



Importance of rearing environment, space and nests for laying hens in cages

Welfare implications of floor space and nest-boxes

A report for the Australian Egg Corporation Limited

By P. Hemsworth, T. Widowski, A. Tilbrook and J. Engel

December 2012

AECL Publication No 1UM091A

© 2012 Australian Egg Corporation Limited.
All rights reserved.

ISBN 1 920835 46 6

Importance of rearing environment, space and nests for laying hens in cages

AECL Project Number 1UM091

The views expressed and the conclusions reached in this publication are those of the author and not necessarily those of persons consulted. AECL shall not be responsible in any way whatsoever to any person who relies in whole or in part on the contents of this report.

This publication is copyright. However, AECL encourages wide dissemination of its research, providing the Corporation is clearly acknowledged. For any other enquiries concerning reproduction, contact the R&D Program Manager on 02 9409 6999.

Researcher/Author Contact Details

Name: Paul H Hemsworth
Address: Animal Welfare Science Centre, The University of Melbourne,
Parkville, VIC 3010
Phone: 03 8344 8383
Fax: 03 8344 5037
Email: phh@unimelb.edu.au

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

AECL Contact Details:

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

Phone: 02 9409 6999
Fax: 02 9954 3133
Email: research@aecl.org
Website: <http://aecl.org/r-and-d/>

Published in December 2012

Foreword

This project was conducted to determine the effects of floor space during rearing (315 and 945cm²/bird) and adulthood (542 and 1650cm²/bird) and access to a nest-box during adulthood on the welfare of laying hens using a broad range of well-accepted welfare indices. There is widespread interest by the general public in the treatment of animals.

From the perspective of the public, captive environments that inhibit or prevent behaviours that are considered to be part of the normal behavioural repertoire of the animal are generally contentious and confinement housing of farm animals appear to be at the forefront of these concerns. Two of the most contentious welfare issues in relation to cage housing of laying hens are floor space allowance and an appropriate nest site.

While a floor space allowance of 542cm²/bird during adulthood resulted in marked behavioural restrictions, there was no evidence based on corticosterone concentrations and heterophil to lymphocyte ratios that this space allowance was stressful. Furthermore, there was no evidence that hens with this reduced space in adulthood preferred additional space over feed based on choice behaviour in Y maze tests. There was no evidence based on corticosterone concentrations and heterophil to lymphocyte ratios that depriving hens of access to a nest-box during adulthood was stressful. However, hens with experience with nest-boxes were at least moderately motivated to choose a nest-box over feed around the time of oviposition.

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

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

Most of our publications are available for viewing or downloading through our website:

<http://aecl.org/r-and-d/>

Printed copies of this report are available for a nominal postage and handling fee and can be requested by phoning (02) 9409 6999 or emailing research@aecl.org.

Dr Angus Crossan
Program Manager – R&D
Australian Egg Corporation Limited

Acknowledgments

The authors would like to acknowledge the technical support of Judy Nash, Tracie Storey, Samantha Borg and Bruce Schirmer and the statistical support of Kym Butler. Advice and support from Philip Szepe are gratefully acknowledged.

The Australian Egg Corporation Limited provided the funds which supported this project.

About the Authors

Joanna Engel, a PhD student in the Animal Welfare Science Centre at the University of Melbourne, conducted her PhD studies within the present research project. She has previously studied the behaviour and physiology of poultry.

Paul Hamilton Hemsworth is Director of the Animal Welfare Science Centre and is professor in the Melbourne School of Land and Environment at the University of Melbourne. He has studied the effects of handling, housing and husbandry practices on animals, particularly farm animals.

Tina Widowski is Director of The Colonel K.L. Campbell Centre for the Study of Animal Welfare and is professor in Animal and Poultry Science at the University of Guelph. Her research and teaching has focused on how housing and management practices affect the behavioural biology and welfare of farm animals.

Alan Tilbrook is Deputy Director of the Animal Welfare Science Centre and Research Chief, Livestock and Farming Systems, South Australian Research and Development Institute. He has studied the behaviour and physiology of farm animals.

Table of Contents

Foreword.....	2
Acknowledgments.....	3
About the Authors.....	3
Table of Contents.....	4
List of Tables.....	5
List of Figures.....	6
Abbreviations.....	7
Executive Summary.....	8
1 Background – Importance of rearing environment, space and nests for laying hens in cages.....	10
1.1 Background to the research.....	10
1.1.1 Public interest in animal welfare.....	10
1.1.2 Welfare of caged laying hens.....	11
1.1.3 Scientific assessment of animal welfare.....	12
1.2 Project aims.....	18
2 Experiment – Effects of space during rearing and adulthood and access to a nest-box during adulthood on the welfare of laying hens.....	19
2.1 Background.....	19
2.2 Objectives.....	19
2.3 Materials and methods.....	20
2.3.1 Facilities.....	20
2.3.2 Measurements.....	22
2.3.3 Statistical analyses.....	30
2.4 Results.....	30
2.4.1 Physiology.....	30
2.4.2 Behaviour.....	32
2.4.3 Productivity and other measures.....	38
2.5 Discussion.....	42
2.6 Conclusions and recommendations.....	50
2.6.1 Assessing the implications of space and nest-box access on hen welfare.....	50
2.6.2 Conclusions.....	52
2.6.3 Recommendations.....	53
3 References.....	54
4 Appendices.....	63
4.1 Appendix 1.....	63
4.2 Appendix 2.....	64
4.3 Appendix 3.....	65
4.4 Appendix 4.....	65
4.5 Appendix 5.....	66
5 Plain English Summary.....	67

List of Tables

Table 2-1 - Space and nest-box treatments	21
Table 2-2 - Form of analyses of variance	22
Table 2-3 - Postures and behaviours measured in the behavioural time budget.....	25
Table 2-4- Orientation definitions to determine consistency of orientation for oviposition observations.....	27
Table 2-5 - Feather condition scoring (Tauson et al., 2005)	30
Table 2-6 - Effect of size of rearing cage on physiology at 26-29 wk of age	31
Table 2-7 - Effect of size of production cage on physiology at 26-29 wk of age.....	31
Table 2-8 - Effect of access to a nest-box on physiology at 26-29 wk of age.....	32
Table 2-9 - Effect of size of rearing cage on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed.....	33
Table 2-10 - Effect of size of production cage on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed.....	34
Table 2-11 - Effect of access to a nest box on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed.....	35
Table 2-12 - Effect of size of rearing cage on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively	36
Table 2-13 - Effect of size of production cage on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively.....	37
Table 2-14 - Effect of access to a nest-box on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively.....	38
Table 2-15- Effect of size of rearing cage on productivity and other measures.....	40
Table 2-16 - Effect of size of production cage on productivity and other measures	41
Table 2-17 - Effect of access to a nest-box on productivity and other measures	42
Table 2-18 - Summary of the significant effects of floor space during adulthood on the postures and behaviours of laying hens	44
Table 4-1 - Vaccination schedule of pullets during the experiment	65



List of Figures

Figure 2-1 - Division of small and large cages for oviposition observations	27
Figure 4-1- Humidity and temperatures within the experimental shed	63
Figure 4-2 - Randomised treatments for (a) replicate 1, (b) replicate 2, (c) replicate 3 and (d) replicate 4	64

Abbreviations

ACTH	Adrenocorticotrophic hormone
AGP	Alpha-1 acid glycoprotein
ANOVA	Analysis of Variance
APP	Acute phase protein
Bn	Barn
CC	Conventional cage
CGB	Corticosteroid- binding globulin
CoVar	Coefficient of variation
CPM	Counts per minute
CNS	Central Nervous System
CRF	Corticotropin releasing factor
d	Day
EIA	Enzyme Immunoassay
FC	Furnished cage
FR	Free Range
h	Hour/hours
HHP	Hen housed production
H/L	Heterophil: Lymphocyte ratio
HPA	Hypothalamic-adrenal-axis
IR	Infra-red
GR	Glucocorticoid receptor
L	Light
Min	Minute/minutes
MR	Mineralocorticoid receptor
NSB	Non specific binding
PEG	Polyethylene glycol
PBS	Phosphate buffered saline
PVN	Paraventricular nucleus
RIA	Radioimmunoassay
s	Seconds
SAM	Sympathetic-adrenal-medullary
SEM	Standard error of the mean
TC	Total counts
TI	Tonic Immobility
wk	Week/weeks

Executive Summary

Captive environments that inhibit or prevent behaviours that are considered to be part of the normal behavioural repertoire of the animal appear to be at the forefront of the public's concern about the treatment of animals. Two of the most contentious welfare issues in relation to cage housing of laying hens are floor space allowance and an appropriate nest site. The main objective of this experiment was to determine the effects of floor space during rearing and adulthood and access to a nest-box during adulthood on the welfare of laying hens.

Animal welfare was assessed in this project by using the two most common approaches, that is, those indices that demonstrate the normality of the animal's biological functioning and fitness (i.e., how well an animal is coping with the challenges it faces) and those indices that demonstrate what resources are perceived to be important to an animal (i.e., animal preferences). There is a clear mandate to avoid animal suffering which is reflected in the prevention of cruelty legislation in many Western countries where cruelty is referred to in terms of "unreasonable pain or suffering".

Furthermore, this legislation often encourages the considerate treatment of animals, which reflects the emerging shift in community values towards enhancing pleasure in these animals. For many a consideration of animal welfare includes not only the avoidance of suffering, but also the presence of positive emotional experiences. Indeed for many, good welfare is not simply the absence of negative experiences, but rather is primarily the presence of positive experiences, such as pleasure.

256 Hy-line Brown hens were studied in a 2x2x2 factorial, incomplete block design consisting of the following three main effects:

1. Rearing space allowance in groups of 8 pullets per cage from 7 wk of age - two levels, 315 and 945cm²/bird;
2. Production space allowance in groups of 6 hens per cage from 16 wk of age - two levels, 542 and 1648cm²/bird;
3. Nest-box access - two levels, presence or absence of access to a nest-box during production. A single nest-box (0.24m (width) x 0.50m (depth) providing 200cm² nest space per bird) was located adjacent to one side of each cage and access to the nest-box was blocked in the treatment involving absence of access to a nest-box.

The main measurements in adulthood were corticosterone concentrations in plasma, faeces, egg albumen and egg yolk, corticosterone response to ACTH challenge, extra-cuticular calcium, heterophil to lymphocyte ratio, time budget of behaviour, pre-laying behaviour, preference for space and a nest-box in Y maze tests, feather condition score, body weight, egg production and egg weight. The experimental unit in this experiment was the cage.

There were no consistent effects of floor space during rearing or adulthood on measures of stress, the preference of hens for space in Y maze tests, body weight and egg production. However, there were some marked effects of reduced floor space allowance during adulthood on the time budget of behaviour: housing hens with a space allowance of 542cm²/bird rather than 1650cm²/bird reduced the display of the behaviours of mobile, inedible pecking, drinking and preening but increased the display of the behaviours of resting and feed pecking and the posture of sitting. Furthermore reduced floor space in adulthood reduced feather condition.

While reduced floor space, particularly during adulthood, imposed considerable behavioural restriction, this effect was not sufficient to elicit a sustained stress response, and in turn, reductions in body weight and egg production. Furthermore, although previous research has shown that hens are strongly motivated to lay their eggs in nest-boxes, deprivation of access to a nest-box during adulthood was also not sufficient to elicit a sustained stress response, and in turn, reductions in body weight and egg production. Hens with reduced floor space in adulthood did not show increased preference to choose additional space over feed in the Y maze tests, but hens provided with access to a nest-box during adulthood choose a nest-box over feed in more Y maze tests than hens deprived of a nest-box in adulthood. Based on the normality of biological functioning and preferences in the Y maze tests, the present results provide no convincing evidence that the lower of the two space allowances provided during either rearing (315cm²/bird) or adulthood (542cm²/bird) or a lack of access to a nest-box in adulthood resulted in suffering in laying hens.

However, reduced space in adulthood resulted in considerable behavioural restriction and that eliminating the opportunity for hens to access a nest site in adulthood eliminated a behaviour that hens are motivated to perform. There appears to be an emerging shift in community values towards not merely minimising suffering in domesticated animals, but also enhancing positive emotional experiences in these animals. The present experiment provides no convincing evidence that reduced space and deprivation of nest-boxes results in suffering, based on normality of biological functioning and preferences for space and nest-boxes. Nevertheless, policy makers in developing animal welfare standards and recommendations should consider the implications of providing commercial laying hens with both increased space to allow more behavioural freedom and nest-boxes, which presumably provide experienced hens with positive emotional experiences based on their motivation to access them in preference tests.

1 Background – Importance of rearing environment, space and nests for laying hens in cages

1.1 Background to the research

1.1.1 Public interest in animal welfare

There is widespread interest by the general public in the treatment of animals. From the perspective of the public, captive environments that inhibit or prevent behaviours that are considered to be part of the normal behavioural repertoire of the animal are generally contentious and confinement housing of farm animals, such as those common in modern poultry and pig farming systems, appear to be at the forefront of these concerns. The livestock industries are sensitive to the issue of the welfare of farm animals (Hemsworth and Coleman, 2011). This arises, in part, because deterioration in the welfare of animals is often associated with reductions in individual animal performance.

Furthermore, local and international agricultural markets are fiercely competitive, and in addition to the necessity of technological improvements, development of new products and marketing expertise to maintain competitiveness and increase sales, livestock industries need to project a welfare-friendly image of their products to maximize their marketing advantage. Food processors and retailers may act on consumer and public concerns to restrict or eliminate contentious welfare issues in farms that supply their animal products. Furthermore, concerns about the welfare of farm animals in a particular industry may influence the buying behaviour of current or potential consumers of the product from that industry. Animal welfare is a social issue often discussed in the public domain and thus the public may be influential, via government decisions, in determining animal welfare standards (Coleman, 2008).

Codes of practice or government regulations may restrict specific practices in a particular livestock industry that the general community finds objectionable on welfare grounds. The image of a welfare-friendly product requires farming practices that minimize the risk to animal welfare and the provision of objective information that positively influences the public's and consumers' beliefs about the welfare implications of the farming practices that produce this product. Indeed the results of welfare research on farming practices will influence both industry practices and the public's and consumers' perception of the product.

On the basis of the popular press, one for example could be led to believe that the only welfare issues in relation to farm animal housing are stall (individual) housing of gestating sows, cage housing of laying hens, and overcrowding of meat chickens. These concerns in themselves raise a number of questions including the following:

- What is the best type of housing to provide and on what basis?
- Is outdoor housing better than indoor housing?
- What are the space allowance requirements of animals?
- What are the adverse consequences of housing laying hens in cages or sows in stalls?
- What are the social requirements of animals?

Assuming we can determine the requirements of different species for space and social contact, what other facilities should be provided? While extensive production farming systems are generally not considered to involve 'housing', extensive systems do impose restrictions on animals, albeit with considerable freedom, and there are different issues raised including frequency of inspections and opportunity for intervention, climatic conditions and natural disasters. Nevertheless, the main focus of welfare concerns by the public has been on intensive confinement systems.

One of the reasons that housing of farm animals changed markedly post-second world war was because consumers and governments in western societies wanted cheap and safe food (Hodge and Han, 2000). Science and the livestock industries responded and, consequently through more industrialized housing and production methods, have increased productivity, improved the quality of food and lowered the cost of food. Furthermore, these changes in animal housing and production methods have reduced or eliminated a number of welfare problems such as predation, thermal stress, some infectious diseases and nutritional stress. However, these changes have exacerbated or created other welfare problems such as overcrowding, social restriction and lameness in livestock.

1.1.2 Welfare of caged laying hens

Many factors may affect the welfare of commercial laying hens housed in cage and non-cage systems (Barnett and Hemsworth, 2003; Widowski et al 2009; Lay et al., 2011). The key welfare issues relate to space allowance, group size, behaviour patterns constrained by conventional cages (nesting, dustbathing, foraging and perching), feather pecking and cannibalism. In addition to these welfare issues that relate to hen behaviour, there are welfare issues that relate to hen health, such as osteoporosis and broken bones, and health and hygiene (e.g., air quality, infectious disease, parasitic infections and foot health), which may be more prominent in non-cage systems.

Arguably, the two of the most contentious issues in relation to cage housing and hen welfare are floor space and the need for a nest. Hens in groups increasingly maximize the distance to their nearest neighbor as floor space allowance increases (Keeling, 1994). For example, Savory et al. (2006) found that average nearest neighbor distances in groups of six hens were around 20, 37, 44, 49, 51 and 53cm between hens at space allowances of 600, 2400, 4800, 7200, 9600 and 12,000cm²/hen, respectively, with the rate of change leveling off at around 5000cm²/hen. These observations suggest that hens have a requirement for physical space to stretch and exercise muscles and may prefer to distance themselves from other birds. Space may also be needed for body care or grooming and assisting in thermoregulation when hot. In addition to spatial requirements for physical size and basic movement, hens may also have additional spatial requirements to perform a range of behaviours that may affect their welfare.

The literature on the effects of space allowance in layer cages shows that in general as floor space decreases, within a range of 300 to 650cm² per caged laying hen, mortality increases, egg production and body weight decrease and efficiency of feed conversion decreases (see Hill, 1977; Hughes, 1983; Adams and Craig, 1985; Sohail et al., 2004). As suggested by Hughes (1983), a reduction in floor area in cages of generally constant depth results in reduced feeding space and, in turn, reduced feed intake. Furthermore, crowding may lead to elevated corticosterone concentrations, which in turn may adversely affect both production efficiency and health.

Mench et al. (1986) reported that reducing space allowance in two-bird cages from 1394 to 697cm²/bird increased plasma corticosterone concentrations. Koelkebeck et al. (1987) reported an 11% increase in plasma corticosterone concentrations in caged hens when

space allowance was decreased from 460 to 350cm² per bird, although the increase was not statistically significant.

The motivation of hens for nesting has been studied extensively and a number of studies using preference and behavioural demand tests concur that most hens prefer and are highly motivated to access an enclosed nest site. Hens have been shown to be willing to squeeze through narrow gaps (Cooper and Appleby, 1997), push open weighted doors (Follensbee et al., 1992), and pass through cages occupied by unfamiliar or dominant hens in order to gain access to a nest-box (Freire et al., 1997), tasks considered costly or aversive to hens. Hens are only weakly motivated to reach the nest site during the searching phase, although motivation to gain access to a nest increases at the end of the searching phase, that is, near the start of the sitting phase preceding oviposition. ISA Brown hens' work-rate (by pushing through a locked door) for a small pen furnished with a nest-box nest pen at 40 min before expected time of egg-laying was shown to be equal to their work-rate to return to their home pen after 4 h of confinement without food, and the work rate to access the nest was double that amount at 20 min prior to oviposition (Cooper and Appleby, 2003).

Although absence of a nest-box results in behavioural differences that may be indicative of a negative affective state, there has been little research to date that assesses whether either an acute or chronic stress response is associated with lack of access to an enclosed nest. Few studies have specifically addressed the effects of furnished versus conventional cages with regard to nesting, on other physiological parameters. In an experiment specifically comparing hens in cages with and without nest-boxes, Cronin et al. (2008) found that hens in cages with a nest-box had 33% higher plasma corticosterone concentrations than hens without nest-boxes early in lay at 23 wk of age and suggested that the elevated stress response in cages with nest-boxes was probably associated with social factors, i.e. competition for the nest-box. When hens that were accustomed to laying in a nest-box were denied access to the nest at 39 wk, egg albumen corticosterone concentrations were not different from controls during the first two days, were significantly higher on day three but were similar again on day seven (Cronin et al., 2008). The authors concluded that there were no long-term adverse effects on stress physiology between hens with or without a nest-box, or those subsequently prevented from laying in a nest-box.

Therefore, the major objective of this project was to determine the effects of floor space allowance and access to a nest-box in cages on a broad range of accepted welfare indices. Since there is limited evidence that early experience may affect preferences for resources such as space, this project also examined the effects of floor space allowance during rearing on subsequent hen welfare. Faure (1991) found that when hens were trained to peck at a key in order to increase their cage size, previous experience had a significant effect. Hens adapted to cages worked less to enlarge their cages than hens from floor pens.

1.1.3 Scientific assessment of animal welfare

1.1.3.1 Scientific concepts of animal welfare

In a broad international examination of livestock production systems, one of the overwhelming impressions is the diversity. Not only is there variation between countries in the mix of housing systems, but there is also variation between countries in the legislated or voluntary welfare standards for housing livestock. Furthermore, there is both between and within country diversity in attitudes to animal welfare. On top of this, there is considerable variation within science in both methodologies used to study animal welfare and the interpretation of these methodologies in terms of animal welfare implications.

Although there is a wide acceptance of the scientific method in problem solving, its ability to contribute to the welfare debate has been limited to some extent by a lack of consensus amongst scientists on the criteria or methodology used in assessing an animal's welfare.

There are basically three prominent concepts of animal welfare in the literature in which scientists assess animal welfare. For many scientists, animal welfare is defined and assessed on the basis of how well the animal is performing from a biological functioning perspective. For others, animal welfare concerns affective states, such as suffering, pain, and other feelings or emotions, and thus animal welfare can be assessed by measuring animal preference on the basis that preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction. One concept in the literature, albeit not well enunciated, promotes the principle that animals should be allowed to express their normal behaviour. For some this also implies that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways.

Although society continues to struggle to identify and agree on minimum welfare standards for its domestic animals, there has been and still remains a clear priority to avoid animal suffering. Suffering is a term commonly used to denote highly negative or noxious subjective or emotional mental experiences (derived from Mellor et al., 2009). The mandate to avoid suffering is prescribed in the prevention of cruelty legislation in many Western countries which specifically refers to cruelty in terms of "unreasonable pain or suffering" (e.g. Victoria, Australia (Anonymous, 2007)) or "unnecessary suffering" (the United Kingdom (Anonymous, 1911)). It should also be recognised though that the legislation in many of these countries refers to its purpose as not only "to prevent cruelty to animals" but also "to encourage the considerate treatment of animals" (e.g. Victoria, Australia (Anonymous, 2007)). Indeed there is an emerging shift in community values towards not merely minimising suffering in domesticated animals, but also enhancing pleasure in these animals (Tannenbaum, 2001). For many a consideration of animal welfare includes not only the avoidance of suffering, but also the presence of positive emotional experiences (Duncan, 2004). Indeed some have suggested that it is widely accepted that "good welfare is not simply the absence of negative experiences, but rather is primarily the presence of positive experiences such as pleasure" (Boissy et al., 2007).

1.1.3.2 Rationale underpinning the assessment of animal welfare

Since this present research project will utilize the two most common approaches to assess animal welfare, the measurement of biological function and affective states using preference testing, it is useful to briefly review the rationale for these two approaches and their measurements. Reviews by several authors (Broom and Johnson, 1993; Fraser and Matthews, 1997; Moberg, 2000; Barnett, 2003; Hemsworth and Coleman, 2011) will be used here.

The biological functioning concept, equates poor welfare to difficult or inadequate adaptation. Broom (1986, 2000) defines the welfare of an animal as "its state as regards its attempts to cope with its environment". The "state as regards attempts to cope" refers to both (1) how much has to be done in order to cope with the environment and includes biological responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses and (2) the extent to which these coping attempts are succeeding.

These behavioural and physiological responses include abnormal behaviours, such as stereotypies and redirected behaviours, and the stress response, respectively, while the success of the coping attempts are measured in terms of lack of biological costs, such as adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-

free (i.e., fitness effects). Thus as Broom (1986) recognises there are two general types of indicators of poor welfare, one demonstrating that an individual has failed to cope with an environment, the other indicating the effort involved and the extent of an individual's attempts at coping.

The stress response commences once the central nervous system firstly perceives a potential challenge (stressor) to homeostasis and secondly develops a biological response or defence that consists of some combination of the four general biological defence responses: behavioural responses, responses of the autonomic nervous system, responses of the neuroendocrine system and responses of the immune system. For many stressors, the first and, at times, the most biologically economical and effective response is a behavioural one. In concert with the behavioural responses, the physiological responses that can be used by the animal are elicited basically in three series of events, with the full elicitation of these dependent on the time of exposure to the stressor and the success of the biological responses in coping with the challenge. Two key physiological responses that involve both neural and hormonal systems are the activation of the sympathetic-adrenal-medullary (SAM) and the hypothalamic-pituitary-adrenal (HPA) axes. Together, the responses of the SAM and HPA axes result in what is commonly termed the stress response, which encompasses one of the body's major coping mechanisms to environmental disturbance.

The SAM axis response is the first series of physiological events and is characterized by a rapid, specific response of the autonomic nervous system and consequent secretions of catecholamines (adrenalin and noradrenalin; these hormones are named epinephrine and norepinephrine in the USA). These physiological adjustments are the immediate or 'emergency' response proposed by Walter Cannon (Cannon, 1914) as the 'fight or flight' response. A major and important function of adrenalin is to quickly provide energy in the form of glucose from liver and muscle glycogen, a process known as glycogenolysis, and free fatty acids from lipolysis of adipose tissue (Murray et al., 2003). Thus the SAM axis response with the secretions of catecholamines (adrenalin and noradrenalin), is the principal regulatory mechanism that allows the animal to immediately meet physical or emotional challenges by its effects on metabolic rate, cardiac function, blood pressure, peripheral circulation, respiration, visual acuity and energy availability and use.

If the responses of the SAM axis to a stressor are insufficient there is another series of events involving the HPA axis and corticosteroid hormones. There are two predominant corticosteroids; cortisol is the predominant corticosteroid of most mammals, including humans, and bony fish, and corticosterone is the major corticosteroid in rodents, birds, reptiles, amphibians and cartilaginous fish (Chester Jones and Henderson, 1976).

The second series of responses, called the acute stress response (Selye, 1946; 1976), is a corticosteroid-dependent mechanism and thus the HPA axis is central to its function. The adrenal cortex and in particular the cortical cells that secrete the corticosteroids are controlled by higher centres of the hormonal system, the pituitary gland, which in turn is controlled by the hypothalamus at the base of the brain. The acute response may last from minutes to hours and has the major function of providing glucose from food or muscle protein (gluconeogenesis) for the required increased metabolic performance. Therefore, during this stage a steady state is achieved in which the increased demand for energy is met by increased metabolic performance. This physiological state of stress disappears on removal of the stressor with generally no ill effects other than a depletion of energy reserves.

The activation of the SAM and HPA axes is obviously an effective mechanism to assist the animal in adapting to changes in its environment. The physiological outcomes include adjustments in metabolic rate, cardiac function, blood pressure, peripheral circulation,

respiration, visual acuity and energy availability and use that allow the animal to meet physical and/or emotional challenges. Corticosteroids in the short-term also reduce some of the damaging effects of the immune response, such as repressing the inflammatory response. There are also some behavioural adaptations as a consequence of the short-term activation of the SAM and HPA axes, such as increased arousal and alertness, and increased cognition, vigilance and focused attention (Mendl, 1999; Kaldas and Chrousos, 2007), that should assist the animal to search, scrutinize and remember threatening or rewarding situations.

If the stressor continues, the response proceeds to the third series of events, which is the chronic stress response and it is this series of events that can have serious consequences for the animal. The chronic stress response is also a corticosteroid-dependent mechanism, but while in the acute phase the effects are potentially beneficial, this chronic activation of the HPA axis comes at a physiological cost to the animal, such as a decreased metabolic efficiency, impaired immunity and reduced reproductive performance. Therefore, the long-term activation of the HPA axis can have marked effects on efficiency of growth with for example the breakdown of muscle protein under the catabolic effects of ACTH and corticosteroids (Elsasser et al., 2000).

Corticosteroids also support the synthesis and action of adrenalin in stimulating glycogenolysis (i.e. provision of glucose from liver and muscle glycogen for the required increased metabolic performance) and lipolysis (provision of energy in the form of free fatty acids from the breakdown of adipose tissue) (Matteri et al., 2000). Stress-induced changes in the secretion of pituitary hormones have also been implicated in failed reproduction (Clarke et al., 1992; Tilbrook et al., 2000) and immune competency (Blecha, 2000). In laying hens, stress as well as reduced feed intake has been implicated in the increased mortality and reduced feed intake, body weight, efficiency of feed conversion and egg production observed with reduced floor space allowance (Hill, 1977; Hughes, 1983; Adams and Craig, 1985; Sohail et al., 2004). Furthermore, exposure of hens to stressors may delay oviposition and short delays in oviposition can result in additional calcium carbonate being deposited on the shell, extra-cuticular calcium, resulting in what is called 'dusting' (Hughes et al., 1986). The amount of dusting on an egg can be measured using reflectometry in which the colour of the egg is measured when it is dry and then again when it is wet, as water turns the calcium carbonate translucent (Mills et al., 1987; Reynard and Savory, 1999). How serious these costs are, depends on how long the animal is required to divert physiological resources to maintain homeostasis.

The concept of biologically active cortisol is important because of its implications for determining the magnitude of a stress response and its consequences. A predominant feature of a chronic activation of the HPA axis is increased basal secretion of corticosteroids with a loss in diurnal regulation of the axis (Harbuz and Lightman, 1992). It is this sustained elevation in free corticosteroids, together with changes in other hormones, cardiovascular function, metabolism and the immune system, that has broad, long-lasting effects on the body such as decreased metabolic efficiency, impaired immunity and reduced reproductive performance. In other words, increased basal secretion of corticosteroids have significant fitness consequences for the animal, and it is these biological and fitness effects that reflect both the magnitude of the stress response and the welfare implications.

While the role and actions of corticosteroids in acute and chronic stress responses are well known, this is not to imply that the HPA axis is the only neuroendocrine axis affected by stressors. There is also involvement of the somatotrophic and thyroid axes and other hypothalamic and pituitary hormones such as arginine vasopressin and prolactin, respectively. Furthermore there is also a direct involvement of the immune system. While corticosteroids can suppress the immune system (Blecha, 2000; Kaldas and Chrousos,

2007), studies using various stress models show that factors other than corticosteroids may also be involved in the stress-induced immunosuppression observed in animals transported, restrained or isolated (Blecha, 2000).

It is clear that the hormones secreted from the HPA axis have broad, long-lasting effects on the body and thus challenges to homeostasis that result in such neuroendocrine responses clearly have implication for animal welfare. While some component of behaviour is likely to be involved in every stress response, behavioural responses may not be appropriate or effective for all situations. Indeed, redirected behaviours and stereotypes, as with long-term neuroendocrine responses, may indicate difficult or inadequate adaptation. Therefore, measuring both these behavioural and physiological responses to a stressor, which reflect the challenge confronting the animal, as well as the fitness consequences of these responses for the animal, clearly affords an insight into the risks to the animal's welfare.

The second concept, often called the affective state or feelings-based concept, defines animal welfare in terms of emotions and emphasizes reductions in negative emotions, such as pain and fear and frustration, and increases in positive emotions such as comfort and pleasure (Duncan and Fraser, 1997). Duncan (2004; 2005) has argued that animal welfare ultimately concerns animal feelings or emotions as follows. All living organisms have certain needs that have to be satisfied for the organism to survive, grow and reproduce and if these needs are not met, the organism will show symptoms of atrophy, ill-health and stress and may even die. Higher organisms (vertebrates and higher invertebrates) have evolved 'feelings' or subjective affective states that provide more flexible means for motivating behaviour to meet these needs. Thus the central argument is that although natural selection has shaped animals to maximize their reproductive success, this is achieved by proximate mechanisms involving affective states (pain, fear, separation distress, etc.) which motivate behaviour (Fraser, 2003b).

Emotions are classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses) and a subjective component (emotional experience or feeling) (Dantzer, 1988, 2002). Animal emotions have in the past been considered inaccessible to scientific investigation because they have been described as human subjective experiences or even as illusory concepts outside the realm of scientific inquiry (Panksepp, 1998). The difficulties in studying emotions as though they were objective states of bodily arousal are well recognized in the literature (Cacioppo et al., 1993). While each emotion may reflect a different pattern of arousal, the visceral response to many emotions is reasonably uniform in animals. Most animals react physiologically, at least in the short term, in essentially the same way whether the arousal is sexual, fear provoking or if there is the anticipation of play or food. It is obviously a major challenge to study and understand emotions in animals, although there have been some promising recent developments in the comparative study of emotions that show that there are many homologous neural systems involved in similar emotional functions in both humans and other mammals, and perhaps other vertebrates (LeDoux, 1996; Panksepp, 1998, 2005). To date, most studies concerned with emotions in laying hens rely primarily on behavioural measures.

It is widely accepted in animal welfare science that good welfare is not simply the absence of negative experiences, but rather is primarily the presence of positive experiences such as pleasure (Boissy et al., 2007; Mellor et al., 2009). While methods to assess pain and suffering have been developed, there is still no agreement on how to assess positive experiences (Boissy et al., 2007). However, preference tests have been used to identify resources and behaviour that might be important to hens (Cooper and Albentosa, 2003).

Furthermore, measuring preferences of animals, using preference tests, aversion learning and behavioural demand testing (Dawkins 1980; Matthews and Ladewig 1994; Kirkden and

Pajor, 2006) has been used by scientists to assess animal welfare predominantly on the basis that these preferences are influenced by the animal's emotions. Preference testing using for example a Y maze apparatus that allows a choice between access to two different resources has been used to provide information about specific features in the animal environment such as flooring (Hughes and Black, 1973; Hutson, 1981), restraint methods (e.g. Pollard et al., 1994), handling treatments (Rushen, 1986) and ramp design (Phillips et al., 1988), with the overriding objective of optimising the environment for animals.

While the consistent choice or preference of one resource over another or others indicates the animal's relative preference, some have argued that in addition to establishing what an animal prefers, it is important to understand the strength of the preference (Dawkins, 1983; Matthews and Ladewig, 1994). To address the question of the strength of an animal's preference, experiments have incorporated varying levels of cost (e.g., work effort, time and relinquishing a desirable resource) associated with gaining access to a resource or avoiding aversive stimulation. For example, Dawkins (1983) varied the price paid for access to litter by increasing the duration of feed withdrawal before the test. She found that although hens preferred litter to wire floors, their preference was not strong enough to outweigh the attraction of food and concluded that in both experiments there was no evidence that hens regarded litter as a necessity.

Furthermore, Dawkins (1983) suggested that quantitative measures of the importance of resources for animals can be derived from measures of demand elasticity. Consequently, 'behavioural demand' studies, using operant conditioning techniques in which the animal must learn to perform a response, such as pecking at a key or pushing through a weighted door, to gain access to a resource, have been used to study the animal's level of motivation to access or avoid the situation being tested. For example, Matthews and Ladewig (1994) studied the behavioural demand functions of pigs for the resources of food, social contact and a stimulus change (door opening). The amount of work, in the form of pushing a plate, required for access to each reinforcer (resource) was systematically varied. It was found that while the demand for opening the pen door was highly elastic (i.e. the willingness of the pigs to access the resource declined as the effort increased), the demand for food was inelastic and the demand for social contact was intermediate.

In concluding on animal preferences, as with biological functioning, clarifying the conceptual link between animal preferences and animal welfare is an issue for some. The individual's concept of animal welfare clearly underscores the methodology used to judge or measure animal welfare. However, as commented by a number of authors (e.g. Fraser and Matthews, 1997), preference research should be integrated with other measures used in animal welfare research. Furthermore, Widowski and Hemsworth (2008) recommend that, while studies of motivation can provide compelling evidence that the performance of some behaviour (or preference) may be important to the animal, additional evidence, particularly on occurrence of abnormal behaviour, stress physiology and health, are necessary to provide a more comprehensive assessment of the impact of restriction on animal welfare.

1.1.3.3 Scientific uncertainty

These different concepts or views on animal welfare can lead scientists to use different criteria or methodology in assessing an animal's welfare. For short-term animal welfare issues involving acute stress, such as painful husbandry procedures, there is considerable agreement on the need to assess animal welfare from a perspective of biological functioning (Mellor et al., 2000). Furthermore, it is generally considered that there is often a close correspondence between affective states and biological functioning, that is the visceral response of a negative emotional response (Dantzer, 1988, 2002). However, for longer term issues disagreement over these welfare concepts, especially when consequent

interpretations conflict, often lead to debates concerning animal welfare and the varying interpretations (Fraser, 2003a,b).

This so-called 'scientific uncertainty' does not necessarily diminish the robustness of the research utilising methodologies or measurements arising from these views or concepts, but it does raise the question of the relatedness of these concepts (Barnett and Hemsworth, 2009). In other words, are situations in which an animal has to resort to the extreme coping attempts (i.e. challenges that may overwhelm an animal's capacity to adapt) associated with, or lead to, negative affective states and vice versa? In a similar context, is an inability to perform normal or 'natural' behaviours associated with extreme coping attempts and/or negative affective states? Therefore, if these concepts are related are the resultant methodologies measuring the same adverse physiological and mental state(s) in the animal? Indeed many authors have raised the commonalities in these concepts (e.g. Fraser, 2003b, 2008).

As suggested by Barnett and Hemsworth (2009), this conceptual convergence suggests a way forward in developing a broader consensus on the study of animal welfare by reducing both conceptual differences and consequently methodological differences in animal welfare science. The validity of the welfare criteria can be tested in several ways: first, with the finding that there are correlations between independent measures of different concepts of animal welfare; and second, with the finding that an intuitively aversive condition reduces animal welfare on the basis of the measures of different concepts of animal welfare. Therefore, research examining the validity of these concepts and, in turn, methodologies is necessary to understand the relationships between the concepts and indeed minimize the conceptual and methodological differences as discussed here. The development of a broader scientific consensus on welfare measures arising from this research should lead to the development of credible measures that can be incorporated into welfare assessment and screening tools in the field. It should be noted that there is indeed some evidence to support this conceptual convergence (Nicol et al., 2009; Stevens et al., 2009; Arnold and Matthews, 2010).

1.2 Project aims

The major objective of this project was to determine the effects of floor space allowance and access to a nest-box in cages on hen welfare on the basis of the two most common approaches to assess animal welfare, the measurement of biological function and affective states using preference testing. Furthermore, the opportunity was taken to study the validity of these two welfare criteria by examining the associations between independent measures of these two different concepts of animal welfare.

2 Experiment – Effects of space during rearing and adulthood and access to a nest-box during adulthood on the welfare of laying hens

2.1 Background

Two of the most contentious issues in relation to cage housing and hen welfare are space and the need for a nest. The literature on the effects of space allowance in layer cages shows that in general as floor space decreases, within a range of 300 to 650cm² per caged laying hen, mortality increases, egg production and body weight decrease and efficiency of feed conversion decreases. Mench et al. (1986) reported that reducing space allowance in two-bird cages from 1394 to 697cm²/bird increased plasma corticosterone concentrations.

Based on preference and behavioural demand tests, hens are highly motivated to access an enclosed nest site (Follensbee, et al. 1992; Cooper and Appleby, 1997; Freire et al., 1997). Furthermore, when a nest-box is unavailable, hens are more active, engage in locomotory behaviour for a longer duration before laying their eggs, and often perform stereotyped pacing that has been interpreted as signs of frustration (see Appleby et al., 2004). This pre-laying activity could also be interpreted in terms searching for a nest site.

However, the effect of a lack of a nest-box on stress physiology has received little scientific attention. Cronin et al. (2012) found that hens that showed longer duration and fewer bouts of pre-laying sitting had lower plasma corticosterone concentrations regardless of whether they had a nestbox. Further, Cronin et al. (2008) found no long-term adverse effects on stress physiology between hens with or without a nest-box, or those subsequently prevented from laying in a nest-box.

Therefore, the major objective of this experiment was to determine the effects of floor space allowance and access to a nest-box in cages on a broad range of accepted welfare indices. Since there is limited evidence that early experience may affect preferences for resources such space (Faure, 1991), this project also examined the effects of floor space allowance during rearing on subsequent hen welfare. Furthermore, the opportunity was taken to study the validity of the two common approaches to animal welfare assessment that were used in this experiment, that is, those indices that demonstrate the normality of the animal's biological functioning and fitness (i.e., how well an animal is coping with the challenges it faces) and those indices that demonstrate what resources are perceived to be important to an animal (i.e., animal preferences). The validity of these two approaches to animal welfare assessment was studied by examining the associations between these two sets of welfare criteria.

2.2 Objectives

The main objective of this experiment was to determine the effects of floor space during rearing and adulthood and access to a nest-box during adulthood on the welfare of laying hens using a broad range of well-accepted welfare indices.

2.3 Materials and methods

2.3.1 Facilities

This experiment was conducted in a facility at Werribee (Victoria, Australia; 37°55'S, 144°40'E) with 66 Victorsson Trivselburen furnished cages (AB Broderna, Victorsson, Sweden) that were modified to meet the experimental requirements. Each standard cage provided three water nipples, shared between the back-to-back cages, and water was available *ad libitum*. Perches and dust baths were removed while nest-box remained in the cages. The nest-boxes were 241 mm wide, as deep as the whole cage (498mm), and 270mm high at the front of the cage. A blue vinyl flap was suspended in front of the nest-box at the cage front to reduce the light levels inside the nest-box and the speed of eggs rolling out of the nest (to reduce cracked eggs). Overlaying and attached to the wire floor of the nest-box was a 15mm thick rectangular piece of brown artificial turf (370 × 220mm).

The cages were located in two controlled-climate sheds for temperature, light, and ventilation and the rearing component of the experiment was conducted in one shed and the production component in the other. The thermostats were set at 21°C with the average temperature maintained at about 17°C during the dark period and 23°C during the light period, however, humidity within the sheds was difficult to maintain. Temperatures and humidity within the sheds are presented in Appendix 1.

Lighting was provided by incandescent bulbs and controlled by a computer. At placement, the birds were on an initial light: dark cycle of 10L: 14D. Day length was increased until the hens were exposed to an ongoing lighting regimen of 14L: 10D at 29 wk of age (see Appendix 3). The time of day in which lights were programmed to come on is presented in Appendix 3. The birds were initially housed at about 20 lx (1.86 foot candles) during lights on, but this was decreased to 5 lx (0.46 foot candles) at 28 wk of age.

During the production component of the experiment (from 16 wk of age), video cameras with built-in infra-red (IR) lights were positioned above and below all cages as well as inside nest-boxes (if access provided) providing video coverage at all times.

Prior ethical approval was obtained for all procedures from the Animal Ethics Committee of the University of Melbourne. The procedures in this experiment were conducted in accordance with the Australian Prevention of Cruelty to Animals Act 1986 and the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organisation/Australian Animal Commission "Australian code of practice for the care and use of animals for scientific purposes".

2.3.1.1 *Animals and treatments*

Hy-line Brown pullets (n=256) over four replicates (64 pullets per replicate) were transported from a commercial unit approximately 1.5 hours to the research facility at 7 wk of age and housed in an environmentally controlled layer shed. The experiment commenced in November 2009 and concluded in July 2012.

As shown in Table 2-1, the experiment was a 2x2x2 factorial, incomplete block design consisting of the following three main effects:

1. Rearing space allowance in groups of 8 pullets per cage from 7 wk of age - two levels, 315 or 945cm²/bird (16 cages per level);
2. Production space allowance in groups of 6 hens per cage from 16 wk of age - two levels, 542 or 1648cm²/bird (16 cages per level);
3. Nest-box access - two levels, presence or absence of access to a nest-box during production (16 cages per level). A single nest-box (0.24m (width) x 0.50m (depth) providing 200cm² nest space per bird) was located adjacent to one side of each cage and access to the nest-box was blocked in the treatment involving absence of access to a nest-box.

Table 2-1 - Space and nest-box treatments

Rearing space allowance	Production space allowance	Nest-box access	Treatment designation
315 cm ² /bird	542 cm ² /bird	Yes	RearsmallProductionsmallNestBox+
		No	RearsmallProductionsmall NestBox-
	1648 cm ² /bird	Yes	RearsmallProductionlargeNestBox+
		No	RearsmallProductionlargeNestBox-
945 cm ² /bird	542 cm ² /bird	Yes	RearlargeProductionsmallNestBox+
		No	RearlargeProductionsmallNestBox-
	1648 cm ² /bird	Yes	RearlargeProductionlargeNestBox+
		No	RearlargeProductionlargeNestBox-

The space allowances chosen meet the standards of the PISC (Primary Industries Ministerial Council, 2002). The Code of Practices requires pullets to have a space allowance of 315cm²/hen at 15 wk of age. This space allowance was used throughout rearing. The smaller space allowance during production is higher than the requirement for cage systems purchased prior to January 2001, but slightly less than that required of cages purchased after January 2001. The larger space allowance (being three times the smaller space allowances) was chosen as it would likely provide ample space for hens to perform their entire repertoire of behaviours (Cooper and Albentosa, 2003). Feeder space for birds in the large cages was restricted to that of the small cages in both the rearing and adult periods.

Cage lay-out for each replicate was randomised according to the Analysis of Variance (ANOVA) table presented in Table 2-2. Using the ANOVA table, the treatments for each replicate were determined and are presented in Appendix 2.

Table 2-2 - Form of analyses of variance

Source of variation	Degress of freedom
Rep stratum	3
Rep.Group stratum	
Rearing Space Allowance (RearSpace)	1
Residual	3
Rep.Group.Row stratum	
Production Space Allowance (Production)	1
RearSpace.Production	1
Residual	6
Rep.Group.Side stratum	
Production.Nestbox Access (NB)	1
RearSpace.Production.NB	1
Residual	6
Rep.Group.Row.Side stratum	
Production.Nestbox Access (NB)	1
NB	1
RearSpace.NB	6
Residual	

During rearing, pullets were vaccinated as per industry standard (see Appendix 3), ad libitum fed a commercial grower pellet (Barastoc Pullet Grower, 15.5% CP), and were exposed to a light regimen of 10L: 14D. At 16 wk of age, hens were randomly allocated from the two rearing treatments to the four adult treatments, ensuring that within each adult cage, all birds had come from the same rearing treatment. Hens were ad libitum fed a commercially available pre-lay pellet (Barastoc Pullet Finisher, 15.5% CP) for 2 wk, followed by a formulated layer diet (formulated and mixed by the source farm, 15% CP). Day length was set at 10 h from 7-15 wk and then increased 30 min bi-weekly thereafter until hens were 29 wk of age and remained so until 34 wk when the experiment terminated (see Appendix 4). All birds were beak-trimmed only once using an infra-red technique at 1 d of age.

2.3.2 Measurements

2.3.2.1 Physiology

The procedure to collect blood samples consisted of inserting a 23 gauge × 1 inch needle attached to a 4.5ml heparin-coated syringe into the brachial vein of the bird and the required volume of blood was withdrawn. Two laboratories analysed the blood, eggs and faeces for corticosterone; one laboratory for replicates 1 and 2 and the other for replicates 3 and 4. The assay used by the first laboratory was a Corticosterone HS Enzyme Immunoassay (EIA) (IDS Ltd, Boldon, UK), validated by Barnett et al. (1994). The second laboratory used a radioimmunoassay performed in duplicate on extracted samples. The standard curve included triplicate tubes for total counts (TC) and non specific bound (NSB), nine replicates of zero standard, three replicates of each standard and six replicates each of two quality control pools containing 0.71 and 2.22ng/ml of corticosterone which were used to estimate the intra-assay coefficients of variation (5 and 5.7%, respectively). The antiserum B3-163 was purchased from Endocrine Sciences Products and the tracer (1, 2, 6, 7-3H) corticosterone was Amersham Biosciences Pty Ltd. The assay was separated using donkey anti rabbit serum.

To the extracted tubes 100µl of 3H-corticosterone tracer and 150µl 1st antibody were added to all tubes except the non-specific binding tubes and 150µl of buffer was added to

these. The tubes were vortexed and incubated at 4°C for 24 hours. On Day 2, normal rabbit serum (100µl, 1:800) was added, followed by 100µl second antibody (anti-rabbit serum; 1:60 in PBS). The tubes were mixed and incubated overnight at 4°C. On Day 3, 1ml of 6% polyethylene glycol (PEG 6000) in PBS was added to all tubes (except TCs). The tubes were centrifuged in a refrigerated (5°C) centrifuge at 1.500xg for 25 minutes, the supernatant aspirated and the pellet was redissolved in 500µl of HCL (0.05M). The solution was dispensed into counting vials and then mixed with 2ml of scintillant (Starcount, Packard Chemicals Operations). The vials were capped, shaken and left in the dark for two hours before counting in a liquid scintillation counter (Packard Tri Carb 1500).

2.3.2.1.1 Corticosterone concentrations

To examine basal plasma corticosterone concentrations, blood was collected from the brachial vein of each bird at 26 and 27 wk of age. The sequence of sampling cages was randomized and one pair of birds from each cage was sampled in each of three rounds of sampling so that the six birds in each cage were sampled in one afternoon in each of the 2 wk. The two birds in a pair were concurrently sampled as follows. The two people approached the cage, and each caught one of the two selected birds (one bird at front and one at rear) for sampling and briefly carried each bird and firmly held it on a table on its side with one wing extended while one of two other people collected the blood sample. All samples were taken within 2 min of the hen being removed from her home cage in order to avoid an acute stress response to handling influencing the concentrations of plasma corticosterone (Broom and Johnson, 1993). Samples taking longer than 2 min to collect were not used for analysis. Whole blood was collected and samples were kept on ice (< 1 hour) until they were centrifuged and the plasma poured into 1ml tubes.

The plasma samples were pooled for each cage over the two collection days to establish a sample for baseline corticosterone concentrations. Samples were frozen at -20°C until analysed for corticosterone concentration using a Corticosterone HS Enzyme Immunoassay (EIA) (IDS Ltd., Boldon, UK). At the second laboratory (replicates 3 and 4), plasma samples (0.1ml) were dispensed into glass culture tubes and 3mL of Diethyl ether added and the contents were shaken for ten minutes. The tube and contents were placed in a -80 °C freezer and when the aqueous phase was frozen, the solvent fraction was poured into a 12x75mm culture tube. The diethyl ether was removed by heating under a constant flow of nitrogen gas. The precipitate was dissolved in 0.1ml PBS and analysed (Downing and Bryden, 2008).

2.3.2.1.2 ACTH challenge

Hens were subjected to an ACTH challenge (Barnett et al., 2009) at 28 wk of age to investigate their maximum corticosterone response. Hens were briefly caught and injected into the thigh muscle with 0.5ml (12.5 IU) ACTH Synacthen, Ciba Geigy, AllHank Trading, South Melbourne, Victoria, Australia) using a 1ml syringe with a 25 gauge needle and then returned to their cage. After 1 h, blood was collected via the wing vein using a 4.5ml heparin-coated syringe with a 23 gauge needle. Samples were kept on ice (< 1 hour) until they were centrifuged and the plasma poured into 1ml tubes. Plasma samples were pooled for each cage and samples were frozen at -20°C until analysed for corticosterone concentration using a Corticosterone HS Enzyme Immunoassay (EIA) (IDS Ltd., Boldon, UK). For the second laboratory, samples were extracted as described for baseline plasma samples.

2.3.2.1.3 Egg yolk and albumen corticosterone concentrations

Eggs were collected over the two days prior to blood collection at 26 and 27 wk of age. Eggs were identified for each cage and were weighed and separated into the albumen and yolk. Each component was weighed and a sample was kept (10-12g and 4-6g for the albumen and yolk, respectively). Samples were pooled for each cage for the four days of collection and frozen at -20°C until analysis.

For analysis of egg yolk in the first laboratory, 0.5g of egg yolk was taken and 1ml of distilled water was added and vortexed until mixed. The mixture was extracted with 3ml hexane: diether (30:70 ratio), vortexed and left to settle before snap freezing with an ethanol/dry ice bath. The supernatant was collected and dried. 1ml of ethanol was added to the samples which were then frozen -20°C overnight. The samples were centrifuged the next day and the supernatant taken and dried once more before being resuspended in PBS and analysed. In the second laboratory, egg yolk (0.1g) was taken and 0.5ml of distilled water was added and vortexed until mixed. The mixture was extracted with 3ml hexane: diether (30:70 ratio) and vortexed and left to settle before snap freezing with an ethanol/dry ice bath. The supernatant was collected and dried. 1ml of ethanol was added to the samples which were then frozen -80°C overnight. The samples were centrifuged the next day and the supernatant taken and dried once more. The samples were then resuspended in PBS and analysed (Cook et al., 2000).

In the first laboratory, 5g of egg albumen sample were taken and 5ml of distilled water was added. These were mixed, and 0.5g of the mixture was taken for extraction with 4ml of diethyl ether, shaken for 10 min then frozen at -80°C, after which the supernatant was collected and dried down. The samples were resuspended in PBS and analysed for corticosterone concentration using a Corticosterone HS Enzyme Immunoassay (EIA) (IDS Ltd., Boldon, UK).

Similarly, in the second laboratory, the albumen samples were thawed and shaken vigorously until completely mixed. Duplicate 0.5g sub-samples of albumen were transferred to glass culture tubes (16x100mm) and to this 1ml of distilled water were added and then the contents shaken. After complete emulsification diethyl ether (4ml) was added and the contents were shaken for 10 min. The tube and contents were placed in a - 80 °C freezer and when the aqueous phase was frozen, the solvent fraction was poured into a 12x75mm culture tube. The diethyl ether was removed by heating under a constant flow of nitrogen gas. The precipitate was dissolved in 0.1mL PBS and analysed (Downing and Bryden, 2008).

2.3.2.1.4 Faecal corticosterone concentrations

Faeces were also collected over the two days prior to baseline blood sampling at 26 and 27 wk of age. Faeces were collected from 09:00-10:00, 12:00-13:00 and 15:00-16:00 h on each day. At this time, manure belts were cleared of faeces and greaseproof paper was placed under each cage. At the end of each hour, faeces were collected into an aluminium container. At the end of the day, samples were weighed and placed in a drying oven at 60°C for 48 hours. Once dry, samples were weighed and ground. Samples for each cage were pooled over the four sampling days and frozen at -20°C until analysis.

For extraction at the first laboratory, 0.1 g of ground faeces was extracted with 1ml of 80% methanol. The samples were then vortexed for 30 min and centrifuged. The supernatant was taken and dried down. The samples were then resuspended in PBS and analysed for corticosterone concentration using a Corticosterone HS Enzyme Immunoassay (EIA) (IDS Ltd., Boldon, UK). In the second laboratory, ground dried faeces (0.1g) were boiled in 3ml of 90% ethanol for 20 min and centrifuged for 10 min at 500 xg. The supernatant was removed and the process repeated. The supernatants were pooled and dried under a stream of nitrogen. The extracts were then resuspended in PBS and diluted 1 in 20 before being analysed (Brown et al., 1994).

2.3.2.1.5 Haematology

Blood was sampled from all hens via the wing vein using a 4.5ml heparin-coated syringe with a 23 gauge needle at 29 wk of age. These blood samples were transported on ice to a commercial Australian laboratory and the absolute numbers of heterophil and lymphocyte cells were measured on individual hen samples in an autoanalyser CellDyn 3700 (Abbott Diagnostic Division, Abbott Park, IL, USA).

2.3.2.2 Behaviour

2.3.2.2.1 Time budgets of behaviour

Table 2-3 - Postures and behaviours measured in the behavioural time budget

Postures	Definition
Sitting	Hen's legs are approximately parallel to the cage floor with plumage of chest and/or belly in contact with the cage floor
Erect	The hen's body is not in contact with the cage floor, legs are extended
Squatting (Penguin Posture)	Keel is up and the cloaca pointing downward while the hen is in oviposition
Behaviours	
Mobile	Walking or running
Stationary	Stationary in one location but can perform other listed behaviours
Resting	Hen immobile and performing no other listed behaviours
Preening	Hen cleaning feathers or scratching at body
Feed pecking	Hen visibly pecking into the feeder
Drinking	Hen visibly pecking at the nipple drinker
Inedible Pecking	Hen pecking at inedible objects (anything other than feed, drink and other birds)
Cohort Pecking	Hen pecking at cage mates, which includes feather and body pecking
Stretching	Hen has fully extended wing or leg, flapping wings, or ruffling feathers
Oviposition	Hen in process of laying egg
Sham Dust Bathing	Hen performs behaviours associated with dust bathing on the cage floor or in the nest-box

Video cameras with built-in infra-red (IR) lights were positioned above and below all cages as well as inside nest-boxes (if access available) providing video coverage of all hens at all times from 16 wk of age. Instantaneous scan sampling utilizing continuous video recordings was used to examine the time budgets (Weeks et al., 2000; Albentosa et al., 2007; Martin and Bateson, 2007) and digital video records for each cage were observed during the first 5 min of each hour during the 13 h of light on one day in 26 wk. During each 5 min sampling period, instantaneous observations were made every 30 s (0, 30, 60, 90, 120, 150, 180, 240, 270, and 300 s). The posture and behaviours recorded are presented in Table 2-3. For each time point, the total number of hens in the cage performing each was recorded and from these records, the average proportion of hens displaying each behaviour in each cage in the first 4 h of the daylight period (morning period), next 5 h (midday period) and last 4 h (afternoon period) over the two days was calculated. Thus data on time budgets of behaviour are presented as the proportion of observations in which hens displayed specific postures and behaviours.

2.3.2.2.2 Oviposition behaviour

Using continuous video recordings, a sample of four consecutive days when the hens were 28 wk old, were used to examine pre-laying sitting behaviour. The number and duration of sitting bouts were recorded for each hen, 1 h prior to oviposition. Hens were recorded as sitting when their legs were about parallel to the wire floor, and the plumage of the breast and/or abdomen was pressed up against the floor. Standing, or moving into a squat in preparation for oviposition, marked the end of a sitting period. Preliminary observations revealed that some hens would shuffle forward whilst remaining in a sitting position, or half stand and sit repeatedly, or stand slightly to assist with preening or readjust position. These behaviours were included as part of the sitting period. If the hen was already sitting when the 1 h observation period commenced, the time of the start of the observation period was recorded as the beginning of the sitting bout.

The same four day sample period was used for the observation of site and hen orientation at oviposition as was used to assess pre-laying sitting behaviour. To determine the site of oviposition, cages were divided into discrete areas (0.22 x 0.5m per area). Small cages were divided into three areas, with the nest-box as an additional fourth area. Large cages were divided into nine areas, with the nest-box as an additional tenth area (Fig. 2-1). The orientation of the hens was recorded as described in Table 2-4. The consistency of orientation or site at oviposition was calculated as the proportion of eggs laid in the most preferred (most often recorded) orientation or site.

$$\text{Orientation Consistency} = \frac{\text{Total eggs laid in most preferred orientation}}{\text{Total eggs laid on observation days}}$$

$$\text{Site Consistency} = \frac{\text{Total eggs laid in most preferred site}}{\text{Total eggs laid on observation days}}$$

The time of oviposition for all eggs laid by each hen throughout the video recording period (26-33 wk) was recorded. There were 53 days of oviposition times available. The times for each oviposition were used to determine the interval between successive eggs laid for each hen and the coefficient of variation (CoVar) of the variable was then calculated. All oviposition data was averaged for each cage.

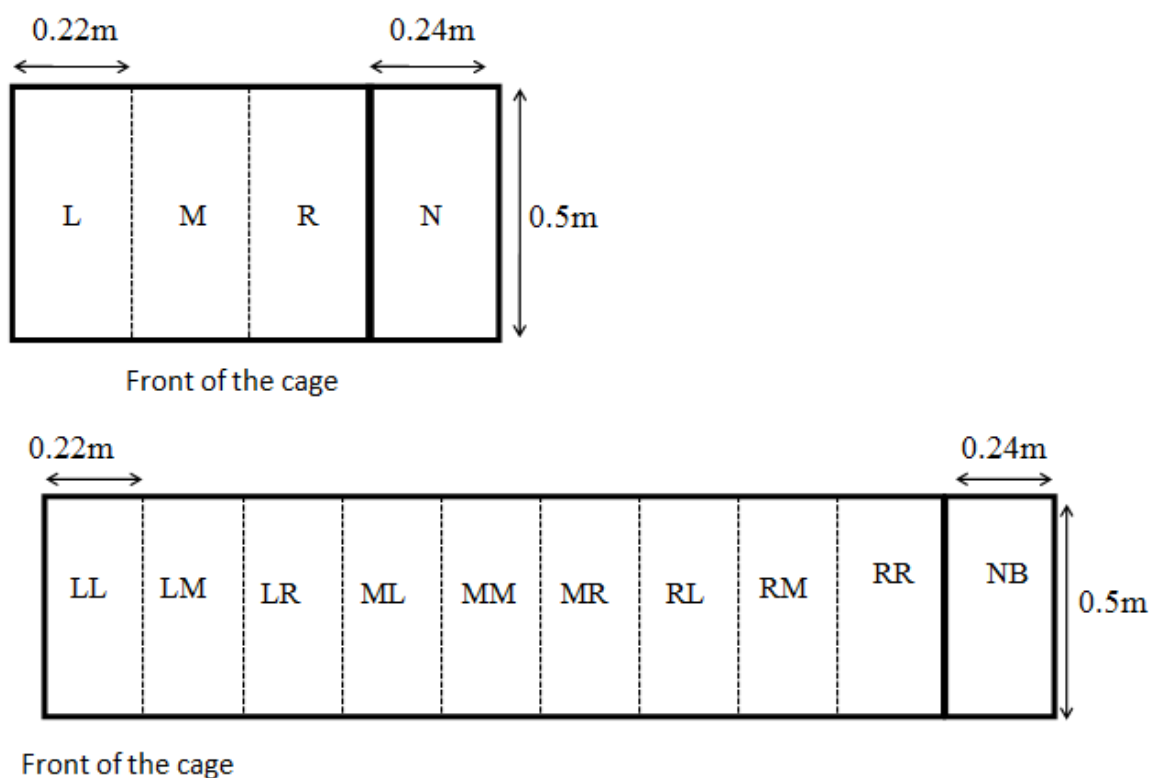


Figure 2-1 - Division of small and large cages for oviposition observations

*L=left side of the cage, M= middle fraction of the cage, R=right side of the cage, NB=nest-box, LL= far left side of the cage LM=middle part of left side of the cage LR=right part of left side of the cage, ML= left part of middle fraction of the cage, MM= middle part of middle fraction of the cage, MR= right part of middle fraction of the cage, RL=left part of right side of the cage, RM= middle part of right side of the cage, RR=right part of right side of the cage.

Table 2-4- Orientation definitions to determine consistency of orientation for oviposition observations

Orientation	Definition
Front	Bird orientated with body facing the front of the cage
Back	Bird orientated with body facing the back of the cage
Nest-box	Bird orientated with body facing the side of the cage where the nest-box is situated
Side	Bird orientated with body facing the side of the cage without the nest-box
Inward	Bird orientated with body facing into the cage. Birds in small cages and birds in the very mid section of the cage are never scored as inward.
Corner	Bird orientated with body facing into one of the back corners
Nest-box front	Bird in the nest-box with body facing the front of the cage
Nest-box back	Bird in the nest-box with body facing the back of the cage

Furthermore, eggs laid in nest-boxes in cages in which hens had access to a nest-box was also monitored in week 26.

2.3.2.2.3 Choice behaviour in Y maze (preference) tests

2.3.2.2.3.1 Predicting Time of Oviposition

Beginning when hens were 22 wk of age, 24 h video footage was observed on a daily basis and the exact time the egg touched the floor of the cage was recorded for each hen. Hens were identified by unique combinations of black and white leg bands and carbon-based ink on their head and back. Therefore, by the onset of Y maze testing, the time that every egg a hen had laid for 8 wk had been recorded. These recorded times of oviposition were used to predict at what time the hens tested for nest-box preference would be tested. If the hen showed a clear pattern, the average amount of time between successive eggs was simply added or subtracted to predict at what time she was likely to lay her next egg. However, if a hen failed to lay an egg on any occasion, the records were checked and note was taken of the last time she failed to lay an egg. The predicted time of oviposition on the next day was predicted in this situation to be approximately the time at which she laid her next egg following the previous occurrence that she did not lay an egg. If a hen laid an egg at a time that varied markedly from her usual pattern, the records were checked for an occasion on which this previously occurred. Using the time following the last egg that was laid at this markedly different time, the time of oviposition was predicted. Once the time of oviposition was predicted, the hen was assigned a testing time approximately 30-40 min prior when a hen was likely to be exhibiting pre-laying behaviours and most motivated to access a nest-box.

2.3.2.2.3.2 Hen selection

At 29 wk of age, 32 (four per cage) of the 48 experimental hens in each replicate were selected for preference testing. These hens were selected based on the regularity of their laying pattern, and therefore, the ease at which their oviposition could be predicted. Hens were not deprived of feed prior to training or testing.

16 (two from each cage) were selected to be tested for nest-box preference over feed. Each of the 16 nest-box-tested hens was then paired with a randomly-selected hen from the same cage to be tested for space preference over feed. As hens were being tested for increased space preference, the Y maze apparatus was specially designed for this experiment to offer as little extra space as possible apart from the arm in which increased space was offered. While the other attachment arm (feed arm or nest-box arm) provided a floor space of 555cm², the space arm attachment was equivalent to the higher space allowance of 1648cm². The feed arm attachment was designed to resemble the front of a cage and the nest-box arm attachment was designed to resemble the nest-boxes present in the experimental cages, with solid walls and artificial turf floor, but were, however, slightly smaller in size (30 x 18.5cm (width x length)).

2.3.2.2.3.3 Training Phase

The purpose of the first training phase was to familiarize hens with the testing apparatus (see Appendix 5). This phase of Y maze testing occurred over 5 consecutive days. A hen was placed into the start box (see Appendix 5) of the Y maze. After 10 s, the gate of the start box was opened and the hen was given 30 s to leave the start box (defined as the hen's entire head and neck being outside of the area). If at 30 s a hen did not leave the start box, she was given a gentle nudge into the choice area (see Appendix 5) and the gate to the start box was closed. At this point, she was given 2 min to explore the rest of the apparatus, including the resources (either feed arm (floor area of 30 x 18.5cm (width x length), 555cm²) and space arm (40 x 36 (maximum width x maximum length), 1648cm²) or

feed arm and nest-box arm (30 x 18.5cm (width x length), 555cm²). Pairs of hens were randomly ordered prior to training and were also randomised as to whether the 'nest-box' hen or 'space' hen would be trained first.

2.3.2.2.3.4 Training Phase 2

The purpose of the second training phase was for hens to associate each arm of the Y maze with a given resource. This phase of testing occurred over 5 consecutive days. Prior to testing, the side in which the hen was first exposed was randomised for each hen and each testing day. The gate of the arm the hen was first exposed to was left open, while the gate of the other arm was closed. A hen was placed into the start box of the Y maze and after 10 s, the gate of the start box was opened and the hen was given 30 s to leave the start box. If at 30 s a hen did not leave the starting box she was given a gentle nudge into the choice area and the gate to the start box was closed. She was then given 30 s to leave the choice area and enter the open arm. If she did not enter the arm after 30 s, she was given a gentle nudge to enter the arm. She was then given 2 min with the resource to familiarize herself with it and the location. This was then repeated for the opposite arm. This phase of Y maze testing took approximately 10 min per bird. Therefore, the nest-box hens were tested in the order in which they were predicted to lay their egg (as described above) to ensure they were introduced to the nest-box when they were most likely to be motivated to lay. Once all nest-box hens were tested, their corresponding space hens were tested in the same order.

2.3.2.2.3.5 Y maze Testing

Hens were divided into two testing groups, consisting of one pair from each cage. Groups were tested from 32 wk of age on alternating days over 14 days (i.e., seven tests/hen) and hens were tested depending on the time the nest-box-tested hen was predicted to lay, but whether the nest-box-tested hen or the space-tested hen was tested first was randomised for each testing day. A hen was placed in the start box of the Y maze for approximately 10 s before the gate was raised. She was then given 30 s to leave the start box and enter the choice area and given a gentle nudge if she had not left.

Subsequently, she was given 30 s to choose either of the resources (Nest-box vs. food or space vs. food) after which she was given a gentle nudge forward but not toward either resource to encourage her to make a choice. Once a resource was chosen, the gate to the other resource was closed so the hen could not enter the other arm. The hen was then given 2 min with the resource unless she chose the nest-box, in which case she was promptly enclosed in the nest-box and the nest-box attachment set aside. She remained in the nest-box for 30 min or until she laid an egg (whichever was longer) before being returned to her cage.

2.3.2.3 Productivity and other measures

2.3.2.3.1 Body weights and egg production

Pullets were weighed weekly from 7-16 wk of age and then weighed at 18, 19, 21, 22, 26, 30 and 34 wk of age. Hen-day egg production was measured from 18 to 34 wk of age.

2.3.2.3.2 Extra-cuticular calcium

The eggs collected for egg yolk and albumen corticosterone analyses at 26 and 27 wk of age were also analysed for extra-cuticular calcium. This analysis was done following the methods of Reynard and Savory (1999). Using a Hunter Lab Miniscan XE, the device was placed over the broad end of the egg where calcium dusting is most likely to occur (Mills et al., 1991). For each egg, three dry readings were taken before the shell was wiped with a damp cloth and three wet readings were taken. Using the L-score (a ranking of white (100)

to black (0)), the dry and wet readings were averaged and the wet reading was subtracted from the dry reading to yield a dusting score.

2.3.2.3.3 Feather condition scores

The methodology for feather condition scoring was adapted from Tauson et al. (2005). Feather condition was assessed at 26, 30 and 34 wk of age using a subjective 4-point scoring system applied to the neck, breast, cloaca/belly, back, wings and tail. The scoring system is described in Table 2-5. Once scores were assigned, the score for each of the 6 body areas were added together to create the following scores: > 20 = good – very good; 15-20 = average; 13-15 = rather poor; and <13 = poor.

Table 2-5 - Feather condition scoring (Tauson et al., 2005)

Area being scored	Score
Neck	1 = naked or naked body part 2= dominating areas of naked skin, e.g., bare patches of an area of more than a diameter of 5 cm on the back or not naked but severely pecked down into only stubs close to the skin spread over the main part of the area. 3 = clearly visible damaged feathers but normally no naked areas. Neck/back can have smaller local naked areas. 4 = intact or very close to intact body part.
Breast	
Cloaca/belly	
Back	
Wings	
Tail	

2.3.3 Statistical analyses

Each measurement was analysed using a multi-strata factorial analysis of variance, with the experimental unit being a cage (Table 2-2). Prior to analysis, the percent of times nest-box was chosen and the percent of time space was chosen were angularly transformed. A number of measurements were transformed prior to the analysis of variance to avoid the residual variation increasing as the mean increased. Mean feather scores at wk 26, 30 and 34 were angularly transformed after linearly transforming the scores to a 0 to 100 scale. The heterophil to lymphocyte ratio was logarithmically transformed. One unusual pen, with a much larger amount of feather damage than all other pens in its time replicate, was treated as a missing value (Payne, 2010).

2.4 Results

There were no mortalities in the experiment but three birds were removed from the study, two for illness and one for injury associated with being wedged under the feeder. These birds were removed during rearing.

2.4.1 Physiology

2.4.1.1 Corticosterone concentrations

As shown in Tables 2-6, 2-7 and 2-8, there were no effects of cage size during rearing, cage size during production or access to nest-box on corticosterone concentrations in plasma, faeces, egg albumen or egg yolk or on corticosterone response to ACTH challenge. There was a significant ($P=0.045$) interaction between cage size during rearing and production on basal plasma corticosterone concentrations: corticosterone

concentrations were highest in hens that remained within the same space allowance level (small or large) in the rearing and production periods.

Table 2-6 - Effect of size of rearing cage on physiology at 26-29 wk of age

Variable	Rearing Treatment (7-15 wk)		SED	P value
	Small	Large		
Corticosterone				
Basal plasma (ng/ml)	1.23	1.14	0.090	0.40
Faecal (ng/g)	49.07	52.20	2.731	0.34
Albumen (ng/g)	0.29	0.31	0.057	0.70
Yolk (ng/g)	2.69	2.79	0.243	0.71
ACTH (ng/ml)	17.56	16.37	1.683	0.53
Haematology				
Heterophil:lymphocyte ratio#	0.03 (1.08)	-0.02 (0.95)	0.088	0.60

#: Heterophil: neutrophil ratios were log₁₀ transformed prior to statistical analysis. Back transformed means presented in parentheses.

2.4.1.2 Haematology

There were no main effects ($P>0.05$) or interactions between main effects ($P>0.05$) on heterophil: lymphocyte ratio (Tables 2-6, 2-7 and 2-8).

Table 2-7 - Effect of size of production cage on physiology at 26-29 wk of age

Variable	Production Treatment (16-34 wk)		SED	P value
	Small	Large		
Corticosterone				
Basal plasma (ng/ml)	1.20	1.17	0.096	0.72
Faecal (ng/g)	51.41	49.86	2.912	0.61
Albumen (ng/g)	0.31	0.30	0.027	0.76
Yolk (ng/g)	2.68	2.80	0.192	0.53
ACTH (ng/ml)	18.13	15.80	2.735	0.43
Haematology				
Heterophil:lymphocyte ratio#	-0.01 (0.98)	0.02 (1.04)	0.04	0.61

#: Heterophil: neutrophil ratios were log₁₀ transformed prior to statistical analysis. Back transformed means presented in parentheses.

Table 2-8 - Effect of access to a nest-box on physiology at 26-29 wk of age

Variable	Nest-box Treatment (16-34 wk)		SED	P value
	No	Yes		
Corticosterone				
Basal plasma (ng/ml)	1.27	1.11	0.093	0.13
Faecal (ng/g)	50.36	50.92	4.967	0.91
Albumen (ng/g)	0.32	0.29	0.033	0.36
Yolk (ng/g)	2.83	2.65	0.099	0.12
ACTH (ng/ml)	16.78	17.14	3.333	0.92
Haematology				
Heterophil:lymphocyte ratio#	-0.02 (0.96)	0.03 (1.06)	0.067	0.56

#: Heterophil: neutrophil ratios were log₁₀ transformed prior to statistical analysis. Back transformed means presented in parentheses.

2.4.2 Behaviour

2.4.2.1 Time budgets of behaviour

Cage size during rearing affected the proportion of hens displaying drinking behaviour at 25 wk of age (Table 2-9). Hens reared in large cages were observed less frequently drinking ($P=0.048$) in adulthood, which was mainly due to reductions in the midday ($P=0.043$) and afternoon ($P=0.0069$) periods. There were no effects of cage size during rearing on postures or other behaviours.

In contrast to cage size during rearing, cage size during production affected numerous bird behaviours at 25 wk of age. As shown in Table 2-10, hens in small cages were observed overall less frequently in an erect posture ($P=0.011$) and being mobile ($P<0.0001$), preening ($P<0.001$) and pecking inedible objects ($P=0.021$). These reductions were particularly evident for mobility in each of the three periods ($P=0.0035$, $P=0.00039$ and $P<0.0001$, respectively) and for pecking inedible objects in the midday and afternoon periods ($P=0.021$ and $P=0.024$, respectively). Hens in small cages were also observed overall less frequently drinking in the morning period ($P=0.023$). However, hens in small cages were observed overall more frequently in a sitting posture ($P=0.0082$), resting ($P=0.0005$) and Feed pecking ($P=0.0013$). These increases were particularly evident for sitting in the midday period ($P=0.0035$), for resting in each of the three periods ($P=0.0009$, $P=0.00069$ and $P=0.0015$, respectively) and for feed pecking in each of the three periods ($P=0.0043$, $P=0.020$ and $P=0.0043$, respectively).

The absence of the nest-box during production affected several bird behaviours at 25 wk of age. As shown in Table 2-10, hens in cages without access to a nest-box were observed less frequently resting ($P=0.046$) but were observed overall more frequently sham dusting bathing ($P=0.044$), particularly in the midday period ($P=0.045$). Furthermore, hens in cages without access to a nest-box were also observed less frequently pecking inedible objects in the midday period ($P=0.037$).

There were no interactions between main effects ($P>0.05$) on hen behaviour.

Table 2-9 - Effect of size of rearing cage on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed

Variables			Rearing treatments (7-15 wk)		SED	P Value
			Small	Large		
Postures						
	Sitting		0.07	0.07	0.011	0.86
	Erect		0.93	0.92	0.011	0.87
Behaviours						
	Mobile		0.07	0.08	0.008	0.60
	Resting		0.32	0.33	0.022	0.80
	Preening		0.11	0.11	0.007	0.35
	Feed pecking		0.24	0.26	0.018	0.43
	Drinking		0.08	0.06	0.006	0.048
		Morning	0.07	0.05	0.017	0.28
		Midday	0.08	0.06	0.005	0.043
		Afternoon	0.10	0.08	0.003	0.0069
	Inedible pecking		0.12	0.11	0.011	0.51
	Cohort pecking		0.03	0.04	0.015	0.72
	Stretching		0.00	0.00	0.000	0.55
	Oviposition		0.00	0.00	0.000	0.34
	Sham dust bathing		0.01	0.01	0.003	0.98

Table 2-10 - Effect of size of production cage on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed

Variables			Production treatments (16-34 wk)		SED	P Value
			Small	Large		
Postures						
	Sitting		0.08	0.06	0.005	0.0082
		Morning	0.07	0.06	0.008	0.59
		Midday	0.13	0.10	0.007	0.0035
		Afternoon	0.04	0.02	0.010	0.11
	Erect		0.92	0.93	0.005	0.011
		Morning	0.93	0.93	0.009	0.64
		Midday	0.87	0.90	0.007	0.005
		Afternoon	0.96	0.98	0.010	0.11
	Squatting		0.00	0.00	0.000	0.802
Behaviours						
	Mobile		0.04	0.11	0.004	2.6 × 10 ⁻⁶
		Morning	0.04	0.11	0.015	0.0035
		Midday	0.03	0.10	0.010	0.00039
		Afternoon	0.04	0.13	0.005	5.1 × 10 ⁻⁶
	Resting		0.37	0.28	0.013	0.0005
		Morning	0.42	0.35	0.019	0.009
		Midday	0.38	0.28	0.016	0.00069
		Afternoon	0.30	0.21	0.017	0.0015
	Preening		0.10	0.12	0.003	0.00039
		Morning	0.10	0.14	0.015	0.064
		Midday	0.13	0.15	0.010	0.081
		Afternoon	0.06	0.07	0.011	0.91
	Feed pecking		0.28	0.22	0.010	0.0013
		Morning	0.28	0.22	0.014	0.0043
		Midday	0.24	0.21	0.011	0.020
		Afternoon	0.32	0.24	0.018	0.0043
	Drinking		0.07	0.07	0.004	0.78
		Morning	0.05	0.07	0.007	0.023
		Midday	0.07	0.06	0.011	0.43
		Afternoon	0.10	0.09	0.008	0.47
	Inedible pecking		0.09	0.14	0.015	0.021
		Morning	0.07	0.08	0.011	0.53
		Midday	0.08	0.13	0.017	0.021
		Afternoon	0.14	0.21	0.026	0.024
	Cohort pecking		0.03	0.04	0.008	0.19
	Stretching		0.00	0.00	0.001	0.13
	Oviposition		0.00	0.00	0.000	0.215
	Sham dust bathing		0.01	0.01	0.002	0.15

Table 2-11 - Effect of access to a nest box on time budgets of behaviour at 25 wk of age. Data presented as proportion of observation sessions in which the individual posture or behaviour was observed.

Variables			Access to nest-box (16-34 wk)		SED	P Value
			No	Yes		
Postures						
	Sitting		0.07	0.07	0.011	0.91
	Erect		0.92	0.93	0.009	0.93
Behaviours						
	Mobile		0.08	0.07	0.006	0.54
	Resting		0.31	0.33	0.008	0.046
		Morning	0.38	0.39	0.007	0.31
		Midday	0.32	0.34	0.014	0.18
		Afternoon	0.24	0.27	0.014	0.076
	Preening		0.11	0.11	0.006	0.24
	Feed pecking		0.26	0.25	0.016	0.60
	Drinking		0.07	0.08	0.004	0.39
	Inedible pecking		0.13	0.10	0.012	0.086
		Morning	0.08	0.07	0.011	0.44
		Midday	0.13	0.09	0.015	0.037
		Afternoon	0.19	0.16	0.020	0.31
	Cohort pecking		0.03	0.04	0.010	0.29
	Stretching		0.00	0.00	0.000	0.63
	Oviposition		0.00	0.00	0.000	0.40
	Sham dust bathing		0.02	0.01	0.003	0.044
		Morning	0.00	0.00	0.001	0.68
		Midday	0.04	0.02	0.006	0.045
		Afternoon	0.00	0.00	0.001	0.213

2.4.2.2 Oviposition behaviour

As shown in Tables 2-12, 2-13 and 2-14 there were no effects of cage size during rearing or during production ($P>0.05$) on oviposition behaviour. However, the absence of the nest-box during production affected the mean duration of sitting behaviour prior to oviposition, as hens without access to a nest-box displayed a shorter duration of sitting behaviour than hens with access to a nest-box ($P=0.023$). Furthermore, there was a tendency ($P=0.074$) for hens without access to a nest-box to display less consistency in their orientation during oviposition.

There was a significant interaction ($P=0.027$) between cage size during production and access to a nest-box on the coefficient of variation for time of oviposition: there was more variation in large cages with access to a nest-box access than large cages without a nest-box and small cages with access to a nest-box.

In cages in which a nest-box was available, a high percentage of eggs (80% of eggs) were laid in nest-boxes in week 26.

2.4.2.3 Choice behaviour in Y maze tests

There were no effects ($P>0.05$) of cage size during rearing or during production on the choice behaviour in Y maze tests for a nest-box over feed or for space over feed (Tables 2-12, 2-13 and 2-14). Furthermore, there were no effects ($P>0.05$) of cage size during rearing or during production on the latencies to choose the nest or space when these two resources were chosen.

In contrast, there was an effect ($P=0.0053$) of access to a nest-box during production on choice of a nest-box in the Y maze tests and a tendency ($P=0.077$) for an effect of access to a nest-box during production on the latency to choose a nest box in the Y maze tests.

There were several interactions between main effects. There was an interaction ($P=0.03$) between cage size during rearing and nest-box access on latency to choose a nest-box, with hens reared in small cages and without access to a nest-box in production taking the most time to choose a nest-box when it was chosen. There was also an interaction ($P=0.03$) between cage size during production and nest-box access on latency to choose the arm with 1648cm² of floor space, with hens housed in small cages during production but without access to a nest-box taking longer to choose a nest-box when it was chosen than hens housed in small cages during production but with access to a nest-box.

Table 2-12 - Effect of size of rearing cage on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively

Variable	Rearing Treatment (7-15 wk)		SED	P value
	Small	Large		
Oviposition behaviour				
Consistency of orientation (proportions)	0.79	0.80	0.041	0.92
Consistency of site (proportions)	0.81	0.81	0.036	0.96
Sitting bouts (/day)	38	38	1.1	0.45
Duration of sitting bouts (s/day)	10650	9920	1009	0.52
Mean duration of sitting bout (s)	307	274	27.3	0.32
Coefficient of variation for time of oviposition	3.49	3.04	0.321	0.25
Choice behaviour in Y maze*				
Nest-box rather than feed chosen (%)	18.8 (10.4)	15.9 (7.5)	3.77	0.49
Latency to choose the nest-box (s)	23.9	21.5	4.40	0.62
Space rather than feed chosen (%)	42.5 (6.0)	34.4 (31.9)	6.01	0.27
Latency to choose space (s)	21.9	18.8	5.21	0.59

*Y maze preferences were angularly transformed, prior to statistical analysis, after the scores were scaled between 0 and 1. Back transformed means presented in parentheses.

Table 2-13 - Effect of size of production cage on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively

Variable	Production Treatment (16-34 wk)		SED	P value
	Small	Large		
Oviposition behaviour				
Consistency of orientation (proportions)	0.81	0.78	0.032	0.38
Consistency of site (proportions)	0.85	0.77	0.051	0.16
Sitting bouts (/day)	38	38	2.9	0.98
Duration of sitting bouts (s/day)	9640	10920	869	0.19
Mean duration of sitting bout (s)	279	302	27.3	0.43
Coefficient of variation for time of oviposition	3.29	3.24	0.487	0.92
Choice behaviour in Y maze*				
Nest-box rather than feed chosen (%)	15.9 (7.5)	18.8 (10.4)	2.92	0.37
Latency to choose the nest-box (s)	22.2	23.2	2.78	0.74
Space rather than feed chosen (%)	32.3 (6.9)	44.6 (49.3)	6.93	0.13
Latency to choose space (s)	20.4	20.3	5.07	0.98

*Y maze preferences were angularly transformed, prior to statistical analysis, after the scores were scaled between 0 and 1. Back transformed means presented in parentheses.

Table 2-14 - Effect of access to a nest-box on oviposition behaviour in home cage and choice behaviour in Y maze tests at 28 and 32-34 wk of age, respectively

Variable	Nest-box Treatment (7-15 wk)		SED	P value
	No	Yes		
Oviposition behaviour				
Consistency of orientation (proportions)	0.73	0.86	0.062	0.074
Consistency of site (proportions)	0.76	0.87	0.070	0.15
Sitting bouts (/day)	37	39	5.70	0.68
Duration of sitting bouts (s/day)	9960	10600	755	0.43
Mean duration of sitting bout (s)	247	334	28.9	0.023
Coefficient of variation for time of oviposition	3.08	3.45	0.270	0.22
Choice behaviour in Y maze*				
Nest-box rather than feed chosen (%)	6.9 (1.4)	27.8 (21.8)	4.90	0.0053
Latency to choose the nest-box (s)	25.0	20.4	2.14	0.077
Space rather than feed chosen (%)	35.9	41.1 (43.2)	7.55	0.52
Latency to choose space (s)	22.8	17.9	3.03	0.16

*Y maze preferences were angularly transformed, prior to statistical analysis, after the scores were scaled between 0 and 1. Back transformed means presented in parentheses.

2.4.3 Productivity and other measures

2.4.3.1 Body weights and egg production

There were both rearing cage and production cage effects on body weight ($P=0.015$ and $P=0.013$, respectively) only at 19 wk of age (Tables 2-15, 2-16 and 2-17). However, there was an interaction between cage size during rearing and nest-box access during production on body weight at 19 wk (see below). There was an effect of rearing cage on egg weight 29 wk, but there was an interaction between cage size during rearing and nest-box access during production on egg weight at 30 wk (see below).

There were no effects of production cage or access to a nest-box on egg weight or hen day production.

There were a number of interactions. There was a significant ($P=0.008$) interaction between cage size during rearing and production on body weight: hens were heavier at 21 wk of age when reared and housed during production in large cages than when reared small cages and then housed during production in large cages. There were interactions between cage size during rearing and nest-box access during production. Hens were lighter at 34 wk when reared in small cages and housed during production without access to a nest-box ($P=0.035$), while hens were heavier at 19 wk when reared in large cages and housed during production with access to nest-boxes ($P=0.039$) and heavier at 30 wk when reared in large cages and

housed during production without access to nest-boxes ($P=0.071$). There were also interactions between cage size during production and nest-box access during production and between cage size during rearing, cage size during production and nest-box access. Hens were heavier at 30 wk when housed in small cages during production with access to a nest-box than when housed in small cages during production without access to a nest-box ($P=0.084$) and hens were lightest at 26 wk when reared in small cages, and then housed in small cages during production without access to a nest-box ($P=0.038$).

There was a tendency ($P=0.085$) for an interaction between cage size during production and access to a nest-box on hen day production: hens tended to have highest hen day production when housed in small cages with access to nest-boxes and when housed in large cages without access to nest-boxes.

2.4.3.2 Extra-cuticular calcium

There were no main effects or interactions between main effects ($P>0.05$) on extra-cuticular calcium.

2.4.3.3 Feather condition score

While there were no effects ($P>0.05$) of rearing cage on feather condition score (Table 2-15), as shown in Tables 2-16 and 2-17 there were effects ($P<0.05$) of both production cage and access to a nest-box on feather condition score. Feather condition score was worse ($P=0.011$) in small cages at 34 wk of age and there was a similar tendency ($P=0.091$) at 30 wk. Feather condition score was worse ($P=0.037$) in cages with access to a nest-box at 34 wk of age and there was also a similar tendency ($P=0.10$) at 30 wk.

Table 2-15- Effect of size of rearing cage on productivity and other measures

Variable	Rearing Treatment (7-15 wk)		SED	P value
	Small	Large		
Body Weight (kg)				
16 wk	1.53	1.52	0.011	0.85
18 wk	1.66	1.69	0.013	0.069
19 wk	1.76	1.79	0.006	0.015
21 wk	1.87	1.86	0.013	0.27
22 wk	1.88	1.89	0.016	0.42
26 wk	1.95	1.97	0.024	0.42
30 wk	2.02	2.01	0.028	0.92
34 wk	2.05	2.07	0.048	0.67
Egg Weight (g)				
26 wk	58.3	58.7	0.54	0.44
27 wk	59.5	59.3	0.32	0.66
28 wk	60.1	60.0	0.45	0.86
29 wk	60.7	59.8	0.20	0.028
Average Hen Day Production (%)	95.8	95.0	1.23	0.58
Extra-cuticular calcium				
26 wk	2.26	2.25	0.132	0.93
27 wk	2.07	2.20	0.141	0.42
28 wk	2.08	2.10	0.112	0.87
29 wk	1.99	1.96	0.108	0.79
Feather condition score*				
26 wk	84.7 (23.8)	82.7 (23.7)	1.26	0.21
30 wk	79.5 (23.4)	78.7 (23.3)	1.87	0.69
34 wk	77.5 (23.2)	77.0 (23.0)	0.71	0.61

* Feather condition scores were angularly transformed prior to statistical analysis. Back transformed means presented in parentheses.

Table 2-16 - Effect of size of production cage on productivity and other measures

Variable	Production Treatment (16-34 wk)		SED	P value
	Small	Large		
Body Weight (kg)				
16 wk	1.53	1.52	0.011	0.42
18 wk	1.68	1.66	0.013	0.16
19 wk	1.79	1.76	0.007	0.013
21 wk	1.87	1.86	0.008	0.23
22 wk	1.89	1.88	0.010	0.38
26 wk	1.96	1.96	0.014	0.89
30 wk	2.01	2.02	0.019	0.73
34 wk	2.06	2.07	0.028	0.63
Egg Weight (g)				
26 wk	58.3	58.7	0.31	0.18
27 wk	59.2	59.5	0.32	0.38
28 wk	59.8	60.2	0.41	0.37
29 wk	59.6	60.9	0.97	0.24
Average Hen Day Production (%)	95.0	95.7	0.61	0.30
Extra-cuticular calcium				
26 wk	2.13	2.37	0.169	0.21
27 wk	2.15	2.12	0.131	0.82
28 wk	2.03	2.14	0.173	0.55
29 wk	1.98	1.96	0.143	0.93
Feather condition score*				
26 wk	84.2 (23.8)	83.2 (23.7)	0.90	0.29
30 wk	78.1 (23.2)	80.2 (23.5)	1.03	0.091
34 wk	74.6 (22.7)	79.9 (23.4)	1.46	0.011

* Feather condition scores were angularly transformed prior to statistical analysis. Back transformed means presented in parentheses.

Table 2-17 - Effect of access to a nest-box on productivity and other measures

Variable	Nest-box Treatment (16-34 wk)		SED	P value
	No	Yes		
Body Weight (kg)				
16 wk	1.52	1.53	0.016	0.53
18 wk	1.68	1.67	0.015	0.84
19 wk	1.78	1.76	0.011	0.15
21 wk	1.86	1.86	0.015	0.87
22 wk	1.89	1.88	0.017	0.46
26 wk	1.95	1.97	0.018	0.31
30 wk	2.00	2.03	0.019	0.29
34 wk	2.05	2.07	0.020	0.25
Egg Weight (g)				
26 wk	58.8	58.2	0.51	0.25
27 wk	59.9	58.8	0.66	0.15
28 wk	60.2	59.8	0.47	0.42
29 wk	60.4	60.1	0.61	0.60
Average Hen Day Production (%)	95.7	95.1	0.80	0.50
Extra-cuticular calcium				
26 wk	2.27	2.23	0.230	0.85
27 wk	2.16	2.11	0.11	0.69
28 wk	2.13	2.05	0.087	0.40
29 wk	2.00	1.94	0.100	0.57
Feather condition score*				
26 wk	84.5 (23.8)	82.9 (23.7)	1.18	0.25
30 wk	80.3 (23.5)	77.9 (23.2)	1.23	0.10
34 wk	78.0 (23.2)	76.5 (23.0)	0.54	0.037

* Feather condition scores were angularly transformed prior to statistical analysis. Back transformed means presented in parentheses.

2.5 Discussion

The main objective of this experiment was to determine the effects of floor space during both rearing and adulthood and access to a nest-box during adulthood on the welfare of laying hens using two common approaches to animal welfare assessment, those indices that demonstrate the normality of the animal's biological functioning, including consequent fitness (i.e., how well an animal is coping with the challenges it faces) and those indices that demonstrate what resources are perceived by the animal to be important (i.e., animal preferences). The biological functioning indices used in this experiment consisted of measurement of the behavioural and physiological responses to the housing treatments as well as fitness variables such body weight, egg production and feather condition, while the animal preference indices consisted of measurements of motivation to access the resources of extra space and a nest-box.

There were no consistent effects of cage size during rearing, cage size during adulthood or access to a nest-box during adulthood on corticosterone concentrations in plasma faeces, egg albumen or egg yolk or on corticosterone response to ACTH challenge at 26-29 weeks of age. There are few reports in the literature on the effects of space on the stress physiology of laying hens. Mench et al. (1986) reported that reducing space allowance from

1394 to 697cm²/hen increased plasma corticosterone concentrations. In this previous experiment, birds were housed in two-bird cages and so caution is required in interpreting these effects since spatial requirements per bird may be greater in small groups because of less total space. Roush et al. (1984) and Mashaly et al. (1984) found that corticosterone concentrations in laying hens increased with reducing space allowance from 516 to 310cm²/hen by increasing group size in standard-sized cages from 3 to 5 hens. Care is also required in interpreting experiments in which stocking density is used to vary space allowance as opposed to pen size since space allowance may be confounded with group size in such designs. Koelkebeck et al. (1987) reported an 11% increase in plasma corticosterone concentrations in caged hens when space allowance was decreased from 460 to 350cm² per bird, although the increase was not statistically significant. Barnett et al. (2009) found that while there were no treatment effects on plasma corticosterone concentrations, hens in groups of 16 with a space allowance of 750cm²/hen had higher egg albumen corticosterone concentrations at 29-46 weeks of age than hens in groups of 8 or 16 with 1500cm²/hen. In contrast, hens in groups of 16 with 750cm²/hen had a lower plasma corticosterone response to ACTH at 29-46 weeks of age than hens in groups of eight with 1500cm²/hen. Mammals that are chronically stressed experience an endogenous down-regulation of the hypothalamic-pituitary-adrenal axis, with endogenous increases in corticosteroids operating on the pituitary (via negative feedback) to suppress ACTH (see Barnett et al., 2009). However in hens the literature on the responsiveness of the hypothalamic-pituitary-adrenal axis in chronically stressed birds is equivocal (Beuving and Vonder, 1986; Beuving et al., 1989).

There is little evidence in the literature that a lack of nest-box results in either an acute or a chronic stress response. In an experiment examining hens in cages with and without nest-boxes, Cronin et al. (2008) found that hens in cages with a nest-box had 33% higher plasma corticosterone concentrations than hens without nest-boxes early in lay at 23 weeks of age and suggested that the elevated stress response in cages with nest-boxes was probably associated with social factors, that is, competition for the nest-box. However, there were no longer term effects of treatment on corticosterone concentrations. Furthermore, when hens that were accustomed to laying in a nest-box were denied access to the nest-box at 39 wk, egg albumen corticosterone concentrations were not different from controls during the first 2 days, were significantly higher on day 3 but were similar again on day 7 (Cronin et al., 2008). In an experiment examining effects of perches, dust baths and nest-boxes, either alone or in combination as used in furnished cages, as well as group size and floor space, Barnett et al. (2009) concluded that any effects of nest-boxes on hen stress physiology and immunology were smaller than effects of group size and space allowance, although nest-boxes when present, were generally well-used.

In an experiment examining the effects of housing laying hens in standard cages (groups of three hens with 730cm²/hen) and furnished cages standard cages (groups of eight hens with 750 cm²/hen), Moe et al. (2010) found no effects of housing on plasma corticosterone concentrations or corticosterone response to an ACTH challenge at 50 weeks of age. However, heterophil to lymphocyte ratios were affected with hens in furnished cages having higher ratios at 62 weeks of age.

A range of stressors has been shown to cause delays in the expected time of oviposition with consequent effects on egg shell quality (Reynard and Savory 1997). Delayed oviposition is generally due to retention of the egg in the shell gland (uterus) which is caused by the release of adrenaline and thus a change in egg shell colour or quality can be used as an indirect measure of delayed oviposition because additional time in the uterus after the cuticle on the egg has been laid down can result in a deposit of extra-cuticular calcium. In the present experiment there were no effects of cage size during rearing, cage size during adulthood or access to a nest-box during adulthood on extra-cuticular calcium. Yue and

Duncan (2003) found no difference in extra-cuticular calcium on shells from hens from cages with or without nest-boxes, or from hens blocked from using their usual nest-box.

It is interesting that in the experiment by Barnett et al. (2009) that, while there were effects of group size and floor space on egg albumen corticosterone concentrations and plasma corticosterone response to ACTH at 29-46 weeks of age, there were no housing effects at 59-66 weeks of age. Recently, Hemsworth et al. (2013) found that the effect of space on stress in group-housed sows was most pronounced early after grouping and the authors suggested sows in groups may adapt over time to reduced space. Increasing density (increasing number of animals per unit of space and thus reduced floor space allowance) increased plasma corticosterone concentrations in male mice at days 1 and 7, but not at day 14, after grouping (Peng et al., 1989). While, there is little evidence in the literature of animals adapting to spatial restriction in groups, measurements on both behavioural and physiological responses early in the adult treatment period of the present experiment would have been valuable in identifying temporal treatment effects.

There is relatively little known of the effects of stressors on the immune system of laying hens (Thaxton, 2004). No treatment effects on the heterophil to lymphocyte ratio were evident in the present experiment. Barnett et al. (2009) found no differences in white cell counts and the differential white cell counts (ratio of granulocytes to lymphocytes plus monocytes) in hens housed in groups of 16 with 750cm²/hen or groups of 8 or 16 with 1500 cm²/hen. As reported earlier, In an experiment examining the effects of housing laying hens in standard cages (groups of three hens with 730cm²/hen) and furnished cages standard cages (groups of eight hens with 750cm²/hen), Moe et al. (2010) found that heterophil to lymphocyte ratios at 62 weeks of age were higher in hens reared in floor pens and then housed in furnished cages than those reared and housed in standard cages. The authors suggested immune response may have been associated with pathogenic load due to environmental complexity in the rearing environment and furnished cages rather than stress due to housing system *per se*.

Table 2-18 - Summary of the significant effects of floor space during adulthood on the postures and behaviours of laying hens

Variables		Change in frequency in small space allowances (%)
Postures		
	Sitting	33% increase
	Erect	1 % reduction
Behaviours		
	Mobile	64% reduction
	Resting	42% increase
	Preening	17% reduction
	Feed pecking	27% increase
	Drinking (morning period)	29% reduction
	Inedible pecking	36% reduction

While there were no consistent effects of cage size during rearing, cage size during adulthood or access to a nest-box during adulthood on corticosterone concentrations in plasma faeces, egg albumen or egg yolk or on corticosterone response to ACTH challenge, there were numerous effects of floor space allowance during adulthood on the time budget of behaviour of hens at 25 weeks of age. As shown in Table 2-18 for those postures and behaviours in which there were significant space effects, housing adult hens with a floor

space allowance of 542cm²/bird rather than 1650cm²/bird resulted in marked reductions in the proportion of observations in which hens displayed the behaviours of mobile (64%), inedible pecking (36%), drinking (29%) and preening (17%). However, housing hens with a floor space allowance of 542cm²/bird resulted in marked increases in the proportion of observations in which hens displayed the behaviours of resting (42%) and feed pecking (27%) and the posture of sitting (33%). A reduction in floor space and thus increased opportunity for physical contact with other birds clearly may restrict locomotion, as well as comfort behaviours, such as preening, and investigation, such as pecking cage features. With a reduction in locomotion, an increase in sitting and resting are expected.

Hens with reduced floor space in adulthood were observed more frequently pecking feed. Displacement behaviours are often defined as usually arising when there is motivational conflict or frustration, and is a normal behaviour that is performed in a different and apparently inappropriate situation (Taylor, 2010). Excessive feeding (Meijsser and Hughes, 1989) and preening (Duncan and Wood-Gush, 1972) have been suggested as displacement behaviours exhibited for example in the absence of a suitable nest site. However, there is no evidence in the current experiment that reduced floor space in adulthood was associated with increased stress. The increase in sham dust bathing during the midday period observed in the absence of a nest-box during adulthood is also difficult to explain. While dust bathing occurs more frequently at midday (Hogan, 2008), it is unclear how the absence of access to a nest-box would lead to an increase in sham dust bathing unless there was less social facilitation of the behaviour when hens were in the nest-box during the mid-day period (Duncan et al., 1998).

Keeling (1994) examined the effects of floor space allowances of 5630, 3000, 1200 and 600cm²/hen of hens in groups of four on litter and found that as space decreased, frequency of walking and ground pecking decreased, frequency of standing increased, but frequency of preening remained unchanged. While aviary systems vary markedly from cage systems, Hansen (1994) examined the time budgets of hens in aviaries (2953cm² of ground area/hen) and conventional cages (720cm² of ground area/hen). In comparison to hens in aviaries, the authors found that those in cages spent less time lying, walking, and object pecking, but more time standing/sitting, food pecking, and drinking at 25 to 26, 40 to 41 and 60 to 61 weeks of age. No differences were found between the two housing systems in comfort behaviours such as preening, scratching, stretching and beak cleaning. Birds in cages performed less wing flapping and fleeing but there was no difference between the two housing systems in the frequency of feather pecking. Reduced floor space in the present experiment also reduced time mobile and pecking inedible objects and increased time sitting and food pecking. While many of the effects of reduced space were similar, in contrast to the observations of Keeling (1994) and Hansen et al. (1994), hens in the present experiment with reduced space spent more time drinking and less time preening but also did not display increased cohort pecking, which included feather and body pecking.

In relation to cohort pecking, as discussed later, while feather condition score in the present experiment was generally good to very good, hens with reduced space had poorer feather condition scores at 34 weeks of age. Nicol et al. (2006) found that mortality was lower but feather loss was worse in small commercial flocks (2450-3150 birds per flock) stocked at 12 birds/m² (833cm²/bird) compared to those stocked at either 7 birds/m² (1428cm²/bird) or 9 birds/m² (1111cm²/bird) in single-tiered aviaries. While feather pecking in the present experiment was low overall and feather pecking behaviour is multifactorial (see recent reviews by Rodenburg and Koene, 2004; Sedlackova et al., 2004; van Krimpen et al., 2005; Rodenburg et al., 2008), further research on the effects of floor space allowance on feather pecking is required, particularly in those situations in which feather pecking is high such as where there is a lack of foraging material, increased light intensity, genetic predisposition, poor availability and design of perches and feeding troughs, large groups, etc.

In relation to preening, this behaviour decreased with reduced floor space, contrary to the observations of Keeling (1994) and Hansen et al. (1994). There is no obvious explanation for this contrary finding. As discussed earlier, displacement behaviour usually arises when there is motivational conflict or frustration and Duncan and Wood-Gush (1972) has been suggested that preening in hens may be a displacement behaviour exhibited for example when thwarted in accessing feed. Keeling (1994) considered that preening is a resilient behaviour, one that will still be performed when the cost is high, since there was no decrease in frequency as the pen size decreased.

The literature on space requirements suggests that hens have a requirement for physical space to stretch and exercise muscles (Fraser and Broom, 1997) and may prefer to distance themselves from other birds (Savory et al., 2006). Furthermore, many species are motivated to separate several important functions, for example nesting away from the feeding area. Space may also be needed for body care or grooming and assisting in thermoregulation, such as when hot. Thus, in addition to spatial requirements for physical size and basic movement, birds also have requirements for space to perform a range of behaviours that are likely to affect their welfare. A behaviour that many authors have proposed that is important to hen welfare is nesting in an enclosed nest site away from the feeding area. Using preference and behavioural demand tests, research has shown that most hens prefer and are highly motivated to access an enclosed nest site. Hens have been shown to be willing to squeeze through narrow gaps (Cooper and Appleby, 1997), push open weighted doors (Follensbee et al., 1992), and pass through cages occupied by unfamiliar or dominant hens in order to gain access to a nest-box (Freire et al., 1997), tasks considered costly or aversive to hens. The present experiment indicated that hens that had access to a nest-box in adulthood more often chose a nest-box during their expected pre-laying period rather than feed in the Y maze tests than hens that were denied access to a nest-box in adulthood (22% of tests vs 1%). Furthermore, hens reared with less space and without access to a nest-box in adulthood were slowest to choose a nest-box when it was chosen and hens with less space during adulthood but without access to a nest-box were slower to choose a nest-box when it was chosen than hens with less space in adulthood but with access to a nest-box.

It should be recognized that procedural differences in tests of preference and motivation can influence the results and therefore the interpretation of these tests (Kirkden and Pajor, 2006), but the influence of specific methodologies has rarely been investigated (Browne et al., 2011). When comparing hens' preferences for three environments in pairs of tests using either a discrete-choice method (T-maze where a hen's choice followed a short period of confinement with that choice) or a free-access method (where hens could freely move between choices), Browne et al. (2011) found that the discrete-choice method was more sensitive for detecting a mild aversion to one of the environments and had greater transitivity (more consistency) in the preference rankings for the three environments.

Furthermore, the choices of individual hens were associated with lower corticosterone concentrations when hens were exposed to these environments during a training period (Nicol et al., 2011). In our study, a discrete choice test was used based on some previous studies (Laine, 2011; Arnold and Hemsworth, 2013), but if and how the results might have differed if we had used a free-access method is unknown. Using the discrete-choice methodology, we were able to detect differences among individual hens in preferences for nest-boxes. It is possible that one specific type of test methodology may be more or less appropriate for a given type of stimulus (e.g. space versus nest-box). Another consideration in interpreting these results is that the handling and novelty of the testing procedure and inaccurate predictions of the timing of oviposition may have affected the hens' motivation to access the nest-box in the present Y maze tests and thus may have underestimated motivation. However, hens were handled considerably in the training phases and consequently were reasonably familiar with both the Y maze apparatus and handling. Food

is generally considered as the “gold standard” in preference testing (Matthews and Ladewig, 1994) and A. Lam and P.H.

Hemsworth (unpublished data) using a similar Y maze apparatus found that choice of feed over peat moss was high (78%) and that level of feed deprivation (0, 2, 3, 4 and 5 h) had no effect on choice of feed. Thus, the present findings suggest that hens with experience with nest-boxes are at least moderately motivated to choose a nest-box over feed around the time of oviposition (nest-box chosen in 22% of tests). Hens without experience with nest-boxes from 16-29 weeks of age chose a nest-box infrequently over food (1% of tests).

The behaviour of the hen around oviposition in the present experiment was also affected by access to a nest-box in adulthood: the absence of the nest-box reduced the mean duration of sitting behaviour prior to oviposition and tended to reduce the consistency of orientation during oviposition. These observations suggest that hens with access to a nest-box were more consistent in their orientation during oviposition and had longer sitting bouts prior to oviposition than hens without access to a nest-box. This is in agreement with the observations that hens in furnished cages exhibit more settled pre-laying behaviour (Appleby et al., 2002). Higher consistency in orientation during oviposition of hens with access to a nest-box may be explained by a combination of a higher motivation to lay their egg in a nest-box together with more protection from interference from other birds in a nest-box. It is unclear why there would be more variation in orientation during oviposition in large cages with access to a nest-box than in large cages without access to a nest-box (as well as small cages with access to a nest-box).

The literature on the effects of space allowance in layer cages shows that in general as floor space decreases, within a range of 300 to 650cm² per caged laying hen, mortality increases, egg production and body weight decrease and efficiency of feed conversion decreases (see Hill, 1977; Hughes, 1983; Adams and Craig, 1985; Sohail et al., 2004). In the present experiment, there were few main effects on body weight or egg production. The main effect of space during adulthood and the interaction between space during rearing and nest-box access on body weight at 19 weeks seems inconsistent with effects at other ages and thus may be chance effects.

There were no effects of space during rearing on feather condition score, but both space during adulthood and access to a nest-box in adulthood affected feather condition. It should be recognized that feather condition scores in all treatments were on average in the good-very good category but nevertheless, feather condition score was worse with a reduction in floor space at 34 weeks of age and there was a similar tendency ($P=0.091$) at 30 weeks. Feather condition score was also worse in cages with access to a nest-box at 34 weeks of age and there was also a similar tendency ($P=0.10$) at 30 weeks. It should also be noted that feather condition is likely to decrease over time with treatment effects and increasing age. While feather pecking was low at 16 wk and there were no effects of space during rearing or in adulthood, or access to a nest-box on cohort pecking at 25 wk of age, the effects of both space during adulthood and access to a nest-box on feather condition score may have been through feather pecking. However, poor feather condition with reduced space may be a result of increased abrasion with cage fittings (Hughes and Black, 1976) and the deterioration in feather condition with access to a nest-box may be due to abrasion with the nest-box associated with access the box, particularly during periods of high use.

In the present experiment birds had 200cm²/bird of nest space. This may be less than the optimal nest space of 300cm²/hen suggested by Appleby (2004) using a theoretical model of nest area requirements but is similar or greater than the nest space allowance in many studies on furnished cages. For example, both Wall and Tauson (2002) and Wall (2011) reported very high degree of use of the nest-box (>95%) when birds were provided 150cm²/bird of nest space in furnished cages similar to the models used in our study but housing 8 or 10 hens per cage. Hens that had access to a nest-box in the present

experiment generally laid their eggs in the nest-box. In contrast, Barnett et al. (2009) found no effects of the presence of a nest-box and no effects of group size and space allowance (groups of 16 with 750cm²/hen and groups of 8 or 16 with 1500cm²/hen) on feather damage and cover at 29 to 36 and 59 to 66 weeks.

Thus in conclusion, in addition to spatial requirements for physical size and basic movement, hens also have requirements for space to perform a range of behaviours that are likely to affect their welfare. The results of the present experiment show no consistent effects of floor space during rearing or adulthood on corticosterone concentrations in plasma faeces, egg albumen or egg yolk, corticosterone response to ACTH challenge, extra-cuticular calcium, heterophil to lymphocyte ratios and preference for space in Y maze tests. Furthermore, while floor space affected body weight and feather condition in adulthood, there were no effects of space during rearing or adulthood on egg production. The effects on body weight and feather condition may be due to reduced floor space affecting feed intake, rather than social stress effects, and abrasion with pen fittings, respectively. Overall, these results indicate that, while reduced floor space, particularly during adulthood, imposed considerable behavioural restrictions, these effects were not sufficient to elicit a prolonged stress response and, in turn, adversely effects on body weight and egg production.

Responses to stress are integral to the ability of an animal to cope and, in turn, the welfare of the animal. A stress response commences once the central nervous system firstly perceives a potential challenge to homeostasis and secondly develops a biological response or defence that consists of some combination of the four general biological defence responses: behavioural responses, responses of the autonomic nervous system, responses of neuroendocrine systems and responses of the immune system (Moberg, 2000; Barnett, 2003). For many stressors, the first and, at times, the most biologically economical and effective response is a behavioural one. Thus behavioural responses to spatial restriction in the birds in the present experiment may have been sufficient to allow adaptation, without the need to resort to physiological responses and consequent fitness costs. There are some limited examples in the literature of animals adapting to spatial restriction (e.g., Peng et al., 1989), but further research is required to determine whether the lack of effects of floor space are due to adaptation. There is also some evidence that rearing experience may affect an animal's motivation for space later in life. For example, Nicol (1986) found that when given free choice among different sized cages, individual hens reared in floor pens spent more time in larger enclosures than in smaller ones, although the hens regularly visited and spent short periods of time in the small cages. Using an operant method, Faure (1991) found that hens that had been housed in cages for some time worked less to enlarge their cages than hens reared in floor pens with more space. However, in the present experiment space during rearing had little or no effects on stress physiology, behaviour in situ and preference for additional space in adulthood.

Similarly, hens also have requirements for other resources to perform behaviours that may affect their welfare. Hens that had access to a nest-box in the present experiment generally laid their eggs in the nest-box. A suitable nest site is a resource that hens are highly motivated to access in preference and behaviour demand tests (Follensbee et al., 1992; Cooper and Appleby, 1997; Freire et al., 1997). In the present experiment, there was no evidence based on corticosterone concentrations, extra-cuticular calcium or heterophil to lymphocyte ratios that depriving hens of access to a nest-box during adulthood was stressful. While hens with experience with nest-boxes were at least moderately motivated to choose a nest-box over feed around the time of oviposition, hens with no previous experience of laying their eggs in a nest-box rarely chose a nest-box over feed, indicating a low preference to access a nest-box. This is in contrast to Cooper and Appleby (1995) who found no effect of previous experience with nest-boxes on the hens' willingness to access a pen with a nest or their nesting behaviour. Apart from reductions in the duration of sitting bouts prior to oviposition and resting, hens without access to a nest-box in the present

experiment showed a similar time budget of behaviour to that of hens with access to a nest-box.

In considering the welfare implications of behaviours that are prevented, or can only be released by very inadequate stimuli, as occurs with dust bathing and nesting behaviour, Beilharz and Zeeb (1981) made the point that if a hen in a cage never sees sand or anything like it, she may only rarely be sufficiently motivated to “want” to sand-bathe. Since an animal’s measured motivation to interact with additional resources are enhanced by stimuli from those resources (Widowski and Duncan, 2000), animals may not suffer when test resources are not available, even if the animals are motivated to work for them when available (European Commission, 2001).

This notion that 'out of sight' is also 'out of mind' is supported by a comparison of two studies on farm mink. Mason et al. (2001) studied the effects of depriving farm mink access to a water pool. Mink were highly motivated to access a pool and, based on cortisol concentrations, denying mink access to the pool increased stress to similar concentrations as caused by food deprivation. The authors concluded that caging mink on fur farms causes them frustration, mainly because they are prevented from swimming. Hansen and Jeppesen (2001) studied farm mink raised with or without access to water suitable for swimming and found no differences between treatments in the frequency of stereotypies. In contrast to the conclusion of Mason et al. (2001), the authors concluded that whether swimming constitutes a behavioural need in farm mink and whether for that reason their welfare should be impaired without water for swimming is still debatable. The European Commission report (European Commission, 2001) recommended that comparisons of the biological responses of animals housed with or without the resource under question are essential complements to motivational studies. The results of this present experiment support the notion 'out of sight' is also 'out of mind' at least in respect to the resource of a nest-box for hens.

A secondary objective of this experiment was to examine the validity of the two common approaches to animal welfare assessment used in this experiment, that is, those indices that demonstrate the normality of the animal’s biological functioning and fitness (i.e., how well an animal is coping with the challenges it faces) and those indices that demonstrate what resources are perceived to be important to an animal (i.e., animal preferences). Some authors (Barnett and Hemsworth, 2009) have proposed that challenges or situations in which an animal has to resort to the extreme coping attempts (i.e., challenges that may overwhelm an animal’s capacity to adapt) are associated with, or lead to, negative affective states. That is, that challenges or situations in which an animal has to resort to extreme coping attempts (i.e., challenges that may overwhelm an animal’s capacity to adapt) are associated with, or lead to, negative affective states. In relation to the findings in the present experiment on the effects of space and nest-box access in adulthood, these two approaches show that reducing space and denying access to a nest-box in adulthood do not markedly disruption biological function and do not result in a high motivation to access either resource. While neither restriction seriously affected biological functioning, there was also no convincing evidence that the restrictions increased the motivation of the birds to access when given the opportunity. This consistency of these two sets of findings adds to those that support this conceptual convergence (Nicol et al., 2009; Stevens et al., 2009; Arnold and Matthews, 2010).

Overall, the results of this experiment suggest that while reduced floor space, particularly during adulthood, imposed considerable behavioural restrictions, these effects were not sufficient to elicit a sustained stress response with adverse consequences on body weight and egg production. There are some limited examples in the literature of animals adapting to spatial restriction. Animals are equipped with a number of neural, physiological and behavioural responses that are utilised in a coordinated fashion to enable it to cope with acute and chronic challenges. Behavioural responses are one of the primary means that

animals have of responding to external challenges, particularly acute challenges. Behavioural responses are often highly sensitive and can be seen in response to those less severe challenges that may not provoke physiological responses (Coe et al., 2000). However, behavioural responses may assist animals to cope in more severe and longer lasting challenges. For example there is some evidence that stereotypies may be an adaptive mechanism in response to frustration, when activities are prevented or do not lead to the expected outcomes (Odberg, 1989; Cooper and Nicol, 1991; Mason and Latham, 2004).

In relation to nest-boxes, motivation for nesting has been studied extensively and a number of studies using preference and behavioural demand tests consistently show that most hens prefer and are highly motivated to access an enclosed nest site. While individual and strain differences in nesting motivation and nest-box use are not well understood, there is no evidence from the present experiment that naïve hens experience chronic stress, with adverse consequences on body weight and egg production, or are highly motivated to access a nest-box when deprived of a nest-box.

2.6 Conclusions and recommendations

2.6.1 Assessing the implications of space and nest-box access on hen welfare

The two common approaches to animal welfare assessment that were used in this experiment were those indices that demonstrate the normality of the animal's biological functioning and fitness (i.e. how well an animal is coping with the challenges it faces) and those indices that demonstrate what resources are perceived to be important to an animal (i.e. animal preferences).

As recognized by Sandoe et al. (2004), scientific uncertainty operates at several levels in the scientific investigation of an animal's welfare. In addition to scientists using different concepts or views on animal welfare and consequently using different criteria or methodology in assessing an animal's welfare, there is often uncertainty surrounding the relative importance of different variables to animal welfare. Furthermore, there are no clearly defined thresholds indicative of acceptable or unacceptable welfare in the measured response. Scientific uncertainty is presently unavoidable and thus it is essential that when scientists give scientific advice, the assumptions on which their advice is based is clear.

2.6.1.1 Biological functioning

There were some marked effects of reduced floor space allowance during adulthood on the time budget of behaviour of hens at 25 weeks of age. Housing hens with a space allowance of 542cm²/bird rather than 1650cm²/bird resulted in marked reductions in the proportion of observations in which hens displayed the behaviours of mobile (64%), inedible pecking (36%), drinking (29%) and preening (17%) but marked increases in the proportion of observations in which hens displayed the behaviours of resting (42%) and feed pecking (27%) and the posture of sitting (33%).

However, there were no consistent effects of cage size during rearing or during adulthood on the measures of stress utilised in this experiment (i.e. corticosterone concentrations in plasma faeces, egg albumen or egg yolk or on corticosterone response to ACTH challenge at 26-29 weeks of age). Furthermore, stress is known to affect the immune system and there was no evidence of spatial effects on haematology in this experiment (i.e. the heterophil to lymphocyte ratio).

There were few effects of access to a nest-box during adulthood on the time budget of behaviour of hens. Hens with access to a nest-box were observed less often resting and sham dust bathing. A range of environmental and psychological stressors has been shown to cause delays in the expected time of oviposition with consequent effects on egg shell quality. The absence of a nest-box during adulthood tended to reduce the consistency of the hen's orientation during oviposition and reduced the duration of sitting bouts prior to oviposition, characteristics of oviposition behaviour that may be disrupted by stressors. However, there were no effects of access to a nest-box on extra-cuticular calcium, as well as no consistent effects of access to a nest-box on corticosterone concentrations or heterophil to lymphocyte ratios.

There were no consistent main effects or interactions between main effects on body weight or egg production. The main effect of space during adulthood and the interaction between space during rearing and nest-box access on body weight at 19 weeks seems inconsistent with effects at other ages and thus may be an anomaly.

Both space during adulthood and access to a nest-box in adulthood affected feather condition. Poor feather condition with reduced space and access to a nest-box may be a result of increased abrasion with cage fittings.

The basis of the biological functioning approach to assess hen welfare in this experiment was that difficult or inadequate adaptation will generate welfare problems for animals (Broom and Johnson, 1993; Barnett and Hemsworth, 2009). How well an animal is coping with the challenges it faces will be reflected in the normality of its biological functioning, and severe risks to welfare will be associated with the most extreme coping attempts (Moberg, 2000; Barnett, 2003). Thus, a broad examination of the behavioural, physiological, health and fitness responses of hens in response to the treatments was undertaken. The magnitude and duration of the elicited biological responses to a stressor and their consequent costs to biological fitness are important considerations in appreciating firstly, the magnitude of the challenge for the animal and secondly, the welfare risks to the animal (Broom and Johnson, 1993; Mellor and Stafford, 2000; Barnett and Hemsworth, 2009).

These results suggest that while reduced floor space, particularly during adulthood, imposed considerable behavioural restrictions and lack of access to a nest-box in adulthood denied hens access to a resource that they are at least moderately motivated to access when experienced, there was no evidence that reduced space and a lack of a nest-box elicited a sustained stress response with adverse effects on body weight and egg production. There are some limited examples in the literature of animals adapting to spatial restriction in groups.

2.6.1.2 Animal preferences

While hens with reduced floor space in adulthood did not show increased preference to choose additional space over feed in the Y maze tests, hens provided with access to a nest-box in their home cage chose a nest-box over feed in more Y maze tests than hens with no previous experience of laying their eggs in a nest-box. These results suggest that experience is important in the hen's preference for nest-boxes. Since food is generally considered the "gold standard" in preference testing, the present findings suggest that hens with experience with nest-boxes are at least moderately motivated to choose a nest-box over feed around the time of oviposition. Hens without experience in adulthood with a nest-box rarely chose a nest-box over feed.

2.6.1.3 Utilizing these two concepts of animal welfare assessment

Although science can provide the factual basis of the impact of a husbandry or housing practice on the biology of the animal, there is some uncertainty amongst scientists on the concept of animal welfare. For many scientists, animal welfare has been assessed on the basis of how well the animal is performing from a biological functioning perspective. For others, animal welfare concerns affective states, such as pain and other negative feelings or emotions that may represent different component of suffering. Some have criticised the biological functioning concept of animal welfare on the basis that it does not adequately include emotions, but this is unlikely since the mental state of an animal is an integral component of its biological state. While it seems likely that animals will avoid aversive stimulation and choose positive stimulation, preference and motivation testing have generated considerable debate relating to conceptual and methodological difficulties. However, as discussed earlier, any argument for impaired welfare due to restriction of a resource or behaviour would be strengthened by both evidence that animals are highly motivated to access or perform the behaviour, respectively, and evidence of disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor health.

While a floor space allowance of 542cm²/bird during adulthood resulted in marked behavioural restrictions, there was no evidence based on corticosterone concentrations and heterophil to lymphocyte ratios, that this space allowance was stressful, or at least stressful enough to activate the hypothalamic-pituitary-adrenal axis. Furthermore, there was no evidence that hens with this reduced space in adulthood preferred additional space over feed based on choice behaviour in Y maze tests.

There was also no evidence based on corticosterone concentrations and heterophil to lymphocyte ratios that depriving hens of access to a nest-box during adulthood was stressful. However, hens with experience with nest-boxes were at least moderately motivated to choose a nest-box over feed around the time of oviposition. These results indicate that the welfare of the majority of hens is not seriously compromised when access to a nest-box is not available in adulthood, even if experienced hens are motivated to choose them over feed.

2.6.2 Conclusions

In addition to spatial requirements for physical size and basic movement, hens also have requirements for space to perform a range of behaviours that are likely to affect their welfare. The results of the present experiment show no consistent effects of floor space during rearing (two levels, 315 and 945cm²/bird) or adulthood (two levels, 542 and 1648cm²/bird) on measures of stress, the preference of hens for space in Y maze tests and egg production. Reduced floor space in adulthood also reduced feather condition, possibly through abrasion with pen fittings. Thus these results indicate that, while reduced floor space during adulthood imposed considerable behavioural restrictions, these effects were not sufficient to elicit a sustained stress response, with adverse effects on body weight and egg production. There are some limited examples in the literature of animals adapting to spatial restriction, but further research is required to determine if there were adverse effects soon after exposure to reduced floor space in adulthood which subsequently disappeared due to adaptation.

Hens also have requirements for other resources to perform behaviours that may affect their welfare. In the present experiment, there was no evidence that depriving hens of access to a nest-box during adulthood was stressful. Hens with experience with nest-boxes were at least moderately motivated to access a nest-box relative to feed in Y maze tests, but hens

denied access to a nest-box during adulthood rarely choose a nest-box in Y maze preference tests. Apart from some disruption to laying behaviour, hens without access to a nest-box in adulthood showed a similar time budget of behaviour to that of hens with access to a nest-box. Therefore, these results indicate that while hens with access to a nest-box during adulthood are at least moderately motivated to access a nest-box, depriving hens of the opportunity to access a nest-box during adulthood results in low motivation to access a nest-box when given the opportunity and does not elicit a sustained stress response.

Based on the normality of biological functioning and preferences, the present experiment provides no convincing evidence that the lower of the two space allowances provided during either rearing (315cm²/bird) or adulthood (542cm²/bird) or a lack of access to a nest-box in adulthood resulted in suffering in laying hens.

2.6.3 Recommendations

1. There were no effects of space and nest-boxes on hens late in the experimental period, but physiological and behavioural measurements early in adulthood were not conducted. There is some limited evidence for poultry, pigs and mice in the literature that animals may adapt to spatial restriction. Video records and egg samples from the present experiment are available for analyses and thus it would be useful to conduct these behavioural and physiological measurements early in adult treatment period.
2. The notion that 'out of sight' is also 'out of mind' is worthy of further discussion and investigation. As conducted in the present experiment and as concluded by the European Commission report (European Commission, 2001), comparisons of the biological responses of animals of housed with or without the resource under question are essential complements to motivational studies. The results of this present experiment support the notion 'out of sight' is also 'out of mind' at least in respect to the resource of a nest-box for hens.
3. Although our society continues to struggle to identify and agree on minimum welfare standards for its domestic animals, there is a clear priority to avoid animal suffering. This mandate to avoid suffering is prescribed in the prevention of cruelty legislation in many Western countries which specifically refers to cruelty in terms of "unreasonable pain or suffering" (e.g. Victoria, Australia (Anonymous, 2007)) or "unnecessary suffering" (the United Kingdom (Anonymous, 1911)). It should also be recognised though that the legislation in many of these countries refers to its purpose as not only "to prevent cruelty to animals" but also "to encourage the considerate treatment of animals" (e.g. Victoria, Australia (Anonymous, 2007)). Indeed there is an emerging shift in community values towards not merely minimising suffering in domesticated animals, but also enhancing pleasure in these animals (Tannenbaum, 2001). For many a consideration of animal welfare includes not only the avoidance of suffering, but also the opportunity for positive emotional experiences (Duncan, 2004).

The present results show that reduced space in adulthood results in considerable behavioural restriction and a reduction in feather condition and that eliminating the opportunity for hens to access a nest site in adulthood eliminates a behaviour that hens are motivated to perform. The present results provide no convincing evidence that reduced space and lack of a nest-box causes suffering based on normality of biological functioning and preferences for space and nest-boxes. Nevertheless, government, industry and Non-Government Organisation (NGO) policy makers in developing animal welfare standards and recommendations should consider the implications of the providing commercial laying hens with both increased space to allow more behavioural freedom and nest-boxes which presumably provide experienced hens with positive emotional experiences based on their motivation to access them in preference tests.

3 References

Adams, A. W. and Craig, J. V. (1985). Effect of crowding and cage shape on productivity and profitability of caged layers: A survey. *Poultry Science* 64, 238-242.

Albentosa, M. J., Cooper, J.J., Luddem, T., Redgate, S.E., Elson, H.A. and Walker, A.W. (2007). Evaluation of the effects of cage height and stocking density on the behaviour of laying hens in furnished cages. *British Poultry Science* 1, 1-11.

Anonymous (1911). Protection of Animals Act, 1911. Government of the United Kingdom.

Anonymous (2007). Prevention of Cruelty to Animals Act, 1986. Government of Victoria, Australia.

Appleby, M.C. (2004). What causes crowding? Effects of space, facilities and group size on behaviour, with particular reference to furnished cages for hens. *Animal Welfare* 13: 313-320.

Appleby, M.C., Walker, A.W., Nicol, C.J., Lindberg, A.C., Freire, R., Hughes, B.O. and Elson, H.A. (2002). Development of furnished cages for laying hens. *British Poultry Science* 43, 489-500.

Appleby, M. C., Mench, J.A. and Hughes, B.O. (2004). *Poultry Behaviour and Welfare*. CAB International Publishing, Wallingford Oxfordshire UK.

Arnold, N.A. and Matthews, L.R. (2010). Measuring what animals 'really want': implications for welfare on dairy farms. Proceedings of the 4th Australasian Dairy Science Symposium (Lincoln University, Christchurch, New Zealand, August-September, 2010), 444-447.

Arnold, N.A. and Hemsworth, P.H. (2013). Examining the usefulness of a Y maze choice method to measure the preferences of laying hens. *Animal Production Science* (in press).

Barnett, J.L. (2003). Studying stress to assess animal welfare. In: Paterson, J.E. (ed.) *Manipulating Pig Production IX*. Australasian Pig Science Association, Werribee, Australia, pp. 107-114 and 116-120.

Barnett, J.L and Hemsworth, P.H. (2003). Science and its application in assessing the welfare of laying hens. *Australian Veterinary Journal* 81, 615-624.

Barnett, J.L and Hemsworth, P.H. (2009). Welfare monitoring schemes: using research to safeguard the welfare of animals on the farm. *Journal of Applied Animal Welfare Science* 12, 114-131.

Barnett, J.L., Hemsworth, P.H., D. P. Hennessy, McCallum, T.M. and Newman, E.A. (1994). The effects of modifying the amount of human contact on the behavioural, physiological and production responses of laying hens. *Applied Animal Behaviour Science* 41, 87-100.

Barnett, J.L., Tauson, R., Downing, J.A, Janardhana, V., Lowenthal, J.W., Butler, K.L. and Cronin, G.M. (2009). The effects of a perch, dust bath and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens. *Poultry Science* 88, 456-470.

Beilharz, R.G. and Zeeb, K. (1981). Applied ethology and animal welfare. *Applied Animal Ethology* 7, 3-10.

Beuving, G. and Vonder, G. M. A. (1986). Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and plasma baseline levels of corticosterone. *General and Comparative Endocrinology* 62, 353–358.

Beuving, G., Jones, R. B. and Blokhuis, H. J. (1989). Adrenocortical and heterophil/lymphocyte responses to challenge in hens showing short or long tonic immobility reactions. *British Poultry Science* 30, 175–184.

Blecha, F. (2000). Immune system response to stress. In: Mench, J.A. and Moberg, G. (eds.) *Biology of Animal Stress*. CAB International, Wallingford, Oxfordshire, UK, pp. 111-122.

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. and Arnaud, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology and Behaviour* 92, 375-397.

Broom, D.M. (1986). Indicators of poor welfare. *British Veterinary Journal* 142, 524-526.

Broom, D.M. (2000). Coping, stress and welfare. In: D.M. Broom (ed) *Coping with Challenge: Welfare in Animals including Humans*. Chapter 1, pp 1-9. Dahlem University Press, Berlin.

Broom, D.M. and Johnson, K.G. (1993). *Stress and Animal Welfare*. Chapman and Hall, London.

Brown, J.L., Wasser, S.K., Wildt, D.E. and Graham L.H. (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of Reproduction* 51, 776–786.

Browne, W.J., Caplen, G., Statham, P. and Nicol, C. J. (2011) Mild environmental aversion is detected by a discrete-choice preference testing method but not by free-access method. *Applied Animal Behaviour Science* 134, 152-163.

Cacioppo, J.T., Klein, D.J., Berston, G. and Hatfield, E. (1993). The psychophysiology of emotion. In: Lewis, M. and Haviland, J.M. (eds.) *Handbook of Emotions*. Guildford Press, New York, USA, pp. 119-142.

Cannon, W.B. (1914). The emergency function of the adrenal medulla in pain and the major emotions. *American Journal of Physiology* 33, 356-372.

Chester Jones, I. and Henderson, I.W. (1976) *General, Comparative and Clinical Endocrinology of the Adrenal Cortex*, Volume 1. Academic Press, London, UK.

Clarke, I.J., Hemsworth, P.H., Barnett, J.L. and Tilbrook, A.J. (1992). Stress and reproduction in farm animals. In: Sheppard, K.E., Boublik, J.H. and Funder, J.W. (eds.) *Stress and Reproduction*. Serono Symposium Publications, Vol 86. Raven Press, New York, USA, pp. 239-251.

Coe, C.L., Dantzer, R., Jensen, S.L., Lightman, S.L., Porges, S.w., Rushen, J., Stefanski, V. And Zanella, A.J. (2000). Group report: key elements of coping. In: D.M. Broom (ed) *Coping with Challenge: Welfare in Animals including Humans*. Chapter 11, pp 151-168. Dahlem University Press, Berlin.

- Coleman, G.J. (2008) Public perceptions of animal pain and animal welfare. OIE Technical Series (World Organisation for Animal Health) 10, 26-37.
- Cook, C.J., Mellor, D.J., Harris, P.J., Ingram, J.R. and Matthews, L.R. (2000). Hands-on and hands-off measurement of stress. In Moberg, G.P. and Mench, J.A. (eds.) *The Biology of Animal Stress*. CABI Publishing, New York, New York, USA, pp. 123–146.
- Cooper, J. J. and M. J. Albentosa. (2003). Behavioural priorities of laying hens. *Avian Poultry Biology Review* 14, 127-149.
- Cooper, J.J. and Appleby, M.C. (1995). The effects of experience on motivation: prelaying behaviour in laying hens. *Animal Behaviour* 42, 283–295.
- Cooper, J.J. and Appleby, M.C. (1997). Motivational aspects of individual variation in response to nestboxes by laying hens. *Animal Behaviour* 54, 1245-1253.
- Cooper, J.J. and Appleby, M.C. (2003). The value of environmental resources to domestic hens: A comparison of the work-rate for food and for nests as a function of time. *Animal Welfare* 12, 39-52.
- Cooper, J. J. and Nicol, C. (1991). Stereotypic behaviour affects environmental preference in bank voles, *Clethrionomys glareolus*. *Animal Behaviour*, 41, 971-977.
- Cronin, G.M., Barnett, J.L., Storey, T.H., Thomson, P.C. and Hemsworth, P.H. (2012). The relationship between pre-laying activity and corticosterone concentrations, and the interpretation for laying hen welfare. In 'Proceedings of the 23rd Australian poultry science symposium'. (Ed. J Roberts) pp. 168–171. (The Poultry Research Foundation, University of Sydney: Camden)
- Cronin, G.M., Downing, J., Borg, S.S., Storey, T.H., Schirmer, B.N., Butler, K.L. and Barnett, J.L. (2008). The importance of nest-boxes to young adult laying hens. CD of Proceedings XXIII World's Poultry Congress, Brisbane.
- Cronin, G.M., Barnett, J.L., Storey T.H. and Hemsworth, P.H. (2012). The relationship between pre-laying activity and corticosterone concentrations, and the interpretation for laying hen welfare. *Proceedings of the Australian Poultry Science Symposium* 23, 168-171.
- Dantzer, R.(1988). *Les émotions*. Presses Universitaires de France, Paris.
- Dantzer, R. (2002) Can farm animal welfare be understood without taking into account the issues of emotion and cognition? *Journal of Animal Science* 80:E1-9.
- Dawkins, M. (1980). *Animal Suffering: The Science of Animal Welfare*. Chapman and Hall, New York, USA.
- Dawkins, M. (1983). Battery hens name their price: consumer demand theory and the measurement of animal needs. *Animal Behaviour* 31, 1195-1205.
- Downing, J. A., and Bryden, W.L. (2008). Determination of corticosterone concentrations in egg albumen: Non-invasive indicator of stress in laying hens. *Physiology and Behaviour* 95, 381–387.
- Duncan I.J.H. and Fraser D. (1997). *Understanding Animal Welfare*. In: Appleby, M.C. and Hughes, B.O. (eds.) *Animal Welfare*. CAB International, Wallingford, Oxfordshire, UK, pp. 19-31.

- Duncan, I.J.H. (2004). A concept of welfare based on feelings, in: Benson, G.J. and Rollin, B.E. (Eds.) *The Well-being of Farm Animals: Challenges and Solutions*, pp. 95-101 (Iowa, USA, Blackwell Publishing).
- Duncan, I.J.H. (2005). Science-based assessment of animal welfare: farm animals. *Revue Scientifique et Technique – Office International des Epizooties* 24, 483-492.
- Duncan, I.J.H. and Wood-Gush, D.G.M. (1972). An analysis of displacement preening in the domestic fowl. *Animal Behaviour* 20, 68-71.
- Duncan, I. J. H., T. M. Widowski, A. E. Malleau, A. C. Lindberg, and Petherick, J. C. (1998) External factors and causation of dustbathing in domestic hens. *Behavioural. Processes* 43, 219-228.
- Elsasser, T.H., Klasing, K.C., Filio, N. and Thompson, F. (2000). The metabolic consequences of stress: targets for stress and priorities of nutrient use. In: Mench, J.A. and Moberg, G. (eds.) *Biology of Animal Stress*. CAB International, Wallingford, Oxfordshire, UK, pp. 77-110.
- European Commission (2001). *The Welfare of Animals kept for Fur Production. Report of the Scientific Animal Health and Animal Welfare. European Commission, Health and Consumer Protection Directorate, General, Directorate C, Scientific Opinions. Adopted 12-13 December 2001.*
- Faure, J.M. (1991). Rearing conditions and needs for space and litter in laying hens. *Applied Animal Behaviour Science* 31, 111-117.
- Follensbee, M.E., Duncan, I.J.H. and Widowski, T.M. (1992). Quantifying nesting motivation of domestic hens. *Journal of Animal Science* 70, 164.
- Fraser, D. (2003a). Emerging animal welfare standards and their implications for animal hygiene. XI ISAH Congress in Animal Health, 23 - 27 February 2003, Mexico City, pp 61-66.
- Fraser, D. (2003b). Assessing animal welfare at the farm and group level: the interplay of science and values. *Animal Welfare* 12, 433-443.
- Fraser, D. (2008). *Understanding Animal Welfare: The Science in its Cultural Context*. Wiley-Blackwell, West Sussex, UK.
- Fraser, A. F. and Broom, D.M. (1997). *Farm Animal Behaviour and Welfare*, 3rd. ed. CAB International, Wallingford, UK.
- Fraser, D. and Matthews, L.R. (1997). Preference and motivation testing. In: Appleby, M.C. and Hughes, B.O. (eds.) *Animal Welfare*. CAB International, Wallingford, Oxfordshire, UK, pp. 159-174.
- Freire, R., Appleby, M.C. and Hughes, B.O. (1997). Assessment of pre-laying motivation in the domestic hen using social interaction. *Animal Behaviour* 54, 313-319. Hemsworth, P.H., and Coleman, G.J. (2011). *“Human-Livestock Interactions: The Stockperson and the Productivity and Welfare of Farmed Animals”*, 2nd Edition CAB International, Oxon UK.
- Hansen, I. (1994). Behavioural expression of laying hens in aviaries and cages: frequencies, time budgets and facility utilisation. *British Poultry Science* 35, 491-508.

- Hansen, C. P. B. and Jeppesen, L. L. (2001). Swimming Activity of Farm Mink (*Mustela vison*) and its Relation to Stereotypies. *Acta Agriculturae Scandinavica*, Section A, Animal Science 51, 71-76.
- Harbuz, M.S. and Lightman, S.L. (1992). Stress and the hypothalamo-pituitary-adrenal axis: acute, chronic and immunological activation. *Journal of Endocrinology* 134, 327-339.
- Hemsworth, P.H., Coleman, G.J. (2011). *Human-Livestock Interactions: The Stockperson and the Productivity and Welfare of Farmed Animals*, 2nd Edition. CAB International, Oxford.
- Hemsworth, P.H., Rice, M., Giri, K., Butler, K.M., Tilbrook, A.J. and Morrison, R. (2013). Effects of group size and floor space allowance on grouped sows: aggression, stress, skin injuries and reproductive performance. *Journal of Animal Science* (in press).
- Hill, A. T. (1977). The effects of space allowance and group size on egg production traits and profitability. *British Poultry Science* 18, 483-492.
- Hill, A.T. (1977). The effects of space allowance and group size on egg production traits and profitability. *British Poultry Science* 18, 483-492.
- Hodge, J. and Han, K. (2000) Preface. In "Livestock, Ethics and Quality of Life", Hodges, J. and Han, K. (Eds.), CAB International, Oxon, UK.
- Hogan, J.A. (2008). Motivation. In "The Behaviour of Animals: Mechanisms, Function and Evolution", Bolhuis, J.J. and Giraldeau, L-C. (eds), pp 41-70. Blackwell Publishing. Malden, USA.
- Hughes, B. O. (1983). Space requirements in poultry. In "Farm Animal Housing and Welfare", S. H. Baxter, Baxter, M. R., and MacCormack, J. A. C. (eds), pp. 121-128. Martinus Nijhoff, The Hague.
- Hughes, B.O. (1983). Space requirements in poultry, in: Baxter, S.H., Baxter, M.R. and MacCormack, J.A.C. (Eds.) *Farm Animal Housing and Welfare*, pp. 121-128 (Martinus Nijhoff, The Hague).
- Hughes, B.O. and Black, A.J. (1973). The preference of domestic hens for different types of battery cage floor. *British Journal of Poultry Science* 14, 615-619.
- Hughes, B. O., and A. J. Black. (1976). Battery cage shape—Its effect on diurnal feeding pattern, egg-shell cracking and feather pecking. *British Poultry Science* 17, 327–336.
- Hughes, B.O., Gilbert, A.B. and Brown , M.F. (1986). Categorisation and causes of abnormal egg shells: relationship with stress. *British Poultry Science* 27, 325– 337.
- Hutson, G. D. (1981). Sheep movement on slated floors. *Australian Journal of Experimental Agriculture and Animal Husbandry* 21, 474-479.
- Hughes, B. O., Gilbert, A.B. and Brown, F.M. (1986). Categorization and causes of abnormal eggshells - relationship with stress. *British Poultry Science* 27, 325-337.
- Kaltas, G.A. and Chrousos, G.P. (2007) The neuroendocrinology of stress. In: Cacioppo, J.T., Tassinari, L.G. and Berntson, G.G. (eds.) *Handbook of Psychophysiology*. Cambridge University Press, Cambridge, UK, pp. 303-318.

Keeling, L. J. (1994). Inter-bird distances and behavioural priorities in laying hens: the effect of spatial restriction. *Applied Animal Behaviour Science* 39, 131-140.

Kirkden, R.D. and Pajor, E.A. (2006). Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. *Applied Animal Behaviour Science* 100, 29-47.

Koelkebeck, K.W., Amoss, M.S.J. and Cain, J.R. (1987). Production, physiological and behavioural responses of laying hens in different management environments. *Poultry Science* 66, 397-407.

Laine, S.M. (2011). Animal preferences: effects of environmental and animal factors on the choice behaviour of laying hens, *Gallus gallus domesticus*. PhD thesis, University of Melbourne, Australia.

Lay Jr., D. C. Fulton, R. M., Hester, P. Y., Karcher, D. M., Kjaer, J. B., Mench, J. A., Mullens, B. A., Newberry, R. C., Nicol, C. J., O' Sullivan, N. P. and Porter, R. E. (2011). Hen welfare in different housing systems. *Poultry Science* 90, 278–294.

LeDoux, J.E. (1996). *The Emotional Brain*. Simon & Schuster, New York, USA.

Martin, P. and Bateson, P. (2007). *Measuring Behaviour. An Introductory Guide*. Cambridge University Press, Cambridge, UK.

Mashaly, M. M., Webb, M. L., Youtz, S. L., Rousch, W. B. and Graves, H. B. (1984). Changes in serum corticosterone concentration of laying hens as a response to increased population density. *Poultry Science* 63, 2271–2274.

Mason, G.J. and Latham, N. (2004). Can't stop, won't stop: is stereotypy a reliable animal welfare indicator. *Animal Welfare* 13 (Suppl), 57–69.

Mason, G. J., Cooper, C. and Clarebrough C. (2001). The welfare of fur-farmed mink. *Nature* 410, 35-36.

Matteri, R.L., Carroll, J.A. and Dyer, C.J. (2000). Neuroendocrine response to stress. In: Mench, J.A. and Moberg, G. (eds.) *Biology of Animal Stress*. CAB International, Wallingford, Oxfordshire, UK, pp. 43-76.

Matthews, L.R. and Ladewig, J. (1994). Environmental requirements of pigs measured by behavioural demand functions. *Animal Behaviour* 47, 713-719.

Meijsser, F.M. and Hughes, B.O. (1989). Comparative analysis of pre-laying behaviour in battery cages and in three alternative systems. *British Poultry Science* 30, 747-760.

Mellor, D.J., Cook, C.J. and Stafford, K.J. (2000). Quantifying some responses to pain as a stressor. In: Mench, M and Moberg, G.O (eds.) *Biology of Animal Stress*. CAB International, Wallingford, Oxfordshire, UK, pp. 171-198.

Mellor, D.J., Patterson-Kane, E. and Stafford, K.J. (2009). *The Sciences of Animal Welfare*, Wiley-Blackwell, Oxford, UK.

Mench, J.A. and Keeling, L.J. (2001) The social behaviour of domestic birds, in: Keeling, L.J. and Gonyou, H.W. (Eds.) *Social Behaviour in Farm Animals*, pp. 177-209 (UK, CABI Publishing).

Mendl, M. (1999). Performing under pressure: stress and cognitive function. *Applied Animal Behaviour Science* 65, 221-244.

- Mills, A. D., Y. Nys, Y., Gautron, J. and Zawadski, J. (1991). Whitening of brown shelled eggs: Individual variation and relationships with age, fearfulness, oviposition interval and stress. *British Poultry Science* 32, 117-129.
- Mills, A.D., Faure, J., Picard, M. and Marche, M. (1987). Reflectometry of wet and dry eggs as a measure of extraneous egg shell calcification. *Medical Science Research* 15, 705–706.
- Moberg, G.P. (2000) Biological response to stress: implications for animal welfare. In: Mench, J.A. and Moberg, G. (eds.) *Biology of Animal Stress*. CAB International, Wallingford, Oxfordshire, UK, pp. 1-21.
- Moe, R. O., Guemene, D., Bakken, M., Larsen, H. J. S., Shini, S., Lervik, S., Skjerve, E., Michel, V. and Tauson, R. (2010). Effects of housing conditions during the rearing and laying period on adrenal reactivity, immune response and heterophil to lymphocyte (H/L) ratios in laying hens. *Animal* 4, 1709-1715.
- Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. (2003). *Harper's Illustrated Biochemistry*. McGraw-Hill, New York, USA.
- Nicol, C.J. (1986). Non-exclusive spatial preference in the laying hen. *Applied Animal Behaviour Science* 15, 337-350.
- Nicol, C. J., Brown, S.N., Glen, E., Pope, S.J., Short, F.J., Warriss, P.D., Zimmerman, P.H. and Wilkins, L.J. (2006). Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. *British Poultry Science* 47, 135-146.
- Nicol, C.J., Caplen, G., Edgar, J. and Browne, W.J (2009). Associations between welfare indicators and environmental choice in laying hens. *Animal Behaviour* 78, 413–424.
- Nicol, C.J., Caplen, G., Statham, P. and Browne, W.J. (2011). Decisions about foraging and risk trade-offs in chickens are associated with individual somatic response profiles. *Animal Behaviour* 82, 255-262.
- Odberg, F. O. (1989). Behavioural coping in chronic stress conditions. In: R. J. Blanchard, P. F. Brain, D. C. Blanchard and S. Parmigiani (eds.) *Ethoexperimental Approaches to the Study of Behaviour*. Kluwer Academic Press, Dordrecht, pp. 229-239.
- Panksepp, J. (1998). *Affective Neuroscience. The Foundation of Human and Animal Emotions*. Oxford University Press, London, UK.
- Panksepp, J. (2005). Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition* 14, 30–80.
- Payne, R.W. (Ed), 2010. *The Guide to GenStat® Release 13. Part 2: Statistics*. VSN International, Hertfordshire, UK, pp 486-487.
- Peng, X., Lang, C.M., Drozdowicz, C.K. and Ohlsson-Wilhelm, B.M. (1989). Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations of laboratory mice. *Laboratory Animals* 23, 302-306.
- Phillips, P.A., Thompson, B.K. and Fraser, D. (1988). Preference tests of ramp designs for young pigs. *Canadian Journal of Animal Science* 68, 41-48.

PISC (Primary Industries Ministerial Council) (2002). Model code of practice for the welfare of animals—domestic poultry, 4th edition. Collingwood, Victoria, Australia: CSIRO Publishing.

Pollard, J.C., Littlejohn, R.P. and Suttie, J.M. (1994). Responses of red deer to restraint in a Y maze preference test. *Applied Animal Behaviour Science* 39, 63-71.

Reynard, M. and Savory, C.J. (1999). Stress-induced oviposition delays in laying hens: duration and consequences for eggshell quality. *British Poultry Science* 40, 585-591.

Rodenburg, T. B., and Koene, P. (2004). Feather pecking and feather loss. In “Welfare of the Laying Hen”, Edited by G. C. Perry, pp. 227-238. CAB International Publishing, Wallingford Oxfordshire UK.

Rodenburg, T. B., Komen, H., Ellen, E.D., Uitdehaag, K.A. and van Arendonk, J.A.M. (2008). Selection method and early-life history affect behavioural development, feather pecking and cannibalism in laying hens: A review. *Applied Animal Behaviour Science* 110, 217-228.

Roush, W.B., Mashaly, M.M. and Graves, H.B. (1984). Effect of increased bird population in a fixed cage area on production and economic responses of single comb White Leghorn laying hens. *Poultry Science* 63, 45-48.

Rushen, J. (1986). Aversion of sheep to handling treatments: Paired-choice studies. *Applied Animal Behaviour Science* 16, 363-370.

Sandøe, P., Forkman, F. and Christiansen, S.B. (2004). Scientific uncertainty - how should it be handled in relation to scientific advice regarding animal welfare issues? *Animal Welfare* 13, 121-126.

Savory, C.J., Jack, M.C. and Sandilands, V. (2006). Behavioural responses to different floor space allowances in small groups of laying hens. *British Poultry Science* 47, 120-124.

Sédlackova, M., Bilcik, B. and Kostal, L. (2004). Feather pecking in laying hens: Environmental and endogenous factors. *Acta Vet. Brno* 73, 521-531.

Selye, H. (1946). The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology* 6, 117-230.

Selye, H. (1976). *Stress in Health and Disease*. Butterworth, Boston, Massachusetts, USA.

Sohail, S. S., Bryant, M. M. and Roland, D.A. (2004). Effect of reducing cage density on performance and economics of second-cycle (force rested) commercial leghorns. *Journal of Applied Poultry Research* 13, 401-405.

Sohail, S.S., Bryant, M.M. and Roland, D.A. (2004). Effect of reducing cage density on performance and economics of second-cycle (force rested) commercial leghorns. *Journal of Applied Poultry Research* 13, 401-405.

Stevens, B., Barnett, J.L., Tilbrook, A. and Hemsworth, P.H. (2009). Effects of deprivation of a preferred resource (feed or social contact) on the biological functioning of pigs. In: *Manipulating Pig Production XII*. Australasian Pig Science Association, van Barneveld, R.J (eds.), Werribee Victoria, Australia, pp. 28.

Tannenbaum, J. (2001). The paradigm shift toward animal happiness: what it is, why it is happening, and what it portends for medical research. In *Why Animal Experimentation*

Matters: The use of Animals in Medical Research. E. F. Paul, and Paul, J., Eds.; Transaction Publishers, Somerset, New Jersey, USA, pp. 93-130.

Tauson, R., Kjaer, J., Maria, G.A., Cepero, R. and Holm, K.-E. (2005). Applied scoring of integument and health in laying hens. Proceedings of the 7th. European symposium on poultry welfare, Lublin, Poland, June 2005. Animal Science Papers and Reports 23, 153-159.

Taylor, K.D. (2010). Displacement behaviour. In The Encyclopaedia of Applied Animal Behaviour and Welfare, 1st ed.; Mills, D.M., Marchant-Forde, J.N., Morton, D.B., Phillips, C.J.C., McGreevy, P.D., Nicol, C.J., Sandoe, P., Swaisgood, R.R., Eds.; CABI Publishing: Wallingford, UK, pg. 180.

Thaxton, P. (2004). Stress and the welfare of laying hens. In Welfare of the Laying Hen; Perry, G.C., Ed.; CABI Publishing: Wallingford, UK, pp. 81–95.

Tilbrook, A.J., Turner, A.I. and Clarke, I.J. (2000). Effects of stress on reproduction in non-rodent mammals: considerations of the role of glucocorticoids and sex differences. Reviews of Reproduction 5, 105-113

van Krimpen, M. M., Kwakkel, R.P., Reuvekamp, B.F.J., van der Peet-Schwering, C.M.C., Den Hartog, L.A. and Verstegen, M.W.A. (2005). Impact of feeding management on feather pecking in laying hens. World's Poultry Science Journal. 61, 663-685.

Wall, H. (2011). Production performance and proportion of nest eggs in layer hybrids housed in different designs of furnished cages. Poultry Science 90, 2153–2161.

Wall, H. and Tauson, R. (2002). Egg quality in furnished cages for laying hens - Effects of crack reduction measures and hybrid. Poultry Science 81, 340-348.

Weeks, C. A., Danbury, T. D., Davies, H. C., Hunt, P. and Kestin, S. C. (2000). The behaviour of broiler chickens and its modification by lameness. Applied Animal Behaviour Science 67, 111–125.

Widowski, T. M. and Duncan, I. J. H. (2000). Working for a dustbath: are hens increasing pleasure rather than reducing suffering? Applied Animal Behaviour Science 68, 39-53.

Widowski, T. M. and Hemsworth, P. H. (2008). Housing hens to suit their 'needs'. In: Proceedings of the XXIII World Poultry Congress. World's Poultry Science Journal and the World's Poultry Science Association, The Netherlands, p. 241.

Widowski, T.M., Hemsworth, P. H., Coleman, G. J., Guein, M. T., Dysart, L. M. and Barnett, J. L. (2009). Welfare Issues and Housing for Laying Hens: International Developments and Perspectives. A Report Submitted to Australian Egg Corporation Ltd, 121 pp.

Yue, S. and Duncan, I. J. H. (2003). Frustrated nesting behaviour: relation to extra-cuticular shell calcium and bone strength in White Leghorn hens. British Poultry Science 44, 171-177.

4 Appendices

4.1 Appendix 1

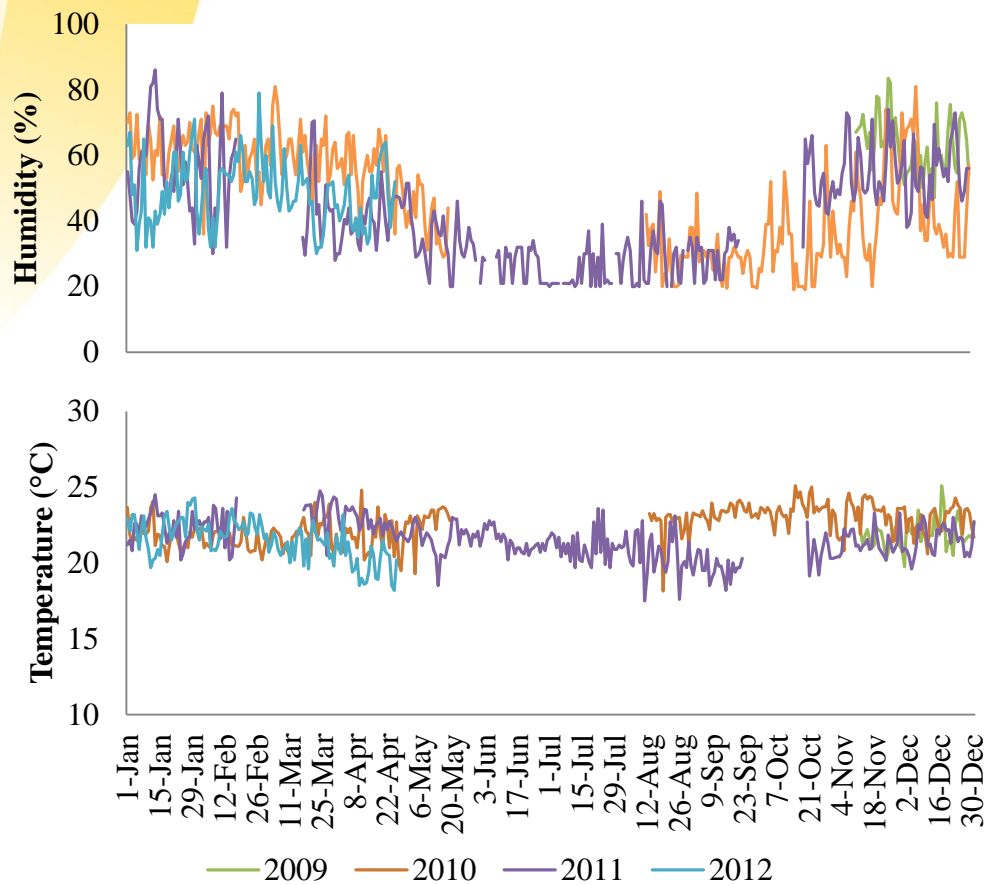


Figure 4-1- Humidity and temperatures within the experimental shed

4.2 Appendix 2

R _s P _s NB-	R _s P _L NB+	R _L P _s NB-	R _L P _L NB+
R _s P _s NB+	R _s P _L NB-	R _L P _s NB+	R _L P _L NB-

R _L P _L NB+	R _L P _s NB-	R _s P _L NB-	R _s P _s NB+
R _L P _L NB-	R _L P _s NB+	R _s P _L NB+	R _s P _s NB-

R _L P _s NB+	R _L P _L NB-	R _s P _s NB-	R _s P _L NB+
R _L P _s NB-	R _L P _L NB+	R _s P _s NB+	R _s P _L NB-

R _s P _L NB+	R _s P _s NB-	R _L P _L NB-	R _L P _s NB+
R _s P _L NB-	R _s P _s NB+	R _L P _L NB+	R _L P _s NB-

Figure 4-2 - Randomised treatments for (a) replicate 1, (b) replicate 2, (c) replicate 3 and (d) replicate 4

4.3 Appendix 3

Table 4-1 - Vaccination schedule of pullets during the experiment

Age	Vaccination or treatment	Serology
Day old	Marek's – Rispens/HVT IBV Chick Papers - Salmonella	
3 weeks	IBV (Vic S) NDV (Live V4)	
6-8 weeks	ILT (SA-2) NDV (Live V4)* AEV MG (ts-11)/MS (MS-H)	
10-12 weeks	NDV/EDS Killed Combination Fowl Pox AEV	
13 weeks	IBV (Vic S)	
18 weeks		MG, MS, IBV, AEV, NDV, EDS

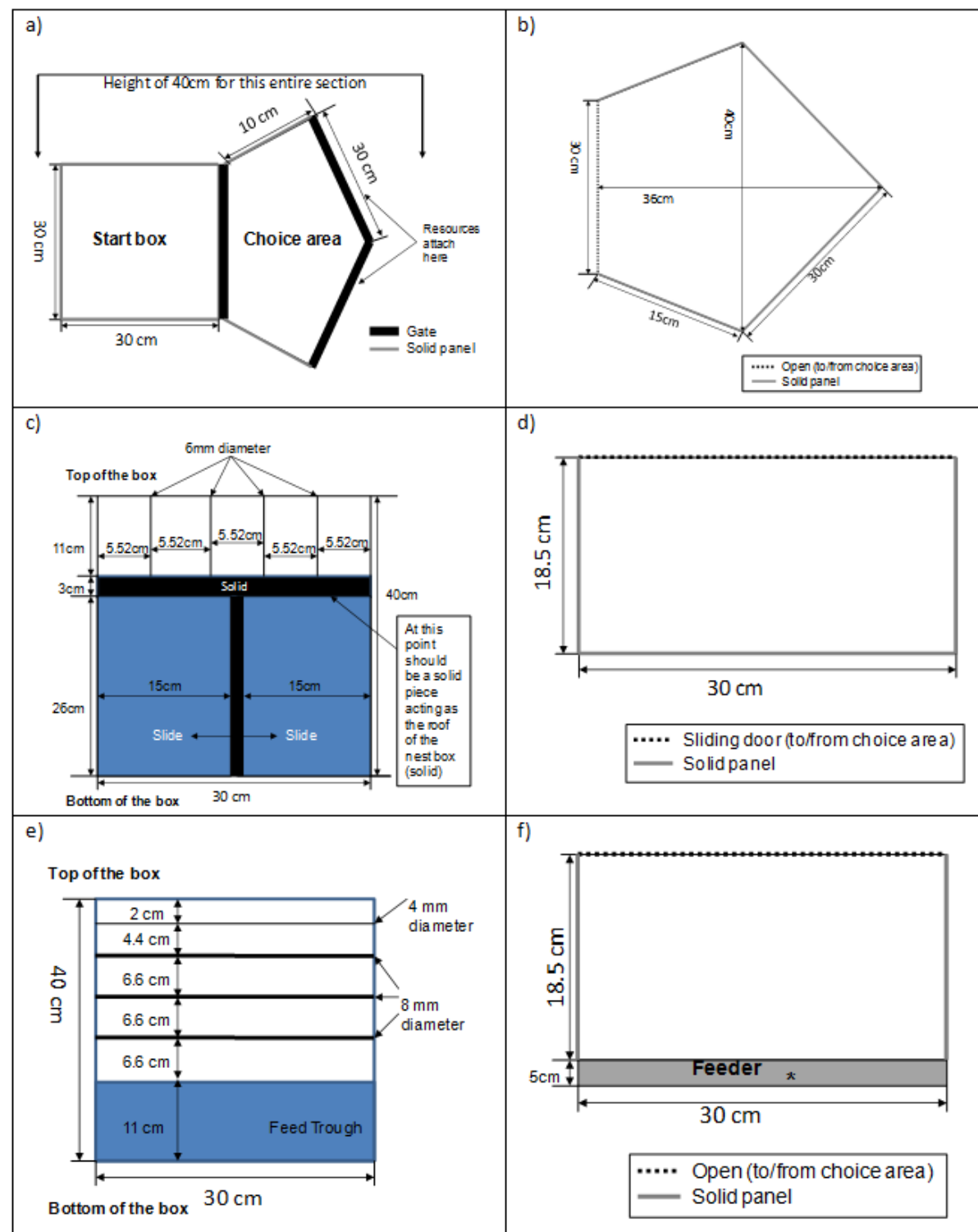
*Vaccine not given to pullets due to small flock with no outdoor exposure.

4.4 Appendix 4

Age (weeks)	Hours of light	Lights on and off times	
		Rep 1	Reps 2, 3 and 4
7-15	10	0630-1630	0930-1930
16	10.5	0630-1630	0900-1930
17	11	0530-1630	0830-1930
19	11.5	0500-1630	0800-1930
21	12	0430-1630	0730-1930
23	12.5	0400-1630	0700-1930
25	13	0400-1700	0700-2000
27	13.5	0400-1730	0700-2030
29-34*	14	0400-1800	0700-2100

*Lights no longer extended after 29 weeks to ensure predictability of oviposition for Y maze testing.

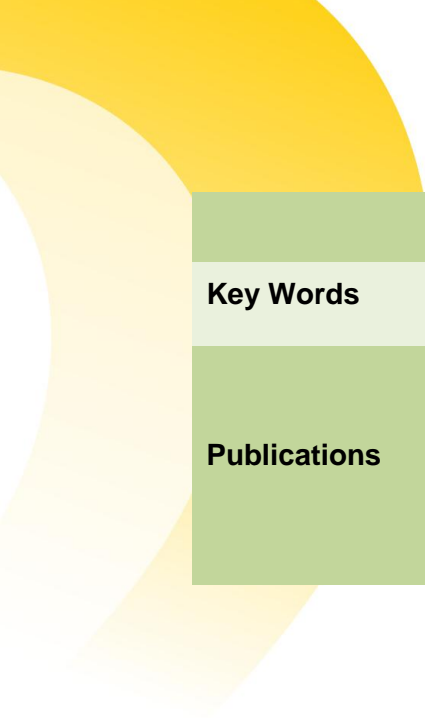
4.5 Appendix 5



Pictured are views of the Y maze from above (a) and the space attachment from above (b), a view of the nest-box attachment from inside the Y maze (c) and above (d), and the feed attachment from inside the Y maze (e) and above (f)

5 Plain English Summary

Project Title:	Importance of rearing environment, space and nests for laying hens in cages: Welfare implications of floor space and nest boxes
AECL Project No	1UM091
Researchers Involved	P.H.Hemsworth, T.M.Widowski, A.J.Tilbrook and J. Engel
Organisations Involved	Animal Welfare Science Centre Melbourne School of Land and Environment Department of Agriculture and Food Systems The University of Melbourne PARKVILLE, 3010, Victoria, Australia
Phone	03 9035 5026
Fax	03 9347 6739
Email	tkendal@unimelb.edu.au
Objectives	The main objective of this experiment was to determine the effects of floor space during rearing and adulthood and access to a nest box during adulthood on the welfare of laying hens.
Background	Captive environments that inhibit or prevent behaviours that are considered to be part of the normal behavioural repertoire of the animal appear to be at the forefront of the public's concern about the treatment of animals. Two of the most contentious welfare issues in relation to cage housing of laying hens are floor space allowance and an appropriate nest site.
Research	256 Hy-line Brown hens were studied in a 2x2x2 factorial design: <ol style="list-style-type: none"> 1. Rearing space allowance - two levels, 315 and 945 cm²/bird; 2. Production space allowance - two levels, 542 and 1648 cm²/bird; 3. Nest box access - two levels, presence or absence of access to a nest box during production. Hen welfare was assessed using measures of stress, preference of hens for space and nest boxes in Y maze tests, body weight and egg production.
Outcomes	There were no consistent effects of floor space or nest box access on measures of stress, preference of hens for space in Y maze tests, body weight or egg production. However, reduced floor space allowance during adulthood reduced the display of the behaviours of mobile, inedible pecking, drinking and preening but increased the display of the behaviours of resting and feed pecking and the posture of sitting. Furthermore reduced floor space in adulthood reduced feather condition.
Implications	The present experiment provides no convincing evidence that reduced space and deprivation of nest boxes results in suffering, based on normality of biological functioning and preferences for space and nest boxes. Nevertheless, policy makers in developing animal welfare standards and recommendations should consider the implications of providing commercial laying hens with both increased space to allow more behavioural freedom and nest boxes, which presumably provide experienced hens with positive emotional experiences based on their motivation to access them in



	preference tests.
Key Words	Hen welfare, behaviour, stress, preferences, floor space allowance, nest box access.
Publications	<p>Engel, J., Widowski, T.M., Tilbrook, A.J. and Hemsworth, P.H. (2011). Further investigation of non-invasive measures of stress in laying hens. Proceedings of the Australian Poultry Science Symposium 22, p.126-129.</p> <p>Engel, J., Bont, Y. and Hemsworth, P.H.H. (2011). Effect of cage design on consistency of orientation and location during oviposition of laying hens. Proceedings of the 45th Congress of the International Society for Applied Ethology, 1-4 August 2011, Indianapolis, USA, p. 118.</p>