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Free range hen welfare: Characterisation of ‘outdoor’ and ‘indoor’ hens and physical features in the range

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Foreword

This project examined the impact of accessing the outdoor range on the behaviour and stress physiology of free range laying hens and the effects of environmental design on the use of the outdoor range.

Free range production systems are increasing in Australia, partly driven by consumer perception of free range systems as animal welfare friendly, presumably because outdoor access is considered conducive to the expression of natural behaviours. Yet, research regarding the implications of allowing outdoor access on hen welfare is surprisingly limited.

This project allowed the conduct of research projects for one PhD student, whose stipend was supported by an Australian Postgraduate Award and top-up stipend was provided by the Poultry Cooperative Research Centre; and two Master of Animal Science students' major research projects at the University of Melbourne.

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

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Abbreviations

AECL	Australian Egg Corporation Limited
ANOVA	Analysis of variance
FAWC	Farm Animal Welfare Committee (UK)
FDR	False discovery rate
FOV	Field of view
GLM	Generalised linear model
GNSS	Global navigation satellite system
GPS	Global positioning system
h	Hour
ha	Hectare
LS-means	Least squares means
min	Minute
OIE	World Organisation for Animal Health
RFID	Radio-frequency identification
s	Second
SE	Standard error
TI	Tonic immobility
UV	Ultraviolet

Executive Summary

Free range egg production currently contributes about 39% in volume and 49% in value of egg grocery sales in Australia (AECL source, 2014/2015). Nevertheless, the use of the outdoor range in free range commercial flocks, which is the main feature of the system in comparison to other non-cage systems (barns, aviaries), remains poorly understood.

This project investigated use of the outdoor range by laying hens in Australian commercial egg free range flocks, with three aims, to:

- examine the impact of accessing the outdoor range on the behaviour and stress physiology of free range laying hens
- investigate if the performance of so-called 'natural' behaviours, such as accessing an outdoor range, has animal welfare implications
- determine the effects of environmental design on the use of the outdoor range.

A series of six experiments was conducted over a three-year period. All experiments were conducted on commercial farms of various sizes (flocks of 120 to 18,000 hens), with a diversity of outdoor range features considered representative of the overall Australian free range egg industry.

In order to elucidate some of the motivational factors to range, Experiment 1 consisted of an observational study investigating hen behaviour around a highly preferred natural structure in the outdoor range (Kangaroo Apple trees). The findings showed that hens performed a variety of behaviours (predominantly foraging, preening and perching) in this shrub-like structure, and that the primary use of these structures changed throughout the day. The search for artificial structures that allow the hens to perform similar behaviours could ultimately optimise range use.

Because free range hens are often noticed spreading unequally across the range, Experiment 2 investigated the behavioural time budget of hens between distinct locations or 'patches' within the outdoor range. The range characteristics were mapped on one commercial free range farm. Four distinct patch types were chosen on the basis of cover and substrate, all at a 20 m distance from the shed and with observation areas of equal sizes. Higher numbers of hens were observed in an area underneath a large Eucalyptus gum tree (30 m high and providing large canopy cover) and an area underneath Acacia wattle trees (providing 1-2 m high dense canopy cover) than a bare sand and gravel ground area, and a bamboo-like dense leafy vegetation area. Highly favoured areas were less subject to diurnal patterns of range use, whereas other areas contained more hens early or late in the day. The most common behaviours were foraging, preening, locomotion, resting and vigilance. A wider variety of behaviours was observed in the highly preferred patches, whereas mostly active behaviours (foraging and locomotion) were performed in areas that were less frequented. Hence, different behaviours are performed in patches that differ in cover or substrate. Furthermore, providing areas of highly preferred patch types could enhance the spatial distribution across the range and minimise the diurnal pattern of range use in commercial settings.

The transition between the shed and the outdoor range is often anecdotally cited as an important determinant of ranging, but has not been scientifically studied. Therefore, Experiment 3 investigated the behaviour of free range laying hens after exiting a pop hole, with a focus on the role of environmental and social factors. The behaviour of focal hens was analysed for 5 min after exiting the pop hole or until they disappeared from the field of view, with the field of view of the cameras covering approximately 300 m². The results showed that at the end of the observation, hens ended up half of the time within the pop

hole area (within 1 m from the shed), and a quarter of the time in the bare earth area (extending from 1 to 9 m from the shed). However, most hens left the field of view within 1 to 3 min, so before the end of the observation (set at 5 min) it could not be excluded that hens may have taken longer to reach the tree cover area if at all. Overall, the findings did not support the hypothesis that the barren environment was used solely as a transitioning area and be the least occupied, or that hens would prefer to join groups after leaving the pop hole. Further, hens ranged more during the morning periods, travelling greater distances.

Free range hens are often observed congregating in the range area closer to the shed or next to structural elements of the range (e.g. trees, fences). Experiment 4 investigated different principles that may underlie the effectiveness of artificial structures to attract hens in the outdoor range: orientation of structures, cover density, and height of shelter. The most important factor was the cover density of these structures, with 90% UV block being most attractive, followed by 50%. The second most important factor was orientation, with combined structure (i.e. horizontal structures with one vertical side) and horizontal structures attracting more hens than vertical structures. Finally, height also mattered but its interaction with orientation prevailed, in that height did not make a difference for combined orientation structures, whereas short horizontal structures were preferred over tall ones but, conversely, tall vertical structures were preferred over short ones. These results highlight the complexity of designing attractive outdoor environments for commercial laying hens. It is evident that hens have retained a preference for areas that provide dense cover as seen in their wild ancestor, the Red Junglefowl.

The hen welfare implication of accessing an outdoor range, despite being an impetus for free range egg demand, crucially lacks science-based evidence. Through individual tracking with RFID technology, Experiment 5 investigated the relationship between ranging pattern and the behaviour and stress physiology of free range laying hens in two commercial flocks, at mid-lay (45 weeks of age) and toward end-of-lay (65 weeks of age). The majority of tracked hens (85 to 97%) accessed the range over the course of the two weeks of tracking. Furthermore, most hens (69% and 82% of two flocks respectively) accessed the range every day. Hens spent about 2 to 5 hours in the range daily, visiting the range between 5 and 20 times a day. Hence, hens make regular moves between the shed and the range, as well as making regular changes between the three zones monitored, about every 15 to 20 min, suggesting active ranging behaviour. Despite the extensive ranging behaviour, there was little and inconsistent evidence that differences in range access led to differences in measures of hen welfare, based on a battery of behavioural and physiological measures: blood corticosterone concentration (reflective of the acute stress response), faecal corticosteroid metabolites (chronic stress response), heterophil:lymphocyte ratio (immune system), plumage condition, weight, keel bone deformation, foot pad dermatitis, comb colour and beak condition. However, there was evidence that the minority of hens that do not range are more fearful. Therefore, it is possible that range access has little effect on these measures of hen welfare. Alternatively, variation in range access observed between individual hens may reflect their decision to use the range to balance their welfare needs.

Finally, Experiment 6 investigated the behaviour displayed by hens in various areas of the range to identify whether the outdoor range stimulated a greater behavioural repertoire, with the performance of so-called ‘natural behaviours’. Whilst in the open range, hens mostly foraged and moved around. The outdoor area was most conducive to exploratory behaviours and greater foraging opportunities where vegetation was present. The behaviour of hens in the wintergarden showed similarities to both indoor and outdoor areas, displaying various comfort behaviours (e.g. preening, dust bathing) similar to indoor locations but also exploring and foraging mostly seen on the range. The covered indoor area was conducive to various comfort behaviours, probably as it provided refuge, which was not offered in the range. Hence, despite the observation that no additional behaviour was performed outdoors that was not seen indoors, the time budget of behaviour changed, being dominated by a more active and exploratory pattern and less comfort behaviours.

Overall Conclusions

The project had three distinct, but related aims.

- Examine the impact of accessing the outdoor range on the behaviour and stress physiology of free range laying hens.

This question was addressed through tracking range access in individual laying hens over a two-week period in two flocks, and comparing hens that differ in their use of the outdoor range within flocks to control for between-flock variation. A large majority of hens on the farm studied accessed the range on a regular basis, although there was considerable variation between individuals in the amount of range access. Despite this variation between individuals, there was little and inconsistent evidence that the extent of ranging was related to hen welfare, based on a range of behavioural and physiological variables. Nevertheless, there was evidence that the minority of hens that do not range may be more fearful.

- Investigate if the performance of so-called ‘natural’ behaviours, such as accessing an outdoor range, has animal welfare implications.

The various behavioural studies conducted in this project showed that hens performed different behaviours outdoors as compared to indoors. Furthermore, these behaviours varied according to the resources offered in the range and the time of day. Nevertheless, there was no evidence that additional behaviours were performed in the range that were not performed inside the shed. The difference was not in the nature of the behaviours displayed (i.e. behavioural repertoire), but rather in the frequency of these behaviours (i.e. behavioural time budget), with more exploratory behaviours in the outdoor range (walking, foraging) and less comfort behaviours (preening, resting). Therefore, outdoor range access does encourage higher expression of exploratory behaviours by laying hens. The welfare implications of these particular behavioural changes remain to be fully examined

- Determine the effects of environmental design on the use of the outdoor range.

In addition to individual differences in ranging behaviour, environmental features offered in the outdoor range were found to have a large influence on the number of hens in these areas as well as on the behaviour displayed by hens while in these areas. Both specific natural structures (in these studies: a large Eucalyptus tree, Acacia wattle trees, or Kangaroo Apple tree) and artificial structures (horizontal structures with dense cover) were found to be highly successful at attracting hens. Furthermore, highly preferred areas remained well used throughout the day, as compared to other areas, which usually experienced a strong time of day peak in hen numbers. These structures offer novel avenues for the design of outdoor range features that attract the hens into the range and stimulate a more uniform use of the range in terms of the area of range use. These findings suggest that modern laying hens have retained preference for areas that provide dense cover as seen in their wild ancestor, the Red Junglefowl.

1 Literature review: the behaviour and welfare of hens in free range systems

1.1 Animal welfare assessment

Animal welfare is defined by the World Organisation for Animal Health (OIE) as “how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. ‘Animal welfare’ refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment” (OIE, 2010). This definition covers quite comprehensively all aspects that can impact on the welfare of an animal. Nevertheless, assessing animal welfare on-farm remains practically challenging.

The assessment of animal welfare requires the use of multiple indicators from multiple disciplines but their relative importance has yet to be clarified. The Five Freedoms (FAWC, 1979) provides a general framework that has been widely accepted among welfare scientists for tackling core welfare components (although it does not specify thresholds indicative of acceptable and unacceptable welfare). This specifies that an animal is in a good state of welfare if it is free from hunger and thirst; discomfort; pain, injury and disease; free to express normal behaviour; and free from fear and distress. However, the use of the Five Freedoms in animal welfare assessment is difficult, given they are an ideal rather than practical aspects (Mellor, 2016).

The fact that animal welfare is ultimately an internal experience results in competing perspectives within the field about the best way to assess it. Three major schools of thought co-exist. The first centres on biological functioning, and considers indicators of physical health, survival, growth and reproduction to be truthful mirrors of the welfare state of an animal (McGlone, 1993). This approach has been historically predominant in animal welfare science, partly as a result of its direct relevance to common forms of animal use where aspects related to health, survival, growth and reproduction are usually closely monitored. The second school is referred to as the feeling-based or affective states approach. This approach emphasises mental health, arguing that welfare is to do with what animals feel and that animals should not experience fear, suffering, or anxiety (Dawkins, 1990). This view has gained momentum in the last two decades, supported by progress in neurobiology and new testing methodologies (Mendl et al., 2009; Panksepp, 2011). A third view, the natural living approach, emphasises the need for animals to behave in natural ways (Kiley-Worthington, 1989). For example, a cow should be able to graze and a duck should have access to water. This view has broad parallels with the widely held understanding that if an animal is able to perform its natural behaviours, its welfare is good. Indeed, it seems close to the perception by the general public that free range systems are natural systems that are assumed to safeguard an animal’s welfare. However, this last approach is poorly defined and draws criticism from the fact that an animal’s natural behaviours include responses to unfavourable conditions and undesirable activities, such as shivering in the cold or escaping predators (Fraser, 2008).

Despite this long-held debate between the three major schools of thought on animal welfare assessment, the three concepts have considerable overlap, and indeed influence each other as complementary aspects, reflecting the various facets of animal welfare. The reader is referred to more comprehensive reviews such as Hemsworth et al. (2015) for more details on animal welfare assessment.

1.2 Implications of outdoor range use on hen welfare

The use of free range production systems is increasing in Australia, partly due to public concern that cage housing restricts birds in terms of space, social contact and environment stimulation. Free range systems allow hens to access an outdoor area that provides the opportunity to perform 'natural' behaviours such as foraging for food and dust bathing. Yet, there is a lack of scientific knowledge relative to the use of the free range area (the main characteristic of this system in comparison to non-cage systems such as barn or aviaries), and its implications, advantages and disadvantages, in terms of animal welfare and productivity.

The outdoor run offers a wide range of environmental stimuli, exercise and foraging opportunities, dust bathing substrates, a diversity of food items (seeds, insects) and various climatic conditions. All these could be seen as potential benefits to hen welfare. Yet, the outdoor run also presents a risk of predation, imbalanced diet, increased exposure to pathogens and inclement weather. All these could seriously compromise the welfare of the hens. The literature is inconsistent in the welfare outcomes of free range systems. For instance, several studies in laying hens have reported that greater outdoor range use is inversely related to the prevalence of feather pecking (e.g. Bestman & Wagenaar, 2003; Nicol et al., 2003). Mahboub et al. (2004) found that, on an individual basis, hens that spent more time outside had less feather damage but for Hegelund et al. (2006), plumage condition was not correlated with use of the outdoor run. Furthermore, the question remains as to whether feather pecking inhibits the willingness to go outside (possibly because of the lack of plumage's insulating effect) or is it the fact of not going outside that leads to feather pecking? The current literature does not allow the identification of causal relationships as these previous studies are based on correlated data. However, as noted by Hegelund et al. (2006), "both feather pecking and use of the outdoor run have been associated with fear, and could therefore be related through this third parameter". Indeed, Grigor et al. (1995) found that 'outside' hens, which spent more than half their time outside, had a shorter tonic immobility righting times than birds that were never seen outside. This suggests that the outside hens had a reduced fear response. This may have been because the outside hens were exposed to a wider variety of stimuli outside, so were less fearful of novel stimuli. Alternatively, the lower fearfulness in outside birds may have been the reason why these birds went outside in the first place.

The welfare implications of free range systems have generally been assessed by comparison to other systems such as barn-housed or cage-housed birds. Nevertheless, the welfare of the birds in free range systems might also vary within the flock since the use of the outdoor range is highly variable. Only 10 to 30% of the flock access the outdoor range at any one time, with large variations between times of the day, flocks and studies (Bubier & Bradshaw, 1998; Dawkins et al., 2003; Zeltner & Hirt, 2003; Hegelund et al., 2005, 2006). A recent study on a UK commercial farm (Richards et al., 2011) confirmed that subpopulations exist within free range flocks regarding outdoor use. In their study, 80% of their focal birds accessed the outdoor range at some point, with a high variation between birds, but 8% of birds never accessed the range. The causes of these differences in outdoor use were not investigated, nor were the implications in terms of welfare or productivity. Few studies to date have taken into account the amount of use of the outdoor range in the assessment of welfare in free range laying hens, except recent Australian research, which investigated the relationship between range access and welfare measures and reported no differences between hens that used the range or not (Hinch & Lee, 2014).

In other studies that did not monitor individual range use, results are probably representative of an average of birds that go out to different extents, possibly explaining the variation in the results or the discrepancy across studies conducted on free range flocks. As an example of variation, van den Brand et al. (2004) found a greater variation in egg external and internal characteristics in an outdoor housing system compared to a cage system, using the same type of birds. It is important to determine the factors that determine the greater fluctuations in egg quality. Variations in the use of the free range could be one major factor. It is generally accepted that environmental factors (such as lighting, nutrition, and environmental temperature) can affect egg quality, possibly through physiological stress (Holst et al., 2011). All of these factors are likely to vary significantly between birds that spend time in the outdoor range and those that do not. For instance, hens with access to an outdoor range area often have greater faecal worm egg counts than hens without access to range (Häne et al., 2000), and high contamination levels of soil outside can increase dioxin content in the hens and their eggs (Kijlstra et al., 2007). These parameters could logically correlate with the amount of time the hen spent outdoor foraging.

Some of the inconsistencies between the welfare performances of free range systems in comparison to other systems might also arise from variations in the particular features of the house, outdoor run, or stockmanship knowledge or skills. Good stockmanship is a major determinant of success in free range systems. The management of free range birds requires careful monitoring and is more complex than indoor environments due to the uncontrolled environmental conditions and heterogeneous diet composition. Differences in stockmanship knowledge or skills may partly explain why some free range operations perform as well or better than conventional cages whereas others perform worse in terms of productivity and mortality. Increasing our understanding of the interactions between the environment and the birds in free range conditions will allow refinement of the management of free range systems.

Studies that compared free range systems and barn systems are probably the best to assess the effects of the outdoor run per se. Mortality has been reported to be higher (Häne et al., 2000) or lower (Sherwin et al., 2010) in free range systems compared to barn systems. In any case, free range birds are likely to be exposed to more pathogens during their lifetime, highlighting the importance of good immune defences and disease control in the flock. A major skeletal health issue of conventionally caged hens is the increased susceptibility to osteoporosis mainly due to lack of exercise (Lay et al., 2011). Since exercise enhances bone strength, increased bone breaking resistance is to be expected in hens with access to an outdoor run. Free range laying hens have higher bone breaking strength than caged birds (Leyendecker et al., 2001 as in Jones et al., 2007). However, no comparisons exist between free range systems and non-cage indoor systems such as barns, which could tell us something about the influence of the outdoor range per se. Furthermore, Gregory et al. (1990) found that while aviary and free range systems had a lower incidence of bone break following catching, these birds had more old fractures than birds from conventional cages. Therefore, the welfare of birds might be affected in different ways in different systems.

The welfare implications of the use of the outdoor range itself remain unclear. No study to date has investigated the behavioural or physiological differences between birds that extensively use the outside and those that do not in commercial conditions. In experimental settings, Grigor et al. (1995) identified differences in fear response, a finding corroborated by Hinch and Lee (2014). One of the limitations has been the technical difficulty to follow individual birds in large flock settings over extended periods of time. However, this technical problem can be overcome today by using technologies such as miniature global positioning system (GPS) devices or radio-frequency identification (RFID) systems.

All studies to date have examined the welfare of free range systems as compared to other housing systems. However, rigorous comparisons could be made within free range systems, according to ranging behaviour, given that ranging behaviour is known to differ between hens within the same flock (Richards et al., 2011).

1.3 Factors affecting outdoor range use

Several causes have been suggested for laying hens not utilising the outdoor range in free range systems: genetics, experience, fear, lack of cover, etc. Yet, this practical issue remains unsolved. The use of the outdoor range is highly variable among hens, with some hens never going outside (e.g. Richards et al., 2011). Furthermore, the hens' distribution is not uniform across the range. The hens are usually observed to stay close to the house or to particular features of their enclosure such as walls and fences. This could be linked to issues in terms of loss of grass cover and increased stocking density in particular areas, which may contribute to feather pecking, land overstocking and parasite contamination although scientific studies are lacking. Furthermore, free range systems in which hens do not or rarely range are at risk of losing public credibility.

The outdoor environment should offer physical features that allow for protection and escape from predators in order to counteract the behavioural inhibition induced by fear. The modern laying hen evolved from the Red Junglefowl, their wild ancestor. The natural habitat of Red Junglefowl is a dense rainforest, which contains abundant vegetation providing both cover from predators and a source of food over a relatively small area of about 1 hectare per bird (Collias & Collias, 1967). Most free range farms offer a large open-field pasture but with comparatively very little overhead cover. As a result, only a maximum of 10 to 30% of the flock access the outdoor at any one time, with large variations between times of the day, flocks and studies (Bubier & Bradshaw, 1998; Dawkins et al., 2003; Zeltner & Hirt, 2003; Hegelund et al., 2005, 2006). Furthermore, most birds do not venture further than 20 to 30 m away from the house (Zeltner & Hirt, 2003; Hegelund et al., 2006).

A comprehensive study on outdoor fowl in commercial settings found that the number of broiler chickens found in the range was positively correlated with the amount of tree cover, the time of the day and the season (Dawkins et al., 2003). Studies on laying hens confirmed that the amount of cover in the range is a crucial factor influencing the willingness of birds to go outside (Zeltner & Hirt, 2003; Hegelund et al., 2005). Cover also allows the birds to gain shelter during inclement weather. Natural trees or bushes would provide cover but present several disadvantages such as slow growth, attractants for wild birds and a stance for aerial predators, hence the search for artificial substitutes. Furthermore, artificial structures have the advantage that they can be moved to enhance a uniform use of the range. Shelterbelts, as natural vertical structures, or the provision of shaded areas have been found to attract more hens in the range (Hegelund et al., 2005; Borland et al., 2010; Glatz et al., 2010), whereas other structures such as roofed boxes with sand increased the distribution, but not the number, of hens in the range (Zeltner & Hirt, 2003). However, birds are not attracted to trees or artificial horizontal structures if these are located too far from the house, indicating the importance of location in the range in addition to the attractiveness of the structure (Mirabito & Lubac, 2001; Dawkins et al., 2003; Hegelund et al., 2005). That 'cover' does not need to be overhead was demonstrated by Taylor et al. (2004), who found that hens spent significantly more time near wire fences covered black plastic (66% of observations) than wire fences (19%) or away from either (11%). While most studies showed the effectiveness of those structures to various extents, the particular features or cues of the structures that fulfil the biological requirements for hens have not been scientifically investigated. For example, offering structures that vary in their features (two-level perches, a "pecking-tree", pine tree and boxes with pine cones) was more effective at increasing the number of hens outside than offering just one type of structure (a shelter), but the authors did not identify which specific features caused more birds to go outside (Zeltner & Hirt, 2008). The effect of artificial structures placed in the enclosure on the bird's willingness to range remains poorly understood. Research is needed to identify which environmental features are biologically relevant to the birds, and should be provided for optimum use of the range.

Artificial vertical panels have been investigated to increase environmental complexity for young broiler chickens kept indoors (Newberry & Shackleton, 1997; Cornetto & Estevez, 2001a,b). Vertical structures changed the behaviour of the birds, increasing resting (Cornetto & Estevez, 2001a; Newberry & Shackleton, 1997) and preening (Newberry & Shackleton, 1997), although the causes of those behavioural changes remain unclear. Vertical structures can attract the birds to open spaces that are usually an underused area, therefore increasing the uniformity of the bird distribution within the pen (Cornetto & Estevez, 2001b). The importance of vertical cover continuity remains to be elucidated, as it has been reported that indoor broiler chickens prefer a frame with a mesh offering medium continuity (67%) to no or full continuity (Newberry & Shackleton, 1997) but others using the same structures showed a frame was as effective with or without mesh (Cornetto & Estevez, 2001b). However, these experiments were conducted indoors on small groups of broiler chickens and may not be representative of the needs of laying hens outdoors. Indoor settings do not have the predation and inclement weather conditions present in outdoor systems. Broiler chickens and laying hens have different physical and behavioural requirements. Most of those experiments have also been performed in small group sizes not exceeding a few hundreds of birds whereas the large group sizes in commercial settings, usually thousands of birds, may lead to different social dynamics, making extrapolation of results difficult.

A number of other factors have also been reported to affect ranging behaviour. Genotype to some extent affects the willingness to range outdoors. In similar conditions, Hy-Line birds spent less time foraging than Red Junglefowl or unselected breeds such as Bantam birds (Schütz & Jensen, 2001). Therefore, genetic selection may be part of the solution but is a rather slow process, which may also involve a cost in terms of lower productivity. Weather and seasonal conditions such as temperature, rain and cloud cover also affect the number of birds found in the range (Dawkins et al., 2003; Hegelund et al., 2005). Nonetheless, no practical options are available to control for those weather conditions in the outdoor range. Increasing flock size has been suggested to reduce the number of birds going outside. However, most studies have been conducted on relatively small flocks (100-2,500 birds), making it difficult to extrapolate to larger commercial flock sizes, in which some studies report no effect of flock size (e.g. Hegelund et al., 2006, looking in the range of 1,200 to 5,000 hens). Furthermore, direct comparisons across studies are difficult because of the use of different strains and different management practices – e.g. large flocks locked inside the house in the morning vs. ad-lib access for small flocks, beak trimmed or not, professionals vs. hobby farmers (for example, see Hirt et al., 2000 or Kijlstra et al., 2007). Hence, evidence is equivocal and it remains to be determined if this is the influence of group size per se or the result of other factors such as differences in management or stockmanship.

1.4 Aims of the current research project

This project has three aims, to:

- examine the impact of accessing the outdoor range on the behaviour and stress physiology of free range laying hens
- investigate if the performance of so-called 'natural' behaviours, such as accessing an outdoor range, has animal welfare implications
- determine the effects of environmental design on the use of the outdoor range.

2 Experiment 1: Go outside and play? Behavioural time budget of free range laying hens in a natural shrub structure

An identical version of this chapter was published as a four-page abstract at the Australian Poultry Science Symposium, 2014, Sydney, Australia, 25: 113-116, and delivered as an oral presentation.

2.1 Abstract

The main feature of free range production systems is the provision of an outdoor area. However, what hens do during their time outdoors, or what they wish to do, remains poorly understood. Some free range farms see low numbers of birds outside, and/or uneven distribution across the range. Environmental enrichment such as the provision of trees, bushes or other types of cover could help solve these problems. However, there is little scientific evidence about what enrichment strategy will work, and most importantly why. We investigated the behaviours performed by free range laying hens in a commercial setting when provided with an attractive outdoor structure, in this study naturally occurring Kangaroo Apple trees. We found that the hens performed a variety of behaviours (predominantly foraging, preening and perching) in these shrub-like structures, and that the primary use of these structures changed throughout the day. The search for structures that allow the hens to perform similar behaviours could ultimately optimise range use.

2.2 Introduction

Although it is assumed that free range production systems provide greater opportunities for laying hens to perform more “natural behaviours”, there is little scientific evidence about what those behaviours are, and when and where the hens are likely to perform them.

Use of the outdoor range by commercial laying hens in free range systems is often limited to a small proportion of the flock at any one time (Hegelund et al., 2006). It is also apparent that the hens’ distribution over the range is not uniform, with the hens usually staying close to the shed (Hegelund et al., 2006). Enrichment of the outdoor range as a means to encourage more hens outside and a more uniform distribution across the range has become an increasingly popular and necessary topic of interest. Ranges that contain natural structures such as trees and shrubs can increase the number of chickens in the range, and with the right placement, could improve the distribution of the flock (Dawkins et al., 2003). However, in many commercial settings there is not a sufficiently established natural biota that will encourage range use. An alternative may be in the form of artificial structures that mimic the important principles of natural structures to the hens, and therefore increase range use and distribution. Items such as hay bales, shelterbelts, shade cloth and sand boxes have been investigated, and showed marginal to significant improvements in either range use or distribution (Hegelund et al., 2005; Nagle and Glatz, 2012; Zeltner and Hirt, 2003; Rault et al., 2013). However, the way hens perceive and utilise these structures is still poorly understood. Elucidating the behaviours performed by hens utilising the outdoor range will help with designing reliable artificial enrichment in commercial settings that fulfil the hens’ needs.

The Kangaroo Apple (*Solanum laciniatum*) is a native shrub that occurs in temperate regions of South Eastern Australia and New Zealand. Kangaroo Apple is a soft wooded, tolerant plant that is fast growing (>2 m in approx. 6 months) and short-lived (5-6 years). On a commercial free range farm in Victoria, Kangaroo Apple shrubs have been allowed to self cultivate and grow randomly throughout the ranges to form large (4 m high x 5 m wide) complex shrubs, that grow in tight groups forming larger shrubberies of up to 200 m^2. After onsite visits and conversations with the farm managers, it became apparent that the laying hens utilise the areas in and around the shrubs more than any other location on the range. An observational pilot study on this farm was designed with the overall goal to use the information gained from observing the hens' behaviour to apply to further research hypotheses on outdoor range design. We had three objectives: 1) to establish the baseline behavioural time budget in an attractive naturally occurring structure; 2) to derive principles from the behaviours observed during this study to aid the design of artificial enrichment structures; and 3) to generate hypotheses on how outdoor range use might impact behaviour of free range laying hens based on hen behaviour around these structures.

2.3 Materials and methods

We observed two flocks of ISA Brown hens on one commercial free range farm in Victoria with naturally occurring Kangaroo Apple shrubs in the range. Flock one contained 120 pullets at 25 weeks of age that had arrived approximately one month before the start of the study. The range for flock one was approximately 0.9 ha and contained a heavily vegetated perimeter fence, multiple Kangaroo Apple shrubs (10-15), and predominately featured grass. Flock two contained 200 hens at 45 weeks of age, arriving approximately four months before the study, and 180 pullets at 25 weeks of age that had arrived one month before the study, combining to make a single group of 380 hens. The range for flock two was approximately 0.8 ha, contained multiple Kangaroo Apple shrubs (~20), and again predominately featured grass. The hens were not beak trimmed and had 24 h access to the range from mobile sheds that contained nesting boxes, feeding and watering areas, and perches. Each flock was protected from ground predators by two Maremma sheep dogs.

Observations were carried out sequentially in each flock using one Canon1000D SLR camera equipped with an intervalometer. The camera, tripod and protective case were placed in the Kangaroo Apple shrubs in an area that allowed for maximum field of view without obstructions from branches or leaves. Each field of view contained an approximate area of 15 m^2, <1% of the total range area available to the hens. Scan samples were taken for three two-hour periods daily: 0730-0930h ('morning'), 1130-1330h ('midday') and 1530-1730h ('evening'). During these periods, the camera was programmed to take continuous photos at two-minute intervals for one second (average of three shots). A total of six days worth of photographs was obtained for each flock, equivalent to 1080 scans per flock.

Photographs were analysed by one observer to identify the number and behaviour of the hens present in the Kangaroo Apple area, in accordance with a behavioural ethogram designed for this study. The main behaviours recorded were foraging, preening, perching, dust bathing, walking, standing upright (head erect and alert, eyes open), lying (body and head on ground or head tucked under wing, not moving) and standing (in non alert position, neck not outstretched, eyes may be open or closed) and vigilance (standing in alert position with neck outstretched). Behaviours were recorded only when they could be positively identified; hens that were obscured by conspecifics, branches, partially in the shot or where the head was not visible, were counted but their behaviour was listed as 'unidentifiable'. Behavioural time budgets were constructed for each flock. Daily weather observations of wind speed, min and max temperature and rainfall were collected from the nearest Australian Bureau of Meteorology weather station. Differences in the proportion of behaviours performed at each time period within each flock were calculated using Fisher's exact test, and P values were corrected for multiple comparisons using the Bonferroni correction method. Results are presented as means ± standard error, and results are considered significant at $P < 0.05$.

2.4 Results

A combined total of 9517 behaviours was recorded from the two flocks over the study period. Flock one and two had a much higher proportion of observed hens present in the Kangaroo Apple area in the midday session (58.4 ± 0.69% and 58.1 ± 0.75% respectively) compared to morning (18.9 ± 0.55% and 22.0 ± 0.63% respectively; both $P < 0.05$) and evening (22.7 ± 0.58% and 19.9 ± 0.60% respectively; both $P < 0.05$). Flock one also had higher number of hens in the evening compared to morning sessions ($P < 0.05$).

Foraging patterns were similar for both flocks, where foraging behaviours significantly increased in the evening session compared to morning and midday sessions ($P < 0.05$; Figure 2-1). Preening behaviours showed a decline throughout the day for both flocks ($P < 0.05$). Differences between the two flocks were observed in their perching and lying behaviours. Perching behaviours for flock one were highest at midday than in the morning, and finally, in the evening ($P < 0.05$). Flock two did not differ significantly in perching behaviour by time period, and had lower proportions of this behaviour overall. However, flock two did show a significant increase in lying behaviour for the midday session over both morning and evening sessions ($P < 0.05$).

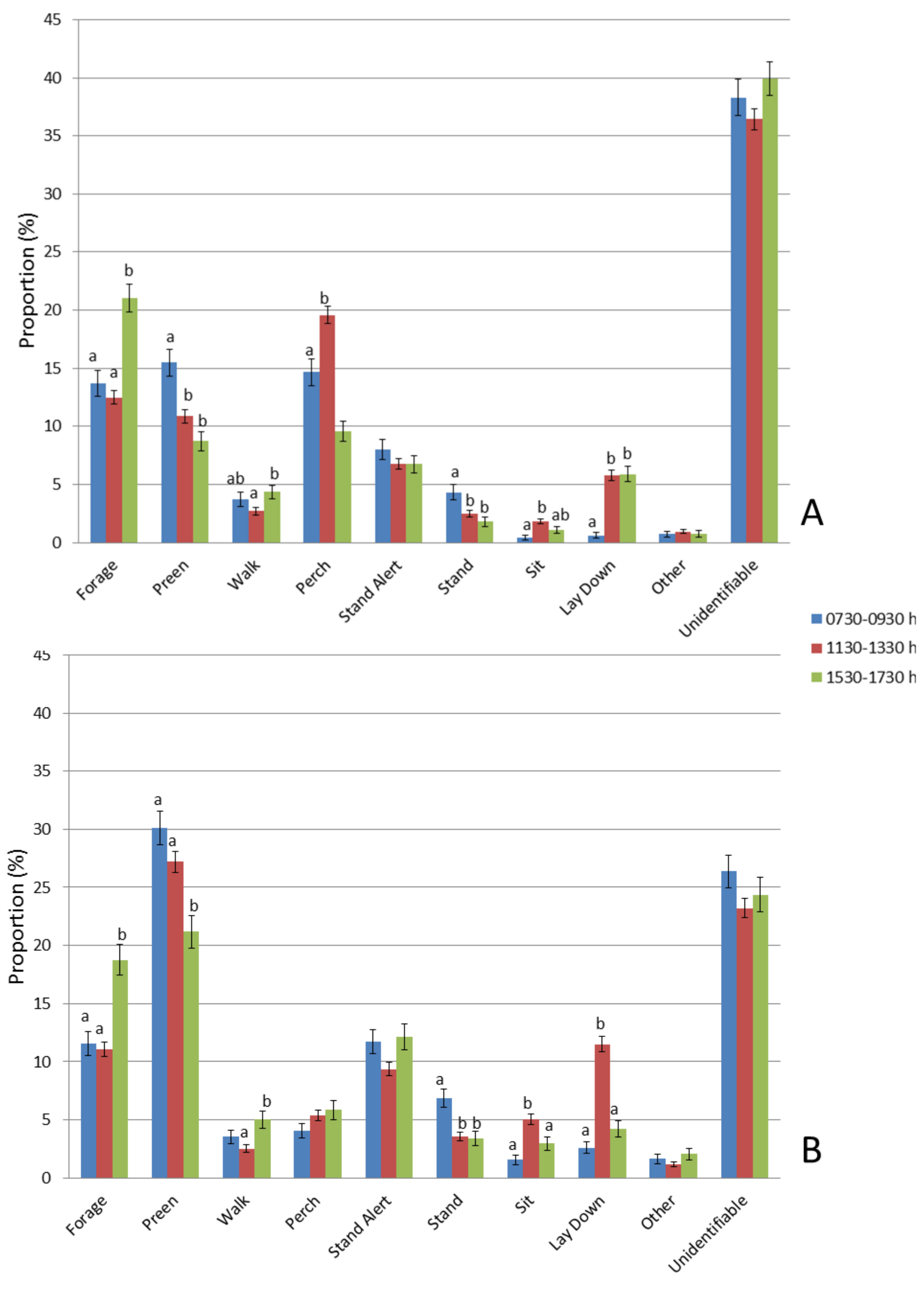


Figure 2-1 Proportion of hens' behaviours (%) in flock one (A) and flock two (B) for each sampling time period

Behaviours with different superscript (a-b) differ (P < 0.05).

The overall proportion of unidentifiable behaviours was greater in flock one than in flock two, likely due to the placement of the camera in the field. The visible area in flock one contained more of the Kangaroo Apple tree than that of flock two, therefore more hens were obscured in photos by branches and leaves. This may also explain other differences in the proportions of behaviours observed, in particular preening. Hens that were categorised as unidentifiable were often stationary, in groups and often had their head tucked.

Observations were conducted during early spring, temperatures were average for the season (min av. 10.5 ± 0.4°C, max av. 17.5 ± 0.7°C) and rainfall low during the study period (1 day at 12 mm, 1 day at 8 mm, 4 days at 1.6-2.2 mm, and the rest dry days). Regression analyses revealed that weather conditions had no significant influence on the number of hens using the structure, but we deliberately chose to avoid particularly poor weather days.

2.5 Discussion

More laying hens utilised the Kangaroo Apple areas at midday. This was somewhat contradictory to previous studies of range use, which have indicated that there is a peak in range use in mornings and evenings (Hegelund et al., 2006; Rault et al., 2013). As numbers were recorded only for the proportion of the Kangaroo Apple area, we cannot judge whether the natural structures alter the diurnal pattern of range use. The hens may have been more attracted to other areas of the range during the morning and evening periods.

Analysis of the behaviours performed by each flock in each of the time periods indicated that the principal uses of the natural structure changed throughout the day. This is particularly evident in flock one, where the morning was dominated by preening, midday by perching and evening by foraging behaviours. Flock two also demonstrated changes, where preening decreased from morning to evening, foraging increased in the evening, lying behaviour increased at midday as well as a decrease in alert vigilance behaviour. This pattern of change suggested that the laying hens may utilise the Kangaroo Apple area more for grooming and comfort behaviours in the morning, resting and shelter in the warm midday periods, and more actively as a secondary foraging source in the evenings.

The overall complexity of the Kangaroo Apple is surely an attractive feature to the hens and our study showed that a large number of behaviours can be performed in this structure. Dust bathing is a behaviour that was conspicuously not observed in this study, possibly because the ground was moist at the time of the study, and may not be ideal for dust bathing. On site visits prior to the study, dust bathing was observed, in addition to the presence of many dust bathing divots in the ground. Our observations also suggest that social facilitation is important in laying hen behaviour: in cases where there were multiple 'groups' of hens that were dispersed throughout the observation area, most of the individuals of each group would be performing the same behaviour, e.g. preening simultaneously, foraging in the same spot, or perching together, etc.

Considerable variation exists between the two flocks, in particular age, group size, and range configuration. However, despite this variation, some important similarities between the two flocks' behaviours indicated that the use of natural structures may follow predictable patterns. The particular behavioural time budget hens performed in this natural structure provides valuable information for further research in the field of artificial enrichment of the range.

2.6 Conclusions

Kangaroo Apple trees are a complex natural structure that facilitate a large number of behaviours for free range laying hens. The structure was utilised mostly in the middle of the day as an area for shelter and rest when the hens were most inactive, however, primary use of the structure changed in the mornings and evenings. This indicates that the most effective artificial enrichment items could be complex enough to allow for changes in key use, as well as large enough to accommodate and shelter large number of hens throughout the day.

3 Experiment 2: Behaviour of free range laying hens in distinct outdoor environments

These results were presented at the 48th Congress of the International Society for Applied Ethology, Vitoria-Gasteiz, Spain, p. 186, and delivered as a poster presentation.

3.1 Introduction

Natural behaviours are those that wild counterparts to domestic or captive animals would display in their natural environment as part of their behavioural repertoire. These behaviours have evolved over time through natural selection and are therefore connected with survival, offspring production and growth (McFarland, 1985) as cited in Duncan (1998). Behavioural observations are used as a tool to assess the welfare of animals, and many researchers propose those animals that display 'natural behaviours' in a captive environment compared to animals that do not, have good welfare (Waiblinger, Baumgartner, Kiley-Worthington, & Niebuhr, 2004). However, as Dawkins (2004) identified, not all natural behaviours are indicative of good welfare (e.g. fleeing a predator), and therefore some context and species-specific attributes must be taken into account. Performing natural behaviours does not necessarily mean that the animals in question have good welfare, in the same manner that animals that have a high level of fitness or health but cannot perform natural behaviours have good welfare (Fraser, 2008). Additionally, when considering domesticated animals, most of the species' behavioural repertoire is retained through the process of domestication; some behaviours, however, are diluted or even exacerbated (Price, 1999).

Free range production of eggs has been designed to provide hens with the opportunity, or choice, to perform more natural behaviours in an intensive farming system. Hens are usually kept in large flocks with open access indoor area that provides feed, water, nest boxes and in some cases other features such as litter or perches. Access to an outdoor range is usually provided once hens are at point of lay until end of lay. The outdoor range features vary greatly between countries, farms and flocks and there are very few standards or guidelines given to producers to assist with range design, animal and land management.

Outdoor ranges in commercial production often differ from natural environments and are not always designed with the emphasis to enable different behaviours to be performed. Hens will utilise the outdoor area more when there is some form of cover (Bright & Joret, 2012; Gilani, Knowles & Nicol, 2014; Hegelund L., Sørensen & Hermansen, 2006), but the number of hens using the range in relation to cover varies within the literature, and differences in cover type preferences are not explicit or addressed. Artificial structural elements are used where natural habitats cannot be provided or are undesirable. These designs are often based on very little information of hen preference and have varying levels of success in achieving the outset goals (e.g. encouraging greater numbers of hens to use the outdoor range or to range further from the shed) (Hegelund L., Sørensen, Kjaer & Kristensen, 2005; Nagle & Glatz, 2012; Rault, van de Wouw & Hemsworth, 2013; Zeltner & Hirt, 2003, 2008). This may be due to a lack of understanding of what additional behaviours the outdoor range would provide for domestic laying hens and what the level of motivation or choice preference hens have for certain range elements.

The Red Junglefowl is the ancestral species of domesticated chicken breeds (Collias N. E. & Collias, 1967) and therefore it is often the model for scientific studies looking at the behaviour and domestication of chickens (Dawkins, 1989; Håkansson & Jensen, 2004; Jensen, 2006). The process of domestication involves the adaptation of a species to a human environment, and the Red Junglefowl being gregarious, male dominated and existing on a diet that is easily provided by humans allowed for easy domestication of this species (Mignon-Grasteau et al., 2005).

Domestication has led to qualitative trait differences between the Red Junglefowl and domesticated chicken (Jensen, 2006). Traits related to productivity such as egg production, growth rate and weight have all been increased in the domestic chicken compared to the Red Junglefowl (Schütz et al., 2002). Behaviourally Red Junglefowl are more fearful of predators and humans (Campler, Jöngren & Jensen, 2009), and spend more energy foraging than domestic breeds of chickens (Schütz, Forkman & Jensen, 2001; Schütz & Jensen, 2001). Domestic chickens still display similar patterns of behaviour to the Red Junglefowl, and have retained the ability to adapt to their environment (Andersson, Nordin & Jensen, 2001), although they may be less adaptable than the Red Junglefowl (Lindqvist & Jensen, 2009). Therefore, it is suitable to use the Red Junglefowl to identify natural behaviours in relation to range use that could be applied to investigations of domestic chicken behaviour. For example, habitat preference and daily behavioural routines of Red Junglefowl can be used as a reference for domestic chickens in commercial settings, particularly in free range production.

In the wild, Red Junglefowl are often found in areas with dense vegetation such as bamboo forests or other tropical forests (Johnson, 1963; McBride, Parer & Foenander, 1969). Collias and collaborators conduct observational studies on Red Junglefowl in their natural environment and found that they rely on clearings, whether they are natural or formed from agricultural land management, to move throughout their territories and forage. Additionally, they are often seen on termite mounds or small fruit trees or in areas of secondary forest where there is plenty of forage from rice, bamboo or grass shoots. Burnt forests and forests with interspersed clearings allow for the Red Junglefowl to move through the area whilst remaining relatively unseen. In the wild the daily routine of the Junglefowl centres around the roost, with each bird having approximately 2.5 acres in which to roam, and consists of morning and evening foraging and resting in cover throughout the middle of the day. In a study on the behavioural time budget of captive bred semi-wild Red Junglefowl, it was found that a very large proportion of their active minutes were spent ground pecking (60%) and ground scratching (34.1%), despite being provided with feed three times daily, suggesting that foraging behaviour is very important for welfare (Dawkins, 1989).

The scientific understanding of the way free range hens use the outdoor-range is limited. The aim of this project was to determine which behaviours free range laying hens express in the outdoor range of a commercial property. We investigated preferences for distinct areas and how time of day influenced the use of each area. We hypothesised that hens would show a clear preference for different outdoor areas that more closely mimicked natural habitats of the Red Junglefowl, and that these areas would also provide for a greater diversity of natural behaviours to be performed.

3.2 Methods

3.2.1 Study site and subjects

One flock of 8000 Hy-Line brown laying hens was observed on a commercial free range layer farm during summer, with average maximum temperatures of 32.8 ± 0.30°C and average minimum temperatures of 13.46 ± 0.23°C during the time of the study.

The flock was 62 weeks of age and had access to the outdoor range from 22 weeks of age. The indoor shed was 80 m in length and 10 m wide, providing an indoor stocking density of 10 hens per square meter, with a deep litter (rice hulls) flooring system. Hens were provided with perches and nest boxes indoors, in compliance with code of practice specifications, and had access to the outdoor range via pop holes on either side of the shed; pop holes were 2.8 m wide and 50 cm tall, spaced approximately every 6 m along the shed. Hens were fed *ad libitum* and had access to the outdoor range just after sunrise and sunset from 0700h to 2100h when data collection took place.

3.2.2 Range characteristics

The outdoor range for the flock was approximately 1.6 ha, giving an outdoor stocking density of approximately 5000 hens per hectare, and contained varied vegetation and topography throughout. Distinct habitat types, hereafter referred to as 'locations', were broadly characterised based on their ground substrate and canopy cover in each range. Each location was 20 m from the indoor shed and had no obstructions in a path directly from the shed (Figure 3-1).

Four distinct location types were identified: location one (gum tree) consisted of one large *Eucalyptus* gum tree with a 30 m high canopy cover; location two (wattle trees) contained *Acacia* wattle trees that provided 1-2 m high dense canopy cover; location three (bare earth) consisted of bare sand and gravel ground with no canopy cover; and location four (saplings) consisted of self-propagated *Acacia* and *Eucalyptus* saplings with a bamboo-like dense (spacing of 10-40 cm between saplings) canopy structure.

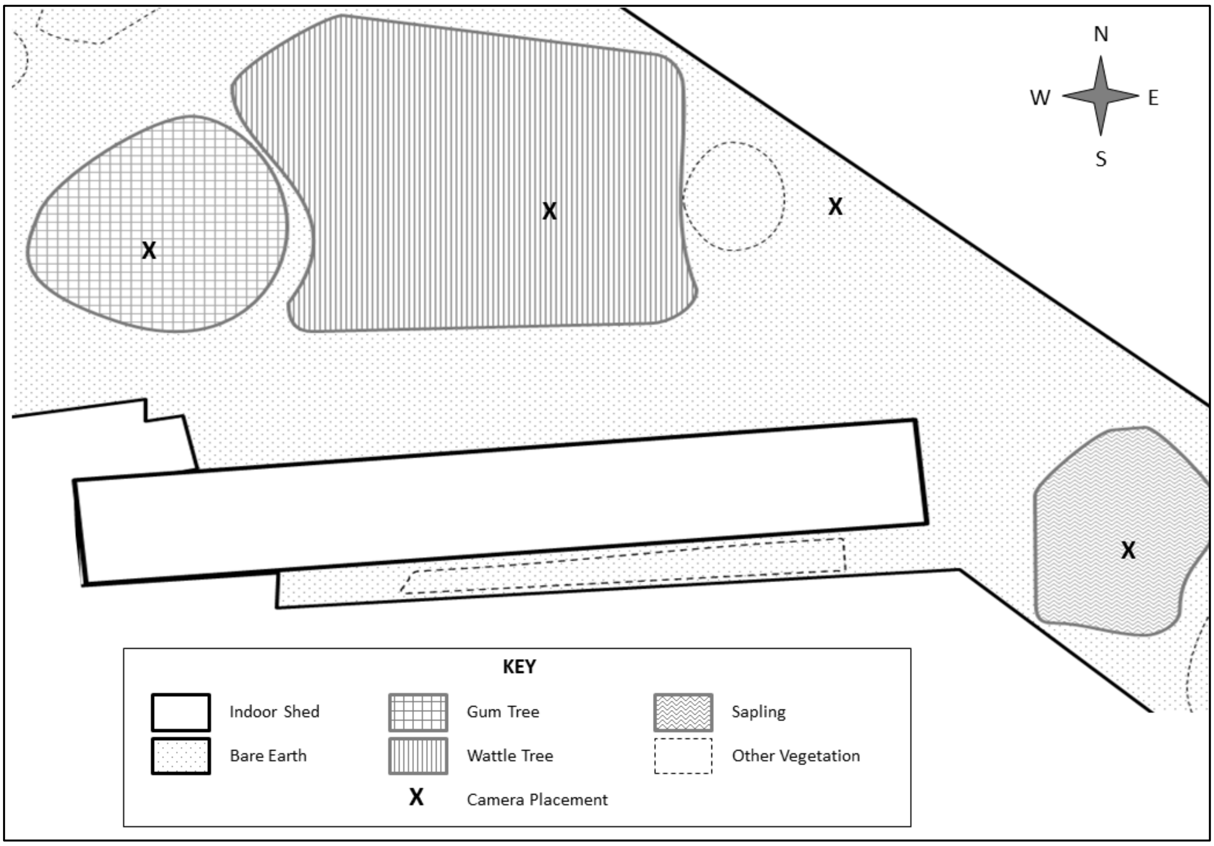


Figure 3-1 Map of the outdoor range showing where the different locations are and placement of cameras

3.2.3 Data collection

Data were collected using Scoutguard Zeroglow 10M cameras (Professional Trapping Supplies, Molendinar, QLD, Australia) that were positioned in each of the locations. The field of view (FOV) for each location was 38 m^2. Cameras captured 10s videos every 15 minutes during the hours of range access over 21 days. Data was then pooled per period of the day for analysis: 0701-1030h, 1031-1400h, 1401-1730h, 1731-2100h. This was based on a clear diurnal pattern of range use, defined as the number of hens in each area, which was standardised for equal time for each period.

One observer collected the behavioural data using a scan sampling technique, whereby the number of hens in each location was counted and the behaviour of each hen recorded according to a behavioural ethogram (Table 1) for all time points. Behaviour was recorded for all hens present in the area, hence behaviour displayed being dependent on the number of birds in that area. If the first behaviour of the hens was unable to be determined, either by obstruction of view by conspecifics or physical range features, then the hens' behaviour was recorded as 'unknown'. If the first behaviour was uncertain based on the first screen shot and the hen was not physically obstructed from view then the rest of the video (up to 10s) was played to determine the first behaviour.

Table 3-1 Ethogram for behavioural observations

Behaviour	Description
Foraging	Pecks directed at the ground or trees, scratching at ground or walking with head below midline. Also includes pecks directed in the air presumably foraging for small insects.
Preening	Grooming of plumage with beak in either sitting or standing position.
Social interaction	Any interaction, aggressive or gentle, with conspecific.
Perching	Perching in or on tree, or other structural element in the range
Vigilance behaviour	Sitting with hocks on ground, or standing, with neck outstretched, head upright and eyes open. Could be still or moving head around in alert manner.
Resting	Sitting or standing in a resting non-vigilant state, head not outstretched and eyes open or closed.
Lying	Head flat on ground or tucked under wing. Eyes open or closed. Body position flat on ground.
Dust bathing	Lying with head rubbing on ground, scratching at ground, wings open and feathers ruffled.
Locomotion	Moving at normal or fast speed (including wings flapping) to or from location/conspecific.
Comfort behaviour	Head shake, wing stretch, wing flap or crop adjustment.

3.2.4 Data Analysis

The average number of hens at each time point in each location was calculated by pooling counts for each time point within a time period within each day. Behavioural data were based on the proportion of hens performing the behaviour at each time period, which was then averaged over each day of data collection. Normality of data and homogeneity of variance residual plots were generated using the GLM function in Minitab (v17, Minitab Pty

Ltd, Sydney, NSW) statistical software and assumptions of normality were considered met for most behaviours and total number of birds. Dust bathing, comfort behaviours and 'other' behaviours did not meet assumptions of normality and were square root transformed prior to analysis, after which they met the assumption of normality. Data are presented as least square means ± standard error. All data analyses were performed using SPSS statistical software (v22, IBM Corp, Armonk, NY, USA). To test for preferences of each location type, number data were analysed with a Linear Mixed Model using the REML method, which included fixed effects of location (gum tree, wattle trees, bare earth and saplings), time period (0701-1030h, 1031-1400h, 1401-1730h, 1731-2100h) and the interaction between location and time period. Day (1-21) was included as a random factor and minimum and maximum outdoor temperatures, minimum and maximum shed temperatures, and wind speed at 0900 h and 1500 h were included as random factors nested within day. Linear Mixed Model including the same fixed and random factors as above was used to test for differences in behaviour time budgets between time points and location types for each type of behaviour. All post-hoc multiple pairwise comparison test p-values were adjusted using the False Discovery Rate (FDR) method as described in Verhoeven et al. (2005), and only corrected values are shown. The relationship between the number of unknown behaviours observed and the number of hens present was tested using Pearson correlation.

3.3 Results

A total of 31,659 observations of the behaviours of hens on the range across all locations were analysed.

3.3.1 Number of hens in each location

There was a significant interaction between location and time on the number of hens observed ($P < 0.001$; Figure 3-2). The wattle tree location had more hens during morning (0701h to 1030h) and evening (1731h to 2100h) than any other location (FDR $P = 0.03$). Both the gum tree and wattle tree locations had more hens in the midday period (1031 h to 1400h) than the bare earth and sapling locations (FDR $P \leq 0.03$), and the gum tree location had more hens in the afternoon period (1400h to 1730h) than the sapling location (FDR $P \leq 0.03$). The combined number of hens in each location dropped significantly in the afternoon and evening periods from the morning and midday periods ($P < 0.05$).

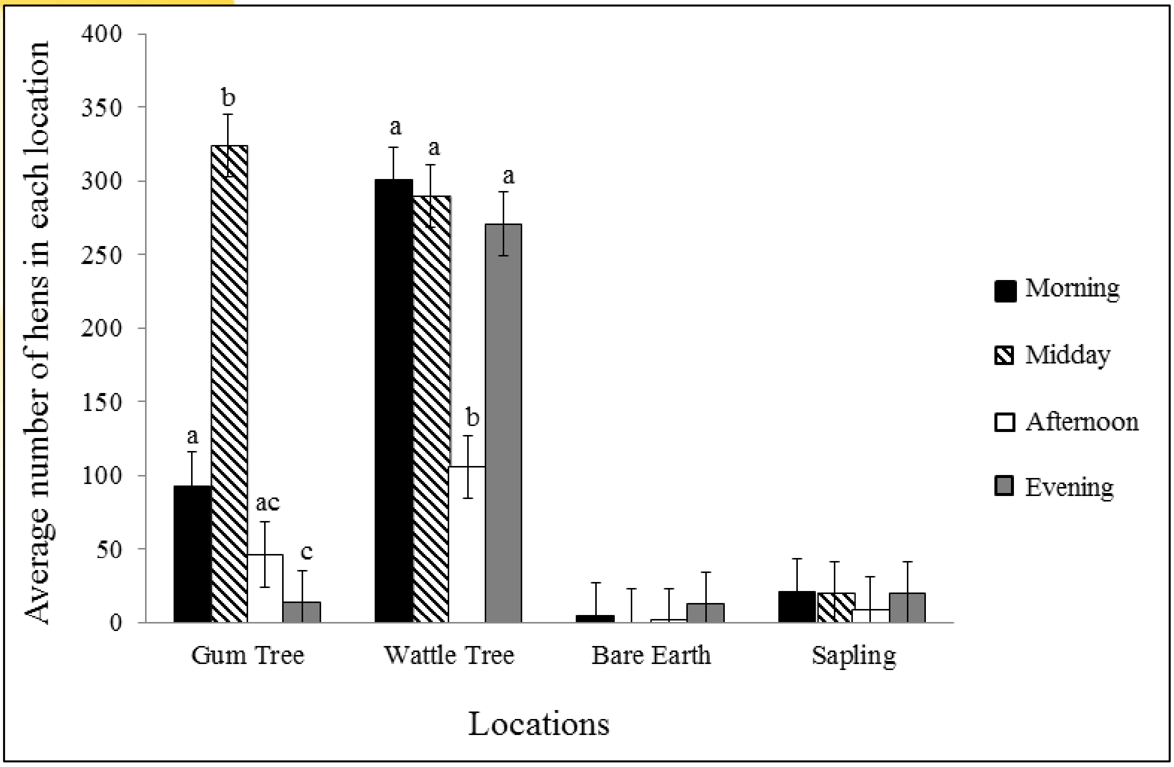


Figure 3-2 Average number of hens per time point ± SE in each location at four different time periods: morning (0701-1030h), midday (1031-1400h), afternoon (1401-1730h), and evening (1731-2100h)

Times within each location with different superscript significantly differ ($P < 0.05$) from each other.

3.3.2 Behaviours within each location

The five most common behaviours seen across all locations were foraging (33.1% ± 1.3), preening (9.4% ± 0.8), locomotion (19.3% ± 1.2), resting (7.1% ± 0.5) and vigilance (6.1% ± 0.4) – see Figure 3-3, which displays behaviour by location. There was a significant interaction between location and time for the following behaviour: foraging ($P = 0.02$), preening ($P = 0.001$), resting ($P < 0.001$), vigilance ($P = 0.02$), and social interactions (P = < 0.001).

In the morning, foraging and preening were reduced in the bare earth location compared to all other locations (FDR $P \leq 0.03$), and hens interacted with conspecifics more in the gum tree and wattle locations than in the bare earth and sapling locations during this period (FDR $P \leq 0.02$).

In the midday period, the proportion of foraging was higher in the gum tree location than both bare earth (FDR $P = 0.03$) and sapling (FDR $P = 0.02$) locations, and higher in the wattle tree compared to bare earth (FDR $P = 0.04$) locations. Preening, resting and vigilance were lower in the bare earth location compared to all other locations during the midday period (FDR $P \leq 0.03$). Hens interacted with conspecifics more in the gum tree and wattle locations than in the bare earth and sapling locations during the midday period (FDR $P \leq 0.02$), and dust bathing was more prevalent in the wattle tree location in this time period than any other location (FDR $P \leq 0.01$).

The proportion of foraging, preening, vigilance and hen interaction behaviours in the afternoon period was lower in the bare earth location compared to all other locations (FDR $P \leq 0.03$). During this period, significantly more hens performed preening behaviour in the sapling location than the wattle tree location (FDR P = 0.01). Resting increased during the afternoon in the wattle tree location compared to any other location (FDR P ≤ 0.02), and was more common in the gum tree and sapling locations than the bare earth (FDR P = 0.02) during this time. Dust bathing was more prevalent in the wattle tree location in this time period than any other location (FDR P ≤ 0.01).

During the evening, the gum tree and sapling locations had higher foraging than the wattle (FDR P = 0.03 and FDR P = 0.01, respectively) and bare earth locations (FDR P = 0.04 and FDR P = 0.01 respectively). Dust bathing and interactions were more prevalent in the wattle tree location during the evening compared to all other locations (FDR P ≤ 0.03).

Expression of behaviours within each location changed throughout the day (Figure 3-3), with foraging and locomotion being more common in the morning and evening periods within most locations, and resting and preening behaviours being more common throughout the midday and afternoon periods. Vigilance behaviours remained fairly consistent throughout the day.

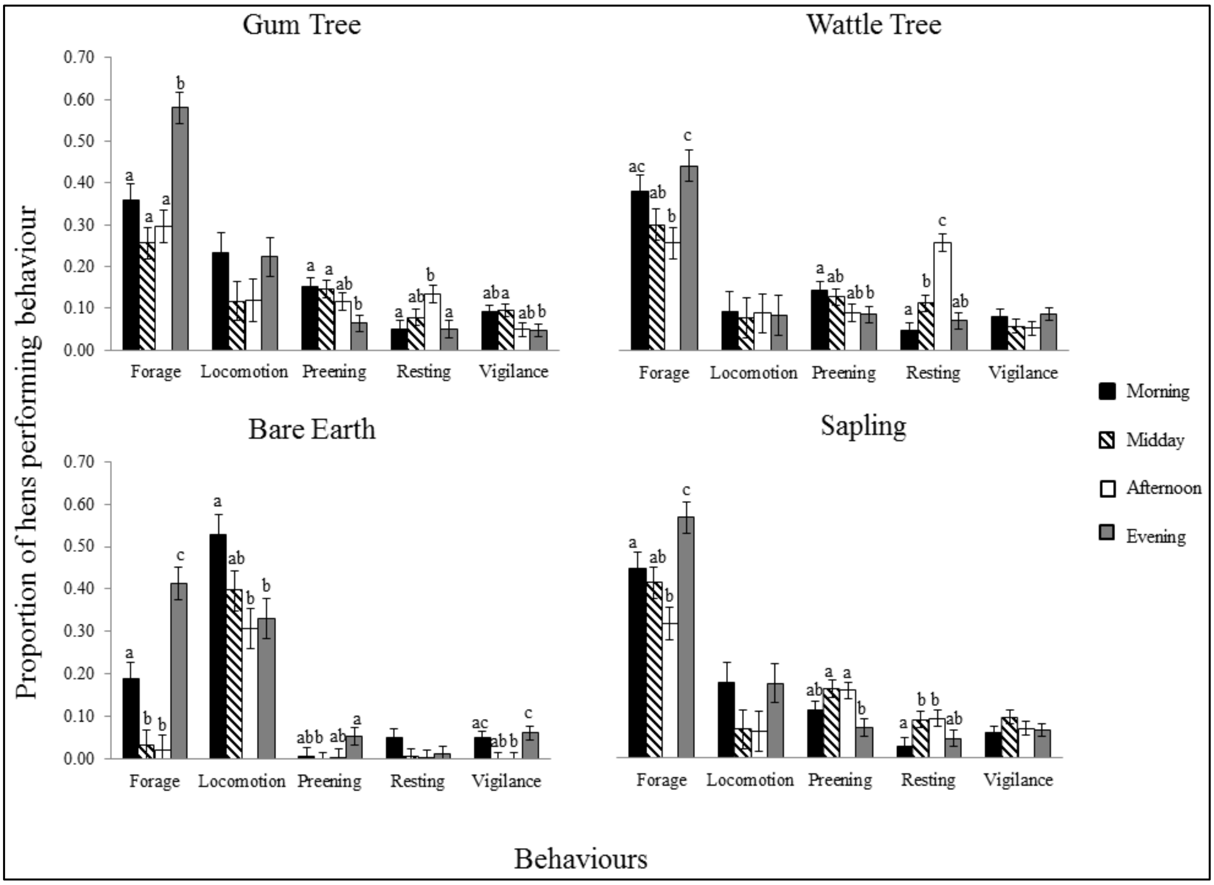


Figure 3-3 Daily time budget of hens in each location

Data shown are average proportion (± SE) of hens per time point performing each behaviour at the four different time periods: morning (0701-1030h), midday (1031-1400h), afternoon (1401-1730h), and evening (1731-2100h).
Behaviours at each location with different superscripts differ significantly (P < 0.05) over time.

Comfort behaviours were influenced by location (P < 0.001) but not by time (P = 0.31) or the interaction between location and time (P = 0.10). Comfort behaviours were more prevalent in the gum tree and wattle tree locations than the bare earth (FDR P = 0.02 and FDR P = 0.04 respectively) and sapling (FDR P = 0.03 and FDR P = 0.05, respectively) locations.

The number of 'unknown' behaviours was positively correlated with the number of hens in each observation ($r = 0.55$, $n = 329$, $P < 0.001$).

Table 3-2 LSM ± SE of all other behaviours recorded in each of the study locations at four different time points – morning (0701-1030h), midday (1031-1400h), afternoon (1401-1730h), and evening (1731-2100h)

Behaviour	Location	Time Period			
		Morning	*Midday*	*Afternoon*	*Evening*
Interaction	*Gum Tree*	0.02 ± 0.004^{a}	0.04 ± 0.004^{b}	0.02 ± 0.004^{a}	0.00 ± 0.004^{c}
	Wattle	0.03 ± 0.004^{a}	0.03 ± 0.004^{a}	0.01 ± 0.004^{b}	0.04 ± 0.004^{a}
	Bare Earth	0.00 ± 0.004	0.00 ± 0.004	0.00 ± 0.004	0.00 ± 0.004
	Sapling	0.00 ± 0.004^{a}	0.01 ± 0.004^{ab}	0.02 ± 0.004^{b}	0.00 ± 0.004^{a}
Dust Bathing*	*Gum Tree*	0.00 ± 0.000^{ab}	0.00 ± 0.000^{ab}	0.00 ± 0.000^{a}	0.00 ± 0.000^{b}
	Wattle	0.01 ± 0.000^{a}	0.07 ± 0.000^{b}	0.06 ± 0.000^{b}	0.01 ± 0.000^{a}
	Bare Earth	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.001	0.00 ± 0.000
	Sapling	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Comfort*	*Gum Tree*	0.01 ± 0.000^{ab}	0.02 ± 0.000^{a}	0.00 ± 0.000^{b}	0.00 ± 0.000^{b}
	Wattle	0.02 ± 0.000	0.01 ±	0.01 ±	0.01 ±

			0.000	0.000	0.000
	Bare Earth	0.00 ± 0.000	0.00 ± 0.001	0.00 ± 0.001	0.00 ± 0.000
	Sapling	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Unknown	*Gum Tree*	0.07 ± 0.020^{a}	0.23 ± 0.019^{b}	0.24 ± 0.020^{b}	0.01 ± 0.019^{a}
	Wattle	0.18 ± 0.020^{a}	0.18 ± 0.019^{a}	0.12 ± 0.019^{b}	0.15 ± 0.019^{ab}
	Bare Earth	0.00 ± 0.020	0.00 ± 0.019	0.00 ± 0.019	0.01 ± 0.019
	Sapling	0.10 ± 0.020	0.08 ± 0.019	0.06 ± 0.019	0.04 ± 0.019

LSM of behaviours with superscripts a-c differ significantly across time within each location.
* indicates behaviours that did not meet assumption of normality, raw means are displayed.

3.4 Discussion

3.4.1 Habitat preference

Laying hens exhibit preferences for different habitat types in the outdoor range, and this preference also varies throughout the day. The wattle tree location with dense, low canopy cover attracted more hens throughout most of the day than any other habitat type. Hen numbers in this location did drop in the afternoon period, but remained higher than all other locations, suggesting that the hens were utilising another area of the range or the indoor shed during the afternoon. The gum tree location, providing high canopy cover, was also highly attractive at midday, but became no more attractive to hens than areas of bare earth during other times of the day. The sapling location with low dense canopy cover, but less space between vegetation was not preferred over the bare earth location at any time of the day and was therefore no more attractive.

Perhaps the most dramatically diurnally influenced location was the gum tree location. In this area hens were seen in greatest numbers during the midday period when the sun was highest and the shadow cast by the gum tree was directly overhead. Hens were still more likely to be present in this area during the early morning and afternoon periods than the bare earth area, but this was not the case of the evening period. During the evening the shadow cast by the canopy cover was far from the tree itself, which possibly explained the lack of hens in this time period as shade is highly influential for attracting hens to an area

(Zeltner & Hirt, 2008). Investigating whether shade attracts hens when there is no adjacent structure (tree trunk) would be the next logical step in this investigation, as it is unknown whether similar numbers of hens were using the shade as it moved throughout the day.

The wattle tree area was, at least one of, the most attractive locations at all time periods despite there being a drop in numbers in the afternoon. This drop in numbers may be related to hens moving indoors to feed or rest indoors, however, we did not record the behaviour or number of hens indoors and thus cannot determine the cause of this decline. This is the only location that was specifically put in place to provide shelter for the hens, and was therefore very evenly distributed and had highly consistent canopy cover. The trunks of the wattle trees were small and spaced approximately five metres apart and often branches were just low enough for hens to jump on or jump to peck at leaves. Spacing of the trunks and the ability to interact more readily with the environment more closely mimics what was seen in the Red Junglefowl natural habitats, excluding the presence of an understory. This area allowed for more behaviours such as dust bathing and sun bathing in patches of sunlight, which would not be suitable with a thick understory, and which are important behavioural priorities in the laying hen (Weeks & Nicol, 2006). An additional explanation of preference in this area could be that within this location the producer had placed sprinklers that would mist birds when temperatures rose to extreme levels. The hens may have become acclimatised to prefer this area during hot weather, which may have continued throughout this study despite sprinklers not being used during this study.

It was expected to find that the bare earth location was not attractive for laying hens as the Red Junglefowl natural habitat consists of areas containing a lot of dense and patchy vegetation (Collias N. E. & Saichuae, 1967; Johnson, 1963). Similar habitat preferences in the domestic chicken compared to the Red Junglefowl are expected to be retained to some degree, whether it is of greater or lesser influence, throughout the domestication process (Price, 1984). Additionally, production systems where the outdoor range is enriched prove to encourage greater numbers of hens outdoors than those that provide no environmental complexity (Bestman, Wagenaar & Nauta, 2002; Bright & Joret, 2012; Gilani et al., 2014). Hens are likely to feel exposed to predators and the elements in these areas, as demonstrated by their lack of stationary behaviours, and the primarily locomotory and foraging behaviours. The speed at which hens move throughout these areas and the number of directional steps while foraging would be interesting to investigate in relation to proximity to cover, as perhaps if this location was in closer proximity to the other resources we would have seen more hens utilising the area similar to the way Red Junglefowl use clearings in forests for foraging and socialising (Collias & Collias, 1967; Collias & Saichuae, 1967; Johnson, 1963).

The finding that the sapling location was no more attractive than the bare earth location was unexpected, however, as it seemed to be more closely related to the habitat type of the Red Junglefowl. One possible explanation for this is that the saplings were too densely grouped, which did not allow for easy movements of the hens, as Red Junglefowl prefer patchy environments with both clearings and cover (Collias & Collias, 1967; Johnson, 1963), and perhaps this did not meet this requirement fully. Additionally, this location was the only one oriented on the east end of the shed (all others being located on the north side), and perhaps there were unknown differences in the number of hens accessing each orientation of the shed.

Determining exactly which factors of each location are causing differences in numbers throughout the day is difficult, particularly with the scan sampling method used. For example, it is difficult to determine if orientation, density or ground cover, or an interaction of all of these factors and more caused the sapling location to be less attractive to hens. However, what is clear is that some locations are highly preferred and that this preference for laying hens can change throughout the day. This could be related to sun orientation, as indicated in the gum tree location, or different behavioural needs throughout the day, where some locations are better suited than others to meet these needs. It is also not clear how the seasons might affect laying hens' preferences for certain areas; this study was performed in summer only and temperatures were relatively high throughout. Furthermore, the indoor shed was naturally ventilated, hence with no cooling system. Seasonal changes can affect laying hen behaviour (Gilani et al., 2014).

3.4.2 Behavioural time budgets

Overall, hens within the most highly preferred locations (wattle tree and gum tree) displayed a greater behavioural repertoire than in the least preferred areas. However, there was also a clear distinction between the two least preferred locations where hens using the sapling areas performed more varied behaviours than in the bare earth location. Almost all behaviours recorded were also influenced not only by location and/or time but the interaction between the two.

Foraging for all locations except the bare earth was the most performed behaviour overall, and each location showed an increase in the performance of foraging in either the morning or evening periods. This result is similar to those found by Larsen and Rault (2014) where foraging in and around a natural shrub structure increased in the evening from all other times, and is closely related to the behavioural time budget of the Red Junglefowl. Interestingly, foraging in the evening was most prevalent in one of the least preferred locations (saplings), which could possibly be explained by the presence of more leaf litter on the ground than in any other area. Chickens are more likely to spend time foraging for food; when presented with the choice to obtain an 'easy' meal or a meal in which they have to 'work' to gain, hens more frequently chose to work suggesting a behavioural need to perform the act of foraging within the litter (Hughes & Duncan, 1972). Conversely, this foraging behaviour has also been shown to be decreased in the more domesticated Hy-Line strains of chicken when compared to junglefowl or bantam strains (Schütz & Jensen, 2001). Foraging is considered to be a behavioural priority for laying hens (Weeks & Nicol, 2006) and, therefore, providing varied and sheltered environments in the outdoor range may be the simplest way to allow for this natural behaviour.

Locomotory behaviours increased in the morning and evening periods in both the gum tree and sapling locations, but had no change in the wattle tree location and decreased over the day in the bare earth location. It is likely that the hens are using the morning and afternoon periods as transition periods from the indoor shed to range and vice versa, or perhaps to locations where foraging or other resources are better, which would be consistent with behaviours seen in the Red Junglefowl in the wild (Collias & Collias, 1996). As the wattle tree area is the most popular during the evening period, this could explain why fewer hens are using this area for locomotion, which is also consistently lower throughout the day. Hens in the wattle tree area may be spending more time in this location per visit than in any other location, which could lead to fewer transitions and less locomotion. Decreased locomotion in the bare earth area could be related to an increase in foraging behaviour, which included a lot of movement already, and a decrease in bright sunlight, allowing hens to spend longer in the area without being subject to extreme temperatures.

Resting occurred most commonly in the wattle tree location and was expectedly uncommon in the bare earth area, suggesting that the presence of lower canopy cover is important for resting to occur. Resting also increased in the afternoon in the wattle tree location which is similar to behaviours seen in Red Junglefowl and other flocks of laying hens (Larsen & Rault, 2014). Preening, like resting, was uncommon within the bare earth location, but did show more diurnal variation in the sapling location where there was an increase in midday and afternoon periods.

Vigilance in all locations was relatively low, especially in the bare earth area where hens did not linger and were perhaps being vigilant while walking or performing other behaviours. Vigilance in animals is generally decreased when there are larger group sizes (Roberts, 1996), which may be reflective in this case of the entire flock, rather than within the smaller locations. Additionally the presence of Maremma guard dogs could have reduced the vigilance behaviour in hens overall, as it is not uncommon for prey animals to use sentinels of other species to be alerted to predators (Rainey et al., 2004; Zuberbühler, 2000).

Overall comfort, social interaction and dust bathing behaviours were more prevalent in the wattle tree location, however, the number of hens performing these was relatively small. These are not behaviours that are performed intensively, so despite the small frequency of occurrence, it does not mean they are insignificant for the overall well-being of the laying hens. When hens that have had previous access to dust bathing material are denied the ability to dust bathe they become stressed, as indicated by an increase in the hormone corticosterone (Vestergaard, Skadhauge & Lawson, 1997), and therefore can have reduced welfare. Dust bathing is also important for plumage condition and parasite control (Van Liere, 1992). Comfort behaviours could relate to anything such as wing stretching or flapping, crop adjustment and ruffling feathers, many of which are denied to hens in cage systems and relate to the ‘natural’ behaviours argument for free range production. Additionally, the inability to perform comfort behaviours for a period of time can lead to a significant increase in the performance of those behaviours, or a rebound effect, suggesting that the hens’ motivation to perform that behaviour may be increasing over the time of restriction (Nicol, 1987).

3.5 Conclusions

Laying hens showed preference for different locations within the outdoor range, and this preference changes throughout the day as did the behavioural time budget of hens in each of those areas. Highly preferred areas allow hens to demonstrate a greater behavioural repertoire, however no single location provides for everything at all times of the day. Low dense canopy cover and shade are major principles relating to the attractiveness of locations and may be instrumental in encouraging hens to explore further within the range; however, varied and complex environments throughout the entire range may be most effective as an overall range design.

4 Experiment 3: Ranging behaviour of free range laying hens after leaving the pop hole: social and environmental factors

The results of this study were presented at the International Society of Applied Ethology 2014 Regional Meeting, Sydney, Australia, and delivered as a poster presentation.

4.1 Abstract

This project investigated the behaviour of free range laying hens after exiting a pop hole, with a focus on external factors that drew hens into the outdoor range. These factors included environmental and social characteristics.

A commercial flock of 8000 Hy-line brown laying hens with access to a range was studied over 14 days in the winter season of Victoria. Behaviour was recorded using four video cameras linked to a digital recording system. Videos were analysed by selecting 5 focal hens to exit a pop hole from 0830h, 1130h, 1430h and 1730h. Observations were made sequentially for 5 min or until the focal hen disappeared from the field of view of ~300 m^2. The main measures recorded included the estimated distance travelled, number of stops, duration of on-screen visibility, destination, whether it joined a group and the group size joined.

Average maximum temperature was 11.6 ± 0.9ºC with cloudy conditions. In total, 258 hens were observed, covering an average distance of 6.4 ± 0.2 m with 1.2 ± 0.1 stops. In terms of destination, 52% of hens stayed within the pop hole area, 1 m alongside the shed whereas 25%, 9% and 14% of the hens ended up in bare earth area (1-9 m), tree coverage (>9 m from the shed) or went back into a pop hole, respectively. Only 28% joined a group of 4.9 ± 0.6 hens, 1.8 ± 0.3 m from the shed. The distance travelled differed according to the destination and the duration of the observation (both $P < 0.01$), but not group size or group distance. Time of day had an effect on the destination ($P < 0.05$).

However, behavioural observations through camera recording in the outdoor range proved very challenging, as only 8% of hens stayed within the field of view for 5 min, reflecting the difficulty of empirical observation on individual hens on a commercial free range farm. Alternative methods for behavioural observations in free range commercial flocks are required, but it is an inherently challenging environment due to the large space to cover and dynamic movements of hens between locations. Furthermore, this study was conducted in the winter season, which may affect range use.

4.2 Introduction

4.2.1 Social factors

Little is known about the influence of social factors on the willingness of hens to access the range and to distribute over the range area, in addition to the factors that may motivate them to explore certain areas more than others. Although it is believed that laying hens prefer to cluster in groups, the degree to which their environment affects social group dynamics is relatively unknown (Collins et al., 2011).

The overall consensus of group behaviour and spatial distribution among laying hens is that birds will adjust their inter-individual distances according to repulsive and attractive forces. Repulsive forces are circumstances in which birds might distance themselves from one another (e.g. competition for resources), while attractive forces are those that encourage birds to be closer to each other (e.g. reduced predation risk facilitated by protection from a group) (Keeling, 1994; Asher et al., 2013; Leone et al., 2007; Leone & Estevez, 2008; Estevez et al., 2007). It is therefore believed that hens distribute themselves far enough from conspecifics to avoid competition for resources and close enough to benefit from the protection that is offered by a group (Estevez et al., 2007).

Studies have suggested that limitations to the movement of poultry are the result of obstacles in the form of conspecifics in the path of movement (Newberry & Hall, 1990; Leone & Estevez, 2008). For instance, in broilers, some individuals can act as physical barriers when another individual is moving from one location to another (Newberry & Hall, 1990). However, most studies have been conducted indoors.

The clustering of chickens is another important area of research (Asher et al., 2013; Collins et al., 2011; Febrer et al., 2006), with the question of whether hens move independently as a result of their own motivations or in a social manner, influenced by the movements of their conspecifics. It is believed that hens prefer to cluster rather than distribute themselves within an open space (Collins et al., 2011). Similarly for broilers, it appears that birds are attracted to one another even while space is locally available to them (Febrer et al., 2006). However, clustering can have detrimental consequences for hen welfare due to the increased risk of injuries and spread of diseases or parasites. It is therefore useful to understand what the importance of conspecifics is to the individual hen out on the range, in order to contribute to housing system recommendations. Asher et al. (2013) have distinguished between homogenous and heterogeneous attraction in animals. Homogenous attraction refers to group cohesion whereby individuals contribute to group movement and decision-making. Heterogeneous attraction can refer to resource-attraction, whereby individuals may leave the group in favour of minimising competition for resources, and heterogeneous social attraction, whereby dominant members of a group ‘lead’ others and are followed by the majority. These types of attractions offer possibilities as to what may influence birds to join a group or not.

Joining a group can facilitate protection for an individual, or it can be disadvantageous when foraging for resources. However, these effects are likely to be variable depending on group size. In general, the view is that in very large groups, hens are likely not able to recognise each other well enough to maintain a hierarchical system, and therefore social tolerance prevails (Estevez et al., 2007). Studies have indeed found a reduction in aggression with incremental increases of group size (Nicol et al., 1999; Estevez et al., 1997). This may explain the occurrence of clustering, whereby protection becomes more important than competing for resources that can lead to aggressive encounters. However, the effect of grouping between hens in free range commercial settings is not extensively studied. Though, it is clear that the presence of other birds can have an impact on an individual’s decision-making and behaviour, which may in turn affect its use of space.

4.2.2 Environmental factors

The environment may be an important factor contributing to spatial use by laying hens. Barren, open environments are often thought to result in poor use of an outdoor range (Nagle & Glatz, 2012), while enriched environments have been found to encourage utilisation and foraging (Nicol et al., 2003; Nagle & Glatz, 2012). There is often a need to draw birds out of and away from the shed and improve ranging behaviour in laying hens (Rault et al., 2013).

There is a strong association between the percentage of birds out on the range and the availability of cover (Nicol et al., 2003; Hegelund et al., 2005). A recent study found that birds were attracted to areas further from the house if cover was provided and if the amount of artificial enrichments was increased on the range (Gilani et al., 2014).

Outdoor range with adequate environmental enrichment could reduce undesirable crowding, encourage exploration and dispersion and draw hens away from the shed area (e.g. Rault et al., 2013; Hegelund et al., 2005). A recent paper (Cronin et al., 2016) also suggests that hens, at least initially, moved away from the shed (i.e. and pop holes) if their view of the range allowed them to see where they were going.

4.2.3 Pop holes

Little is known in the scientific literature about the behaviours or motivations of hens after leaving the pop hole into the range. Hegelund et al. (2006) reported that the majority of hens that were already outside stayed relatively close to the shed. However, some farm owners had been successful in attracting the birds away from the shed thereby reducing the proportion of hens close to the shed (Hegelund et al., 2006).

Pop hole size and number are often cited as possible impediments for range access. In terms of pop hole length, in a study on 33 UK commercial flocks, Gilani et al. (2014) reported that use of the range increased as pop hole length per bird increased, studying a variation between 0.07 to 1.88 cm of pop hole per hen (the Australian Model Code of Practice recommends 500 birds per m, equivalent to 0.20 cm per hen). Unfortunately, Gilani et al. (2014) did not report their raw data and therefore it can only be said that there is a positive relationship between pop hole length per bird and use of the range in the scale of 0.07 to 1.88 cm per hen, without being able to determine a critical threshold. Keeling et al. (1988) and Harlander-Matauschek et al. (2006) in experimental settings found no influence of pop hole size on range use, studying a variation between 0.23 and 1.17 cm per hen for the latter study. In terms of pop hole number, Gilani et al. (2014) did not report a significant effect of the number of pop holes on range access.

4.3 Aims and hypotheses

The study aimed to analyse the behaviour of laying hens after exiting the pop hole into the outdoor range, and identify social and environmental factors that influence the movement and distribution of hens in the range.

We hypothesised that hens would stay close to the shed. We also hypothesised that hens that ventured out onto the bare earth would not spend a significant amount of time in that area but rather use it as a transition area to the tree coverage area. We also hypothesised that hens would join a group after leaving the pop hole, given their disposition to cluster and seek protection.

4.4 Materials and methods

4.4.1 Data collection

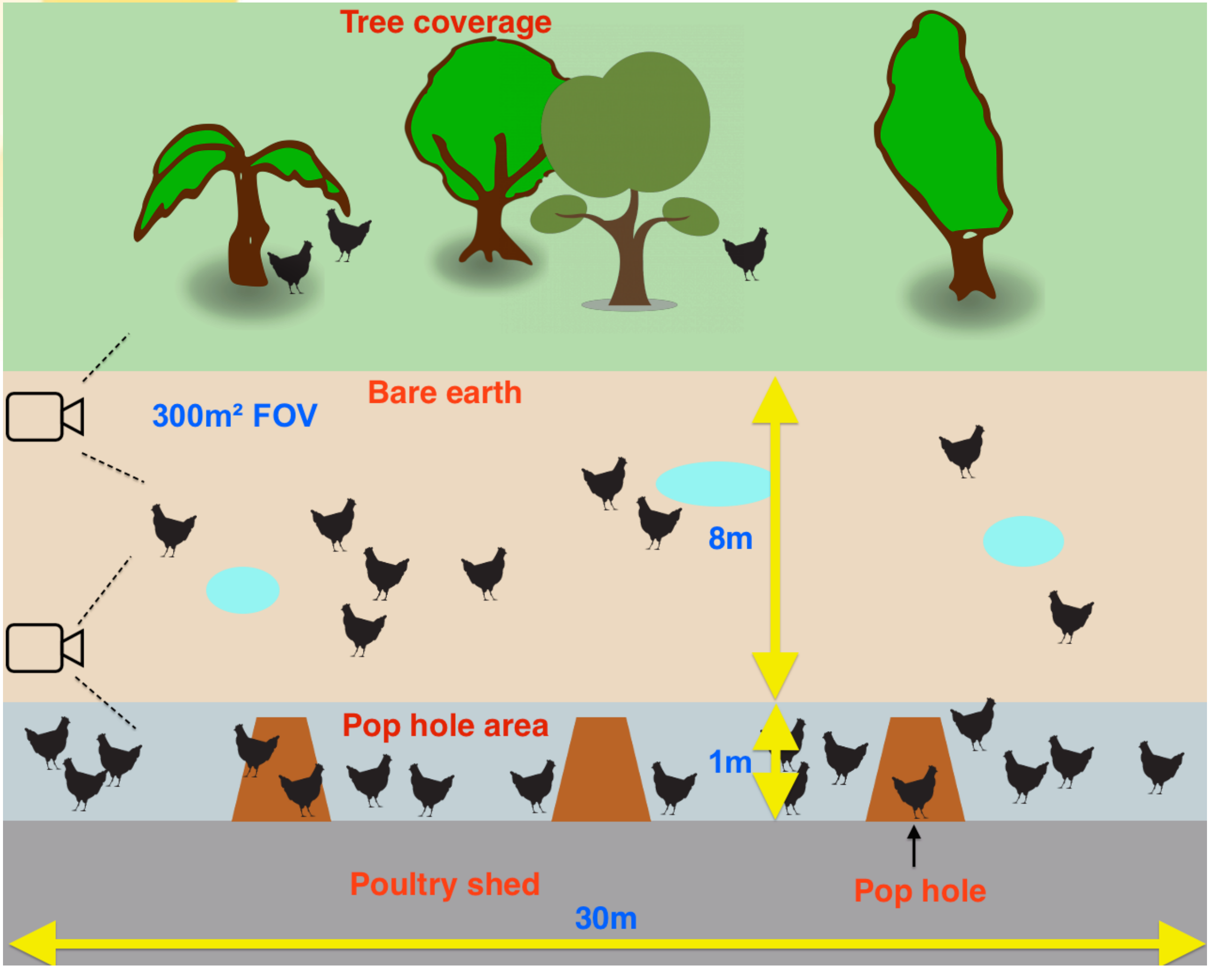


Figure 4-1 Schematic range layout

A flock of 8000, 60-week-old Hy-Line Brown laying hens was studied on one commercial farm. The indoor shed was 80 × 10 m (l × w), providing an indoor stocking density of 10 hens per square metre, with a deep litter (rice hulls) flooring system. Hens were provided with perches and nest boxes indoors. The flock had been given access to the range for approximately 40 weeks prior to the beginning of the study. The range comprised a barren earth area, which extended from the shed 8 m into the range, and tree cover further than 8 m from the shed (Figure 4-1). Hens had access to the range via pop holes on either side of the shed, pop holes were 90 cm wide, 45 cm high and 70 cm off the ground with ramp access to the range. The pop holes were placed approximately 6.5 m apart all down the shed.

Three main areas of the range were observed: the pop hole area, bare earth area, and tree coverage (Figure 4-2). A fourth location 'pop' was included to signify when birds went back into the pop hole rather than remained on the range.

Two cameras were set up at the end of the range with an observable distance of 30 m parallel to the shed. The camera closer to the shed recorded a view of the pop holes and bare earth area, while the second camera recorded bare earth and the beginning of vegetative cover for the birds. The field of view of the cameras covered approximately

300 m². There was a 1 m overlap of view between the two cameras, so as to locate and keep track of a bird that may have transitioned from one camera view to the other. Due to necessary compromise in camera placement, it was difficult to see all pop holes along the shed. Therefore, only one pop hole was chosen to record from, the closest visible from the camera, which was the last pop hole located on the end of the shed (Figure 4-2). Videos were recorded continuously from 0600h to 1800h over 14 days.

Measures taken included social factors: whether or not a bird joined a group, group size, group distance from the shed; and environmental factors: zones occupied, distance travelled, number of stops (Table 4-1). Weather conditions were recorded: minimum and maximum temperature (°C), and rainfall (mm) for each day using Bureau of Meteorology data. Weather conditions were also described according to what was seen in video footage during observation.

An unforeseen event occurred whereby a number of puddles formed within the bare earth area during and after rainy conditions. Because birds had a tendency to drink from these puddles, or they could be attracted to this resource in that particular area, we recorded days as puddle or dry range.

Figure 4-2 Video still of pop hole area (red zone), bare earth (green zone), and selected pop hole (circled)

Table 4-1 Ethogram used to record bird activity after leaving the pop hole

Variable	Description
On-screen duration (sec)	The time at which a bird was no longer viewable due to reaching the maximum observation time, proceeding off-screen, or becoming hidden by a pop hole or by other birds.
Total distance travelled	An estimation of this distance was determined by using a scaled grid placed over the screen. The focal bird's movements were then tracked and its distance approximated in metres.
Group distance from the shed	Approximation of a group's distance from the shed in metres.
Destination	Location of the bird at the time that it was no longer viewable, whether it ventured off-screen, got lost in a cluster of birds or went beneath the ramp of a pop hole. There were one of four possible destinations: 'Pop hole area', 'Bare earth', 'Tree coverage', or 'Pop', whereby a bird re-entered a pop hole.
Join group	Joined a group of at least two other hens. Hens were required to be within at least 1 m of each other to be classified as a 'group'. Passing through a group did not count, and birds that formed a group around the focal bird did not count.
Stops	Number of times a bird was immobile for at least 5 seconds. A new stop was recorded when the focal bird walked to an area at least 2 m away from its last stop.
Group size	If a bird joined a group, the number of hens in that group.

Time sampling methods were used to observe a total of five individual birds (to avoid observing the same bird over time) at 0830h, 1130h, 1430h and 1730h, totaling approximately 20 focal birds per day. Birds were selected according to their emergence from the pop hole. The focal bird was selected based on the first bird to come out of the pop hole closest to the camera at the start of each time period at 5-minute intervals. The focal bird was then observed for 5 minutes, or for as long as it was on screen. If a bird was on screen for 5 minutes, the next bird could not be chosen from the overlapping 5-minute mark. If the focal bird disappeared from the field of view, the last location was recorded as the destination.

4.4.2 Statistical analyses

Minitab software was used to conduct all statistical analyses. Chi-squared tests for association were used to find effects between categorical data such as destination and time periods, and nominal data such as whether hens joined a group, and days with puddles and days without puddles. General linear models were used for continuous variables such as total distance travelled and duration of observation, with the fixed effects of number of stops, group size nested within joining a group, time on screen, and final destination; and day as a random effect. Testing quantitative discrete variables such as the number of stops and group size against continuous variables required Spearman rank order and linear regression tests to be performed, respectively. A binary logistic regression was conducted between whether hens joined a group and the duration of observation. A Kruskal-Wallis test was performed for group size and destination. Pearson correlations were used between distance travelled and duration, maximum, minimum temperature and rainfall.

4.5 Results

4.5.1 Environmental effects

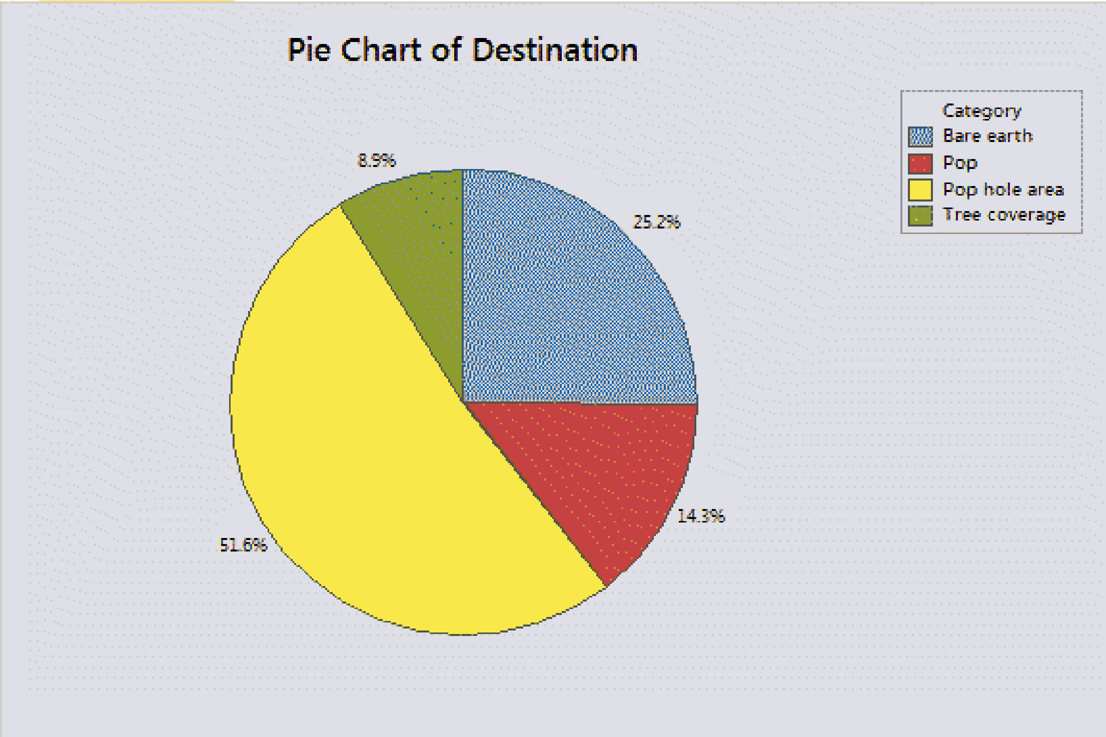


Figure 4-3 Destination at the end of observation

In terms of destination, the majority of hens remained within the pop hole area near the shed before they ventured off-screen, followed by bare earth, re-entering a pop hole, and finally tree coverage (Figure 4-3).

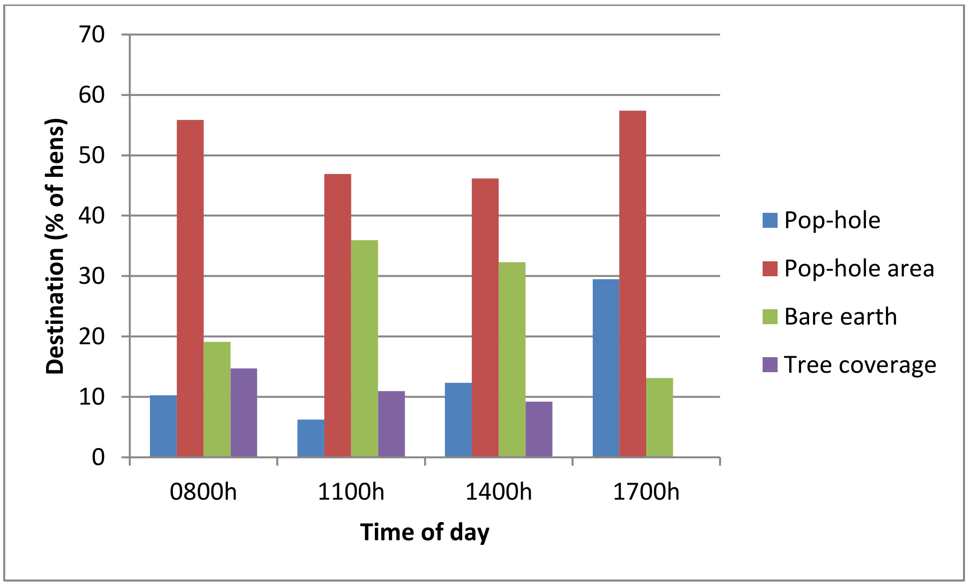


Figure 4-4 Destination according to time of day

Destination also varied according to time of day ($P < 0.05$; Figure 4-4). Half of the hens ended up in the pop hole area, but ventured more into the bare earth area at midday. Few went to the tree coverage area, and re-entering the pop hole was much more common in the evening.

Given that the various destinations were at different distances from the shed, total distance travelled and the duration of observation varied according to destination (both $P < 0.0001$), with the exception of returning into pop hole, which was higher than when hens stayed in the pop hole area (Figures 4-5 and 4-6).

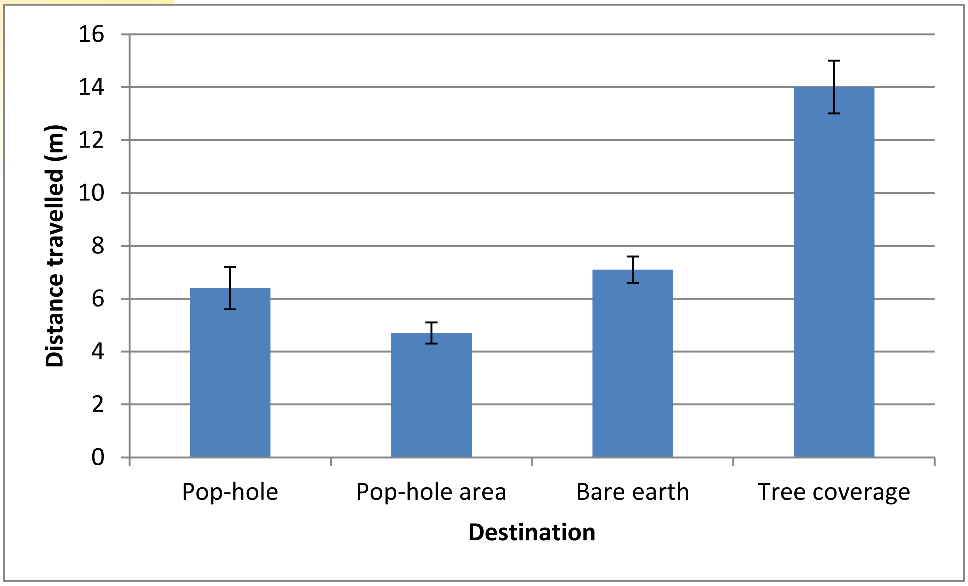


Figure 4-5 Distance travelled according to destination

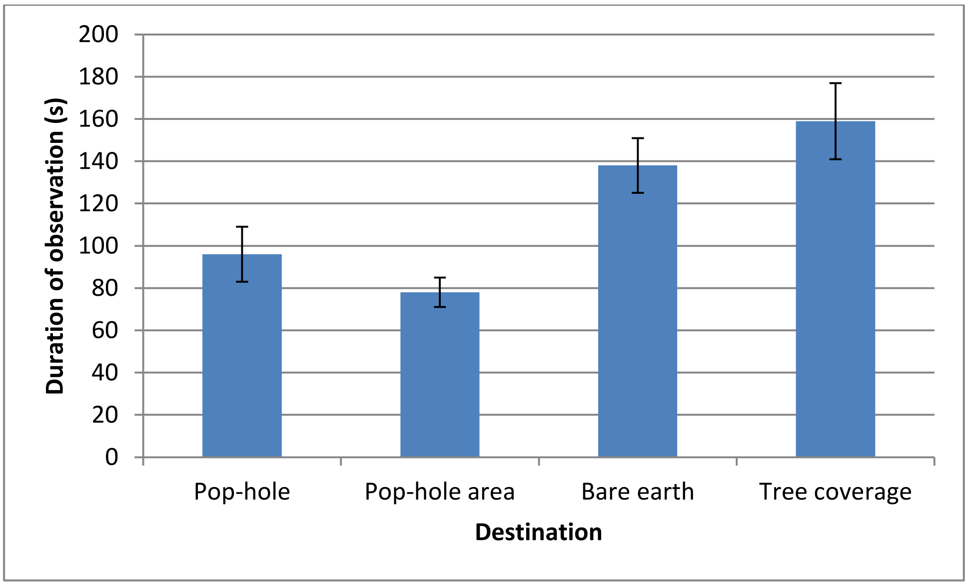


Figure 4-6 Duration of observation according to destination

Total distance travelled was positively correlated with the number of stops that were taken by a bird ($r = 0.459$, $p < 0.0001$, Figure 4-7).

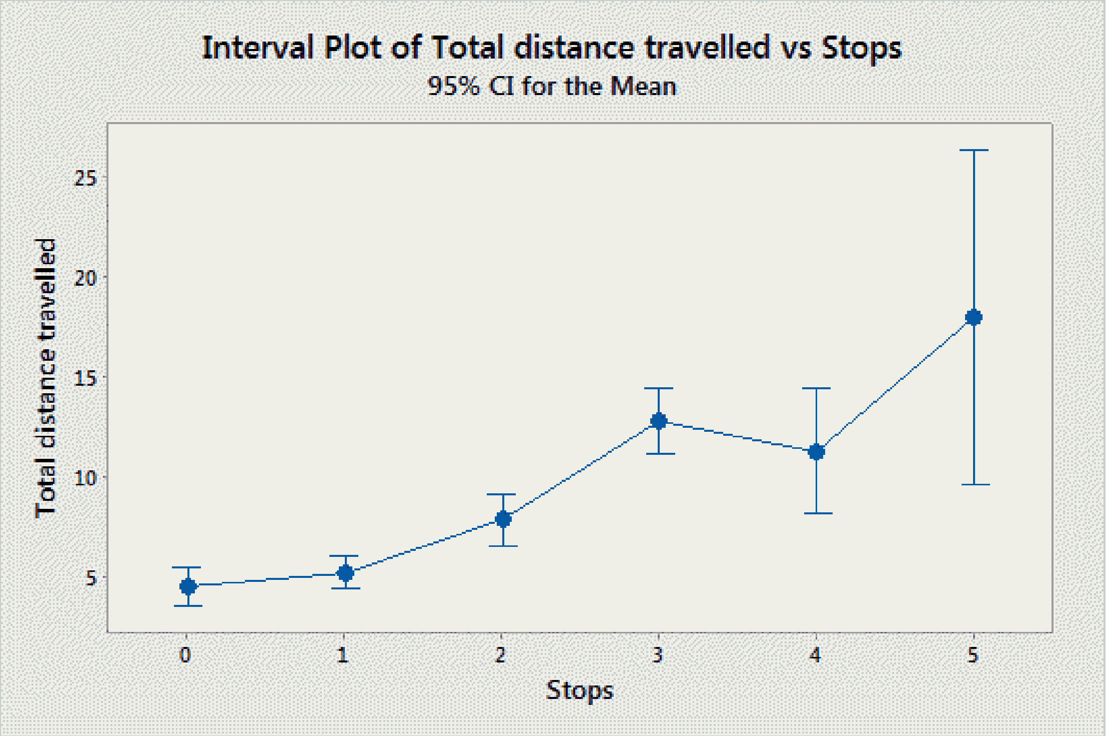


Figure 4-7 Distance travelled according to the number of stops

Distance travelled also differed according to time period (P < 0.05, Figure 4-8), with greater distance travelled in the morning.

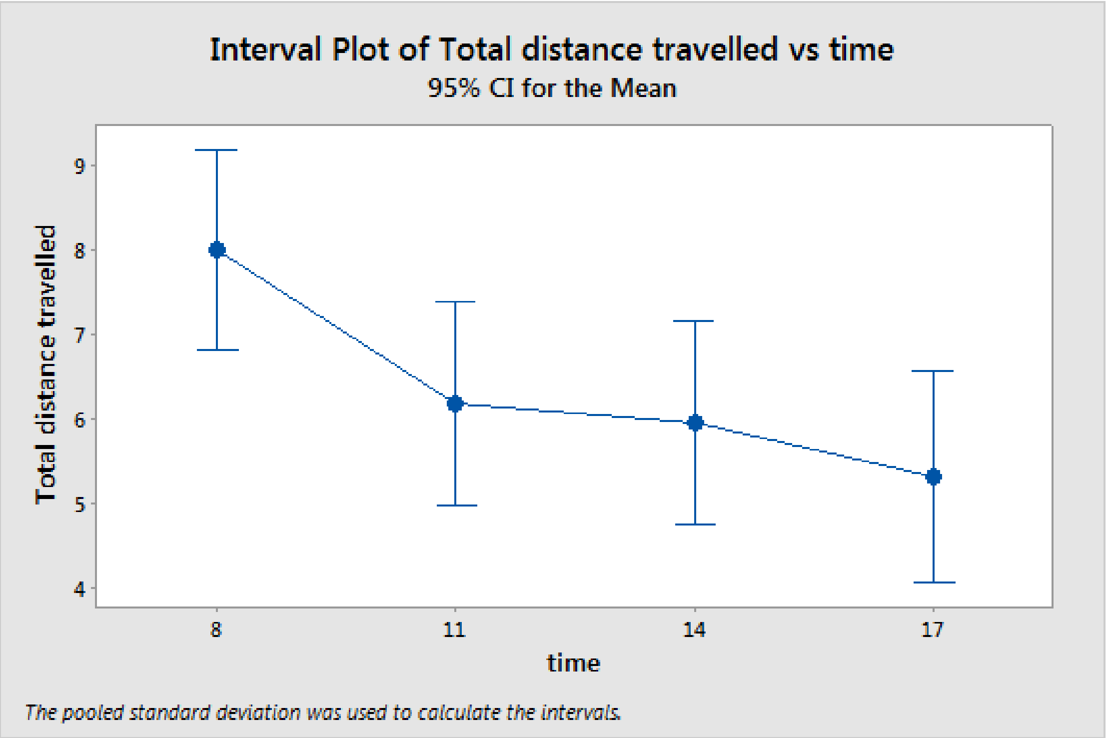


Figure 4-8 Distance travelled according to time period

Time in x-axis as hour of the day – 8: 0800h, 11: 1100h, 14: 1400h and 17: 1700h.

Minimum temperature was positively correlated with distance travelled ($r = 0.215$, $P < 0.0001$) but not maximum temperature (average ± SE: 11.6 ± 0.09) or rainfall (average ± SE: 1.8 ± 0.15).

Out of the 14 observation days, 8 had puddles present, but this did not influence significantly the destination or distance travelled ($P > 0.05$).

4.5.2 Social effect

Only 27.5% of observed hens joined a group after leaving the pop hole. The mean group size was 4.9 ± 0.3 hens, which were located on average 1.8 ± 0.3 m from the shed. Of the birds that joined a group, 16% joined a second group with a mean group size of 5.8 ± 0.7 hens, 3.4 ± 1.0 m from the shed.

The likelihood that hens joined a group did not differ according to time of day or destination.

Distance travelled did not differ depending on whether hens joined a group or not. Group size also did not differ significantly according to destination (Figure 4-9).

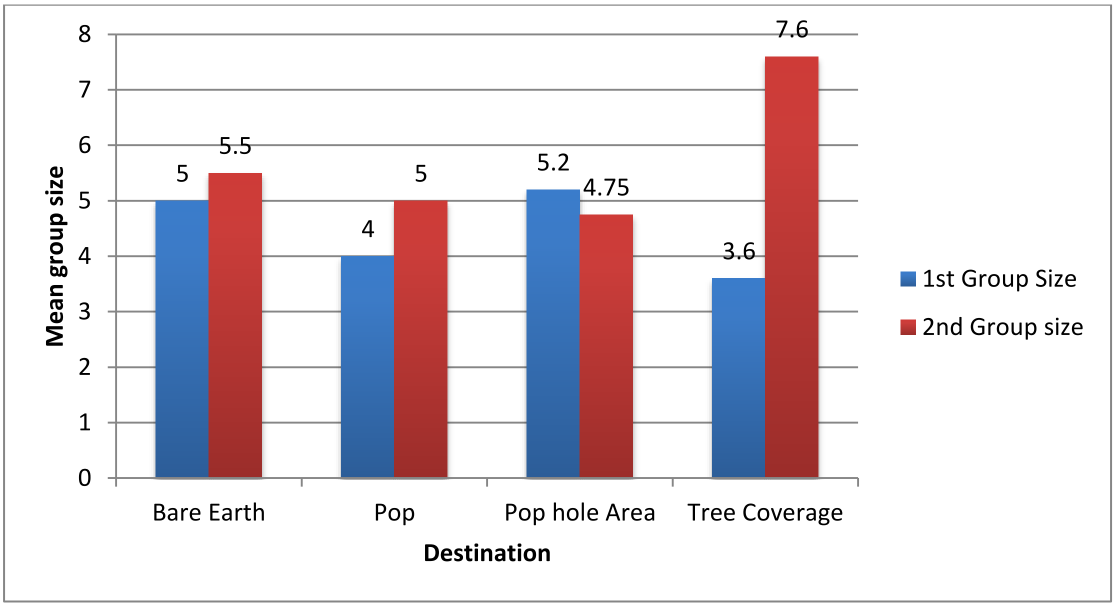


Figure 4-9 Group size according to destination

4.6 Discussion

These findings confirmed that the environment can affect ranging behaviour in laying hens. Most of the time, hens preferred to stay close to the shed. The study did not support the hypothesis that hens would prefer to join a group after leaving the pop hole, or that social group could attract hens in particular areas. This study was only performed on 1 flock in the winter season. Yet, it provides knowledge about the ranging behaviour of hens over the first 5 min after exiting the pop hole, although only 8% of hens stayed within the field of view for the entire 5 min, and most disappeared within 1 to 3 min. The limitations of the behavioural observation method are highlighted below.

4.6.1 Environmental effects

Environmental enrichment is shown in the literature to promote ranging behaviour. Enriched environments encourage exploration. As the range used in the study maintained both cover and bare environments, it was hypothesised that the bare area between the shed and tree coverage would be occupied the least. This would have indicated that bare patches may merely act as a transition area for hens to access tree cover. However, observations of the hens after exiting the pop hole revealed that hens ended up half of the time within the pop hole area, within 1 m from the shed, and a quarter of the time in the bare earth area, extending from 1 to 9 m from the shed. Free range hens are known to prefer to remain close to the shed (Hegelund et al., 2006).

The hens rarely ventured further into the tree coverage area. The findings also indicated that hens moved away from the shed gradually over time, with a number of stops, as opposed to alternating between the pop hole area and the tree coverage. Given that the observation time was restricted to 5 min, and that most hens left the field of view within 1 to 3 min, it cannot be excluded that hens may have taken longer to reach the tree cover area. However, camera placement made it difficult to observe hens for longer periods of time.

4.6.2 Distance travelled

Total distance travelled was affected by destination, especially for the tree coverage area 9 m away. This was expected, as distance from the shed differed according to the various areas. However, the path taken during these trips was not necessarily direct, that is in straight lines. For example, recordings of hen movement showed that for a hen that originated at the pop hole area and then returned into the pop hole, the hen walked about 4 to 6 m.

As distance travelled increased, the number of stops increased. Although we did not record the specific behaviour displayed, this likely suggests the occurrence of foraging and other exploratory activity in the range.

4.6.3 Duration of observation

Only 8% of hens stayed within the field of view for the entire 5 min. Most hens disappeared from the field of view within 1 to 3 min. The average hen stayed on screen for 108 s before it became no longer visible to the observer within cluster, or the view was impeded by objects. However, the results suggested that the longer a hen was observed, the more likely it was to move away from the shed. Hence, it cannot be ruled out that more birds could have ventured to the tree coverage area, had they not left the field of view. This was a limitation of the (static) camera and the unpredictable nature of the focal hens.

4.6.4 Time

Distance travelled by the hens differed according to time of day, confirming that birds are more active in the morning periods than in the evening (Mahboub et al., 2004). Furthermore, there was an effect of time of day on the destination of hens. Half of the hens ended up in the pop hole area, but ventured more into the bare earth area at midday. Few went to the tree coverage area, but, as pointed out earlier, the short observation time may have influenced the final destination recorded for the hen.

Re-entering the pop hole was much more common in the evening. This result may appear contradictory to studies reporting that poultry preferred to go out in the evening (Dawkins et al., 2003; Bubier & Bradshaw, 1998). However, the study by Dawkins et al. (2003) for instance was conducted in the summer period whereas the present study was conducted in the winter, with cool evenings. Furthermore, at the time of study, sunset occurred around 1700h, whereas the Dawkins et al. (2003) study would have also included a long twilight period in the UK summer.

4.6.5 Social effects and the impact of groups

Throughout the literature, laying hens have shown a tendency to cluster in groups (Febrer, 2005; Collins et al., 2011). However, these social group dynamics can be greatly influenced by the environment in which they co-exist. Competition for resources and protection from predators can facilitate an individual's motivation to cluster or disperse (Keeling, 1994; Asher et al., 2013; Leone et al., 2007; Leone & Estevez, 2008; Estevez et al., 2007). It has been found that open spaces may encourage this clustering (Febrer, 2005). The study hypothesised that after leaving the pop hole most hens would join a group, given the open space that they were presented with. However, results revealed that only 28% of all hens that left a pop hole actually joined a group, and only 16% of those hens joined a second group. This result largely disputes findings that hens prefer to join a group when faced with open spaces. Furthermore, there was no preference to joining a group at different destinations on the range. It is also possible that the formation of a group was merely linked to the increased density of hens in an area, bringing hens in closer contact because of a resource or location, rather than social motivation to be in a group. The average first group size was made up of 5 hens, approximately 1.8 m from the shed. This may have been because there were often clusters of hens near the shed at any given time.

4.6.6 Effects of weather and puddles

Weather conditions have been known to affect motivation to range (Richards et al., 2011; Keeling et al., 1988; Hegelund et al., 2005). The results revealed that minimum temperature affected the total distance travelled by birds, but not maximum temperature or rainfall. This finding suggests that hens may change their ranging activity according to how cool it was in the morning, rather than how high the temperature could reach throughout the day. This is consistent with the finding that hens range more in the morning compared to the afternoon or evening. However, the study was conducted in winter where temperatures stayed consistently and relatively low. Conditions may be different during summer.

4.6.7 Limitations

There are numerous limitations to this study, which should be considered a preliminary, exploratory study. It is difficult to extrapolate these findings to other farm layouts, other seasons, etc.

The difficulty of recording behaviour continuously in an outdoor range setting was a major hurdle for this study. Live observations are not possible, as laying hens are highly attracted to human observers, which would then disturb their normal behaviour.

While we attempted to maximise the field of view, only 8% of birds were successfully followed for the entire 5 minutes of observation. Hens could exit the field of view, hide amongst clusters of hens, or disappear underneath the pop hole ramps. More cameras to cover a larger area of the range may make hens easier to track, or to track for a longer period of time. Nonetheless, the large area offered in free range represents a technical difficulty for video coverage, and it was difficult to predict the behaviour of birds and their motivations prior to the study being conducted.

This reflects the great challenge of observing the behaviour of hens in free range production systems, which comprise multiple and diverse environments in extensive spaces that extend often beyond the technical capacity of video recording. We therefore decided for further projects to either focus on particular areas of the range (e.g. structures, areas in various parts of the range), or to use other technologies (e.g. RFID) that allowed individual tracking of hens.

Due to the difficulty of obtaining a clear depth of view with the camera, only one pop hole was studied, located at the end of the shed. This may have been a possible confounding factor as the distribution of birds within the poultry shed was unknown. It was possible that more birds preferred one end of the barn as opposed to the other, or that certain types of birds occupied this area. It has previously been suggested that dominant birds occupy central regions of a range while subordinate birds are inclined to inhabit peripheral locations (Estevez et al., 2007). Therefore, it is possible that the only birds that emerged from the chosen pop hole were subordinate birds and therefore were more inclined to congregate at the shed upon leaving this pop hole.

The definition of joining a group was subjective and arbitrary. Due to the restriction that birds needed to pause there in the group to be considered joining one, passing through a cluster of hens did not count. Upon deliberating this restriction, the proportion of hens that joined a group would have been higher if passing through a group without stopping was considered 'joining' a group. However, this may have conflicted with the term to 'join' a group, and would have been better described as momentarily joining in a group. Furthermore, birds had to be within 1 m^2 of one another, which was based on an assumption that groups were only formed within this spatial constriction. The spatial restriction had been placed in order to emphasise close proximity between birds in a group. However, it was entirely possible that interindividual distances were much larger for hens that considered themselves part of a group, considering all attractive and repulsive forces that might have influenced this (Keeling, 1994). This could also have affected the group size of hens if the actual group size expanded beyond a 1 m^2 spatial restriction. Furthermore, as pointed out earlier, hens may congregate around the same areas, creating an area of higher hen density without necessarily reflecting a voluntary motivation to be in a group, but rather common attraction to an area.

The focal birds under observation were part of a relatively old flock and depopulation occurred shortly after video recording had ceased. For this reason the physical condition of these birds may not have been optimal. Upon reviewing video footage, some birds were observed limping, potentially indicating bone weaknesses or keel fractures. However, older flocks such as these were more acclimatised to the range than younger flocks, and this was considered advantageous, as younger birds may not have ventured out onto the range as frequently, due to a novel environmental experience.

4.7 Conclusions

The current study confirmed environmental effects on the ranging behaviour of laying hens, but did not support the initial hypotheses that the barren environment would be used as a transitioning area and be the least occupied, or that hens would prefer to join groups after leaving the pop hole.

Hens ranged more during the morning periods, travelling greater distances. Nonetheless, most hens after leaving the pop hole remained close to the poultry shed for the first few minutes when we could observe the hens. However, given that 92% of observed hens left the field of view, it is possible that these hens could have moved away from the shed and towards tree cover, had their movements been tracked continuously.

The fact that many hens moved out of the field of view demonstrated the difficulty when attempting to perform observational studies in challenging environments such as the structure and area of a commercial outdoor range.

5 Experiment 4: Artificial structures as range enrichment to encourage increased range use and distribution

These results were presented at the Behaviour 2015, International Ethological Conference, Cairns, Australia, and delivered as an oral presentation.

5.1 Summary

This experiment investigates different principles that may underlie the effectiveness of artificial structures to attract hens in the outdoor range: vertical vs. horizontal structures, cover density, and height of shelter. A significant three-way interaction between all factors was found. The most important principle was cover density, with 90% UV block being most attractive, followed by 50%. The second most important factor was orientation, with combined structure (i.e. horizontal structures with one vertical side) and horizontal structures attracting more hens than vertical structures. Finally, height also mattered, but its interaction with orientation prevailed, in that height did not make a difference for combined orientation structures, whereas short horizontal structures were preferred over tall ones; conversely tall vertical structures were preferred over short ones. These results highlight the complexity of designing attractive outdoor environments for commercial laying hens. It is evident that hens have retained preference for areas that provide dense cover, as seen in their wild ancestors.

5.2 Introduction

The modern laying hen evolved from the Red Junglefowl, their wild ancestor. The natural habitat of Red Junglefowl is a dense rainforest, which contains abundant vegetation providing both cover from predators and a source of food (Collias & Collias, 1967). The outdoor environment should offer physical features that allow for protection and escape from predators in order to counteract the possible behavioural inhibition induced by fear. Most free range farms offer a large open-field pasture but with comparatively very little overhead cover. A comprehensive study on free range broilers in commercial settings showed that the number of birds observed in the range was positively correlated with the amount of tree cover (Dawkins et al., 2003). Studies on laying hens confirmed that the amount of cover in the range is a crucial factor influencing the willingness of birds to go outside (Zeltner & Hirt, 2003; Hegelund et al., 2005). Cover also allows the birds to gain shelter during inclement weather.

Natural trees or bushes provide cover but present several disadvantages such as slow growth, attractants for wild birds and a stance for aerial predators, hence the search for artificial substitutes. Furthermore, artificial structures have the advantage that they can be moved to enhance a uniform use of the range. Shelterbelts, as natural vertical structures, or the provision of shaded areas have been found to attract more hens in the range (Hegelund et al., 2005; Borland et al., 2010; Glatz et al., 2010), whereas other structures such as roofed boxes with sand increased the distribution, but not the number, of hens in the range (Zeltner & Hirt, 2003). That ‘cover’ does not need to be overhead was demonstrated by Taylor et al. (2004) and Rault et al. (2013), who found that more hens are present in the range when vertical fences are provided.

While most studies showed the effectiveness of those structures to various extents, the particular features or cues of the structures that fulfil the biological requirements for hens have not been scientifically investigated. For example, offering structures that vary in their features (two-level perches, a "pecking-tree", pine tree and boxes with pine cones) was more effective at increasing the number of hens outside than offering just one type of structure (a shelter), but the authors did not identify which specific features caused more birds to go outside (Zeltner & Hirt, 2008). The effect of artificial structures placed in the enclosure on the bird's willingness to range remains poorly understood. Research is needed to identify which environmental features are biologically relevant to the birds and should be provided for optimum use of the range.

This study aimed to elucidate the principles underlying the attractiveness of these structures to laying hens. We hypothesised that hens would prefer structures that provided the greatest cover, i.e. with the greatest visual density and surface area of shade cloth.

5.3 Methods

5.3.1 Flock

This experiment took place on a Victorian commercial Hy-Line Brown layer flock consisting of 18,000 24-week old hens during summer. Structures were placed in the outdoor range prior to hens having access to the range at 20 weeks of age, hence allowing for four weeks of establishing ranging behaviour in the presence of structures. Hens were fed standard layer diet *ad libitum* and were given access to the outdoor range from 1100h to 2000h, which was during daylight hours due to daylight saving over summer.

5.3.2 Structure design

Three principles of cover design (height, orientation and cover density) were tested for laying hen preference using a 2x3x3 factorial design. Factors included height: 0.5 m or 1.5 m; orientation: vertical, horizontal or horizontal cover with one vertical side; cover density: 0% (control), 50% or 90% UV blocking cloth (Figure 5-1). These combinations resulted in 18 different structures, with nine replicates of each height and six replicates of each visual density and orientation including controls. Structures were positioned in the outdoor range on either the east facing or west facing side of the indoor shed, at a distance of 4 m from the shed and spaced approximately 12.5 m apart. Structure position was chosen randomly to control for effects on shed orientation and positioning. All 'vertical' structures were oriented so that the two vertical panels ran perpendicular to the shed, and all 'horizontal with one vertical panel' structures were oriented with the vertical panel running parallel with the shed. All structures were based on a 2.4 x 2.4 m square shape and made of hardwood, pine and dark green shade sail cloth. Shade cloth was stretched tight over the structure frame to minimise movement in the wind and was re-tightened if necessary after hens loosened the cloth from perching on top of it.

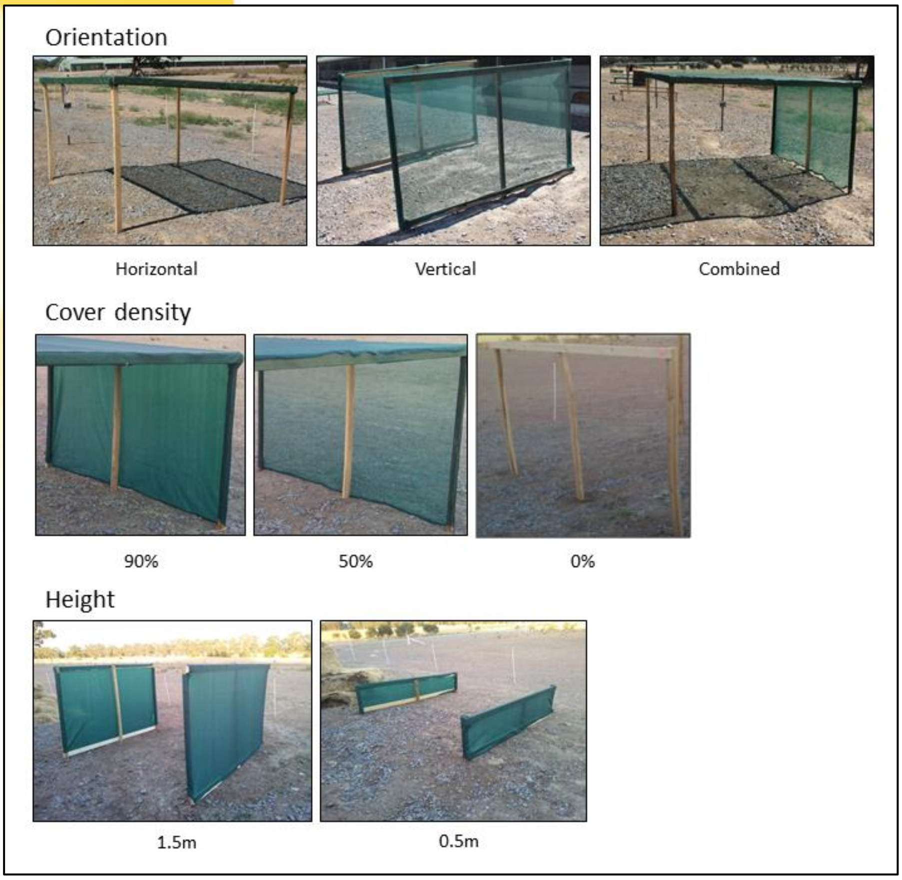


Figure 5-1 Combination of the three principles tested: orientation, cover density and height

5.3.3 Data collection

After four weeks acclimatisation GoPro Hero 3 cameras were placed in the range, prior to hens being given access, for a total of ten days in December and January. Using a scan sampling technique, the number of hens under and around a 1 m perimeter of each structure was counted every 15 minutes from 1115h to 2000h for each of these days. As the number of hens under and around each structure varied and the distribution of hens obstructed the view of some hens, when the exact number could not be counted it was estimated to the nearest five for counts of 30 or under, and to the nearest ten for counts over 30.

5.3.4 Statistical analysis

The number of hens at each structure over ten days was analysed with a mixed model in SAS, including as fixed effects orientation, cover density, height, and all their interactions to the third level, accounting for day and with structures as the experimental unit at a single time of the day (i.e. observation). Because interactions were significant to the third level, results were compared according to two-way interactions, keeping the third factor constant, with the resulting interaction plots displayed.

5.4 Results

Figure 5-2 Example of the attractiveness of the artificial structures

As illustrated (Figure 5-2), the artificial structures were highly successful at attracting a large number of hens to the areas where they were placed.

The number of hens observed under the different structures differed significantly ($P<0.0001$, Figure 5-1), with a three-way interaction between the three factors of height, orientation and cover density. Given that a three-way interaction is complex to interpret, data were visualised according to an interaction plot to facilitate interpretation, keeping one of the factors constant to interpret the interaction between the two remaining factors (Figures 5-3 and 5-4). From the attribution of variation in statistical results, the most important factor was cover density ($F_{(2,119)}=883.34$, $P<0.001$), with more hens under artificial structures with cover density of 90% UV block, followed by 50%, than the control with no cover. The second most important factor was orientation ($F_{(2,119)}=122.24$, $P<0.001$), with combined structure (i.e. horizontal structures with one vertical side) and horizontal structures attracting more hens than vertical structures. Finally, height was also found to matter ($F_{(1,119)}=51.94$, $P<0.001$), but its interaction with orientation prevailed ($F_{(2,119)}=169.28$, $P<0.001$), in that height did not make a difference for combined orientation structures, whereas short horizontal structures were preferred over tall ones, but conversely tall vertical structures were preferred over short ones.

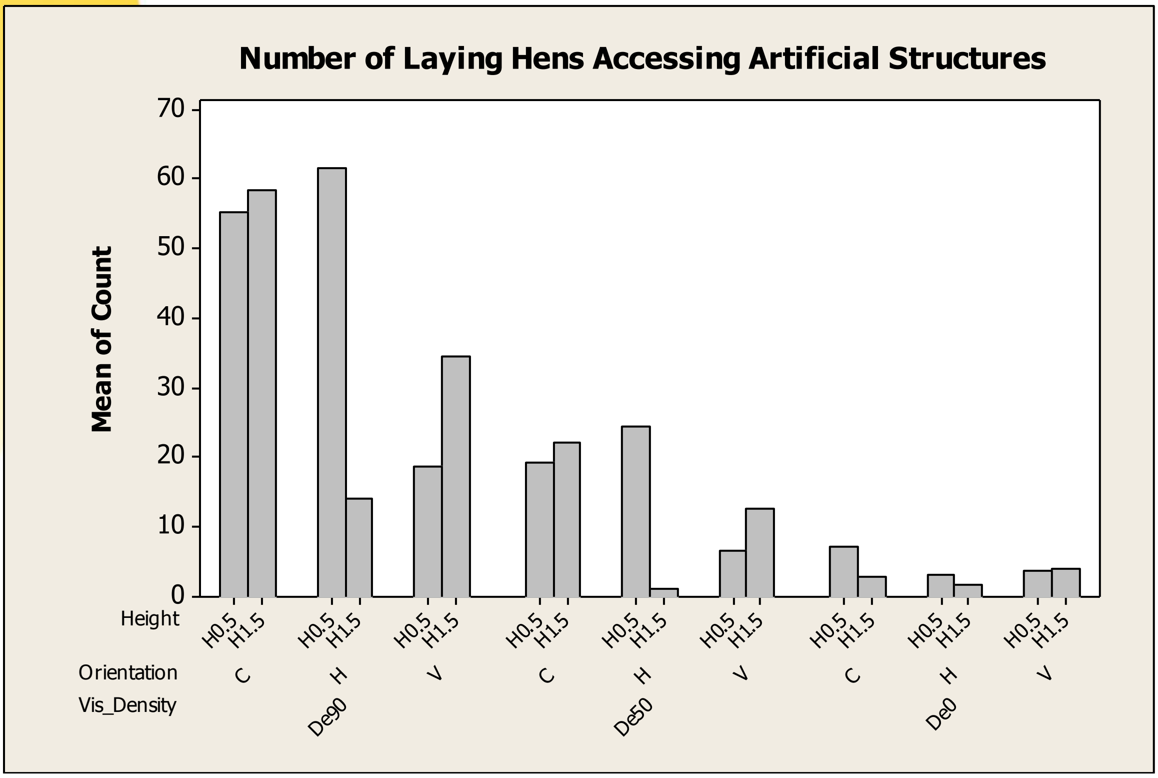


Figure 5-3 Mean count per day, across 10 days, of the number of laying hens accessing the artificial structure area according to cover density (90, 50 or 0% UV block), orientation (C: combined, H: horizontal, V: vertical) and height (H0.5: 0.5 m, H1.5: 1.5m)

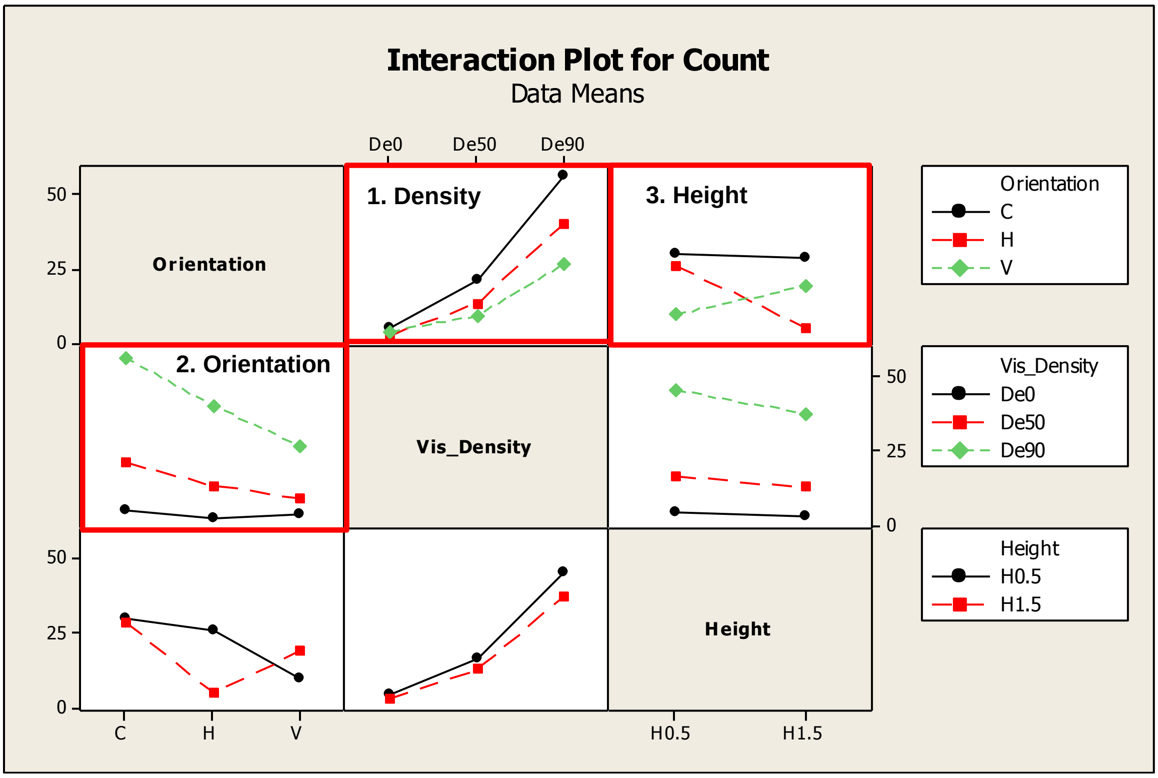


Figure 5-4 Interaction plot representing the count of the number of laying hens accessing the artificial structure area according to cover density (De90, De50 or De0% UV block), orientation (C: combined, H: horizontal, V: vertical) and height (H0.5: 0.5 m, H1.5: 1.5m)

5.5 Discussion

There was a significant interaction effect between all three factors (height, orientation and visual density) on the number of hens using the structures. Greater visual density was highly attractive to laying hens in this study, suggesting this to be one of the most important factors in our design. Few studies have examined preference for varying visual densities in laying hens in terms of outdoor cover. However, our results are supported by others that have shown that when given a choice between 0%, 33.3%, 67.7% and 100% vertical visual cover between areas, indoor hens would spend more time in the area with 67.7% visual cover, and the least in 0% (Newberry et al., 1997). Similarly, more commercial laying hens were found in zones with vertical panels of 84-90% UV blocking than in zones with panels of ~60% UV blocking shade cloth, and even less in zones with 5% UV blocking panels (Rault et al., 2013). However, visual density in this study was also confounded with distance from the shed, which has been removed as a factor in the present study to control for this potential confounding effect.

Preference for the combined structures could be indicating a variation in hens' needs that might be met by more diverse structures. Greater diversity in artificial structures in terms of the type of structure provided successfully attracted significantly more hens into the outdoor range (38.6 ± 6.4% compared to 29.2 ± 5.0% of the flock), and improved distribution of hens in the range (Zeltner & Hirt, 2008), suggesting diversity in structure type is important to hens. When examining the use of shade, additional forage and shelterbelts in an outdoor range, hens did not use the range significantly more when provided shade, but did so when provided with shelterbelts, which were used for protection, foraging, scratching and dust bathing, suggesting that forms of shelter that allow for more behaviours are more attractive for laying hens (Glatz et al., 2010).

Height had a pronounced interaction with orientation when the effect of the combined structure was kept constant, indicating that short horizontal cover is preferred over tall horizontal cover, but that tall vertical cover is preferred over short vertical cover. This study is the first to examine the effect that structure height has on hen preference. We interpreted these results as identifying preferences for structure types that provide the greatest protection from predators. Short vertical structures provide hens with an object to interact with, but little shade and little cover above head height, leaving the hens exposed, whereas tall horizontal structures provide overhead shade, but still leave hens exposed and do not provide any interaction opportunities.

5.6 Conclusion

These results highlight the complexity of designing attractive outdoor environments for commercial laying hens. It is evident that hens have retained preference for areas that provide dense cover, as seen in their wild ancestors. The principles investigated in this study, orientation, cover density and height, were all found to be important in the design of attractive artificial structures in the outdoor ranges on a commercial farm. The influence of the distance from the shed at which these artificial structures are placed remains to be investigated, for its potential to attract hens further into the range.

6 Experiment 5: Monitoring ranging behaviour and its welfare implications in commercial free range laying hens using RFID technology

Some of these results were published as a one-page abstract at the Australian Poultry Science Symposium, 2016, Sydney, Australia, v. 27: p. 77, and delivered as an oral presentation; at the 2016 Spatially Enabled Livestock Management Symposium, Camden, NSW, and delivered as an oral presentation; and at the 50th Congress of the International Society of Applied Ethology, Edinburgh, UK, and delivered as a poster presentation.

6.1 Introduction

Little is known about the ranging behaviour of laying hens on free range commercial egg farms. Previous methods aiming to assess range access have relied upon scan sampling and survey methods, with studies reporting between 5 and 46% of the flock observed in the range at any one time, and large variations between times of the day, flocks and studies (Bubier & Bradshaw, 1998; Nicol et al., 2003; Zeltner & Hirt, 2003; Hegelund et al., 2005, 2006).

Until recently, researchers studied the flock as a whole due to the technical difficulty of following individual birds in large flock settings over extended periods of time. However, these difficulties can now largely be overcome by using technologies such as radio-frequency identification (RFID) systems. Using RFID tags, Richards et al. (2011) reported that different sub-populations of hens can be identified in a flock based on their range use, with 10% of heavy users, 80% of regular users, and 10% of the flock that never ventured outside. Recent research in experimental settings (Hinch et al., 2014; Hartcher et al. 2016) and on commercial farms (Gebhardt-Henrich et al. 2014a) confirmed those observations.

Until now, there has been no study on commercial flocks in Australian settings using RFID technology to assess how many hens access the outdoor range on a regular basis. Furthermore, none of the previous RFID studies have investigated the relationship between individual ranging behaviour and hen welfare on commercially housed laying hens.

The aims of this study were to determine the ranging behaviour by individual laying hens on an Australian commercial farm through RFID tracking, and to investigate the relationship with measures of hen welfare.

6.2 Methods

6.2.1 Animals and husbandry

Two flocks of 18,000 Hy-Line Brown hens on one commercial free range layer farm were studied, housed in two different sheds. Each flock had *ad libitum* access to feed and access to an outdoor area from approximately 1000h to 1800h, with first access at 21 weeks of age. Both flocks complied with the model code of practice for laying hen housing and RSPCA free range standards at the time of study. Flock A was 41 weeks of age at the commencement of the study and egg production levels were 93.6% according to producer records. Flock B was 63 weeks of age and egg production levels were 85.3% at the commencement of the study. Both flocks had access to nesting boxes and perches in the slatted indoor environment; however, Flock B had access to a greater number of perches than Flock A. Flock A had only one laser beak trim at day-old, whereas Flock B had a laser beak trim at day-old and a second beak trim with hot blade at a later age.

6.2.2 Study area

Within each flock a group of 2000 hens was segregated using temporary fencing in the shed and outdoor range to allow for the RFID equipment to be installed in the study area, as we did not have enough equipment to cover all exits from the whole shed. The stocking density of the hens in this area was the same as the remainder of the flock. Antennas for the RFID system were placed at two pop holes giving access from the indoor shed to the wintergarden area, three pop holes giving access from the wintergarden area to the close range (0-9 m from shed), and across a gate that led from the close range to the far range (9-46 m from shed; Figure 6-1). Hens were segregated and antennas fitted to all pop holes two weeks prior to data collection in Flock A and 1.5 weeks prior to data collection in Flock B to allow for habituation to the fencing and antennas.

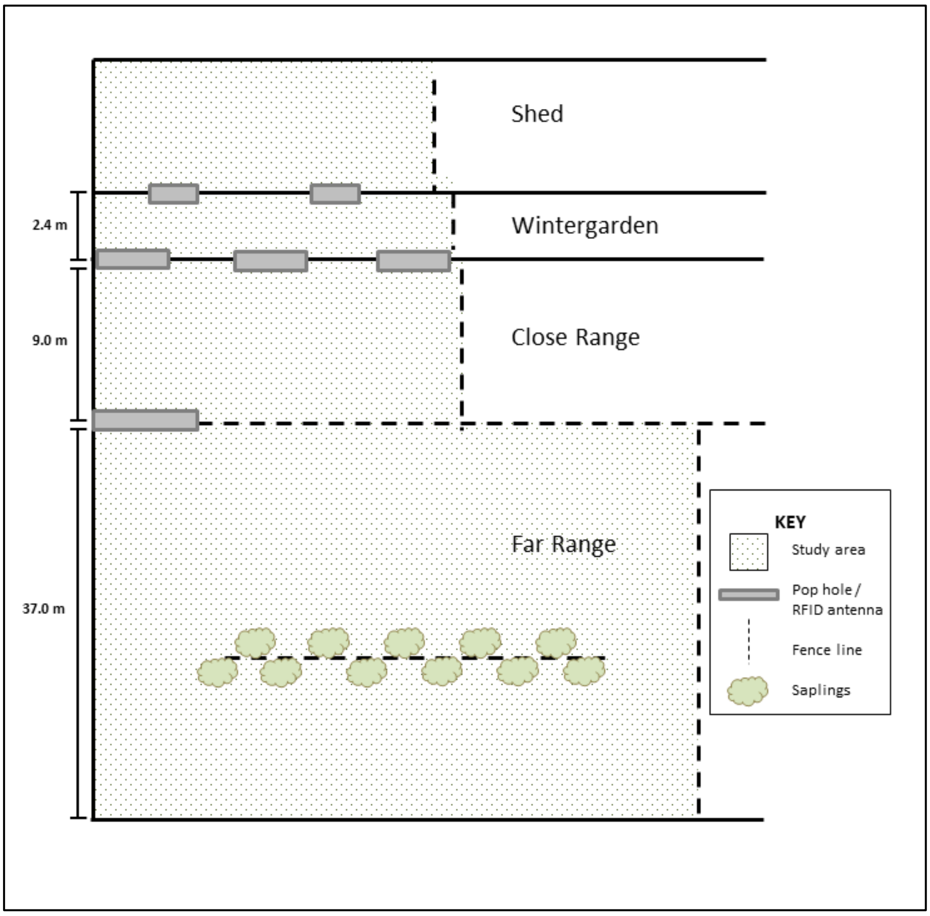


Figure 6-1 Layout of the shed RFID area, with the three outdoor zones

6.2.3 RFID antennas

The RFID system used was the *Gantner Pigeon System,* with a bespoke program, *Chicken Tracker* (© 2015 Gantner Pigeon Systems GmbH), that was developed for the use of tracking commercial chickens. It had been previously validated on farm and successfully used to track chickens overseas (Gebhardt-Henrich et al., 2014a, b). Antennas were attached to both sides of all pop holes in order to determine the number and the direction of movements by each tagged bird.

6.2.4 Ranging data collection

Data were collected for Flock A one month prior to collecting data from Flock B, as the equipment could not be used on both flocks simultaneously. Laying hens were caught with a net and fitted with silicone leg bands each containing one ID chip (Ø 4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered on the antennas as hens walked across them. Leg bands containing RFID chips were retrieved from the hens after 19 days, in which there was no experimental disturbance, to assess ranging behaviour in a normal production environment. In Flock A, 441 hens were tagged and 450 hens were tagged in Flock B.

At the end of the study 353 working chips (80%) were retrieved from the hens in Flock A and 309 working chips (69%) were retrieved in Flock B. In Flock A, six of the 19 days (32%) of ranging data were excluded from the final analysis due to the system failing and missing data points, and nine of 19 days (47%) were excluded in Flock B, leaving 13 full days of ranging data for Flock A, and 10 full days of ranging data for Flock B. System failures were not related to any specific events that may have influenced flock behaviour such as adverse weather conditions.

6.2.5 Welfare data collection

Welfare data were collected at two time points during the study, the first immediately prior to tagging hens with leg bands, and the second immediately prior to removing the leg bands. During these two time points, hens' access to the outdoor range was restricted to the wintergarden to allow for easier selection and capture of the hens. At the first time point, 150 randomly selected hens were subjected to a tonic immobility test to assess general fearfulness. At the second time point another 150 randomly selected hens were subjected to four behavioural tests, performed in the same order for each hen, to assess fearfulness. Hens were randomly selected by observers by first entering a randomly selected section of the shed or wintergarden, as determined by a numbered grid and random number generator. Hens were then visually selected within that section, and to ensure that visually conspicuous hens (very large or small, or poor plumage condition) were not biased the observer then attempted to catch the hen that was two individuals to the left of the first hen. Behaviour tests were followed immediately by a blood sample to test for plasma corticosterone concentration as a measure of acute stress and heterophil:lymphocyte ratio as a measure of the immune system mobilisation. Finally, body condition scores were taken as a further measure of physiological fitness. Faecal samples were also opportunistically collected during the behavioural tests, to test for faecal corticosterone metabolites (chronic stress).

6.2.5.1 Tonic immobility

Randomly selected hens were caught from either the shed or wintergarden area with a catching net and immediately taken to one of four covered testing arenas outside of the flock area. Methods for tonic immobility testing were adapted from Jones and Faure (1985). In each arena there was a table with a U-shaped cradle and hens were inverted and placed on their backs in the cradle. Hens were then restrained for 15 s with gentle contact with hands over head and torso. After 15 s of restraint the hens were released and the tester slowly moved back to a distance greater than 1 m from the hen and averted eyes to the ground. If the hen righted itself or attempted to right itself in the first 15 s of the test, tonic immobility (TI) was considered not induced and the tester attempted to induce TI again. A maximum of five attempts to induce TI per hen was allowed before the test was aborted, and hens were 'woken' from the TI state by the tester if hens remained induced for 5 minutes. Hens that righted prior to 5 minutes were then taken back to the shed and their leg bands attached before being returned to the flock.

6.2.5.2 *Behavioural tests*

At the second data collection time point, randomly selected hens were caught from either the shed or wintergarden area with a catching net and immediately taken to one of four covered testing arenas outside of the flock area. Each arena was 3 × 3 m and consisted of bare earth, wooden walls and a fabric roof, with white chalk paint markings on the wall to identify nine 1 × 1 m grid squares. For the first test, open field (OF) modified from Gallup & Suarez (1980), hens were placed into the centre of the arena, and left for four minutes to assess behaviour via a video camera. The latency of hens to move was recorded, as well as the number of 'grids/lines' crossed in the pen to measure mobility. Immediately after the OF test a novel object (NO) test, modified from methods described in Campler et al. (2009), was performed for two minutes where an orange traffic cone was placed in the centre grid space of the arena door edge and left for two minutes while video was recorded for behavioural analysis. Again, the latency for hens to move and the number of 'grids/lines' crossed were recorded, as well as the latency for hens to peck the novel object (if it occurred). After the NO test the human avoidance test (HA) and human approach (HAP) tests, modified from methods as described in Hemsworth and Barnett (1989), were performed (results not reported) where for HA an experimenter entered the arena, removed the traffic cone, and stood in the corner that was furthest away from the hen for one minute. For the HAP test, which was the last test in the sequence, the experimenter moved again to the furthest corner from the hen (if necessary) and walked directly towards the hen at a pace of one step per two seconds. Immediately after the sequence of behavioural tests was performed, faecal samples were collected from the arena and hens taken to a work station where blood samples were collected and body condition was scored.

6.2.5.3 *Physiological fitness scores*

Physiological fitness scores were conducted by two observers after ranging data had been collected and behavioural fear tests conducted, immediately prior to removing leg bands (Table 6-1). Hens were weighed using small animal veterinary scales and scores were collected on plumage condition, beak condition, footpad condition, comb colour and keel bone deformations. Plumage condition, keel bone deformation and footpad condition were scored using the same protocol as described in Welfare Quality (Welfare Quality, 2009).

Table 6-1 Fitness assessment scores as derived from Welfare Quality (2009)

Fitness assessment	Score	Description
Plumage condition	0	No or slight wear, (nearly) complete feathering
	1	Moderate wear, i.e. damaged feathers (worn, deformed) or one or more featherless areas < 5 cm in diameter
	2	At least one featherless area ≥ 5 cm in diameter
Keel bone deformation	0	No deviations, deformations or thickened sections, keel bone completely straight
	1	Deviation or deformation of keel bone (including thickened sections)
Footpad condition	0	Feet intact, no or minimal proliferation of epithelium
	1	Necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling
	2	Swollen (dorsally visible)

Beak condition was scored using a modified version of the beak trimming score from Welfare Quality (Welfare Quality, 2009; Table 6-2), where the score of zero was removed (due to all hens being beak trimmed), and an additional score was added to indicate a midlevel score between the original scores of 1 and 2.

Table 6-2 Beak condition classification as modified from Welfare Quality (2009)

Classification	Description
1	Light to moderate trimming, with no abnormalities
2	Moderate to severe trimming, with no abnormalities
3	Severe trimming, with clear abnormalities

Comb colour was scored using the ‘comb colour scale’ from the Bristol Welfare Assurance Programme hen assessment (Leeb et al., 2005), using a seven point scoring system and colour scale (Figure 6-2).

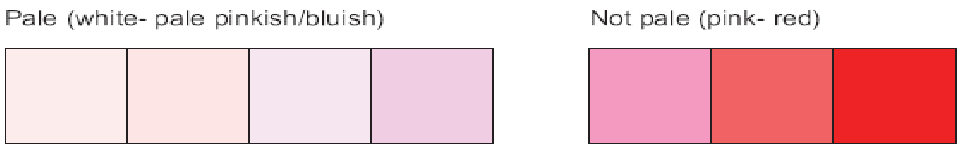


Figure 6-2 Comb colour scoring chart

6.2.5.4   Corticosterone analysis

Faecal samples were analysed separately to test for glucocorticoid metabolite extracts as a baseline physiological measure of stress, and plasma corticosterone was analysed separately as a measure of stress response to behavioural tests.

All faecal samples were homogenised and dried at 103°C overnight. After cooling at room temperature, the dried samples were milled to a fine powder. Following drying and milling, 0.2 gm of faecal sample was mixed with ethanol, vortexed for 30 mins and centrifuged at 10000 gm for 15 mins. The supernatant was then dried using nitrogen dryer and stored at -20°C. The dried extracted samples were stored at -20°C. Immediately before the immunoassay, the extract was dissolved in ethanol. Samples were analysed for corticosterone metabolite concentrations using an RIA kit (ImmuChemTM Double Antibody corticosterone 125I RIA Kit, MP Biomedicals LLC, Orangeburg, NY, USA), following the manufacturer’s manual and using a 1:100 dilution. Sample results with coefficient of variation superior to 5% between duplicates were rerun. The results were interpreted as ng/ml.

Blood samples were spun at 1500 g for 15 min and plasma was transferred to a 1 mL Eppendorf tube and subsequently stored at -20°C. Samples were then analysed using the same RIA kit as for faecal corticosterone, but using a 1:50 dilution.

6.2.6 Statistical analysis

Data obtained were first cleaned with SAS™ statistical program (v9.3) using two macros modified from Gebhardt-Henrich et al. (2014a) to identify the time and duration of hen movements between zones. Any dates where a full reliable dataset could not be obtained (e.g. due to known power failures, known failures in writing data to computer or missing minutes of time from data set, percentage of points with missing values exceeding 35% of the total data set) were discarded from the analysis. The RFID system used has previously been validated in commercial layer flocks and reliably registers hens crossing over the antennas at a speed up to 1.5 m/s (Gebhardt-Henrich et al., 2014b). Therefore the obtained dataset may contain missing data for hens travelling in excess of this speed over the antenna. Missing values of data, as characterised as an entry or exit data point for a hen without a corresponding exit or entry data point were excluded from the dataset. All data points that indicate a time period less than 10 seconds were excluded from the data set to eliminate the chance of including false data points created from hens sitting or walking on the pop hole but not entering a designated zone.

Generation of descriptive ranging data was performed using Microsoft Excel (2010). All statistical data analysis was performed using SPSS statistical software (v22, IBM Corp, Armonk, NY, USA). To test for relationships between ranging data and welfare assessment, correlations were performed using Spearman's rho analysis for non-parametric correlations. Tests according to hen classification were conducted using ANOVA.

TI durations and latency to move durations for open field and novel object tests and latency to peck a novel object were analysed using Kaplan-Meier survival analysis in SPSS, with ranging duration (non-rangers, occasional rangers, high rangers) as the fixed factor. Movement in the open field and novel object tests were analysed according to hen group using one-way ANOVA.

6.3 Results and discussion

6.3.1 Ranging

6.3.1.1 Overall time in the range

The overall time spent in the range over the course of the study varied greatly within each flock, from no time outside to up to 6 h per day (Flock A) or 3.5 h per day (Flock B), with a spread distribution in between these extremes (Figure 6-3).

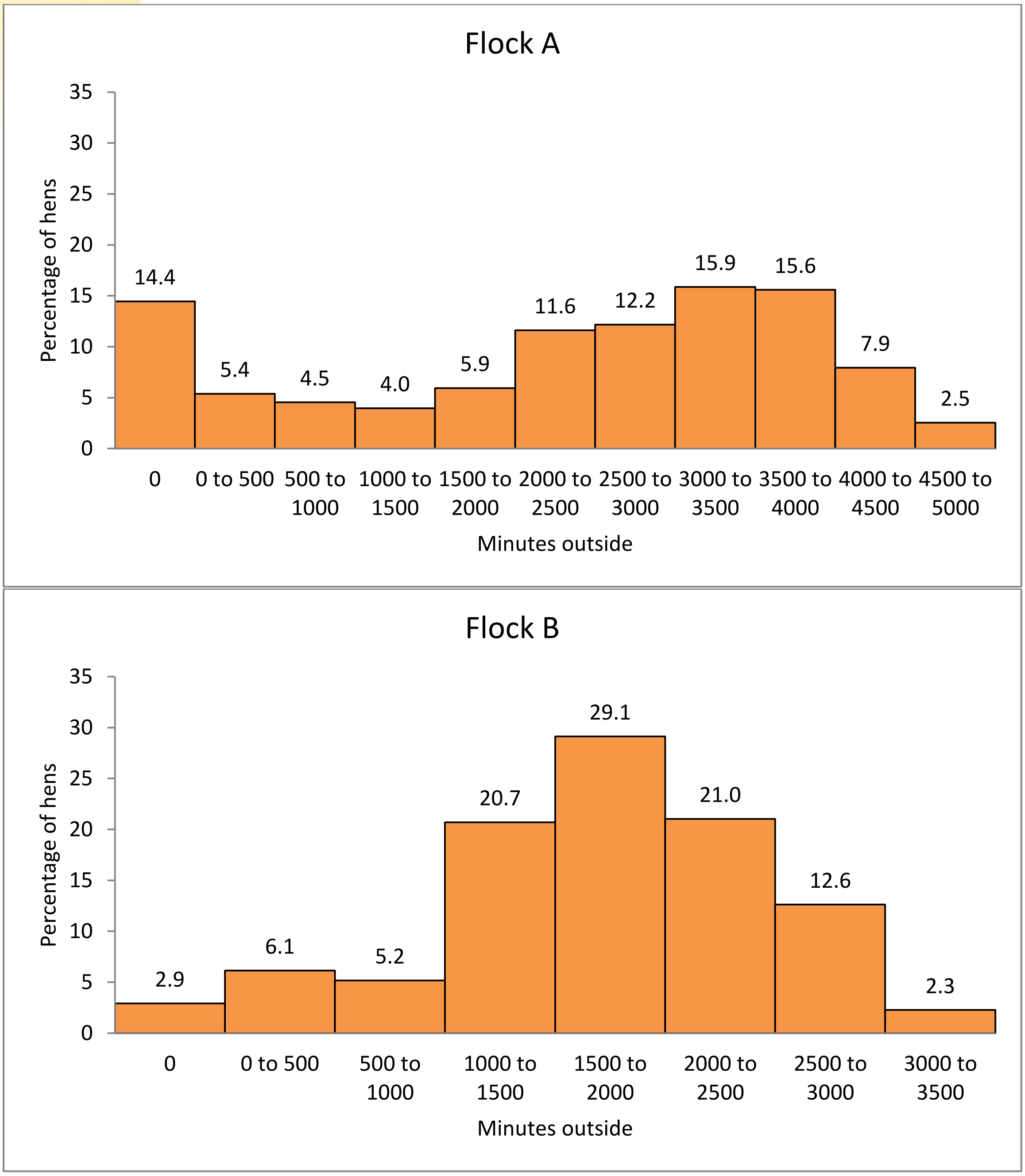


Figure 6-3 Total time spent in the range over the course of the study (min; Flock A over 13 days of range access and Flock B was studied over 10 days of range access)

6.3.1.2 *Frequency of range access*

Most tagged hens in the flocks accessed the outdoor range on a regular basis (Figure 6-4). More precisely, in Flock A 68.6% of the hens were every day rangers, 17.0% occasional users, and 14.4% non-rangers; and in Flock B 82.2% every day rangers, 14.8% occasional users, and 2.9% non-rangers over the course of the study.

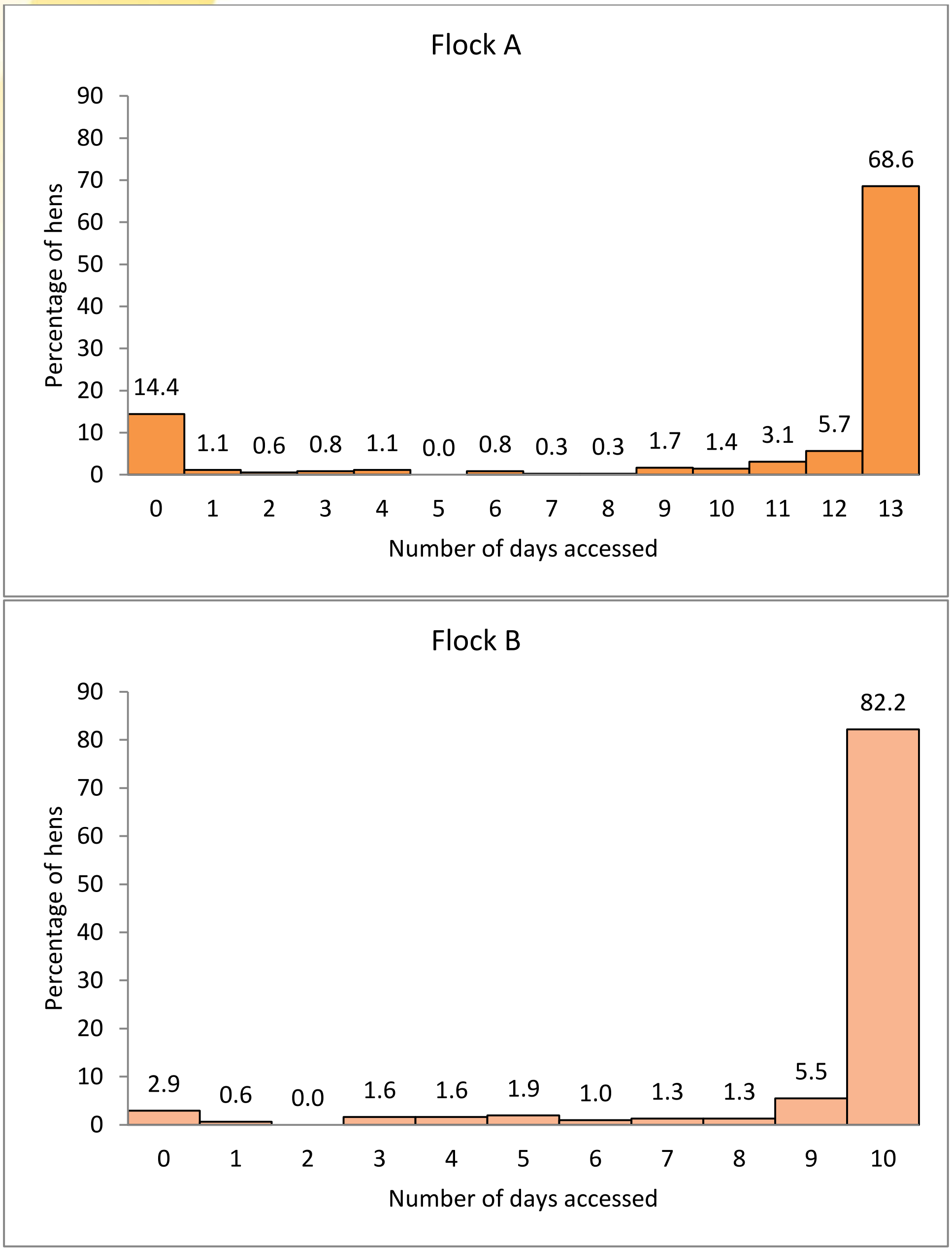


Figure 6-4 Number of days tagged hens accessed the range (out of 13 and 10 days for flocks A and B, respectively)

The frequency of daily visits was calculated by dividing the overall number of visits to the range by the number of days the range was accessed (Figure 6-5). The frequency of daily visits was spread quite evenly in Flock A from none to 30 visits daily, whereas in Flock B the variation was narrower with most hens making between 5 and 20 daily visits to the range.

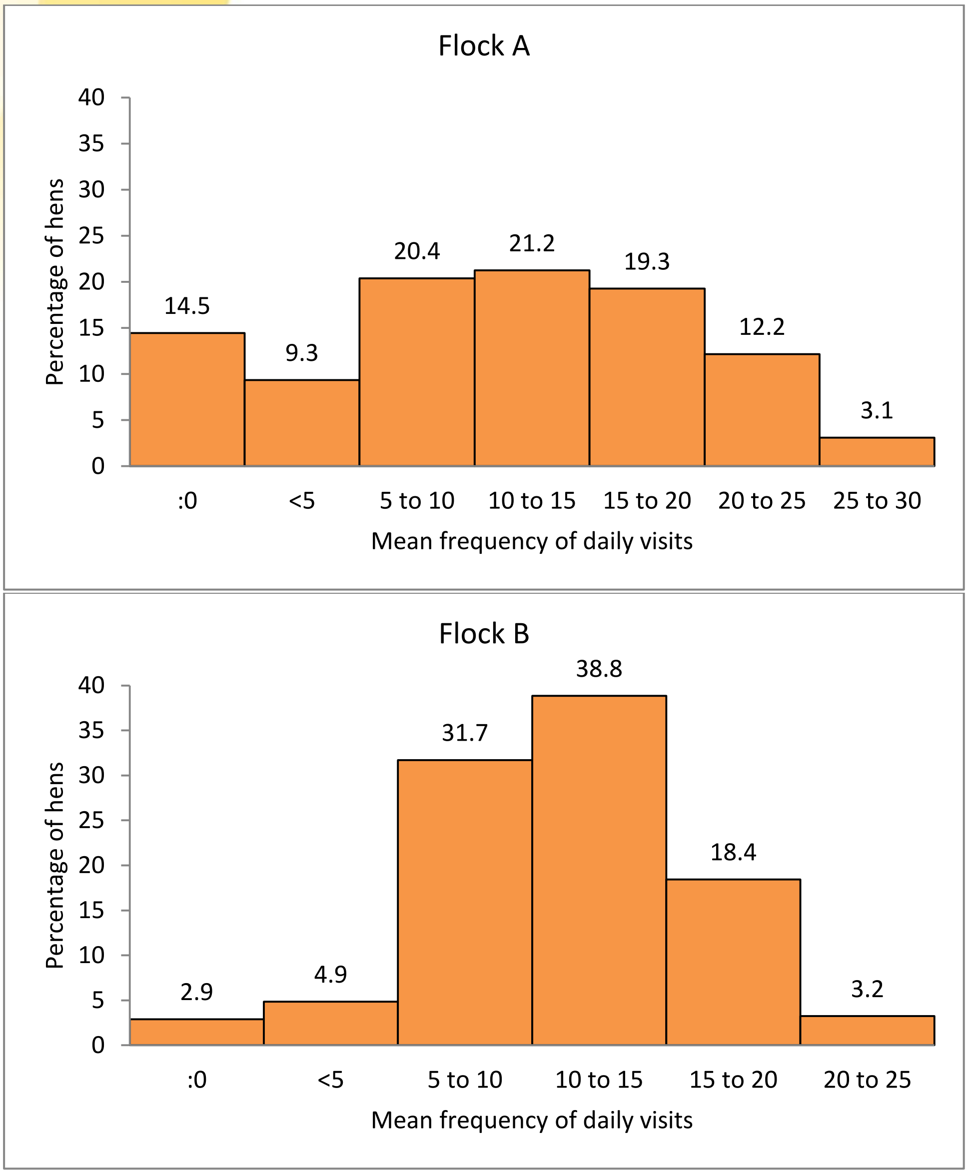


Figure 6-5 Mean frequency of daily visits to the outdoor range for hens in both flocks

The x-axis represents mean daily visits to the outdoor range, and the y-axis represents the proportion of hens in the flock that on accessed the range within those frequencies.

6.3.1.3 Duration of range access

Hens also differed in terms of the time they spent on the range (Figure 6-6). In Flock A, daily time spent in the range varied between 0 and up to 6 h. In Flock B, the variation was narrower, with most hens spending between 2 and 4 h in the range daily.

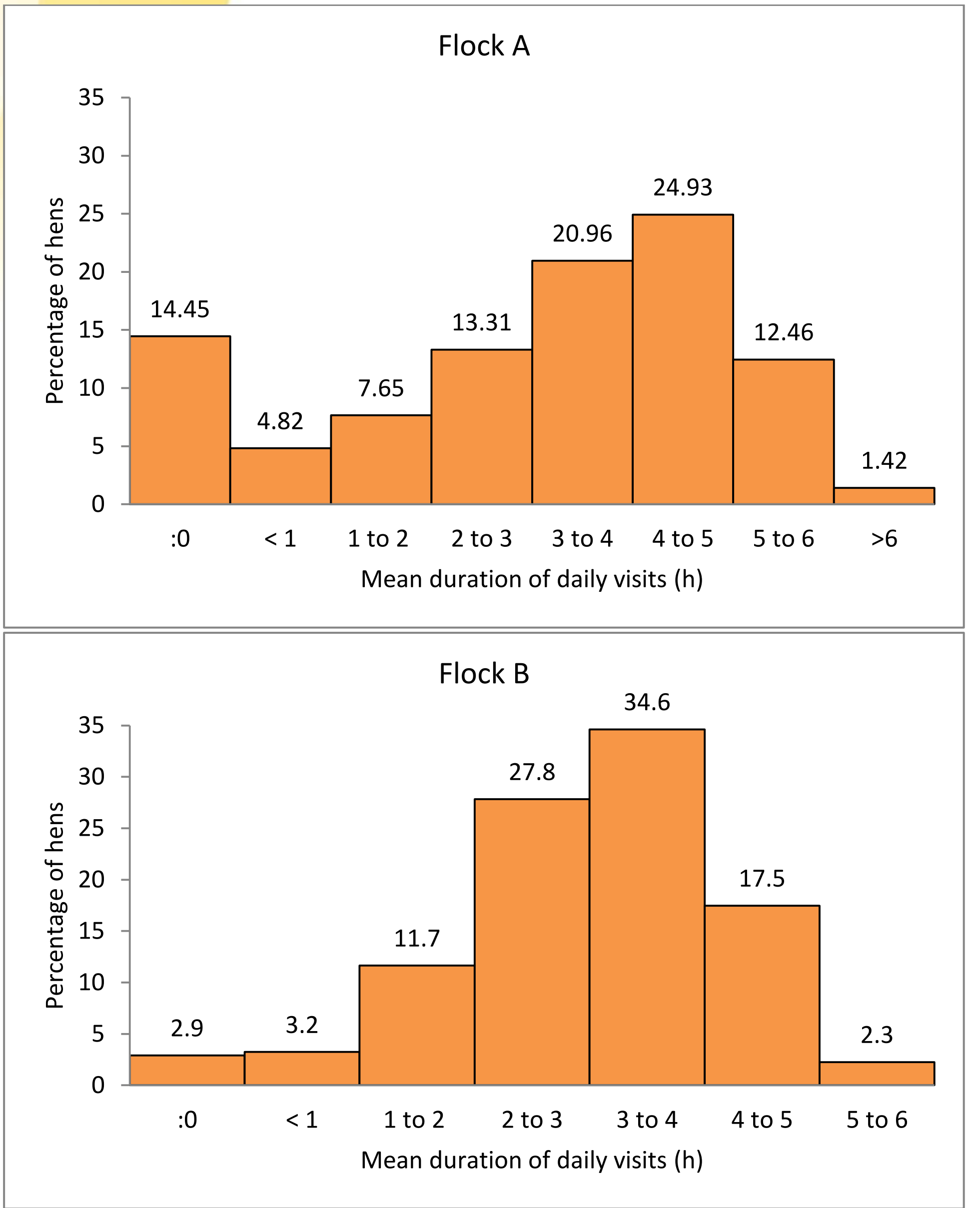


Figure 6-6 Mean daily duration of range access to the outdoor range for hens in both flocks

The x-axis represents mean duration of daily visits to the outdoor range, and the y-axis represents the proportion of hens in the flock that on accessed the range within those durations.

6.3.1.4 Pattern of range visits

Hens did not vary too much in terms of their patterns of range visits, as there was an overall positive correlation between duration and frequency of range visits (Figure 6-7). Hence, as they visited the range more often, they spent more time in the range.

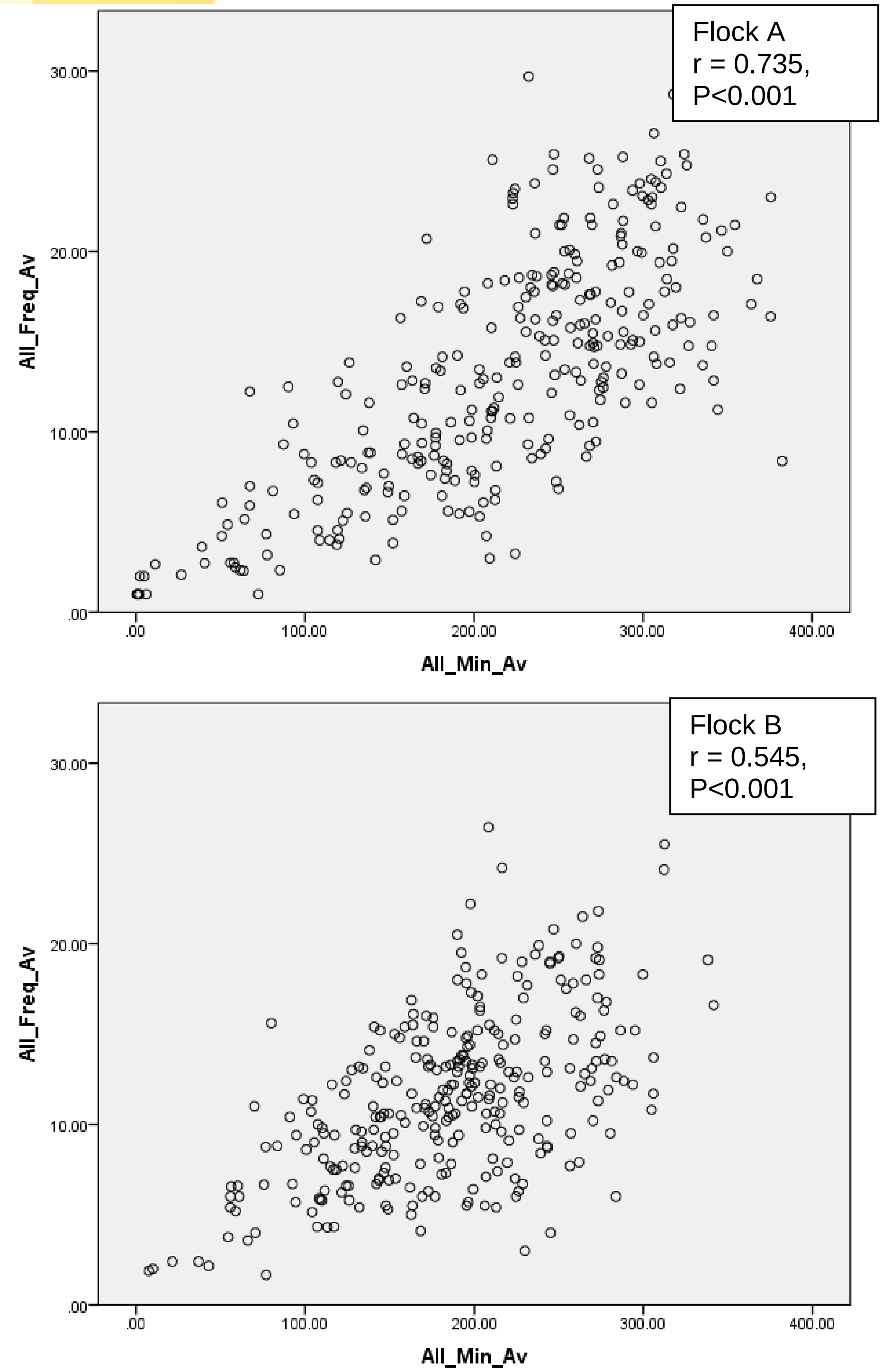
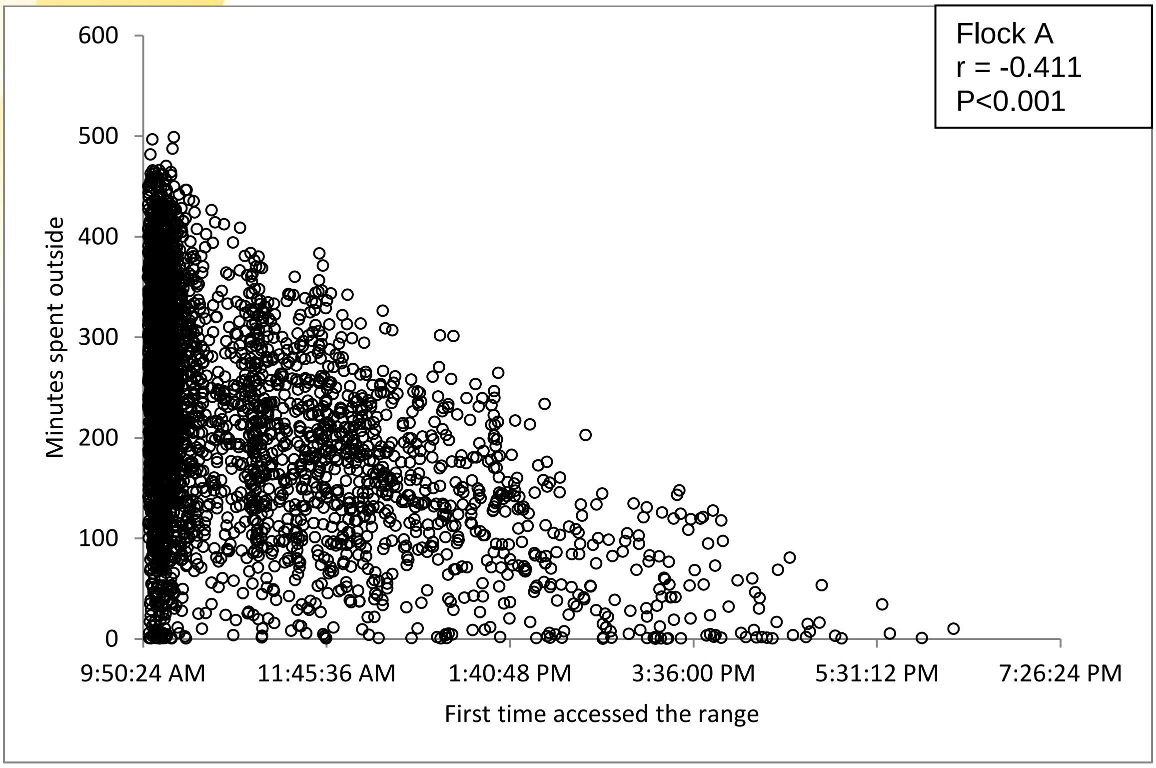


Figure 6-7 Correlation between duration and frequency of range visits on the basis of daily mean

The x-axis represents the average total number of minutes that each hen accessed the outdoor range for the duration of the study, and the y-axis represents the total number of times (frequency) that each hen accessed the outdoor range for the duration of the study for both Flock A (302 hens, 13 days) and Flock B (300 hens, 10 days).

6.3.1.5   Time of day for first range access

As expected, the time of day for the first visit to the range was negatively correlated with time spent in the range (Figure 6-8) and frequency of visits (Figure 6-9). This means that visits made later during the day were associated with less time spent outside. Doors were opened around 1000h in this experiment.



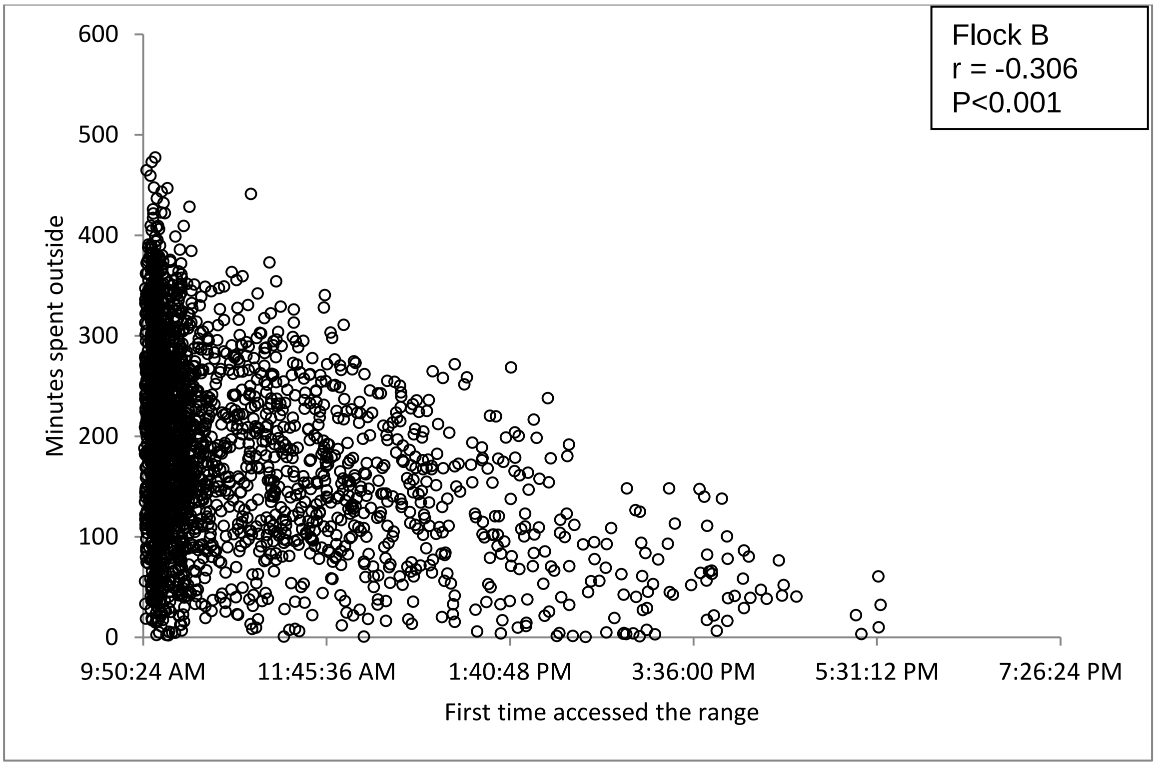
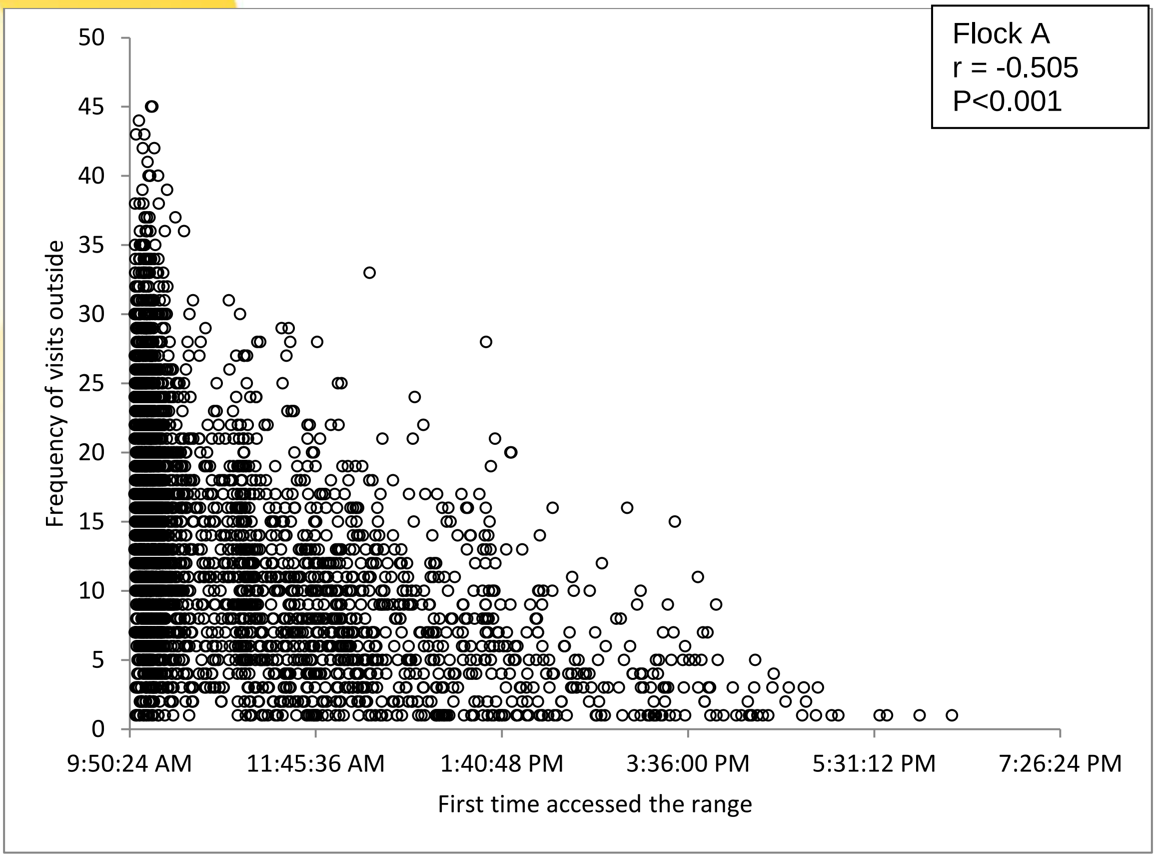


Figure 6-8 Correlation between time of first access daily and duration of range visits on the basis of overall duration



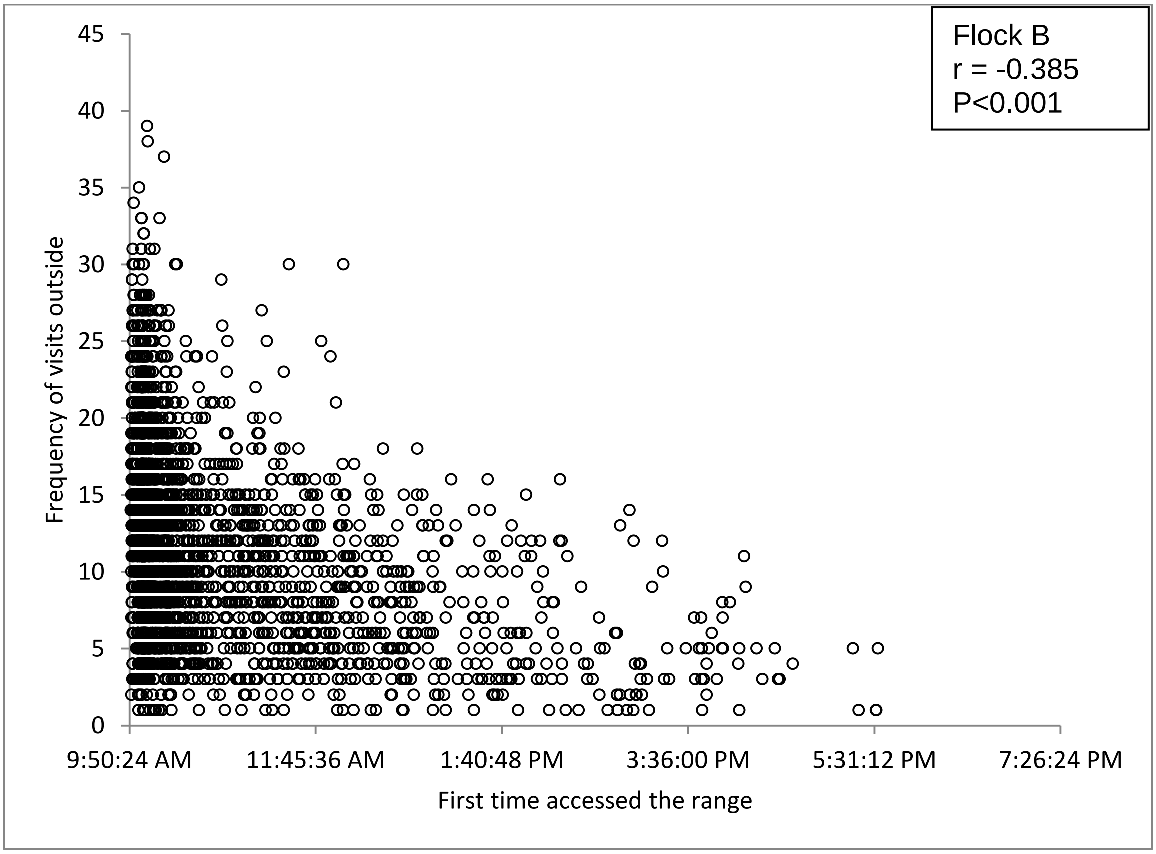


Figure 6-9 Correlation between time of first access daily and frequency of range visits on the basis of overall frequency

6.3.1.6 *Movement between zones*

Most hens in both flocks accessed all zones monitored in the range, further than 9 m from the shed (Table 6-3).

Table 6-3 Range zones accessed

Zones accessed	% Hens Flock A	% Hens Flock B
None	14.45	2.9
Wintergarden only	2.83	0.3
Wintergarden + close range	9.07	12.3
All	73.65	84.5

In terms of time spent in each zone, hens in Flock A made significantly longer visits in the wintergarden than in the zone 9-46 m from the shed, and the shortest visits in the intermediate zone 0-9 m from the shed (all $P<0.001$). Hens in Flock B made significantly longer visits in the zone 9-46 m from the shed, then 0-9 m from the shed and the wintergarden (all $P<0.001$) (Table 6-4). When comparing relative time spent in the various zones of the range, as a proportion of total time spent in the range, hens in both flocks spent about half the time in the wintergarden. Hens visited the wintergarden more often.

Table 6-4 Mean duration in the different range zones

Zones	Flock A		Flock B	
	Average duration in Zone (min ± SE)	% duration in Zone	Average duration in Zone (min ± SE)	% duration in Zone
Wintergarden	19.43 ± 0.20[a]	57.75	13.23 ± 0.17[a]	47.40
0-9 m from the shed	11.85 ± 0.15[b]	25.85	17.70 ± 0.24[b]	32.09
9-46 m from the shed	16.18 ± 0.12[c]	16.41	25.24 ± 0.13[c]	20.50

Values with different superscripts (a-c) differ significantly.

6.3.1.7 *Relationship between the number of days ranging and ranging patterns*

The number of days hens accessed the range was correlated with accessing more outdoor zones, therefore increasing the distance travelled from the shed (Flock A: $r=0.458$, $P<0.001$; Flock B: $r=0.393$, $P<0.001$).

The number of days hens accessed the range was correlated with frequency (Flock A: $r=0.75$, $P<0.001$; Flock B: $r=0.49$, $P<0.001$), and duration of range access (Flock A: $r=0.73$, $P<0.001$; Flock B: 0.58, $P<0.001$; Tables 6-5 and 6-6).

6.3.1.8 *Weather conditions*

A regression analysis was conducted to assess the effect of weather conditions on ranging behaviour. None of the weather variables was significant in either flock for the number of individual hens accessing the range daily, or the total time spent outside (all $P>0.05$).

The average weather conditions were:

- outdoor maximum daily ambient temperature
  Flock A: 11.2 ± 0.6°C, Flock B: 17.3 ± 0.4°C
- outdoor minimum daily ambient temperature
  Flock A: 2.1 ± 0.9°C, Flock B: 1.7 ±1.1°C
- indoor maximum daily ambient temperature
  Flock A: 18.1 ± 0.3°C, Flock B: 21.1 ± 0.5°C
- indoor minimum daily ambient temperature
  Flock A: 13.1 ± 0.4°C, Flock B: 16.3 ± 0.5°C
- daily rainfall
  Flock A: 1.2 ± 0.6 mm. Flock B: 0 mm
- outdoor morning relative humidity
  Flock A: 85.2 ± 3.7%, Flock B: 72.6 ± 3.8%
- outdoor morning wind speed
  Flock A: 4.3 ± 1.0 km/h, Flock B: 6.4 ± 1.8 km/h
- indoor light intensity
  Flock A: 94.7 ± 4.1 Lux, Flock B: 83.5 ± 1.4 Lux.

6.3.2 Hen welfare: physiological and fitness measures

Plasma corticosterone concentrations for Flock A averaged 6.2 ± 0.3 ng/ml (Figure 6-10A) and faecal corticosterone concentrations averaged 36.4 ± 1.7 ng/ml (Figure 6-10B). Flock B plasma corticosterone concentrations averaged 11.4 ± 0.4 ng/ml (Figure 6-10C) and faecal corticosterone concentrations averaged 34.9 ± 1.7 ng/ml (Figure 6-10D).

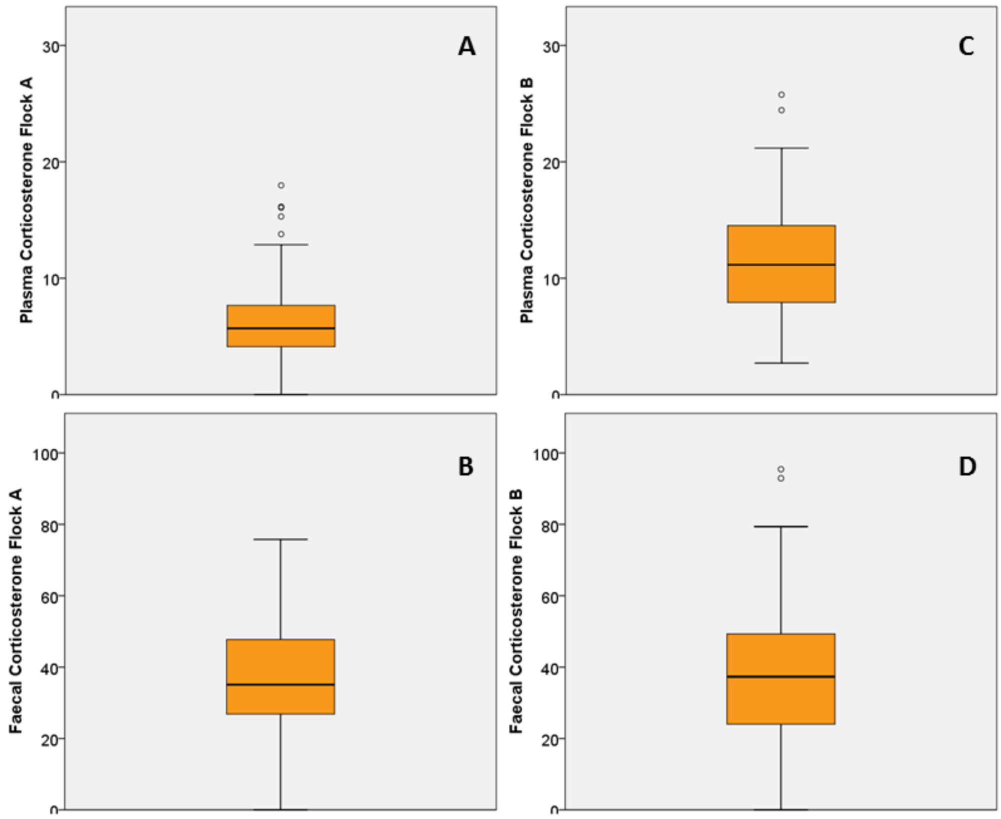


Figure 6-10 Mean plasma coriticosterone concentrations (ng/ml) for Flock A (A) and Flock B (C), and mean faecal corticosterone concentrations (ng/ml) for Flock A (B) and Flock B (D)

The middle line indicates the mean, outer edges of the box the 1st and 3rd quartile and the whiskers represent the range.
Extreme values (outliers) are represented by open circles.

There was little variation in other physiological welfare assessments for both Flock A (Table 6-5) and Flock B (Table 6-6).

Flock A	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Std. Error
Plumage Score (0-2)	0	2	1.0	0.03
Footpad Score (0-2)	0	2	0.3	0.04
Keel Bone Score (0-1)	0	1	0.6	0.04
Weight (kg)	1.5	2.3	2.0	0.01
Comb Score (1-7)	3	7	5.8	0.07
Beak (1-3)	1	3	2.0	0.07

Table 6-5 Descriptive statistics of physiological welfare scores excluding corticosterone concentrations for Flock A

Flock B	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Std. Error
Plumage Score (0-2)	0	2	1.6	0.04
Footpad Score (0-2)	0	2	0.4	0.05
Keel Bone Score (0-1)	0	1	0.4	0.04
Weight (kg)	1.1	2.6	2.0	0.02
Comb Score (1-7)	5	6	5.6	0.04
Beak (1-3)	1	3	1.8	0.07

Table 6-6 Descriptive statistics of physiological welfare scores excluding corticosterone concentrations for Flock B

For Flock A, comb colour was correlated with duration and frequency of range access, meaning that hens that spent more time in the range or accessed it more frequently had darker comb (Table 6-7). Weight was also positively correlated with the number of days of range access. None of the other measures was significantly correlated with duration or frequency of range access.

Between physiological and anatomical measures for Flock A, faecal and plasma corticosterone concentrations were correlated, meaning that hens with a higher baseline level of corticosterone also had a higher level of acute stress response (10 min post-stressor). Plumage condition was correlated with plasma and faecal corticosterone concentration, meaning that hens with higher levels of chronic stress and higher levels of acute stress response to a stressor had worse plumage condition. Keel bone deformation was negatively correlated with food pad dermatitis, meaning that hens had either some evidence of keel bone deformation or some evidence of foot pad dermatitis, which could be related to perching or activity.

For Flock B, beak condition was negatively correlated with duration and frequency of range access, meaning that hens with poorer beak condition accessed the range less (Table 6-8). Plumage condition was negatively correlated with duration of range access, meaning that hens with poorer plumage condition accessed the range for shorter periods of time. Plasma corticosterone concentration was positively correlated with frequency of range access, meaning that hens that accessed the range more often overall had a higher level of acute stress response to a stressor. Conversely, faecal corticosteroid metabolites' concentration was negatively correlated with the total duration of range access, meaning that hens that spent more time in the range overall showed evidence of a reduced chronic stress response. Weight was also positively correlated with the number of days of range access and the frequency of range access.

Between physiological and anatomical measures for Flock B, keel bone deformation was negatively correlated with beak condition, meaning that hens had either some evidence of keel bone deformation or some evidence of poor beak condition.

Correlations between frequency and duration of range access differ from those presented in Section 6.3.1, because not all hens were sampled for welfare measures – Flock A: 353 hens ranging data and 141 hens welfare data (including 18 non-rangers); Flock B: 309 hens ranging data and 150 hens welfare data (including 5 non-rangers).

Table 6-7 Correlations (r-values) between physiological and anatomical measures and overall range access for Flock A

Correlations

	H_L	Plasma_Cort	Faecal_Cort	Comb	Beak	Plumage	Footpad	Keel	Weight	Ranging_ Days	Ranging_ Min	Ranging_ Freq
H_L	1.000	-.056	-.084	.010	-.130	-.013	.013	.026	-.062	-.154	-.055	-.029
Plasma_Cort	-.056	1.000	.340**	.026	-.122	.213*	.124	-.127	-.137	.064	.051	-.039
Faecal_Cort	-.084	.340**	1.000	-.086	-.117	.230*	.047	.084	.014	.110	-.034	-.044
Comb	.010	.026	-.086	1.000	-.105	-.045	-.057	.041	.104	.319**	.289**	.485**
Beak	-.130	-.122	-.117	-.105	1.000	-.049	.043	.134	.089	-.030	.045	-.072
Plumage	-.013	.213*	.230*	-.045	-.049	1.000	.020	.080	.037	.058	-.073	-.098
Footpad	.013	.124	.047	-.057	.043	.020	1.000	-.174*	-.101	.019	-.021	-.078
Keel	.026	-.127	.084	.041	.134	.080	-.174*	1.000	.015	-.036	-.066	-.078
Weight	-.062	-.137	.014	.104	.089	.037	-.101	.015	1.000	.243**	.072	.117
Ranging_Days	-.154	.064	.110	.319**	-.030	.058	.019	-.036	.243**	1.000	.734**	.745**
Ranging_Min	-.055	.051	-.034	.289**	.045	-.073	-.021	-.066	.072	.734**	1.000	.846**
Ranging_Freq	-.029	-.039	-.044	.485**	-.072	-.098	-.078	-.078	.117	.745**	.846**	1.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 6-8 Correlations (r-values) between physiological and anatomical measures and overall range access for Flock B

Correlations

	H_L	Plasma_Cort	Faecal_Cort	Comb	Beak	Plumage	Footpad	Keel	Weight	Ranging_Days	Ranging_Min	Ranging_Freq
H_L	1.000	.095	.041	-.010	.086	.097	-.017	-.030	-.128	-.057	.018	.000
Plasma_Cort	.095	1.000	.156	-.145	.075	-.036	.039	.058	.003	.013	.111	.163*
Faecal_Cort	.041	.156	1.000	-.130	.105	.042	.102	.055	.061	-.134	-.198*	-.168
Comb	-.010	-.145	-.130	1.000	.127	-.080	.112	-.007	.150	.045	.073	.033
Beak	.086	.075	.105	.127	1.000	.128	.079	-.172*	-.029	-.158	-.198*	-.238**
Plumage	.097	-.036	.042	-.080	.128	1.000	.086	.100	.100	-.111	-.247**	-.156
Footpad	-.017	.039	.102	.112	.079	.086	1.000	-.016	-.053	-.046	.077	.130
Keel	-.030	.058	.055	-.007	-.172*	.100	-.016	1.000	.033	-.057	-.023	.035
Weight	-.128	.003	.061	.150	-.029	.100	-.053	.033	1.000	.215**	.043	.168*
Ranging_Days	-.057	.013	-.134	.045	-.158	-.111	-.046	-.057	.215**	1.000	.581**	.490**
Ranging_Min	.018	.111	-.198*	.073	-.198*	-.247**	.077	-.023	.043	.581**	1.000	.555**
Ranging_Freq	.000	.163*	-.168	.033	-.238**	-.156	.130	.035	.168*	.490**	.555**	1.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

6.3.3 Ranging categories: non-ranger, occasional ranger, high ranger

6.3.3.1 Hen categorisation

Few differences were found when hens were categorised on the basis of the overall time spent on the range, with the classification being non-ranger (never outside over the course of the study), occasional ranger (low to medium total duration spent in the range), high ranger (high duration spent in the outdoor range over the course of the study).

For Flock A, non-rangers (n=22) never accessed the outdoor range (including wintergarden), occasional ranger (n=34) spent up to 41 h outside over the course of the study and high rangers (n=73) spent from 41 to 82 h outside.

Comb colour differed between ranging categories ($P < 0.001$), with high rangers having darker combs than occasional rangers (6.05 ± 0.06 vs. 5.60 ± 0.13, P = 0.001), which themselves had darker combs than non-rangers (4.71 ± 1.19, $P < 0.001$).

For Flock B, non-rangers constituted only 4 hens, occasional rangers spent up to 28 h outside (n=59), and high rangers spent between 28 and 57 h (n=86) outside.

Weight differed between ranging categories (P = 0.009), with high rangers being heavier than occasional rangers (1.99 ± 0.02 vs. 1.88 ± 0.05, P = 0.035), non-rangers being no different from either groups (1.82 ± 0.09).

6.3.3.2 Fear behaviour: tonic immobility, open field and novel object tests

Tonic immobility tests were performed on randomly selected hens prior to assessing range use (Flock A: n=129, including 22 non-rangers; Flock B: n=94, including 4 non-rangers), and welfare assessments, open field and novel object tests were performed on randomly selected hens after assessing range use (Flock A: n=141, including 18 non-rangers; Flock B: n=150, including 5 non-rangers).

Tonic immobility durations in Flock A were higher for non-rangers (254.3 ± 14.0 s) than occasional ranger (159.4 ± 18.0 s; X^2 = 8.55, P = 0.003) and high ranger hens (185.6 ± 13.0 s; X^2 = 5.13, P = 0.024). However, there was no significant difference in TI durations between occasional and high ranger hens (X^2 = 1.66, P = 0.198). In Flock B there were no significant differences tonic immobility durations between any of the groups, but it is important to note that only 4 non-rangers were present, hence an insufficient sample size.

Latency to move in the open field test was higher for non-rangers hens (115.9 ± 19.9 s) than the occasional ranger (37.4 ± 6.1 s) and the high ranger hens (31.5 ± 5.5 s, $P < 0.001$), but there was no difference between the occasional and high ranger hens. High ranger hens crossed more lines (i.e. moved more) in the open field test (8.6 ± 1.1 lines crossed) than the non-ranger hens (1.5 ± 0.5 lines crossed, P = 0.001), but not the occasional ranger hens (5.7 ± 0.9 lines crossed, P = 0.08). There was no difference between the occasional ranger and non-ranger hens

Latency to move in the novel object test was also higher for non-ranger hens (63.6 ± 13.4 s) than the occasional ranger (11.8 ± 2.0 s, $P < 0.001$) and the high ranger hens (22.2 ± 4.7 s, P = 0.004). There was no difference in the latency to move between the high and occasional ranger hens (P = 0.06). The occasional ranger (4.5 ± 0.7 lines crossed) and high ranger (4.35 ± 0.7 lines crossed) hens crossed more lines (i.e. moved more) than the non-rangers (0.8 ± 0.3 lines crossed, P = 0.03 and P = 0.04, respectively), occasional and high ranger hens not being different from each other. There were no differences in the latency to peck the novel object between all groups; however, there were only seven hens that pecked the novel object. Open field and novel object tests for Flock B are yet to be analysed.

6.4 Summary of ranging behaviour and implications for hen welfare

This experiment provided the first detailed scientific account of ranging behaviour on an Australian commercial egg farm through RFID tracking of individual free range laying hens in a sub-flock population.

The large majority of tracked hens (85 to 97%) accessed the range over the course of the 10 to 13 days of tracking. Furthermore, a majority (69% and 82%) accessed the range every day. These findings are similar to previous RFID studies conducted using experimental flocks (Campbell et al., 2016; Gebhardt-Henrich et al., 2014; Richards et al., 2011; Hartcher et al., 2015; Hinch et al., 2014). It supports the body of evidence that assessing range use at individual hen level reveals much larger estimates of range use than assessment at flock level (i.e. the proportion of the flock seen outside at any one time).

The majority of hens spent about 2 to 5 hours in the range daily, out of 8 hours permissible access, visiting the range between 5 and 20 times a day. Hence, hens make regular moves between the shed and the range. Furthermore, through the placement of antennas at various distances from the shed, we also found that the majority of hens (74% and 85%) ranged further than 9 m from the shed, and made regular changes between the three zones monitored, about every 15 to 20 min. This suggests that hens are quite mobile while ranging. However, as reported in the literature (Zeltner & Hirt, 2003; Hegelund et al., 2006), they spend most of their time close to the shed, in the wintergarden area. It would be interesting to assess the frequency of range access or time spent close to the shed between sheds that offer a wintergarden vs. those that do not.

We observed variation in the frequency and duration of range access between hens. Nevertheless, there was no evidence that hens differ noticeably in their ranging pattern, in terms of some hens that could have preferred short frequent visits or conversely sparser but longer visits.

This study provided new knowledge in terms of identifying the relationship between individual ranging behaviour and hen welfare measures, through a comprehensive assessment with the most widely-accepted behavioural and physiological measures of hen welfare: blood corticosterone concentration (reflective of the acute stress response), faecal corticosteroid metabolites (chronic stress response), heterophil:lymphocyte ratio (immune system response), plumage condition, weight, keel bone deformation, foot pad dermatitis, tonic immobility and fear tests (fear responses), comb colour and beak condition. Despite this battery of measures, relatively few significant associations were found with ranging behaviour in terms of the number of days the range was accessed, or the frequency or duration of range access. Furthermore, most significant correlations were relatively weak (r values around 0.20), hence with ranging behaviour only explaining only about 4% of the variation in these welfare measures, and inconsistent across flocks. Therefore, these data could be interpreted to suggest that range access had little effect on the measures of hen welfare collected in this study. This is also supported by RFID tracked hens in experimental flocks showing only subtle differences in physiological and behavioural measurements between outdoor preferring hens, indoor preferring hens and weak outdoor preferring hens (Campbell et al., 2016; Hinch & Lee, 2014). Alternatively, it cannot be ruled out that hens accessed the range as a means of self-regulating their welfare needs. In other words, it could be hypothesised that the variation in range access observed between individual hens reflected their decision to use the range to balance their needs. This is similar to studies showing that meat chickens and other animals can self-medicate by consuming food with analgesics when in pain (Danbury et al., 2000).

A few behavioural (e.g. hen characteristic) differences were found between hens that did not use the range and those that did, in terms of fear. In Flock A, hens that did not range had higher tonic immobility duration and a longer latency to move and less movements in the open field and novel object tests. This supports the hypothesis that hens that do not venture outdoor are more fearful, in agreement with Hartcher et al. (2015). Campbell et al. (2016) also found that indoor preferring and moderate-outdoor hens took longer to move, and moved less overall in open field tests compared to outdoor preferring hens, but found no difference in physiological or behavioural measurements of fearfulness in manual restraint or tonic immobility tests.

Although some parameters varied quite consistently between flocks (such as a narrower variation between hens in Flock B, 65 weeks of age, compared to Flock A, 45 weeks of age), it is not possible to compare the two flocks or conclude that these differences are age-related, because these flocks, despite being on the same farm, differed in more than one variable (e.g. history of the flock, shed cardinal orientation, range characteristics, management, time of the year).

This study contained limitations that should be kept in mind. Both flocks were followed for a period of two weeks, and therefore greater differences could possibly be observed if hens were followed over a longer period. Unfortunately, we were limited by equipment availability in the present study. Because of this short period, we could only assess correlation between ranging behaviour and hen welfare, but not infer causality (i.e. that ranging did cause such and such changes). This could be circumvented in the future by providing access to some hens and not others, or follow a flock starting prior to range access, although there will always be an age-related confounding factor in that last experimental design. Several other factors may affect ranging behaviour (e.g. weather conditions, breed, rearing period and initial range access training, presence of wintergarden or not, vegetation and other range characteristics, pop hole design and placement, etc.). Therefore, this result cannot be extrapolated to all other free range egg farms.

The detailed knowledge derived from this study revealed that the ranging behaviour of free range hens on a commercial farm is complex. Overall, a large majority of free range hens on the farm studied access the range regularly, for extended periods of time and with regular movements between various areas of the range. Despite this extensive ranging behaviour, there was little and inconsistent evidence that range access led to differences in measures of hen welfare.

7 Experiment 6: Behaviour of free range laying hens in various areas

This study has been submitted for peer-review publication to the journal Applied Animal Behaviour Science, but subsequently rejected, principally on the basis that the study only comprised of one flock. It will be resubmitted as a short communication to a different journal.

7.1 Abstract

Despite the increasing demand for free range eggs, the way laying hens utilise the range remains poorly understood. Due to the differing features of various areas in free range systems, it is important to understand the utilisation of these areas by the hens to optimise free range design. This study investigated the behaviour of free range laying hens in various areas of a commercial free range farm. A flock of 2000 Hy-line Brown laying hens were observed in six distinctive areas of the farm. Video footage was recorded every 20 minutes from 1000h to 1800h in two indoor areas (next to the indoor pop hole and in the middle of the shed) and four outdoor areas (wintergarden, close range, mid range, far range) using cameras over seven days and a scan focal sampling method. Whilst in the open range, hens mostly foraged and moved around ($P < 0.05$). The behaviour of hens in the wintergarden showed similarities to both indoor and outdoor areas, spending on average 34% of their time displaying various comfort behaviours (e.g. preening, dust bathing) and 36% of their time exploring and foraging. Preening behaviour was observed predominantly indoors and in the wintergarden ($P < 0.05$). In conclusion, free range laying hens do utilise various areas of the range differently. The outdoor area was most conducive to exploratory behaviours and greater foraging opportunities where vegetation was present. The covered indoor area was conducive to various comfort behaviours, probably as it provided refuge, which was not offered in the range.

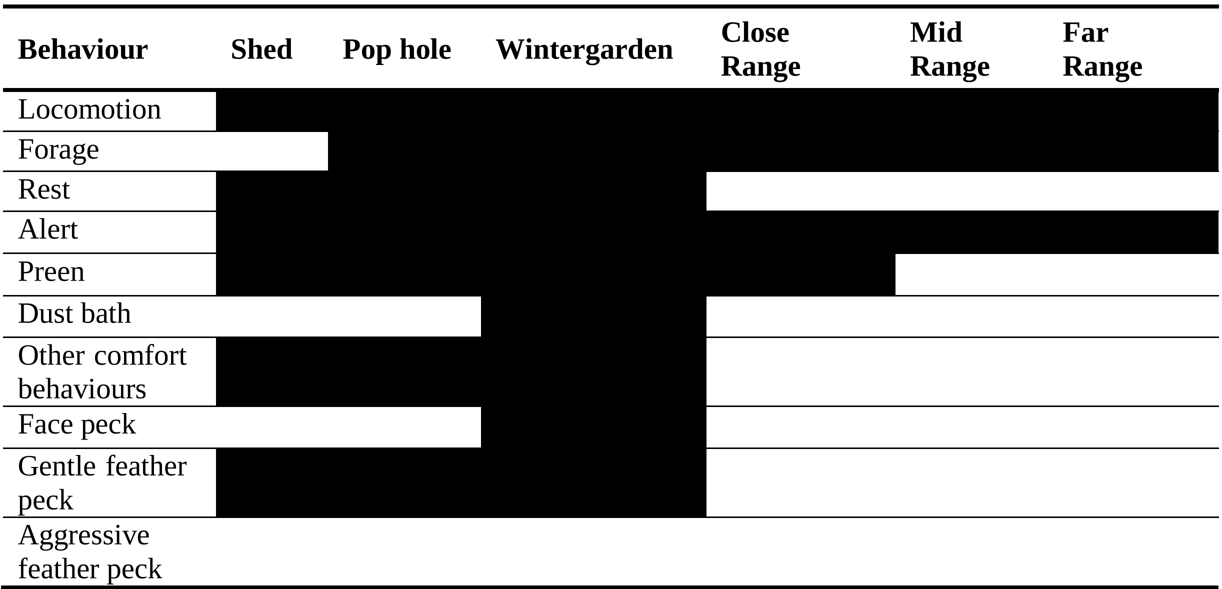


Figure 7-1 Graphical abstract of hen behaviour indoor and outdoor

Presence (filled) or absence (blank, <0.01% of focal hens) of the behaviours displayed according to the indoor (shed, pop hole) and outdoor (wintergarden, close range, mid range, far range) areas. Behaviours that were area-specific due to resource placement are not shown (feed, drink, perch, enter or exit pop hole).

7.2 Introduction

Free range systems allow animals access to an outdoor range supposedly to promote the expression of a wider range of behaviours. Free range egg production systems have been recently expanding worldwide – for example, contributing 49% of the Australian retail market value (AECL, 2015). Free range systems provide hens the opportunity to perform various 'natural' behaviours such as foraging and dust bathing. Therefore, consumers perceive that providing opportunities for such behaviours can provide animal welfare benefits. However, the actual use of the outdoor range and the 'natural' behaviours displayed by laying hens in free range systems remain poorly understood.

Foraging behaviour contributes to a large proportion of the hens' daily activities (Dawkins, 1989; Cooper & Albentosa, 2003). When pasture or litter is present, birds will forage despite being offered regular feed (Duncan & Hughes, 1972; Appleby et al., 1989). The diversity of vegetation present in the range may promote a variety of foraging behaviours including pecking and scratching whilst the presence of insects and other small animals may motivate predation and digging. However, the willingness to explore and forage the range is dependent on the hens' perception of the range. For example, hens may be reluctant to utilise the range when there are greater perceived costs than benefits. Such costs include the lack of refuge or shelter from predation and severe climatic conditions (Hegelund et al., 2005). The presence of novel features in the range can also elicit a fear response characterised by avoidance and withdrawal from entering the range (Murphy and Wood-Gush, 1978). Despite being regarded as high priority behaviour in hens, the readiness to forage and explore is influenced by numerous factors including the presence of shelter and fearfulness; hence it could be said to be context-dependent, especially dependent on the availability and quality of foraging substrates.

The motivations for comfort and grooming behaviours are influenced by internal (e.g. hormones, neuropeptides, opioids) and external (e.g. environmental moisture, dirt, parasites) factors (Vezzoli et al., 2015). Comfort and grooming behaviours are performed whilst hens can afford to be non-vigilant, often in groups and sheltered areas. However, when present in high-density groups, aggressive feather pecking and cannibalism can become major concerns that greatly compromise animal welfare in such systems (Rodenburg et al., 2013). Aggressive feather pecking results in loss of feathers or tissue damage leading to more severe welfare concerns and even mortality. The current cause of feather pecking remains unknown, and has been hypothesised as a redirected foraging behaviour where there is a lack of foraging opportunities (Huber-Eicher & Wechsler, 1998), which therefore could be influenced by range use or range quality, or hyperactivity (Kjaer et al., 2015).

Behaviour is a useful approach in determining how laying hens interact with and utilise the environment. Behavioural responses provide an indication of positive (e.g. dust bathing) and negative welfare states (e.g. fear-related behaviour), and as such are useful to assess the implications of keeping laying hens in free range production systems. The aim of this study was to determine how hens utilise various areas of a free range farm, using behavioural observations both indoors and outdoors, in order to elucidate the welfare implications of keeping laying hens in free range systems and to suggest improvements.

7.3 Materials and methods

This experiment was approved by the University of Melbourne Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

7.3.1 Subjects and study site

The study was conducted on a free range egg commercial farm, in winter with an average maximum temperature of 10°C during the study period according to the Australian Bureau of Meteorology closest weather station. Out of the overall flock of 18,000 38 week-old Hy-line Brown laying hens, a fence was installed to segregate a study flock of approximately 2000 hens, keeping an indoor stocking density of approximately 12.1 hens per square meter (5.5 × 30 m shed, not including nest box space), with plastic slatted floors and access to perches inside. The flock was given two weeks to habituate to this change. Access to feed and water was provided only in the shed *ad libitum* through an automated dispensing system. The hens were fed a feed source of complete layer pellet, sourced by the farm to meet all nutritional requirements. Nest boxes were provided only in the shed.

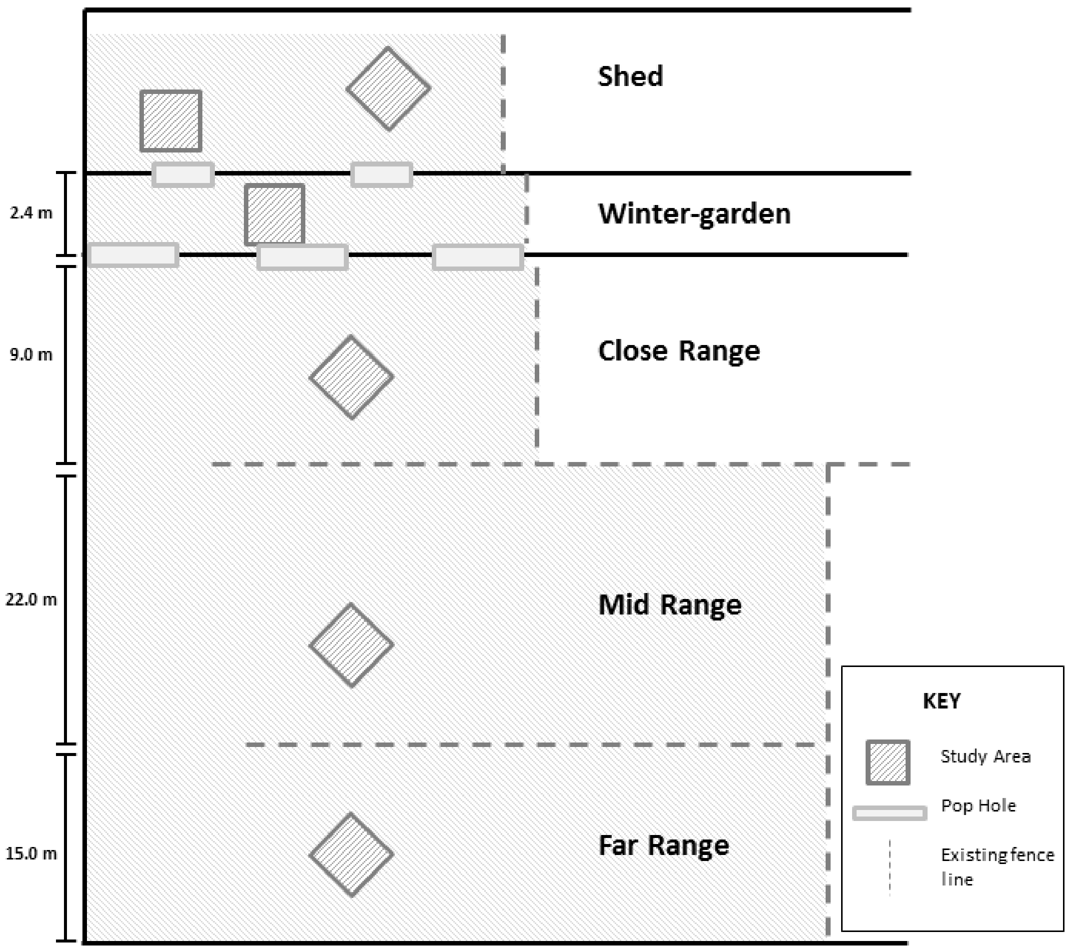


Figure 7-2 Diagram of the study site indicating sub-ranges and specific study areas within the range

Diagram is not drawn to scale.

Access to the wintergarden (2.4 × 22.5 m for the study flock), through two pop holes (2 × 0.45 m), and from the wintergarden to the outdoor range (46 × 22.5 to 56.7 m), through three pop holes (3.65 × 0.45 m) (Figure 7-2) were opened between 1000h to 1800h from the time hens were 21 weeks of age. The wintergarden, also called veranda, served as an intermediate zone between the indoor and the outdoor environment, providing shade and shelter for the hens whilst remaining outdoors. In the wintergarden were two 1 m × 0.5 m haystacks that were evenly spaced and litter ground cover, which consisted of manure mixed with straw. The close range started immediately outside the wintergarden and extended 9 m into the range with no vegetation but large stones of varying sizes and gravel distributed unevenly on bare earth. The mid range followed expanding a further 22 m into the range, covered with large stones of varying sizes distributed unevenly on bare earth along the fence lines and small grasses and Juncaceae plants sparsely distributed closer towards the far range. Due to the wet weather season for the study period, there were rain puddles (0.5 m^2 to 5 m^2) randomly distributed throughout the mid range. The far range, extending a further 15 m, possessed denser vegetation comprising again of small grasses and Juncaceae plants spaced every 50-100 cm, and was used according to a rotational pasture schedule to allow that vegetation to regenerate. The outdoor range stocking density was 1500 hens per hectare.

7.3.2 Experimental set up

Six Buckeye Cam X80 video cameras (Buckeye Cam, Athens, OH, USA) were used. Two cameras were set up inside the shed. One camera was attached to existing indoor shed structures on the roof, aimed downwards to observe the area around the pop hole inside the shed to identify how hens utilised this area; whether hens exclusively used the area to enter and exit the shed or other additional behaviours that were displayed. This indoor pop hole area included the feeder line which was unavoidable due to the farm layout. The second indoor camera for the shed area was attached to the temporary fence approximately 1.2 m from the ground, pointing down toward the middle of the shed, and including the drinker line in the field of view. The remaining four cameras were set up in the wintergarden (approximately 63 cm from the shed), close range (approximately 7.4 m from the shed), mid range (approximately 29 m from the shed) and far range (approximately 32 m from the shed) (Figure 7-2). The camera in the wintergarden was attached to the roof and all remaining outdoor cameras were attached to a pole approximately 1.5 m high. The study areas were chosen to best represent the different environments. Fences and physical structures present in the range (i.e. haystacks) were avoided in the study areas to control for variations due to resources, as hens tend to be attracted to and utilise physical structures and resources (Rault et al., 2013), which was not the focus of this study.

All cameras were placed so that the field of view observed a 2 × 2 m area. Study areas were marked using traffic cones to specify the area of interest during sampling and to record the 2 × 2 m area covered on the video screen, and cones were subsequently removed during behavioural recording. At the onset of data collection, recordings began at 1000h for 10 seconds every 20 minutes until 1800h for all cameras. The purpose of 10-second recordings was to assist in behaviour identification, as it can be difficult to score behaviours correctly at times using still pictures. Video recordings were collected over seven consecutive days.

7.3.3 Behavioural analysis

Once all recordings were collected, data analyses were conducted by one observer. Video footage was played and behavioural scorings were conducted using scan focal sampling. For each video, five focal hens were haphazardly chosen using the grid method (Dawkins, 2007). An online random number generator was used to specify focal hens, to reduce observer bias. If the same focal hen was randomly chosen again within the same recording, the nearest hen was used as the new focal bird. The number of focal birds was also recorded, as some time points had less than five focal birds present, in which case the maximum number of birds present was studied.

Scoring of behaviours was based on a behavioural ethogram (Table 7-1). Behaviours that could not be identified due to the positioning of the bird (i.e. the head was not clearly visible due to the positioning of the bird) or being hidden behind another bird were scored as unidentified.

Table 7-1 Ethogram used for behavioural observations

Behaviour	Description
Locomotion	Movement at any speed including wing flapping to or from a conspecific/location, but movement as a component of foraging or entering/exiting pop hole not included.
Exiting pop hole	Movement at any speed including wing flapping to exit pop hole in the direction out of the shed.
Entering pop hole	Movement at any speed including wing flapping to enter pop hole in the direction into the shed.
Foraging	Pecking or scratching the ground, tree or air whilst in a still position or walking with head situated below midline of the body.
Alert	Standing, lying down or sitting still with neck extended vertically and eyes opened.
Resting	Standing, sitting or lying down in a non-vigilant state, head not outstretched and eyes can be opened or closed. Head may be tucked under wing or flat on ground.
Preening	Raising feathers and arranging the feathers with the beak in either a sitting or standing position.
Dust bathing	Lying with head and neck rubbing on floor, scratching the floor, open wings and vertical wing shaking.
‘Other’ comfort behaviour	Head shake, tail wag, wing flap, wing stretch, scratch self with leg or feather raise/crop adjustment excluding resting, perching, preening and dust bathing behaviours.
Perching	Resting standing up or sitting with both feet on the drinker or feeder line.
Aggressive feather pecking	Forceful downward peck at any part of conspecifics leading to feather loss or tissue pecking.
Gentle feather pecking	Gentle downward peck at any part of conspecific’s body excluding the head without removal of feathers.
Face pecking	Gentle pecking at any part of conspecific’s head.
Feeding	Neck extended, pecking at the feeder. Only present in the pop hole area where feeder is provided.
Drinking	Neck extended, drinking from the drinker or puddles present in the range.
Unidentified	Behaviours that could not be identified due to the positioning of the bird, or the bird was partially hidden by conspecifics or other structures.

A number of behaviours were adapted and modified from Larsen and Rault (2014).

7.3.4 Statistical analyses

Data were analysed using Minitab (v17, Minitab Pty Ltd, Sydney NSW, Australia). The proportions of focal birds displaying each behaviour were tested in a general linear model ANOVA, which included the effects of area (shed, indoor pop hole, wintergarden, close range, mid range, far range), day (D1-D7), time (1000h-1800h), the total number of birds and the number of focal birds present. Day and time never showed significant effects. Residual plots were constructed using the general linear model ANOVA to check that the criteria of normality of data and homogeneity of variance were met. The significance level for all analyses was set at $P < 0.05$, and where significant differences between areas were detected, the Tukey's pair wise comparison method was applied to determine significant differences between specific areas, accounting for the number of comparisons. The correlations between the average proportion of hens displaying each behaviour and the total number of birds present at each area were analysed using Pearson's correlation. For data that were not normally distributed ('other' comfort behaviours, dust bathing, face pecking and gentle pecking), a Kruskal-Wallis non-parametric test was used.

7.4 Results

7.4.1 Number of hens present at each zone

In total, 1050 video recording scans were collected. A sum of approximately 10,841 hens was observed for all recordings and 2780 focal individuals were sampled for behavioural observation.

Table 7-2 Mean number of hens present at each area (± SE of means) at any given time

Area	Shed	Pop hole	Wintergarden	Close Range	Mid Range	Far Range
Mean number of hens observed	20.6 ± 1.1^{a}	18.8 ± 0.9^{b}	21.4 ± 1.2^{a}	0.3 ± 0.1^{c}	0.6 ± 0.2^{c}	0.3 ± 0.1^{c}

Significant differences ($P < 0.05$) between areas are indicated by different letters (a-c).

Hen distribution differed between areas ($P < 0.001$; Table 7-2), with more hens present in the wintergarden and shed, followed by the indoor pop hole area, and much fewer hens in the various areas of the range: close range, mid range and far range (all $P < 0.001$). Indeed, in the various areas of the range, a maximum of five hens or more at any given time was only seen in 0.01% of the observations.

7.4.2 Active and exploratory behaviours

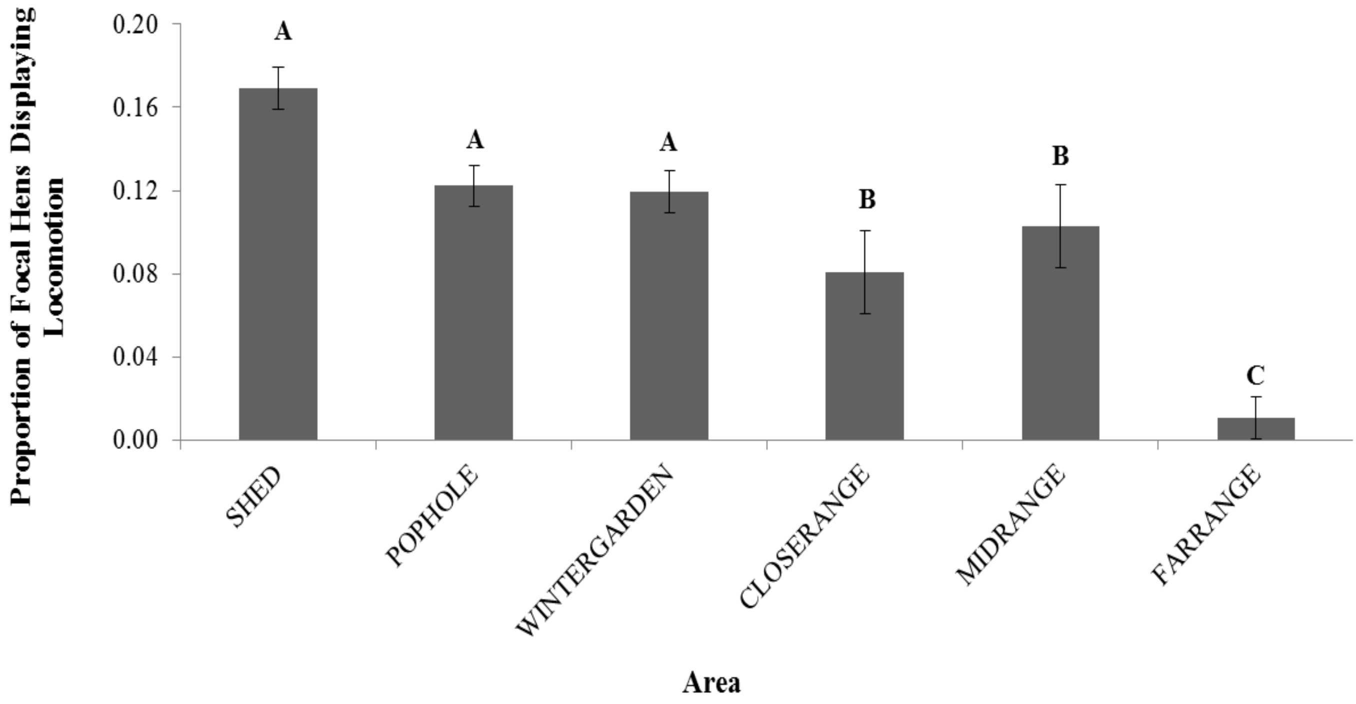


Figure 7-3 Proportion of focal hens displaying locomotion at each area (± SE of means)

Significant differences ($P < 0.05$) between areas are indicated by different letters (A-C).

Locomotion differed between areas ($P < 0.001$, Figure 7-3), with hens moving around more frequently in the shed, indoor pop hole and wintergarden areas than in the close range and mid range areas (all $P \leq 0.04$), and least in the far range area (all $P < 0.04$). The proportion of focal hens displaying locomotion decreased as the total number of hens present increased ($r = -0.64$, $P = 0.005$).

Foraging differed between areas (P < 0.001; Figure 7-4), with hens foraging more frequently in the wintergarden and far range areas, followed by the close range and mid range, and finally the indoor pop and shed areas (all P < 0.02). The proportion of focal hens foraging tended to decrease as the total number of hens present increased (r = -0.64, P = 0.07).

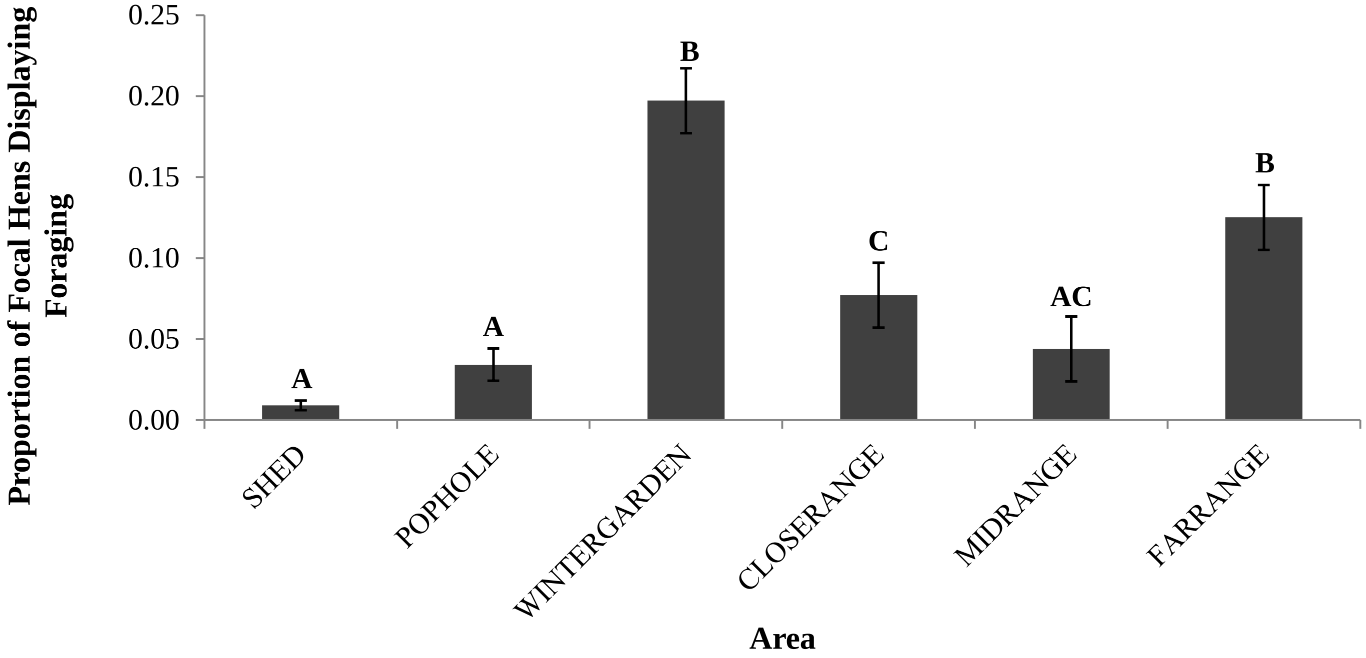


Figure 7-4 Proportion of focal hens displaying foraging at each area (± SE of means)
Significant differences (P < 0.05) between areas are indicated by different letters (A-C).

7.4.3 Comfort and maintenance behaviours

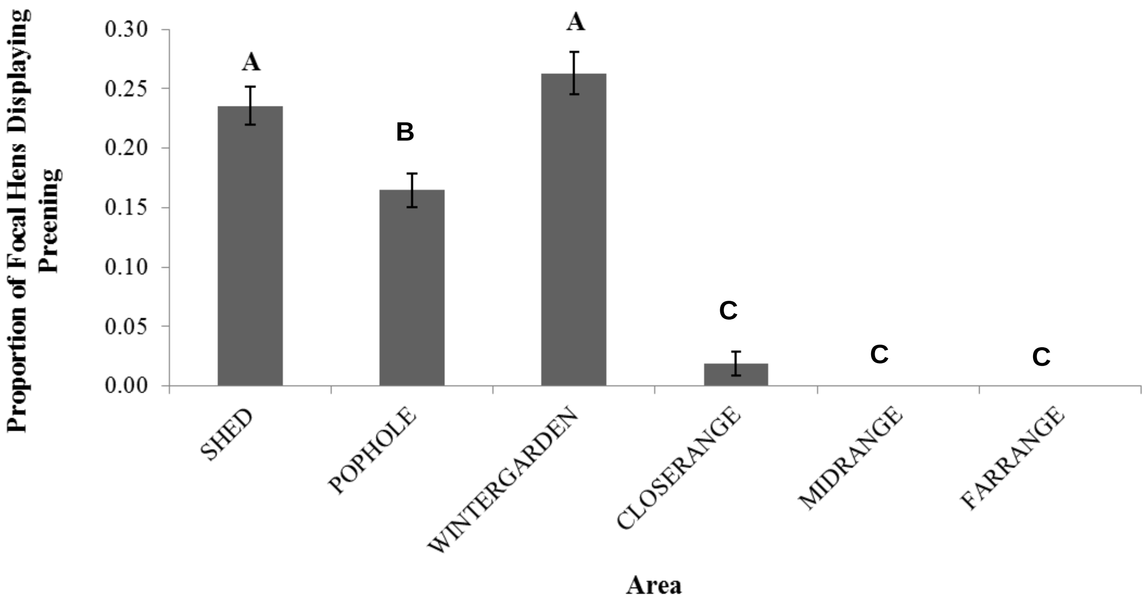


Figure 7-5 Proportion of focal hens displaying preening at each area (± SE of means)
Significant differences (P < 0.05) between areas are indicated by different letters (A-C).

Preening differed between areas ($P < 0.001$, Figure 7-5), with hens preening more frequently in the wintergarden and shed areas than in the close range, mid range and far range areas where it was very rarely seen (all $P < 0.0003$), the indoor pop hole area being intermediate. The proportion of focal hens preening increased as the total number of hens present increased ($r = 0.51$, $P < 0.001$).

Resting differed between areas ($P < 0.001$, Table 7-3), with hens resting more frequently in the wintergarden than in shed or indoor pop hole areas, and being lowest in the range areas.

Dust bathing and other comfort behaviours did not differ between areas (Table 7-3).

Table 7-3 Behaviours performed at each area (LS-means ± SEM)

Behaviours	Shed	Indoor pop hole	Wintergarden	Close Range	Mid Range	Far Range	P-Value
Alert	0.04 ± 0.019	0.03 ± 0.018	0.04 ± 0.018	0.04 ± 0.032	0.04 ± 0.031	0.05 ± 0.032	0.77
Resting	0.02 ± 0.009^{a}	0.02 ± 0.009^{a}	0.04 ± 0.008^{b}	0.01 ± 0.015^{c}	0.01 ± 0.015^{c}	0.01 ± 0.015^{c}	< 0.001
Dust bathing*	0.00 ± 0.000	0.00 ± 0.000	0.03 ± 0.008	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.28
'Other' comfort*	0.02 ± 0.004	0.01 ± 0.003	0.01 ± 0.003	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.69
Perching	0.00 ± 0.012^{a}	0.08 ± 0.012^{b}	0.00 ± 0.012^{a}	0.00 ± 0.021^{a}	0.00 ± 0.020^{a}	0.00 ± 0.021^{a}	< 0.001
Gentle feather pecking*	0.01 ± 0.004^{a}	0.01 ± 0.004^{a}	0.04 ± 0.007^{b}	0.00 ± 0.000^{c}	0.00 ± 0.000^{c}	0.00 ± 0.000^{c}	0.03
Face pecking*	0.00 ± 0.001	0.00 ± 0.000	0.01 ± 0.003	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.98
Unidentified behaviour	0.17 ± 0.032^{a}	0.12 ± 0.032^{b}	0.06 ± 0.031^{c}	0.01 ± 0.055^{abc}	0.01 ± 0.054^{bc}	0.02 ± 0.055^{abc}	< 0.001

* A nonparametric Kruskal-Wallis test was used, with the means presented as raw means.
a-c Means in a row not sharing a common superscript differ ($P < 0.05$).

7.4.4 Social interactions: pecking behaviours

Gentle feather pecking differed between areas (P = 0.03, Table 7-3), with hens performing more gentle feather pecking toward a conspecific in the wintergarden than in the shed and indoor pop hole areas ($P < 0.05$), whereas it was not observed in the close range, mid range and far range ($P < 0.05$). The proportion of focal hens displaying gentle feather pecking increased as the total number of hens present increased (r = 0.25, $P < 0.001$).

Aggressive feather pecking was never observed. However, few occurrences of face pecking were observed but did not differ between areas (Table 7-3).

7.4.5 Other behaviours

Behaviours that were recorded as unidentified (i.e. the view of the focal hen was obstructed by conspecifics or the positioning of the focal hen) differed between areas ($P < 0.001$, Table 7-3), being higher in the shed area, then pop hole area followed by wintergarden, and lowest in the close range, mid range and far range areas (all $P < 0.05$). The proportion of unidentified behaviours increased as the total number of hens present increased (r = 0.70, $P < 0.001$).

All other behaviours were relatively rare or area-specific for obvious reasons. Alert did not differ between areas. In the indoor pop hole area, entering the shed (0.019 ± 0.002%) and exiting the shed (0.018 ± 0.002%) were both relatively infrequent behaviours. Perching differed between areas ($P < 0.001$), being higher in the indoor pop hole area where perches were provided, compared to other areas. Feeding was only seen in the indoor pop hole area (0.20 ± 0.007%), as the only area which had the feeder line in the field of view. Drinking was observed inside the shed (0.18 ± 0.01%), as the only area which had the drinker line in the field of view, but also in the mid range area (0.13 ± 0.01%), where temporary rain puddles were present.

7.5 Discussion

7.5.1 The outdoor range

Hens predominantly utilised the outdoor range area for foraging and exploration. In the close range, similar frequencies of locomotion and foraging behaviour were observed. However, in the mid range there was twice as much locomotion as foraging, whilst this trend was reversed in the far range where foraging was more prominent than locomotion. Increased locomotion in the mid range suggests that hens used it as a transition zone while foraging in the far range. Foraging is high priority behaviour in the Red Junglefowl and domesticated laying hens seem to have conserved that trait (Dawkins, 1989) and expend the extra efforts foraging even when provided regular feed, a phenomenon known as contrafree-loading (Duncan & Hughes, 1972). Foraging in the far range may have been motivated by the presence of more vegetation, as foraging was lower in the mid range, only a few metres away but with much sparser vegetation. The available space provided in the range may also enable the display of foraging, and exploratory behaviours as these behaviours require a larger area to perform (Keeling, 1994), which is also supported by the negative correlation between the number of hens present in the area and both foraging and locomotion behaviours.

7.5.2 The wintergarden

Current literature and regulatory policies are contradictory when referring to the wintergarden, as it has been considered both as an indoor or outdoor environment for the hens. Findings from this study suggest similarities to both environments based on the hens' behaviour. The wintergarden promoted both comfort and exploratory behaviours. The wintergarden was also characterised by a large bird density suggesting that hens have a high preference for this area. Cover provided birds with refuge (Larsen et al., 2014; Rodriguez-Aurrekoetxea et al., 2015), which in combination with high bird density enables hens to be non-vigilant whilst performing various comfort behaviours. Furthermore, the wintergarden also promoted high foraging activities likely due to the presence of litter (Appleby et al., 1989).

A distinctive difference observed in this study, between the wintergarden and other areas, is the display of dust bathing behaviour. Dust bathing behaviour is influenced by the quality and availability of suitable dust bathing substrate, which usually consists of small, dry particles such as dust or sand to assist in the removal of excess lipids from plumage (Campbell et al., 2015). Consequently, flocks that were only provided wire flooring were found to dust bathe less frequently than when provided with sand (Nørgaard-Nielsen, 1997). Therefore the presence of dry litter cover in the wintergarden may motivate dust bathing in hens, although it did not differ from other areas in our study and was rarely observed, possibly due to the recording method used, which may have missed dust bathing bouts with the 20-min scan sampling interval.

7.5.3 The indoor environment

In this study, hens tended to utilise the indoor areas and the wintergarden in larger numbers than the outdoor range, likely a reflection of their time budget. The high bird density portrayed in the shed may indicate the reluctance in hens to roam the outdoor environment or the high preference in hens to remain in a social group as individuals deem this environment the safer option (Rodriguez-Aurrekoetxea et al., 2015). However, overcrowding in hens can lead to various negative impacts and ultimately compromise hen welfare. Despite no observation of aggressive feather pecking, it cannot be concluded that agonistic behaviours between conspecifics did not occur in the flock, considering the large number of unidentified behaviours. Previous studies suggested that hens are more likely to display agonistic behaviours in high density areas, as overcrowding in hens can promote aggressive feather pecking (Rodenburg et al., 2013). However, foraging behaviour, which consists of a pecking movement by the hens, can be redirected into feather pecking when foraging opportunities are limited (Huber-Eicher & Wechsler, 1998; Rodenburg et al., 2013). Hence, the high proportion of foraging activity observed in this flock may have reduced the motivation for hens to display aggressive feather pecking toward each other.

Hens predominantly displayed comfort behaviours indoors, but also in the wintergarden. Hence, this may be linked to the provision of shelter, which reduces the need for vigilance and allows performing comfort behaviours, notwithstanding that hens display more preening when present in a social group at lower inter-individual distance (Keeling, 1994), concomitant with the higher number of hens in this area, and possibly a result of social facilitation (Hoppitt et al., 2007). Whether access to a range also motivates preening is unknown as there has been no study on the relationship between time spent outdoors and the amount of contracted ectoparasites on hens, to our knowledge. Nevertheless, 25% of focal hens display preening in the shed and wintergarden in our study, which is comparatively higher than the 13% of the daily time budget spent preening reported by Dawkins (1989).

Pop holes allow hens to access the range and to move in and out of the shed freely. Richards et al. (2011) reported that 80% of hens, monitored using RFID technology, moved through the pop holes regularly whilst only 4% sat in the pop holes. During our behavioural observations, only a small proportion of focal hens moved through the pop hole at any given time whilst a larger proportion of hens were performing other behaviours in the indoor area adjacent to the pop hole. Therefore, pop holes do not only allow access to the range but also serve as an area for the performance of other behaviours. The presence of the wintergarden may have helped by enabling hens to stay around the pop hole without being exposed to the rapid transition between the indoor and outdoor environments.

4.4 Implications for current practices

This study site was characterised by an open field with a lack of canopy cover or artificial shelter. Poultry can perceive the range as a risk due to the lack of refuge in cases of predation or severe weather events (Dawkins et al., 2003; Rodriguez-Aurrekoetxea et al., 2015), supporting the low use of the range in previous studies (Dawkins et al., 2003; Hegelund et al., 2005; Singh & Cowieson, 2013). Additionally, range use has also been reported to decrease in wet cold weather (Dawkins et al., 2003; Reiter et al., 2006). Therefore, the low number of hens present observed in the range could have been explained by the lack of shelter for individuals or the fact that this study was conducted during the Australian winter. Nevertheless, a complementary study conducted by Larsen et al. (2016) using RFID tracking on individual hens from the same study flock revealed that 69% of the hens accessed the wintergarden and outdoor range on a daily basis, an additional 17% irregularly, and 86% of the hens that utilised the range frequented all outdoor zones (i.e. wintergarden, close range, mid range and far range). Therefore, it is reasonable to conclude that a large proportion of the hens did utilise the outdoor area frequently despite few shelters in the range, in agreement with Hinch and Lee (2014) and Campbell et al. (2016).

Hens observed in this study also ranged as far as the far range located further than 32 m from the shed. Previous studies found that hens rarely utilised the range greater than 20 to 30 m from the shed (Rault et al., 2013; Zeltner & Hirt, 2003), indicating that hens are reluctant to venture further into the range. However, at this site, a reduction in the distance that hens need to travel to the far range where they spent most time foraging may encourage hens to utilise the range optimally. To reduce the transition zone, more vegetation could also be provided closer to the shed.

The range promoted the expression of foraging and locomotion behaviours, which were less prominent indoors. The wintergarden possessed features similar to both the indoor shed and the range, conducive to both comfort and exploratory behaviours in the wintergarden, and was a highly frequented area. Therefore, free range commercial farms could benefit from providing a wintergarden. An extension of the wintergarden may also assist in reducing the bird density and the risk of overcrowding which can promote agonistic behaviours and compromise hen welfare.

7.6 Conclusions

Free range laying hens utilise the various areas of the range differently. In the shed, hens perform greater comfort and maintenance behaviours whereas hens utilise the outdoor range predominantly for exploratory behaviours. Foraging was greatest when litter or vegetation was provided in the area. The presence of the outdoor range did contribute to the hens' behavioural repertoire, though various grooming behaviours were displayed preferentially inside the shed and wintergarden.

8 Implications for industry practices

8.1 Overall findings and remaining questions

The demand for free range eggs has experienced a dramatic increase in recent years, partly driven by consumer perception of free range systems as animal welfare friendly, presumably because outdoor access is considered conducive to the expression of natural behaviours. However, the definition of what free range implies and requires has been the subject of recent debate.

Overall, the data obtained with this project showed that, at least in the flocks studied and through individual RFID tracking, most hens did access the outdoor range on a regular basis and for extended periods of time. They also moved a lot between the shed and the range (5 to 20 times) and between different areas of the range, every 15 to 20 min. This means that it is difficult to accurately judge the ranging behaviour of individual hens while looking at the large flocks kept in commercial operations, whether it is a few hundred or a few thousand hens.

Nevertheless, there was only little and inconsistent evidence that differences in ranging behaviour were associated with differences in hen welfare, assessed on the basis of behavioural and physiological variables. It is not to say that the behaviour and physiology of free range hens do not differ from laying hens in other systems (this project did not compare the welfare of free range hens to hens in other systems such as barns, aviaries or cages). Nevertheless, there was indication that the minority of hens that do not range may be more fearful. A longer term study, following a flock from prior to range access to later in the laying cycle, could offer different insights.

The various behavioural studies conducted in this project showed that hens performed different behaviours outdoors as compared to indoors. Nevertheless, there was no evidence that additional behaviours were performed in the range that were not performed inside the shed. The difference was therefore not in the behavioural repertoire displayed, but rather in the behavioural time budget, with more exploratory behaviours in the outdoor range (walking, foraging) and less comfort behaviours (preening, resting).

This project also demonstrated the importance of environmental design on the use of the outdoor range. Various features were found to be attractive to the hens. Artificial structures could constitute an effective substitute for natural cover (e.g. trees, bushes), and present the advantage of being mobile, able to be moved across different areas of the range. The most important factor is an artificial structure with a dense (90% UV block) and overhead cover. Height mattered, in that 0.5 m was more attractive than 1.5 m, but monitoring of the hens and maintaining the range (e.g. grass mowing) could be more challenging. An important aspect that remains to be investigated is the effects of distance from the shed – the maximum distance that these artificial structures can be placed from the shed, and whether they could encourage hens to venture further into the range. Hens also spent a lot of time in the wintergarden when provided with one, with behaviours that are a mix of comfort behaviours usually displayed inside the shed and exploratory behaviour usually displayed more in the outdoor range.

A number of factors still remain to be clarified, such as whether animal, management or housing characteristics influence the propensity of hens to range – such as: rearing experience, breed, flock size, stocking density (in the shed and on the range), training program at the time of first access to the range, season (at placement or at the time ranging is assessed), time of access, mobile vs. fixed sheds, pop hole number and size, vegetation on the range, etc.

In particular, the effect of providing a wintergarden on hen ranging behaviour is an important topic for further research. In this project, we have studied farms with or without a wintergarden, but comparisons across farms contain multiple cofounding factors in addition to the presence or absence of a wintergarden. As such, the value of providing a wintergarden should be approached through further controlled experiments aimed at specifically elucidating the implications of offering a wintergarden. It is hypothesised that a wintergarden would affect the distribution of hens on the range, as well as possibly their ranging patterns (distance from the shed, frequency or duration of range use) with implications for hen welfare but also environmental impact and possibly bird health (parasite load in heavily used parts of the range).

8.2 Range use: RFID applications on commercial flocks

Using RFID technology to assess the movements of individual hens on commercial laying hen flocks allowed us to understand how frequently and for how long hens are accessing the range, and to determine what sort of variation in range use is occurring throughout the flock. In this design, we were also able to assess the use of different areas in the outdoor range. However, in order to assess how individuals were utilising the outdoor areas, all hens that had been tagged had to be re-captured at the end of the study and the leg band removed to ensure that the ID chip was still working. A number of hens either removed or lost their leg bands by the end of the study and others avoided re-capture, requiring these hens to be excluded from the analysis. The likelihood of these leg bands remaining on and the chips still working until depopulation is low. Covering all access or movements between areas through antenna placement on a commercial scale farm would be highly challenging, due to the cost and daily maintenance of the system. This is why we used an alternative approach by segregating part of the flock. Finally, the technology itself is not failsafe, hens that move too quickly through the pop holes may not be registered (Gebhardt-Henrich et al., 2014b) and the power required to run the system fluctuates on the frequency of tag readings, which can cause system failures and loss of data. RFID technology is a useful tool for scientific purposes, providing novel insight that was not feasible before, but at present it is not necessarily suitable for commercial applications.

9 Communication arising from this project

9.1 Peer-reviewed conference abstracts

- H. Larsen, J.-L. Rault. 2014. Go outside and play? Behavioural time budget of free-range laying hens in a natural bush structure. Australian Poultry Science Symposium 2014, Sydney, Australia, 25: 113-116, available at:
  https://sydney.edu.au/vetscience/apss/documents/2014/APSS%20Proceedings%202014.pdf
- H. Larsen, G. Cronin, P. Hemsworth, C. Smith, J.-L. Rault. 2014. Behaviour of free-range laying hens in distinct outdoor environments. 48th Congress of the International Society for Applied Ethology, Vitoria-Gasteiz, Spain, p. 186, available at:
  http://www.applied-ethology.org/hres/ISAE%202014.pdf
- L. Ly, H. Larsen and J.-L. Rault. 2014. Should I stay or should I go? Ranging distance in free-range laying hens, International Society of Applied Ethology, Regional meeting, Sydney, Australia, available at:
  http://www.applied-ethology.org/hres/08JAN15%20ISAE%20regional%20abstract%20booklet%202014.pdf
- H. Larsen, G. Cronin, P. Hemsworth, C. Smith, J.-L. Rault. 2015. What are hens looking for? Preference testing for structural elements in free-range chickens. Behaviour2015, International Ethological Conference, Cairns, Australia, available at:
  http://behaviour-2015.m.asnevents.com.au/schedule/session/6848/abstract/24631
- H. Larsen, G. Cronin, C. Smith, P. Hemsworth and J-L. Rault. 2016. Use of different outdoor areas in commercial free-range layers using RFID technology. Australian Poultry Science Symposium, Sydney, Australia, p. 77, available at:
  https://sydney.edu.au/vetscience/apss/documents/2016/SymposiumProgram2016.pdf
- H. Larsen, G. Cronin, P. Hemsworth, C. Smith, S. Gebhardt-Henrich and J-L. Rault. 2016. Fearfulness and access to the outdoor range in commercial free-range laying hens. 50th Congress of the International Society of Applied Ethology, Edinburgh, UK.

9.2 Industry and technical communications

- H. Larsen, G. Cronin, S. Gebhardt-Henrich, P. Hemsworth, C. Smith and J-L. Rault. 2016. Conference abstract. Individual tracking of free-range laying hens on an Australian commercial farm using Radio Frequency Identification. Spatially Enabled Livestock Management Symposium, Camden, NSW.
- J.-L. Rault. PIX 2014 Invited presentation and proceedings abstract: The implications of outdoor range use on hen welfare in free-range flocks.
- J.-L. Rault. Putting the chicken before the eggs. University of Melbourne Pursuit website, April 1st, 2016, available at:
  https://pursuit.unimelb.edu.au/articles/free-range-debate-skips-the-science-of-animal-welfare
- H. Larsen, G. Cronin, P. Hemsworth, C. Smith and J-L. Rault. PIX 2016 Invited presentation and proceedings abstract: Radio frequency identification as a tool to monitor access to the outdoor range on commercial farms.

- J.-L. Rault. 2016. Free-range and poultry welfare: win-win situation or fragile equilibrium. Australasian Veterinary Poultry Association, Queenstown, New Zealand, 12-15 October 2015.
- J.-L. Rault. 2014. Update of current research projects on free range system. Presentation at the Poultry Health and Welfare Liaison Group-Victoria.
- H. Larsen, P. Hemsworth, G. Cronin, K. Smith, J-L. Rault. 2015. Determining behaviour of free-range laying hens in the outdoor environment. Australasian Veterinary Poultry Association Conference. The University of Sydney, NSW.
- J.-L. Rault. The Pulse radio (94.7 Geelong), “Free-range layers definition”, April 5th 2016.
- Hannah Larsen’s 3 min thesis presentation: “Why did the hens cross the range”, Finalist for Victorian competition.
- H. Larsen. The Project (Channel 10) "How free is free range?", June 10th 2015.
- H. Larsen. *Einstein a Go Go,* 3RRR’s Live Science show (102.7FM Melbourne). October 5th 2014.

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11 Plain English Summary

Project Title:	Free range hen welfare: Characterisation of 'outdoor' and 'indoor' hens and physical features in the range
AECL Project No	1UM121
Researchers Involved	J-L. Rault, P. Hemsworth, G. Cronin
Organisations Involved	University of Melbourne, Parkville, VIC; University of Sydney, Camden, NSW
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Objectives	This project examined the impact of accessing the outdoor range on the behaviour and stress physiology of free range laying hens and the effects of environmental design on the use of the range.
Background	Free range production systems are increasing in Australia, partly driven by consumer perception that outdoor systems are more animal welfare friendly and allow for the expression of natural behaviours. Research on the implications of allowing outdoor access on hen welfare is surprisingly limited.
Research	Six experiments were conducted, combining tracking of individual hens using radio-frequency identification, behavioural studies of ranging in varying locations and conditions, and animal welfare assessment using behavioural and physiological measures.
Outcomes	A large majority of hens on the farm studied accessed the range on a regular basis and for extended periods of time. Nevertheless, there was no evidence that the frequency or amount of ranging were related to differences in hen welfare. The various behavioural studies conducted within this project showed that hens performed different behaviours outdoors as compared to indoors. However, these differences were in terms of frequency rather than the type of behaviours displayed. Hens performed more exploratory behaviours in the range and more comfort and resting behaviours inside the shed. Environmental features offered in the outdoor range were found to have a great influence on the number of hens in these areas as well as on the behaviour displayed while in these areas. Artificial structures could be designed that were attractive to the hens, with the most successful offering overhead cover and dense cover blocking UV light and sight.
Implications	A large majority of free range laying hens were found to make regular and extensive visits to the range. Nevertheless, there was no evidence that the amount of ranging related to hen welfare outcomes. The features of the outdoor environment were found to be important, influencing hens' number and behaviour. This offers novel avenues for the design of outdoor range features that attract the hens into the range.

Key Words	Behaviour; free range; range; natural; outdoor; hen welfare
Publications	No peer-reviewed scientific publications to date.

12 Appendix 1 – Vertical structures influence distribution and behaviour of laying hens in an outdoor range

Australian VETERINARY JOURNAL THE JOURNAL OF THE AUSTRALIAN VETERINARY ASSOCIATION LTD

AVIAN

Fly the coop! Vertical structures influence the distribution and behaviour of laying hens in an outdoor range

J-L Rault,[a]* A van de Wouw[b] and P Hemsworth[a]

AVIAN

Background The number of free-range farms has greatly increased in the Australian egg industry, up by 64% over the past 5 years and representing 34% of the retail egg sales last year. Nonetheless, free-range systems offer particular challenges to farmers. The use of the outdoor range is variable among hens; their distribution is usually not uniform across the range and they tend to stay close to features such as walls or fences, resulting in high stocking density in particular areas, with associated welfare and environmental concerns.

Methods Using video recordings, we investigated the effect of erecting a series of vertical structures in the range on the hens' numbers, distribution and behaviour.

Results Hens were very attracted to the structures, which altered their distribution and behaviour. Up to 160 hens were seen around each structure, giving a density of 6.4 hens/m². The hens spent 40% of their time pecking at the structures and standing in these areas and less time walking, preening or ground pecking.

Conclusion Elucidating which physical features fulfil hens' biological needs could improve their use of outdoor ranges.

Keywords animal welfare; behaviour; chickens; free-range farms

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Laying hens show varied use of the outdoor range in free-range systems. Some hens never go outside[1] and only 10–30% of the flock access the range at any one time.[3–7] Their distribution is not uniform across the range and hens usually stay close to the shed or to particular features of the enclosure, such as walls and fences, and most birds do not venture further than 20–30 m from the shed.[5,7] This causes issues in terms of loss of grass cover and increased stocking density in particular areas, which may contribute to feather pecking, overstocking and parasite contamination. Several causes have been suggested for this, including genetics, experience, fear or lack of cover, but this practical issue remains unsolved.

Exposure to novelty and the risk of predation are powerful fear-eliciting situations. The modern laying hen evolved from the red jungle fowl, which lives in dense rainforest with abundant vegetation providing cover from predators.[2] Most free-range farms offer a large, open pasture with comparatively little cover. A comprehensive study of outdoor broiler chickens found that the number of birds using the range positively correlated with the amount of tree cover, time of day and season.[4] Studies of laying hens confirm that the amount of cover in the range is important.[5,6] Natural trees or bushes provide cover, but present disadvantages for the farmer such as slow growth, attraction of wild birds and a perch for aerial predators. Hence the search for artificial substitutes that can also be moved to ensure uniform use of the range by the flock.

The provision of shelterbelts or shaded areas can attract hens into the range.[6,8–10] Such 'cover' does not need to be overhead; Taylor et al. reported that hens spent more time near fences covered with black plastic (66% of observations) than near wire fences (19%) or away from either (11%).[11] Most studies show the effectiveness, to various degrees, of artificial structures, but the behavioural changes as a result of the presence of these structures have rarely been investigated.[10–13] A systematic research approach is needed to identify which particular features of the structures fulfil the hens' biological requirements and thus ensure optimal use of the range. As a first step toward this goal, we hypothesised that providing a gradual reduction in the visual continuity of the vertical structures rather than an abrupt change from the shed to the open outdoor area could increase use of the range.

Materials and methods

Animals and housing

The study took place during summer and the group comprised a flock of 67-week-old Hy-Line browns (n = 17,000). The hens had access to the outdoor range from 1200 h to 2200 h daily and from 28 weeks of age. The shed (130 × 15 m) had 11 pop holes (2 m × 45 cm) on each side through which the hens had access to a winter garden (130 × 2.5 m) and an outdoor range area (130 × 60 m) (Figure 1). The outdoor range consisted of sandy soil immediately outside the winter garden (0–20 m from the shed), loose straw, which was provided every 6–8 weeks, at approximately 20 m from the shed (2 m away from the end of the structure) and from 30 m onwards there was medium-high grass. Outdoor range access was provided continuously, with no rotation of pasture. The hens could exit from either side of the shed when the pop holes were opened. Feed, water and nests were in the shed only. Feeding times were 0400 h, 1400 h and 1900 h.

Treatments

The two 'treatments' were the presence or absence (control) of vertical structures in two blocks (i.e. two matched-pairs) on one side of the shed facing east (Figure 1). The vertical structures were erected as two parallel, 1-m apart, 0.8-m high fences that extended 17.4 m into the

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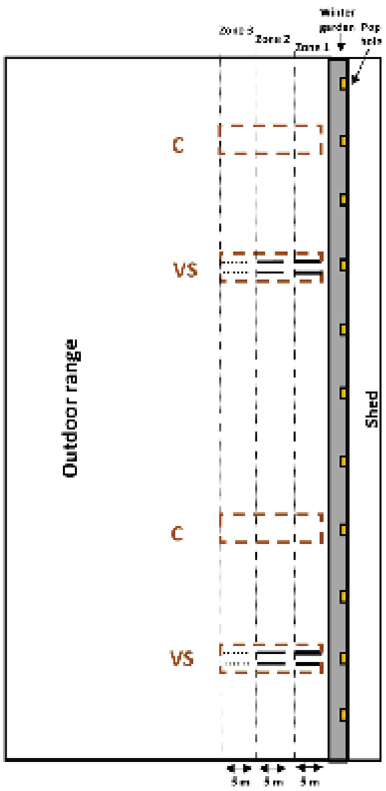

Figure 1. Experimental design of the treatments. The outdoor range area included the two sets of vertical structure (VS), each of which was compared with an adjacent control area (C). The three types of vertical structure materials (heavy shade cloth, moderate shade cloth and conventional chicken wire) are represented by lines of different sizes in the VS treatment in zones 1, 2 and 3 respectively. Only one side of the shed to which the treatments were applied is represented, with the pop holes allowing the hens access between the shed and the winter garden.

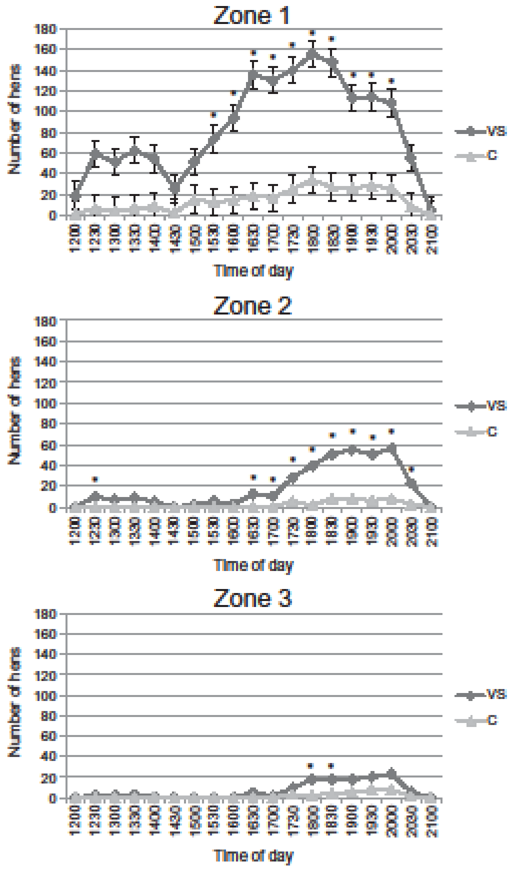

Figure 2. Average number of hens in each 5 × 5 m zone throughout the day. VS: vertical structure (zones 1, 2 and 3 consist of heavy shade cloth, moderate shade cloth and conventional chicken wire, respectively); C: control with no structure; *P < 0.05. Data for zones 2 and 3 were analysed using the square root transformation and are presented as back-transformed.

range. Starting 1 m from the wintergarden, the first 5 m of each fence was heavy shade cloth ('zone 1'; Coolaroo Green Extra Heavy brand; blocking 84–90% of ultraviolet light), the next 5 m was moderate shade cloth ('zone 2'; 'mosquito-net' type, Cyclone Miniweave brand; blocking ~60% of ultraviolet light) and the last 5 m was conventional chicken wire ('zone 3'; blocking ~5% of ultraviolet light). A gap of 0.7 m was left between each structure to allow hens to leave or enter the path.

Observations

Cameras were set up on day 0 while erecting the vertical structures and no people were present while the filming was conducted. Behavioural observations started after a 10-day adaptation period and were performed by one observer from day 11 to day 15 using video recordings. The numbers and distribution of hens were analysed by counting the hens present in each 5× 5 m zone, with the 5 m width corresponding to the 1 m between the vertical structures plus 2 m on each side. The observations were made using instantaneous scan sampling at 30-min intervals from 1200 h to 2100 h.[14] For each scan, hens were counted individually up to 10 birds and in increments of 10 above that. Hen behaviour was analysed in a 1 × 1 m² area only in zone 1 using instantaneous scan sampling at 5-min intervals during three time periods: for the first hour after pop-hole opening (1200–1300 h), in the mid-afternoon (1530–1630 h) and in the evening (1900–2000 h).

Statistical analysis

The study used a matched-pairs design with repeated measures. Square root transformation was applied if the data did not fulfil the criteria of normality and homogeneity of variance. Data were pooled by treatment and analysed using Proc MIXED in SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Data were analysed separately for each zone with a model that included treatment, time of day and their interaction, and accounted as repeated measures over days. When significant interactions were detected ($P < 0.05$), Bonferroni corrected post-hoc tests were performed.

Results

Hen distribution

Within treatment, for the vertical structures, more hens were present in zone 1 (86.2 ± 6.2) than in zone 2 (22.3 ± 6.2) and finally, zone 3 (8.23 ± 6.2; all $P < 0.001$) (Figure 2). However, no differences in hen numbers existed among zones for the control treatment (zone 1: 14.9 ± 6.2; zone 2: 2.8 ± 6.2; zone 3: 2.6 ± 6.3; all $P > 0.05$).

Between treatments, the vertical structures in zone 1 attracted more birds than the control areas, but this effect varied with time of day (treatment × time: $P < 0.001$), with the vertical structures attracting more hens from 1530 h to 2000 h ($P < 0.05$). For zone 2, the vertical structures attracted more birds than the control areas (treatment × time: $P < 0.001$) at 1230 h and from 1630 h to 2030 h ($P < 0.05$). For zone 3, the vertical structures attracted more birds than the control areas (treatment × time: $P = 0.02$), but only from 1800 h to 1830 h ($P < 0.05$).

Hen behaviour

The hens were observed pecking at the structures for 40% of the time (Table 1). The shorter time spent in locomotion, lying and pecking at the ground, and conversely longer time spent standing in the areas around the vertical structures, likely reflected this interest in pecking at the structure. Hens spent more time preening in the control areas. Pecking at conspecifics was rarely observed, although the density of hens was usually high around the structures, especially in the evening (Figure 2). Wing flapping was rarely observed and dust bathing was never observed.

Weather conditions were stable over the 5 days, with moderately warm conditions that had no significant effects on the distribution or behaviour of the hens (av. temperature: 27.33 ± 0.46°C; av. humidity: 33.91 ± 1.36%; all $P > 0.1$).

Discussion

The vertical structures were highly successful at attracting the hens and the high frequency of pecking at the structures suggested that their presence stimulated the hens' interest, possibly providing environmental enrichment.

Few hens ventured further than 6 m into the outdoor range (zone 1) for the control treatment. The provision of vertical structures attracted more hens and further into the range. We observed a gradient along the vertical structures: zone 1 was more attractive than zone 2, which in turn was more attractive than zone 3. The vertical structures in each zone were made of different materials, but the zones were

Table 1. Proportion (%) of hens' behaviours within 0.5 m of the heavy shade cloth vertical structure (VS1) or in the control area (C) for each period of the day, adjusted for the number of hens present

Behaviour	Treatment	Period of day			Treatment effect	Period effect	Treatment × period effect
		1200–1300 h	1530–1630 h	1900–2000 h	(P value)	(P value)	(P value)
Lie	VS1	$7.1 \pm 1.1^{a,x}$	0.3 ± 1.1^{b}	0.5 ± 1.1^{b}	0.007	<0.001	0.01
	C	$20.5 \pm 2.1^{a,y}$	6.4 ± 1.6^{b}	3.2 ± 1.5^{b}			
Stand	VS1	19.8 ± 2.1^{a}	$38.9 \pm 2.0^{b,x}$	28.6 ± 2.0^{c}	0.06	0.005	0.001
	C	17.6 ± 3.9^{a}	$17.4 \pm 2.9^{a,y}$	30.2 ± 2.9^{b}			
Locomotion	VS1	$17.4 \pm 1.4^{a,x}$	$7.6 \pm 1.3^{b,x}$	$9.5 \pm 1.3^{b,x}$	<0.001	0.009	0.05
	C	32.2 ± 3.0^{y}	30.6 ± 1.9^{y}	22.0 ± 1.9^{y}			
Wing flap	VS1	0.8 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.01	>0.1	>0.1
	C	2.5 ± 0.9	1.0 ± 0.5	1.2 ± 0.5			
Peck ground	VS1	12.5 ± 1.8	10.0 ± 1.7^{x}	12.8 ± 1.7^{x}	0.006	0.04	0.008
	C	15.7 ± 3.4^{a}	$32.0 \pm 2.5^{b,y}$	$25.7 \pm 2.5^{ab,y}$			
Peck structure	VS1	40.8 ± 1.9	38.9 ± 1.9	44.4 ± 1.9	<0.001	>0.1	>0.1
	C	–	–	–			
Peck conspecific	VS1	0.5 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	>0.1	>0.1	>0.1
	C	0.1 ± 0.5	0.7 ± 0.4	0.9 ± 0.4			
Preen	VS1	1.2 ± 1.4	3.4 ± 1.3	3.3 ± 1.3^{x}	0.006	0.05	>0.1
	C	11.3 ± 2.4	12.0 ± 1.9	16.9 ± 1.9^{y}			

$^{a-c}$Least square-means within a row with different superscript differ and ^{x,y}least square-means within a column with different superscript differ ($P < 0.05$).

also defined by different distances from the shed. Randomizing the order of the type of materials used for the structures across distance from the shed would be necessary to disentangle these effects.

The number of hens in a range is usually found to decrease as the distance from the shed increases.[5,7] In the present study, the vertical structures resulted in hens moving up to 18 m (i.e. the length of the structures) from the shed, but very few hens were seen to move beyond that distance. Furthermore, a maximum of 160 hens (equivalent to a density of 6.4 hens/m^2) were seen around each structure in zone 1, which is a small subpopulation relative to the total flock of 17,000 hens, although only two sets of structures were erected. The potential for longer or different types of structures to enhance the distribution of the hens further away from the shed and over greater areas of the range requires additional research.

Most hens were observed around the structures in the late afternoon and evening, but few were seen in the early afternoon after pop-hole opening. It is well known that the time of the day has a strong influence on the number of birds present in the range,[4,6,12] so although the structures successfully attracted more hens, there was no change in the diurnal pattern of range use.

The structures did alter the hens' behaviour. They spent a large amount of time pecking at the structure. No evident stimuli, such as flies or dust, were seen on the structures to explain this pecking behaviour. The vertical structures may have provided environmental enrichment, a possible reason for their attractiveness. However, this interpretation is different from the 'fear and cover from predator' hypothesis for explaining the advantage of placing structures in the range. Whether hens' interest in these structures diminishes over time is unknown, as we only observed the hens for 2 weeks after erection of the structures. No study has investigated the behaviour of free-range laying hens around cover or structures provided in the outdoor range. Artificial vertical panels have been shown to increase environmental complexity for chickens kept indoors, and to increase resting and preening.[13,15] Further research is needed to elucidate the reasons behind these behavioural changes, especially as these structures should encourage ranging and not simply aggregation of birds, which would merely relocate the issue of high density to particular areas. This study using a small sample size, but under commercial conditions, showed promising results but requires replication to allow more thorough interpretation of the results.

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13 Appendix 2 – Poultry Information Exchange (PIX) 2014

THE IMPLICATIONS OF OUTDOOR RANGE USE ON HEN WELFARE IN FREE-RANGE FLOCKS

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THE CONTEXT

The use of free-range production systems is increasing in Australia, with public concern that cage housing restricts hens in terms of space, social contact and environment stimulation, or the perception that free-range provides healthier or safer products (Harper & Makatouni, 2002). Free-range systems allow hens to access an outdoor area that provides the opportunity to perform 'natural' behaviours such as foraging for food and dust-bathing. However, there is a lack of scientific knowledge relating to the use of the outdoor range area, which is the main characteristic of this system compared to other non-cage systems such as barn or aviaries. Furthermore, the implications of accessing an outdoor range on hen welfare and productivity remain poorly understood.

RANGE USE

Number and spatial distribution in the range

The frequency of access to the outdoor range is highly variable, with studies reporting between 5 and 46% of the flock observed in the range at any one time, and large variations between times of the day, flocks and studies (Bubier & Bradshaw, 1998; Nicol et al. 2003; Zeltner & Hirt, 2003; Hegelund et al., 2005, 2006). Furthermore, hens' distribution is not uniform across the range as hens rarely venture further than 20 to 30 m away from the shed (Zeltner & Hirt, 2003; Hegelund et al., 2006). The hens are usually observed to stay close to the house or to particular features of their enclosure such as trees, walls and fences (Rault et al., 2013). This causes issues in terms of loss of grass cover and increased stocking density in particular areas, which may contribute to feather pecking, land degradation and parasite contamination.

Individual range use vs. flock range use

Until recently, researchers studied the flock as a whole due to the technical difficulty of following individual birds in large flock settings over extended periods of time. However, these difficulties can now largely be overcome by using technologies such as radio-frequency identification (RFID) systems. Using RFID tags, Richards et al. (2011) reported that different sub-populations of hens can be identified in a flock based on their range use, with 10% of heavy users, 80% of regular users, and 10% of the flock which never ventured outside. Recent research at the University of New England (Hinch, Hernandez et al., pers. comm.) confirmed those observations. The reason behind these differences in range use across individuals remains to be elucidated.

Factors influencing range use

A number of factors have been reported to affect ranging behaviour. Genotype affects the willingness to range outdoor. For example, Hy-Line birds spent less time foraging than red jungle fowl or unselected breeds such as Bentham birds (Schütz & Jensen, 2001). Therefore, genetic selection may be part of the solution but is a rather slow process which may also involve a cost in terms of lower productivity traits. Weather and seasonal conditions such as temperature, rain and cloud cover affect the number of birds found in the range (Hegelund et al., 2005; Richards et al., 2011), but no practical options are available to control for weather conditions in the outdoor range. Increasing flock size has been suggested to reduce the number of birds going outside. However, most studies have been conducted on relatively small flocks (100-2,500 hens), making it difficult to extrapolate to larger commercial flock sizes, in which some studies report no effect of flock size (e.g. Hegelund et al., 2006 looking in the range of 1,200 to 5,000 hens). Furthermore, direct comparisons across studies are difficult because of the use of different strains and different management practices (e.g. restricted vs. *ad libitum* range access, professionals vs. hobby farmers, etc.; for example see Hirt et al., 2000 or Kijlstra et al., 2007). Hence, the evidence is equivocal and it remains to be determined if range use is affected by group size per se or by other confounded factors such as differences in management or stockmanship.

WELFARE IMPLICATIONS OF FREE-RANGE SYSTEMS

The outdoor run offers a wide range of environmental stimuli, exercise, dust bathing substrates, and foraging opportunities with a diversity of food items (seeds, insects). All these could be seen as potential benefits to hen welfare. Yet, the outdoor run also presents a risk of predation, imbalanced diet, increased exposure to pathogens and inclement weather. These risks could seriously compromise the welfare of free-range hens. The literature is inconsistent in the welfare outcomes of free-range systems. For instance, several studies in laying hens have reported that greater outdoor range use is inversely related to the prevalence of feather pecking (e.g. Bestman & Wagenaar, 2003; Nicol et al., 2003). Mahboub et al. (2004) found that, on an individual basis, hens that spent more time outside had less feather damage but Hegelund et al. (2006) reported that plumage condition was not correlated with range use. Furthermore, the question remains as to whether feather pecking inhibits the willingness to go outside, possibly because of the lack of plumage's insulating effect, or if rather the fact of not going outside share common underlying causes with feather pecking. Whether fear affects range use is unknown.

Mortality clearly is the ultimate parameter of compromised health. Mortality has been reported to be higher (Häne et al., 2000) or lower (Sherwin et al., 2010) in free-range systems compared to barn systems. In any case, free-range birds are likely to be exposed to more pathogens during their lifetime, highlighting the importance of good immune defences and disease control in the flock. For instance, free-range hens have greater faecal worm egg counts than barn hens (Häne et al., 2000), and high contamination levels of soil outside can increase dioxin content in the hens and their eggs (Kijlstra et al., 2007). Nevertheless, studies on the immune system of free-range hens that vary in their range use are inexistent, to the author's knowledge.

A major skeletal health issue of caged hens is the increased susceptibility to osteoporosis (Lay et al., 2011). Since exercise enhances bone strength, increased bone breaking resistance is to be expected in hens kept in non-cage systems. Free-range hens have been shown to have higher bone breaking strength than caged hens (Leyendecker et al., 2001). However, no comparisons exist between free-range systems and non-cage indoor system such as barns, or between hens that varied in range use, which could tell us something about the influence of the outdoor range per se. Furthermore, Gregory et al. (1990) found that while aviary and free-range systems had a lower incidence of bone break following catching, these hens had more old fractures than birds from conventional cages. Therefore, the welfare of hens is likely affected in different ways in different housing systems.

IMPROVING RANGE USE

The modern laying hen evolved from the red jungle fowl, their wild ancestor. The natural habitat of red jungle fowl is a dense rainforest, which contains abundant vegetation providing both cover from predators and a source of food (Collias & Collias, 1967). The outdoor environment should offer physical features that allow for protection and escape from predators in order to counteract the possible behavioural inhibition induced by fear. Most free-range farms offer a large open-field pasture but with comparatively very little overhead cover. A comprehensive study on free-range broilers in commercial settings showed that the number of birds observed in the range was positively correlated with the amount of tree cover (Dawkins et al., 2003). Studies on laying hens confirmed that the amount of cover in the range is a crucial factor influencing the willingness of birds to go outside (Zeltner & Hirt, 2003; Hegelund et al., 2005). Cover also allows the birds to gain shelter during inclement weather.

Natural trees or bushes provide cover but present several disadvantages such as slow growth, attractants for wild birds and a stance for aerial predators, hence the search for artificial substitutes. Furthermore, artificial structures have the advantage that they can be moved to enhance a uniform use of the range. Shelterbelts, as natural vertical structures, or the provision of shaded areas have been found to attract more hens in the range (Hegelund et al., 2005; Borland et al., 2010; Glatz et al., 2010), whereas other structures such as roofed boxes with sand increased the distribution, but not the number, of hens in the range (Zeltner & Hirt, 2003). That ‘cover’ does not need to be overhead as was demonstrated by Taylor et al. (2004) and Rault et al. (2013), who found that more hens are present in the range when vertical fences are provided.

While most studies showed the effectiveness of those structures to various extents, the particular features or cues of the structures that fulfil the biological requirements for hens have not been scientifically investigated. For example, offering structures that vary in their features (two-level perches, a “pecking-tree”, pine tree and boxes with pine cones) was more effective at increasing the number of hens outside than offering just one type of structure (a shelter), but the authors did not identify which specific features caused more birds to go outside (Zeltner & Hirt, 2008). The effect of artificial structures placed in the enclosure on the bird’s willingness to range remains poorly understood. Research is needed to identify which environmental features are biologically relevant to the birds and should be provided for optimum use of the range. There is little scientific knowledge of the activity time budget of hens in the outdoor range. Our group started a series of studies investigating the effects of various structures in the range (Rault et al., 2013; Larsen & Rault, 2014), and the principles underlying the attractiveness of these structures to the hens. Vertical structures can attract more hens in the range, and further into the range (Figure 1), possibly providing some forms of environmental enrichment. Furthermore, it appears that hens use attractive structures differently depending on the time of the day, suggesting that the outdoor range has different functions (Table 1). Our most recent research showed that the behavioural time budget of hens differed between distinct locations or ‘patches’ within the outdoor range that differ in substrate or tree cover, possibly explaining the observed pattern of spatial distribution across the range. Specifically, highly preferred areas were less subjected to diurnal patterns of range use whereas less preferred areas saw more hens early or late in the day (Larsen et al., 2014).

Figure 1 Average number of hens in each 5 × 5 m outdoor range zone throughout the day
VS: vertical structure; C: control with no structure; *P < 0.05 (Extracted from Rault et al., 2013)

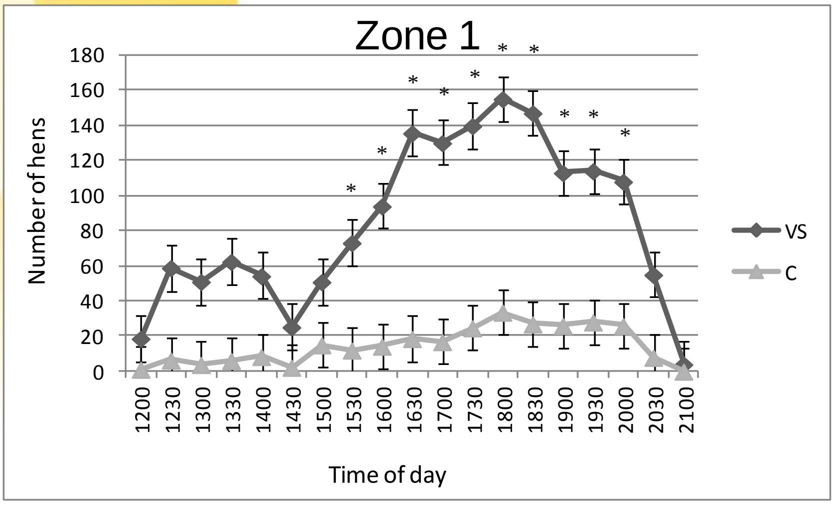

Table 1 Variation in the behavioural time budget of hens in a kangaroo apple tree as a natural structure according to the time of day (% of time; extracted from Larsen & Rault, 2014)

Behaviour	Forage	Preen	Walk	Perch	Alert	Stand	Lay down
0730-0930h	13.7 ± 1.1	15.5 ± 1.2	3.7 ± 0.6	14.7 ± 1.1	8.0 ± 0.9	4.3 ± 0.7	1 ± 0.3
1130-1330h	12.5 ± 0.6	10.9 ± 0.6	2.7 ± 0.3	19.6 ± 0.7	6.8 ± 0.5	2.5 ± 0.3	7.6 ± 0.3
1530-1730h	21.1 ± 1.2	8.7 ± 0.8	4.4 ± 0.6	9.6 ± 0.9	6.8 ± 0.7	1.8 ± 0.4	7.0 ± 0.5

STOCKMANSHIP

Some of the inconsistencies between the welfare and productive performances of free-range systems in comparison to other systems might also originate from variations in the particular features of the shed, outdoor range, or stockmanship knowledge or skills. Good stockmanship is a major determinant of animal behaviour and welfare (Hemsworth & Coleman, 2011). The management of free-range birds requires careful monitoring and is more complex than indoor environments due in parts to the uncontrolled environmental conditions and heterogeneous diet composition. Increasing our understanding of the interactions between the environment and the birds in free-range conditions will allow refinement of the management of free-range systems.

CONCLUSION

Few studies to date have investigated the effects of outdoor range use on the welfare of individual birds. Furthermore, the reasons for which laying hens decide to access the range, and the function of providing hens with outdoor access on their behaviour and welfare, remain poorly understood. It appears that hens vary in their use of the outdoor range depending on numerous factors, including the quality of the space provided in the range. Understanding the principles that attract hens in particular areas of the range, and its associated welfare implications, will ensure that hen welfare be safeguarded while improving the system in terms of management and productivity. It is important to investigate the impact of outdoor use on Australian hens because local climatic conditions may be quite different from other parts of the world. Most studies to date have focused on the behavioural changes observed in free-range hens, but the welfare and particularly health implications of allowing outdoor access require urgent investigation, for the welfare of the hens and the sustainability of free-range production systems.

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14 Appendix 3 – Testing of GPS tracking systems

Miniature GPS have been reported in the literature to follow broiler chickens at pasture (Dal Bosco et al., 2010 J. Appl. Poult. Res. 19: 213-218). The advantage of this technology over RFID tracking systems is that it could provide the coordinates of monitored hens in the range, whereas RFID systems most often are restricted to telling us whether a hen is out in the range or not, given RFID systems rely on antenna detection, with therefore a need to have more antennae than is practically or economically feasible to cover the range area.

Therefore, two commercially-available GPS tracking devices were purchased. The first GPS device was the TrackStick mini (Trackstick, CA, USA), with dimensions (88.9 x 38.1 x 9.5 mm), a weight of 62.2 g, and an accuracy of ± 2.5m. The second GPS device was a lighter and cheaper alternative: i-gotU GT-600 (Mobile Action Technology Inc., Taiwan), with dimensions (46 x 41.5 x 14 mm), a weight of 37 g, and no estimated accuracy. Both devices were tested for their sensitivity and accuracy in detecting movements between various outside locations, and movements between outdoor and indoor, as GPS are notably inaccurate in indoor situations as they rely on satellite-based location estimates.

Both GPS devices were found to provide an accuracy lower than what would be required to track hens (Figure 1), between 10 and 20 m. Furthermore, the sensitivity of both devices was questionable, with many outliers, especially when the device remained immobile for a period of time (2 min) in one location.

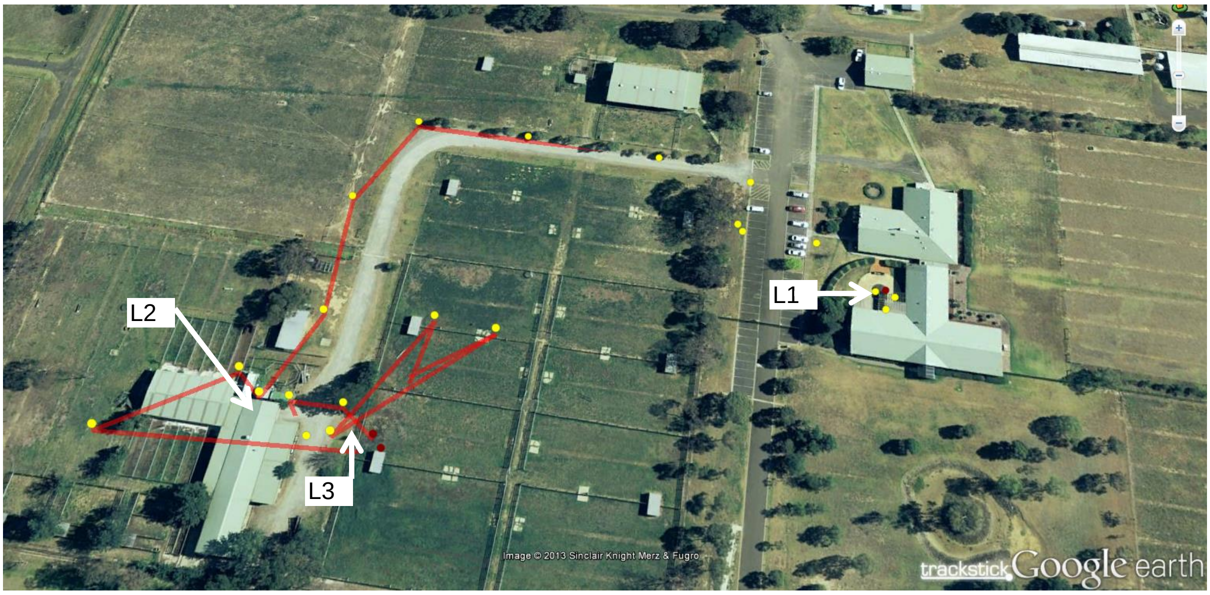

Figure 1 One of the TrackStick mini testing

The recorded movements appear in red between estimates locations marked as yellow dots.
The experimenter started from Location (‘L1’) outside, followed the gravel path in the middle to L2, which consisted of coming inside the metal shed and staying there for 2 min, and finally moving to L3, just along the paddock, and remaining there for 2 min.

Figure 2 One of the TrackStick mini testing, with the device real position being Location 1 ('L1') inside a house at the bottom right corner

As was expected, the GPS also showed a low tolerance for movement between outdoor and indoor locations (Figure 2).

The i-gotU GT-600 device always performed worse than the TrackStick mini device, and indeed stopped turning on after 3 weeks.

After consultations with experts in geo-localisation, we learnt that GPS devices work at their best when moving at a constant rate, ideally in straight lines, as GPS predict where their next point may be; hence why they work well for car travel, but conversely why they probably would not work well for hens, moving at a comparatively slow pace, in irregular fashion, and only over relatively small distances.

Based on these results, the attempt to use GPS devices to track the hens was deemed unsuccessful, as the reliability and accuracy were not sufficient for our purpose. Based on other researchers' experience with GPS (actually GNSS) devices, to improve accuracy the device needs to be set at a frequent fix rate; GPS signals are also unreliable close to buildings and under (wet) trees (Greg Cronin, personal communication).