AUSTRALIAN EGG®
CORPORATION LIMITED

Welfare of Laying hens in Furnished Cages

A report for the Australian Egg Corporation Limited

by John L Barnett and Gregory M Cronin

Co researchers:
Ragnar Tauson, Jeff Downing,
Vijaya Janardhana, John Lowenthal and
Kym Butler

June 2005

AECL Project No: DAV-197A

ISSN 1448-1316

Welfare of Laying Hens in Furnished Cages
Publication No. 05/08
Project No DAV-197A

Researcher Contact Details
Name: John Barnett
Address: Animal Welfare Science Centre, DPI, 600 Sneydes Road, Werribee Vic 3030

Phone: 03 9742 0433
Fax: 03 9742 0400
Email: john.barnett@dpi.vic.gov.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
Suite402, Level 4
107 Mount Street
NORTH SYDNEY NSW 2060

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

Published in June 2005
Appears only on the web.

Foreword

There is increasing pressure in Europe to find alternatives to conventional cage systems for egg production and one such alternative is the furnished cage. For example, the European Union agreed in 1999 that conventional cages are to be replaced with furnished cages by 2013; furnished cages contain a perch, nest box and dust bath. Although there appears to be an inability both within and outside of science to agree on the need for non-cage systems, nevertheless alternative housing systems are being actively promoted in some countries. For example, Switzerland and Sweden have banned the conventional cage, The Netherlands has been encouraging producers to use non-cage systems and in the UK, marketing initiatives surrounding programs such as 'Freedom Foods' preclude the use of conventional cages. While it is sometimes difficult to fully understand the motives for some of these initiatives, as they are often a complex intermingling of economics, public perceptions and political expediency, there is a desire either to improve the welfare of birds or remove the issue from the political agenda. Some Australian producers recognise the 'pressures' on conventional cage housing and are pursuing alternatives to the conventional cage eg. barn systems. The proponents of alternatives to conventional cages are in two main camps: those proposing non-cage systems and those proposing furnished cage systems.

This project focused on the welfare of hens in furnished cages. It includes a review of the literature on furniture in cages and a review of the different approaches to welfare assessment; the latter is important to put into perspective the approach that was used in the experimental component to assess welfare. There were two experiments, the first was a major experiment that examined the presence of a perch, a dust bath and a nest box in a cage both alone and in various combinations. The additional space that was provided by the dust bath and nest box was also included as an experimental treatment. Furthermore, additional treatments were included in the same experiment to provide some preliminary data on the welfare implications of space allowance and group size. The second experiment examined egg laying behaviour in hens that were either experienced or inexperienced with a nest box.

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

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

www.aecl.org

Printed copies can be purchased by faxing or emailing the downloadable order form from the web site or by phoning (02) 9409 6999.

James Kellaway
Managing Director
Australian Egg Corporation Limited

Acknowledgments

We are grateful to the following co-researchers who contributed to the major aim of this project, particularly experiment 1 (Chapter 3): Dr. Ragnar Tauson (Swedish University of Agricultural Science, Uppsala, Sweden) for his assistance in obtaining the cages and his inputs into experimental design and morphological measurements, Dr. Jeff Downing (University of Sydney) for undertaking the assays for corticosterone concentrations in egg albumen and Drs. John Lowenthal and Vijaya Janardhana (CSIRO, Livestock Industries, Geelong) for their assistance in deciding on immunological measurements and for supervising the immunological assays and measurements. We thank Mary Broadway and Matt Bruce (CSIRO, Livestock Industries, Geelong) for their assistance with conducting the proliferation assays and the haematology measurements, respectively and Dr. Ian McCauley and his staff for the plasma corticosterone assays. We also thank Marion Desnoyers (Institut National Agronomique, Paris-Grignon, France) for her major contributions to the additional work on nesting behaviour described in Chapter 4. We are also grateful for the excellent technical support provided by Lisa Newman, Samantha Borg, Bruce Schirmer and Tracie McCallum.

About the Authors

Dr. John Barnett completed his undergraduate degree in the UK and then completed a PhD at Monash University, in 1973. He subsequently held a post-doctoral position in the UK and a teaching/research position at La Trobe University, before joining the Department of Agriculture (Victoria) in the late 1970s. His research career spans 30 years in both wildlife research and domestic animal research. His area of expertise is in stress physiology and its application to environmental physiology of mammals and birds. He has published over 120 papers in refereed scientific journals and chapters in books, plus over 200 additional publications including addresses to conferences, reports to funding bodies and extension articles.

He has recently given up the mantle of Head, Animal Welfare Department at the Department of Primary Industries' Werribee campus and his current position is leading scientist. In this position he is responsible for scientific standards and project development in the area of animal welfare. Some current and recent appointments are chair of the institute's Animal Ethics Committee, member of the Scientific Advisory Panels for both the Royal Society for the Prevention of Cruelty to Animals and the World Society for the Protection of Animals Scientific and a member of a State Government Animal Welfare Advisory working group.

His recent and current research program includes collaborative projects on tail docking in dairy cows, beak trimming in poultry, alternative housing of laying hens, stall housing of pigs examining stall dimensions and humane vertebrate pest control. A current program is developing comprehensive welfare audits for a number of animal industries, including the chicken meat, pork and dairy industries for both the on-farm and processing sectors.

He is a member of the Animal Welfare Science Centre, which is a joint Centre of the Department of Primary Industries, Victoria, University of Melbourne (Institute of Land and Food Resources) and Monash University (Departments of Psychology and Physiology). He has an honorary position of Associate Professor at the Institute of Land and Food Resources at the University of Melbourne. He is the program leader of the welfare program for the recently funded Australian Poultry Cooperative Research Centre.

Dr Greg Cronin completed a PhD in 1985 at the Agricultural University of Wageningen, The Netherlands, under the supervision of Professor Piet Wiepkema. The theme of the PhD was the development of stereotyped behaviour in tethered sows, and implications for sow productivity of the performance of the behaviours. Since completing his PhD studies, Greg Cronin has been employed as a research scientist with the Department of Primary Industries, Victoria, Werribee Centre, where he has been involved in research programs on animal behaviour, production and welfare. Dr Cronin currently holds the position of Statewide Leader Animal Welfare Science, in the Animal Production Science Platform of Primary Industries Research Victoria.

A major research area was the study of maternal behaviour in sows to investigate the role of the physical environment on maternal behaviour and the effects of the environment x maternal behaviour interactions on piglet survival. An outcome of the research was the development of the Werribee Farrowing Pen, which has been evaluated on-farm and is a potential alternative to full-confinement farrowing crates. Other areas of pig research have involved studies to improve suckling behaviour of sows and growth of piglets, the interaction between social behaviour and aggression in finisher pigs and the effects of aggression on reducing feeding behaviour and growth, the effects of gestation housing systems on sow welfare and production, methods of mixing sows for reduced aggression and injury risk to improve their welfare and alternative housing systems for gestating and farrowing/lactating sows. Dr Cronin has also conducted research on the effects of housing systems on behaviour and welfare of laying hens and a small number of projects have also been undertaken on other species such as dogs, sheep and cattle. Greg Cronin has more than 120 scientific publications including 66 in refereed scientific journal papers, and books/chapters in books as well as extension articles in the farmer press.

TABLE OF CONTENTS

Page number

List of Tables

List of Figures

Abbreviations

Abbreviation	Meaning
ACTH	Adrenocorticotrophic hormone (a hormone from the pituitary gland that stimulates the synthesis and release of corticosterone from the adrenal glands)
AE	Avian encephalomyelitis (a viral infection primarily affecting young birds). The disease is characterised by a variety of neurological signs including incoordination, ataxia, and tremors of the head and neck
Ci	Curies
CO_2	Carbon dioxide
Con A	Concanavalin A
cpm	Counts per minute
EDS	Egg drop syndrome (a viral infection resulting in thin-shelled or shell-less eggs or a marked reduction in egg production)
G	Gravitational force
h	Hour
HPA-axis	Hypothalamic/pituitary/adrenal axis
IB	Infectious bronchitis (an acute, highly contagious respiratory disease of chickens caused by a coronavirus)
ID	Identification
IgG	Immunoglobulin fraction G
IL-6	Interleukin 6 (a cytokine which is an intercellular chemical messenger protein released by white blood cells. Cytokines facilitate communication among immune system cells and between immune system cells and the rest of the body.) IL-6 has direct immunoregulatory effects and also plays a role in communication between the neuroendocrine and immune systems. It activates the HPA-axis and is under the feedback control of adrenal corticosteroids
ILT	Infectious Laryngotracheitis is a highly contagious viral infection of the respiratory tract of chickens.
IR	Infra-red
IU	International units

LED	Light-emitting diode
LPS	Lipopolysaccharide
M	Molar
min	Minute
MG	*Mycoplasma gallicepticum* (a bacteria organism which typically causes respiratory disease)
mm	Millimetre
mM	Millimolar
N	Newtons
NaOH	Sodium hydroxide
NM	Nanomolar
REML	Restricted maximum likelihood (the model used for statistical analysis)
RIA	Radioimmunoassay

Executive Summary

This project focused on the welfare of hens in furnished cages. Chapter 1 provides a review of the literature on furniture in cages and a review of the different approaches to welfare assessment; the latter is important to put the homeostasis or functional approach to welfare assessment that was used in the experimental component into perspective. There were two experiments, the first was a major experiment that examined the presence of a perch, a dust bath and a nest box in a cage both alone and in various combinations. The additional space that was provided by the dust bath and nest box was also included as an experimental treatment. Furthermore, additional treatments were included in the same experiment to provide some preliminary data on the welfare implications of space allowance and group size. The second experiment examined egg laying behaviour in hens that were either experienced or inexperienced with a nest.

Experiment 1
Despite the presence of furnished cages in the market place and the considerable support for such designs of cage, particularly in Europe, there has been little comprehensive assessment of hen welfare as a consequence of such furniture. Indeed, because of the opportunity they provide for increased behavioural repertoire they fulfil the intentions of the 'Five Freedoms' and consequently there has been an assumption that welfare is improved. Probably the most discussed issue for laying hens is the requirement for a nest, indeed the lack of a nest site in conventional cages is considered, by some, to be the biggest welfare problem in conventional cage housing systems.

This experiment examined the welfare of hens housed in furnished cages and the effects of the individual items of furniture in a factorial experiment with 8 birds per cage. At the same time it also examined, in a preliminary experiment, the effect of increasing space per bird (8 birds in double-width cages) and the effect of stocking density (16 birds in double-width cages). The experiment involved 20 treatments using 66 Victorsson Trivselburen Furnished Cages (AB Bröderna Victorsson, Sweden) that were modified to meet the experimental requirements. The cages were 1206 mm wide, 498 mm deep and 455 mm high at the rear of the cage. Double-width cages were formed by removing one side panel to provide a cage approximately 2400 mm wide. The cages were located in a controlled climate shed with control for temperature, light and ventilation. The birds were Hy-Line Brown that had been floor-reared and were housed in the cages at 15+ weeks of age. The experimental design was a 3 (nest box) x 3 (dust bath) x 2 (perch) factorial + 2 added controls (space and group size) in a rectangular lattice. The 3 levels of nest box and dust bath were 1) nest box or dust bath provided, 2) no nest box or dust bath, but the equivalent space and site of the nest box or dust bath was available to the hens and 3) no nest box or dust bath and the extra cage space due to the nest box or dust bath was not available to hens. The 2 levels of perch treatment were either perches or no perches. The added control treatments were either double cages housing 8 hens (space control) or 16 hens (group size control). The experimental unit was a cage of hens. Floor space of a single cage with and without the nest/dust bath was 950 x 498 and 1206 x 498 mm, respectively.

There were 2 sampling periods, commencing at 29 and 59 weeks of age and each was conducted over an 8-week period. There were 5 sets of measurements/observations taken in the following order at both sampling periods: 1) video observations, 2) blood sampling for immunology, 3) collection of eggs for determination of corticosterone concentrations, 4) blood sampling for determination of corticosterone concentrations and 5) body weight and feather damage and cover scoring, claw length, foot condition scoring, injuries on the comb, around the cloaca and on the hen's back, keel bone deformation and blood sampling 60 min after injection of ACTH for determination of the corticosterone response. In addition, at the end of the experiment commencing at 67 weeks of age, 3 birds were randomly selected from each cage and euthanased and bones extracted for bone strength determination.

The normality of the birds in this experiment was evidenced by the production data that showed egg production was not dissimilar from industry targets. Hen day egg production figures from 22-29 weeks and 48-55 weeks of age from the company website (www.hyline.com.au) for Hy-Line Brown hens are 90 and 86 %; in the furniture treatments mean hen day egg production for the two ages was 91.5 and 80 %, respectively. Similarly, industry figures for body weight at 48-55 weeks of age is 2.24 kg, with the birds in this study being slightly heavier with a mean body weight of 2.54 kg.

This experiment has shown, based on both mean values and the relatively small standard errors, that with the exception of a benefit of a perch to bone strength, any effects of the three items of furniture, that is a perch, dust bath and nest box, were relatively small. There were some minor effects on bird feather and foot condition and feather damage/cover, with dirtier and more damaged feathers in the perch treatment but better foot condition in this treatment. There were also some behavioural changes as a consequence of changing the environment with effects of a nest on egg laying behaviour. Over 30 % of eggs were laid on the wire floor and over 80 % of those eggs laid in the nest box were laid on the low side ie. closest to the egg roll-out tray. Hens did not generally use the dust bath unless a perch was also present. As reported in the literature, bone strength was significantly improved if a perch was present. The tibia, humerus and coracoid were stronger in the perch treatment and the coracoid was stronger in the larger cages.

In contrast to the general lack of effect of furniture, there were significant physiological effects as a consequence of group size and stocking density with some evidence that birds housed at 16 hens/cage (space allowance of 750 cm^2/bird) were stressed compared to birds housed at 8 hens/cage with the same space allowance. This was based on evidence of immunosuppression and higher egg corticosterone concentrations at 32 and 33 weeks of age, respectively. However, the data for plasma corticosterone concentrations both at rest and in response to ACTH make it equivocal whether the stress response was an acute or a chronic response. Further research to resolve the important issue of space allowance and its interaction with group size is clearly warranted.

Probably the most discussed issue in relation to the welfare of hens in cages is the lack of a suitable nest site. One objective of furnished cages was to address this shortcoming. Nevertheless, it can be argued that the data do not allay this concern. There is no doubt that in the presence of a nest there is behavioural change. Egg laying was affected by the presence of a nest and interacted with other items of furniture. For example, in both sampling periods, more eggs were laid in the nest if there was also a perch present and in the second sampling period, more eggs were laid in the nest in the absence of a dust bath. However, there were no effects on the interval between eggs being laid and the maximum interval between eggs being laid. In contrast, the maximum interval between eggs being laid was lowest in the 8 hens/cage treatment with a space allowance of 1500 cm^2/hen. It has been suggested that egg laying behaviour is disturbed in conventional cages, with evidence of frustration in the absence of a nest. While this experiment suggests that the disturbance may be reduced with more space, the presence of a nest in the furniture treatments neither affected the duration of egg laying nor the maximum interval between eggs, within a cage.

Experiment 2

This preliminary experiment identified and measured the different behaviours of hens around oviposition, particularly in relation to the “searching” and “sitting” phases of nesting, and examined the effects of the presence or absence of a nest box on pre- and post-laying behaviours of hens that were experienced with laying under the respective test conditions. There were two treatments: a Nest Box treatment and a Control (no nest box) treatment. Prior to transfer to the two test cages which were identical to the home cage, other than being equipped for video recording, hens were housed in groups of 8 from the point of lay either in cages with a nest or without a nest. Data from nine replicates of hens housed in

pairs either in a cage with a nest or a cage with no nest were transcribed from video records for the period of 2 h pre-laying to 2-h post-laying in hens aged from 45-48 weeks of age.

Of the 18 focal hens in the Nest Box treatment, nine laid eggs in the nest box and nine lay outside the nest box on the wire floor. These hens were thus termed “nest layers” and “floor layers”, respectively ie. 50 % of hens chose to lay outside of the nest box. Based on observations of these hens on consecutive days, only one hen was observed to change her location of laying between the two days, ie. laid in the nest box and on wire floor on different days. These data are not inconsistent with either the data from experiment 1 where 30 % of eggs were laid on the wire when a nest box was available or with the literature which indicates a large variation in use of a nest box when available in a cage.

Hen activity level around oviposition was assessed by the time hens spent walking and the frequency of entering 9 prescribed areas of the cages. Darkness strongly reduced hen activity. As the 2-h pre-laying period included darkness for four hens, the time in the dark was used as a co-variate in the analysis. These four hens alternated between standing and sitting in the dark and none were observed to locomote. After adjusting for darkness, there were no differences in the activity measures in the period 120-60 min pre-laying. However, in the 1 h before egg-laying, nest layers were less active than floor layers; nest layers performed less ($P<0.001$) walking behaviour (excluding following behaviour) and entered areas 1-9 less frequently ($P<0.01$) than floor layers. A new behaviour, “following behaviour” was observed but not analysed because it only occurred in the floor layers. Following behaviour (the focal hen was walking or running, but followed the other hen as she moved about the cage) was observed for over 55% of the floor layers, and occupied about 5% of the total time in the period 60 min pre-laying to 30 min post-laying and the peak frequency of occurrence was in the 30 min prior to oviposition.

This small experiment that involved observations on egg-laying behaviour in 18 pairs of hens has raised more questions than it has provided answers. A new finding was “following behaviour” which only occurred in hens that laid eggs on the wire floor, irrespective of the presence of a nest box. This behaviour occurred during pre- and post-laying periods and involved the hen (follower) appearing to attempt to remain close to the other hen (followed), including when the followed hen was locomoting. When the followed hen was stationary and standing, the follower would often sit next to her and the follower would put her head under the body of the followed hen. The reasons for this behaviour are unknown as are the reasons why it only occurred in 55% of floor layers and also occurred post-oviposition. A possible explanation was that when a nest box was present and the hen utilised the nest box for egg laying, this environment provided appropriate cues for nest site selection. In contrast, the follower hen may have derived cues for nest site selection from the followed hen.

This experiment has shown, as reported in other studies, that the use of the nest box for egg laying is highly variable between birds. In this experiment where experienced (with a nest box) hens were housed in pairs in a cage with a nest box, 50% of hens laid in the nest box and 50% laid on the wire cage floor outside the nest box. These data raise the question of hens’ preference for egg laying location. While the literature suggests hens are motivated to seek a “preferred” location for egg laying, the data from this experiment could be interpreted to suggest that either the nest box or the wire cage floor were both preferred locations. Alternatively, by one hen making a choice, this may or may not force the other hen into a less-preferred location. This experiment only used pairs of hens in a cage and the possible combinations for preference presumably become more complex in commercial settings of group sizes of 5 to 20 hens, particularly when the number of nest sites (boxes) is limited. Clearly further research is required to answer the following types of questions: What is the biological significance of following behaviour, including any relationship with nest site selection? Is consistency of nest site selection associated with a preference for that site, or

are some birds forced to choose a less-preferred site? Are these behaviours the same with larger group sizes? Are there any implications for welfare?

Conclusion

In conclusion, this project focused on the welfare implications of providing furniture, *viz* a perch, nest box and dust bath, in cages for laying hens and also provided a limited examination on the welfare implications of space allowance and group size. The space and group size treatments were external to the main experiment and had limited replication and consequently the data should be treated with some caution. Nevertheless, they indicated that the physiological criteria were sufficiently sensitive to distinguish treatment effects. Hence, in relation to the larger experiment on furniture in cages, if there was an effect of any of the items of furniture, either alone or in combination, they were relatively minor, except for the positive impact of the presence of a perch on bone strength.

Furthermore, this project has provided evidence that, within the range of group size and space allowance treatments used in this experiment, space allowance in particular was probably more important for welfare than group size and items of cage furniture, such as a perch, dust bath or nest box. While there were some behavioural changes as a result of the furniture, particularly the presence of a perch, such as effects on foot condition, feather cleanliness, feather damage/cover, and egg laying intervals, these data are difficult to interpret in terms of welfare. Dust bath occupancy generally could not occur if the cage did not have a perch, and the presence of a perch increased the proportion of eggs laid in the nest box from 29-31 weeks of age. These data are difficult to interpret in terms of welfare and this highlights the importance of some basic research to clarify the methodologies used to assess welfare. A perch appeared to have a greater impact on improved bone strength than increased space. The choice of a preferred site for egg laying appeared highly variable with 30-50 % of hens apparently “choosing” to lay on the wire floor even when a nest was present. Further research is clearly required to determine the welfare consequences of birds laying eggs in either preferred or non-preferred sites.

Chapter 1

Introduction

There is increasing pressure in Europe to find alternatives to conventional cage systems for egg production and one such alternative is the furnished cage. For example the EU has agreed (Directive 99/74/EC; EU Directive, 1999) that conventional cages are to be replaced with furnished cages by 2013; furnished cages contain a perch, nest box and dust bath. Although there appears to be an inability both within and outside of science to agree on the need for non-cage systems (eg. see Carter and Carter, 1992), nevertheless alternative housing systems are being actively promoted in some countries. For example, Switzerland and Sweden have banned the conventional cage, The Netherlands have been encouraging producers to use non-cage systems and in the UK, marketing initiatives surrounding programs such as 'Freedom Foods' preclude the use of conventional cages. While it is sometimes difficult to fully understand the motives for some of these initiatives, as they are often a complex intermingling of economics, public perceptions and political expediency, there is a desire either to improve the welfare of birds or remove the issue from the political agenda. Some Australian producers recognise the 'pressures' on conventional cage housing and are pursuing alternatives to the conventional cage eg. barn systems.

Overseas, the proponents of alternatives to conventional cages are in two main camps: Those proposing non-cage systems and those proposing furnished cage systems (ie. cages that may include perches, dust baths and nest boxes). Again, these persuasions can be affected by the political agenda. For example, in Sweden where conventional cages are to be banned, there has been on-going research on non-cage alternatives (Tauson *et al.*, 1992; Abrahamsson and Tauson, 1995). Nevertheless, as Sweden does not permit beak trimming, the performance and welfare of birds in the non-cage systems (in the above studies) was poorer than in cages. The result is support, by default, for furnished modified cages as an acceptable alternative to the conventional cage (Tauson and Abrahamsson, 1994a; Tauson, 1995) and in the UK, where furnished modified cages were developed (Appleby, 1993a; Hughes and Sherwin, 1994) there appears to be considerable support for this system(s). Also, on the basis that the five freedoms (see Appleby, 1991) prescribed in 'Freedom Foods' (UK, RSPCA marketing initiative, see above) cannot all be met in conventional cages (Appleby, 1993b; Baxter, 1994), this tends to lend support for furnished cages. However, it must be recognised there is some circularity in this latter argument as included in the five freedoms concept is the perception that close confinement is unacceptable. In the USA the welfare pressures on the laying industry appear less than in Europe (see Craig and Swanson, 1994).

Furniture in cages

There has been considerable research on modifying conventional cages. This has included simple modifications such as inclusion of a perch to reduce the risk of bone breakage by increasing bone strength or volume (Appleby *et al.,* 1992; Abrahamsson and Tauson, 1993; Hughes *et al.*, 1993; Sherwin, 1993; Wilson *et al.*, 1993; Alvey and Tucker, 1994; Fleming *et al.*, 1994), an abrasive strip to maintain claw length to reduce the risk of entrapment, based on the work of Tauson (1986) and Niekerk and Reuvekamp (1994) or modifying cage fronts, by having horizontal bars to increase concurrent feeding behaviour (Sherwin *et al.*, 1993; Tanaka *et al.*, 1993). There are more sophisticated systems that also include nest boxes and/or dust baths, to provide birds with the opportunity to perform nesting and dust bathing behaviours (Nicol, 1992; Petersen, 1992; Reed and Nicol, 1992; Sherwin and Nicol, 1992; Appleby *et al.*, 1993, 1994; Sherwin, 1993; Reed, 1994; Hughes and Sherwin, 1994;

Appleby and Hughes, 1995; Petherick *et al.*, 1995; Abrahamsson and Tauson, 1997; Tauson, 2002; Tauson and Holm, 2002).

Perches and bone strength

There is a recent comprehensive review of perches in conventional cages and nest boxes and dust baths in modified cages (Ekstrand and Keeling, 1994), thus it is not necessary to reiterate all that literature in this review. These authors conclude that, because of the potential benefits of perches in relation to increasing leg bone strength, reducing feed intake and keeping birds calmer, and their low cost of installation, cages should contain a suitable perch. However, the perch has to be correctly positioned, although the data are equivocal and there is still the risk of bone breakage during the depopulation process. Abrahamsson and Tauson (1993) suggest that perches should be 17 cm from the back rather than centrally placed (24 cm from the back) to improve cage hygiene without restricting bird movement. However, Alvey and Tucker (1994) showed reduced bone breakages on depopulation when perches were 18 *vs* 13 cm from the back of the cage (cage dimensions were not reported). In the latter study, the presence of perches had no effect on the strength of the tibia. Thus, while the mechanism for reducing bone breakages is unclear, Knowles *et al.* (1993) have shown a reduced risk of bone breakages (from birds in cages) in birds with stronger bones. Also, to add to the confusion in the literature, birds reared up to 18 weeks of age in cages had stronger humeri bones and fewer broken bones at the end of lay compared to floor reared birds (Gregory *et al.*, 1991).

A study by Barnett *et al.* (1997a) confirmed that perches (21 and 24 cm from the back and front of the cage, respectively) resulted in increased strength of the tibia and also resulted in increased dirty and cracked eggs (Glatz and Barnett, 1996). While placing the perch further back in the cage may reduce the incidence of dirty eggs, most studies agree that the incidence of cracked eggs is increased. An economic assessment of the production data from this experiment based on the variable: egg income minus feed costs, showed that perches on their own reduced the financial return compared to conventional cages (financial returns were 1.49 *vs* 1.52 cents/bird/day), although if solid sides were also included in the cage returns were improved (1.68 cents/bird/day) (Barnett *et al.,* 1997b). Further validation of this economic analysis of perches in cages under Australian conditions is required if the welfare advantages of incorporating perches into cages are to be maximised by industry. As mentioned previously, the risk of bone breakage is still generally apparent when cages are depopulated, notwithstanding the presence of a perch. Removing birds from cages by both legs significantly reduced the percent of femur breakages from 7.4 to 0.6 % of birds (Gregory *et al.*, 1992) and if validated in the Australian industry should be a recommended procedure during depopulation. Other factors such as lighting regimes have not affected bone strength (Gregory *et al.*, 1993), while using drugs (bisphosphonates) developed for treatment of osteoporosis in humans improved bone morphology (Thorp *et al.*, 1993) and a study by Koelkebeck *et al.* (1993) showed an increase in bone strength by providing carbonated drinking water during warm weather. Also, the relationships between diet, growth rate, egg production and osteoporosis, being developed into a model of osteoporosis by Parkinson *et al.* (1996), should result in practical methods of dietary manipulation to reduce the incidence of osteoporosis. Innovations such as these should be researched to help minimise the impact of low bone strength on bird welfare.

Current recommendations for perches are for elliptical wooden perches with flattened tops and bottoms (vertical cross section of 3.1 cm and horizontal cross section of 3.6 cm) installed 17 cm from the back of a 48 cm deep cage and 7-7.5 cm above the floor and with sufficient perch space (15 to 18 cm per hen, although Appleby (1995) indicates 14 cm is adequate for medium weight hybrids) so that all birds can perch simultaneously. This shape of perch reduces the incidence of bumble-foot (inflamed foot pads) compared to rectangular perches. Plastic perches increase the incidence of bumble-foot (Oester, 1994). In the study of Glatz and Barnett (1996) where rectangular perches were used, the foot condition which was subjectively assessed using the 4 point scoring system of Tauson (1984a), was worse

in cages with perches, but the differences were only small (3.6 *vs* 3.8 for birds in cages with and without perches, respectively).

Nest boxes, dust baths and environmental enrichment

The review by Ekstrand and Keeling (1994) also provides evidence to support the inclusion of nest boxes and dust baths in cages ie. furnished modified cages. Duncan (1992) considers the lack of a nest site in conventional cages is the biggest welfare problem in this system of housing. The importance of the nest box is based on evidence of preference tests and evidence of frustration in the absence of a nest box (see review by Ekstrand and Keeling 1994) and the strong motivation of hens to use a nest (Smith *et al.* 1990). Cooper and Appleby (1995) have considered the controversy as to whether animals can be frustrated or experience a sense of deprivation by not having certain resources they have never experienced. For nesting, they found no differences in the motivation of birds to use a nest between birds previously experienced or inexperienced with a nest, although it is not known if this leads to chronic frustration. However, Hughes *et al.* (1994) showed that naive birds did not recognise a visual stimulus with some features of a nest, although it must be recognised that the birds in this study were unable to physically interact with the 'nest'. A study by Webster and Hurnik (1994) suggests that birds may synchronise their behaviours within cages and this may have welfare implications if nest sites are limited. However, as indicated above, while there has been considerable work on nest boxes, there are no current clearly accepted design recommendations that satisfy both the hen and industry requirements. Aspects examined have been nesting material where a preference was shown for artificial-turf nests over roll-away nests or those with litter (Appleby *et al.* 1993a), nest floor preferences (plastic floors were preferred although there was no aversion to wire floors; Sherwin and Nicol, 1994) and size and quantity of nests, nest height, nest floor surface and nest partitions (Reed, 1994). The latter author has provided design recommendations on a nest/cage design for 4 birds: Three nests were incorporated at the rear of the cage, in the form of pre-moulded, roll-away, plastic, flat-floored nests with hollows (25 x 31 cm/nest) and with the floors flush with the cage floors. Attractiveness was enhanced by lining the hollows with smooth neoprene rubber. To compensate for the lack of a peckable substrate, strips of artificial grass were attached to the rear of each nest. A perch was provided to reduce nest soiling.

Nevertheless, in spite of the above recommendations, the problems to be overcome include roosting in the nest boxes, laying eggs outside of nest boxes, the higher incidence of cracked eggs and using the nesting material, when it is provided, for a dust bath. To reduce the use of the nest box as a dust bath, dust baths have generally been provided in furnished cages although the welfare evidence for their inclusion appears less than for nest boxes. For example, Petherick *et al.* (1993) suggest birds are not highly motivated to dustbathe, while Liere (1992) suggests that dust baths are essential to maintain feather integrity and for welfare. Notwithstanding any possible direct effects of dust baths on welfare, dust baths appear to increase the effectiveness of nest boxes, by separating nesting and dust bathing behaviours to different areas of the cage. Studies have shown that hens do not make any great effort to obtain access to litter or sand (Faure, 1991; Faure and Lagadic, 1994), although they prefer litter to wire mesh (Lagadic, 1992). Also, in experiments with young chickens, Sanotra *et al.* (1995) indicate a risk of pathological feather pecking when straw or wood-shavings are used as a substrate, although Norgaard-Nielsen *et al.* (1993) showed that rearing with access to sand or peat reduced subsequent feather pecking and that access to straw, as an environmental enrichment, during the layer phase also reduced feather pecking. Rudkin (1996) has also shown positive effects of hay, both during rearing and the laying period in reducing feather pecking. Other forms of environmental enrichment such as adding objects to feed troughs are considered to improve welfare (Sherwin, 1995) and coloured plastic enrichment devices placed in cages reduced mortalities through a redirection of pecking behaviour (Bell *et al.*, 1995). If these enrichment devices are practical, they may be a simple way of reducing mortalities.

While many of the practical problems of nest boxes and dust baths have been overcome, furnished cages appear to require some development prior to their introduction into the commercial industry. Studies are currently underway in the UK in a semi-commercial environment (see Elson, 2004). It has been estimated (Elson, 1994) that these modifications will increase egg production costs by 10-20 % over conventional cages. Also, surveys of public opinion suggest that furnished cages are only slightly more acceptable than conventional cages (Rogers *et al.,*1989) and this needs to be taken into account in a cost benefit analysis for the Australian industry, although as indicated by Duncan (1992) more focus should be put on bird welfare rather than public perceptions. Nevertheless, the Australian industry has been under pressure to adopt furnished cages.

Abrasive claw shorteners

As well as the above recommendations, another recommendation, based on the work of Tauson (1986), is the inclusion of abrasive strips on the manure deflector in front of the feed trough, to reduce both the risk of entrapment and of claws overgrowing and breaking; the strip should be 8 mm wide. A number of claw shortening devices/materials have been developed, including abrasive paste, ceramic strip, coined steel plate, tungsten carbide coated plate, small holes punched in steel plate, emery cloth and abrasive tape and are available from several manufacturers (Elson, 2002) and if correctly fitted, most appear effective (Elson, 2002, 2003), although some claw shorteners appear more durable than others (Elson, 2004).

While the above indicates there has been considerable effort put into getting furnished cages to work, and there is some research on design criteria for perches and nest boxes, the welfare benefits appear to have been largely assumed. On the one hand this is somewhat surprising considering the likely costs, but on the other hand fits a predominantly European perspective of assessing welfare based on the concept of the 'five freedoms' and the underlying rationale that an increased behavioural repertoire promotes improved welfare. This is predicated by the belief of the UK Farm Animal Welfare Council that animal welfare should be considered with reference to the 'five freedoms' (FAWC, 1992, 1997). In the broader context of poultry housing systems and the 'five freedoms', it has been broadly claimed that cages are unable to meet the 'freedom to express normal behaviour'. However, as indicated by Elson (2004), three other freedoms, namely freedom from discomfort, freedom from pain, injury and disease and freedom from fear and distress, are compromised to varying degrees in non-cage systems. He indicates that on balance, alternative systems are unlikely to satisfy the five freedoms any better than cages (Elson, 2004).

The following experiment examines the welfare benefits of the items of cage furniture in furnished cages using the functional (homeostasis) approach to assessing animal welfare. To assist put this approach in perspective it is worthwhile to briefly consider the current approaches to welfare assessment.

Welfare Assessment

Our value systems for animals tend to operate at two levels (Broom and Johnson, 1993). Private attitudes, which may be difficult to enunciate, may be ascribed to belief, intuition, learning or experience. Communal value systems, which may arise from family, culture or some other social belief, are rarely upheld unanimously in the population, but may be supported by a sizeable proportion of it (for example, bull-fighting, particular practice of

animal slaughter, and so on) and perhaps be reinforced by legislative control or codes of practice.

Some have argued that science and ethics cannot be separated in any discussion of animal welfare. For example, some authors have used the term 'animal welfare' to refer to an animal's quality of life (Duncan and Fraser, 1997). Furthermore, they consider that our conception of animal welfare involves values as well as information, and others consider that a conventional definition of animal welfare does little more than establish the general area of discourse (Duncan and Dawkins, 1983). The widespread variation in science, philosophy and the general community in the definition of animal welfare has created considerable confusion and controversy that has hindered attempts to study animal welfare. Without a clear definition, welfare cannot be studied because it cannot be measured either directly or indirectly.

Barnett and Hemsworth (2003) believe that the most credible scientific definition of animal welfare is the following one: "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986). Using this definition, welfare risks can be assessed in terms of firstly, how much has to be done by the animal in order to cope with the environmental imposition and secondly, the extent to which the animal's coping attempts are succeeding. The rationale for this definition is considered in more detail later.

Therefore, with a satisfactory scientific definition of welfare, and this is presently open to considerable discussion within science, it should be the aim of science to provide an objective assessment of the welfare risks associated with the practice under question. Science therefore should aim to provide the facts on how well animals adapt to a housing or husbandry practice (that is, the welfare risks). Such information, together with the individual's value system, assists the individual in deciding whether or not a particular practice that imposes on an animal's welfare is acceptable (that is, whether or not the welfare risks are acceptable).

There is an ongoing debate about the role of science in the welfare debate and whether welfare involves both facts and ethics (Rollin, 1995). We suggest that an individual's opinion of an animal welfare issue is influenced by their values (for example, moral duties to the animals) together with their knowledge of how the issue in question imposes on the animals. Science is a descriptive discipline aimed at explanation and then prediction. Using science we try to discover and articulate natural laws and regularities that govern the behaviour and relationships of objects in the natural world (Comstock, 2000). Thus the role of science in addressing the welfare of domestic animals is to generate facts on how animals respond to the practice/conditions under question, while individuals will make value judgements to determine whether or not the practice is acceptable to them. A continuing difficulty confronting scientists studying animal welfare has been the definition of animal welfare. Although some may not agree, a solution to this impasse is that the role of science is to provide facts on how well animals cope with their environment. Such a consideration includes the issues of emotions, natural behaviours in natural settings and preferences. The issue of whether or not animals require environments that provide more than those that address their 'basic' biological requirements (that is, pleasure) is another level of discussion that needs to occur. Nevertheless, if we can develop a consensus that those conditions that create biological dysfunction are the most serious for animals, then we can probably reach some agreement that it is these issues that are the most important to be promptly addressed. Notwithstanding the possible conservative nature of this approach, it will allow significant progress to be made in improving animal welfare.

There are 5 broad approaches used by scientists in studying animal welfare:

- the 'feelings-based' approach,
- the 'animal-choices' approach,

- the ‘nature of the species’ approach,
- the ‘ freedoms for animals’ approach, and
- the ‘functioning-based’ approach or the ‘homeostasis-based’ approach.

These 5 approaches will be briefly considered here.

Feelings

This approach defines animal welfare in terms of emotions and thus it emphasises reductions in negative emotions such as pain and fear, and increases in positive emotions such as comfort and pleasure (Duncan and Fraser, 1997).

The modern notion of emotions in both the animal behaviour and psychology literature highlights the linkage between visceral or bodily arousal and cognitive processes (Bolles, 1981; Mandler, 1981). Any discrepancy or any interruption of expectations or of intended actions, produces undifferentiated visceral (autonomic) arousal and the associated sensation of the emotion, whether positive or negative, depends on the cognitive evaluation of this discrepancy or conflict between the state of the world and the expectations of the individual. While it is accepted that humans have a great variety of emotions, animal behaviourists generally consider that animals are restricted to a few basic emotions such as anger, fear, joy and happiness. This is predicated on the view that animals probably only have emotions to deal with certain kinds of survival problems, for which there is some strong evolutionary benefit. For example, while we might expect animals to show fear because of the adaptive value of being frightened in a dangerous situation, there is no reason to expect animals for example, to show pity to other species because there would be no clear adaptive advantage if they did (Bolles, 1981).

The difficulties in studying emotions as though they were objective states of bodily arousal is well recognised 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 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, however there are some examples in the literature that indicate that it is possible to assess the strength of emotions in animals in intuitively negative and positive emotionally arousing situations. Behavioural and physiological correlates of fear of humans by pigs demonstrate our ability to quantify the level of fear towards a specific stimulus in pigs (Hemsworth and Barnett, 1987). Some of the motor patterns and neural changes presumably associated with emotions in humans and animals appear to be highly specific. Brain lesion studies and studies involving electrical stimulation of the brain indicate that particular neural circuits such as components of the limbic system appear to mediate or control emotions (Rosenzweig *et al.,* 1999). For example, rats learn to press a lever when the reward (reinforcement) was a brief burst of electrical stimulation of the septal area of the limbic system. Such studies indicate the potential to associate positive and negative emotions with specific behavioural and neural changes. Defining emotions to further develop the feelings-based assessment of welfare is likely to occur in the next 10 years and will provide a major contribution to the welfare debate (see Broom and Zanella, 2004).

Animal choices or preferences

Animals have functional systems controlling, for example, body temperature, nutritional state and social interactions. By investigating these functional systems and the associated motivational mechanisms, there is opportunity to identify the resources or stimuli in the environment that are required by or are important to animals, and thereby learn something about an animal's needs (Broom and Johnson, 1993). Some of these motivational systems can be regulated by physiological consequences (such as consumption of food), whereas others require the display of a particular behaviour (such as rooting behaviour in pigs).

Driven by the view that animal choices may indicate the existence of important underlying needs, there has been and continues to be considerable interest in studying the preferences of animals for resources, such as space, flooring and a parturition or nest site. The preferences of animals for resources can be studied by allowing the animals to choose between resources with preference being measured by either the time the animal spends with the resources or the resource that is selected. The simplest preference study involves allowing the animal to make a choice between two situations in which the resource is varied. For example, it was found that laying hens preferred a spacious cage to a confined cage and that neither time of day nor strain of bird was influential in this choice (Hughes, 1975). Observing animals in complex environments that provide a range of activities will also provide details of the animal's preference for habitats and resources (Broom and Johnson, 1993).

In an attempt to measure the strength of an animal's choice, scientists have incorporated tasks in which the animal has to expend energy or take risks in gaining access to an alternative resource. For example, operant conditioning techniques, in which an animal learns to perform a response, such as a lever pressing, to gain access to an alternative resource, have been used to measure the value that the animal puts on the resource. Pre-parturient sows worked harder on the basis of lever lifting to gain food than access to straw (Hutson, 1992). Consumer demand theory has been used with preference testing to put a value on the animal's choice (Dawkins, 1983): The strength of motivation ('need') for a resource can be measured through the animal's willingness to consume ('work' for) the resource as the 'price' of the resource increases. Thus, by measuring consumption at increasing prices, needs can be classified as necessities where the animal works harder to maintain consumption (called an inelastic demand function) or luxuries where the animal does not maintain consumption by working harder (elastic demand function). Using this approach needs can be ranked in terms of their demand functions.

Preference or choice testing has been criticised on several grounds and further research, not only on methodological issues, but also on understanding the principles underpinning the animal's decision is required (Lawrence and Illius, 1997). For example, one of the most serious challenges to this approach is that an animal's short term choice may reflect its proximate (immediate) needs, which are likely to vary markedly over time, rather than the animal's ultimate needs or those necessary for survival, growth and reproduction (Lawrence and Illius, 1997). For example, since an animal's choice between feed and space will be markedly affected by short term changes in hunger, the choice of space is more likely immediately after feeding rather than before.

In attempting to determine what animals need it is worthwhile to consider a need as being indicative of having a deficiency, often manifested as a homeostatic maladjustment. A need can therefore be defined as a requirement, which is fundamental in the biology of an animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus (Broom and Johnson, 1993). Hence, some needs are for food, water or heat, but others are for a certain behaviour such as grooming, exercising or nest building to occur. When an animal has an unsatisfied need, its motivational state will usually elicit behavioural and physiological responses that remedy that need, so the individual will be able to cope with its environment. If a need cannot be satisfied, the consequence in either the short term or the long term will be poor welfare.

A problem associated with the use of the word 'need', especially in legislation, is that the deficiencies involved range from the rapidly life-threatening to those which are relatively harmless in the short term. Most of what is strongly avoided is harmful and most of what is strongly preferred is beneficial. However, some of what is wished for is not necessary, in the sense of essential for life, so the references to 'fundamental to the biology of the animal' and to 'deficiency' in the definition and understanding of 'need' are valuable.

Nature of the species

The principle underlying this approach is that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways (Duncan and Fraser, 1997). This approach is reflected in the much quoted proposal that has been incorporated into the 'five freedoms' requirement for animals: animals should have the 'freedom to perform natural behaviour' (Webster and Nicol, 1998). However, of all the approaches to assess welfare, the nature of the species approach has least scientific credibility because it fails to define both 'natural' and the welfare risks if such 'natural' conditions are not provided. Until these attributes are rigorously defined for both the 'nature' approach to welfare assessment and elements of the five freedoms, such approaches may be used to reflect an ethical position but are not open to scientific scrutiny.

The view that animals should perform their full 'repertoire' of behaviour was common in early welfare research, but there are a number of shortcomings as a criterion for animal welfare (Dawkins, 1980). 'Wild' behaviour often represents an animal's efforts to survive in a life and death struggle and therefore many of these responses are adaptations to cope with extreme adverse situations. Such situations clearly reduce animal welfare and are thus situations from which domestic animals should be spared. Furthermore, mortality is generally higher in wild populations than domestic ones. For example, piglet mortality in the wild boar (*Sus scrofa*) often exceeds 25% (Kirkwood *et al.,* 1987), a situation that clearly would be unacceptable in commercially raised pigs. Thus the 'natural behaviours' that are desirable or undesirable in terms of animal welfare require definition together with the rationale for their inclusion or exclusion. To date there are no such agreed definitions or rationales.

The notion that we can improve animal welfare by respecting the 'nature' of animals is intuitively appealing (Duncan and Fraser, 1977). However modern domestic animals are the product of thousands of generations of selective breeding and consequently the behaviour and physiology of domestic animals have been modified during domestication (Mills *et al.,* 1997). While the behaviour of domestic animals in wild or semi-wild conditions is often similar to their wild relatives, there are differences in the behavioural responses and thresholds to a number of stimuli such as sexual stimuli, novel stimuli, humans and environmental conditions (Broom and Johnson, 1993; Craig, 1981).

The Five Freedoms

The starting point for the Five Freedoms was the UK Report of the Brambell Committee (Brambell *et al.,* 1965), which concluded, amongst other things, that all intensively housed animals should be provided with sufficient space to be able to stand up, lie down, turn around, groom themselves and stretch. With developments over subsequent years, such behavioural requirements became known as the Five Freedoms. The UK Farm Animal Welfare Council proposed in 1992 that the welfare of animals can be protected by recognising the Five Freedoms (FAWC, 1992):

- Freedom from hunger and thirst,
- Freedom from discomfort,
- Freedom from pain, injury and disease,
- Freedom to express normal behaviour,
- Freedom from fear and distress.

While most would agree with the ethical basis of this general approach, it requires a number of definitions, which are often not provided by proponents. For example, freedoms 2 and 4 clearly require definition. Discomfort, particularly in relation to consequences for welfare, requires definition, and as discussed in the previous section on the 'nature of the species' approach, the normal behaviours that are both desirable and undesirable, from a welfare perspective, require definition. Similarly, the levels of hunger, fear and stress that may impinge on animal welfare require clarification. Until these definitions and clarifications are

made and widely agreed, as indicated in the previous section, the five freedoms approach to welfare assessment may be used to reflect an ethical position, but is not open to scientific scrutiny.

Homeostasis

The definition of animal welfare that underpins this approach is "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986). In this definition, the "state as regards attempts to cope" refers to both how much has to be done by the animal in order to cope with the environment and the extent to which the animal's coping attempts are succeeding. Attempts to cope include the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses. The extent to which coping attempts are succeeding refers to the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, health and freedom from injury. Therefore, using such a definition, the risks to the welfare of an animal by an environmental challenge can be assessed at two levels: firstly the magnitude of the behavioural and physiological responses and secondly the biological or fitness costs of these responses (Broom and Johnson, 1993; Barnett and Hutson, 1987; Hemsworth and Coleman, 1998; Barnett, 2003).

A subtle but important component of this approach, therefore, is that welfare is considered within the concept of biological fitness (Broom and Johnson, 1993; Hemsworth *et al.,* 1996; Fraser and Broom, 1990). This concept of biological fitness generally applies to natural populations and refers to 'fitter' animals having a greater genetic contribution to subsequent generations (Pianka, 1974); this is based on their abilities to successfully survive, grow and reproduce. While the last attribute may not always apply to individual farm animals since reproduction is either controlled or absent for many farm animals, the ability to grow, survive and reproduce could be considered measurements of 'fitness' within the limits of the management system. Most production systems in agriculture have breeding and growing components and these can generate considerable data on reproductive success of individuals. For example, conception rates and mortality, morbidity and growth of offspring can be used as a measure of 'fitness'. Similarly, reproductive performance of domestic species has been linked with welfare (Beilharz and Zeeb, 1981; Beilharz, 1982).

An attribute of the 'homeostasis' approach that affords this approach credibility within scientific circles is that it contains some widely accepted criteria of poor welfare. Furthermore, there are some excellent examples of the value of this 'homeostasis' approach in assessing animal welfare (Hemsworth and Coleman, 1998). For example, handling studies on both young and adult pigs have shown that fearful pigs have a sustained elevation of plasma free corticosteroid concentrations; the consequences of this chronic stress response in these fearful animals include depressions in the growth and reproductive performance (Hemsworth and Barnett, 1991; Hemsworth *et al.,* 1981, 1986).

A counter argument is that this example involves extreme effects and our current knowledge may not allow detection of more subtle or less serious risks to welfare. The example of fearful pigs clearly demonstrates the consequences of animals failing to cope with an environmental change: such biological changes and biological costs for the animal clearly enable the interpretation with some considerable degree of confidence that the welfare of these animals is seriously compromised. Nevertheless, less serious challenges should be reflected in biological changes, admittedly of lower magnitude, with consequent effects on fitness variables such as growth, reproduction, injury and health. Short term challenges can also be studied with this approach. The behavioural and physiological responses of cattle to two branding procedures were studied to assess the relative aversiveness of the procedures (Lay *et al.,* 1992), while behavioural and physiological responses together with growth performance were utilised to assess the welfare implications of a husbandry procedure regularly imposed (daily injections) on pigs (Hemsworth *et al.,* 1996).

Repetitive and stereotyped behaviours are part of the biological response of animals to a long term challenge and it is appropriate to consider stereotypies within the homeostasis approach to welfare assessment. Stereotypic behaviour can be defined as those behaviours that consist of morphologically identical movements that are regularly repeated, have no obvious function, or are unusual in the context of their performance (Cronin *et al.,* 1986). Examples of these behaviours are chain pulling, bar biting, sham chewing, head weaving and excessive drinking.

There has and continues to be considerable controversy on the causation and function of stereotypies in farm animals. A brief review of some examples from the literature demonstrates this controversy. Excessive chain manipulation by sows is a stereotypy seen in gestating sows housed on tethers and it has been shown that food restriction contributes to the development of this stereotypy (Terlouw *et al.,* 1991). The authors have postulated that the appetitive behaviour of foraging may persist and develop into a stereotypy in these sows because these appetitive sequences are positively reinforcing and there is also insufficient negative feedback from the consummatory behaviour (feeding) and its functional components (food). Unavoidable fear or stress and barren and restrictive environments have also been implicated in the development of other stereotypies. Examples are body-rocking in mentally handicapped patients when distressed and where the occurrence of stereotypies increases with increasing confinement (Mason, 1991). It has also been proposed that some forms of stereotypies reduce responses to aversion by affecting the animal's perception of the situation (Cooper and Nicol, 1991). Thus it is clear that different forms of stereotypies may have different causes, such as frustration, stress and lack of control and stimulation, however our understanding of the motivational basis of stereotypies is poor.

A similar controversy exists in relation to the function of stereotypies. Based on early evidence of associations between stereotypies and physiological signs of coping such as reduced corticosteroid concentrations, reduced adrenal gland weights and reduced ulceration, there is a view that stereotypies may be a coping response. However more recent studies and re-interpretation of some of the early evidence, questions this general coping hypothesis for at least some forms of stereotypic behaviours (Mason, 1991; Rushen, 1993). Furthermore, while some evidence exists to indicate that stereotypies may be coping mechanisms in the short term, it is unknown whether they exert benefits in the long term. Irrespective of the function of stereotypies, the existence of a stereotypy is indicative at the least of a past problem for the animal in coping with its conditions. Stereotypies that result in physical damage to or illness in the animal (such as the development of lesions in stall-housed sows that persistently rub their tail roots from side to side against stall fittings or wind-sucking in horses where persistent wind-sucking can lead to colic) have obvious and immediate implications for the welfare of farm animals. Thus stereotypies should not be used alone to assess risks to animal welfare: they should be used together with other biological responses and consequent effects on biological fitness.

There are two contentious issues that we recognise in using the homeostasis approach to scientifically assess animal welfare. One involves the definition of animal welfare and the other is whether the homeostasis approach for the assessment of animal welfare adequately includes feelings. The homeostasis approach utilises the definition of animal welfare proposed by Broom (1986) and is based on the premise that maladaptation generates animal welfare problems. Adaptation is considered, at the individual animal level, to involve behavioural and physiological responses that assist the individual to cope with its environmental conditions (Broom and Johnson, 1993). There may be some disagreement within science on the appropriateness of this definition. For example, some argue that animal welfare only concerns animal feelings (Duncan, 1996). Nevertheless, as discussed earlier, we and others believe that Broom’s definition is more widely accepted both within science and by the wider community. Without a consensus on a definition of animal welfare

there can be no consensus on the scientific assessment of animal welfare. In relation to the issue of feelings, Broom has emphasised the evolutionary advantage of feelings (Broom, 1998). As others have proposed (Cabanac, 1979; Broom, 1998, 2001; Rolls, 1999), Barnett and Hemsworth believe that feelings are part of the body's regulatory system and function to either remove animals from harmful situations or attract animals to beneficial situations (Barnett and Hemsworth, 2003).

Another criticism of the homeostasis approach is that it is conservative and, because of methodological limitations, a lack of difference using this approach may not mean that welfare is unaffected. Nevertheless, its conservatism should be seen as a strength, because, if differences are found, they are likely to be of importance to the animal. This approach has been successfully used to demonstrate changes in welfare due to physical and social environments and in situations that affect an animal emotion, namely fear (Hemsworth and Coleman, 1998). There is a wide acceptance of this approach when it demonstrates risks to welfare, for example, due to overcrowding or chronic fear responses. However, the lack of responses, particularly in situations to which humans have some degree of antipathy, such as confinement housing systems, is often seen as indicative of inadequate methodology. That is, if the data do not align with some individuals' perceptions, common responses are to criticise the methodology or change the definition of welfare. There is no argument that better methodology is required, however, it is not logical to accept or deny the same methodology on the basis of its fit with human perceptions. Similarly, changing the definition of welfare from something that can both be defined and measured, to include words such as 'suffering' or 'ethical values' that cannot be assessed using the scientific method, because of a lack of definition, cannot be seen as a forward step in the welfare debate. Indeed, at the present state of knowledge, to attempt to measure suffering as an entity could be considered a misdirection of limited resources, both in terms of monies and in terms of improving animal welfare. It could lead to similar open-ended arguments that occur in the definition of welfare and could provide yet another excuse for making only limited gains for animals' welfare. Peter Medawar (1986) suggests "there is no limit upon the ability of science to answer the kind of questions that science can answer". The question of animal suffering and ethical values are holistic entities not open to scientific investigation.

Conclusions on welfare assessment

With our present knowledge, the 'homeostasis' approach appears to offer science the best assessment of the welfare of animals. As a research tool, this approach involves comparing housing systems or husbandry procedures and risks to welfare are assessed on the basis of relative changes in biological (behavioural and physiological) responses and corresponding decreases in fitness. Assessing motivation using preference testing has the potential to measure the animal's important underlying needs, and thus provides a valuable addition to the homeostasis approach in studying animal welfare.

In the future, there are obvious opportunities to integrate the 'feelings' approach within the 'homeostasis' approach. If we accept that emotions in animals are important adaptive responses that assist survival, it is an easy step to recognise that the visceral or bodily arousal, the cognitive processes and the associated sensation of the emotion are part of the animal's biological response to the challenge. Indeed, emotions may have some adaptive advantage such as acting as a reinforcer (Broom, 1998), which makes it more likely that the individual will learn to carry out the adaptive action. Further indication of the adaptive function of emotions is that they can modulate memory formation in several ways (Reisberg and Heuer, 1995). Studies principally on laboratory rodents have shown that a fear-provoking stressor, presumably via its effects on hormones in the sympathetic-adrenomedullary axis and hypothalamic-pituitary-adrenal axis, may play an important role in memory formation and recall (Mendl, 1999). Some of these effects can be viewed as having adaptive value in helping the animal to search, scrutinise and remember threatening stimuli or situations.

Along similar lines, it has been proposed that feelings or emotions are involved in monitoring the effectiveness of regulatory actions, being positive when the regulation is successful (homeostasis is achieved) and negative when it is not (Wiepkema, 1985). Similarly an emotion, such as pleasure or anxiety, has been considered as a functional state of the animal induced by specific signals which rapidly organise response systems (approach or avoidance) relevant to broad categories of relevant stimuli (Spruijt and Frijtag, 1999). Interestingly, this general view has been extended by suggesting that the animal's tolerance or sensitivity to rewarding and aversive stimuli may be closely related to the state of the animal in terms of welfare (Spruijt and Frijtag, 1999). In fact, the authors have proposed that, together with neurobiological knowledge, an increased insight into the welfare of the animal can be gained by measuring the anticipatory behaviour of the animal for rewards in a Pavlovian conditioning paradigm: animals deprived of essential stimuli react more readily not only to stimuli that they are deprived of but also rewarding and aversive stimuli in general. Such philosophical discussions accompanied by experimental validation will assist in further developing the concept of welfare. These attempts to conceptualise animal welfare will lead to further development and refinement of the methodology to study animal welfare. This limited discussion on integrating the two research approaches, the feelings and homeostasis approaches, demonstrates not only how the concept of welfare has and will continue to develop, but that increased agreement amongst scientists on the concept of welfare will lead to greater consensus on ways to study animal welfare. There is a wide acceptance of the scientific method in problem solving and its ability to contribute to our understanding of the factors that contribute to welfare. It would be unfortunate, in relation to improving animal welfare, if agreement cannot be reached on a single definition of animal welfare. There would appear to be no benefits in having a scientific definition and another that includes aspects that cannot be resolved by the scientific method. Public perceptions are not ignored in the welfare debate. They are a quite rightly a significant driver in raising questions, but from a scientific perspective they are not part of the answer. With our present knowledge, the most scientifically credible approach to welfare assessment involves measuring the magnitude of the biological responses to the challenge and also the consequences of these behavioural and physiological responses on the animal's ability to grow, reproduce and remain healthy. Information on the animal's preferences for resources should provide valuable information complementing this approach. This is the approach that is utilised in this report.

Chapter 2

Objectives

- To determine the relative welfare and productivity of laying hens in furnished cages and modern conventional cages by undertaking detailed experiments on the behaviour and physiology of birds in furnished and conventional cages in a controlled research environment.

- To determine the effects of the contribution of items of furniture in furnished cages on the welfare of hens.

Chapter 3

Experiment 1 – Welfare of Hens in Furnished Cages

Introduction

There has been considerable research on modifying conventional cages. This has included simple modifications such as inclusion of a perch to reduce the risk of bone breakage by increasing bone strength or volume (Appleby *et al.*, 1992; Abrahamsson and Tauson, 1993; Hughes *et al.*, 1993; Sherwin, 1993; Wilson *et al.*, 1993; Alvey and Tucker, 1994; Fleming *et al.*, 1994), an abrasive strip to maintain claw length to reduce the risk of entrapment, based on the work of Tauson (1986) and Niekerk and Reuvekamp (1994) or modifying cage fronts, by having horizontal bars to increase concurrent feeding behaviour (Sherwin *et al.*, 1993; Tanaka *et al.*, 1993). There are more sophisticated systems that also include nest boxes and/or dust baths, to provide birds with the opportunity to perform nesting and dust bathing behaviours ie. furnished cages (Nicol, 1992; Petersen, 1992; Reed and Nicol, 1992; Sherwin and Nicol, 1992; Appleby *et al.*, 1993, 1994; Sherwin, 1993; Reed, 1994; Hughes and Sherwin, 1994; Appleby and Hughes, 1995; Petherick *et al.*, 1995; Abrahamsson and Tauson, 1997; Tauson, 2002; Tauson and Holm, 2002). More recently, there has been research using furnished cages to examine issues such as cage height and group size (Drakley *et al.*, 2002; Albentosa and Cooper, 2004; Niekerk, *et al.*, 2002). Despite, the relatively few in-depth studies on furnished cages, there is considerable support for such designs of cage, particularly in Europe, based on the increased behavioural repertoire they permit. Probably the most discussed issue is the requirement for a nest, indeed Duncan (1992) considers the lack of a nest site in conventional cages is the biggest welfare problem in this system of housing. Past research on nests is detailed in Chapter 1. Despite this apparent support, particularly in Europe, there is still resistance to the introduction of furnished cages from animal welfare groups (Wilkins, 2004).

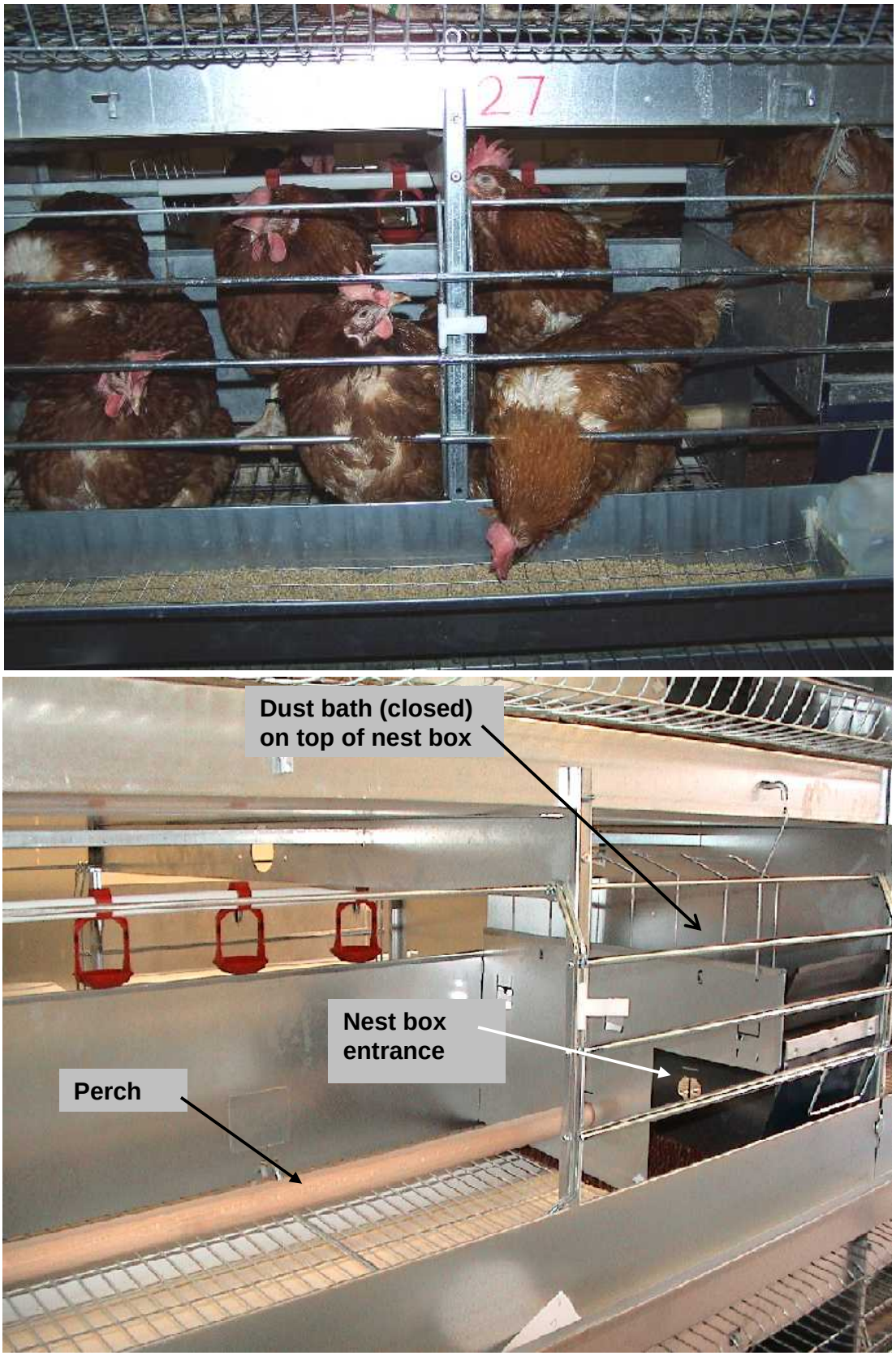
This experiment examined the welfare of hens housed in furnished cages and the effects of the individual items of furniture in a factorial experiment with 8 birds per cage. At the same time, in a preliminary experiment, it also examined the effect of increasing space per bird (8 birds in double-width cages) and the effect of stocking density (16 birds in double-width cages).

Methods

This experiment involved 20 treatments using 66 Victorsson Trivselburen furnished cages. The cages were imported from Sweden and during their installation they were modified to meet the experimental requirements. The cages were 1206 mm wide, 498 mm deep and 455 mm high at the rear of the cage. Double-width cages were formed by removing one side panel to provide a cage approximately 2.4 m wide. Each standard cage provided 3 water nipples, shared between the back-to-back cages and water was available *ad libitum*. The cages were located in a controlled climate shed with control for temperature, light and ventilation. The thermostats were set to 21 °C and average temperature was maintained at about 17 °C during the dark period and 23 °C during the light period. Lighting was provided by incandescent globes and controlled by a computer. At placement, the birds were on an initial light:dark cycle of 11:13 h. Day length was increased each week by 30 min, until the hens were exposed to an ongoing light:dark regime of 16 h light and 8 h dark at 26 weeks of age. At this stage, lights were programmed to come on at 0500 h. There was a daily

‘sunrise’ and ‘sunset’ of 30 min duration commencing at 0500 h and 2030 h, where the light level either slowly increased from dark or slowly decreased from light to dark, respectively. The birds were initially housed at about 20 lux during lights on, but this was decreased to 5 lux at 28 weeks of age, due to a slight increase in cannibalism. Humidity was maintained at ~40%.

Figure 1. Victorsson Furnished Cages (from Sweden) used in the Experiment



The birds were Hy-Line Brown and were floor reared and vaccinated at the appropriate ages for ILT, IB, Marek's disease, AE, MG and EDS. The pullets were transported in poultry crates to Werribee, Victoria (37°55'S, 144°40'E) at 15 weeks and 3 days of age and transferred to the cages. Initially 9 birds were housed in each cage to allow for mortalities in the first 6 weeks. The birds were weighed, identified with spiral coloured leg bands and housed at 8 birds per cage at 21 weeks of age; at this stage some birds were reallocated to other cages of the same treatment, so that body weight was consistent across treatments. The hens were initially fed a commercial (Ridley Agriproducts Ltd, Pakenham) grower crumble (16 % protein) and subsequently a commercial ration from the same company with 17 % crude protein. There were 1 or 2 eggs being laid each day by 18 weeks of age and about 20 % of birds were laying by 20 weeks of age.

Treatments were allocated to cages according to a 2 perch (perch *vs* no perch) by 3 nest box (nest box *vs* space *vs* no nest box) by 3 dust bath (dust bath *vs* space *vs* no dust bath) factorial plus 2 added large cage controls (8 hens or 16 hens) within a 3 replicate 4 x 5 rectangular lattice (Cochran and Cox 1957). The 'space' treatment within the furniture factorial experiment refers to the provision of the equivalent space and site of the nest box/dust bath without providing the items of furniture *per se*. This experimental design provided 3 separate replicates of each treatment and a further blocking of cages into groups of 4 cages within each replicate; there was an associated hidden replication (see discussion) which increased replication to 18 or 27, depending on the number of levels of the treatment. Replicates and blocks corresponded, as much as possible, to position of cage within the shed, order of filling cages with hens, order of husbandry operations, order of measurement procedures and order of assay procedures. The experimental unit was a cage of hens.

Description of the cages
Photographs of cages with furniture are shown in Figure 1. Floor space of a single cage with and without the nest/dust bath was 950 x 498 and 1200 x 498 mm, respectively. All cages were fitted with claw shorteners.

Description of the nest box
The nest box was 241 mm wide, as deep as the whole cage (498 mm) and about 270 mm high at the front of the cage. A blue vinyl egg flap was suspended in front of the nest box at the cage front to reduce light levels inside the nest box and to reduce the speed of eggs rolling out of the nest to reduce cracked eggs. Overlain and attached to the wire floor of the nest box was a 15 mm thick, rectangular piece of "astro turf" (370 x 220 mm).

Description of the dust bath
In the two treatments in which the dust bath was available to hens, the wire exclusion mechanism opened at about mid-day and remained open for 6.3 h daily. For the treatment that received "litter" in the dust bath, approximately 2 cups (500 mL) of hardwood sawdust was placed in the dust bath tray as required. To ensure that the "no litter" dust bath treatment (ie. dust bath space) did not have litter generated from settled air borne dust, the litter tray was cleaned using a vacuum cleaner approximately once per week.

Description of the perch
Perches were made from wood. Their "oval-shaped" cross-section measurements were 36 mm x 30 mm and the top and bottom surfaces were flat. The bottom of the perch was positioned about 90 mm above the wire floor and 190 mm from the rear and 230 mm from the claw-trimming plate below the feed tray at the front of the cage.

Hen and shed maintenance activities commenced daily at about 0930 h and were generally completed within 4 h. Routine activities included visual inspection to detect ill/unhealthy birds, monitoring shed temperature and humidity, testing water lines and drinkers,

assessing level of feed in the trough and if necessary, adding a weighed quantity of feed to the trough. The latter ensured feed was available *ad libitum* but limited feed wastage. Eggs were collected between 11.00 and 12.00 h and production was recorded from 20 weeks of age. Air-conditioner filters were cleaned of dust and the floor of the shed was vacuumed daily to minimise air-borne dust levels. The production parameters recorded each day were number of eggs/cage and the location of each egg ie. whether it was laid in the dust bath or appeared in front of the nest box or in another area of the roll-out tray. Feed added to each feed trough was recorded daily and feed left in the trough of each cage was recorded monthly.

There were 2 sampling periods, each conducted over an 8 week period. The first commenced at 29 weeks of age and the second at 59 weeks of age. There were 5 sets of measurements/observations taken in the following order at both sampling periods: 1) behaviour observations using video, 2) blood sampling for immunology, 3) collection of eggs for determination of corticosterone concentrations, 4) blood sampling for determination of corticosterone concentrations and 5) body weight and feather damage and cover scoring, claw length, foot condition scoring, injuries on the comb, around the cloaca and on the hen's back, keel bone deformation and blood sampling 60 min after injection of ACTH for determination of the corticosterone response. In addition, at the end of the experiment commencing at 67 weeks of age, 3 birds were randomly selected from each cage, euthanased and bones extracted for subsequent bone strength determination. All eggs were collected on two non-consecutive days when the hens were 54 and 55 weeks of age and the number of dirty or cracked eggs was determined.

Video recording

Time-lapse video recorders were used to continuously record the hens over a 3 week period at the start of the two sampling periods. Video observations were conducted using four sets of video equipment, so that on any observation day up to four cages were concurrently video recorded. Video recording commenced each observation day by 1600 h and concluded at around 1530 h the following day. The order of observation of cages was randomised within blocks and replicates.

To record the behaviour of the 8 hens in single cages, up to four DC-powered mini black and white video cameras with in-built infra-red (IR) lights for recording in the dark were used. The set of four video cameras were positioned to provide different views of the hens as follows:

- Camera 1 was located approximately 1 m from the front of the cage, to provide a view of the entire front of the cage including the egg tray and feed tray.
- Camera 2 was located approximately 10 cm inside the upper left corner of the cage to provide a general view of the mid-area of the cage.
- Camera 3 was located approximately 10 cm inside the cage, in the mid-cage region just below the cage ceiling, to provide a view of the dust bath.
- Camera 4 was located inside a small wooden receptacle attached to the cage wires in front of the nest box so that it provided a view of the interior of the nest box without intruding into the nest space. The blue vinyl egg flap was positioned over the wooden box. Thus, entry of visible light into the nest was unaffected by placement of the camera. In treatments without nest boxes, the nest box camera was not used.

For recording of either 8 or 16 hens in the double cages (dust baths, nest boxes and perches were absent in these cages), two cameras were located opposite the front of the cage and two cameras were located inside the cage at the upper left and upper mid-cage positions. These video records were used to record the timing of egg laying (appearance of eggs on the roll-out tray).

Camera 1 had a CCTV lens with 12 forward facing infra-red (IR) light-emitting diodes (LEDs) for illumination, whereas Cameras 2-4 were "miniature" cameras measuring 52 mm (W) x 42 mm (H) x 16 mm (D) with six IR LEDs embedded in the front of the black plastic camera case. Cameras 2 and 3 were mounted on spring clamps that could be easily clipped into position inside the cage from outside the cage. For recording hens in the double cages, which did not have dust baths or nest boxes, two video cameras (as per Camera 1 above) were located opposite the front of the cage and two mini-cameras were located inside the cage at the upper left and upper mid-cage positions.

The three or four cameras per set were connected to a 4-channel, black and white quad video processor unit that arranged the four video images in the four quadrants of the video image field. This video image was then streamed via a 20 m video cable to one of four time-lapse video recorders (Panasonic AG-6124) situated in an adjacent shed. To accustom the hens to the video cameras, "dummy" cameras were positioned in the locations described above for 24 h prior to video recording. Behaviour data were transcribed from the video records using a Panasonic AG-7355 Video Cassette Recorder with jog-and-shuttle control that enabled frame by frame analysis of the video record. The observer entered the data into a computer using MS Excel.

Behaviour observations

Table 1
Hen behaviours recorded from time-lapse video records.

Behaviour	Definition
General activity of hens	
feeding behaviour[1]	A hen was recorded as 'feeding' when its head was outside the front of the cage, while the neck was bent down so that the head was within about 50 mm of the feed, ie. the hen's beak did not necessarily need to be in the feed.
head movements[2]	Once the other scan sample observations were completed, the observer selected 1 hen in the upper left quadrant of the screen (Camera 2) and performed the following: With the video image paused, the observer sought any hen's heads in a side-on orientation (with the eye visible) in mid-cage. If none was in the required orientation, the video tape was rolled forwards until a head was in clear view. The observer then placed a clear acetate sheet against the TV monitor and drew a circle around the head of the hen, such that the eye of the hen was in the middle of the circle and the tip of the beak was at the perimeter of the circle, ie. to produce a circle about the size of the hen's head. The video tape was rolled forward for 5 s and the observer recorded whether the hen's head moved outside the circle. A "yes" implied the hen was active while "no" implied the hen was inactive.
Perch use[1]	Number of hens on the main perch (not on edge of dust bath).
Perch on edge of dust bath[1]	Number of hens on the edge of the dust bath.
Dust bath use[1,3]	Hen(s) in the dust bath tray. A hen was considered to be within the constraints of a dust bath when either or both of her feet were on the floor of the dust bath. If the hen had both feet on the side of the dust bath, then it was deemed to be perching on the dust bath, but as soon as any part of the

	hen's foot was in the dust bath, it was counted as being in the dust bath.
Nest box use[1,3]	Hen(s) in the nest box. A hen was considered to be within a nest box if there was any part of the hen visible on the camera image of the nest interior. A hen was considered out of the nest box when no part of her could be seen on the camera inside the nest box (Camera 4).

[1]Scan sample recording technique, sampled at 60-min intervals to provide 24 observations per cage.
The observer recorded the number of hens performing the specified behaviour; [2]One hen per cage sampled per hour; [3]Continuous recording technique; the observer recorded the time of entry to and exit from the dust bath or nest box.

The various hen behaviours that are shown in Table 1 were collated from the second observation of each time-lapse video tape. At the first viewing of the video tapes the time and location in the egg tray, nest box or dust bath, that each egg was first sighted was recorded.

Blood sampling for immunology

This was conducted on the first day of the fourth week of each sampling period for 3 birds per cage to provide a total of 180 samples collected in 6 h commencing at 0800 h. Where possible, the same birds were sampled at both sampling periods. Approximately 2.5 mL of blood was collected into a heparinised syringe (sampling period 1) or a 4.5 mL Monovette closed blood collection system (Sarsted Australia, Technology Park, SA; period 2), with separation beads coated with lithium-heparin as an anticoagulant. The needle was removed and the blood was inverted 3 times to ensure adequate mixing of the blood and anticoagulant and the blood (period 1) was transferred into a plastic tube or remained in the Monovette (period 2) and placed on ice. The whole blood on ice was transferred to another laboratory for white blood cell counts and immunology assays, within 1.5 h of the final sample being collected.

After each bird was sampled it was placed in a holding crate until sampling was completed for the cage. On approaching the cage a bird was visually selected for sampling and it was this bird that was caught. The bird was removed from its cage and carried against the catcher's body or under his/her arm with its legs held together in one hand. The bird was passed to a holder and placed on a table on its side with its legs extended and held firmly. The holder's other hand was used to extend the wing and hold any large feathers covering the femoral vein out of the way; on a few occasions it was necessary to remove (pluck) small feathers that obscured a clear view of the vein. The bird's head was away from the blood sampler but the exact angle that the bird was presented to the blood sampler depended on his/her preferences. The 23 gauge x 1 inch attached to the syringe or Monovette was inserted into the vein and the required volume of blood was withdrawn. The time taken from opening of the cage to collection of the sample was recorded. The samples reached the laboratory within an hour of sampling being completed.

Following withdrawal of the needle, pressure was applied to the puncture site for a few seconds, then the bird was returned to a holding crate or its cage (last bird to be sampled from the cage) by the catcher, ensuring that its wings were restrained while being carried to prevent flapping and reduce the incidence of haematoma formation. During blood sampling, the catcher caught another bird from the same or another cage and swapped birds with the holder for a repeat procedure.

The following cells were measured in an autoanalyser CellDyn 3700 (Abbott Diagnostic Division): total white cell count, heterophils, lymphocytes, basophils, eosinophils and monocytes and cell ratios were calculated. Haemographs from the first sampling period appeared normal and conformed to the typical chicken pattern. However, most of the scatter plots from the second sampling period were different. The cells were smaller and less granular resulting in a misidentification of most monocytes as lymphocytes, and the

distinction between heterophil and eosinophil was also not clear. While the reasons for this change are unknown, one variable was the sampling method where the Monovette sampling system with coated beads was used at the second sampling period. Thus, it was not possible at the second sampling period to reliably calculate the heterophil:lymphocyte ratio. Instead the ratio of granulocytes (heterophils + eosinophils + basophils) : peripheral blood mononuclear cells (lymphocytes + monocytes was calculated at both sampling periods.

From the blood sample taken for immunology, the responsiveness of the immune system was assessed in two ways, by the ability of white blood cells to produce interleukin-6 (IL-6) in response to stimulation with a lipopolysaccharide (LPS) and a proliferation test to determine the lymphocyte response, on the basis of incorporation of tritiated thymidine, to stimulation with a mitogen, concanavalin A (Con A), over a 4 day period. For the IL-6 response test, whole blood was cultured with or without LPS (100 μg/mL; from *E. coli*, K-235, Sigma Pharmaceuticals catalogue number L-2018) in duplicates in 96 well plates (Nunclon tissue culture plates, catalogue number 167008) with glass fibre filter mats for 24 hours at 37 °C in a humidified incubator with 5% CO_2. After 24 h the culture plates (Corning, catalogue number 3511) were attached over another round bottom Nunclon tissue culture plate with sticky tape. Supernatants from the upper plates were collected into the bottom plate by centrifuging the assembly at 1000 G for 1 h. The plate with supernatants was sealed and stored at 4 °C until assayed for IL-6. IL-6 produced in whole blood culture supernatants were quantified in a 7TD1cell (Murine hybridoma cell line) proliferation assay based on the methods described by Asai *et al*. (1994). Briefly, IL-6 dependent 7TD1 cells were initially grown to a log phase with recombinant chicken IL-6 and then starved of IL-6 by washing the cells three times in medium and further culturing the cells for three more days. These starved cells ($2x10^3$) were added to a 1:40 dilution (in DMEM {Dulbecco's Modified Eagle Media}) supplemented with 5% foetal calf serum (FCS), 2 mM glutamine, 0.55mM arginine, 0.24mM asparagine and 100 units penicillin/streptomycin) of supernatant from LPS stimulated whole blood, incubated at 37 °C, in a humidified CO_2 incubator for 48 h. 1 μCi of tritiated thymidine was added to each well for the last 5 h of incubation. Cells were harvested on to glass fibre filter mats in a Tomtec cell harvester and counts per minute (cpm) of tritiated thymidine incorporated into the cells was determined and used for statistical analysis.

For the Con A stimulation test, heparinised chicken blood was diluted 1:40 in the same medium as used for the IL-6 response test and cultured in triplicate with (10 and 50 μg/mL) or without Con A (Sigma Pharmaceuticals, catalogue number C 2010) in a humidified 37 °C incubator with 5% CO_2 for three days and pulsed with 0.5μCi tritiated thymidine and further cultured for 18-24 h. On day 4 the culture plates (as described in the IL-6 response test) were shaken on a plate shaker for 2-3 min to resuspend the cells and the cells were harvested onto glass fibre filter mats using a Tomtec cell harvester. Counts per minute (cpm) of tritiated thymidine incorporated into the cells was determined and used for statistical analysis. The assay procedure is described by Talebi *et al.* (1995). The use of 10 and 50 μg/mL of Con A was based on preliminary tests with samples from 10 birds (from spare cages) at 17 weeks of age. Blood was tested with a range of Con A concentrations from 1-100 μg/mL and there was considerable variation in responses between hens. Nevertheless, Con A concentrations of 10 and 50 μg/mL were effective for high and low responding hens, respectively and were subsequently used in the assays.

Egg collection for determination of corticosterone concentrations

This was conducted on two consecutive days in the fifth week of each sampling period for all eggs laid in that period. On the evening before eggs were to be collected all eggs were removed from the egg shed. Commencing at 0600 h, eggs were identified to cage and collected hourly until 1700 h. Any eggs laid after this time on day 1 of collection were included in the 0600 h collection of day 2. The eggs were weighed, the albumen and yolks

separated and the albumen weighed. The albumen was then stored frozen and subsequently transported to the laboratory where its frozen state was maintained until analysed for corticosterone concentrations (Downing and Bryden, 2002).

Blood sampling for corticosterone assays

This was conducted between 1300-1600 h each day for 8 days in the sixth and seventh week of each sampling period for all birds in the experiment. The blood was sampled as described above for the immunology assays with the addition that the blood was transferred into a plastic microcentrifuge tube and stored on ice. Similarly, the birds were handled as described above for the immunology assays, with the following exceptions: A maximum of two birds were sampled from each cage each day with the birds being sampled one after the other and the sequence of cages (within block structure for the experiment) was selected to minimise walking in front of cages that contained birds that were yet to be sampled on the day. The blood was centrifuged in a refrigerated microcentrifuge with a relative centrifugal force of 11,290 G for 5 min in period 1 and with a relative centrifugal force of 2,524 G in a refrigerated centrifuge for 15 min in period 2. The plasma was separated and stored frozen until assayed for total corticosterone concentrations using a commercial diagnostic kit (ICN ImmuChem Double antibody RIA from ICN, Seven Hills, NSW). Some assay characteristics were a within assay coefficient of variation of 14.1 and 8.4 % for 0.44 and 2.1 nM standards, respectively and a between assay coefficient of variation of 8.5 and 14.1 % for high and low sample variances of 9.1 and 1.7 nM, respectively; the lower limit of detection was 0.3 nM. The corticosterone assay first involved the treatment of the sample with a solvent to precipitate binding proteins, followed by RIA. Fifty microlitres of plasma was mixed with 50 µL of 0.1 M NaOH in a 1.5 mL 'Eppendorf' centrifuge tube and gently mixed. For high concentration samples (ie. those treated with ACTH), this step was modified to the addition of 10 µL of sample to 90 µL of 50:40 v:v 0.1 M NaOH:water. To this alkaline mixture was added 100 µL of 90:10 v:v acetonitrile:water containing a pre-titrated amount of acetic acid sufficient to neutralise the pH of the resulting mixture to a pH of between 6 and 8. The tube was mixed and centrifuged at 11,290 G for 3 min. The supernatant was used for subsequent analysis. The remainder of the assay was conducted according to the manufacturer's instructions except that all reagent volumes were reduced by 50%. All values were corrected by a factor of two or ten for the normal and high concentration samples, respectively.

Feather damage and cover, claw length, foot condition, injuries on the comb, around the cloaca and on the back, keel bone deformation, body weight and ACTH responsiveness

These measurements were conducted over 4 days in period 1 and 5 days in period 2 during the eighth week of each sampling period on all birds in the experiment. The cages to be sampled for the day were identified within the block structure of the experiment. Birds were removed from the cage and handled in a similar manner to that described for the immunology sampling. It was possible to conduct the morphological scoring, weigh and blood sample the birds at the one session, as the only time constraint was to collect a blood sample 60 min after the intramuscular ACTH injection. On each day a maximum of 16 cages (4 blocks) were sampled.

A bird was removed from the cage by the catcher, weighed and passed to another person (holder) who held and scored the various aspects; a third person (recorder) recorded body weight, cage number, bird ID and the verbal scores and measurements. The recorder then gave an intramuscular injection of 12.5 IU ACTH (Synacthen, Ciba Geigy) into the muscle of the leg while it was being held by the holder and recorded the time of injection and the time that a blood sample was required. The bird was then held in a crate and its cage mates were added as they were scored and injected. After a number of birds were handled as above (21-55 birds/h), the 60 min period had nearly elapsed and the recorder then identified the bird for the catcher to retrieve from the crate for blood sampling which occurred at 60

min post injection. The blood was handled as described for the corticosterone assays. Once the birds had been blood sampled, the procedure was repeated on another batch of birds.

Feather cover and damage was assessed using a subjective 4 point scoring system applied to the neck, breast, cloaca, back, wings, and tail (Tauson *et al.,* 1984). The scores were as follows: 1 for part of the body that had heavy damage with no or only very small areas being covered with feathers ie. with naked or almost naked body area, 2 for part of the body that showed clear deterioration of feathers and/or with large naked areas (more than a circle of 5 cm), 3 for part of the body where feathers were deteriorated but the skin was still completely or almost completely covered by feathers and 4 for very good plumage with few or no worn or deformed feathers. The length of the claw on the middle toe of each foot was measured with vernier callipers and the values were averaged and claw condition for all claws on both feet was scored: 1 for no damage to any claws, 2 for mild chipping but no broken claws, 3 for 1 claw broken, but could be long and straight, and 4 for more than 1 claw broken and/or twisted. Foot condition was scored as follows: 0 for normal, 1 for mild hyperkeratosis, 2 for severe hyperkeratosis, 3 for very severe hyperkeratosis, 4 for severe hyperkeratosis and other lesions and 5 for bumble-foot (inflamed foot pads). The cleanliness of plumage for body areas and the feet were scored as follows: 1 for clean, 2 for mildly dirty, 3 for moderately dirty and 4 for very dirty. Wounds (from pecking) on the comb were scored as follows: 1 for many over the whole of comb, 2 for more than 4, 3 for 1-4 and 4 for none. Pecking wounds and scratches on the rear part of the back and around the cloaca were scored as follows: 1 for very severe, 2 for some severe and obvious, 3 for few and obvious and 4 for none. The scores for pecking wounds and scratches were averaged over the 3 body parts. Keel bone damage was assessed for any deformation (from straight) by touch and vision; the score was 4 for normal, 3 for slightly flattened and 2 for twisted. For ease of understanding the tabulated data, the scores for feather cover and damage, comb pecking injuries and keel bone deformation, described above have been reversed for presentation so that for all tabulated variables a higher score indicates a poorer condition or more damage.

Bone strength

At the end of the experiment, over a 4-week period commencing at 67 weeks of age, 3 birds per cage were sampled for bone strength determinations. The birds were killed by an intravenous injection of 2 mL of a mixture of sodium pentobarbitone (325 mg/mL; Lethobarb; Virbac Australia Pty Ltd, Peakhurst, NSW):saline (1:1). Once the birds were dead, the femur, tibia, humerus and coracoid bones were removed from the carcase. The flesh was removed from the bones with a knife and then the bones are scraped free of excess tissue with a scalpel blade. The bones were wrapped in cling wrap, identified with a number and bone type with a tie-on tag and sealed in a small plastic bag. The small bags were placed in a larger storage bag (within bone types) and stored frozen at minus 18 °C until analysed for bone strength.

Bone strength was measured using a Lloyd 1000K Tensile Testing Machine and a 3-point bending jig (Lloyd Instruments Ltd., Fareham Hants, England). The instrument parameters were a 5 kN load cell accelerating at 10 mm/min. Bones were taken out of the cling wrap and placed in a single layer on a plastic slate tray to enable good air flow, thawed at 4 ^{o}C for approximately 7 h and placed in a humidity room (20 ^{o}C) overnight (16 h). The middle point of the exterior bone face (the outside edge of the bone while the chicken was alive) was the impact test point, as this was the 'face' that would have received the greatest breaking force in the live bird. The bone was supported on the bending jig at the 2 points that represented 55% of the bone length, measured from the mid point (this support distance was chosen as it represented the maximum length of a relatively consistent diameter of the shortest bones {coracoid and humerus}).

Statistical analyses

In general, measurements were analysed as a 3 nest box treatment by 3 dust bath treatment by 2 perch treatment factorial plus 2 added control treatments, using a restricted maximum likelihood analysis (GenStat Committee, 2000) with random effects for the replicates and blocks within replicates. When the variance components for the blocking structure were estimated as being negative, this was allowed to stand. In some cases, when numerical convergence could not be obtained with these random effects, a simpler blocking structure, such as a block random effect not nested within replicates or only a random replicate effect, was used. In every case, individual cages were used as the experimental unit. Where appropriate, the data for each cage were transformed so that the residuals from a saturated treatment model were homogeneous. With a few measurements, particular cages were deleted from the analysis due to their unrepresentative nature. In the second sampling period there was a concern over the haemographs and most of the scatter plots, with the cells being smaller and less granular resulting in a misidentification of most monocytes as lymphocytes and an unclear distinction between heterophils and eosinophils. While these data have been presented in the tables, for the purposes of cautious interpretation of the data they have not been included in either the description of the results or the discussion.

Starting with a fully saturated model for each measurement, the factorial model was reduced, in a stepwise manner, to the simplest model that reasonably fitted the data using Wald tests. With most measurements it was reasonable to consider a model with no treatment effects of nest box, dust bath or perch. In these cases, predicted means, and back-transformed means as appropriate, are presented for nest box, dust bath and perch treatments using models that only have a fixed effect of the treatment being examined and a fixed effect to account for the two larger cage size control treatments. Also, a treatment comparison was made of predicted means between the 16 hen large cage, the 8 hen large cage and the combined small cage pens using an analysis that only included these treatments. A likelihood ratio test for fixed effects in REML models (Welham and Thompson, 1997) was used to test for the effects of nest box, dust bath and perch main effects. These tests compared a model with only fixed effects for cage size and allocated hen number with models that also included fixed effects of nest box, dust bath and perch, respectively. A similar type of likelihood ratio test for fixed effects was carried out for testing cage size and allocated hen number effects against a model with no treatment effect at all. In some cases it was not possible to calculate the likelihood ratio test due to non-convergence, and in these cases the Wald test was used instead.

Main effects of perch or nest box were observed for some measurements. In these cases the predicted means for all other main effects were adjusted for the main effect observed. The hypotheses tested of the other main effects were made using a comparison of models with the observed main effect included. Large cage size treatments were compared to the predicted mean of all cages with either no perch, or no nest box and nest box space, depending on whether a perch or nest box main effect was observed.

Many of the behavioural measurements were restricted to a subset of the cages. With these measurements analogous statistical analyses were carried out, but with the analysis restricted to the appropriate set of cages. At both sampling periods the number of bouts of dust bathing was 0 in nearly all cages without a perch, and a positive number in all cages with a perch. In these two cases, the effect of perch was formally tested using a 2-side non-parametric within-block permutation test.

Results

A few interactions between the perch, nest box and dust bath main effects were found and these are described in the text below. However, a number of second- and third-order interactions occurred for which there were no meaningful interpretations.

Behaviour observations - egg laying parameters from the video records

The time of appearance and location in the egg tray or nest box, of 443 and 321 eggs, respectively, were recorded from the video records at 29-31 and 59-61 weeks of age, respectively. The first eggs observed on each day were recorded at 0408 h and 0351 h, while the last eggs were recorded at 2033 h and 1806 h, respectively. The median times of the first egg per cage were 0633 h and 0620 h, at 29-31 and 59-61 weeks of age, respectively. The median time interval between eggs laid per cage (ie. the time from first to last egg divided by the number of eggs minus 1) was 32 and 37 min, respectively (mean values were 55 and 56 min, respectively). The quartile intervals are shown in Table 2 for the pooled egg data and in Figures 2a and 2b for hens in the 8-bird standard sized, unfurnished cages, differentiated according to the three nest box treatments. While the largest proportion of eggs were laid in the early morning, represented by the short intervals for quartiles 1 and 2, there were no differences due to any of the main effects on the overall interval of lay per day or the quartile values.

Table 2

Median time interval (min) between eggs per cage and the quartile intervals based on pooled data for all cages, at sampling periods commencing at 29 and 59 weeks of age. Mean values are shown in parentheses.

Egg laying time periods	29-31 Weeks of Age	59-61 Weeks of Age
Inter-egg interval (all eggs)	32 (55)	37 (56)
Interval between eggs		
First quartile	19 (20)	11 (15)
Second quartile	32 (37)	37 (36)
Third quartile	57 (68)	71 (78)
Fourth quartile	118 (156)	143 (163)

Figures 2a & 2b
Median time intervals between eggs laid in each time quartile on video observation days, for cages of hens based on nest box treatment. Upper and lower graphs, show the data for sampling periods 1 and 2, respectively.

Figure 2a

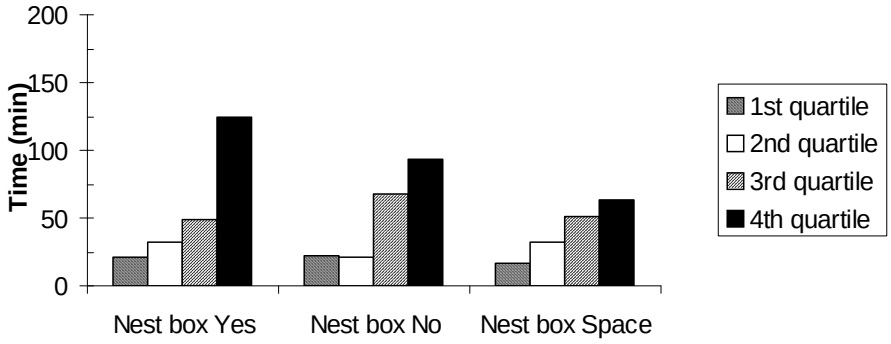
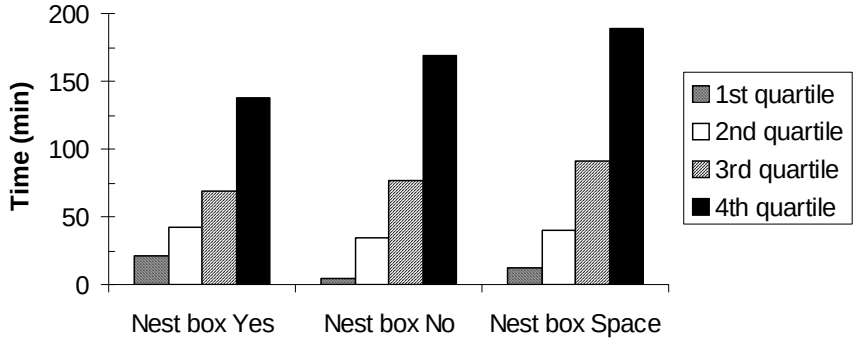


Figure 2b



When a nest box was available for use the majority of eggs were laid in the nest box (Figure 3a); this difference was statistically significant when a perch was present at 29-31 weeks of age ($P = 0.0089$) with a similar trend ($P > 0.05$) at 59-61 weeks of age (Table 3a). Nevertheless, over 30 % of eggs were laid elsewhere in the cage. The next preferred site for laying was against a (solid) side wall of the cage (Figures 3a, 3b and 3c). While there were no other effects of items of furniture on egg laying parameters ($P > 0.05$; Table 3a), from 29-31 weeks of age, hens in cages with nest boxes were more ($P < 0.01$) likely to lay in the nest box (67% of eggs) if the cage also contained a perch than no perch (47% of eggs; Table 3a). While this relationship was not found from 59-61 weeks of age, there was a difference in the proportion of eggs laid in the nest box due to the dust bath main effect. As shown in Table 3a, eggs were more likely to be laid in the nest box if either a dust bath with litter was available (82% of eggs) or no dust bath was available (75% of eggs) compared to the dust bath space treatment (51% of eggs). In the larger space allowance treatment (double cages with 8 hens) the maximum time between consecutive eggs being laid was of considerably shorter duration from 59-61 weeks of age ($P = 0.033$; Table 3b).

Figures 3a-c
The percent of eggs laid in each location within the cage at sample periods 1 (left) and 2 (right); NB indicates nest box. Values in parentheses within NB indicate the proportion of NB eggs that were laid on the lower half of the NB. Note: drawing not to scale

Figure 3a
Cages with a nest box

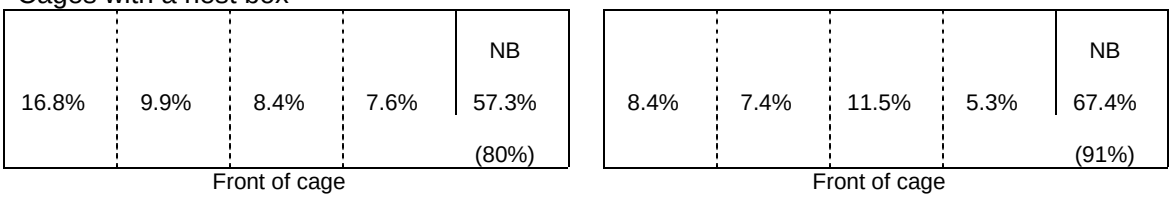


Figure 3b
Cages with no nest box and where the space that would have been occupied by the nest box was blocked off

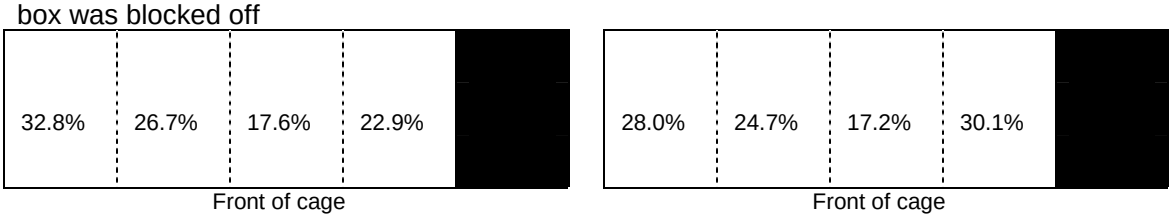
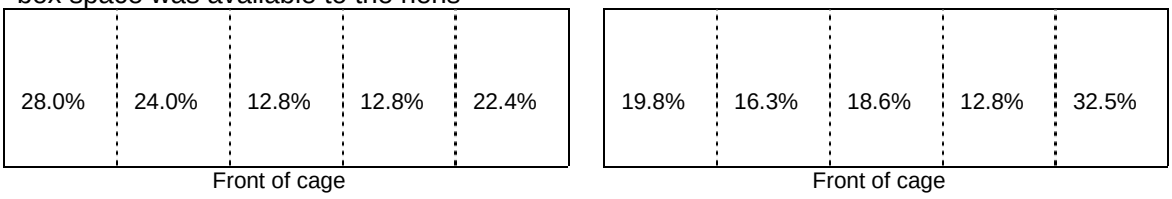


Figure 3c
Cages with no nest box and where the space that would have been occupied by the nest box space was available to the hens



There were no effects of treatment on the use of perches by hens, although there was a trend from 59-61 weeks of age (P=0.078) for reduced use of the perch in the Space compared to the Nest Box and No Nest Box treatments (Table 4). It is to be noted that the perch in the Space treatment did not transverse the space that would have been occupied by the nest box if it was present. In general, hens were observed to use the perches less at 29-31 than 59-61 weeks of age (pooled means were 27 % *vs* 43 % of observations over 24 h, respectively) and less in the light than dark period (pooled means were 21 % and 30 % of observations in the light, and 37 % and 66 % of observations in the dark, from 29-31 and 49-51 weeks of age, respectively).

However, there was an effect of the perch treatment on use of the dust bath at both time periods, in that the dust bath was generally only used if a perch was present. For example, at 29-31 weeks of age there were no bouts of dust bathing in any cage without a perch, except for one cage in which 4 bouts of dust bathing were observed. Every cage with a perch had at least one bout of dust bathing. In every block all the cages without a perch had fewer bouts of dust bathing than all cages with a perch. Within standard cages from 29-31 weeks of age (as an example), the chance of this occurring if the perch had no effect was:

$$\frac{1}{4}\times\frac{1}{3}\times\frac{1}{3}\times\frac{1}{3}\times\frac{1}{2}\times\frac{1}{2}\times\frac{1}{2}\times\frac{1}{3}\times\left(\frac{2}{4}\times\frac{1}{3}\right)=\frac{1}{4}\times\left(\frac{1}{3}\right)^{5}\times\left(\frac{1}{2}\right)^{4}=\frac{1}{15552}$$

Thus a 2-sided non-parametric within-blocks permutation test for no perch effect has a P value of :

$$2\text{ x}\frac{1}{15552}\quad=\quad\frac{2}{15552}\quad=0.00013$$

Table 3a
Effects of furnished cage treatments on body weight (at 36 and 66 weeks of age), egg production (at 22-29 and 48-55 weeks of age) and egg laying parameters (29-31 and 59-61 weeks of age) in hens. The younger and older ages are periods 1 and 2, respectively.

Parameter	Factor[1]	Mean values (back-transformed values in parentheses)			SE	P value
		Yes	No	Space		
Period 1						
Body weight *(kg)*	Perch	2.32	2.36	-	0.022	0.087
	Dust bath	2.35	2.33	2.34	0.027	0.867
	Nest box	2.32	2.34	2.37	0.026-0.027	0.219
Egg production per hen-day	Perch	0.93	0.93	-	0.010	0.817
	Dust bath	0.93	0.93	0.93	0.012-0.013	0.989
	Nest box	0.94	0.92	0.94	0.012	0.172
Eggs laid in the nest box *(%)*[2]	Perch	67[b]	47[a]	-	7.4	**0.0089**
	Dust bath	57	61	52	9.4-10.3	0.669
	Nest box	-	-	-	-	-
Interval of egg laying *(min)*[3]	Perch	2.52 (333)	2.42 (265)	-	0.053	0.087
	Dust bath	2.45 (283)	2.46 (289)	2.51 (322)	0.064-0.065	0.674
	Nest box	2.50 (317)	2.47 (298)	2.44 (276)	0.062-0.065	0.669
Maximum interval of lay *(h)*[4]	Perch	0.38 (2.4)	0.27 (1.9)	-	0.069	0.147
	Dust bath	0.32 (2.1)	0.26 (1.8)	0.40 (2.1)	0.082	0.297
	Nest box	0.36 (2.3)	0.31 (2.0)	0.31 (2.0)	0.083-0.085	0.765
Period 2						
Body weight *(kg)*	Perch	2.52	2.56	-	0.024	0.076
	Dust bath	2.56	2.54	2.52	0.031	0.413
	Nest box	2.54	2.52	2.55	0.031	0.656
Egg production per hen-day	Perch	0.83	0.76	-	0.035	0.076
	Dust bath	0.76	0.80	0.82	0.044	0.339
	Nest box	0.75	0.82	0.81	0.044	0.208
Eggs laid in the nest box *(%)*[2]	Perch	78	61	-	8.3	0.085
	Dust bath	75[b]	82[b]	51[a]	10.0-10.9	**0.037**
	Nest box	-	-	-	-	-
Interval of egg laying *(min)*	Perch	320	377	-	41.0	0.173
	Dust bath	331	331	388	50.2-50.9	0.439
	Nest box	347	359	340	50.8-51.6	0.932
Maximum interval of lay *(h)*[3]	Perch	0.42 (2.6)	0.47 (2.9)	-	0.063	0.480
	Dust bath	0.44 (2.8)	0.38 (2.4)	0.51 (3.3)	0.076-0.078	0.277
	Nest box	0.42 (2.6)	0.46 (2.9)	0.44 (2.8)	0.078-0.080	0.873

[1]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [2]Only analysed for those treatments with nest boxes; [3]Log_{10} values; [ab]Different letters denote a significant treatment effect.

Table 3b
Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on

body weight (at 36 and 66 weeks of age), egg production (at 22-29 and 48-55 weeks of age) and egg laying parameters (29-31 and 59-61 weeks of age). The younger and older ages are periods 1 and 2, respectively.

Parameter	Mean values (back-transformed values in parentheses)			SE (between double cages)	SE (between double and single cages)	P value
	Double 16	Double 8	Single 8			
Period 1						
Body weight *(kg)*	2.28	2.34	2.34	0.068	0.040-0.058	0.347
Egg production per hen-day	0.95	1.00[b]	0.93[a]	0.034	0.020-0.026	**0.030**
Interval of egg laying *(min)*[1]	2.45 (279)	2.79 (561)	2.47 (298)	0.150	0.086-0.132	0.136
Maximum interval of lay *(h)*[1]	0.26 (1.8)	0.56 (1.8)	0.33 (2.1)	0.217	0.156	0.284
Period 2						
Body weight *(kg)*	2.43	2.48	2.54	0.081	0.049-0.066	0.075
Egg production per hen-day	0.87	0.81	0.79	0.106	0.070-0.095	0.559
Interval of egg laying *(min)*	140	350	350	123.3	75.6-104.2	0.146
Maximum interval of lay *(h)*[1]	0.26 (1.8)	0.07[a] (1.2)	0.44[b] (2.8)	0.186	0.135	**0.033**

[1]$Log_{10}(1-y+0.2)$ values; [ab]Different letters denote a significant treatment effect ($P < 0.05$).

Table 4
Effects of furnished cage treatments on hen behaviour

Parameter	Factor[1]	Mean values			SE	P value
		Yes	No	Space		
29-31 weeks of age						
Hen feeding activity[2]	Perch	1.60	1.57	-	0.099	0.964
	Dust bath	1.50	1.67	1.59	0.119-0.120	0.386
	Nest box	1.60	1.56	1.60	0.120-0.123	0.923
Hen feeding activity[2] in the light period	Perch	2.45	2.42	-	0.161	0.832
	Dust bath	2.33	2.55	2.44	0.194-0.197	0.550
	Nest box	2.41	2.40	2.50	0.196-0.199	0.864
Head movement per observation in 24 h period[3]	Perch	0.51	0.52	-	0.017	0.950
	Dust bath	0.52	0.50	0.53	0.020	0.508
	Nest box	0.53	0.50	0.51	0.020	0.173
Head movement per observation in lights-on period[3]	Perch	0.75	0.74	-	0.023	0.505
	Dust bath	0.73	0.75	0.75	0.029	0.741
	Nest box	0.76	0.72	0.76	0.028-0.029	0.248
Use of perch per 24 h	Perch	-	-	-		
	Dust bath	2.21	2.16	2.10	0.177-0.195	0.843
	Nest box	2.10	2.21	2.15	0.183-0.190	0.832
Use of perch per h in light period	Perch	-	-	-		
	Dust bath	1.76	1.71	1.47	0.149-0.158	0.157
	Nest box	1.64	1.60	1.67	0.160-0.166	0.929
Use of perch per h in dark period	Perch	-	-	-		
	Dust bath	2.89	2.75	3.24	0.433-0.463	0.553
	Nest box	2.72	3.15	3.01	0.446-0.453	0.634
59-61weeks of age						
Hen feeding activity[2,4]	Perch	1.01	1.00	-	0.074	0.887
	Dust bath	0.96	1.02	1.04	0.0090-0.0092	0.679
	Nest box	0.92[a]	0.97[a]	1.14[b]	0.0084-0.0085	**0.034**
Hen feeding activity[2,4] in the light period	Perch	1.45	1.44	-	0.105	0.915
	Dust bath	1.37	1.48	1.49	0.126-0.128	0.618
	Nest box	1.30[a]	1.40[ab]	1.63[b]	0.117-0.118	**0.025**
Head movement per observation in 24 h period[3]	Perch	0.46	0.46	-	0.024	0.928
	Dust bath	0.45	0.45	0.48	0.029-0.030	0.569
	Nest box	0.47	0.47	0.44	0.029-0.30	0.563
Head movement per observation in lights-on period[4]	Perch	0.64	0.63	-	0.030	0.787
	Dust bath	0.63	0.61	0.66	0.036-0.037	0.412
	Nest box	0.65	0.65	0.62	0.037	0.658
Use of perch per 24 h	Perch	-	-	-		
	Dust bath	3.49	3.32	3.60	0.291-0.306	0.741
	Nest box	3.51	3.75	3.11	0.269	0.078
Use of perch per h in light period	Perch	-	-	-		
	Dust bath	2.43	2.45	2.29	0.253-0.272	0.783
	Nest box	2.24	2.71	2.22	0.241-0.248	0.127
Use of perch per h in dark period	Perch	-	-	-		
	Dust bath	5.16	4.80	5.71	0.472-0.480	0.205
	Nest box	5.65	5.45	4.62	0.462-0.463	0.104

[1]Within each factor the mean values for the presence or absence, respectively, of a perch, dust box or nest box, are presented in the 'Yes' and 'No' columns. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [2]Estimated on the basis of number of hens feeding at each hourly observation period averaged over 24 h; [3]Head movement of the hen was used as an

estimate of activity; [4]At the second observation period the data have been adjusted for the number of hens/cage. The observer recorded head movement for one hen per hour from the video record; [ab]Different letters denote a significant treatment effect ($P < 0.05$).

In nest box treatment cages, access to the nest box was available the whole time. At 29-31 and 59-61 weeks of age, respectively, nest boxes were occupied by hens during an average of 17.4 % and 22.9% of the time (Table 5). However, in the latter sampling period, hens in one cage occupied the nest box during about 21 h. If the data for this cage were removed, the mean occupancy time was reduced to 19.0 % of the time. The frequency of bouts in the nest box and mean bout times by hens are shown in Table 5. The hourly scan sampling of nest-box occupancy indicated that on occasions more than one hen occupied the nest box as shown in Table 5. However, the occurrence of multiple nest box occupants was lower at 29-31 than 59-61 weeks of age (possibly due to behaviours such as in one cage in which some hens seemed to 'hide' in the nest box). As also indicated in Table 5, there was a low incidence of nest box occupancy in the dark period (6.7 % and 20.4 % of occurrences in sample times 1 and 2, respectively). The furniture treatments did not affect the use of the nest box ($P > 0.05$; Table 6). Only data for effects of furniture on nest box use from 29-31 weeks of age are fully tabulated.

Table 5

Nest box (NB) occupancy by hens in cages over 23.5 h of observation. Values shown are the pooled mean values, and the minimum and maximum cage means, for all cages with a NB, and the number of occasions per hour when there was 1 or more hens in the nest box

Parameter	29-31 Weeks of Age			59-61 Weeks of Age		
	Mean	Min.	Max.	Mean	Min.	Max.
Total time occupied (min)	245.6 (17.4%)	131.6	464.0	323.3 (22.9%)	71.1	1257.8
• one cage with ~21 h occupancy excluded	-	-	-	268.6 (19.0%)	71.1	586.7
Number of bouts of occupancy	14.6	6	27	31.0	15	54
Mean bout time (min)	18.4	7.5	29.4	11.7	3.7	66.2
Hens in NB[1]						
• 1 hen (occasions)	3.0	1.0	6.0	4.9	1	22
• more than 1 hen (occasions)	0.5	0	1	3.9	0	1
Hens in NB in the light[2]						
• 1 hen (occasions)	2.8	1.0	6.0	3.9	1.0	13.0
• more than 1 hen (occasions)	0.5	0	1.0	0.1	0	1.0
Hens in NB in the dark[3]						
• 1 hen (occasions)	0.2	0	2.0	1.0	0	9.0
• more than 1 hen (occasions)	0	0	0	0	0	0

[1]Number of hens in nest box when scan-sampled once per hour over 24 observations; [2]Number of hens in nest box when scan-sampled once per hour in the light period; [3]Number of hens in nest box when scan-sampled once per hour in the dark period.

The presence of a nest box also influenced the number of bouts of use of the dust bath at both 29-31 and 59-61 weeks of age. The number of bouts of dust bath use was higher ($P < 0.05$) in the absence of a nest box (ie. nest box space was blocked off) than the treatment in which the nest box was not available but the floor space that it occupied was available, at both time periods (Table 6). From 59-61 weeks of age the number of bouts of use of the dust bath was higher in the absence of the nest box than when the nest box was present ($P = 0.019$). The total duration of use of the dust bath from 29-31 weeks of age was higher ($P = 0.018$) in the presence or absence (floor space restricted) of a nest box compared to no nest box but floor space available (Table 6). From 59-61 weeks of age the latency to first use of the dust bath after it opened was lower in the absence of the nest box (floor space restricted) than when there was additional floor space available in the absence of a nest box.

Table 6
Effects of furnished cage treatments on use of the dust bath[1] and nest box[2] by hens

Parameter	Factor[3]	Mean values (back-transformed values in parentheses)			SE	P value
		Yes	No	Space		
29-31 weeks of age						
Dust bath						
Number of bouts of use[4]	Dust bath	3.31 (10.9)	-	3.55 (12.6)	0.490	0.627
	Nest box	3.64[b] (13.3)	4.24[b] (18.0)	2.39[a] (5.7)	0.584-0.590	**0.023**
Latency to first use after opening *(s)*[5]	Dust bath	0.38 (2.4)	-	0.67 (4.7)	0.267	0.310
	Nest box	0.65 (4.5)	0.35 (2.2)	0.58 (3.8)	0.391-0.436	0.733
Mean bout duration *(min)*[5]	Dust bath	0.96 (9.2)	-	0.87 (7.4)	0.095	0.395
	Nest box	0.90 (8.0)	0.97 (9.4)	0.80 (6.3)	0.051-0.064	0.535
Total duration of use/day *(min)*	Dust bath	2.02 (104)	-	1.83 (67)	0.099	0.103
	Nest box	2.02[b] (105)	2.12[b] (131)	1.66[a] (46)	0.142-0.143	**0.018**
Nest box						
Number of bouts of use[4]	Perch	3.93 (15.5)	3.62 (13.1)	-	0.360	0.386**
	Dust bath	3.98 (15.9)	3.92 (15.4)	3.45 (11.9)	0.399-0.445	0.341[†]
Total duration of use/day *(min)*[5]	Perch	2.42 (265)	2.30 (200)	-	0.077	0.112*
	Dust bath	2.40 (251)	2.41 (257)	2.28 (188)	0.097-0.098	0.349
Mean bout duration *(min)*	Perch	19.1	17.7	-	3.59	0.703
	Dust bath	18.1	18.3	18.8	4.58	0.989
59-61 weeks of age						
Dust bath						
Number of bouts of use[4]	Dust bath	2.03 (4.1)	-	2.22 (4.9)	0.457	0.706
	Nest box	1.97[a] (3.9)	3.08[b] (9.5)	1.21[a] (1.5)	0.528-0552	**0.019**
Latency to first use after opening *(s)*[5]	Dust bath	0.57 (3.7)	-	0.67 (4.7)	0.195	0.310
	Nest box	0.63[ab] (4.3)	0.21[a] (1.6)	1.04[b] (11.0)	0.212-0.242	**0.014**
Mean bout duration *(min)*[5]	Dust bath	1.09 (12.4)	-	0.92 (8.3)	0.121	0.176
	Nest box	1.06 (11.6)	1.00 (10.1)	0.95 (9.0)	0.191-0.219	0.878
Total duration of use/day *(min)*	Dust bath	1.51 (31)	-	1.45 (27)	0.244	0.830
	Nest box	1.52 (32)	1.85 (69)	1.08 (11)	0.269-0.299	0.082
Nest box						
Number of bouts of use[4]	Perch	5.74 (32.9)	-	5.18 (26.8)	0.456	0.695
	Dust bath	5.21 (27.1)	5.65 (31.9)	5.43 (29.4)	0.544-0.605	0.249

[1]The statistical analysis was restricted to cages with a perch; [2]The statistical analysis was restricted to cages with a nest box; [3]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [4]Square root transformation; [5]Log_{10} transformation; *****Using Wald Test since model fitted did not numerically converge using Fisher algorithm; [†]The model used for these results had only one random effect for blocks rather than a random effect for replicates and blocks. A model with the 2 random effects did not numerically converge; [ab]Different letters denote a significant treatment effect ($P < 0.05$).

The analyses of hens occupying the dust bath were restricted to cages containing a perch. At 29-31 and 59-61 weeks of age, the dust baths were open to the hens for about 380 and 346 min, respectively per day. The raw data describing occupancy of the dust baths are presented in Table 7. As indicated in the table, hens occupied the dust baths on average for about 25 % of the available time. While the number of bouts of occupancy per cage halved between sample period 1 and 2, the mean bout time almost doubled. The average latency for the first visit by a hen to the dust bath per cage upon the dust bath opening for the day was 21.9 min at time 1 compared to 14.4 min at time 2. This hen occupied the dust bath on the first occasion for the day for about 17.6 min in both sample periods. On occasions during the hourly scan sampling procedure, hens were also observed perched on the edge of the dust bath. In sample periods 1 and 2, respectively, this was observed for an average of 0.7 (9 cages) and 0.1 (2 cages), respectively during 24 h.

Table 7
Dust bath (DB) occupancy by hens in cages with perches over 23.5 h of observation. Values shown are the pooled mean values, and the minimum and maximum cage means, for all cages with perches and a DB, and cages that contained a 'functional' dust bath ie including litter and the dust bath 'space' treatment, in which cages contained a dust bath without litter

Parameter	29-31 Weeks of Age			59-61 Weeks of Age		
	Mean	Min	Max	Mean	Min	Max
Total time open per day (min)	380	-	-	346	-	-
Total time occupied (min)	105.6 (27.8%)	14.9	244.5	81.3 (23.5%)	0	267.6
• DB with litter (min)	122.7 (32.3%)	38.6	244.5	91.3 (26.4%)	0	267.6
• DB space / no litter (min)	88.6 (23.3%)	14.9	165.7	71.4 (20.6%)	4.4	172.0
Number of bouts of occupancy	13.3	1	35	5.9	0	20
• DB with litter	12.4	3	35	6.1	0	20
• DB space / no litter	14.1	1	27	5.8	1	11
Mean bout time (min)	10.0	3.0	32.2	17.5	0.9	85.8
• DB with litter	12.4	5.6	32.3	25.2	2.5	85.8
• DB space / no litter	7.5	3.0	14.9	11.5	0.9	19.1
Latency to enter dust bath (min)[1]	21.9	0.1	165.3	14.4	0.6	94.5
• DB with litter	16.5	0.1	93.9	7.6	0.7	33.3
• DB space / no litter	27.2	0.3	163.3	19.7	0.6	94.5
Duration of 1st bout (min)[2]	17.6	3.1	44.9	17.7	0.6	85.8
• DB with litter	19.2	4.2	44.9	29.3	0.6	85.8
• DB space / no litter	15.9	3.1	35.5	8.7	0.6	22.6

[1]Time after dust bath initially opens around mid-day until the first hen occupies the dust bath; [2]Duration of the first bout by a hen in the dust bath after the dust bath opens for the day.

Hen feeding behaviour and general activity level
The activity level of hens was estimated by measuring the occurrence of hens feeding at the trough per hour and the occurrence of a focal hen moving her head through a set distance in 5 s, also measured once per hour. There were no differences due to the main effects on feeding activity of hens or head movement from 29-31 weeks of age (Table 4). However, from 59-61 weeks of age there were differences due to the nest box main effect on the occurrence of feeding activity. In the daily interval when lights were on, hens with access to a nest box were observed to feed less ($P<0.05$) than hens with the nest box space treatment (Table 4). For the other estimate of hen activity, based on head movement, there were no differences due to any of the main effects from 59-61 weeks of age.

Production

While there were no effects of furniture on hen day egg production (Table 3a), production was slightly higher from 22-29 weeks of age in the larger cage with 8 hens (Table 3b). Overall hen day production figures from 22-29 weeks and 48-55 weeks of age were 93 and 80 %, respectively (see Tables 3a and 3b). The overall number of cracked or dirty eggs collected over 2 days when the hens were 54 and 55 weeks of age was 12.8 %; 34 % of these were from a treatment with a perch. Mean body weight at 48-55 weeks of age was 2.54 kg (see Tables 3a and 3b). Overall mortalities in the experiment were 7 % found dead and a further 5.8 % culled due to being pecked or unwell. While half the cages in the furnished cage treatments had a perch, only about one-third of cracked and dirty eggs were from a treatment with a perch, thus in this experiment the incidence of cracked and dirty eggs was not exacerbated by the presence of a perch.

Immunology

There were no significant effects of the furniture treatments of perch, nest box and dust bath on white blood cell counts, differential white cell counts, cell count ratios ($P > 0.05$; Tables 8a and 8b) or immunological responsiveness ($P > 0.05$; Table 9a), except for a higher white blood cell count at 32 weeks of age in the dust bath treatment when only the space provided by the dust bath was provided (ie. without dust) ($P = 0.021$; Table 8a). However, there was no difference in white cell count in the dust bath treatment when the dust bath (with sawdust) was either present or absent ($P > 0.05$; Table 8a). In contrast, while group size and space allowance treatments had no effects on white cell and differential white cell counts ($P > 0.05$; Table 8b), there were significant effects on immunological responsiveness (Table 9b). In the cage with 16 hens, the ability of cells to produce IL-6 was reduced at 32 weeks of age ($P = 0.051$; Table 8b).

Corticosterone

While the corticosterone concentration as a result of an ACTH challenge was higher if a dust bath ($P = 0.016$) or a nest box ($P = 0.041$) was present in the cage at 64 weeks of age, this was in part due to higher corticosterone concentrations in the base-line sample ($P > 0.05$) and thus the corticosterone response, calculated as a per cent change was not affected by the furniture treatments ($P > 0.05$; Table 10a). There were no other effects of furniture treatments, perch, dust bath and nest box on plasma or egg corticosterone concentrations or the adrenal response to exogenous ACTH ($P > 0.05$; Table 10a). In contrast there were some significant effects of the space allowance and group size treatments. Egg corticosterone concentration was significantly higher at 33 weeks of age in the 16 hen treatment ($P = 0.021$), although there was no effect at 63 weeks of age ($P = 0.086$). While plasma corticosterone concentrations were higher in the 16 bird double cage and 8 bird single cage
treatments, the effects were not statistically different ($P > 0.05$; Table 10b). There was evidence of a reduced adrenal responsiveness to exogenous ACTH at 36 weeks of age ($P = 0.034$) due to both slightly higher baseline corticosterone concentrations and lower corticosterone concentrations following the ACTH injection ($P > 0.05$; Table 10b).

Table 8a
Effects of furnished cage treatments on haematology of laying hens

Parameter	Factor[1]	Mean values Yes	No	Space	SE	P value
32 weeks						
[2]WBC count	Perch	17.8	16.7	-	1.07	0.343
	Dust bath	16.7	15.8	19.2	1.21-1.22	**0.021**
	Nest box	17.8	15.8	18.2	1.26-1.28	0.137
[2]Heterophil : lymphocyte ratio	Perch	1.38	1.29	-	0.184	0.665
	Dust bath	1.35	1.40	1.25	0.223-0.227	0.795
	Nest box	1.24	1.51	1.25	0.220-0.225	0.417
Grans : Pbmnc ratio[3]	Perch	0.91	0.97	-	0.068	0.390
	Dust bath	0.96	0.92	0.95	0.084-0.085	0.877
	Nest box	0.94	1.03	0.85	0.081	0.092
[2]Heterophil + eosinophil + basophil count	Perch	8.94	8.37	-	0.502	0.261
	Dust bath	8.31	8.30	9.37	0.603	0.136
	Nest box	8.90	8.12	8.94	0.611-0.613	0.327
[2]Lymphocyte + monocyte count	Perch	9.67	9.00	-	2.384	0.449
	Dust bath	9.29	8.34	10.38	1.054-1.057	0.169
	Nest box	9.77	8.23	10.00	1.061-1.065	0.211
62 weeks						
[2]WBC count	Perch	9.6	8.8	-	0.77	0.348
	Dust bath	8.9	9.0	9.7	0.97-0.98	0.721
	Nest box	9.0	9.2	9.4	0.97-1.01	0.945
[2]Heterophil : lymphocyte ratio	Perch	*	*	-	*	*
	Dust bath	*	*	*	*	*
	Nest box	*	*	*	*	*
Grans : Pbmnc ratio[3]	Perch	2.51	2.31	-	0.164	0.228
	Dust bath	2.35	2.38	2.52	0.208-0.211	0.705
	Nest box	2.22	2.47	2.55	0.203-0.210	0.280
[2]Heterophil + eosinophil + basophil count	Perch	6.57	6.15	-	0.664	0.528
	Dust bath	5.96	6.28	6.87	0.827-0.838	0.550
	Nest box	5.82	6.32	6.93	0.828-0.857	0.443
[2]Lymphocyte + monocyte count	Perch	3.03	3.04	-	0.305	0.984
	Dust bath	2.88	2.94	3.29	0.379-0.384	0.526
	Nest box	2.88	2.93	3.29	0.380-0.393	0.523

[1]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [2]10^6cells/mL; [3]Ratio of 'granulocytes' (heterophils + eosinophil + basophils): 'peripheral blood mononuclear cells' (lymphocytes + monocytes)**;**
*Data not analysed due to misidentification of cells in samples at 62 weeks of age.

Table 8b
Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on total and differential white cell counts

Parameter	Mean values			SE between double cages	SE between double and single cages	P value
	Double 16	Double 8	Single 8			
32 weeks						
[1]WBC count	16.4	16.4	17.2	3.33	1.95-2.81	0.873
Heterophil : Lymphocyte ratio	1.88	0.90	1.34	0.528	0.324-0.459	0.135
Grans : Pbmnc ratio[2]	0.86	1.06	0.94	0.229	0.148-0.180	0.699
[1]Heterophil + eosinophil + basophil count	7.92	9.41	8.66	1.61	0.953-1.338	0.627
[1]Lymphocyte + monocyte count	8.55	7.66	9.33	2.790	1.645-2.334	0.706
62 weeks						
[1]WBC count	11.1	9.8	9.2	2.61	1.59-2.08	0.479
Heterophil : Lymphocyte ratio	*	*	*	*	*	*
Grans : Pbmnc ratio[2]	2.71	2.29	2.42	0.561	0.341-0.449	0.669
[1]Heterophil + eosinophil + basophil count	7.80	5.65	6.37	2.232	1.362-1.784	0.543
[1]Lymphocyte + monocyte count	2.05	2.62	3.04	1.024	0.624-0.819	0.881

[1]10^6cells/mL; [2]Ratio of 'granulocytes' (heterophils + eosinophil + basophils): 'peripheral blood mononuclear cells' (lymphocytes + monocytes)**;** *Data not analysed due to misidentification of cells in samples at 62 weeks of age.

Table 9a
Effects of furnished cage treatments on immunological responsiveness of laying hens

Parameter	Factor[1]	Mean values (back-transformed values in parentheses)			SE	P value
		Yes	No	Space		
32 weeks						
Proliferation test (10 μg of Con A) *(cpm)*[2]	Perch	2.31 (153)	2.35 (173)	-	0.098	0.693
	Dust bath	2.33 (162)	2.34 (168)	2.32 (157)	0.121	0.986
	Nest box	2.29 (143)	2.38 (190)	2.32 (157)	0.121	0.725
Proliferation test (50 μg of Con A) *(cpm)*	Perch	2410	2410	-	417	0.997
	Dust bath	2530	2270	2420	502-507	0.877
	Nest box	2520	2440	2270	488-504	0.878
IL-6 production test *(cpm)*[2]	Perch	3.96 (9060)	3.86 (7190)	-	0.056	0.077
	Dust bath	3.97 (9270)	3.88 (7640)	3.87 (7410)	0.070-0.071	0.338
	Nest box	3.87 (7380)	3.92 (8360)	2.93 (8530)	0.071-0.072	0.638
62 weeks						
Proliferation test (10 μg of Con A) *(cpm)*[2]	Perch	2.26 (130)	2.37 (184)	-	0.091	0.229
	Dust bath	2.29 (145)	2.21 (112)	2.45 (229)	0.109-0.111	0.124
	Nest box	2.28 (140)	2.21 (112)		0.109-0.110	0.093
Proliferation test (50 μg of Con A) *(cpm)*[2]	Perch	2.74 (501)	2.91 (759)	-	0.129	0.202
	Dust bath	2.85 (655)	2.72 (480)	2.91 (763)	0.159-0.162	0.506
	Nest box	2/74 (501)	2.98 (894)	2.77 (540)	0.159-0.165	0.295
IL-6 production test *(cpm)*[2]	Perch	4.01 (10210)	4.07 (11690)	-	0.055	0.297
	Dust bath	4.05 (11320)	4.01 (10210)	4.05 (11270)	0.069-0.070	0.767
	Nest box	4.04 (11040)	4.06 (11560)	4.01 (10210)	0.069-0.071	0.757

[1]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [2]$Log_{10}(y+50)$ values; a lower response in these tests indicates immunosuppression.

Table 9b
Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on immunological responsiveness

Parameter	Mean values (back-transformed values in parentheses)			SE between double cages	SE between double and single cages	P value
	Double 16	Double 8	Single 8			
32 weeks						
Proliferation test (10 μg of Con A) *(cpm)*[1]	2.13 (85)	2.52 (283)	2.33 (162)	0.309	0.185-0.257	0.424
Proliferation test (50 μg of Con A) *(cpm)*	1680	3480	2410	1240	720-1066	0.339
IL-6 production test *(cpm)*[1]	3.63 (4280)	3.79 (6180)	3.91 (8070)	0.187	0.113-0.153	**0.051**
62 weeks						
Proliferation test (10 μg of Con A) *(cpm)*[1]	1.87[a] (24)	2.32[b] (160)	2.31[b] (156)	0.291	0.174-0.241	**0.047**
Proliferation test (50 μg of Con A) *(cpm)*[1]	2.53 (287)	2.74 (505)	2.74 (620)	0.427	0.257-0.346	0.501
IL-6 production test *(cpm)*[1]	3.96 (9060)	3.94 (8650)	4.04 (10910)	0.183	0.111-0.148	0.619

[1]$Log_{10}(y+50)$ values; [ab]Different letters denote a significant treatment effect ($P < 0.05$); a lower response in these tests indicates immunosuppression.

Table 10a
Effects of furnished cage treatments on corticosterone concentrations in plasma and eggs and ACTH responsiveness

Parameter	Factor[1]	Mean values (back-transformed values in parentheses)			SE	P value
		Yes	No	Space		
At 33, 34-35 and 36 weeks of age for egg and plasma corticosterone and ACTH response, respectively						
Plasma corticosterone concentrations *(ng mL^{-1})*	Perch	3.21	3.27	-	0.126	0.649
	Dust bath	3.33	3.04	3.35	0.149-0.150	0.073
	Nest box	3.27	3.33	3.10	0.152-0.154	0.317
Egg corticosterone concentrations *(ng mL^{-1})*	Perch	1.52	1.54	-	0.041	0.704
	Dust bath	1.49	1.54	1.55	0.049-0.050	0.471
	Nest box	1.53	1.50	1.56	0.049-0.050	0.548
Corticosterone response to ACTH *(ng mL^{-1})*	Perch	37.7	36.6	-	2.60	0.672
	Dust bath	39.3	37.1	35.0	3.15-3.20	0.404*
	Nest box	39.6	33.1	39.1	3.05-3.09	0.065*
Corticosterone response to ACTH *(% change)*[2]	Perch	3.02 (1040)	3.00 (990)	-	0.040	0.624
	Dust bath	3.02 (1040)	3.03 (1070)	2.98 (950)	0.049-0.050	0.613
	Nest box	2.83 (670)	2.74 (550)	2.83 (670)	0.051-0.052	0.172
At 63, 64-65 and 66 weeks of age for egg and plasma corticosterone and ACTH response, respectively						
Plasma corticosterone concentrations *(ng mL^{-1})*	Perch	3.42	3.34	-	0.115	0.502
	Dust bath	3.48	3.28	3.37	0.140	0.363
	Nest box	3.53	3.34	3.26	0.137-0.138	0.152
Egg corticosterone concentrations *(ng mL^{-1})*	Perch	1.14	1.10	-	0.024	0.084
	Dust bath	1.10	1.12	1.12	0.030	0.772
	Nest box	1.10	1.10	1.15	0.029	0.239
Corticosterone response to ACTH *(ng mL^{-1})*	Perch	26.0	25.3	-	1.85	0.682
	Dust bath	29.1^{b}	22.6^{a}	25.4^{ab}	2.15-2.17	**0.016**
	Nest box	28.4^{b}	26.0^{ab}	22.7^{a}	2.17-2.22	**0.041**
Corticosterone response to ACTH *(% change)*[2]	Perch	2.79 (620)	2.80 (630)	-	0.043	0.896
	Dust bath	2.85 (710)	2.75 (550)	2.79 (660)	0.051-0.052	0.127
	Nest box	2.83 (670)	2.74 (550)	2.83 (670)	0.051-0.052	0.172

[1]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [2]Log_{10} values; [ab]Different letters denote a significant treatment effect ($P < 0.05$); *****Using Wald Test since submodel fitted for Likelihood Ratio Analogue Test failed to numerically converge.

Table 10b
Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on corticosterone concentrations in plasma and eggs and ACTH responsiveness

	Mean values (back-transformed values in parentheses)			SE between double cages	SE between double and single cages	P value
	Double 16	Double 8	Single 8			
At 33, 34-35 and 36 weeks of age for egg and plasma corticosterone and ACTH response, respectively						
Plasma corticosterone concentrations *(ng mL^{-1})*	3.47	2.58	3.24	0.404	0.244-0.332	0.095
Egg corticosterone concentrations *(ng mL^{-1})*	1.75^{ap}	1.48^{bq}	1.53^{q}	0.128	0.076-0.107	**0.021**
Corticosterone response to ACTH *(ng mL^{-1})*	29.0	41.3	47.2	8.27	4.94-6.82	0.251
Corticosterone response to ACTH *(% change)*[1]	2.84^{ap} (690)	3.23^{bq} (1680)	3.01^{pq} (1020)	0.129	0.078-0.105	**0.034**
At 63, 64-65 and 66 weeks of age for egg and plasma corticosterone and ACTH response, respectively						
Plasma corticosterone concentrations *(ng mL^{-1})*	3.39	2.81	3.38	0.337	0.213-0.288	0.155
Egg corticosterone concentrations *(ng mL^{-1})*	1.17	1.24	1.12	0.076	0.045-0.064	0.086
Corticosterone response to ACTH *(ng mL^{-1})*	23.5	21.5	25.7	6.08	3.69-4.93	0.611
Corticosterone response to ACTH *(% change)*[1]	2.75 (560)	2.82 (670)	2.80 (630)	0.137	0.084-0.112	0.830

[1]Log_{10} values; $^{ab, pq}$different letters denote a significant treatment effect ($P < 0.05$ and $P < 0.01$, respectively).

Feather damage and cover, claw length, foot condition, injuries on the comb, around the cloaca and on the back, keel bone deformation

While feathers were significantly dirtier in the perch treatment at both 36 and 66 weeks of age ($P = 6.1 \times 10^{-6}$ and 0.002, respectively; Table 11a), the differences were only small (< 0.4 on a 4 point scale) and the mean values suggested feathers got dirtier with age. In contrast there was a significant effect of the perch treatment on foot condition at both 36 and 66 weeks of age ($P = 0.003$ and 0.0004, respectively); condition was better when a perch was present. Foot condition appeared to be poorer in the older hens. Feather damage and cover was slightly worse in the perch treatment at 66 weeks of age ($P = 0.012$; Table 11a). There were no effects of group size and space allowance on any condition measurements ($P > 0.05$; Table 11b).

Table 11a
Effects of furnished cage treatments on feather damage and cover and foot and claw condition of laying hens

Parameter	Factor[1]	Mean values Yes	No	Space	SE	P value
36 weeks of age						
Feather cover and damage score[2]	Perch	1.27	1.26	-	0.044	0.803
	Dust bath	1.27	1.26	1.26	0.023-0.024	0.974
	Nest box	1.28	1.27	1.24	0.023-0.024	0.283
Feather cleanliness[2]	Perch	1.45[a]	1.23[b]	-	0.042	**6.1×10^{-6}**
	Dust bath	1.33	1.35	1.35	0.053-0.054	0.865
	Nest box	1.34	1.35	1.33	0.054-0.055	0.933
Claw length *(mm)*	Perch	17.2	17.2	-	0.17	0.856
	Dust bath	17.0	17.2	17.3	0.20	0.173
	Nest box	17.1	17.0	17.4	0.20	0.104
Claw condition[2]	Perch	3.93	3.92	-	0.041	0.753
	Dust bath	3.88	3.94	3.96	0.050-0.051	0.251
	Nest box	3.93	3.91	3.93	0.052-0.053	0.895
Foot cleanliness[2]	Perch	1.65	1.56	-	0.067	0.165
	Dust bath	1.63	1.60	1.59	0.083-0.085	0.887
	Nest box	1.61	1.65	1.56	0.082-0.084	0.535
Foot condition[2]	Perch	0.61[a]	0.79[b]	-	0.058	**0.0030**
	Dust bath	0.65	0.79	0.66	0.070-0.071	0.098
	Nest box	0.72	0.73	0.64	0.0734-	0.483
66 weeks of age						
Feather cover and damage score[2]	Perch	2.32[a]	2.13[b]	-	0.072	**0.012**
	Dust bath	2.12	2.32	2.23	0.087	0.095
	Nest box	2.17	2.32	2.19	0.087-0.088	0.193
Feather cleanliness[2]	Perch	2.05[b]	1.83[a]	-	0.068	**0.0020**
	Dust bath	1.98	1.89	1.94	0.084-0.085	0.573
	Nest box	1.91	1.95	1.95	0.086-0.087	0.852
Claw length *(mm)*	Perch	20.5	20.5	-	0.30	0.874
	Dust bath	20.3	20.6	20.5	0.37	0.729
	Nest box	20.4	20.2	20.8	0.36	0.271
Claw condition[2]	Perch	2.63	2.60	-	0.062	0.664
	Dust bath	2.56	2.62	2.66	0.077	0.445
	Nest box	2.62	2.67	2.55	0.075-0.077	0.360
Foot cleanlines[2]	Perch	1.97	1.99	-	0.048	0.792
	Dust bath	2.56	2.62	2.66	0.077	0.445
	Nest box	2.62	2.67	2.55	0.075-0.077	0.360
Foot condition[2]	Perch	1.85[a]	2.20[b]	-	0.091	**0.00042**
	Dust bath	1.94	2.02	2.11	0.114-0.115	0.326*
	Nest box	2.00	2.12	1.96	0.113-0.116	0.349
Pecking injuries on comb[2]	Perch	1.57	1.64	-	0.087	0.435
	Dust bath	1.68	1.65	1.49	0.103	0.131
	Nest box	1.52	1.60	1.69	0.105	0.310

[1]Within each factor the mean values for the presence or absence, respectively of a perch, dust box or nest box, are presented in the 'Yes' and 'No' columns. The mean values for the factor, space, provided by the absence of these items of furniture are presented in the 'Space' column.; [2]Score varies from 0-5 for foot condition and 1-4 for comb pecks and feather cover and damage (comb peck injuries and feather cover/damage scores reversed for clarity – see methods), claw condition and feather and foot cleanliness with a high score indicating increased damage or poorer cleanliness; [ab]Different letters denote a significant treatment effect ($P < 0.05$); *Using Wald Test since submodel fitted for Likelihood Ratio Analogue Test failed to numerically converge.

Table 11b
Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on feather damage and cover and foot and claw condition of laying hens

Parameter	Mean values			SE between double cages	SE between double and single cages	P value
	Double 16	Double 8	Single 8			
36 weeks of age						
Feather cover and damage score[1]	1.28	1.20	1.26	0.062	0.037-0.050	0.445
Feather cleanliness[1]	1.24	0.99	1.23	0.141	0.089-0.116	0.123
Claw length *(mm)*	17.0	18.0	17.2	0.54	0.32-0.44	0.159
Claw condition[1]	3.93	4.00	3.92	0.132	0.80-0.109	0.790
Foot cleanliness[1]	1.73	1.51	1.61	0.214	0.126-0.178	0.522
Foot condition[1]	0.86	0.41	0.79	0.194	0.123-0.159	0.056
66 weeks of age						
Feather cover and damage score[1]	2.29	1.98	2.13	0.234	0.146-0.195	0.397
Feather cleanliness[1]	1.69	1.44	1.83	0.226	0.143-0.186	0.129
Claw length *(mm)*	20.0	19.6	20.5	0.89	0.57-0.75	0.421
Claw condition[1]	2.63	2.51	2.61	0.206	0.124-0.167	0.821
Foot cleanliness[1]	1.93	1.77	1.98	0.139	0.079-0.120	0.228
Foot condition[1]	2.44	2.08	2.20	0.306	0.195-0.250	0.400
Pecking injuries on comb[1]	1.84	1.54	1.61	0.266	0.159-0.225	0.320

[1]score varies from 0-5 for foot condition and 1-4 for comb pecks and feather cover and damage (comb peck injuries and feather cover/damage scores reversed for clarity – see methods section), claw condition and feather and foot cleanliness with a high score indicating increased damage or poorer cleanliness.

The number of pecking wounds and injuries on the back and around the cloaca were low at both 36 and 66 weeks of age (Tables 11a and 11b for hens at 66 weeks of age); the assessment of wounds was only made in live hens and does not include any birds that died or were culled and for which pecking may have been a contributing factor. For example, the mean overall wound/injury score at 66 weeks of age was 3.94 ± 0.165 (SD), where a score of 4 was for 0 wounds/injuries. At 36 weeks of age there was only 1 hen given a score of 3 for a few obvious pecking wounds/injuries on the back/cloaca and all other hens were scored as 4. There were more injuries/wounds at 66 weeks of age; all hens were scored as 4 except for 3 hens given a score of 2 and 8 hens a score of 3 for the cloaca and 3 hens were given a score of 1, 5 hens a score of 2 and 15 hens a score of 3 for the back. Pecking scores on the combs were not analysed in the period commencing at 36 weeks of age as 79 % of all hens were given a score of 4 (ie. no pecks). At 66 weeks of age 43 % of hens were scored as 4, 49 % were scored as 3 (1-4 pecks/comb) and 33 % scored as 2 (> 4 pecks/ comb). There were no effects of treatment at 66 weeks of age (Tables 11a and 11b; note the scores in the tables have been reversed so that a higher score indicates increased damage). There were no instances of cages with keel bone deformations at 36 weeks of age (all cages had an average score of 4 indicating normal keel shape). At 66 weeks of age the overall mean score was 3.64; 10 cages had an average score of 3 indicating their keel bones were slightly flattened. Of these 10 cages, 60 % were from cages with perches. On an individual hen basis, there were 15.5 % of hens scored with twisted keel bones and 3.7 % with slightly flattened keel bones. Of the hens with twisted keel bones, 54.9 % were from a cage with a perch and 11.2 % from double cages. Of the hens with slightly flattened keel bones, 70 % were from a cage with a perch.

Bone strength

The major effect on bone strength was due to the perch treatment with some lesser effect due to cage size (Tables 12a and 12b).The tibia, humerus and coracoid were stronger in the perch treatment ($P < 0.05$; Table 12a) and the coracoid was stronger in the larger cages ($P = 0.046$; Table 12b).

Table 12a

Effects of furnished cage treatments on bone strength (N) in hens at 67-70 weeks of age

Parameter	Factor[1]	Mean values			SE	P value
		Yes	No	Space		
Tibia	Perch	162.7[b]	151.0[a]	-	4.57	**0.0090**
	Dust bath	161.6	159.8	151.6	5.50-5.51	0.181
	Nest box	159.8	157.9	155.2	5.54-5.63	0.705
Femur	Perch	199.8	195.3	-	6.43	0.486*
	Dust bath	203.7	198.2	191.2	7.8	0.283[†]
	Nest box	198.1	198.1	196.5	8.0	0.975*
Humerus	Perch	116.6[b]	102.1[a]	-	5.11	**0.0063**
	Dust bath	115.5	104.5	114.3	6.15-6.25	0.169
	Nest box	106.3	112.8	115.1	6.24-6.34	0.393
Coracoid	Perch	159.0[b]	144.5[a]	-	6.54	**0.031**
	Dust bath	161.0	146.0	147.8	7.68-7.74	0.543
	Nest box	154.5	153.9	146.3	7.76-7.90	0.542

[1]Within each factor the presence or absence, respectively of a perch, dust box or nest box, data are presented in the 'Yes' and 'No' columns of mean values. The data for the factor, space, provided by these items of furniture are presented in the 'Space' column of mean values; [ab]Different letters denote a significant treatment effect ($P < 0.05$); *****Using Wald Test since model fitted did not numerically converge using Fisher algorithm; [†]Using Wald Test since submodel fitted for Likelihood Ratio Analogue Test failed to numerically converge.

Table 12b

Effects of housing hens in single cages with 8 birds and double cages with 8 or 16 birds on bone strength (N) at 67-70 weeks of age

Parameter	Mean values			SE between double cages	SE between double and single cages	P value
	Double 16	Double 8	Single 8[1]			
Tibia	175.5	158.6	151.0	13.90	8.44-12.05	0.082
Femur	214.5	207.8	197.6	20.4	12.2-17.0	0.380
Humerus	109.2	127.1	102.1	16.3	10.2-13.7	0.184
Coracoid	170.6[b]	171.8[b]	144.5[a]	18.7	11.8-16.6	**0.046**

[1]Standard size cages with a perch were excluded from the single cage analysis so that all comparisons across treatments were of similarly configured cages ie. without perches; [ab]Different letters denote a significant treatment effect ($P < 0.05$).

Discussion

The limited production data in this experiment showed that egg production was not dissimilar from industry targets. Hen day egg production figures from 22-29 weeks and 48-55 weeks of age from the company website (www.hyline.com.au) for Hy-Line Brown hens are 90 and 86 %; in the furniture treatments mean hen day egg production for the two ages was 91.5 and 80 %, respectively (see Tables 3a and 3b). Similarly, industry figures for body weight at 48-55 weeks of age is 2.24 kg, with the birds in this study being slightly heavier with a mean body weight of 2.54 kg (see Tables 3a and 3b). Overall mortalities in the experiment were 7 % found dead and a further 5.8 % culled due to being pecked or unwell. The overall number of cracked or dirty eggs collected over 2 days when the hens were 54 and 55 weeks of age was 12.8 %. Thirty four per cent of these were from a treatment with a perch, suggesting that cracked and dirty eggs were not exacerbated due to the presence of a perch, as has been reported in other studies where an appropriate placed and designed perch was present (Tauson, 1984b; Appleby *et al.*, 1992; Duncan *et al.*, 1992; Abrahamsson and Tauson, 1993, Abrahamsson *et al.*, 1995). The incidence of cracked and dirty eggs was not dissimilar to that found in previous studies, for example Abrahamsson *et al.* (1995) found 9.8 and 11.2 % cracked and dirty eggs in two trials. However, the size of the present experiment was too small for rigorous conclusions to be drawn from the production data and thus most of these data were not statistically analysed. Nevertheless, they have been presented to indicate the normality of the birds in the present experiment.

This experiment has shown, based on both mean values and the relatively small standard errors, that any effects of the three items of furniture, that is, a perch, dust bath and nest box, on the physiological measurements used in this experiment were relatively small. There were some minor effects of furniture on bird feather and foot condition and feather damage/cover, with dirtier and more damaged feathers in the perch treatment but better food condition in this treatment. There were also some behavioural changes as a consequence of changing the environment with effects of a nest on egg laying behaviour. While over 30 % of eggs were laid on the wire floor, over 80 % of those eggs laid in the nest box were laid on the low side ie. closest to the egg roll-out tray. Hens did not generally use the dust bath unless a perch was also present, but this was probably due to the configuration of the cage used for this experiment whereby the dust bath was on top of the nest box and access was probably inhibited/prevented in the absence of a perch. Other cage designs of different configurations ie. with the dust bath at floor level may have increased dust bath use. In contrast there were significant physiological effects of group size and space allowance with some evidence that birds housed at 16 hens/cage (space allowance of 750 cm^2/bird) were stressed compared to birds housed at 8 hens/cage with the same space allowance. This was based on higher egg corticosterone concentrations at 33 weeks of age and evidence of immunosuppression at 32 weeks of age. While the group size/space allowance data suggest that, within the range of space allowance and group size treatments used in this experiment, group size had a greater potential effect on welfare related criteria than space allowance, the data for plasma corticosterone concentrations both at rest and in response to ACTH make it equivocal whether the stress response was an acute or a chronic response. It is also of interest that in the larger space allowance treatment (double cages with 8 hens) the daily period of egg laying was shorter ($P > 0.1$) and the maximum time between consecutive eggs being laid was considerably shorter from 48-55 weeks of age ($P = 0.032$) and tended to be shorted in time from 29-31 weeks of age ($P = 0.054$) than other treatments. This could indicate less disturbance of egg laying behaviour due to the increased space, or improved “nest-site selection”. A number of factors such as aggression, limited nest space and the presence of humans can lead to a disturbance of egg laying (Appleby *et al.*, 2004). Further research on group size, space allowance and their interactions is clearly warranted. While these space and group size control treatments were external to the main experiment and consequently the data should be treated with some caution, they nevertheless indicate that the physiological criteria were

sufficiently sensitive to distinguish treatment effects. Hence, in relation to the larger experiment on furniture in cages, if there was an effect of any of the items of furniture, either alone or in combination, they were relatively minor.

In this experiment, the experimental design and statistical analysis have been carefully implemented to maximise the power of the experiment in relation to the amount of resources used. The furniture treatments were arranged in factorial treatment replications to obtain the benefits of hidden replication (Mead *et al.*, 1993). This meant that when there was no evidence of interactions between furniture treatments, as occurred with this experiment, the effective number of replications of each treatment increased dramatically. In the case of this experiment, the replication increased from 3 to 18 or 27 depending on the number of levels of the treatment. A rectangular lattice blocking structure was used so that the effects of spatial location within sheds, order of filling birds to cages, temporal effects related to husbandry and temporal effects related to measurement could, as much as possible, be confounded with blocks, without a large compromise in the efficiency of treatment comparisons. A REML (restricted maximum likelihood) mixed model analysis was used to extract information from both between blocks and within blocks. Interactions were examined with every measurement, so that more complex effects of furniture types, such as those producing synergistic or compensatory effects, would not be overlooked. Where appropriate the data has been analysed after a transformation, to avoid spurious effects caused by the statistical distribution assumptions not being in accord with the data. The combination of these techniques allowed definitive and rigorous examination of the effect of furniture within a moderately sized experiment.

This experiment was unique in that it has examined a range of items of furniture considered important to bird welfare in the one experiment. The relatively low standard errors, across a number of variables indicate the rigour of the experimental design and that any main effects of items of furniture on physiological criteria related to bird welfare were small. Previous experiments have examined a range of physiological variables associated with cage/pen effects, including one or more of plasma corticosterone, glucose and cholesterol concentrations, weights of adrenal, thyroid and pituitary glands and white cell (leukocyte) numbers (Wolford and Ringer, 1962, Bareham, 1972, Eskeland, 1976, Craig and Craig, 1985, Mench *et al*., 1986). Differences were generally few with one study showing adrenal hypertrophy in hens housed in cages (Eskeland, 1976) and increased corticosterone concentrations in one of two studies in hens housed in pens (Koelkebeck and Cain, 1984a, 1984b). While a study by Thaxton and Odhiambo (2003) found no initial stress effects of housing individually in cages, in groups in cages or floor pens, if the birds were stressed (infused ACTH over a 6-day period) there was evidence of increased stress in the individually housed hens. More recent studies on furnished cages or aspects of furnished cages have examined some cage modifications on physiological variables. These cage modifications included perches (Barnett *et al.*, 1997a) and solid sides (Barnett *et al.*, 1997b), with the only effect being reduced corticosterone concentrations and lower heterophil:lymphocyte ratios in cages with solid sides (Barnett *et al.*, 1997b). Most research on hens in furnished cages has focussed on behavioural or production indicators of welfare (Appleby, 1998; Niekerk *et al.*, 2002), although Elson (2004) reports recent work in the UK that is also utilising physiological indicators.

As indicated in the results it was necessary to change the way in which white blood cell ratios were calculated from samples at 62 weeks of age. While haemographs from the samples at 32 weeks of age appeared normal and conformed to the typical chicken pattern, most of the scatter plots from the samples at 62 weeks of age were different. The cells were smaller and less granular resulting in the machine miss-identifying most monocytes as lymphocytes, and the distinction between heterophil and eosinophil was also not clear. The reasons for this change are unknown. It could have been due to equipment malfunction, age of the birds or the sampling method. At 32 weeks of age blood was collected into an anti-coagulant coated syringe, mixed and transferred to a vial, while at 62 weeks of age, the

Monovette sampling system with anti-coagulant coated beads was used. For the purpose of cautious interpretation of the data from this experiment the data for the immunological measurements and assays from hens at 62 weeks of age are not discussed.

The H/L ratio at 32 weeks of age was typical of the range seen in other studies for adult hens (Beuving *et al.*, 1989; El-Lethey *et al.*, 2000), although they are higher than the reference values suggested by Siegel and Gross (2000). There is some evidence (Mench *et al.*, 1986) that the H/L ratio is lower in old birds. These authors found a ratio of < 0.2 for birds at 98 weeks of age compared to typical ratios of 1.3-2.5 (see Beuving *et al.*, 1989; El-Lethey *et al.*, 2000) and 0.6-1.2 (Siegel and Gross, 2000). While there were no effects of items of furniture on any immunological measures the data from the present experiment suggest a reduced ability of white blood cells *in vitro* to produce interleukin-6 at 32 weeks of age, suggesting either an acute or chronic stress response. As reviewed by Thaxton (2004) there has been a limited amount of research on the effects of stress on the immune system of laying hens, although heterophil numbers are affected by diet, bacterial infection, light and trauma (Maxwell and Robertson, 1998). Birds reared under thermoneutral conditions experienced immunosuppression when exposed to acute hot or cold episodes (Hester *et al.* 1996). Lymphocyte proliferation was suppressed in response to heat in *in vitro* assays to the mitogen concanavalin A and a lipopolysaccharide fraction, similar to those used in this experiment (Puthpongsiriporn *et al.*, 2001). Immune responsiveness to human IgG was reduced in birds in floor pens compared to those in cages (Erhard *et al.*, 2000). The ability of hens to produce antibodies to both sheep red blood cells and tetanus toxoid antigens was greater in hens in floor pens with litter compared to hens in similar pens without litter (El-Lethey *et al.*, 2000).

Generally, there is an expectation in mammals that are chronically stressed of an endogenous down-regulation of the HPA axis, with endogenous increases in corticosterone operating on the pituitary (via negative feedback) to suppress ACTH. If this system is stimulated with exogenous ACTH there is often an increased corticosteroid response in chronically stressed animals (Dantzer *et al.*, 1983). This has been shown in rats (Riegle, 1973), dairy cows (Friend *et al*., 1979) and pigs (Borell and Hurnik, 1991; Barnett and Taylor, 1995). However, the methodology has been used and shown few effects. For example, in pigs Hemsworth *et al*., (1996) showed only a trend for an increase in response to a daily injection treatment and Cronin *et al*., (1991) and Levis *et al*. (1995) showed no effects in response to farrowing and boar housing treatments, respectively. These data have either been inconsistent with other data or have been used as evidence for a lack of a chronic stress response. In hens the data are equivocal, with considerable variation in hens in responsiveness of the HPA axis and corticosterone concentrations *per se* (Beuving and Vonder, 1978, 1986; Beuving *et al*., 1980). However, there are examples, as appears to be occurring in the present study, where there is potentially a reduced responsiveness. In poultry there were no differences in response to ACTH in hens classified as either high fear or low fear (Beuving *et al*., 1980). However, in that study while the ‘at rest’ corticosterone concentrations for the ACTH response test were not provided, they had elevated corticosterone concentrations in response to a feeding frustration, although there were no differences in corticosterone concentrations at the start of the feeding trial. If high fear birds had elevated corticosterone, this could have resulted in a decreased response to ACTH. This issue of responsiveness to ACTH requires further clarification across and within species. In the present experiment the corticosterone response to exogenous ACTH only varied ($P < 0.05$) in the space allowance/group size subset of data. In conjunction with the plasma corticosterone data which ‘at rest’ was highest ($P > 0.05$) in the 16 hen/cage treatment at 34-35 weeks of age, a cautious interpretation of these ACTH data is there is some evidence of an acute stress response, perhaps due to social factors interacting with other factors associated with the time around peak egg production. Further research is clearly warranted on this topic.

Probably the most discussed issue in relation to the welfare of hens in cages is the lack of a suitable nest site. One objective of furnished cages was to address this shortcoming. Nevertheless, it can be argued that the data do not confirm this concern. There is no doubt that in the presence of a nest there is behavioural change. In the present experiment, egg laying was affected by the presence of a nest and interacted with other items of furniture. For example, from 29-31 weeks of age more eggs were laid in the nest if there was also a perch present, and from 59-61 weeks of age more eggs were laid in the nest in the absence of a dust bath. However, there were no effects on the interval between eggs being laid and the maximum interval between eggs being laid ($P > 0.05$). In contrast, the maximum interval between eggs being laid was lowest in the 8 hens/cage treatment with a space allowance of 1500 cm^2/hen. It has been suggested that egg laying behaviour is disturbed in conventional cages, with evidence of frustration in the absence of a nest (see review by Ekstrand and Keeling 1994). This experiment suggests that the disturbance may be reduced with more space, but the presence of a nest does not appear to have on impact on this behaviour. It is noteworthy that in another experiment on nesting behaviour (see Chapter 4) only half of the birds that had experience of a nest in their cage chose to lay in the nest when the opportunity was provided. Also, there was little difference (in 2-bird cages with approximately 3000 cm^2/hen) in nesting behaviour between birds that previously had either experience or no experience with a nest. Laying outside of nest boxes is well known and there is also considerable individual variation in preferences for nest design (Sherwin and Nicol, 1993). Other studies report on floor laying in furnished cages and this varies from 0-80 % of eggs (see Sherwin and Nicol, 1993), although other studies report a high incidence of laying in nests (> 90 %; see Abrahamsson *et al.* (1996)). Similarly, Appleby (1995) found no differences in the motivation of birds to use a nest between birds previously experienced or inexperienced with a nest. In both Appleby's experiment and the one described in Chapter 4 it is not known if there are any adverse welfare consequences if birds are unable to lay in their preferred site ie. in a nest if they want to lay in a nest (see Keeling, 2004). Factors affecting nest site selection include rearing and housing conditions, nest box design and management and human intervention, although most of these factors have been shown to be influential in non-cage systems (see Appleby *et al.*, 2004) rather than in furnished cages. However, it is likely that similar principles will apply.

The dust bath was only used (with the exception of one cage) if a perch was also present in the cage. For cages with both a perch and a dust bath, the dust bath was open for about 380 min per day and on average it was occupied for about 28 % of the available time. An interesting finding was the generally higher use of the dust bath in cages where there was either a nest box available or access to the nest box was blocked off, compared to the treatment that provided additional floor space because the nest box was removed. The latter treatment differed from the others in that one end of the perch was attached by a vertical bracket to the cage floor compared to the other 2 treatments where it was attached to the solid wall of the nest box (see upper photo in Figure 1). The lack of a solid wall in the 'nest box space' treatment between the end of the perch and the dust bath may have resulted in a different visual perspective of the dust bath and perhaps it was not recognised as a dust bath. Alternatively, because the dust bath provided a cover above the additional 'space' in the nest box space treatment, this area may have been perceived as more secluded. In this experiment, dust bathing behaviour could only be measured in the dust bath because of positions of the video cameras. Thirdly, the reduced 'free' space due to a solid wall when the nest box was available for use, or the reduced absolute space when the nest box was blocked off, may have increased the hens' motivation to occupy the dust bath because space was actually or apparently restricted (ie. the hens perceived and utilised the dust bath and the "space" differently).

The major effects of the furniture treatments on bird condition were due to the perch treatment ($P < 0.05$). However, in all cases the differences were only small. When a perch was present, the feathers were slightly dirtier at both 36 and 66 weeks of age, foot condition was slightly better at 36 and 66 weeks of age while feather cover and damage were slightly

worse at 66 weeks of age. Overall, in this experiment, while the differences were statistically different there was no great deterioration due to treatment. For foot condition the hens averaged between normal to mild hyperkeratosis across treatment at both ages. For feather cover and damage at 36 weeks of age the scores indicated a range from very good to parts of the body with deteriorated feathers and almost no loss of feather cover. At 66 weeks of age feather cover and damage varied from parts of the body with deteriorated feathers and almost no loss of feather cover to parts of the body that showed a clear deterioration of feathers and/or with large naked areas (more than a circle of 5 cm). The only effect of the nest box treatment was slightly more body wounds in cages with nest boxes, although the number of wounds averaged <1/bird. The dust bath treatment had no significant effects on any of the condition measurements. The design of the perch used in this experiment (oval-round in cross section with flattened top and bottom surfaces) has been recommended to reduce the incidence of foot damage (Tauson and Abrahamsson, 1994b). A previously reported negative aspect of a perch is that of deformed keel bones (Tauson and Abrahamsson, 1994; Abrahamsson *et al.*, 1996). However, in the present experiment there was no keel bone damage at period 1, but at the second sampling period this had increased to an average score of 3.64 (where a score of 4 was no damage). These scores are in the range of those reported by Tauson and Abrahamsson (1994) for hens at 35 and 55 weeks of age. As in that study the damage was higher in treatments where a perch was present.

The major effect on bone strength was due to the perch treatment with some lesser effect due to cage size. The tibia, humerus and coracoid were stronger in the perch treatment while the coracoid was stronger in the larger cages, irrespective of whether there were 8 or 16 hens in the cage. The latter may reflect the larger absolute space for wing flapping in the 1500 cm^2/hen 8-hen cages or the increased free space in the 750 cm^2/hen 16-hen cages, although it is unclear why the effect was restricted to the coracoid with no effect ($P = 0.184$) on the strength of the humerus. Increased bone strength has been shown previously as a consequence of a perch, although the bones affected vary across studies. For example, the humerus (Tauson and Abrahamsson, 1994b), femur (Barnett *et al.*, 1997a) and tibia, humerus and coracoid (this study) had increased strength as a consequence of a perch in the referenced studies.

In conclusion, this experiment has shown that, with the exception of the positive effects of a perch on bone strength, any effects of items of furniture in furnished cages are relatively small. While there were changes in behaviour, it is unclear whether they have any implications for welfare. In contrast there was a significant increase in egg corticosterone concentration in the double cage treatment of 16 hens at 33 weeks of age and evidence of immunosuppression at 32 weeks of age. While the group size/space allowance data suggest that, within the range of space allowance and group size treatments used in this experiment, group size had a greater potential effect on welfare related criteria than space allowance, the data for plasma corticosterone concentrations both at rest and in response to ACTH make it equivocal whether the stress response was an acute or a chronic response. Further research on these aspects is clearly warranted. Indeed, as indicated by Cooper and Albentosa (2004), depending on the value that hens place on activities such as foraging, dust bathing and wing flapping, it is not clear if the increased space provided in furnished cages will allow adequate expression of such behaviours.

Chapter 4

Experiment 2 – A Comparison of Behaviour Around Oviposition of Hens Laying in Nests and on the Wire Cage Floor

This experiment was largely conducted by a visiting student, Marion Desnoyers, Institut National Agronomique, Paris-Grignon, France during a 4 month period in 2003.

Introduction

From studies of the pre-laying activities of domestic hens, it is generally accepted that there are two phases of behaviour involved in oviposition (Wood-Gush, 1972, 1975; Meijsser and Hughes, 1989; Sherwin and Nicol, 1992; Yue and Duncan, 2003). Beginning about one hour prior to oviposition, the activity level of hens increases in a phase of behaviour termed "searching" in which hens appear motivated to seek a nest site. In this phase, hens increase their level of locomotion, including stereotyped pacing, and perform other behaviours such as visual inspections of potential nests, although Freire *et al*. (1996) have concluded that nest examination behaviour cannot be termed as part of searching behaviour, but rather as the onset of the sitting phase. Once hens have selected the preferred nest site, the "sitting" phase commences. This phase includes the adoption of a sitting posture interspersed with nest-building activities such as scratching the floor/litter, rotating the body on the nest and collecting litter if available.

Activities performed in the searching phase are goal-directed or appetitive behaviours. These behaviours occur when hens are motivated to find a suitable nest for oviposition (the consummatory behaviour). Thus, Appleby and McRae (1986) and Duncan and Kite (1989) showed that hens were motivated to lay their egg in a nest box, and if a nest box was not available hens performed more nest-searching behaviour (Cooper and Appleby, 1995; Freire *et al*., 1996). While an increased occurrence of appetitive behaviour may indicate a stronger motivation to achieve the consummatory phase, it does not necessarily indicate that increased locomotion by hens in the pre-laying period reflects increased frustration and thus a potential welfare problem. For example, using an aversive task approach, Freire *et al.* (1997) suggested that hens were only weakly motivated to reach the nest site during the searching phase, although the motivation to gain access to a nest site increased near the start of the sitting phase. Further, Cooper and Appleby (1997) compared hens that were consistent and inconsistent in their use of nest boxes. While no apparent difference between the two classes of hens was detected in hens' motivation to use a nest box, the inconsistent hens were less responsive to the cues provided by nest boxes than consistent hens. Based on evidence of motivation of hens to lay in a nest box, increased time spent in pre-laying behaviours in the absence of a nest and increased vocalisations indicative of frustration when access to a nest is blocked, it has been concluded that there is convincing evidence of the importance of a suitable nest site (see Keeling, 2004). However, it is also known that even in the presence of a nest not all birds lay in the nest with reports of 0-80 % of eggs being laid on the floor (see Sherwin and Nicol, 1993; this report) and the question remains of what do hens perceive as a suitable nest site?

Clearly, while there is reasonable knowledge of the environmental cues that influence pre-laying behaviour by hens, such as the presence of a nest box, litter compared to wire flooring, light, genotype, social position, etc. (Appleby *et al.*, 1993; Freire et al., 1996), the importance of pre-laying searching behaviour and sitting posture to the welfare of laying hens is less-well understood. The objectives of this preliminary experiment were to identify and measure the different behaviours of hens around oviposition, and if possible group them according to the "searching" and "sitting" phases of nesting, and examine the effects

of the presence or absence of a nest box on these behaviours in pre- and post-laying behaviour of hens that were experienced with laying under the respective test conditions.

Methods

Animals

The hens used in this experiment were selected from 48 commercial Hyline Brown hens aged between 45 and 48 weeks. The hens were part of a larger, factorial experiment involving a total of 504 hens, in which the effects of the different components of cage "furniture" (ie nest box, dust bath and perch), plus group size and space allowance, on hen welfare and productivity were being evaluated. For the present experiment however, the 48 hens were housed in 6 "home" cages: Three cages contained a nest box but neither a dust bath nor perch, while the other 3 cages were without a nest box, dust bath and perch.

Housing and treatments

The cages used were 8-bird Victorsson Trivselburen Furnished Cages (Sweden) modified for use in experiment 1. The cages measured 1206 mm wide, 498 mm deep and 455 mm high at the rear of the cage and were situated in rows 3 tiers high. Three water nipples, shared between the back-to-back cages provided water *ad libitum*. The cages were located in a controlled climate shed in which the average temperature was maintained at ~17°C during the dark period and ~23°C during the light period. Lighting was controlled by a computer so that lights came on at 0500 h and the light intensity increased gradually in a "sunrise" effect over 30 min. From 0530 to 2030 h the light level was 5 lux, except during the period of stockperson activity to maintain the hens and the shed. A "sunset" period of 30 min commenced at 2030 h and the shed was dark at 2100 h. Humidity was maintained at ~40%.

Hen and shed maintenance activities commenced daily at 0930 h and were generally completed within 4 h. Routine activities included visual inspection to detect ill/unhealthy animals, monitoring shed temperature and humidity, testing water lines and drinkers, assessing level of feed in the trough and if necessary, adding a weighed quantity of feed to the trough. The latter ensured feed was available *ad libitum* but limited feed wastage. Eggs were collected between 1100 and 1200 h. Air-conditioner filters were cleaned of dust and the floor of the shed was vacuumed daily to minimise air-borne dust levels.

Two experimental cages, situated back-to-back on the third (top) tier at the end of a row of cages in the controlled climate shed, were used in the experiment. These were "spare" cages not required for the larger experiment. Twelve replicates in time were conducted and there were 2 treatments:

Nest box treatment

The cage was fitted with a nest box which was 241 mm wide, as deep as the whole cage (498 mm) and about 270 mm high at the front of the cage. The floor of the nest box was overlain with a 15 mm thick rectangular piece of "astro turf" (370 x 220 mm), attached to the wire floor.

Control treatment

There was no furniture in the cage and the hens had access to the whole area occupied by the nest box in the other treatment. The position of the Nest box and Control treatments in each replicate was randomised.

Behaviour observation procedure

A total of 6 black and white video cameras with in-built infra-red (IR) lights were used to continuously record the activities of the hens in the experiment. Two video cameras were fixed to the ceiling of the shed, one above each experimental cage. These two cameras were connected to individual time-lapse video recorders (Panasonic AG-6124) situated in an adjacent shed. The images from the remaining 4 video cameras were connected to a 4-channel, black and white quad video processor unit and continuously recorded on a third time-lapse video recorder. Two cameras were positioned to provide views of the front of each cage, showing the egg trays into which eggs normally rolled for later collection. The third camera was placed inside the nest box. The fourth camera that occupied the final channel of the quad-splitter unit, was placed inside one of the cages at floor level, at random, to provide an additional perspective to assist in determining hen behaviour if required. A "dummy" camera was positioned in a similar position to this fourth camera in the other experimental cage.

On the first day of each of the ten replicates, 2 hens from the same home cage containing a nest box were transferred to the experimental cage in which an identical nest box had been fitted. Similarly, 2 hens from the same non-furnished home cage were moved into the other experimental cage. While the home cages hens were selected from were chosen in random order, the pairs of hens were selected on an *ad-hoc* basis and each hen was only used on one occasion. Hens were moved into and out of the experimental cages at about 1600 h, in order not to disturb egg laying behaviour, which generally occurred in the early morning.

Prior to placing the hens in the experimental cages, the hens were marked on the feathers of the back with carbon-based black ink so that they could be differentiated on the video record under normal light as well as IR light. In each experimental cage, one hen was marked with a line and the other hen with two dots (~4 cm diameter); these two hens were then respectively identified as hens 1 and 2. The hens remained in these cages during two to three days. If all four hens did not lay an egg during the second day in treatment, they remained in the cages for an additional day in the anticipation of both hens in the respective pair laying eggs on that day. Nine replicates were completed in which eggs were laid by both hens in the pair within the same day. In the other replicate, one hen did not lay any eggs and the data for the entire replicate were not collated.

Data collection and collation

Although the four hens per replicate were continuously video-recorded from 16.00 h on the day the hens entered the cages, the data from about the first 20 h were only used to determine the timing and location of oviposition. This allowed the hens about 24 h to become accustomed to the new environment before observations occurred. Behaviour data, as defined in Table 13, were transcribed from the video records using The Observer behaviour recording program, supplemented with the Support Package for Video Analysis (version 4.0 for Windows; Noldus Information Technology, 1997). A Panasonic AG-7355 Video Cassette Recorder with jog-and-shuttle control which enabled frame by frame analysis of the video record was linked directly to a computer, which could then read the time track on the video tape record. Movement of the focal hens within the experimental cages was also tracked by continuously recording the location of the hen during the observation period.

For each focal hen, the time of oviposition was first roughly determined using the view of the egg tray and then precisely determined using the overhead view of the whole cage or the view inside the nest box. Videotapes were then replayed and data transcription commenced 2 h prior to the time of oviposition. The experimenter continuously followed the image of each focal hen individually on the video monitor for a 4-h period, that is until 2 h post-egg laying. For the Nest box treatment, when the focal hen was located inside the nest box, data were transcribed from the other videotape showing the inside of the nest box. The data from the two electronic files generated in the Observer program were combined using Microsoft Notepad, to form a single electronic file for each hen.

Hen location in the cage

The video images of the experimental cages were divided into similar sized areas, both within and between the cages, to facilitate measurement of activity, based on the number of times the focal hen crossed a boundary to enter a different area. This was achieved by marking lines on an acetate sheet attached to the TV monitor at data transcription (see Figure 4). For the cage without a nest box, the total area was divided into 12 locations, while the cage with nest box was divided into nine locations (of similar surface area as for the other cage) plus the nest box, containing 3 areas (Figure 5). From the video record, the location of each focal hen was continuously tracked during the 4-h observation time. The focal hen was determined to be in a location when her head and shoulders were within that area. The sum of the frequencies of hens in areas 1 to 9 was used as an estimate of hen activity.

Table 13

List of hen postures and activities recorded from the video record, with definitions

A) Postures	
Stand	the focal hen was standing and not walking (ie not locomoting)
Walk	the focal hen was walking (or running).
Follow	the focal hen was walking (or running), but followed the other hen as she moved about the cage.
Sit	the focal hen was sitting (ie squatted).
B) Activities	
Eat	All activity by the focal hen directed at the feed in the trough, including ingestion of, pecking at and manipulation of the feed.
Drink	All pecking activity directed at the drinkers.
Preen	The beak was brought into contact with the feathers or skin with one or more different movements: pecking, combing, stroking or nibbling.
Nest box observation	The focal hen was inside the nest box and appeared to look with care at the wall or the astro-turf floor covering.
Scratch nest box floor	The focal hen was in the nest box and scratched at the astro-turf floor covering with her feet.
Scratch wire floor	The focal hen was located in the cage (ie not in the nest box) and scraped the wire floor of the cage with her feet.
Idle	The focal hen stood or squatted without locomoting and appeared not to be doing anything.
Sleep	The focal hen was idle with her head positioned towards her back; the head may be under one wing.
Other activity	Any other activities not defined here.
Comfort behaviours	Movements of the focal hen such as shaking, stretching (one or both wings), extension of one leg or wing, head scratch, wing flap, vacuum dust bath and ground scratching.
Aggressive pecks	The focal hen aggressively pecked at the cage mate or hens located in the cage at the rear of the focal cage.
Receive pecks	The focal hen received pecks from the cage mate or from hens located in the other cage (at the rear of the focal cage).
Cage pecks	The focal hen directed pecks at the cage structure including walls, floor, wire cage front or ceiling or the feed trough (excluded pecks at the feed).
Turns body	The focal hen changed her orientation about the body's long axis by $>90^{o}$.
Avoids other hen	The focal hen withdrew from the other hen in response to being pecked by or to the close proximity of the other hen.
Head outside cage	The head of the focal hen was visible in the other cage (at the rear of the focal cage) or outside the front of the cage, but the focal hen was neither feeding nor drinking.
Head outside nest box	The focal hen was located in the nest box and she extended her head outside the nest box entrance without exiting the nest box.
Nest box inspection	The focal hen was located outside the nest box and extended her head through the nest box entrance without entering the nest box.

Nest box facing	The focal hen was located outside the nest box, facing toward the nest box entrance and appeared to glance at the nest box.
Oviposition	The focal hen laid an egg. Direction hen faced when oviposition occurred was also recorded as a modifier:
Egg look	The focal hen that laid the egg was facing toward her egg.
Egg manipulation	The focal hen interacted with her egg.
Head underneath	The head of the focal hen was under her body and thus unsighted on the video record.

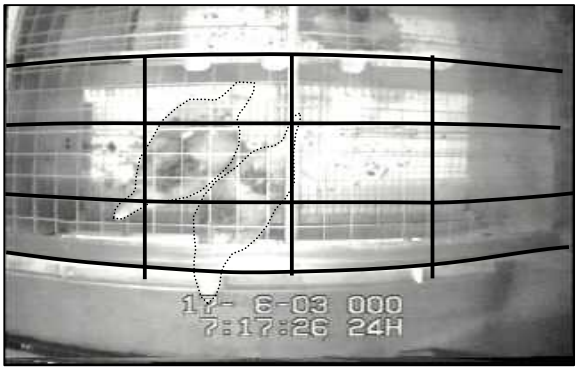
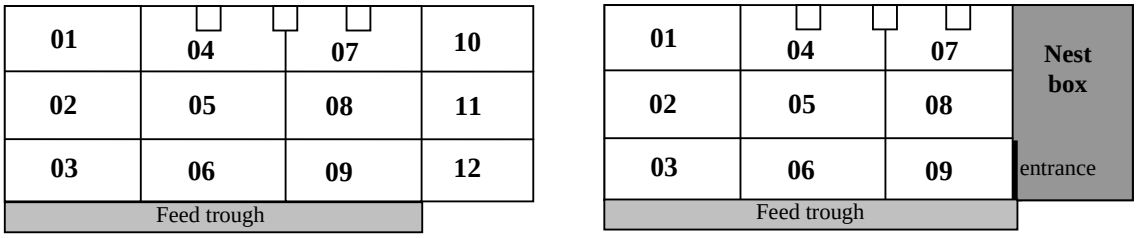


Figure 4
Digital image captured from the infra-red videograph of two hens in the experimental cage without nest box, taken from above the cage. The outline of the hens is enhanced with a dotted line. The solid black lines over the image represent the boundaries of the different locations in the cage. The curvature of the lines is due to the wide-angle lens used with the video camera.

Figure 5

Schematic floor plan of the 2 experimental cages, showing the 12 locations plus the position of the nest box when it was provided. The Control treatment cage is shown on the left. The small squares shown in areas 04 and 07 represent drinkers



Statistical analysis

The 4-h observation period recorded for each hen was divided into eight intervals of 30 min using an iterative analysis step in the Observer 4.1 behaviour recording program. This facilitated analysis to distinguish the pre- and post-laying periods for more-detailed behavioural analysis. In the Nest box treatment, one half of the hens did not lay in the nest box. Thus, data were analysed according to where oviposition occurred, ie. hens were classed as either nest-layers or floor-layers. For the analysis, the total number of hens in each analysis group was thus nine nest-layers, nine floor-layers for the Nest Box treatment and 18 (floor-layers) hens in the Control treatment, ie a total of 27 floor-layers.

In general, individual focal bird measurements were analysed using a restricted maximum likelihood analysis (REML; GenStat Committee, 2000) with a random effect for the home cage of the birds (homecage), a random effect for replicate (rep), and a random effect for each pair of birds examined (cage within rep). In each case, the unit of analysis was an individual focal bird.

Birds were assigned a fixed effect of laying status with levels determined by whether,

1) The focal bird was in a cage without a nest box (denoted cage)
2) The focal bird was in a cage with a nest box, and both birds nested on the floor (denoted floor-floor)
3) The focal bird was in a cage with a nest box, the focus bird nested on the floor, and the other bird nested in the nest box (denoted floor-nest)
4) The focal bird was in a cage with a nest box, the focus bird nested in the nest box, and the other bird nested on the floor (denoted nest-floor)
5) The focal bird was in a cage with a nest box, and both birds nested in the nest box (denoted nest-nest).

The 5 levels of status were divided into a two level factor (location) depending on whether the focal bird nested in a nest box (corresponding to the levels nest-floor and nest-nest of status) or on the floor (corresponding to the levels cage, floor-floor and floor-nest of status). For some measurements, over some time periods, a fixed effect covariate of the amount of time the focal hen was in the dark for the period related to the measurement, was included in the model. Nesting location, and then laying status, of the focal hen were tested using likelihood ratio tests for fixed effects in REML models (Welham and Thompson, 1997). Where appropriate, these tests were adjusted for the time in dark covariate. Where appropriate, a similar type of test was used to test for a time in dark covariate, after adjusting for the five levels of laying status.

When the variance components of the random effects were estimated as being negative, this was allowed to stand. In some cases numerical non-convergence precluded using the fixed effect test with the general random model. In these cases, a simpler random model

was used, with either additive effects of homecage and replicate (home cage + rep), or random effects of replicate and pair of birds within replicate (rep/cage).

With a few measurements, certain birds were deleted from the analysis due to their unrepresentative nature. Where appropriate, the data for each focal bird were transformed before statistical analysis so that the residuals, for the model including all fixed effects, were homogeneous. Since there was only weak evidence for any laying status effect other than whether the focal bird laid on the floor or in a nest, transformed and back-transformed predicted means are presented for comparing the two laying locations. For every measurement, the means were obtained using the same random model as that used for testing the location effect. As hens were inactive in the dark, the means were adjusted for a time in dark covariate, where the covariate was included in the model. In general, hen behaviour was similar in Time periods 1 and 2 and thus were analysed together. Time periods 3 and 4, which covered the periods 60-30 and 30-0 min pre-oviposition, respectively, were analysed individually. There were no differences in hen behaviour in the four, 30-min periods post-oviposition, so data for these periods were generally combined for analysis.

Results

The 40 focal hens selected for the experiment were observed on the video record to lay a total of 80 eggs over the 2 or 3 days that hens occupied the experimental cages. In the NB treatment, 25 of 40 eggs (62.5%) were laid in the NB. Figure 6 shows the locations where eggs were observed to be laid in the two treatment cages over all days of the experiment. The areas shown in Figure 6 correspond to the areas identified in Figure 5.

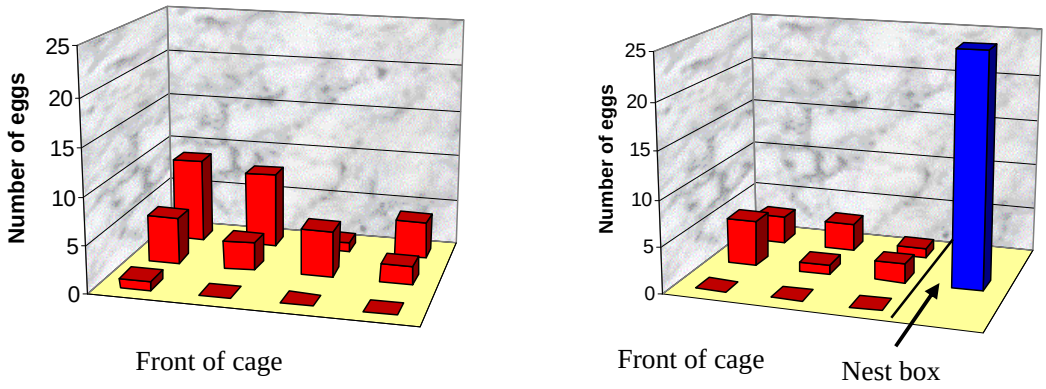


Figure 6
The number of eggs laid by hens in the Control (left) and Nest box (right) treatments during the experiment, according to location in the cage where oviposition occurred

In one of the ten replicates of the experiment, the video data were not transcribed because one hen did not lay any eggs over three consecutive days. Data from the other nine replicates were transcribed from the video records and analysed for inclusion in the present report. Seven replicates were video recorded over two observation days and two replicates were recorded over three days in order to comply with the requirement that both hens of a pair laid an egg on the same day. Of the 18 focal hens in the Nest box treatment, nine lay in the nest box and nine lay outside the nest box on the wire floor. These hens were subsequently termed “nest layers” and “floor layers”, respectively. Based on observation of these hens on consecutive days, only one hen was observed to change her location of laying between the two days, ie. the hen laid in the nest box on day 1 and on the wire floor outside the nest box on day 2.

Activity of hens

Hen activity level was assessed in two ways: 1) locomotory behaviour, especially walking and 2) frequency of entering areas 1-9 of the cages, as depicted in Figs 4 and 5. Darkness strongly reduced hen activity such that hens were inactive in the dark. As the 2-h pre-laying period included darkness for four hens, the time in the dark was used as a co-variate in the REML analysis. After adjusting for darkness, there were no differences in the activity measures in the period 120-60 min pre-laying. However, in the hour before laying, nest layers were less active than floor layers. As shown in Table 14, nest layers performed less ($P < 0.001$) walking behaviour (excluding following behaviour) and entered areas 1-9 less frequently ($P < 0.01$) in the hour prior to oviposition than floor layers.

Table 14

Measures of hen activity. Duration (D) of the main behaviours and frequency (F) of entering different areas of the cage, by focal hens around oviposition that laid in the nest box (Nest layers) and outside the nest box on the wire floor (Floor layers). Values shown are the transformed means and the standard error of difference between the means (sed). Back-transformed means are presented in parentheses. Oviposition occurred at the start of time period T5

Behaviour And time period (T)	Transformation used	Nest layers (n = 9)	Floor layers (n = 27)	sed	P value
Stand stationary (D)					
T1 + T2	Ang (100/60*Y)	60.1 (45.8 min)	53.3 (38.6 min)	3.69	0.122
T3	Ang (100/30*Y)	47.7 (16.4 min)	51.1 (18.1 min)	3.64	0.395
T4	Log_{10} (Y)	0.74 (5.5 min)	1.06 (11.5 min)	0.083	**0.00053**
T5 to T8	Log_{10} (120-Y)	1.03 (109.2 min)	1.25 (102.2 min)	0.102	0.245[†]
Sit stationary (D)					
T1 + T2	Ang (100/60*Y)	16.4 (4.8 min)	17.6 (5.5 min)	5.17	0.819
T3	Ang (100/30*Y)	32.5 (8.7 min)	22.7 (4.5 min)	6.41	0.076
T4	Ang (100/30*Y)	65.6 (24.9 min)	35.7 (10.2 min)	5.35	**1.2×10^{-5}**
T5 to T8	Log_{10} (Y+1)	0.3 (0.9 min)	0.5 (2.0 min)	0.21	0.337
Walk (D)					
T1 + T2	Ang (100/60*Y)	16.4 (4.8 min)	17.3 (5.3 min)	2.17	0.718
T3	Ang (100/30*Y)	13.9 (1.7 min)	21.9 (4.2 min)	2.14	**0.0028**[#]
T4	Ang (100/30*Y)	5.5 (0.3 min)	22.7 (4.5 min)	2.77	**1.8×10^{-6}**
T5 to T8	Log_{10} (Y)	0.96 (9.2 min)	0.99 (9.7 min)	0.113	0.841
Areas entered (F)					
T1 + T2	Square root (Y)	9.7 (93)	9.9 (98)	1.45	0.869

T3	Square root (Y)	5.1 (26)	8.5 (73)	0.97	**0.0031**[#]
T4	Square root (Y)	1.7 (3)	9.5 (90)	0.93	**7.4×10^{-9}**
T5	Log_{10} (Y)	1.45 (28)	1.59 (39)	0.167	0.419
T5 to T8	Log_{10} (Y)	2.37 (233)	2.26 (183)	0.131	0.447

[†]Tested with random model homecage+rep; [#]Tested with random model rep/cage; T1 = 2.0-1.5 h pre-oviposition; T2 = 1.5-1.0 h pre-oviposition; T3 = 1.0-0.5 h pre-oviposition; T4 = 0.5-0.0 h pre-oviposition; T5 = 0.0-0.5 h post-oviposition; T6 = 0.5-1.0 h post-oviposition; T7 = 1.0-1.5 h post-oviposition; T8 = 1.5-2.0 h post-oviposition.

Following behaviour

Following behaviour was a behaviour in which the focal hen appeared to follow the other hen around the cage. When the other hen stopped moving, the focal hen would usually become stationary and sit next to the hen, often in contact with the hen. In addition, the focal hen might then lower her head and hold it under the body of the other hen if she was standing. Following behaviour was not analysed as this behaviour was not recorded for hens in the Nest box treatment that were nest layers. The occurrence of following behaviour was greatest in time periods 3, 4 and 5. In these time periods, 9, 15 and 8 of 27 floor layers, respectively, were recorded to perform the behaviour and the average proportion of time these hens spent in the performance of the behaviour was 3.9%, 6.4% and 3.0%, respectively, for T3, T4 and T5.

Sitting posture

In the 30-min pre-oviposition, sitting posture, in which the hen would sit or squat inactive on the floor, was recorded for significantly more ($P < 0.001$) of the time for nest layers (83 % of the time) than floor layers (34 % of the time; Table 14). However, there was no difference in the mean number of bouts of sitting posture by hens before oviposition (T4: mean values following square root transformation were 4.0 *vs* 3.3, respectively, sed = 0.463, $P = 0.22$; back-transformed means were 15.6 and 11.0 bouts).

Scratching the floor

While the combined frequency of occurrence of focal hens scratching the wire floor of the cage or the astro-turf floor inside the nest box was greater in the 30 min prior to oviposition for the nest layers (14.7 times/hen) than floor layers (9.8 times/hen), the differences were not statistically different ($P > 0.1$). The mean values for the nest layers and floor layers are presented in Table 15. The incidence of hens scratching at the floor was low apart from in the 1 h pre-laying.

Table 15

Duration (D) of the feeding and preening behaviours and frequency (F) of occurrence of scratching at the floor (sum of the wire cage floor and nest box floor) around oviposition by hens that laid in the nest box (Nest layers) and outside the nest box on the wire floor (Floor layers). Values shown are the transformed means with back-transformed means (BMT) in parentheses and the standard error of difference between the means (sed). Oviposition occurred at the start of time period T5. The frequency of scratch floor was not analysed in T1, T2 and T5-8 due to low frequency of occurrence

Behaviour and time period (T)	Transformation used	Nest layers (BTM)	Floor layers (BTM)	sed	P value
Feed (D)					
T1 + T2	Ang (100/60*Y)	19.2 (10.9)	18.0 (9.5)	3.70	0.742
T3	Ang (100/30*Y)	16.0 (7.6)	14.3 (6.1)	4.42	0.693
T4	Ang (100/30*Y)	3.3 (0.3)	9.8 (3.6)	1.83	**0.0075**[†]
T5 to T8	Ang (100/120*Y)	26.1 (19.3)	29.5 (24.3)	2.04	0.179
Preen (D)					
T1 + T2	Ang (100/60*Y)	17.8 (9.4)	18.2 (9.7)	3.61	0.918 #

T3	Ang (100/30*Y)	8.6 (2.2)	14.3 (6.1)	3.41	0.137
T4	Ang (100/30*Y)	8.0 (1.9)	10.9 (3.6)	4.69	0.560
T5 to T8	Ang (100/120*Y)	7.1 (8.6)	18.3 (9.8)	2.38	0.620 #
Scratch floor (F)					
T3	Square root (Y)	1.85 (3.4)	1.72 (3.0)	0.690	0.865
T4	Square root (Y)	3.83 (14.7)	3.12 (9.8)	0.570	0.301

†Tested with random model homecage+rep; #Tested with random model rep/cage; T1 = 2.0-1.5 h pre-oviposition; T2 = 1.5-1.0 h pre-oviposition; T3 = 1.0-0.5 h pre-oviposition; T4 = 0.5-0.0 h pre-oviposition; T5 = 0.0-0.5 h post-oviposition; T6 = 0.5-1.0 h post-oviposition; T7 = 1.0-1.5 h post-oviposition; T8 = 1.5-2.0 h post-oviposition.

Other activities of hens

Feeding and preening behaviour

Nest layers spent less ($P < 0.01$) time feeding in the 30 min pre-laying compared to floor layers (Table 15). As shown in the table, nest layers spent 0.3 % compared to 2.9 % of the time feeding. The occurrence of feeding behaviour in other time periods however was not affected by the hens' choice of laying in the nest box or outside the nest box on the wire floor. Similarly, there were no effects of egg location class on the time hens spent preening around oviposition (Table 15).

Discussion

The hen's choice to lay her egg in the nest box compared to on the wire floor outside the nest box had a large effect on her behaviour in the hour prior to oviposition. In the present experiment, the time spent walking was significantly reduced if hens were nest layers, supporting previous findings of Cooper and Appleby (1995) and Freire *et al.* (1996) that if a nest box was not available hens performed more nest-searching behaviour. The second estimate of hen activity in the present experiment was the frequency of hens entering areas 1-9 in the cages. Using this parameter, the nest layers were also significantly less active in the hour before laying than floor layers. Nest layers were less active during the hour before laying because they spent more time sitting. Similarly, because nest layers spent more time sitting (in the nest box) before laying, analysis of the data also confirmed that nest layers spent less time feeding in the 30 minutes pre-laying. Preening behaviour however, which occurred during about the same proportion of observation time as feeding before egg laying, was not affected by choice of egg laying site. This finding was probably due to the fact that hens could preen at any location, whereas feed was only available outside the next box.

In eight of the nine pairs of hens, egg laying by the two hens was separated by a minimum of 80 minutes (range 80 to 583 min). Thus it appears unlikely that either of the hens in the pairs was excluded from using the nest box. In one pair, egg laying occurred two minutes apart with one hen laying in the nest box and the other on the floor outside the nest box. In only one pair of hens, both hens laid in the nest box and for this pair the time between ovipositions was 80 minutes. During the video observation period for this pair of hens (320 min), at no time were both hens in the nest box together. While these data could suggest that hens, on a daily basis, "choose" their laying site, it needs to be remembered that by the time these data were collected, the majority of the hens were already consistently laying at one site (for each hen). Thus further research is required on the development of choice of egg laying site.

This small experiment that involved observations on egg-laying behaviour in 18 pairs of hens has provided more questions than answers. A new finding was "following behaviour" which only occurred in hens that laid eggs on the wire floor, irrespective of the presence of a nest box. This behaviour occurred during pre- and post-laying periods and involved the hen (follower) appearing to attempt to remain close to the other hen (followed), including when the followed hen was locomoting. When the followed hen was stationary and

standing, the follower would often sit next to her and the follower would put her head under the body of the followed hen. The reasons for this behaviour are unknown as are the reasons why it only occurred in 55% of floor layers and also occurred post-oviposition. A possible explanation was that when a nest box was present and the hen utilised the nest box for egg laying, this environment provided appropriate cues for nest site selection. In contrast, the follower hen may have derived cues for nest site selection from the followed hen.

This experiment has shown, as reported in other studies, that the use of the nest box for egg laying is highly variable between birds (Cooper and Appleby, 1997). In this experiment where experienced (with a nest box) hens were housed in pairs in a cage with a nest box, 50% of hens laid in the nest box and 50% laid on the wire cage floor outside the nest box. These data raise the question of hens' preference for egg laying location. While the literature suggests hens are motivated to seek a "preferred" location for egg laying, the data from this experiment could be interpreted to suggest that either the nest box or the wire cage floor are both preferred locations. Alternatively, by one hen making a choice, this may or may not force the other hen into a less-preferred location. This experiment only used a pair of hens as the experimental treatment and the possible combinations for preference presumably become more complex in commercial settings of group sizes of 5 to 20 hens, particularly when the number of nest sites (boxes) is limited. Clearly further research is required to answer the following types of questions: What is the biological significance of following behaviour, including any relationship with nest site selection? Is consistency of nest site selection associated with a preference for that site, or are some birds forced to choose a less-preferred site? How does choice of nest site develop? Are these behaviours the same with larger group sizes? Are there any implications for welfare?

References

Abrahamsson, P. and Tauson, R. (1993). Effect of perches at different positions in conventional cages for laying hens of two different strains. *Acta Agriculturae Scandinavica,* **43**: 228-235.

Abrahamsson, P. and Tauson, R. (1995). Aviary systems and conventional cages for laying hens. *Acta Agriculturae Scandinavica,* **45**: 191-203.

Abrahamsson, P. and Tauson, R. (1997). Effects of group size on performance, health and behaviour in furnished cages for laying hens. *Acta Agriculturae Scandinavica, Section A, Animal Science,* **47**: 254-260.

Abrahamsson, P., Tauson, R. and Appleby, M.C. (1995). Performance of four hybrids of laying hens in modified and conventional cages. *Agriculturae Scandinavica,* **45**: 286-296.

Abrahamsson, P., Tauson, R. and Appleby, M.C. (1996). Behaviour, health and integument of four hybirds of laying hens in modified and conventional cages. *British Poultry Science,* **37**: 521-540.

Albentosa, M.J. and Cooper, J.J. (2004). Distribution of groups of hens when housed in adjoining high and low furnished cages. *Proceedings of the 38th International Society for Applied Ethology*, (Helsinki, Finland), p. 106.

Alvey, D.M. and Tucker, S.A. (1994). Cage design and laying hen welfare. In: C.M. Sherwin, editor, *Modified Cages for Laying Hens*. (Universities Federation for Animal Welfare, Potters Bar, UK), pp. 55-61.

Appleby, M.C. (1991). *'Do Hens Suffer in Battery Cages?'* (Athene Trust, Edinburgh, UK).

Appleby, M.C. (1993a). Welfare-enhanced cages for laying hens. In: E. Collins and C. Boon, editors, *Livestock Environment IV*. (Conference Proceedings, Coventry, UK. American Society of Agricultural Engineers, Michigan, USA), pp. 225-227.

Appleby, M.C. (1993b). Should cages for laying hens be banned or modified? *Animal Welfare,* **2**: 67-80.

Appleby, M. C. (1994). The Edinburgh system for laying hens. In: Sherwin, C.M., editor, *Modified Cages for Laying Hens* (Universities Federation for Animal Welfare, Potters Bar: UK), pp. 35-9.

Appleby, M.C. (1995). Perch length in cages for medium hybrid laying hens. *British Poultry Science,* **36**: 23-31.

Appleby, M. C. (1998). The Edinburgh modified cage: effects of group size and space allowance on brown laying hens. *Journal of Applied Poultry Research* **7**: 152-161.

Appleby, M.C. and Hughes, B.O. (1995). The Edinburgh modified cage for laying hens. *British Poultry*, **36**: 707-718.

Appleby, M.C., Hughes, B.O. and Savory, C.J. (1994). Current state of poultry welfare: Progress, problems and strategies. *British Poultry Science,* **35**: 467-475.

Appleby, M.C. and McRae, H.E. (1986). The individual nest box as a superstimulus for domestic hens. *Applied Animal Behaviour Science,* **15**: 169-176.

Appleby, M.C., Mench, J.A. and Hughes, B.O. (2004).*Poultry Behaviour and Welfare*. (CABI, UK).

Appleby, M.C., Smith, S.F. and Hughes, B.O. (1992). Individual perching behaviour of laying hens and its effects in cages. *British Poultry Science,* **33**: 227-238.

Appleby, M.C., Smith, S.F. and Hughes, B.O. (1993). Nesting, dust bathing and perching by laying hens in cages: Effects of design on behaviour and welfare. *British Poultry Science,* **34**: 835-847.

Asai, T., Okada, M., Ono, M., Mori, Y., Yokomizo, Y., and Sato, S. (1994). Detection of interleukin-6 and prostaglandin E2 in bronchoalveolar lavage fluids of pigs experimentally infected with *Mycoplasma hyponeumoniae*. *Veterinary Immunology and Immunopathology*, **44**: 97-102.

Bareham, J.R. (1972). Effects of cages and semi-intensive deep litter pens on the behaviour, adrenal response and production in two strains of laying hens. *British Veterinary Journal*, **128**: 153-162.

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

Barnett, J.L and Hemsworth, P.H. (2003). Science and its application in assessing the welfare of laying hens. *Australian Veterinary Journal,* **81**: 615-623.

Barnett, J.L. and Hutson, G.D. (1987). Objective assessment of welfare in the pig: contributions from physiology and behaviour. In: APSA Committee, editors, *Manipulating Pig Production*. (Australasian Pig Science Association, Werribee, Victoria), pp. 1-22.

Barnett, J.L. and Taylor, I.A. (1995). Turn-around stalls and the welfare of pigs. In "Manipulating Pig Production, V", Hennessy, D.P. and Cranwell, P.D. (eds.) Australasian Pig Science Association, Werribee, p. 22.

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

Barnett, J.L., Glatz, P.C., Newman, E.A. and Cronin, G.M. (1997a). Effects of modifying layer cages with perches on stress physiology, plumage, pecking and bone strength of hens. *Australian Journal of Experimental Agriculture,* **37**: 523-529.

Barnett, J.L., Glatz, P.C., Newman, E.A. and Cronin, G.M. (1997b). Effects of modifying layer cages with solid sides on stress physiology, plumage, pecking and bone strength of hens. *Australian Journal of Experimental Agriculture,* **37**: 11-18.

Baxter, M.R. (1994). The welfare problems of laying hens in battery cages. *Veterinary Record,* **134**: 614-619.

Beilharz, R.G. (1982). Genetic adaptation in relation to animal welfare. *International Journal for the Study of Animal Problems,* **3**:117-124.

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

Bell, D.D., Adams, C.J. and Gvaryahu, G. (1995). The effect of environment enrichment devices on performance in caged table egg layer flocks. *84th Poultry Science Association Abstracts,* **74** (suppl. 1): 97.

Beuving, G. and Vonder, G.M.A. (1978). Effect of stressing factors on corticosterone levels in the plasma of laying hens. *General and Comparative Endocrinology* **35**: 153-159.

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.

Bolles, R. (1981). Emotion. In: McFarland, D.J., editor, *The Oxford companion to Animal Behaviour* (Oxford University Press, Oxford), pp. 149-152.

Borell, E. von, and Hurnik, J.F. (1991). Stereotypies, adrenal function and neurophysiological aspects of gestating sows. *Applied Animal Behaviour Science*, **30**: 174-175.

Brambell, F.W.R., Barbour, D.S. Barnett, M.B., Ewer, T.K., Hobson, A., Pitchforth, H., Smith, W.R., Thorpe, W.H. and Winship, F.J.W. (1965). Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Husbandry Systems. (Her Majesty's Stationery Office, London, UK).

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

Broom, D.M. (1998). Welfare, stress and the evolution of feelings. *Advances in the Study of Behaviour,* **27**: 371-403.

Broom, D.M. (2001). Coping, stress and welfare. In: Broom, D.M., editor, *Coping with Challenge: Welfare in Animals Including Man* (Dahlem University Press, Berlin, Germany), pp. 1-9.

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

Broom, D.M. and Zanella, A.J. Brain measures which tell us about animal welfare. *Animal Welfare*, **13**:S41-45.

Cabanac, M. (1979). Sensory pleasure. *Quarterly Review of Biology,* **54**: 1-29.

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

Carter, V. and Carter, H. (1992). *The Laying Hen*. (European Conference Group on the Protection of Farm Animals, The Manor House, Horsham, West Sussex, UK).

Cochran, W. G. and Cox, G.M. (1957). Experimental Designs, 2nd edition (John Wiley and Sons, New York).

Comstock, G.L. (2000). An alternative ethic for animals. In: Hodge J. and Han In. K., editors, *Livestock, Ethics and Quality of Life* (CAB International Oxon), pp. 99-118.

Cooper, J.J. and Albentosa, M.J. (2004). Social space for laying hens. In: Perry G.C., editor, *Welfare of the Laying Hen* (CABI, UK), pp. 191-202.

Cooper, J.J. and Appleby, M.C. (1995). Nesting behaviour of hens: Effects of experience on motivation. *Applied Animal Behaviour Science,* **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,* 5**4**: 1245-1253.

Cooper, J.J. and Nicol, C.J. (1991). Stereotypic behaviour affects environmental preference in bank voles, *Clethrionomys glareolus*. *Animal Behaviour,* **41**: 971-977.

Craig, J.V. (1981). *Domestic Animal Behaviour. Causes and Implications for Animal Care and Management*. (Prentice-Hall, New Jersey).

Craig, J. V. and Craig, J.A. (1985). Corticosteroid levels in White Leghorn hens as affected by handling, laying-house environment, and genetic stock. *Poultry Science*, **64**: 809-816.

Craig, J.V. and Swanson, J.C. (1994). Review: Welfare perspectives on hens kept for egg production. *Poultry Science,* **73**: 921-938.

Cronin, G.M., Wiepkema, P.R. and van Ree, J.M. (1986). Endorphins implicated in stereotypies of tethered sows. *Experientia,* **42**: 198-199.

Cronin, G.M., Barnett, J.L., Hodge, F.M., Smith, J.A. and McCallum, T.H. (1991). The welfare of pigs in two farrowing/lactation environments: cortisol responses of sows. *Applied Animal Behaviour Science,* **32**: 117-127.

Dantzer, R. Moremède, P. and Henry, J.P. (1983). Physiological assessment of adaptation in farm animals. In: Baxter, S.H., Baxter, M.R. and MacCormack, J.A.C., editors, *Farm Animal Housing and Welfare* (Martinus Nijhoff Publishers) pp. 8-19.

Dawkins, M. (1980). *Animal Suffering: The Science of Animal Welfare*. (Chapman and Hall, London).

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. (2002). A non-invasive test of stress in laying hens. *Report to the Rural Industries Research and Development Corporation,* (RIRDC, Canberra), project US-71A.

Drakley, C. Elson, H.A. and Walker, A.W. (2002). Production efficiency of laying hens at four stocking densities in furnished cages of two heights. *Proceedings of the 11th European Poultry Conference* (Bremen, Germany {August}).

Duncan, I.J.H. (1992). Guest editorial - Designing environments for animals - not for public perceptions. *British Veterinary Journal,* **148**: 475-477.

Duncan, I.J.H. (1996). Animal welfare defined in terms of feelings. *Acta Agriculturae Scandinavica,* (supplement) **27**: 29-35.

Duncan, I.J.H. and Dawkins, M.S. (1983). The problem of assessing "well-being" and "suffering" in farm animals. In: Smidt D, editor, *Indicators Relevant to Farm Animal Welfare* (Martinus Nijhoff, Boston), pp. 13-24.

Duncan, I.J.H. and Fraser, D. (1997). Understanding animal welfare. In: Appleby MC, Hughes BO, editors, *Animal Welfare* (CAB International, Oxon, UK), pp.19-31.

Duncan, I.J.H. and Kite, V.G. (1989). Nest site selection and nest building behaviour in domestic fowl. *Animal Behaviour,* **37**: 215-231.

Duncan, E.T., Appleby, M.C. and Hughes, B.O. (1992). Effects of perchies in laying cages on welfare and production of hens. *British Poultry Science*, **33**: 25-35.

El-Lethey, H., Aerni, V., Jungi, T.W. and Weschler, B. (2000). Stress and feather pecking in laying hens in relation to housing conditions. *British Poultry Science*, **41**: 22-28.

Elson, A. (2002). Claw shortener monitoring exercise: interim results. *Poultry World,* **156** (11): 27.

Elson, A. (2003). Compliance of laying cages with new regulations. *ADAS Poultry Progress*, pp. 5-7.

Ekstrand, C. and Keeling, L. (1994). Modified cages and get-away cages for laying hens. A literature review. Research Report No. 34, Swedish University of Agricultural Sciences (Skara, Sweden).

Elson, H.A. (1994). The economics of modified enriched cages (MEC) compared to other systems of production. In: C.M. Sherwin, editor, *Modified Cages for Laying Hens* (Universities Federation for Animal Welfare, Potters Bar, UK), pp. 91-94.

Elson, A. (2004). The laying hen: systems of egg production. In 'Welfare of the Laying Hen', ed. Perry, G.C. (CABI, UK), pp. 67-80.

Eskeland, B. (1976). Methods of observation and measurement of different parameters as an assessment of bird welfare. *Fifth European Poultry Conference,* (Malta), pp. 988-1001.

Erhard, M.H., Ozpinar, H., Bilal, T., Abbas, Y., Kutay, C., Eseceli, H. and Stangassinger, M. (2000). The humoral immune response and the productivity of laying hens kept on the ground or in cages. *Alternatives to Laboratory Animals,* **28**: 699-705.

EU Directive. (1999). Council Directive 99/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. *Official Journal of the European Communities,* No. L 203, 03.08.1999, pp. 53-57.

FAWC (Farm Animal Welfare Council). (1992). *Report on the Welfare of Broiler Chickens*. (FAWC, Tolworth, Surrey, UK).

FAWC (Farm Animal Welfare Council). (1997). *Report on the Welfare of Laying Hens*. (FAWC, Tolworth, Surrey, UK).

Faure, J.M. (1991). Rearing conditions and needs for space and litter in laying hens. *Applied Animal Behaviour Science,* **31**: 111-117.

Faure, J.M., and Lagadic, H. (1994). Elasticity of demand for food and sand in laying hens subjected to variable wind speed. *Applied Animal Behaviour Science,* **42**: 49-59.

Fleming, R.H., Whitehead, C.C., Alvey, D., Gregory, N.G. and Wilkins, L. J. (1994). Bone structure and breaking strength in laying hens housed in different husbandry systems. *British Poultry Science,* **35**: 651-662.

Fraser, A.F. and Broom, D.M. (1990). *Farm Animal Behaviour and Welfare*. (CAB International, Oxon, UK).

Freire, R. Appleby, M.C. and Hughes, B.O. (1996). Effects of nest quality and other cues for exploration on pre-laying behaviour. *Applied Animal Behaviour Science*, **48**: 37-46.

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.

Friend, T.H., Gwazdauskas, F.C. and Polan, C.E. (1979). Change in adrenal response from free stall competition. *Journal of Dairy Science*, **62**: 768-771.

GenStat Committee (2000) *The Guide to GenStat®, Part 2: Statistics*. Lawes Agricultural Trust, Rothamsted, United Kingdom.

Glatz, P.C. and Barnett, J.L. (1996). Effect of perches and solid sides in conventional cages on production, plumage and foot condition of laying hens housed in a naturally ventilated shed. *Australian Journal of Agricultural Research,* **36**: 269-275.

Gregory, N.G., Wilkins, L.J., Austin, D., Belyavin, C.G., Alvey, D.M. and Tucker, S.A. (1992). Effect of catching method on the prevalence of broken bones in end of lay hens. *Avian Pathology*, **21**: 717-722.

Gregory, N.G., Wilkins, L.J., Kestin, S.C., Belyavin, C.G. and Alvey, D.M. (1991). Effect of husbandry system on broken bones and bone strength in hens. *Veterinary Record,* **128**: 397-399.

Gregory, N.G., Wilkins, L.J., Tucker, S.A., Alvey, D.M. and Belyavin, C.G. (1993). Effect of lighting pattern during lay on bone strength and the prevalence of broken bones in end of lay hens. *Journal of Applied Poultry Research,* **2**: 103-106.

Hemsworth, P.H. and Barnett, J.L. (1987). Human-animal interactions. In: Price, E.O., editor, *The Veterinary Clinics of North America, Farm Animal Behavior* (Saunders, Philadelphia), pp. 339-356.

Hemsworth, P.H. and Barnett, J.L. (1991). The effects of aversively handling pigs either individually or in groups on their behaviour, growth and corticosteroids. *Applied Animal Behaviour Science,* **30**: 61-72.

Hemsworth, P.H. and Coleman, G.J. (1998). *Human-Livestock Interactions. The Stockperson and the Productivity and Welfare of Intensively Farmed Animals*. (CAB International, Oxon, UK).

Hemsworth, P.H., Barnett, J.L. and Hansen, C. (1981). The influence of handling by humans on the behaviour, growth and corticosteroids in the juvenile female pig. *Hormones and Behaviour,* **15**: 96-403.

Hemsworth, P.H., Barnett, J.L. and Hansen C. (1986). The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. *Applied Animal Behaviour Science,* **15**: 303-314.

Hemsworth, P.H., Barnett, J.L. and Campbell, R.G. (1996). A study of the relative aversiveness of a new daily injection procedure for pigs. *Applied Animal Behaviour Science,* **49**: 389-401.

Hester, P.Y., Muir, W.M. and Craig, J.V. (1996). Group selection for adaptation to multiple-hen cages: humoral immune response. *British Poultry Science,* **75**: 1315-1320.

Hughes, B.O. (1975). Spatial preference in the domestic hen. *British Veterinary Journal,* **131**: 560-564.

Hughes, B.O. and Sherwin, C.M. (1994). Origins and development of modified cages for laying hens. In: C.M. Sherwin, editor, *Modified Cages for Laying Hens* (Universities Federation for Animal Welfare, Potters Bar, UK), pp. 1-9.

Hughes, B.O., Petherick, J.C., Brown, M.F. and Waddington, D. (1994). Visual recognition of key nest site stimuli by laying hens in cages. *Applied Animal Behaviour Science,* **42**: 271-281.

Hughes, B.O., Wilson, S., Appleby, M.C., and Smith, S.F. (1993). Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. *Research in Veterinary Science,* **54**: 202-206.

Hutson, G.D. (1992). A comparison of operant responding by farrowing sows for food and nest-building material. *Applied Animal Behaviour Science,***34**: 221-230.

Keeling, L.J. (2004). Nesting, perching and dustbathing. In: Perry, G.C., editor, *Welfare of the Laying Hen* (CABI, UK), pp. 203-213.

Kirkwood, J.K., Gaskin, C.D. and Markham, J. (1987). Perinatal mortality and season of birth in captive wild ungulates. *The Veterinary Record,* **120**: 386-390.

Knowles, T.G., Broom, D.M., Gregory, N.G. and Wilkins, L.J. (1993). Effect of bone strength on the frequency of broken bones in hens. *Research in Veterinary Science,* **54**: 15-19.

Koelkebeck, K.W. and Cain, J.R. (1984a). Plasma concentrations of six steroids and LH during the ovulatory cycle of the hen, *Gallus domesticus*. *Biology of Reproduction*, **63**: 2123-2131.

Koelkebeck, K.W. and Cain, J.R. (1984b). Quantitating laying hens responses to several management alternatives. *Poultry Science (abstract)*, **63**: 130.

Koelkebeck, K.W., Harrison, P.C. and Madindou, T. (1993). Research note: Effect of carbonated drinking water on production performance and bone characteristics of laying hens exposed to high environmental temperatures. *Poultry Science,* **72**: 1800-1803.

Lagadic, H. (1992). Étude du besoin en espace chez la poule pondeuse. Thesis, University of Rennes, no. 784.

Lawrence, A.B. and Illius, A.W. (1997). Measuring preferences and the problems of identifying proximate needs. *Animal Choices*. *Occasional Publication of the British Society of Animal Science,* **20**: 19-26.

Lay, D.C. Jr., Friend, T.H., Grissom, K.K., Bowers, C.L. and Mal, M.E. (1992). Effects of freeze or hot-iron branding of Angus calves on some physiological and behavioural indicators of stress. *Applied Animal Behaviour Science,* **33**: 137-147.

Liere, D. W. van (1992). The significance of fowls' bathing in dust. *Animal Welfare,* **1**: 187-202.

Mandler, G. (1981). Emotion. In: Gregory, R.L., editor, *The Oxford companion to the Mind* (Oxford University Press, Oxford), pp. 219-220.

Mason, G.J. (1991). Stereotypies and suffering. *Behavioural Processes,***25**: 103-115.

Maxwell, M.H. and Robertson, G.W. (1998). The avian heterophil leucocyte: a review. *World's Poultry Science Journal*, **54**: 155-178.

Mead, R., Curnow, R.N., and Hasted, A.M. (1993). *Statistical methods in agriculture and experimental biology*. (Chapman and Hall, London, UK).

Medawar, P. (1986). *The Limits of Science.* (Oxford University Press, Oxford).

Mench, J.A., Tienhoven, van A., Marsh, J.A. McCormick, C.C., Cunningham, D.L. and Baker, R.C. (1986). Effects of cage and floor pen management on behavior, production and physiological stress responses of laying hens. *Poultry Science*, **65**: 1058-1069.

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

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.

Mills, A.D., Beilharz, R.G. and Hocking, P.M. (1997). Genetic selection. In: Appleby, M.C. and Hughes, B.O., editors, *Animal Welfare* (CAB International, Oxon). pp. 219-231.

Nicol, C.J. (1992). Poultry cage design - An update. *Veterinary Annual,* **32**: 293-298.

Nicol, C., and Saville-Weeks, C. (1993). Poultry handling and transport. In: Grandin, T., editor, *Livestock Handling and Transport* (CAB International, UK), pp. 273-287.

Niekerk, T.G.C.M. van and Reuvekamp, B.F.J. (1994). Alternative housing systems for laying hens. *Proceedings 9th European Poultry Conference*. (Glasgow, UK, volume 1), pp. 159-160.

Niekerk-Fiks, T.G.C.M. van, Reuvekamp, B.F.J. and Emous, R.A. von. (2002). Experiences with different models of enriched cages. *Proceedings of the 11th European Poultry Conference* (Bremen, Germany {August}).

Norgaard-Nielsen, G., Vestergaard, K. and Simonsen, H.B. (1993). Effects of rearing experience and stimulus enrichment on feather damage in laying hens. *Applied Animal Behaviour Science,* **38**: 345-352.

Oester, H. (1994). Different types of perches and their influence of the development of bumble feet in laying hens. *Archiv für Geflugelkunde,* **58**: 231-238.

Parkinson, G., Jongman, E., Almond, A. and Goldsmith, J. (1996). An experimental model of osteoporosis in the laying hen. *Australian Poultry Science Symposium,* **8**: 114-117.

Petersen, J. (1992). Housing systems with regard to animal welfare in poultry. *Zuchtungskunde,* **64**: 217-224.

Petherick, J.C., Seawright, E. and Waddington, D. (1993). Influence of motivational state on choice of food or a dustbathing/foraging substrate by domestic hens. *Behavioural Processes,* **28**: 209-220.

Petherick, J.C., Seawright, E., Waddington, D., Duncan, I.J.H. and Murphy, L.B. (1995). The role of perception in the causation of dustbathing behaviour in domestic fowl. *Animal Behaviour,* **49**: 1521-1530.

Pianka, E.R. (1974). *Evolutionary Ecology*. (Harper and Row, New York).

Puthpongsiriporn,, U., Scheideler, S.E., Sell, J.L. and Beck, M.M. (2001). Effects of vitamin E and C supplementation on performance, *in vitro* lymphocyte proliferation, and antioxidant status of laying hens during heat stress. *Poultry Science,* **80**: 1190-1200.

Reed, H.J. (1994). Designing a nest for a battery cage. In: C.M. Sherwin, editor, *Modified Cages for Laying Hens* (Universities Federation for Animal Welfare, Potters Bar, UK), pp. 27-34.

Reed, H.J., and Nicol, C.J. (1992). Effects of spatial allowance, group size and perches on the behaviour of hens in cages with nests. *British Veterinary Journal,* **148**: 529-534.

Reisberg, D. and Heuer, F. (1995). Emotions' multiple effects on memory. In: McGaugh, J.L., Weiberger, N. and Lynch, G., editors, *Brain and Memory: Modulation and Mediation of Plasticity* (Oxford University Press, New York), pp. 84-92.

Riegle, G.D. (1973). Chronic stress effects on adrenocortical responsiveness in young and aged rats. *Neuroendocrinology*, **11**: 1-10.

Rogers, C.S., Appleby, M.C., Keeling, L., Robertson, E.S. and Hughes, B.O. (1989). Assessing public opinion on commercial methods of egg production: A pilot study. *Research and Development in Agriculture,* **6**: 19-24.

Rollin, B.E. (1995). *Farm Animal Welfare. Social Bioethical, and Research Issues.* (Iowa State University Press, Ames).

Rosenzweig, M.R., Leiman, A.L. and Breedlove, S.M. (1999). *Biological Psychology: An Introduction to Behavioral, Cognitive and Clinical Neuroscience*. (Sinauer Associates Inc, Massachusetts).

Rudkin, C. (1996). Pecking enrichment for intensively housed chickens. In: Advances in Animal Behaviour and Welfare in Australasia and Africa. *Proceedings of the 1st Regional Meeting of the International Society of Applied Ethology* (Christchurch, New Zealand), p. 12.

Rushen, J. (1993). The coping hypothesis of stereotypic behaviour. *Animal Behaviour,* **45**: 613-615.

Sanotra, G.S., Vestergaard, K.S., Agger, J.F. and Lawson, L.G. (1995). The relative preferences for feathers, straw, wood-shavings and sand for dustbathing, pecking and scratching in domestic chicks. *Applied Animal Behaviour Science,* **43**: 263-277.

Sherwin, C.M. (1993). Behaviour of laying hens in modified cages with nests and perches. In: E. Collins and C. Boon, editors, *Livestock Environment IV. Conference Proceedings*, Coventry, UK (American Society of Agricultural Engineers, Michigan, USA), pp. 258-264.

Sherwin, C.M. (1995). Environmental enrichment for laying hens - spherical objects in the feed trough. *Animal Welfare,* **4**: 41-51.

Sherwin, C.M. and Nicol, C.J. (1992). Behaviour and production of laying hens in three prototypes of cages incorporating nests. *Applied Animal Behaviour Science,* **35**: 41-54.

Sherwin, C.M. and Nicol, C.J. (1993). Factors influencing floor-laying by hens in modified cages. *Applied Animal Behaviour Science*, **36**: 211-222.

Sherwin, C.M. and Nicol, C.J. (1994). Dichotomy in choice of nest characteristics by caged laying hens. *Animal Welfare,* **3**: 313-320.

Sherwin, C.M., Alvey, D.M. and Williamson, J.D. (1993). Effects of cage-front design on the feeding behaviour of laying hens. *Applied Animal Behaviour Science,* **38**: 3-4.

Siegel, P.B. and Gross, W.B. (2000). General principles of stress and well-being. In: Grandin, T., editor, *Livestock, Handling and Transport,* 2nd edition (CABI, Wallingford, UK), pp. 21-34.

Smith, S.F., Appleby, M.C. and Hughes, B.O. (1990). Problem solving by domestic hens: Opening doors to reach nest sites. *Applied Animal Behaviour Science,* **28**: 287-292.

Spruijt, B.M. and von Frijtag, J.D.K. (1999). How is welfare represented in the brain? In: Bøe, K.E., Bakken, M. and Braastad, B.O., editors, *Proceedings 33rd International Congress of the International Society for Applied Ethology* (Lillehammer, Norway), pp. 17-21.

Talebi, A., Torgerson, P.R. and Mulcahy, G. (1995). Optimal conditions for measurement of blastogenic responses of chickens to concanavalin A in whole blood assays. *Veterinary Immunology and Immunopathology,* **46**: 293-301

Tanaka, T., Hosoya, H., Watanabe, T., Tanida, H. and Yoshimoto, T. (1993). Effects of type of cage front on behaviour and performance of laying hens. *Japanese Poultry Science,* **30**: 24-30.

Tauson, R. (1984a). Plumage condition in SCWL laying hens kept in conventional cages of different designs. *Acta Agriculturae Scandinavica,* **34**: 221-230.

Tauson, R. (1984b). Effects of a perch in conventional cages for laying hens. *Acta Agriculturae Scandinavica,* **34**: 193-209.

Tauson, R. (1986). Avoiding excessive growth of claws in caged hens. *Acta Agriculturae Scandinavica,* **36**: 95-106.

Tauson, R. (1995). Comparative evaluation and development of housing systems for laying hens. In: Animal Behavior and the Design of Livestock and Poultry Systems. *Proceedings from the Animal Behavior and the Design of Livestock and Poultry Systems International Conference*. (Northeast Region Agricultural Engineering Service, Ithaca, USA), pp. 83-93.

Tauson, R. (2002). Furnished cages and aviaries: production and health. *World's Poultry Science Journal*, **58**:49-63.

Tauson, R. and Abrahamsson, P. (1994a). Effects on production, health and behaviour in three SCWL strains in an EMC model in comparison with other modified and conventional cages. A. Preliminary report on experiences after one batch of birds. In: C.M. Sherwin, editor, *Modified Cages for Laying Hens* (Universities Federation for Animal Welfare, Potters Bar, UK), pp. 41-54.

Tauson, R. and Abrahamsson, P. (1994b). Foot and skeletal disorders in laying hens. *Acta Agriculturae Scandinavica, Section A, Animal Science,* **44**: 110-119.

Tauson, R. and Holm K.-E. (2002). Evaluation of Victorrson furnished cage for 8 laying hens according to the 7§ *(Section)* of the Swedish Animal Welfare Ordinance and according to the New-Technique Evaluation Program at the Swedish Board of Agriculture. Report 251, Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden.

Tauson, R., Ambrosen, T., and Elwinger, K. (1984). Evaluation of procedures for scoring the integument of laying hens - independent scoring of plumage condition. *Acta Agriculturae Scandinavica,* **34**, 400-408.

Tauson, R., Jansson, L. and Abrahamsson, P. (1992). Studies on alternative keepings systems for laying hens in Sweden at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala. Report No. 209.

Thaxton, J. P. and Odhiambo, J. (2003). Physiological stress in laying hens. Ramada Plaza Hotel, Bristol. *Proceedings of the WPSA UK Branch's 27th Poultry Science Symposium* 'Welfare of the Laying Hen' p. 27.

Terlouw, E.M.C., Lawrence, A.B. and Illus, A.W. (1991). Influences of feeding level and physical restriction on development of stereotypes in sows. *Animal Behaviour,* **42**: 981.

Thaxton, P. (2004). Stress and the welfare of laying hens. In: Perry, G.C., editor, *Welfare of the Laying Hen (*CABI, UK), pp. 81-95.

Thorp, B.H., Wilson, S., Rennie, S. and Solomon, S.E. (1993). The effect of a bisphosphonate on bone volume and eggshell structure in the hen. *Avian Pathology,* **22**: 671-82.

Webster, A.B. and Hurnik, J.F. (1994). Synchronization of behavior among laying hens in battery cages. *Applied Animal Behaviour Science,* **40**: 153-65.

Webster, A.B. and Nicol, C.J. (1998). The case for welfare. *Cages for the Future*. (Cambridge Poultry Conference, ADAS), pp. 11-21.

Welham, S.J. and Thompson, R. (1977). Likelihood ratio tests for fixed model terms using residual maximum likelihood. *Journal of the Royal Statistical Society B*, **59**: 701-714.

Wiepkema, P. (1985). Abnormal behaviours in farm animals: ethological implications. *Netherlands Journal of Zoology,* **35**: 279-299.

Wilkins, D. (2004). The politics of hen welfare. In: Perry, G.C. editor, *Welfare of the Laying Hen* (CABI, UK), pp. 31-37.

Wilson, S., Hughes, B.O., Appleby, M.C. and Smith, S. F. (1993). Effects of perches on trabecular bone volume in laying hens. *Research in Veterinary Science,* **54**: 207-11.

Wolford, J.H. and Ringer, R.K. (1962). Adrenal cortical activity and differential leucocyte picture of S.C. White Leghorns housed in cages, on a slatted floor and on a conventional floor. *Quarterly Bulletin of the Michigan Agricultural Experimental Station*, **45**: 38-44.

Wood-Gush, D.G.M. (1972). Strain differences in response to sub-optimal stimuli in the fowl. *Animal Behaviour,* **20**: 72-76.

Wood-Gush, D.G.M. (1975). The effect of cage floor modification on pre-laying behaviour in poultry. *Applied Animal Ethology,* **1**: 113-118.

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**: 175-181.