}
4 (70 weeks)	0.47 ± 0.02^{c}	0.40 ± 0.02^{c}	0.55 ± 0.03^{c}

Table 8.5: The overall and treatment mean (± SEM) plasma corticosterone concentrations for hens housed in single cages or moved to group cages on four occasions during their production cycle. Within columns values with different superscripts are significantly different ($P <0.05$).

Day	Corticosterone concentration (ng/ml)		
	Overall	Single	Group
-2	0.67 ± 0.03^{a}	0.72 ± 0.05^{a}	0.64 ± 0.04^{bc}
0	0.67 ± 0.04^{a}	0.49 ± 0.03^{c}	0.88 ± 0.06^{a}
1	0.55 ± 0.03^{b}	0.55 ± 0.04^{bc}	0.55 ± 0.04^{c}
5	0.57 ± 0.03^{ab}	0.55 ± 0.03^{bc}	0.65 ± 0.04^{bc}
9	0.69 ± 0.03^{a}	0.64 ± 0.04^{a}	0.75 ± 0.05^{ab}

Table 8.6: The overall and treatment mean (± SEM) plasma corticosterone concentrations for hens housed in single cages or moved to group cages on four occasions during their production cycle. Blood samples were collected two days before the group hens were moved (day –2) and then on days 0, 1, 5, and 9 after they were moved, with day 0 being the day hens were moved. Within columns values with different superscripts are significantly different ($P < 0.05$).

Egg albumen corticosterone concentration: The egg albumen corticosterone concentrations are shown in Figure 8.2. There were significant differences due to treatment ($P<0.0001$), period of collection ($P<0.0001$), day of collection ($P<0.0001$) and interaction between period and day of collection ($P<0.0001$). Other interactions were not significant. Over the course of the study, the corticosterone concentrations was significantly higher in the hens that were moved to group cages compared to the hens that remained in their individual cages (0.96 ± 0.01 vs 0.87 ± 0.01 ng/g).

There was a decrease in corticosterone concentration over the course of the study. The overall means and treatment means for each period are given in Table 8.7. The concentration at 24 weeks (period 1) was significantly higher than for all other periods and at 34 weeks (period 2) the concentration was higher than at 70 weeks (period 4). These differences were evident in the single treatment group whereas the difference between period 2 and 4 was not apparent in the group treatment.

Because of the experimental protocol a significant effect of day of collection on corticosterone concentration was expected. The overall means and treatment means for each day are given in Table 8.8. The single and group treatments had similar concentrations on the day before the group hens were moved. The concentrations increased in both treatments when the hens were moved, however the increase for the single treatment was not significant. In the group treatment the concentration on the day following moving (day 1) was significantly higher than for all other days.

As stated previously, the interaction between period and day of collection was significant with no significant interactions with treatment. On day –1 the corticosterone concentrations varied little between collection periods. On days 1 and 6 the corticosterone concentrations decreased from period 1 to period 2 and then were similar for the remaining periods. On day 2, corticosterone concentrations decreased from period 1 to period 2 and remained similar in period 3 then decreased

again in period 4. On day 10 corticosterone concentrations decreased slightly from period 1 through to period 4.

Egg albumen corticosterone concentration: Effects within periods
Period 1 (24 weeks): There were significant treatment ($P<0.05$) and day ($P<0.001$) effects. The concentration of corticosterone was not different on days -1, 1 and 2, however on days 6 and 10 they were higher in the group housed hens compared to the single caged hens. For the hens that were moved, the corticosterone concentration on day –1 was significantly lower than on days 1, 2 and 6. For the single treatment group the corticosterone concentration was higher on day 2 compared to days –1 and 10. The mean increase when the hens were moved (day –1 vs 1) was 0.3 ng/mg. In the single housed hens the corticosterone concentration on days –1 and 5 are significantly lower than on day 1. The increase from day –1 to 1 was 0.28 ng/ml and was similar to the hens that were moved.

Period 2 (34 weeks): There was a significant treatment ($P<0.05$) effect and no day effect with a significant treatment and day interaction. Corticosterone concentrations were similar for treatments on days –1, 6 and 10 whereas on days 1 and 2 the concentration was significantly higher in the group housed hens. The mean increase for hens that were moved (day –1 vs 1) was 0.18 ng/ml. In the single housed hens there was a decrease in corticosterone concentration from days –1 to 1 of 0.11 ng/ml. This was largely the result of the single treatment group tending to have a higher concentration on day –1.

Period 3 (54 weeks): There was a significant treatment ($P<0.001$) effect but no day effect. There were significantly higher corticosterone concentrations in the group treatment on days –1 and 10 compared to the single treatment group. No other differences were significant although they approached significance on day 1 ($P=0.06$) where concentrations for the group housed hens tended to be higher. The mean increase for hens that were moved (day –1 vs 1) was 0.10 ng/ml. In the single housed hens the increase in corticosterone concentration from day –1 to 1 was 0.06 ng/ml.

Period 4 (70 weeks): There were significant treatment and day effects ($P<0.001$). The concentration of corticosterone was not different on days 1, 2 and 10, however on days 1 and 6 they were higher in the group housed hens compared to the single caged hens. For the hens that were moved, the corticosterone concentration on day –1 was significantly lower than on day 6 and the concentration on day 1 was significantly higher than on days 2, 6 and 10. For the control treatment the corticosterone concentration was higher on day 2 compared to day 6. The mean increase for hens that were moved (day –1 vs 1) was 0.12 ng/ml. In the single housed hens the increase in corticosterone concentration from days –1 to 1 was 0.07 ng/ml.

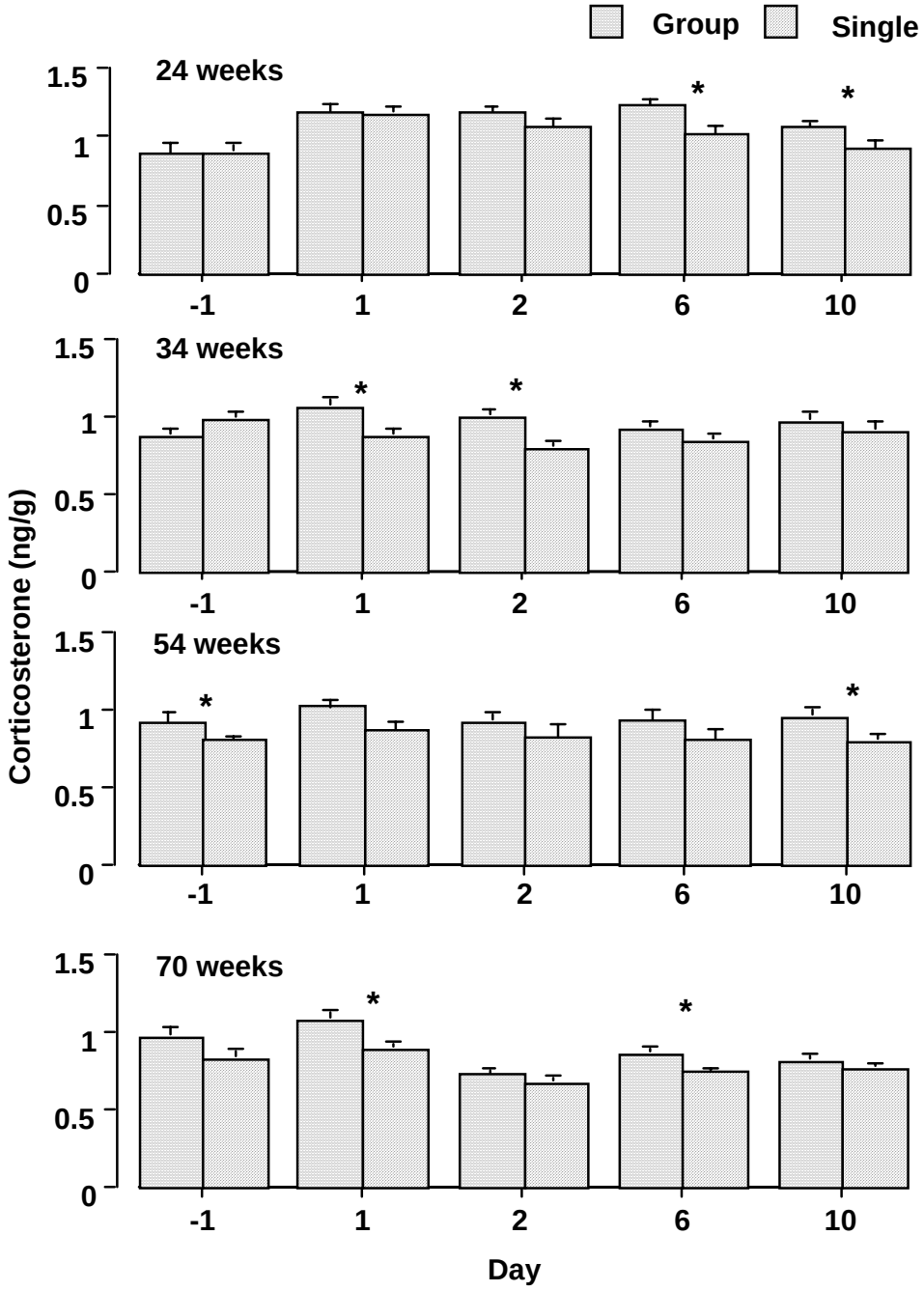

Figure 8.2: The mean (± SEM) egg albumen corticosterone concentrations for hens housed in individual cages (single) or in individual cages and then moved to group cages (5 hens/cage) for 10 days at 24, 34, 54 and 70 weeks of age. Eggs were collected the day before (day –1) moving and then 1, 2, 6 and 10 days after the hens were moved. On individual days for columns marked with an asterisk treatments are significantly different ($p < 0.05$).

Period	Corticosterone concentration (ng/g)		
	Overall	Single	Group
1 (24 weeks)	1.03 ± 0.02^{a}	1.00 ± 0.03^{a}	1.06 ± 0.03^{a}
2 (34 weeks)	0.91 ± 0.02^{b}	0.88 ± 0.02^{b}	0.95 ± 0.03^{b}
3 (54 weeks)	0.87 ± 0.02^{bc}	0.82 ± 0.02^{bc}	0.93 ± 0.02^{b}
4 (70 weeks)	0.84 ± 0.02^{c}	0.78 ± 0.02^{c}	0.90 ± 0.02^{b}

Table 8.7: The overall and treatment mean (± SEM) corticosterone concentrations in egg albumen for hens housed in single cages or moved to group cages on four occasions during their production cycle. Within columns values with different superscripts are significantly different ($P < 0.05$).

Day	Corticosterone concentration (ng/g)		
	Overall	Single	Group
-1	0.90 ± 0.02^{b}	0.87 ± 0.03	0.91 ± 0.02^{b}
1	1.00 ± 0.02^{a}	0.94 ± 0.03	1.08 ± 0.03^{a}
2	0.89 ± 0.02^{b}	0.85 ± 0.03	0.96 ± 0.03^{b}
6	0.91 ± 0.02^{b}	0.87 ± 0.03	0.96 ± 0.03^{b}
10	0.89 ± 0.02^{b}	0.85 ± 0.03	0.95 ± 0.02^{b}

Table 8.8: The overall and treatment mean (± SEM) egg albumen corticosterone concentrations for hens housed in single cages or moved to group cages on four occasions during their production cycle. Eggs were collected on the day before the group hens were moved and then on days 1, 2, 6, and 10 after they were moved. Within columns values with different superscripts are significantly different ($P < 0.05$).

Total corticosterone in albumen: The total amount of corticosterone in egg albumen is given in Figure 8.3. There were significant differences due to treatment ($P<0.0001$), period of collection ($P<0.001$), day of collection ($P<0.0001$) and interaction between period and day of collection ($P<0.0001$). Other interactions were not significant. Over the course of the study, the total amount of corticosterone in egg albumen was significantly higher in the hens that were moved to group cages compared to the hens that remained in their individual cages (33.8 ± 8 vs 30.2 ± 8 ng/g).

There was a gradual decrease in the total amount of corticosterone in albumen over the course of the study. The overall means and treatment means for each period are given in Table 8.9. The total amount of corticosterone in egg albumen at 70 weeks (period 4) was significantly lower than in all other periods. This difference was evident in the single treatment group but was not apparent in the group treatment.

Because of the experimental protocol a significant effect of day of collection on total amount of corticosterone in albumen was expected. The overall means and treatment means for each day are given in Table 8.10. The single and group treatments had similar amounts of corticosterone in albumen on the day before the group hens were moved. The amount of corticosterone in albumen on different days was not different for the single treatment. The amount of corticosterone in albumen on day –1 was significantly lower than on days 1 and 10 for the group treatment. The concentrations increased in both treatments when the hens were moved (day –1 vs day 1), however the increase for the single treatment was not significant whereas it was for the group treatment.

As stated previously, the interaction between period and day of collection was significant with no significant interactions with treatment. On day –1 the total amount of corticosterone increased from period 1 to period 2 and then remained at the level. On all other days the amount of corticosterone decreased from period 1 to period 2 and remained similar for period 3. Between period 3 and 4 the amount of corticosterone decreased on days 2, 6 and 10 and increased slightly on day 1.

Total corticosterone in albumen: *Effects within periods*
Period 1 (24 weeks): There was a significant treatment ($P<0.05$) and day effect ($P<0.001$). The concentration of corticosterone was not different on days -1, 1 and 2, however on days 6 and 10 they were higher in the group caged hens compared to the single caged hens. For both treatments the total amount of corticosterone on day –1 was significantly lower than on all other days. The mean increase when the hens were moved (day –1 vs 1) was 13.4 ng and 12.1 ng for the control hens.

Period 2 (34 weeks): There was a significant treatment ($P<0.05$) effect and no day or treatment and day interaction. The total amounts of corticosterone were similar for treatments on days –1, 2, 6 and 10 whereas on day 1 the amount was significantly higher in the group housed hens. The mean increase for hens that were moved (day –1 vs 1) was 5.9 ng. In the single treatment there was a decrease in total amount of corticosterone from days –1 to 1 of 4.2 ng. This was largely the result of the single treatment group tending to have a higher amount of corticosterone in albumen on day -1.

Period 3 (54 weeks): There was a significant treatment ($P<0.001$) effect but no day effect or interaction between treatment and day. There were significantly higher corticosterone concentrations in the group treatment on days 2 and 10 compared to the single treatment group with no other differences being significant. The mean increase for hens that were moved (day –1 vs 1) was 3.4 ng. In the single housed hens the increase in corticosterone concentration from days –1 to 1 was 2.3 ng.

Period 4 (70 weeks): There were significant treatment ($P<0.001$) and day ($P<0.0001$) effects and no interaction between these. The total amount of corticosterone in albumen was significantly higher in the group caged hens on day 1 and almost different on day –1 ($P=0.052$). For both treatments the amount of corticosterone in albumen on day 2 was significantly less than on day 1. In the group housed hens the amount was significantly lower on day 1 than day –1 and on day 10 than day 1. The mean increase in total corticosterone for hens that were moved (day –1 vs 1) was 3.6 ng and for the hens remaining in their single pens was 2.5 ng.

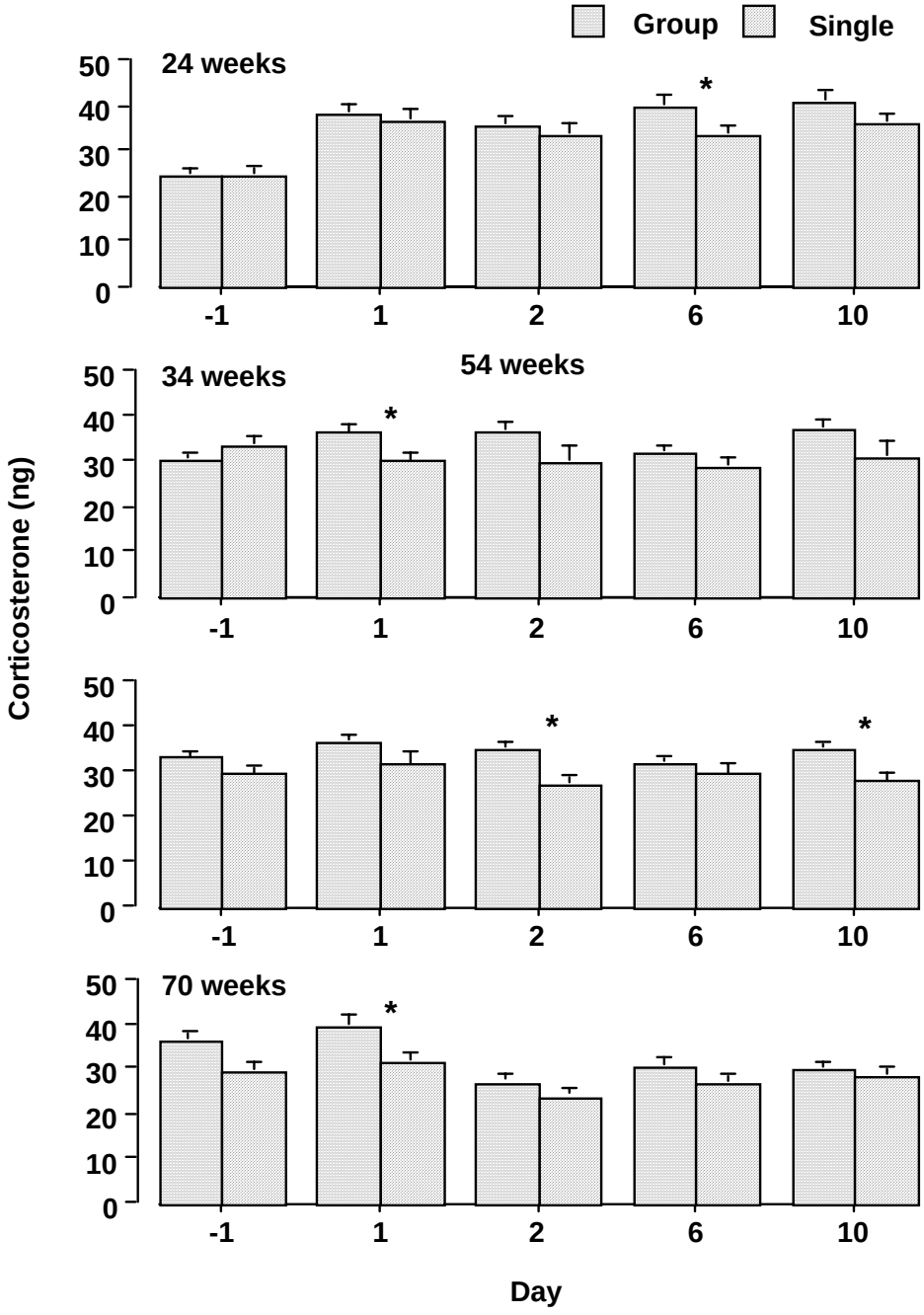

Figure 8.3: The mean (± SEM) total amount of corticosterone in egg albumen for hens maintained in individual cages (single) or in individual cages and then moved to group cages (5 hens/cage) for 10 days at 24, 34, 54 and 70 weeks of age. Eggs were collected the day before (day –1) moving and then 1, 2, 6 and 10 days after the hens were moved. Within treatment periods the columns with an asterisk indicate that treatments are significantly different from one another ($P<0.05$).

Period	Corticosterone concentration (ng)		
	Overall	Single	Group
1 (24 weeks)	33.4 ± 0.7[a]	32.7 ± 1.1[a]	33.9 ± 1.0
2 (34 weeks)	32.0 ± 0.8[a]	30.7 ± 1.1[a]	33.5 ± 1.0
3 (54 weeks)	31.4 ± 0.7[a]	28.9 ± 1.0[a]	33.9 ± 0.9
4 (70 weeks)	30.8 ± 0.7[b]	28.1 ± 0.9[b]	33.5 ± 1.1

Table 8.9: The overall and treatment mean (± SEM) total amount of corticosterone in egg albumen for hens housed in single cages or moved to group cages on four occasions during their production cycle. Within columns values with different superscripts are significantly different (P <0.05).

Day	Total amount of corticosterone (ng)		
	Overall	Single	Group
-1	30.5 ± 0.7[b]	29.3 ± 1.1	31.2 ± 1.0[b]
1	34.5 ± 0.8[a]	32.0 ± 1.1	37.6 ± 1.0[a]
2	30.9 ± 0. 9[b]	28.8 ± 1.3	33.6 ± 0.9[ab]
6	31.4 ± 0.8[ab]	29.8 ± 1.1	33.2 ± 1.1[ab]
10	32.9 ± 0.9[ab]	31.0 ± 1.2	35.6 ± 1.0[a]

Table 8.10: The overall and treatment mean (± SEM) total amount of corticosterone in egg albumen for hens housed in single cages or moved to group cages on four occasions during their production cycle. Eggs were collected on the day (day –1) before the group hens were moved and then on days 1, 2, 6, and 10 after they were moved. Within columns values with different superscripts are significantly different (P <0.05).

8.5. Discussion

The age of the hen has been implicated in modifying various physiological responses. Early stressful events experienced by animals can influence responses to subsequent stressors (Sapolsky, 1992). While some of these effects are related to acclimitisation there are probably components associated with maturation. Regular handling in early life has been reported to diminish the response to fear in later life (Jones *et al*., 1991a). At high temperatures the capacity for hens to increase heat loss is limited and so acclimatisation probably involves physiological adjustments that decrease heat production (Sykes and Fataftah, 1986). Such acclimatisation is crucial for survival when faced with adverse conditions. The tests for fear indicate that they can be a situation-dependent variable that is modified by factors such as age. Age influences how hens respond to various tests of fearfulness (Albentosa *et al*., 2003). The tonic immobility duration has been shown to increase (Campo and Carnicer, 1993) with age. Using the Hansen test (Hansen, 1976) as a fear measure, level of fear increased (Anderson *et al*., 2004) or decreased (Anderson and Adams, 1991) with age. Fear and stress are associated, therefore if fear is modified with age then the response to stress may also be modified with age.

In the present study, hens were maintained in conventional cages for one production cycle and then subjected to relocation stress on four occasions during the production cycle. Egg production was significantly lower in period 4 (70 weeks of age) compared to earlier periods. In the production cycle egg output peaks at a round 32 weeks of age and then declines by the end of production (Etches, 1990). In the hens maintained in single cages production was highest in period 2 (34 weeks) and so the pattern in egg production seen in this study seems normal. Hens moved to group cages had lower egg production in all periods compared to hens not moved. This difference was close to being significant. Reproductive rate is one of the criteria used to assess stress and welfare in hens. On this basis, the decrease in production for hens relocated suggests that they were stressed. This was expected, as two of the most potentially fear-provoking stimuli for hens are a sudden change in the environment (novelty) and exposure to humans (Murphy, 1978; Duncan, 1989; Jones, 1987c; Wiepkema and Koolhaas, 1993; Jones, 1996). In addition to the stress of relocation, when moved and housed as groups, hens would also have been subjected to the stress associated with establishing a social hierarchy. During group housing, there is competition for space and, importantly, social interactions occur continually but especially early on (Keeling, 1995). For hens housed as groups the space available is limited by others in the group as well as by any limits imposed by the size of the enclosure. Social structures, familiarity and competition are key issues responsible for the level of aggressive behaviour in groups of hens housed together.

There were no effects of moving hens on egg weight. Egg weight increased significantly with age. While the normal pattern is for egg production to decrease with age it is also normal for egg weight to increase with age (Etches, 1996) and so what was observed in the present study was anticipated. Also, it would be expected that albumen weight would increase as egg weight increased and this was also observed in the present study. While egg weight was significantly lower at 24 weeks compared to other times and higher at 70 weeks than other times the difference between weeks 34 and 54 was not significant although it tended to be higher at 54 weeks. At 70 weeks albumen weight was higher for the hens moved to group pens. This may be related to the tendency for egg weight to be higher in group housed hens at this time.

Oviposition time was longer in group housed hens. Stressors imposed on hens result in delayed oviposition (Carter, 1977: Hughes and Gilbert, 1984; Hughes *et al*., 1986; Mills *et al*., 1991; Reynard and Savory, 1999). These delays often result in eggs with abnormal shells. On the first day after moving, there was an increased number of abnormal eggs collected and these ranged from those with small amounts of extra calcium deposited on the cuticle to soft-shelled eggs and eggs without shells. The oviposition times were significantly longer in periods 1 (24 weeks) and 2 (34 weeks) than periods 3 (54 weeks) and 4 (70 weeks). If it is accepted that oviposition time delays are related to

stress, than it would suggest that the relocation stress was less extreme in aged hens. Differences within individual periods tend to support this. There were greater differences on individual collection days in periods 1 and 2 than there were in periods 3 and 4.

Plasma corticosterone concentration is thought to be a non-specific response to stress. The variation in corticosterone responses to stressors is probably an inherent characteristic (Littin and Cockrem, 2001) which might change intrinsically with age. During the course of the study plasma corticosterone concentration was higher in the hens housed as groups than hens remaining in their individual cages. As mentioned earlier, novelty is a stressor of hens. Moving hens to a new cage has been reported previously to cause an increase in plasma corticosterone (Cunnigham *et al*., 1988; Downing and Bryden, 2002). The plasma corticosterone concentration decreased as the hens aged. In the hens which remained in their individual pens, the corticosterone concentration in period 1 (24 weeks) was higher than at other periods while significantly lower in period 4 (70 weeks) than other periods and remained similar in periods 2 (24 weeks) and 3 (54 weeks). A similar pattern was seen in the group housed hens except the difference between 54 and 70 weeks was not significant. Similar patterns in egg albumen corticosterone concentration were observed as those seen for plasma corticosterone with some discrepancies. The egg albumen corticosterone concentration was higher in group housed hens and these were significantly higher in period 1 (24 weeks) than other periods. In the individually housed hens (control) the concentration was also higher in period 2 (34 weeks) compared to period 4 (week 70) but this was not so for the group housed hens. Overall, the total amount of corticosterone in albumen was lower in period 4 (70 weeks) than other periods. This was the same for those hens that remained in their individual cages but not for the hens moved to group cages. For these hens, the total amount of corticosterone was the same for all periods. This difference may be influenced by the relocation stress masking the intrinsic pattern of corticosterone seen in the control hens as they aged or related to subtle differences in egg and albumen weights in period 4.

When hens were moved, plasma corticosterone increased when compared to the control hens that were not moved in periods 1, 2 and 3 but not period 4. The failure to observe any increase on the day hens were moved in period 4 (70 weeks) may be related to the diminished responsiveness to this stressor as hens age. On other days in each period there were limited differences in plasma corticosterone. On some occasions the plasma corticosterone concentration was higher in the control hens and this was especially evident on the day before (day-2) hens were moved in period 3. This highlights one of the problems with using point samples to make any clear assessment of what happens with changing physiological parameters. Differences like this are difficult to account for but often are the influence of the large individual variations seen in hens for physiological measures such as corticosterone concentration (Littin and Cockrem, 2001). For the hens moved, the difference in plasma corticosterone from the day before moving to the day of moving was higher in period 1 than period 4 (0.45 vs 0.13 ng/ml). This suggests that hens were more responsive to the relocation stress when they were younger. The corticosterone response seen when hens were moved was not large even at the younger ages. While it is clear that relocation is a stress provoking stimuli, it would seem that this strain of laying hen are not easily stressed by the degree of novelty associated with cage relocation. Selection for production in cages has probably indirectly resulted in selection for less fearful hens. Increased fearfulness has been associated with decreased production (Craig *et al*., 1983; Bessi, 1984). High corticosterone release has been associated with behaviours that indicate fear in some species (Dantzer *et al*., 1980; Katz *et al*., 1981; Harvey *et al*., 1984: Jones *et al*., 1988). As measures of fear increase so do plasma corticosterone concentration and H/L ratio in hens (Davis *et al*., 2000). Frightening stimuli result in the release of catecholamines (Dantzer and Mormede, 1983; Harvey and Phillips. 1984) and corticosterone (Beuving, 1980; Harvey *et al*., 1984). Selection in general doesn't act to eliminate a trait but rather modify the threshold before an effect is observed (Faure *et al*., 2003). A diminished fear response would, because of the suggested association with adrenal function, result in diminished corticosterone responses.

In period 1 (24 weeks) the hens remaining in their cages had an increased egg albumen corticosterone concentration on day 1 that was of a similar magnitude to that seen in the hens that were moved. As a general observation vocalisation appeared to be greater when hens were moved at younger ages

especially at 24 weeks. This general disturbance within the shed may account for the response in egg albumen corticosterone concentration seen in the control hens in period 1. At 54 and 70 weeks of age there was less vocalisation, however there appeared to be more aggressive attacks when hens were first moved.

The higher corticosterone seen at the younger ages may be related to the increase in production as hens achieve their peak performance. Corticosterone concentrations are higher at peak production (Davis *et al*., 2000). Significantly higher levels of fear were recorded at peak production (Anderson and Adams, 1992) than at other stages of the production cycle. The observation that the corticosterone response to relocation diminished with age, suggests that rather than being related to production, the reduced response to stress is more likely the result of decreased fearfulness as hens age.

8.6. Implications

As a general conclusion the patterns of plasma and egg albumen corticosterone concentrations suggest that as hens age their basal plasma corticosterone concentration decreases and their corticosterone responsiveness to a novel stressor also diminishes. This could be related to observations that indicate that fearfulness decreases with age because higher levels of fearfulness are associated with increased corticosterone concentration. Both the higher basal corticosterone levels and responsiveness of young hens to stress could predispose these birds to more readily develop a pathological condition.

8.7. Recommendations

Any stress imposed on hens is likely to be more severe at a younger age. There is a need for increased emphasis at this time on using husbandry practices that minimise stress, especially leading up to peak production. The individual inherent responsiveness of hens to stress might compound the effects of age for some hens. The effects of stressors are additive and hens with high responsiveness to stress are more likely to be the ones predisposed to conditions resulting in pathological states. There is a need to identify individual hens that are most likely to suffer stress. In any production system, the hen’s welfare needs to be ensured and therefore selecting hens that are better able to cope adequately in a particular system should be considered an important goal.

Chapter 9

The relationship between fearfulness and the response to stress in laying hens

9.1. Introduction

Fearfulness is the 'adaptive psycho-physiological (emotional) response to real or perceived danger' (Jones, 1996). In the wild it is a state that acts to protect an animal from physiochemical damage and injury. In intensive production systems extreme fear responses are harmful to the animal and in general are an undesirable state leading to poor welfare. While in any poultry production systems fear can't be completely eliminated, it should be reduced where possible. Reducing fearfulness and increasing the hen adaptability to its environment should improve welfare. This could be particularly so in alternative (barn and free range) production systems where adverse social interactions can result in poor welfare for some of the hens. Indirectly the long period of selection for production in conventional cages has decreased the level of fear in commercial strains of laying hens. These same strains when used in the alternative husbandry systems could potentially have their welfare compromised. This is especially so for those individuals having enhanced fear responses to some of the environmental stimuli inherent in these systems.

Fear can be measured by determining the hen's avoidance of danger, either perceived or real (Duncan, 1981). Tests have been developed which assess a hen's avoidance of novel objects and environments or innate responses when subjected to restraint (Jones, 1987). The tests are based on sudden changes in the hen's physical and social environment (Jones, 1996). Tonic immobility (TI) is a commonly used measure of fearfulness (Gallup, 1979; Jones, 1986). It is believed to have a genetic basis because selection for TI has produced divergent lines differing in TI duration (Mills and Faure, 1991). It is a state of immobilisation induced by mild restraint in which the bird has reduced responsiveness to external stimuli (Jones, 1996). The hen is held on its back or side, usually in a U- or V-shaped cradle for support and held with a hand across the breast area for a short period (usually 15-20 sec). The hen's immobility is a response to fear and the time taken for the bird to right itself is determined as TI duration. This period can vary from a few seconds to hours (Jones, 1996). Hens having the longer TI duration are considered to be the most fearful. This is based on the presumption that the most fearful hens adopt the 'freeze and withdrawal' behaviour when faced with real or perceived danger. The less fearful hens adopt the 'fight/flight response and have short TI durations.

The novel object test measures the degree to which a hen will avoid a novel object placed in its environment. Often a multi-coloured rod is used and placed in the feed trough of the hen's home cage (Craig, *et al*., 1983; Jones, 1985). The amount of avoidance reflects the bird's fearfulness. This is determined as the time taken for a hen to approach the rod and peck at the feed trough. Both the TI and novel object test are used in the present study as measures of fearfulness in hens.

In any production system, a hen is exposed to many stressors, factors that influence its behaviour, physiology and emotions. These include human intervention, husbandry practices, environmental conditions, social interactions, confinement, nutrition and other less defined stressors. When conditions are not ideal, the hen makes various adjustments in an effort to maintain homeostasis. Those hens that maintain homeostasis, cope and their welfare is considered to be good. Those failing

to adequately adjust and re-establish homeostasis, don't cope and their welfare is likely to be poor. Failure to cope very often results in elevated plasma corticosterone concentrations, this is one of the mechanisms an animal needs in its effort to make adjustments needed to regain homeostasis. In any production system it appears that some hens fail to 'cope' adequately as determined by various behaviour and physiological measures of stress.

Stress is distinguished by changes in physiology, behaviour, emotional state and morphology. Emotional stressors are more potent stress provoking stimuli than environmental stimuli (Mason, 1975). Non-specific responses to stress involve physiological changes to many organs. In hens, adrenocortical activation and consequent secretion of corticosterone is a consistent feature characteristic of the non-specific stress response (Siegel, 1980; Harvey, 1984). Behavioural and adrenocortical responses tend to be tightly linked with metabolic and environmental stressors. Studies in hens and quail indicated that there are also links between adverse behaviour and corticosterone. Chronic elevation of plasma corticosterone increases fearfulness (Jones *et al*., 1998). Lines of quail selected for long (LT) or short tonic (ST) immobility duration or high (HS) and low corticosterone (LS) response to immobilisation, had modified responses to a variety of stressors (Mills and Faure, 1991). Chronically elevated corticosterone concentrations are believed to be associated with poor welfare and failure of hens to cope with their environment.

9.2. Objective

The objective of the present study was to determine if there is a relationship between behavioural measures of fearfulness and the hen's adrenal response to stress. If there is an association then simple behavioural measures of fear could be of value in identifying those hens whose welfare could be potentially compromised in various production systems. It could also be possible to use such tests as selection criteria when attempting to establish strains of hens more suited to the extensive production systems gaining increasing popularity. For individual hens, fearfulness is determined using TI and the novel object tests and their corticosterone response to relocation stress measured. Moving hens to a new environment is stress provoking (Jones, 1996; Littern and Cockrem, 2001; Downing and Bryden, 2002).

9.3. Materials and methods

Birds and husbandry: One hundred and fifty Isa-Brown laying hens were obtained from a commercial source and delivered to the University of Sydney poultry research facility at 16 weeks of age. Birds were floor reared and then acclimatised to cages and nipple drinkers for 4 weeks before delivery. Hens were vaccinated against Marek's disease, infectious bronchitis, fowl pox, avian encephalomyelitis, infectious laryngotracheitis and Newcastle disease. Birds were beak-trimmed at 12 weeks of age. From the time of delivery hens were housed individually in conventional cages (47 cm x 28 cm x 48 cm). Throughout the study hens were fed *ad libitum* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h of light with lights on at 0500h.

Behavioural tests:
(i) The tonic immobility test: The tonic immobility test was carried out by removing individual hens from their cage and transferring them to a separate room, where noise and disturbances were minimal. Here, the hen was placed on her back in a shallow V-shaped cradle and restrained for 15 seconds by holding the legs and firmly pressing down on the breast area. The bird was then released and the length of time taken for the hen to right herself was recorded. For any bird that righted herself within the first five seconds, the test was repeated. Any hen that had not righted herself after 20 minutes was assigned a maximum latency value of 1200 seconds. All tonic immobility tests were carried out between 1500h and 1700h.

All hens were subjected to the tonic immobility test on three occasions with a mean time calculated for each bird. The hens were ranked according to the mean time for these three observations. Two groups were selected according to these rankings. Group 1, short immobility times, consisted of hens with a tonic immobility time of less than 70 seconds. Group 2, slow tonic immobility times, consisted of hens with a tonic immobility time of greater than 300 seconds.

ii) The novel object test: A video camera was placed on a stand approximately two metres in front of the cages so that 12 cages were visible at a time. This allowed video taping of 6 hens, 3 in the top row and three in the bottom row. Hens were separated by an empty cage to minimise any influence one hen was likely to have on an adjacent hen. A 20 cm rod, painted red, blue and yellow, was placed in the feed trough of the selected hens. Video recording was commenced immediately after the rod was placed in the trough and continued for 1h during which the hens were left undisturbed. From the recordings, the time it took for each hen to put her head through the cage and peck at the feed in the trough was determined.

Experimental protocol: The tonic immobility test was conducted on all hens between 24 and 26 weeks of age. At 28 weeks of age, the selected hens (total of 49) were exposed to a situation know to be stressful and levels of corticosterone in egg albumen and plasma were measured. This part of the study was conducted over 7 days. At 0500h on day 1, the selected hens were removed from their individual cages and placed in a transport crate, and after 4 hours, were then transferred to a new individual cage located on the other side of the shed. All water and food was kept the same.

Blood sampling: A blood sample (1ml) was taken from all selected hens starting at 1600h on day 1. Blood was centrifuged within 40 minutes and the plasma collected and stored at -20˚C until assayed. All eggs that were laid were collected every hour on the hour from 0600-1700h. Eggs were collected on the day before hens were moved (day-1) and then days 2, 3, 5 and 7. While eggs were laid on these days they equated to days –2, 1, 2, 4 and 6 of albumen formation. All eggs were labelled and any abnormalities in egg size, shell texture, degree of shell calcification, soft-shells or membranous eggs were recorded. After collection, all eggs were weighed, broken open and the albumin weighed. Samples were stored at -20˚C until assayed.

Corticosterone measurement: The corticosterone concentration in egg albumen and plasma were determined by RIA as described in section 2.3.2.

Statistical analysis: Differences in egg albumen corticosterone concentration and the total amount of corticosterone in albumen were determined by ANOVA and if differences were significant, individual comparisons were made using the Fisher's PLSD test. Differences between tonic immobility test, the novel object test, and the plasma corticosterone concentration were analysed using the Student's t-test. Simple regression analysis was used to determine the significance of any relationship between the behavioural tests and individual responses to stress. Analyses were performed using the 'Statview' computer program (SAS Institute Inc, NC, USA).

9.3. Results

Tonic immobility duration: The tonic immobility duration for individual hens is shown in Figure 9.1. There was a wide variation in individual TI times ranging from 13 to 1200 sec. Two groups of hens were selected based on these TI values. One group consisted of 29 hens having a short TI duration of 47+/- 3 sec and the other group consisted of 20 hens having a long TI duration of 575+/- 56 sec.

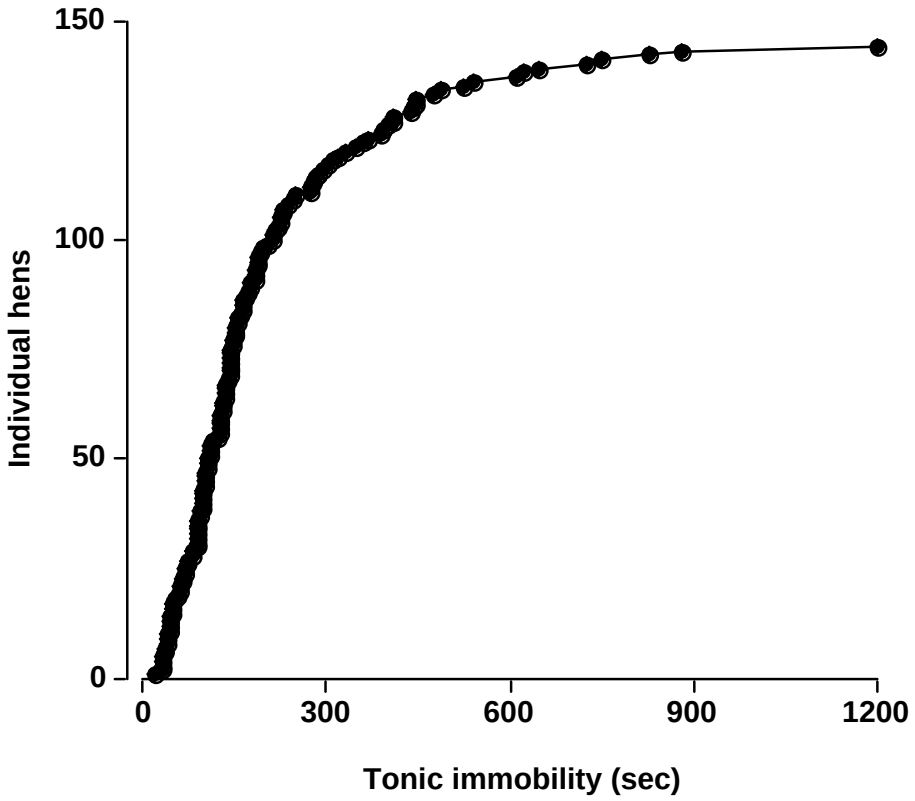

Figure 9.1. The tonic immobility (TI) duration for individual hens. Two groups of hens were selected, group 1 had short TI duration and Group2 had long TI duration.

Novel object test: The novel object test was conducted on the 49 hens selected from the TI test. The time taken for hens to peck at the base of a novel objected placed in the feed trough are shown in Figure 9.2. The individual values ranged from 0.03 to 60 min with 60 min being the maximum observation time. The mean time for group 1 hens was 4.34 +/- 1.61 min and for group 2 hens was 1.76 +/- 0.51 min. There was no relationship between the TI test and the NO test for individual hens or groups.

Plasma corticosterone: On the day the hens were moved, no difference in plasma corticosterone concentration was observed, 1.02 + 0.16 ng/ml for the group 1 hens (fast TI time) and 0.96 ± 0.16 ng/ml for the group 2 hens (slow TI time).

Egg albumen corticosterone concentration: The concentration of corticosterone in egg albumen is shown in Figure 9.3. There was no significant difference between treatments. There was a significant day effect ($P<0.0001$) and no interaction between treatment and day. The concentration on day 1 was significantly higher than on all other days. Also the concentration on day 2 was significantly higher than on day 5.

Total amount of corticosterone in egg: The total amount of corticosterone in albumen in egg albumen is shown in Figure 9.3. There was no significant difference between treatments. There was a significant day effect ($P<0.0001$) and no interaction between treatment and day. The amount of

corticosterone in albumen was significantly higher on day 1 compared to all other days. Also the total amount of corticosterone was significantly lower on day 5 than day –1.

The relationship between corticosterone and TI: The relationship between the TI times and the plasma corticosterone concentration, egg albumen corticosterone concentration and the total amount of corticosterone in egg albumen are given in Figure 9.4. There were no significant correlations between TI and any measures of corticosterone.

Abnormal eggs: On day 2 the first egg collection after the hens were moved, there were a large number of abnormal eggs laid (personal observations). These abnormalities included extra calcium deposited on the cuticle, soft-shelled eggs, eggs without shells and double-yolked eggs.

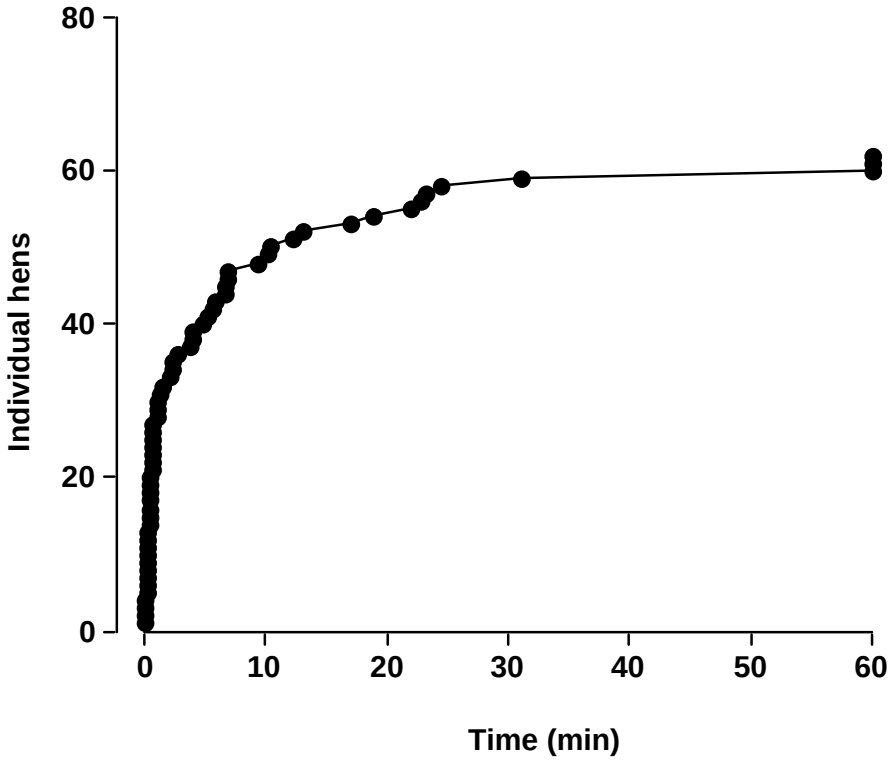

Figure 9.2: The time taken for hens to peck at the base of a novel object placed in the feed trough.

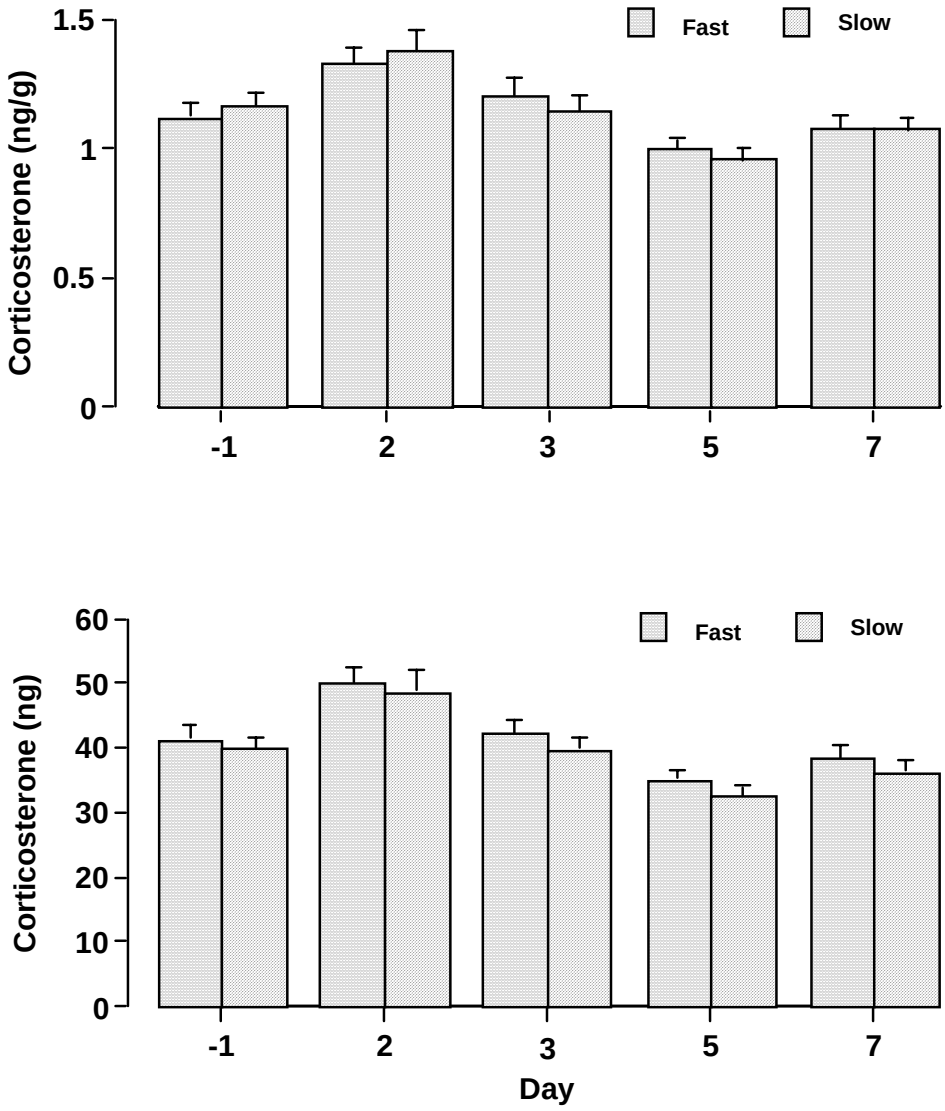

Figure 9.3: The corticosterone concentration (upper panel) in egg albumen and the total amount of corticosterone in albumen (lower panel) for hens grouped according to their tonic immobility duration and then transferred from their home cage to a new cage. Eggs were collected on the day before hens were moved (day-1) and then on days 2, 3, 5 and 7 after moving, with the day of moving designated as day 1 of the study.

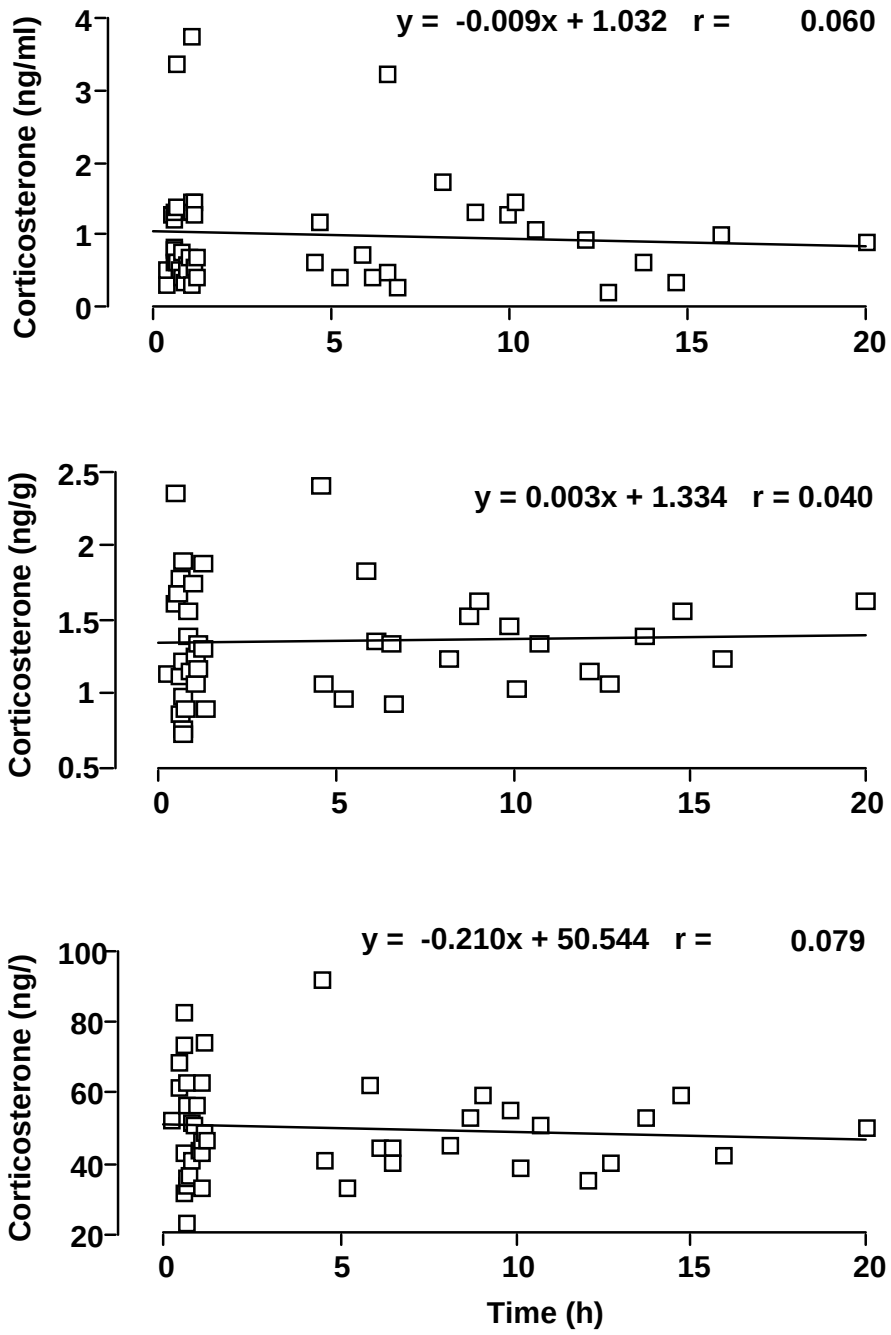

Figure 9.4: The relationship between tonic immobility duration and plasma corticosterone concentration (upper panel), egg albumen corticosterone concentration (middle panel) and total amount of corticosterone in albumen (lower panel).

9.5. Discussion

As part of the evolution to increase the efficiency of egg production, hens are maintained in cages, barns and free range production systems. In each system there are differing concerns for hen welfare because hens are exposed to stressors which require changes to maintain homeostasis. In each system

it appears that some hens are more vulnerable than others to the adverse consequences of stress. Establishing a link between measures of behaviour and an individual response to stress could provide a means of identifying hens susceptible to poor welfare.

In the present study, for individual hens, there was no correlation between the TI duration and the plasma corticosterone response to crating and relocation stress. Similarly there was no correlation between the novel object test and the plasma corticosterone response. Also, there was no correlation between the TI duration and novel object test for individual hens and the group values conflicted.

Tests for fear may measure different aspects of behaviour (Rushen, 2000). Strong strain and line differences in TI have been reported (Craig *et al*., 1984; Compo and Alvarez, 1991; Albentosa *et al*., 2003). Also in some studies, tests used across different genetic lines have produced inconsistent results (Craig and Muir, 1996; Albentosa *et al*., 2003). However, for individual birds, good correlations between different measures of fear have been reported in some studies (Jones and Mills, 1983; Mill and Faure, 1987; Jones *et al*., 1987, 1988, 1991; Gerken and Petersen, 1992), although a recent report indicates that this relationship is not strong (Albentosa *et al*., 2003) and agrees with the finding in the present study where no relationship was found between the TI and novel object tests. The tests may not be measuring the same trait with fear being a very situation-dependent variable that is modified by factors such as age.

Studies in Japanese quail (*Coturnix coturnix japonica*) indicate that there is a correlation between various behavioural tests for fear and the corticosterone response to stress (Mills and Faure, 1986; Jones *et al*., 1991; Gerken and Peterson, 1992). Japanese quail are considered as an appropriate experimental model for predicting effects in hens (Faure and Mills, 1998). Using mini-osmotic pumps implanted subcutaneously to deliver corticosterone at 15 ug/h, significantly increased plasma corticosterone concentration in hens from 0.15-0.48 ng/ml to 1.1-2.9 ng/ml (Jones *et al*., 1988). The increase in plasma corticosterone significantly increased the TI duration and H/L ratio. High corticosterone release is associated with behaviours that indicate fear in some species (Dantzer *et al*., 1980; Katz *et al*., 1981; Harvey *et al*., 1984: Jones *et al*., 1988). Chickens selected for high activity in a novel environment were found to have lower basal and stress-induced corticosterone concentrations (Faure, 1980).

In the present study, the two treatment groups were selected to have very different mean tonic immobility durations. This was done to ensure that there were two distinct populations so that any difference in corticosterone would have been maximal. Both the plasma corticosterone concentration and the corticosterone in egg albumen were similar in both groups. The increase in corticosterone concentrations following the crating and relocation stress was small. In general, much larger responses in corticosterone were anticipated following the imposition of the stress. The relocation stress did not result in a chronic elevation of corticosterone concentration, however the large number of abnormal eggs indicated that there was a major disturbance to egg formation. This is probably the result of adrenomedullary activation and the release of adrenalin and noradrenaline. Hughes and Black, (1976) predicted that adrenalin affected uterine motility and shell gland contraction and that this resulted in poor shell quality and other types of egg deformities. Adrenalin (0.1-0.25 mg) given as a subcutaneous injection produced a dose-dependent increase in the number of abnormal eggs laid (Hughes *et al.*, 1986). Similarly the injection of ACTH causes a dose-dependent increase in the number of abnormal eggs laid (Flickinger, 1966). Corticosterone plays a role in the ovulatory cycle (Beuving and Vonder, 1977). There is evidence indicating that corticosterone can also affect oviposition time and the incidence of abnormal eggs (Downing and Bryden, 2002). Plasma corticosterone concentrations were higher (approximately 1.0 ng/ml) in hens laying soft-shelled or membranous eggs compared to hens producing normal hard-shelled eggs (Klingensmith *et al*., 1984). The extent of abnormal eggs laid in the present study was probably not related to the corticosterone response because the increase was too small.

The failure to find a relationship between the corticosterone response to stress and the behavioural tests of fear could be related to the layer strain used. A probable consequence of selection for

production in cages has also been selection for decreased fearfulness. Increased fearfulness has been associated with decreased production (Craig *et al*., 1983; Bessi, 1984). For the strain of hens used the stress imposed may not have caused a major perturbation and the non-specific adrenocortical response needed to help re-establish homeostasis was small. If this were so then the experimental protocol may not have been adequate to properly test for the relationship between the behavioural test and corticosterone response. If it was possible to measure the adrenomedullary response (adrenalin and noradrenaline) it may give a clearer evaluation of any relationship between the fear tests and stress.

Production level is considered to be a good measure of welfare. In the F2 generation from parent lines of quail selected, one for egg production (Minvielle *et al*., 2000) and the other for long TI duration (Mills and Faure, 1991), no relationship between TI and egg production was found (Grasteau and Minvielle, 2003). The lack of a relationship between egg production and fear probably lends support to the concept that behavioural measures of fear are probably not good indicators of the adrenocortical responses to stress.

9.6. Implications

While fear is reported to be associated with physiological and morphological measures often related to stress, the tests often used to measure it don't seem to be related to stress-induced corticosterone responses in this strain of laying hen. However, these tests may still have a relationship to adrenomedullary activation.

9.7. Recommendations

Chronic elevation of corticosterone suggests that hens are failing to cope with their environment and are probably suffering from poor welfare. In any production system, some hens are more likely to have trouble coping with the stresses imposed on them when compared to flock mates. Identifying these individual hens would seem important if their welfare is to be improved. The stress response from the hens used in the present study would indicate that they are well equipped to cope with what is considered to be a major stress provoking stimuli (cage relocation). As suggested in the discussion, this could be the result of intensive selection for production in cages and therefore selection for a particular environment. If similar strains of hens are used in more extensive production systems their ability to cope with the new environments and the stressors they impose, may not be as well refined as those seen when housed in cages. Any behavioural measure that is related to their stress response may be helpful in identifying hens that could better cope in extensive production systems. The behavioural measures of fear used in this study do not seem to be suitable for this purpose.

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