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Optimisation of Welfare in Free-Range Laying Hens

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EXECUTIVE SUMMARY

The Australian free-range egg consumer market has driven a new egg labelling information standard regarding outdoor stocking density but little scientific data has been available to inform these decisions. There are also few data on alternative feeding practices for industry sustainability, including the use of insect larvae. Finally, early rearing interventions are shown to improve adult adaptability to the layer housing environment within indoor systems, but few data are available on interventions for free-range laying hen chicks.

This research comprised three experiments:

Experiment 1: We applied radio-frequency identification technology, basic health and behavioural measures, behavioural tests and production assessment to hens housed with access to ranges of varying sizes simulating three different outdoor stocking densities (2000, 10 000 or 20 000 hens/ha). We showed that hens used the range more with more space available and were able to spend more time resting both outside and inside. The majority of hens in the small flock sizes were in good visible health condition but at the end of the trial hens stocked at the highest density showed highest concentrations of albumen corticosterone. All hens surpassed breed standards for production but there were some differences between densities in egg quality, which could be related to the variation in range use and thus diet. Commercial trials at a larger scale would be needed to confirm these findings and determine if greater differentiation between densities is observed with larger flock sizes.

Experiment 2: We provided hens an option of choice-feeding on the range with dried black soldier fly (BSF) larvae or no supplementary insects. On average, hens consumed 14g BSF/day. Consumption of larvae did not affect the flock performance after six weeks of provision but after 12 weeks hens consuming the BSF had lower egg weight and darker yolk colour but other measures of egg quality were not affected. This study demonstrated the feasibility of using BSF larvae for choice-feeding in free range hens but BSF larvae are currently too expensive to be used on a large scale in the feed industry. Insect feeding may be an option for small-scale producers but more research is needed on aspects such as food safety.

Experiment 3: We reared chicks from 4 to 21 days in two separate groups with either multiple enrichments (physical, environmental, visual, auditory) or standard rearing conditions (non-enriched). Following transfer to the laying facility RFID technology was used to measure range usage, video observations were made of natural range disturbance behaviour and all birds were regularly assessed for basic health and welfare measures including albumen corticosterone. From 38 to 42 weeks of age birds were subject to two stressor events: locked inside for 2 days,

then 80% range-size reduction. There were no differences between the rearing treatment groups in disturbance behaviours on the range. On average, enriched birds spent less hours outside daily with more visits of shorter duration. There were few differences between treatment groups in health and welfare measures although enriched birds had marginally higher body weight and marginally lower albumen corticosterone concentrations. The non-enriched birds showed a higher albumen corticosterone response to the range-size reduction. The enriched-rearing treatment had some effects on range use and welfare but practical enrichments during rearing for industry now need to be investigated.

Table of Contents

Experiment 1 – Impacts of Outdoor Stocking Density on Range Use, Welfare, Behavioural Traits and Egg Production*

*Full publications of some of this research are available:

'Outdoor stocking density in free-range laying hens: Radio-frequency identification of impacts on range use'*. Animal DOI: 10.1017/S1751731116001154*

'Outdoor stocking density in free-range laying hens: Effects on behaviour and welfare'. *Animal*, *DOI: 10.1017/S1751731116002342.*

'Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens'*. Applied Animal Behaviour Science, doi.org/10.1016/j.applanim.2016.09.004.*

1.0 RECOMMENDATIONS

The aim of this experiment was to assess impacts of different outdoor stocking densities on hens' range use, behaviour, welfare and production. Having more space outdoors increased the time hens spent out on the range with hens in the highest stocking density showing poorer welfare by some measures. The variation in range use may have affected birds' diet and subsequently egg quality. If birds are kept at higher densities adequate space should be provided indoors for resting. Research is needed at the commercial scale, including further data on the health benefits/impacts of ranging.

1.1 INTRODUCTION

Consumer concern for animal welfare is driving changes in the laying hen (*Gallus gallus domesticus*) industry both within Australia and globally leading to growth in alternative housing to the conventional cage such as free-range production systems which are viewed as more natural and ethical (Schröder and McEachern, 2004). The current Australian Model Code of Practice for the Welfare of Animals - Domestic Poultry (Primary Industries Standing Committee, 2002) states a density of 1500 hens per hectare outdoors (at maximum occupancy) with higher hen densities requiring regular range rotation and no maximum density stated. Thus, free-range systems within Australia currently span from a few hundred hens per hectare to several thousand hens per hectare. Subsequently, the Australian Commonwealth Government released a new free-range egg labelling information standard on March 31, 2016 requiring hens to have '*meaningful and regular access to the outdoors, with outdoor stocking of no more than one hen per square metre (maximum 10,000 hens per hectare)*'. In addition to the national information standard on egg-labelling, the Australian Egg Corporation Limited will be releasing new Australian Poultry Standards and Guidelines during 2016. However, there are currently a lack of scientific data available from both Australian and international free-range systems on impacts of outdoor range stocking density on hen range-use behaviour (Pettersson et al., 2016), thus negating objective determination of optimal outdoor space requirements for free-range laying hens.

The free-range system is a dynamic environment where hens have a daily choice of whether to access the outdoor resource. Therefore, to determine the preferred space requirements outdoors, it is imperative to measure how frequently individual hens choose to access the range. An inverse relationship between range use and/or indoor stocking density and flock size has been supported by group-level direct observational studies within the UK and EU with similar low percentages of birds seen on the range simultaneously (e.g., Bubier and Bradshaw, 1998; Harlander-Matauschek et al., 2001; Hegelund et al., 2005).

To document individual patterns of range use, researchers have employed the use of radio-frequency identification (RFID) technology to track range use of microchip-tagged hens (e.g., Richards et al., 2011; Gebhardt-Henrich et al., 2014). These studies consistently show that a much higher proportion of birds use the range than live observations document.

Range access is perceived to improve hen welfare (Knierim, 2006) but few studies have assessed the relationship between time spent on the range and measures of hen health and welfare, particularly for individual hens. Greater use of the outdoor range has been correlated with lower incidences of footpad dermatitis (Rodriguez-Aurrekoetxea and Estevez, 2016) and less plumage damage (Mahboub et al., 2004; Chielo et al., 2016; Rodriguez-Aurrekoetxea and Estevez, 2016) although Hartcher et al., (2016) found no association between range use and plumage damage.

The outdoor range is also perceived to improve hen welfare by providing a suitable environment for hens to express behaviours deemed ethologically important such as dust bathing and foraging (Cooper and Albentosa, 2003) but there are few data on what hens do while ranging. Higher stocking densities in indoor perchery systems have been associated with decreased foraging, dust bathing and mobility (Carmichael et al., 1999 but see Zimmerman et al., 2006).

There are also limited data available to indicate why individual hens of the same flock, housed under the same conditions, vary in their range use, from some hens visiting the range daily to others not visiting it at all. Finally, research with hens in caged systems has shown stocking density or cage size can impact egg production and egg quality to varying degrees across different studies. There are currently no data on the effects of outdoor stocking density on free-range production and egg quality.

1.1.2 Experimental Objectives

- The first objective: to use RFID tracking of individual ISA Brown hens in an experimental free-range system to measure the impact of three different outdoor range stocking densities (2000 hens per hectare (ha), 10 000 hens/ha and 20 000 hens/ha) on average daily time spent outside, average number of daily visits outside, maximum visit durations and percentage of available ranging days that individual hens ventured outside, including video decoding of total numbers of hens outside and present in different areas of the range across the day.

- The second objective: to assess the impacts of outdoor stocking density on multiple parameters of hen health and welfare indicators.

- The third objective: to use tests of fear and coping style to determine if individual hens that differed in their range access habits could be consistently categorised as showing different personality traits to aid understanding of individual ranging variation.

- The final objective was to compare egg production and egg quality of hens housed at different outdoor stocking densities.

1.2 METHODS

1.2.1 Animals and Housing

All experiments were approved by the University of New England Animal Ethics Committee (AEC14-100). Nine hundred ISA Brown pullet laying hens (*Gallus gallus domesticus*) were placed at 16 weeks of age (May 2015) into the University of New England's Laureldale experimental free-range facility located in Armidale, Australia. Floor-raised pullets were obtained from a commercial supplier. Birds were infrared beak-trimmed at 1 day old with a hot-blade re-trim at 11 weeks of age.

The hens were evenly distributed between six indoor floor pens (150 birds per pen) with equal indoor stocking densities of nine birds per m^2 (Figure 1.2.1). Indoor resources per bird were provided to meet or exceed the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002) (Figure 1.2.1). Birds were fed a commercial layer mash (Barastoc - Premium Top Layer Mash, Melbourne, VIC) available *ad libitum*. Rice hulls at an initial depth of approximately 4 cm were provided as a litter substrate.

The shed was fan-ventilated but not temperature or humidity controlled with an average indoor temperature of 8.8 °C ± 4.09 (range: -2.2 to 19.4 °C) across the trial period, as measured at bird height. Incandescent lighting gradually increased from 15 h to 16 h of light by 20 weeks of age (lights on at 0400, lights off at 2000 h). The lux (Lutron Light Meter, LX-112850) inside the pen when the pop holes were closed, measured at bird height in three locations within the pen (front, middle and back), ranged from 4 to 21 lux. This range increased to 5 to 190 lux when the pop holes were open as measured on one cloudy and one sunny day.

Each indoor pen was associated with a designated fenced (2 m high to prevent birds flying over but birds were not visually isolated between ranges) outdoor area which was initially 100% covered (prior to bird access) with a variety of grass and weeds typical to the region. To minimise the variables associated with birds accessing the outdoor areas, no shade or shelter structures that have been shown to encourage range use were present (Hegelund et al., 2005; Nagle and Glatz, 2012). The impact of three outdoor stocking density treatments were assessed with two replicates per

treatment (maximum replicates able to fit within the experimental range area) (Figure 1.2.2). The pop holes containing four RFID passageways (Figure 1.2.1) that provided range access were first opened at 21 weeks of age (~ 20% production) with subsequent daily access from 0900 – 1630 h across 15 weeks over winter. Birds were not forced onto the range as measuring natural range usage (per commercial practice) was the objective of this research. Assessing outdoor access over the winter period provided ideal conditions for frequent range usage as days were typically dry (sunny/cloudy with rain on 12% and snow on 2% of days) and outdoor temperatures generally mild (average outdoor temperatures during range access hours were 14.3°C ± 5.34; range: -3.5 to 27.9 °C). Photos of each range were taken weekly to document hen degradation of ground cover with visual estimations made each week on percentage of ground cover remaining (green vs. brown area). Hens were encouraged to return inside each afternoon using 350 g of poultry grain mix per pen (Barastoc – Poultry Grain Mix, Melbourne, VIC) and all birds were held inside each night.

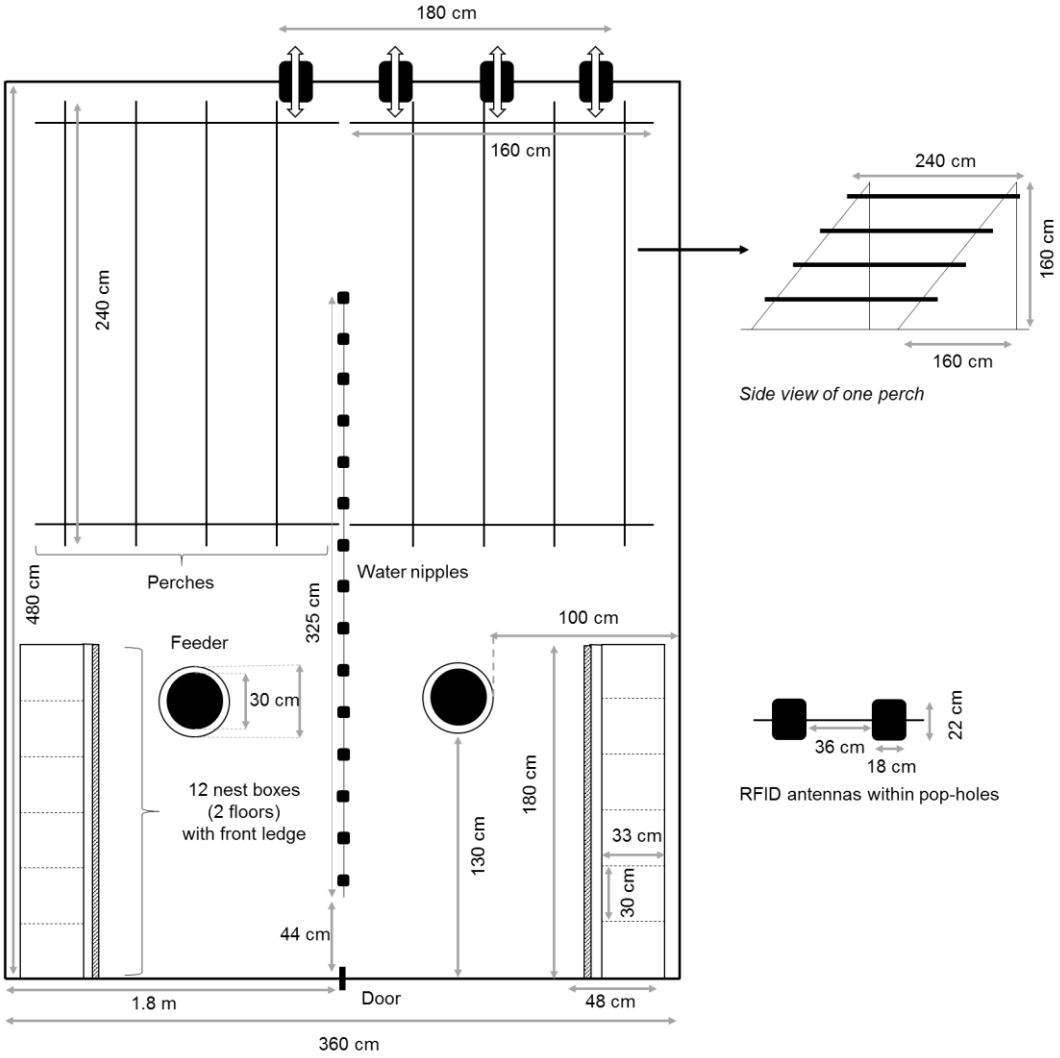

Figure 1.2.1 A top-down schematic of the hens' indoor pen set-up showing location of the range pop holes (including radio-frequency identification antennas), perches

(side view included), nest boxes, feed and water. Each indoor pen had identical resources and configuration.

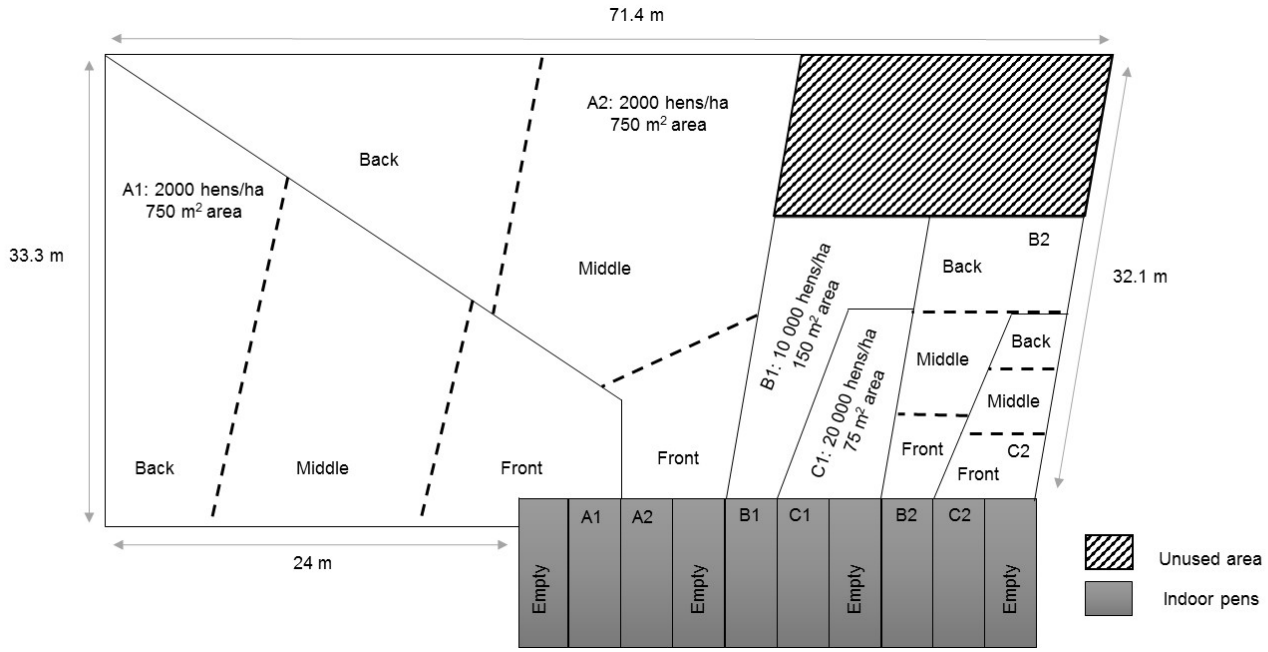

Figure 1.2.2 The six indoor pens and their designated outdoor range areas for the two replicates of each outdoor stocking density treatment (2 000, 10 000, 20 000 hens/ha), including the Front, Middle and Back range delimitations used during behavioural observations (range delimitations were equal between both 'B' and both 'C' pens respectively).

1.2.2 Radio-Frequency Identification Tracking

All birds were leg-banded (plastic numbered split-ring; Roxan Developments Ltd, Selkirk, Scotland) at 17 weeks of age with 75 randomly-selected birds in each pen (50%) also fitted with an adjustable leg band (Roxan Developments Ltd) containing a glued RFID microchip (Trovan® Unique ID 100 (FDX-A): operating frequency 128 kHz). All microchips within leg bands were tested prior to being fitted to hens, and all microchips that were never registered on the system during the trial were re-tested to confirm their functionality. Three RFID systems were purchased from Microchips Australia Pty Ltd (Keysborough, VIC) with equipment developed and built by Dorset Identification B.V. (Aalten, Netherlands) using Trovan® technology. Each RFID system consisted of four passageways (36 cm H x 18 cm W) situated within the pop holes (Figure 1.2.1), each with paired optical beam sensors at either side of an RFID antenna plate and all connected to an RFID decoder downloading directly to a USB flash drive. Each passageway registered and recorded the date and time each tagged bird passed through and in which direction (onto the range, or into the pen) with a precision of 0.024 seconds (maximum detection velocity 9.3 m/s). These passageways were situated at a height ranging from 17 – 23 cm inside the pens and at heights ranging from 33 – 42 cm outside the pens depending on litter build-up depth inside or ground topography outside (as measured at 24 weeks). The RFID systems were placed in the indoor pens 2 weeks prior to pop holes first opening and RFID tracking occurred daily from 22 to 36 weeks (1 week permitted to acclimate to the pop hole passageways and the range). However, due to having three RFID

systems only, one replicate from each stocking density was recorded for 2 weeks, then the three RFID units were swapped to record the second replicate for 2 weeks. In total (excluding days of system failure), daily tracking data were recorded for each stocking density as follows: 2000 hens/ha: 91 days; 10 000 hens/ha: 91 days; 20 000 hens/ha: 88 days.

1.2.3 Welfare Scoring

Birds were weighed 1 week following placement (BAT1, VEIT Electronics, Moravany, Czech Republic). Using a modified version of the Welfare Quality® scoring protocol (Welfare Quality®, 2009), basic health and welfare measures were made on all birds prior to release onto the range (20 weeks of age), then at 5 (26 weeks of age), 10 (31 weeks of age) and 15 (36 weeks of age) weeks following range access. Over 2 days at each sampling time, all birds were weighed, the length of the middle toenail of the right foot was measured with a seamstress tape measure (fitted to the curve of the toenail and measured to the nearest mm), and then feet were checked for broken, missing or injured toes. Any footpad dermatitis was determined using a scale where 1 = footpad dermatitis on one or both feet with no swelling, 2 = dermatitis on one or both feet with moderate swelling, 3 = footpad dermatitis on one or both feet with dorsally visible swelling. At the same time birds were manually palpated for keel damage with the scoring being N for no damage and Y for any damage (deviations or indication of fracture) and at 36 weeks an additional category (Y*) was added because of the comparatively more severe keel damage. Visual checks were made for comb abnormalities, comb wounds, skin pecking wounds, presence of external parasites, feather damage and feather loss but no evidence of these conditions were observed (except for one bird having a nude head at 36 weeks of age). The same experimenter (who was aware of the treatment groups) did all visual health scoring and keel palpations.

1.2.4 Albumen Corticosterone

Ninety eggs from each pen were sampled across 2 days (45 eggs per day) at 23 (hens were at 50-60% lay), 29 and 35 weeks of age for assessment of albumen corticosterone concentrations. On the day of collection, all eggs were weighed, broken open, the albumen was separated from the yolk then weighed and stored at -20° C until processing via radioimmunoassay following procedures reported by Downing and Bryden (2008). All albumen corticosterone analyses were conducted blind to outdoor stocking density treatment.

1.2.5 Video Recordings and Decoding

When weather permitted, a series of video cameras (Panasonic HC-V110, Panasonic HDC-SD40 (Panasonic Australia Pty Ltd), Sony HDR-XR260E, Sony HDR-XR550 (Sony Electronics Inc., San Diego, CA, USA)) recorded each range area during 25, 26 and 27 weeks of age (peak production period and following 3 weeks of acclimation to the range area). Six sampling days of recording were made per range area (only 3 range areas, one pen replicate from each stocking density could be recorded on any one day). On each sampling day, the entire range area was video recorded during range access hours (0900 – 1630 h). Six Hikvision (DS-2CD2T42WD-14 Outdoor EXIR Bullet; iCam Security, Forest Lake, QLD, Australia) cameras were used to simultaneously record the indoor shed pens. Resulting videos were used to count the total number of birds outside in three different parts of the

range (Figure 1.2.2): one close to the pop holes (Front), one in the middle (Middle) and one at the back of the range (Back). Sampling occurred 10 minutes after pop hole opening, and every 20 minutes thereafter until pop hole closing (three observers decoded videos, inter-observer reliability 93%). These range delimitations were designed to describe how the hens used the available outdoor area between different sized ranges, but due to the size variation between and within pens, it was not possible to make any statistical comparisons between the areas.

Videos recordings were also used to count the proportion of birds performing three behaviours outside in the 'Front' and 'Middle' (Figure 1.2.2) of the range area and throughout the indoor shed pen. The observed behaviours included dust bathing, foraging (scratching followed by ground-pecking) or resting (including both sitting down or perching (indoors only)) while awake but performing no other behaviour or asleep with head tucked under the wing). Observations were made for 1 minute starting at 10 minutes after pop hole opening, and then every 20 minutes thereafter with one observer working with the range videos and another observer working with the indoor shed videos. Upon watching the video recordings, the behaviour of hens at the 'Back' of the larger range areas could not be reliably observed and thus this area was excluded from observations across all of the experimental ranges.

1.2.6 Behavioural Tests

From the total of 900 hens, 104 were selected at 37 weeks of age, based on the percentage of available days that the birds accessed the range. All selected hens (and the majority of hens in the total flock) were in visibly good health condition with no feather pecking damage or footpad dermatitis and there were no obvious physical differences between the selected birds. From the total group of tagged birds (448 hens) 5% used the range on less than 10% of available days (including 2% of birds that never went outside), 25% used the range from 10-99% of days and 73% used the range on 100% of available days. Birds from each of these three ranging groups were present in each stocking density treatment. Where many birds were available to select from, i.e., the group that accessed the range on 100% of available days, focal birds were randomly selected from all stocking densities and a larger sample size was selected. Indoor-preferring birds accessed the range 0 – 10% of available days (n = 21). The moderate-outdoor birds were selected as those that accessed the range on 30 – 60% of available days (n = 18); these were birds that initially never went outside but as the trial progressed they began to use the range daily, versus some birds that sporadically went outside across the entire trial duration, or used the range on the majority of days (but not all days). This group was selected as representing birds that may have been initially fearful for several weeks following pop hole opening, but appeared to overcome their fear as the trial progressed. Outdoor-preferring birds accessed the range on 100% of available days (n = 65). The average daily mean (± SEM) time outside for selected indoor-preferring individuals was 26 ± 10 mins; for moderate-outdoor individuals was 101 ± 22 mins, and for outdoor-preferring individuals was 282 ± SE 7 mins.

At 37-38 weeks of age, focal birds from one pen only were tested each day in tests of both induced TI and MR with stocking density treatment, replicate and order of the two behavioural tests alternated across the six testing days. Focal birds were selected from their home pen after being weighed as part of a separate study on hen

health and welfare using the same set of birds, and placed into a temporary holding pen with feed and water. Birds were permitted to rest for 1 hour after being handled. Birds were then caught in no specific order and carried in hand, with a small cloth over their heads to shield their eyes and induce calmness, to a separate room isolated from flock mates for the behavioural tests. Birds were caught in pairs by the same two operators with simultaneous testing occurring in two separate rooms. The same operators induced TI or held the bird in the MR position across all testing days with all operators blind to the range access status (but not outdoor stocking density) of the individual hens. After the initial test (TI or MR), hens were given approximately 1 hour to rest in the temporary holding pen before the second test was initiated. Upon completion of the second test, hens were returned to their home pen. All focal birds were tested in the OFT on a separate day at 39 weeks of age with two pens of birds tested per day. Birds from each pen were caught in no specific order, placed in the open field box (isolated from remaining flock mates) and then on completion returned to their home pen.

1.2.6.1 Tonic Immobility Test

TI was induced by placing the hen in a supine position in a cradle with their head hanging over one end. The right hand of the experimenter was placed on the breast of the bird, while the left hand gently held the bird's head down. Birds were restrained in this position for 10 seconds then released and the experimenter stepped aside out of direct view of the hen with eyes averted downwards. If the hen remained in the supine position for at least 10 seconds, TI duration was recorded until the hen returned to an upright position or until a maximum of 5 minutes elapsed. If the hen self-righted herself within 10 seconds of release, TI was induced again, with a maximum of five attempts made at inducing TI. The number of induction attempts and the duration of tonic immobility (i.e. latency to self-righting) were recorded.

1.2.6.2 Manual Restraint Test

Birds were individually manually restrained for 5 minutes by holding the bird in a right lateral recumbent position on top of a table. The right hand of the experimenter held the hen’s side and the left hand gently stretched the hen's legs. The number of struggles the hen made (i.e. attempting to pull their legs up) and number of vocalisations (individual vocalisations not bouts) were recorded by an observer. At the end of the 5 min test, the hens were moved to an individual cage to allow the corticosterone response to reach its peak (Downing and Bryden, 2008). Twenty minutes following capture a 2 mL blood sample was collected from the brachial vein to measure peak plasma corticosterone response to the manual restraint. Blood samples were collected in EDTA-coated tubes, centrifuged at 700 x g for 15 minutes on the day of collection to extract plasma, which was stored at −20 C until the radioimmunoassay. Plasma corticosterone concentrations were measured using the protocols of Downing and Bryden (2008).

1.2.6.3 Open Field Test

The wooden open field box was a square of 1.25 m length and 1.22 m height, elevated 0.24 m off the ground with an opaque roof, three opaque side panels, and a clear frontal Perspex sliding panel. At 39 weeks of age, each bird was placed in the centre of the box in the dark, lights were then turned on and the test began for a

duration of 5 minutes. The behaviour of the birds in the OFT was video recorded with the operator out of sight but located within the testing room. After 5 minutes, the test concluded, the lights were turned off and the bird was caught and returned to its home pen. Later, a single operator viewed all video recordings to analyse the bird behaviours. The open field box was divided into a grid of 121 squares to assess walking activity. The latency to first move (seconds), total quadrants crossed, latency to first vocalise (seconds) and total number of vocalisations made (individual vocalisations, not bouts) were counted. The operator was blind to the range access status but not the outdoor stocking density status of the birds.

1.2.7 Egg Production

All eggs were collected by hand each morning and counted per pen from the beginning of lay (19-20 weeks) through to trial conclusion when hens were 35 weeks of age. All eggs were visually graded (but not candled) to record the numbers of eggs per pen that were normal or deformed (body-checked, misshapen, pimpled (≥ 1 pimple), rough-shelled or soft-shelled). Across the trial period, only 15 eggs were found laid out on the range (and only in the largest sized ranges). From 21 to 35 weeks of age, all eggs laid within each pen on one day per week were weighed together to obtain the average egg weight per pen.

1.2.8 Egg Quality

At 25, 30 and 36 weeks of age 30 eggs were collected on one day from each pen (60 eggs per stocking density treatment) to conduct egg quality measurements. Eggs were unwashed and eggs with substantial shell contamination were not included for analysis. These eggs were analysed for egg shell quality measurements: shell colour by percentage reflectivity, egg shell breaking strength by quasi-static compression (Newtons), shell deformation to breaking point (µm) and shell weight (g) (egg quality equipment, Technical Services and Supplies (TSS), Dunnington, York, UK). Shell thickness (µm) was measured at the eggshell equator using a custom-made gauge based on a Mitutoyo Dial Comparator gauge (Model 2109-10). Percentage shell was calculated from shell weight (g) and egg weight (g). Egg yolk colour was measured using the DSM YolkFan^{TM} (TSS equipment). All analyses were conducted on the day of egg collection by one person blind to the stocking density treatments.

1.3 DATA AND STATISTICAL ANALYSES

1.3.1 RFID and Video Counts

Discounting any data from birds that died part way through the trial, the individual hen sample sizes for daily RFID tracking data were as follows: 2000 hens/ha: N = 148; 10 000 hens/ha: N = 149; 20 000 hens/ha: N = 150. RFID data were separated into individual hens' daily range use from weeks 22-26, weeks 27-31 and weeks 32-36. These periods incorporated approximately 2 weeks of RFID tracking from each replicate within the three stocking densities. Although individual range use may be affected by activities of the group, space requirements are typically stated per bird, and it was the variation at the individual level within the group environment that was the focus for this study. All RFID data were run through a custom-built software program written in the 'Delphi' language that filtered out any unpaired false readings, such as if a bird sat in the pop hole and triggered continuous readings, or jumped into the pop hole but never completed a full transition either onto the range or back

into the pen. The program then summarised the daily data per hen from within the three sampling time blocks across the experimental period to provide the daily time spent outdoors, the number of daily visits, maximum time per visit, and overall, how many days the range was accessed by each tagged hen (converted to percentage of total available days that each hen visited the range).

The daily time outdoors, and maximum time per visit were $\log_{10}$ transformed to normality with number of daily visits square-root transformed. Data were analysed in JMP® 12.1.1 (SAS Institute Inc., Cary, NC, USA) using General Linear Models (GLM) with α set at 0.05. GLMs were first used to compare the changes in range use parameters between the three sampling week periods by all stocking densities combined. GLMs were then used to compare the effects of individual hens nested within pen replicate nested within stocking density, pen replicate nested within stocking density and stocking density on range use parameters (daily hours outside, daily visits, maximum time per visit), separately within each sampling time period (weeks 22-26, weeks 27-31 and weeks 32-36). The main effects were still present when the interaction term of 'sampling weeks' was fitted to the model. We chose to analyse the sampling periods separately to focus on the differences between stocking density treatments within specific time periods as per the main objective of the study. Where significant differences were present between stocking densities, Student's t-tests were applied to the least squares means with a Bonferroni correction applied to the α level to account for multiple post-hoc comparisons. The percentages of available days that hens accessed the range could not be transformed to normality, thus, non-parametric Kruskal-Wallis tests were used to compare percentages of days outside between stocking densities separately for each sampling week period, and to compare all stocking densities combined across sampling weeks. Post-hoc comparisons for significant effects were made using the Bonferroni-Dunn method. Spearman's rho for non-parametric data were used to compare the relationship between average daily hours spent outdoors and total percentage of available days the range was accessed across the entire trial period, separately for each stocking density (excluding those birds that never went outside).

Total hen counts per day from the video decoding were converted to percentages of birds on the range simultaneously. These percentages were then averaged across all sampling times of day to provide average daily values for 26, 27 and 28 weeks of age for each stocking density. A GLM with repeated measures was used to compare the effect of sampling week and stocking density on the average percentage of hens out on the range. Where significant differences were present between stocking densities, Student's t-tests were applied to the least squares means with a Bonferroni correction applied to the α level to account for multiple post-hoc comparisons. Data from video decoding were also compiled graphically (only) to show the average percentages of hens out on the range within the three different range divides (Front, Middle, Back) at each hour across the day, averaged across all sampling weeks, separately for each stocking density.

1.3.2 Welfare Scoring and Albumen Corticosterone

For individual hens within each outdoor stocking density treatment, at each sampling time point (20, 26, 31 and 36 weeks of age) values were compiled for keel damage, body weight and toenail length, with the latter two measures also separated by pen

replicate. The effect of pen replicate was not assessed for keel damage due to low sample numbers in some categories at different bird ages (i.e. little damage present at 20 and 26 weeks of age), thus data from each replicate were combined into outdoor stocking density treatment only. Individual egg albumen corticosterone concentrations (ng/g) were compiled for each pen replicate within each outdoor stocking density treatment for the three sampling ages. Pearson's chi-squared tests compared frequencies of keel damage between outdoor stocking densities, separately at each sampling age. General Linear Models (GLM) were applied to compare separately for each sampling age, the effects of pen replicate nested within outdoor stocking density, and outdoor stocking density on hens' toenail length (n = 890), hens' body weight (n = 890) and albumen corticosterone (n = 540). Data were analysed in JMP® 12.1.0 with α set at 0.05. Where significant differences were present, Student's T-tests were used to compare the Least Squares Means with α level adjusted using the Bonferroni correction for multiple post-hoc comparisons.
Pearson Product-Moment Correlations were applied to assess the relationship between daily hours outside and toenail length and between daily hours outside and body weight of the RFID-tagged birds within each pen replicate of each outdoor stocking density treatment (50% of birds), separately for each sampling age (e.g., toenail length or body weight at 26 weeks was correlated with range use from 22-26 weeks of age, toenail length or body weight at 31 weeks was correlated with range use from 27-31 weeks and so forth).

1.3.3 Video Behaviours

The video data was averaged across each day to compile the average daily proportion of birds dust bathing, foraging or resting on the range and indoors for six days per outdoor stocking density replicate. Proportions were arcsine square-root transformed and GLM's with repeated measures applied to compare the effect of sampling week of age and outdoor stocking density on behavioural measures separately for outdoors and indoors. The proportion of birds dust bathing, foraging or resting, were also compared between indoor and outdoor areas for each outdoor stocking density across all sampled weeks. Where significant differences were present, Student's t-tests were used to compare the Least Squares Means with α level adjusted using the Bonferroni correction for multiple post-hoc comparisons.

1.3.4 Egg Production and Quality

The numbers of eggs laid were converted to daily percentage production for each pen, taking into account any hen mortality. The daily values were averaged to provide a weekly percentage production value for each pen of birds within each stocking density treatment during the range access period (weeks 21 to 35). Daily grading of normal vs. deformed eggs was converted to a percentage of daily eggs that were deformed and averaged per week during the range access period (weeks 21 to 35) for each pen within each stocking density. Weekly egg weights were compiled to show the average weekly egg weight for each pen within each stocking density treatment from weeks 21 to 35. The percentage production and percentage abnormal eggs were converted to proportions and arcsine square-root transformed for analysis. Egg quality parameters (percentage shell color reflectivity, egg shell breaking strength (N), shell deformation (µm), shell weight (g), shell thickness (µm), percentage shell and yolk color) were compiled based on hen age and stocking density.

General Linear Models with α set at 0.05 were used in JMP® 12.1.0, to assess the effects of stocking density, hen age and the interaction between stocking density and hen age on production, grading and egg quality parameters. Where significant differences were present, post-hoc Student's t-tests were applied to the least squares means with Bonferroni correction applied to the alpha level for greater than 3 comparisons.

1.4 RESULTS

Weekly photos taken of the range showed ground cover decreased from 100% to 0% coverage within 5 weeks in the 20 000 hens/ha ranges, within 6 weeks in the 10 000 hens/ha ranges, and only dropped as low at 20% in the 2000 hens/ha ranges by 8 weeks. Data collection occurred over the winter period where minimal pasture growth would be expected.

1.4.1 Range Use – RFID Tracking

Within all stocking densities combined, hours spent outdoors increased across trial duration ($P < 0.001$, weeks 22-26: raw values mean 3.44 ± s.e. 0.03; weeks 27-31: 4.01 ± 0.03; weeks 32-36: 4.20 ± 0.03, Figure 1.4.1). Within each sample period, there were differences in the daily hours outdoors between individual hens within each replicate of each stocking density treatment ($P < 0.001$) and differences between replicates within stocking densities ($P < 0.001$).These differences in replicates might be expected given ranging changed across time and replicates were assessed separately. There were also differences between stocking densities with hens in the 2000 hens/ha density treatment spending more time outside than hens from both the 10 000 hens/ha and 20 000 hens/ha densities during weeks 22-26 ($P < 0.001$, Figure 1.4.2). Within weeks 27-31 and weeks 32-36 hens from the 2000 hens/ha density spent the most time outdoors and hens from the 20 000 hens/ha density spent the least ($P < 0.001$, Figure 1.4.2).

Across all stocking densities combined, the number of daily visits outdoors differed between sample weeks with the fewest visits during weeks 22-26 and the most visits during weeks 32-26 ($P < 0.001$, weeks 22-26: raw values mean 11.39 ± s.e. 0.10; weeks 27-31: 12.30 ± 0.10; weeks 32-36: 13.91 ± 0.10, Figure 1.4.3). Within each sample period, there were differences in the number of daily visits between individual hens within each replicate of each stocking density treatment ($P < 0.001$) and differences between replicates within stocking densities ($P < 0.001$). There were also differences between stocking densities, with hens in the 2000 hens/ha density showing the most visits outdoors during weeks 22-26 ($P < 0.001$, Figure 1.4.3). Within weeks 27-31, hens in the 2000 hens/ha densities showed the fewest visits outdoors ($P < 0.001$, Figure 1.4.3). During weeks 32-36 hens from the 2000 hens/ha density showed the fewest visits outdoors and hens from the 20 000 hens/ha density the most ($P < 0.001$, Figure 1.4.3).

Across all stocking densities combined, the maximum time (minutes) per visit did not differ between sample weeks ($P = 0.29$, Figure 1.4.4). Within each sample period, there were differences in the maximum time per visit between individual hens within each replicate of each stocking density treatment ($P < 0.001$) and differences

between replicates within stocking densities ($P < 0.001$). There were also differences between stocking densities with hens in the 10 000 hens/ha density showing longer maximum times per visit than hens from the 2000 hens/ha density during weeks 22-26, but neither density group differed from the 20 000 hens/ha density ($P < 0.03$, Figure 1.4.4). Within weeks 27-31 hens from the 2000 hens/ha density spent the longest time per visit outdoors ($P < 0.001$) and within weeks 32-36 hens from the 2000 hens/ha density spent the longest time per visit outdoors and hens from the 20 000 hens/ha stocking density treatment spent the shortest ($P < 0.001$, Figure 1.4.4).

Within all stocking density treatments within all sampling periods the individual hens varied in the percentage of total available days they accessed the range (Figure 1.4.5) but there were no differences between stocking densities within each sampling period in the percentage of available days that individual hens accessed the range (all $P \geq 0.14$, Figure 1.4.5). Across the trial duration there was a very small percentage of hens from each density that never went outdoors (although most did trigger false readings on the RFID system indicating functional tags; all treatments: 2% of tagged hens) and a small percentage that visited the range on 1 – 10% of available days (2000 hens/ha: 1.3%; 10 000 hens/ha: 1.3%; 20 000 hens/ha: 6% (Figure 4.5). However, there were a large proportion of hens that visited the range on a daily basis across the entire trial period (2000 hens/ha: 80.5%; 10 000 hens/ha: 66.5%; 20 000 hens/ha: 71.4%; Figure 1.4.5). Within all stocking densities combined, there were differences across the sampling periods with more hens using the range during the final 32-36 weeks sampling period ($P < 0.001$, Figure 1.4.5).

Finally, there were positive relationships within all stocking densities between average time spent outdoors and the percentage of available days the range was accessed (2000 hens/ha: $r_S = 0.45$, $P < 0.001$; 10 000 hens/ha: $r_S = 0.43$, $P < 0.001$; 20 000 hens/ha: $r_S = 0.42$, $P < 0.001$).

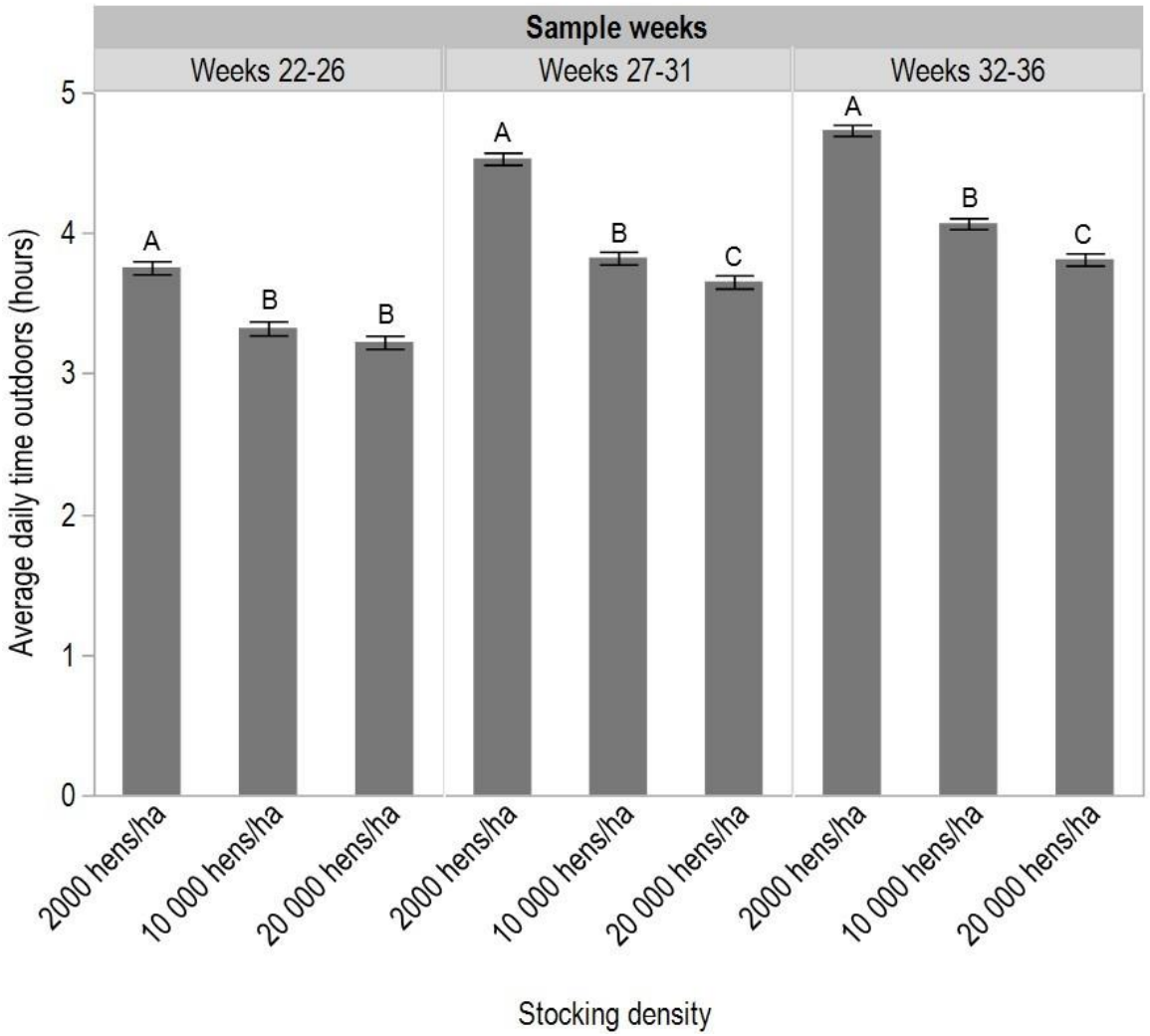

Figure 1.4.1 The average time (hours) ± s.e. (of the raw values) spent outdoors per day for hens from the three stocking density treatments (2000 hens per hectare (ha), 10 000 hens/ha, 20 000 hens/ha) within the three sample periods (weeks 22-26, weeks 27-31 and weeks 32-36). Dissimilar letters indicate differences between stocking densities within sample weeks.

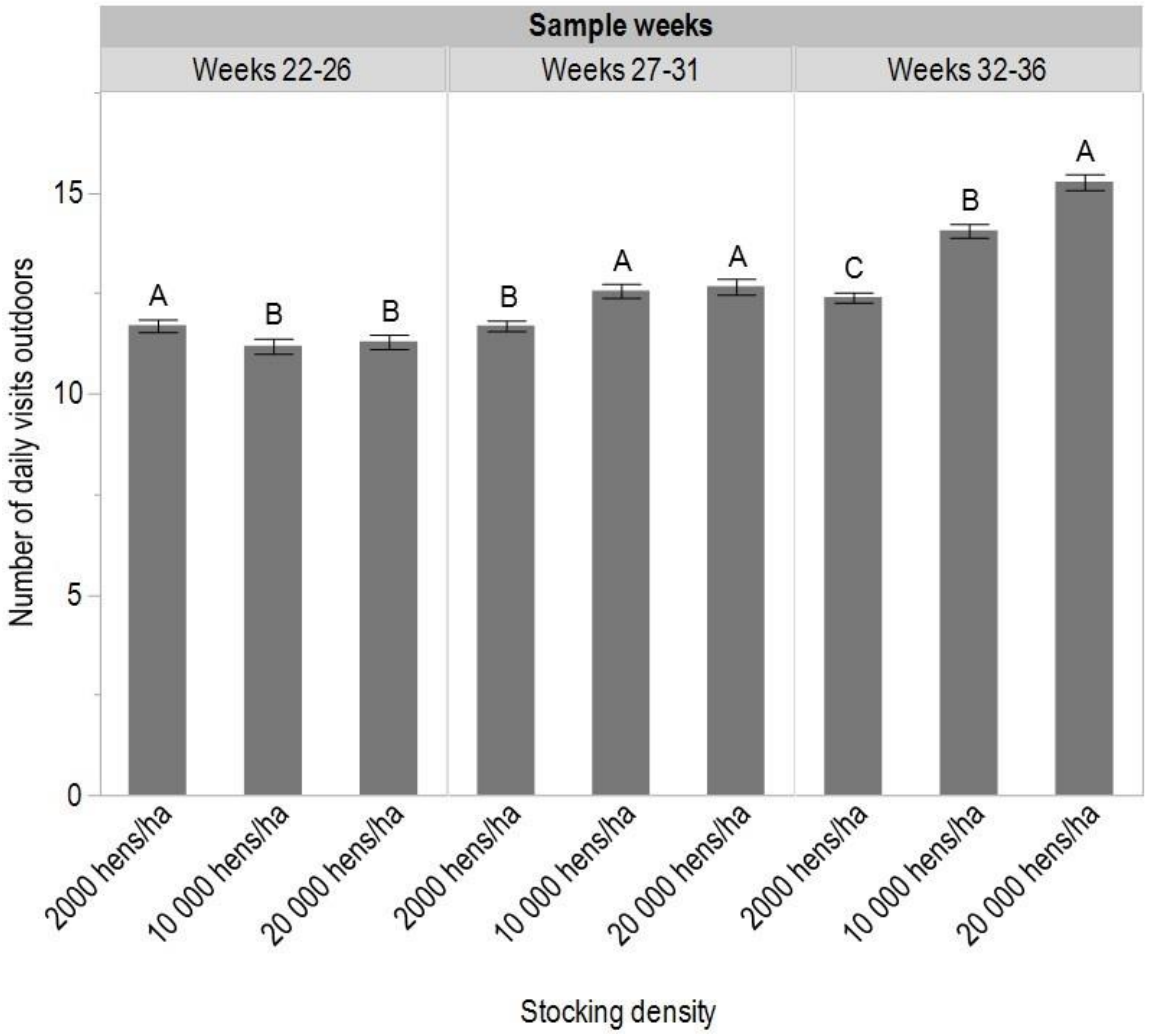

Figure 1.4.2 The average number ± s.e. (of the raw values) of daily visits outdoors by hens from the three stocking density treatments (2000 hens per hectare (ha), 10 000 hens/ha, 20 000 hens/ha) within the three sample periods (weeks 22-26, weeks 27-31 and weeks 32-36). Dissimilar letters indicate differences between stocking densities within sample weeks.

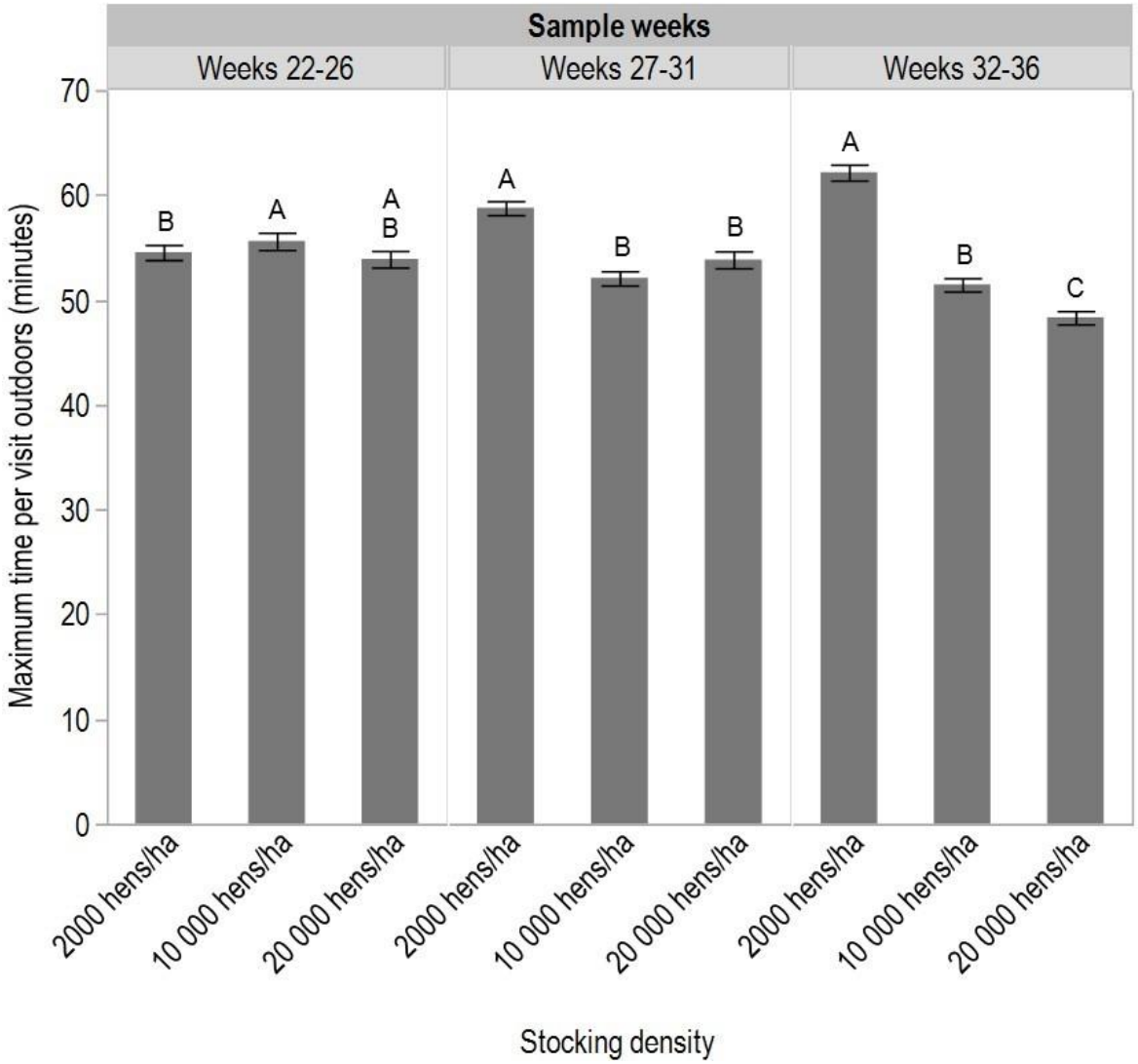

Figure 1.4.3 The average maximum time (minutes) ± s.e. (of the raw values) spent outdoors per visit for hens from the three stocking density treatments (2000 hens per hectare (ha), 10 000 hens/ha, 20 000 hens/ha) within the three sample periods (weeks 22-26, weeks 27-31 and weeks 32-36). Dissimilar letters indicate differences between stocking densities within sample weeks.

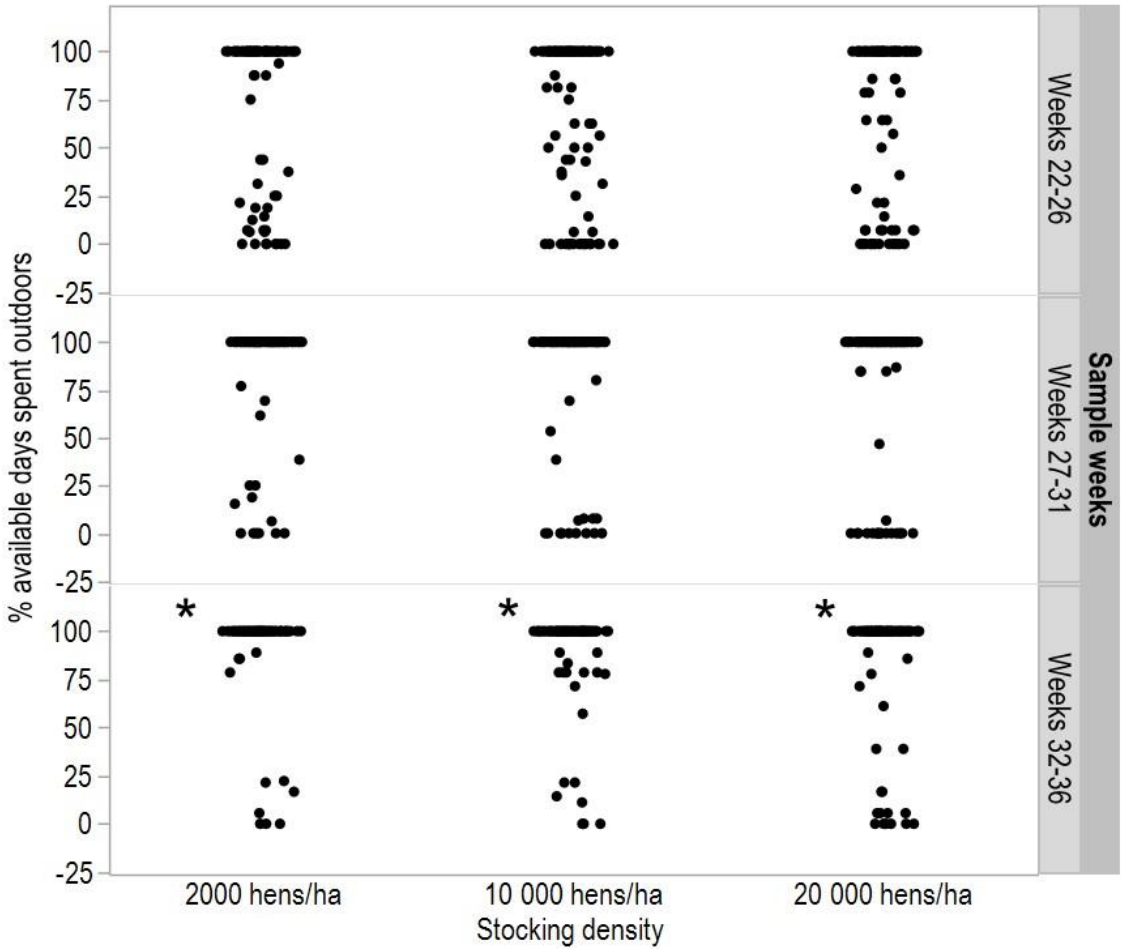

Figure 1.4.4 The percentage of available days that individual hens from each of the three stocking density treatments (2000 hens per hectare (ha), 10 000 hens/ha, 20 000 hens/ha) accessed the range within the three sample periods (weeks 22-26, weeks 27-31 and weeks 32-36) where differences between sampling weeks are indicated by '*'.

1.4.2 Range Use - Video Observations

Total counts of all hens on the range at 26 - 28 weeks showed the highest percentage of hens used the range in the 2000 hens/ha stocking density and the lowest percentage of birds in the 20 000 hens/ha stocking density but neither of these densities differed from the 10 000 hens/ha density treatment ($P < 0.001$, 2000 hens/ha: LSM 48.87 ± s.e. 1.72; 10 000 hens/ha: 41.81 ± 1.79, 20 000 hens/ha: 36.88 ± 1.76). On average, less than half of the birds were on the range at any point in time within each stocking density (range of all individual sampling counts 2000 hens/ha: 2.67 – 78%; 10 000 hens/ha: 10 – 74.67%; 20 000 hens/ha: 2.67 – 64%). There tended to be an effect of sampling week ($P = 0.05$) but no interaction between stocking density and sampling week ($P = 0.69$). Hens within each stocking density used all areas of the range across all times of day (Figure 1.4.5).

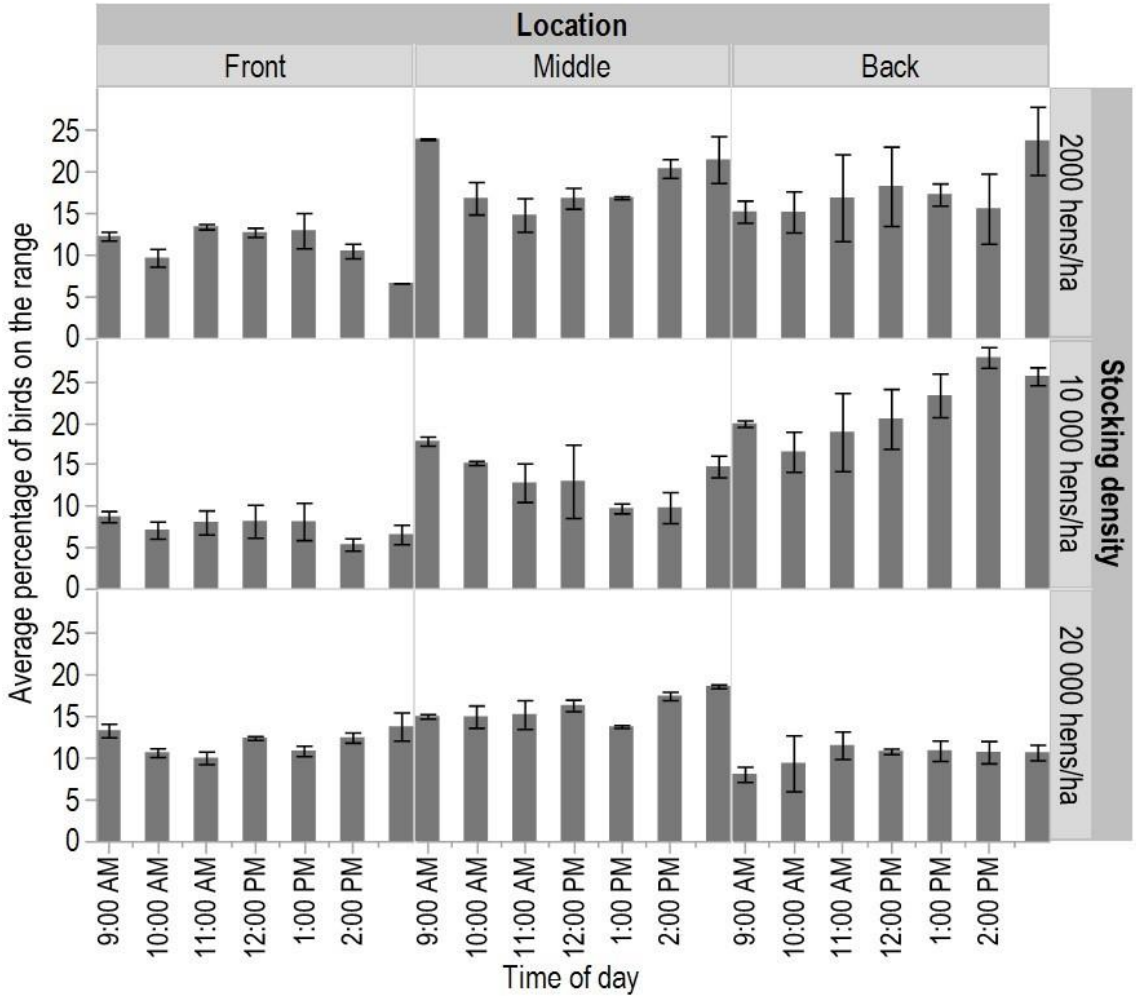

Figure 1.4.5 The average percentage (± s.e.) of hens in different locations on the range (Front, Middle, Back) for each stocking density treatment (2000 hens per hectare (ha), 10 000 hens/ha, 20 000 hens/ha) within each hour across the day from 0900 until 1500 h.

1.4.3 Welfare Scoring

Total flock mortality was low at 0.8% across the trial period (2 000 hens/ha: n = 5; 10 000 hens/ha: n = 2; 20 000 hens/ha: n = 3). By 36 weeks of age, cumulatively, 5.5% of birds across all pens had signs of toe damage (broken toes) (2 000 hens/ha: 3.1%; 10 000 hens/ha: 1.7%; 20 000 hens/ha: 0.7%), and only 0.3% of birds showed any footpad dermatitis with all being category 3 lesions (2 000 hens/ha: n = 1; 20 000 hens/ha: n = 2). There were differences between outdoor stocking densities at 36 weeks of age where hens from the 10 000 hens/ha outdoor stocking density showed the least overall keel damage ($P < 0.007$; 2 000 hens/ha: 19.7% damaged, 2.7% severely damaged; 10 000 hens/ha: 9.4% damaged, 1.7% severely damaged; 20 000 hens/ha: 16.5% damaged, 2.4% severely damaged). In contrast, there were no differences between outdoor stocking densities at other sampling ages although the percentage damaged increased with age (20 weeks of age: 2 000 hens/ha: 0.7% damaged, 10 000 hens/ha: 1.7% damaged, 20 000 hens/ha: 0% damaged; 26 weeks: 2 000 hens/ha: 1.7% damaged, 10 000 hens/ha: 2.7% damaged, 20 000

hens/ha: 1.01% damaged; 31 weeks: 2000 hens/ha: 4.08% damaged; 10 000 hens/ha: 6.04% damaged; 20 000 hens/ha: 5.05% damaged, all $P > 0.20$ for differences between outdoor densities within each sampling age).

Bird weight for pullets (17 weeks of age) assigned to different outdoor stocking density pens at bird placement were unexpectedly found to be different after placement occurred ($P < 0.001$, Figure 1.4.6) and between replicates for pullets assigned to the 10 000 hens/ha treatment ($P = 0.03$). This was indicative of poor flock uniformity during rearing. Approximately 13 crates of pullets were placed into each pen (12 birds/crate). Eight personnel removed crates from the delivery truck and placed them into each pen in no intentional order. It is possible the majority of the first crates off the truck were placed into the first pens which were assigned to the 2 000 hens/ha outdoor stocking density treatment and that these birds were the smallest. Birds were not remixed to avoid social stress. There were still differences in average body weight between the pens assigned to outdoor stocking density treatments at pre-release (20 weeks of age) with birds assigned to the 2 000 hens/ha outdoor stocking density treatment having lower body weight ($P < 0.001$, Figure 1.4.6) but only a trend for differences between pen replicates ($P \geq 0.07$). At 26 weeks of age, after 5 weeks of range access, hens from the 2 000 hens/ha outdoor stocking densities were still of a lower body weight ($P < 0.001$, Figure 1.4.6) and there was an effect of pen replicate within outdoor stocking density ($P = 0.02$) with pen replicates differing within both the 2 000 and 10 000 hens/ha outdoor stocking densities ($P < 0.017$). At 31 weeks of age there were no differences in body weight between outdoor stocking density treatments ($P = 0.10$, Figure 1.4.6) but there continued to be an effect of pen replicate ($P = 0.001$) within the 2 000 and 10 000 hens/ha densities ($P = 0.017$). At the final 36 weeks of age sampling there was no effect of outdoor stocking density on body weight ($P = 0.96$, Figure 1.4.6) but there was a pen replicate effect within the 10 000 hens/ha outdoor stocking density treatment ($P = 0.003$).

There was a negative correlation between body weight and average daily hours spent outside for pen replicate one of the 10 000 hens/ha outdoor stocking density at 26 weeks of age ($r = -0.24$, $P = 0.04$). There tended to be positive associations between body weight and average daily hours spent on the range within both pen replicates of the 2 000 hens/ha outdoor stocking density at 36 weeks ($P \leq 0.07$). There was no relationship between body weight and average daily hours spent outside within the 20 000 hens/ha outdoor stocking density or at the other sampling ages for the 2 000 and 10 000 hens/ha treatments ($P \geq 0.11$).

There was an effect of outdoor stocking density ($P = 0.001$) and pen replicate ($P = 0.003$) on toenail length at 31 weeks of age, with the longest toenails found on the hens from the 2 000 hens/ha outdoor stocking density (LSM ± SEM: 2 000 hens/ha: 1.51 ± 0.007, 10 00 hens/ha: 1.48 ± 0.007, 20 000 hens/ha: 1.48 ± 0.007). But there were no differences between the outdoor stocking density treatments in toenail length at 20 weeks of age (pre-release sampling LSM ± SEM 2 000 hens/ha: 1.46 ± 0.007, 10 00 hens/ha: 1.47 ± 0.007, 20 000 hens/ha: 1.48 ± 0.007, $P = 0.23$), 26 weeks of age (LSM ± SEM 2 000 hens/ha: 1.48 ± 0.007, 10 000 hens/ha: 1.48 ± 0.006, 20 000 hens/ha: 1.48 ± 0.007, $P = 0.74$) or 36 weeks of age (LSM ± SEM 2 000 hens/ha: 1.54 ± 0.007, 10 000 hens/ha: 1.52 ± 0.006, 20 000 hens/ha: 1.53 ±

0.007, *P* = 0.27). There were differences between some of the outdoor stocking density pen replicates at 20 and 26 weeks of age (*P* < 0.001) but not at 36 weeks of age (*P* = 0.18).

Within all pen replicates for all outdoor stocking densities at 36 weeks of age, toenail length was shorter for birds that spent a longer time on the range (all *r* ≥ -0.23, *P* ≤ 0.04). Toenail length was not correlated with daily time (hours) on the range at 26 weeks of age for all pen replicates within all outdoor stocking densities (all *P* ≥ 0.25), or at 31 weeks of age for the 2 000 and 20 000 hens/ha densities (all *P* ≥ 0.10), but there was a significant negative relationship for pen replicate one of the 10 000 hens/ha density (*r* = -0.27, *P* = 0.02).

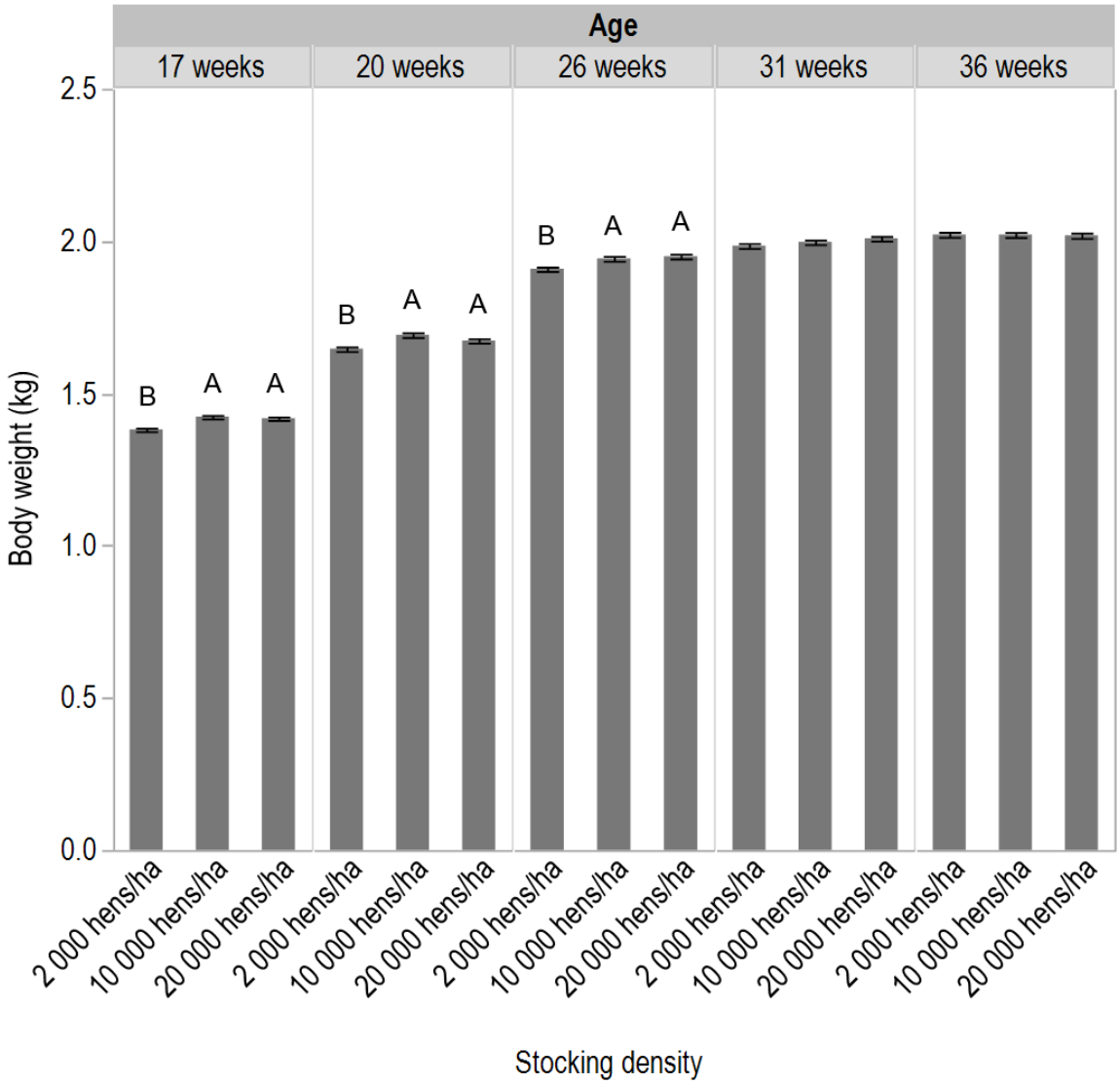

Figure 1.4.6 The average body weight (kg) ± SEM measured at 17 weeks (1 week after pullet placement), then 20 weeks (1 week prior to range access) 26, 31 and 36 weeks of age for all hens of each outdoor stocking density treatment (2 000, 10 000 and 20 000 hens/ha). Stocking densities without common letters were significantly different (adjusted *P* < 0.017).

1.4.4 Albumen Corticosterone

Concentrations of albumen corticosterone increased with age when combined across all stocking density treatments ($P < 0.001$, the LSM ± SEM for 23 weeks: 0.63 ± 0.017 ng/g; 29 weeks: 0.94 ± 0.015 ng/g and 35 weeks: 1.76 ± 0.15 ng/g, Figure 1.4.7). At 23 weeks of age there were no differences in corticosterone concentrations between outdoor stocking densities ($P = 0.44$), but there were differences between the pen replicates within the 10 000 and 20 000 hens/ha outdoor stocking densities ($P = 0.006$, Figure 1.4.7). At 29 weeks of age there was an effect of outdoor stocking density ($P < 0.001$) with the highest albumen corticosterone concentrations seen in the 10 000 hens/ha density ($P < 0.017$, Figure 1.4.7). There were also significant differences between each pen replicate within outdoor stocking density ($P < 0.001$, Figure 1.4.7). At 35 weeks the significant effect of outdoor stocking density ($P < 0.001$) showed hens from the 20 000 hens/ha treatment had the highest albumen corticosterone concentrations and hens from the 10 000 hens/ha treatment had the lowest ($P < 0.017$, Figure 1.4.7). There were also significant differences between each replicate within density ($P < 0.001$, Figure 1.4.7). Overall, eggs from the 10 000 hens/ha outdoor stocking density showed the smallest change in corticosterone concentrations between 23 and 35 weeks (the mean ± SEM for differences between 23 and 35 weeks were: 2000 hens/ha: 1.16 ± 0.09 ng/g; 10 000 hens/ha: 0.89 ± 0.16 ng/g and 20 000 hens/ha: 1.33 ± 0.16 ng/g).

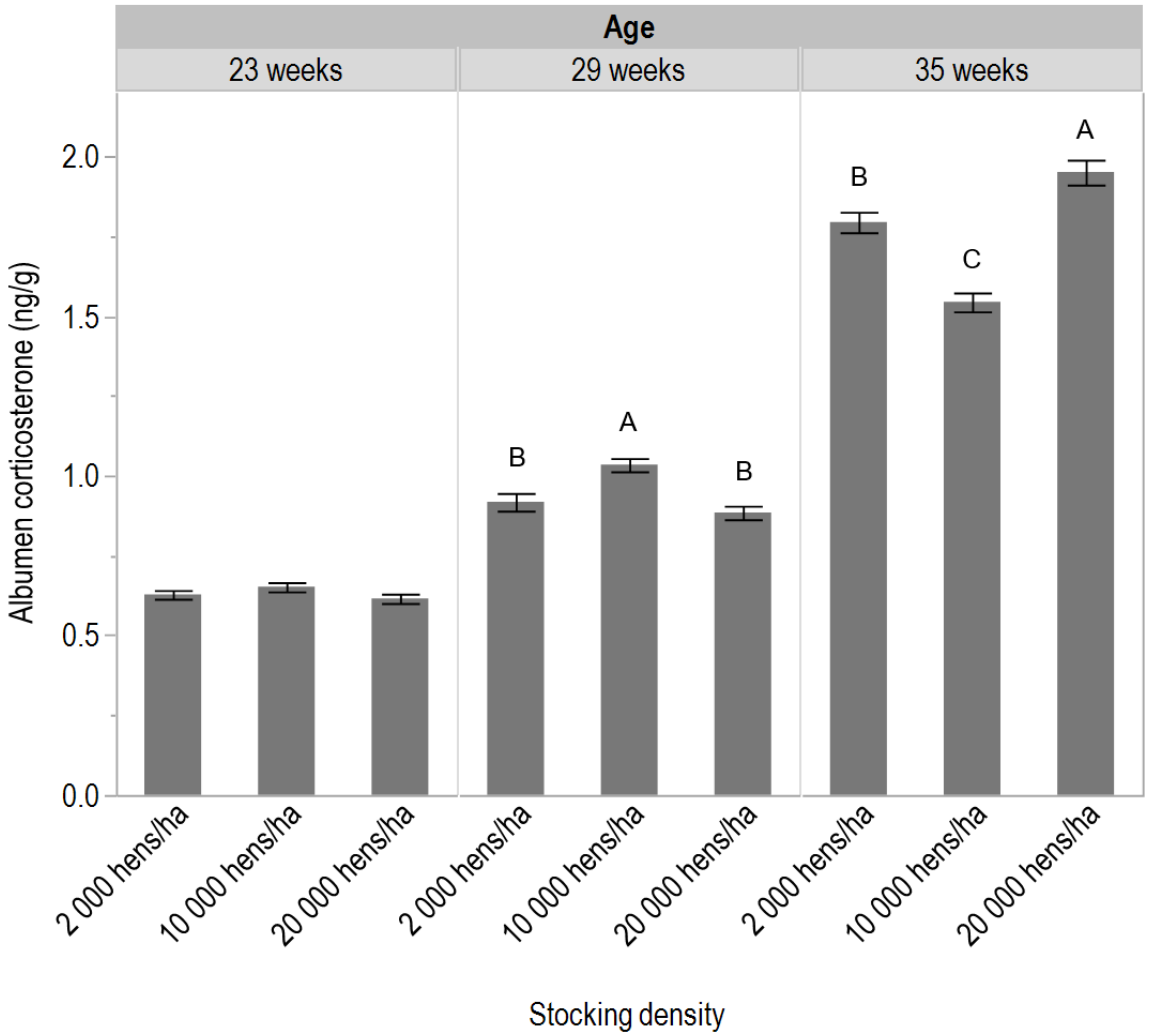

Figure 1.4.7 The mean ± SEM albumen corticosterone concentrations (ng/g) for eggs from hens within the three outdoor stocking density treatments (2 000, 10 000 and 20 000 hens/ha) at each sampling age point (23 weeks, 29 weeks, 35 weeks). Stocking densities without common letters were significantly different (adjusted $P < 0.017$).

1.4.5 Behavioural Observations

Across all sampling weeks and for all outdoor stocking densities, more hens were observed dust bathing and foraging outdoors, and more resting indoors (all $P < 0.001$, LSM ± SEM of recorded values: indoor dust bathing 1.69% ± 0.31, outdoor dust bathing 3.53% ± 0.31; indoor foraging 1.51% ± 0.78, outdoor foraging 35.59% ± 0.78; indoor resting 6.94% ± 0.32, outdoor resting 1.37% ± 0.32).

There was an effect of outdoor stocking density ($P < 0.01$) on proportions of hens dust bathing while indoors with more dust bathing observed in the 2 000 hens/ha densities than in the 20 000 hens/ha densities ($P < 0.017$, Figure 1.4.8). There was also an effect of pen replicate within outdoor stocking density ($P < 0.001$) for the 2000 hens/ha densities ($P < 0.017$, Figure 1.4.8). There was an effect of week of age ($P = 0.002$) with the most dust bathing seen indoors at 25 weeks of age ($P < 0.017$) but no interaction between outdoor stocking density and week of age ($P = 0.87$).

There was only a trend for differences between outdoor stocking densities in the proportion of hens dust bathing outdoors ($P = 0.08$, Figure 1.4.8) and no differences between pen replicates within outdoor stocking densities ($P = 0.21$). There was an effect of week of age ($P = 0.004$) with the least dust bathing seen outdoors at 27 weeks of age ($P < 0.017$) but no interaction between outdoor stocking density and week of age ($P = 0.99$).

There was a trend for differences between stocking densities in the proportions of hens observed foraging indoors ($P = 0.07$, Figure 1.4.9) but no differences between pen replicates or week of age (all $P \geq 0.26$) and no interaction between outdoor stocking density and week of age ($P = 0.52$). There were differences between outdoor stocking densities in the proportions of hens observed foraging outdoors ($P < 0.001$) with the least hens observed foraging outdoors in the 2 000 hens/ha density ($P < 0.017$, Figure 1.4.9). There was trend for differences between pen replicates ($P = 0.06$), specifically within the 20 000 hens/ha density. There was also an effect of week of age ($P = 0.007$) with the least birds foraging at 25 weeks of age ($P < 0.017$) but no interaction between outdoor stocking density and week of age ($P = 0.96$).

The proportion of hens resting indoors differed between outdoor stocking densities ($P < 0.001$) with the greatest proportion of birds resting indoors for the 2 000 hens/ha treatment ($P < 0.017$, Figure 1.4.10). The proportion of hens resting indoors also differed between pen replicates ($P = 0.02$) for the 2 000 and 20 000 hens/ha densities ($P < 0.008$) but there were no differences between weeks of age ($P = 0.11$) and no interaction between outdoor stocking density and week of age ($P = 0.61$).

The proportion of hens resting outdoors also differed between outdoor stocking densities ($P < 0.001$) with the 2 000 hens/ha having the highest proportions of hens resting and the 20 000 hens/ha the least ($P < 0.017$, Figure 1.4.10). But pen replicate within outdoor stocking density did not vary ($P = 0.12$) with no effect of week of age, or interaction between outdoor stocking density and week of age (all $P \geq 0.42$).

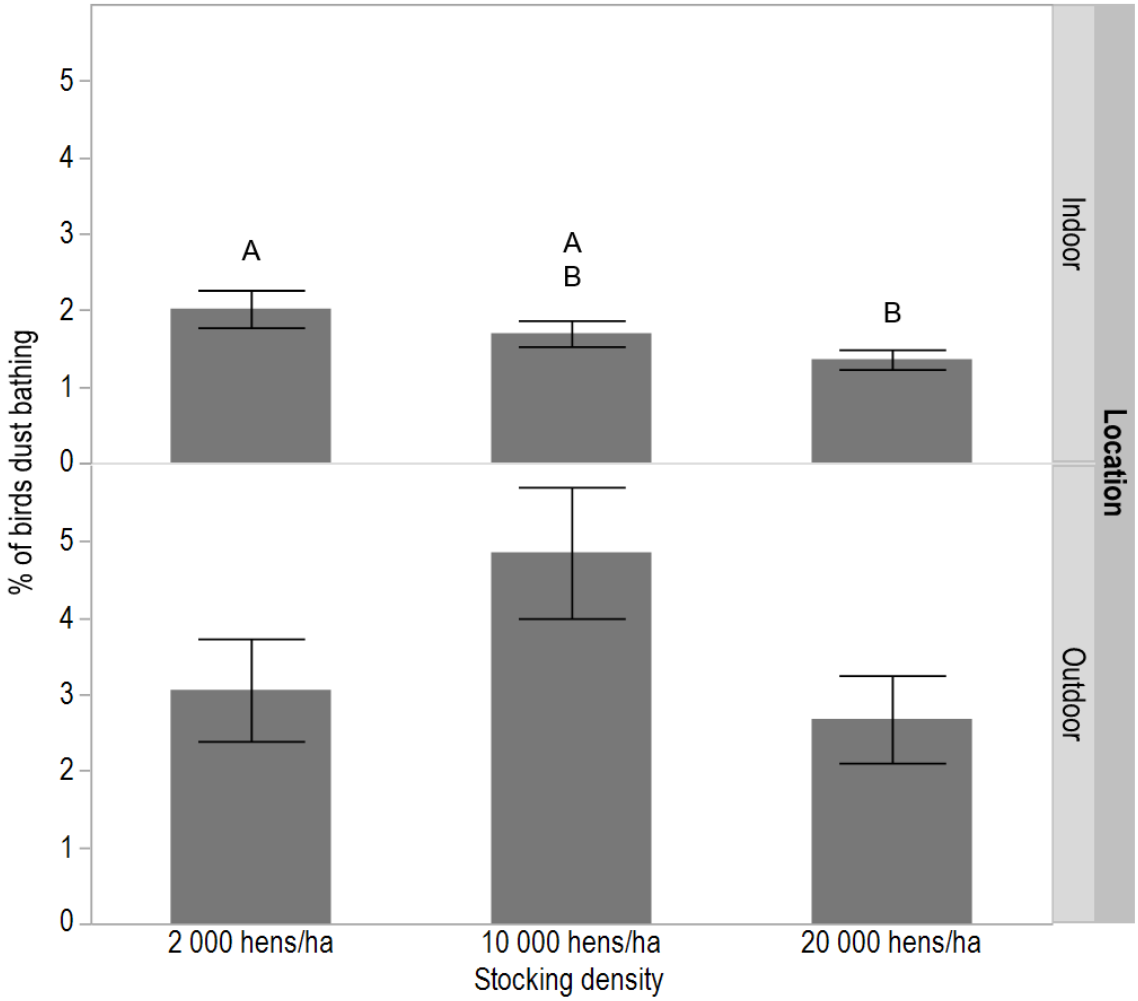

Figure 1.4.8 The mean percentages ± SEM of hens observed dust bathing indoors or outdoors on the range (raw values) for each outdoor stocking density treatment (2 000, 10 000 and 20 000 hens/ha). Stocking densities without common letters were significantly different (adjusted $P < 0.017$).

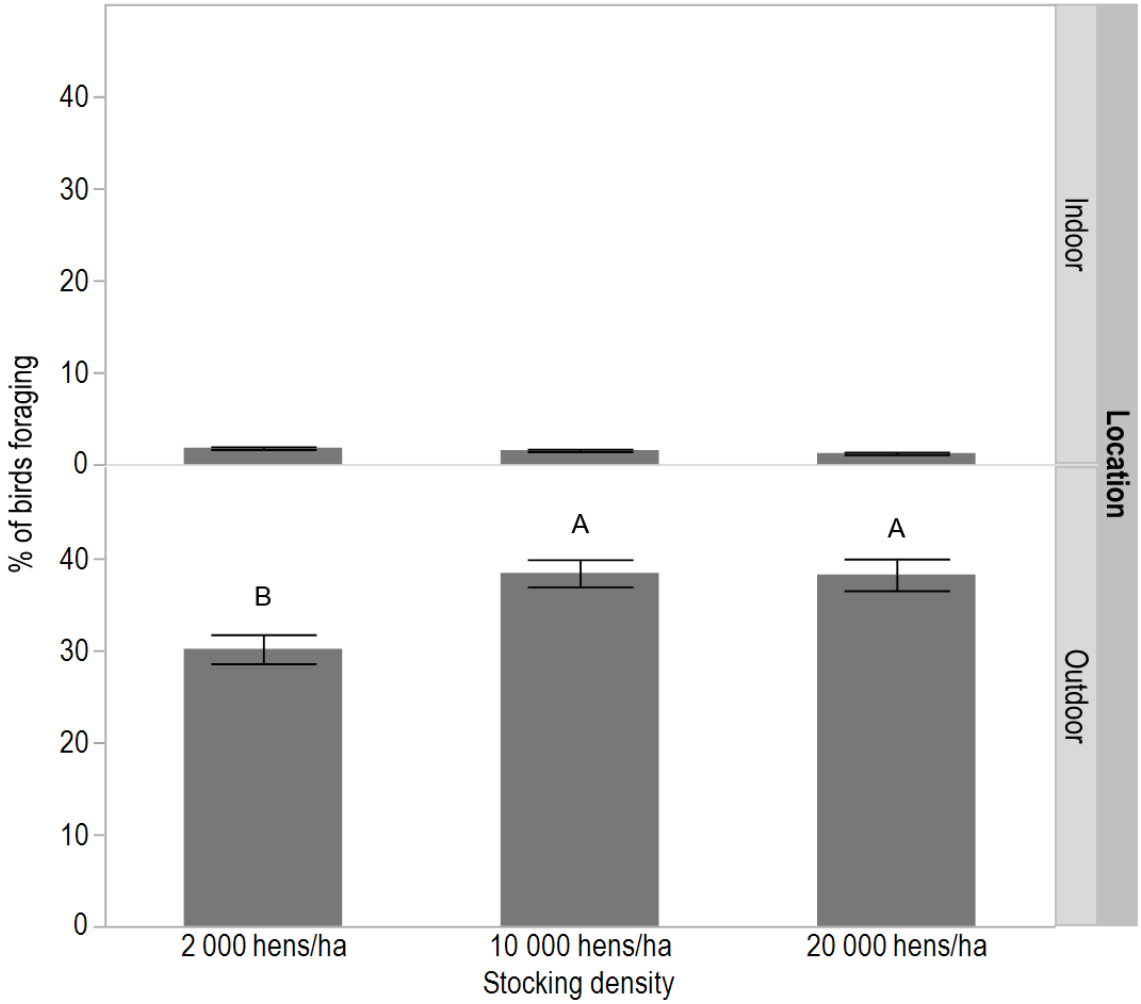

Figure 1.4.9 The mean percentages ± SEM of hens observed foraging indoors or outdoors on the range (raw values) for each outdoor stocking density treatment (2 000, 10 000 and 20 000 hens/ha). Stocking densities without common letters were significantly different (adjusted $P < 0.017$).

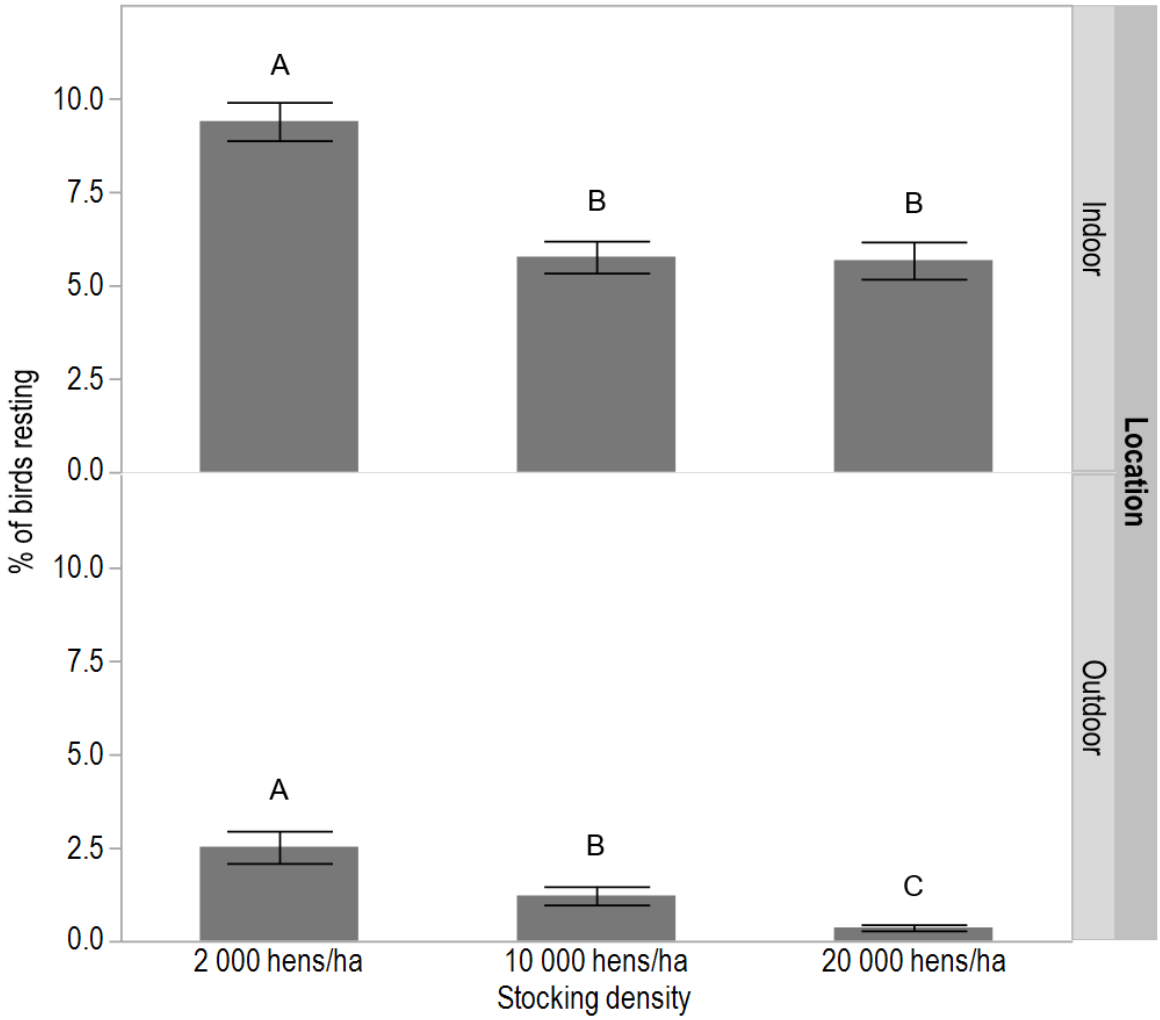

Figure 1.4.10 The mean percentages ± SEM of hens observed resting indoors or outdoors on the range (raw values) for each outdoor stocking density treatment (2 000, 10 000 and 20 000 hens/ha). Stocking densities without common letters were significantly different (adjusted $P < 0.017$).

1.4.6 Behavioural Tests

There were no differences between range access groups in the number of attempts to induce TI (H = 0.17, DF = 2, $P = 0.92$) or the duration of TI (H = 3.96, DF = 2, $P = 0.14$, Table 1.4.1). There were also no differences between range access groups in the latency to first struggle (H = 1.21, DF = 2, $P = 0.55$), number of struggles (H = 1.63, DF = 2, $P = 0.44$) in the MR test, or the 20-min plasma corticosterone concentrations (H = 0.39, DF = 2, $P = 0.82$, Table 1.4.1). However, there were differences between range access groups in total vocalisations produced during MR tests (H = 11.29, DF = 2, $P = 0.004$, Table 1.4.1) with indoor-preferring birds showing the fewest total vocalisations ($P = 0.002$). There was also a trend for differences between range access groups in the latencies to first vocalise (H = 5.07, DF = 2, $P = 0.08$, Table 1.4.1). There were no differences between range access groups in the latency to first vocalise (H = 0.64, DF = 2, $P = 0.72$) or total number of vocalisations made during the OFT (H = 0.19, DF = 2, $P = 0.91$, Table 1.4.1). But there were significant differences in the latency to first movement (H = 9.65, DF = 2, $P = 0.008$, Table 1.4.1) and total number of squares crossed (H = 9.48, DF = 2, $P = 0.009$,

Table 1.4.1) with indoor-preferring and moderate-outdoor hens being slowest to first move (P = 0.02) and crossing fewer squares (P = 0.02) than the outdoor-preferring hens.

		Indoor	Moderate-outdoor	Outdoor
TI	# attempts	1.90 ± 0.25	2.17 ± 0.35	1.95 ± 0.13
	Duration (secs)	98.52 ± 16.32	151.56 ± 22.16	134.48 ± 11.06
MR	Lat. vocalise (secs)	194.57 ± 26.10	121.56 ± 27.89	139.09 ± 13.99
	# vocals	*1.57 ± 0.61*	*10.67 ± 3.24*	*7.14 ± 1.05*
	Lat. struggle (secs)	125.90 ± 29.05	67.39 ± 16.65	113.66 ± 14.13
	# struggles	5.86 ± 1.47	8.28 ± 1.76	8.83 ± 1.28
	Cort. response (ng/ml)	1.94 ± 0.19	2.03 ± 0.33	1.86 ± 0.10
OFT	Lat. vocalise (secs)	63.33 ± 22.70	45.72 ± 22.06	40.72 ± 8.63
	# vocals	37.48 ± 11.85	44.72 ± 18.09	33.12 ± 5.15
	Lat. move (secs)	*124.33 ± 20.77*	*123.28 ± 19.70*	*77.35 ± 8.41*
	# squares crossed	*25.57 ± 4.0*	*24.56 ± 4.16*	*45.23 ± 4.58*

Table 1.4.1 The mean ± SEM for measured variables during behavioural tests. Tonic immobility (TI): number of attempts to induce TI and duration of TI (secs); manual restraint (MR): latency to first vocalise (seconds), the total number of vocalisations made, latency to first struggle (seconds), total number of struggles made and 20-minute elevated plasma corticosterone response (ng/mL); open field test (OFT): latency to first vocalise (seconds), total number of vocalisations made, the latency to first move (seconds) and total number of squares crossed. Values are shown for the three different range access groups of hens: Indoor (accessed the range on 0 – 10% of available days), moderate-outdoor (accessed the range on 30-60% of available days) and outdoor (accessed the range on 100% of available days). Values italicized indicate significant differences between range access groups with all $P \leq 0.02$.

1.4.7 Egg Production

Across the range access period there were no differences in production level between stocking densities ($F_{(2,45)} = 0.41$, $P = 0.67$) and no interaction between hen age and stocking density ($F_{(28,45)} = 0.35$, $P = 0.99$). However, as expected, production increased as the hens aged with birds in the 2,000 hens/ha stocking density reaching 97.67% production, birds in the 10,000 hens/ha stocking densities reaching 97.71% production and birds in the 20,000 hens/ha stocking densities reaching 98.65% production. There were also no differences in average weekly egg weights between stocking density treatments ($F_{(2,45)} = 2.52$, $P = 0.09$) and no interaction between hen age and stocking density ($F_{(28,45)} = 1.27$, $P = 0.23$) but as expected, egg weights increased weekly across the trial period ($F_{(14,45)} = 430.0$, $P < 0.0001$; 23 weeks: LSM ± SE 51.83 ± 0.23, 35 weeks: 67.07 ± 0.23).

Similarly, there were also no differences between stocking densities in the proportion of deformed eggs ($F_{(2,45)} = 1.24$, $P = 0.30$) and no interaction between hen age and stocking density ($F_{(28,45)} = 0.61$, $P = 0.92$). There was significant variation between

the weeks across the trial duration but in no consistent pattern ($F_{(14,45)}$ = 7.01, P < 0.0001, Figure 1.4.11).

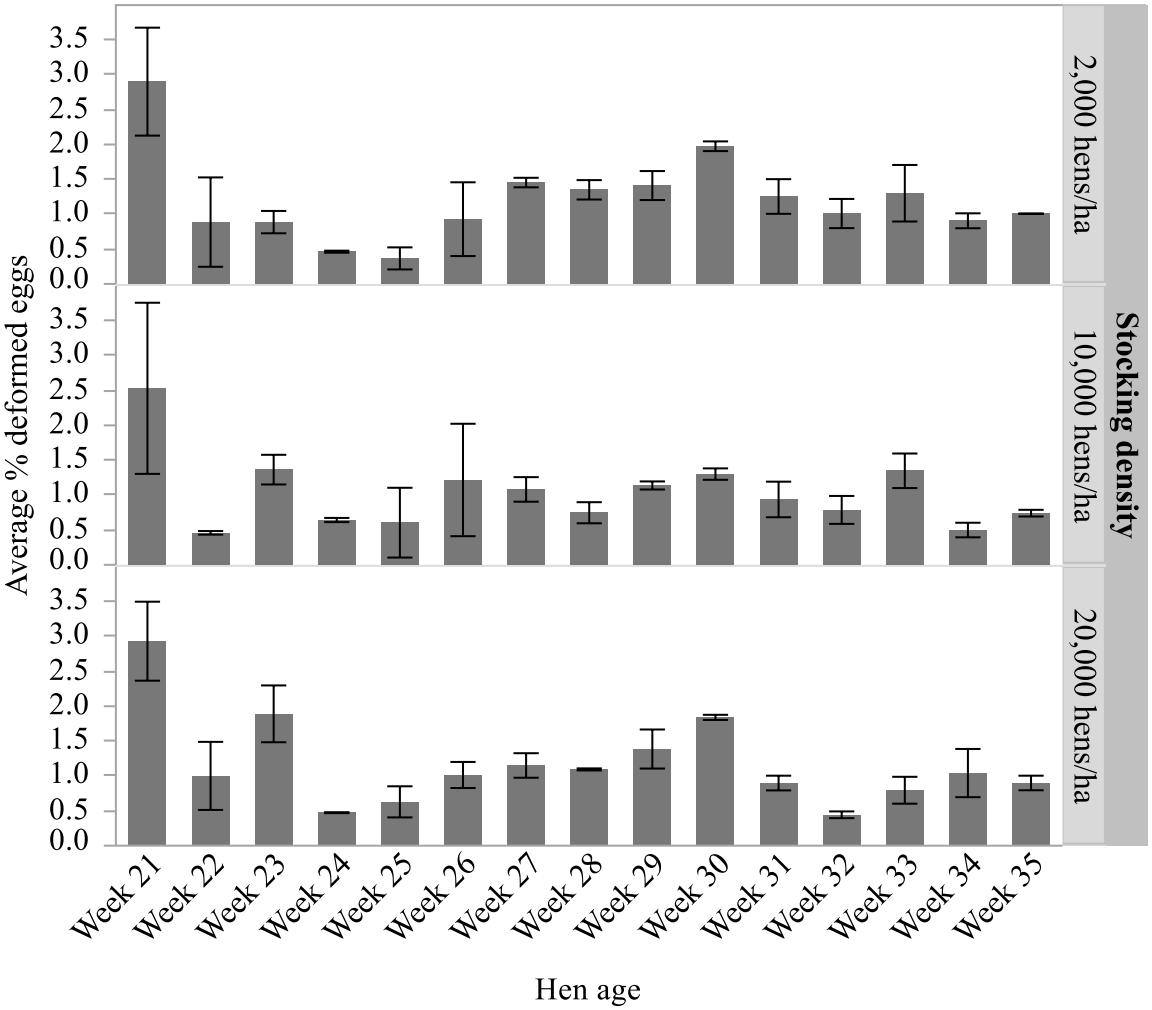

Figure 1.4.11 The raw values showing the total average percentage (±SE) of deformed eggs (body-checked, misshapen, pimpled, rough-shelled or soft-shelled) laid weekly during the range access period for hens from the three stocking density treatments (2,000 hens per hectare (ha), 10,000 hens/ha, 20,000 hens/ha). Differences were present between weeks of age, but not between stocking densities.

1.4.8 Egg Quality

There were no differences between stocking densities in the percentage shell reflectivity ($F_{(2,531)}$ = 0.30, P = 0.74, Table 1.4.2), but there was an overall effect of hen age with the percentage reflectivity being lowest, and thus egg shells darkest, in eggs laid at 25 weeks of age ($F_{(2,531)}$ = 15.71, P < 0.0001, Table 1.4.2). There was no interaction between stocking density and hen age ($F_{(4,531)}$ = 0.88, P = 0.48).

There was a trend for differences in shell breaking strength across the stocking densities ($F_{(2,531)}$ = 2.66, P = 0.07, Table 1.4.2), but no changes with hen age ($F_{(2,531)}$ = 1.87, P = 0.16, Table 1.4.2) and no interaction between stocking density and hen age ($F_{(4,531)}$ = 1.13, P = 0.34).

There were no differences between stocking densities in shell deformation (µm) ($F_{(2,531)}$ = 0.18, P = 0.83) but there was an effect of hen age (($F_{(2,531)}$ = 9.26, P < 0.0001) with the shortest breaking distance in eggs laid from hens at 36 weeks of age (Table 1.4.2). There was no interaction between stocking density and hen age ($F_{(4,531)}$ = 0.17, P = 0.95).

There was an effect of stocking density on yolk color ($F_{(2,531)}$ = 33.41, P < 0.0001) with eggs laid from hens housed at the lowest stocking density showing the highest colour score (Table 1.4.2) and an effect of hen age ($F_{(2,531)}$ = 41.54, P < 0.0001) with the highest colour score in eggs laid at 25 weeks of age and the lowest in eggs laid at 30 weeks of age (Table 1.4.2). There was no interaction between stocking density and hen age ($F_{(4,531)}$ = 0.77, P = 0.54).

There was no effect of stocking density on shell weight (g) ($F_{(2,531)}$ = 2.20, P = 0.11) but there was an effect of hen age ($F_{(4,531)}$ = 78.35, P < 0.0001) with the highest weight in eggs laid at 36 weeks of age and the lowest in eggs laid at 26 weeks of age (Table 1.4.2). There was no interaction between hen age and stocking density ($F_{(4,531)}$ = 0.79, P = 0.53).

In contrast, there was an effect of stocking density on the percentage shell weight ($F_{(2,531)}$ = 5.57, P < 0.004) where eggs laid from hens housed at the highest density showed the highest percentage shell weight (Table 1.4.2). There was also an effect of hen age ($F_{(2,531)}$ = 9.06, P = 0.0001) with eggs laid from hens at 36 weeks of age showing the lowest percentage shell weight (Table 1.4.2), but there was no interaction between stocking density and hen age ($F_{(4,531)}$ = 0.60, P = 0.67, Table 1.4.2).

There was no effect of stocking density on shell thickness (µm) ($F_{(2,531)}$ = 1.43, P = 0.24) but there was a trend for a difference in shell thickness with hen age ($F_{(2,531)}$ = 2.38, P = 0.09, Table 1.4.2). There was no interaction between stocking density and hen age ($F_{(4,531)}$ = 0.25, P = 0.91).

Hen age	Stocking density	% reflectivity	Breaking strength (N)	Shell deformation (µm)	Shell weight (g)	% Shell weight	Shell thickness µm	Yolk Score
25 wk	2,000 hens/ha	20.65 ± 0.46	50.58 ± 0.95	310.83 ± 4.78	6.06 ± 0.06	10.17 ± 0.08	453.95 ± 2.95	12.72 ± 0.10
	10,000 hens/ha	21.58 ± 0.47	52.60 ± 0.95	312.83 ± 4.78	6.10 ± 0.06	10.11 ± 0.08	455.87 ± 2.95	12.27 ± 0.10
	20,000 hens/ha	21.58 ± 0.46	52.79 ± 0.95	306.61 ± 4.81	6.20 ± 0.06	10.35 ± 0.08	458.70 ± 2.97	12.03 ± 0.10
30 wk	2,000 hens/ha	23.36 ± 0.46	51.85 ± 0.95	314.67 ± 4.78	6.50 ± 0.06	10.0 ± 0.08	460.06 ± 2.95	12.05 ± 0.10
	10,000 hens/ha	23.32 ± 0.46	52.62 ± 0.95	314.16 ± 4.78	6.62 ± 0.06	10.09 ± 0.08	461.07 ± 2.95	11.38 ± 0.10
	20,000 hens/ha	22.74 ± 0.45	54.23 ± 0.94	315.74 ± 4.74	6.60 ± 0.06	10.22 ± 0.08	462.08 ± 2.92	11.36 ± 0.10
36 wk	2,000 hens/ha	22.95 ± 0.46	51.87 ± 0.95	298.67 ± 4.77	6.74 ± 0.06	9.96 ± 0.08	456.21 ± 2.95	12.23 ± 0.10
	10,000 hens/ha	22. 93 ± 0.46	50.08 ± 0.95	299.67 ± 4.77	6.66 ± 0.06	9.82 ± 0.08	454.29 ± 2.95	11.89 ± 0.10
	20,000 hens/ha	23.18 ± 0.46	52.33 ± 0.95	297.33 ± 4.77	6.81 ± 0.06	10.05 ± 0.08	460.48 ± 2.95	11.70 ± 0.10

Table 1.4.2 The mean ± SEM values for egg quality measures sampled from hens at three ages (25, 30, 36 weeks) and housed at one of three different outdoor stocking densities (2000, 10 000, 20 000 hens/ha).

1.5 DISCUSSION AND IMPLICATIONS

1.5.1 Range Use – RFID Tracking and Video Counts

The RFID tracking of individual free-range laying hens housed with access to different-sized ranges that simulated three different outdoor stocking densities, showed a linear relationship between stocking density and range use through most of the trial. Hens housed in the lowest outdoor stocking density (2000 hens/ha), spent more time outdoors with fewer visits of longer maximum duration and hens housed at the highest stocking density spent less time outdoors with more visits of shorter duration. Within all stocking densities individual hens showed great variation in the percentage of days they accessed the range, including hens that never went outside and hens that used the range daily with on average, less than 50% of birds on the range simultaneously.

The average daily time outdoors of approximately 3 - 5 h across all densities is comparable to other studies of hens in wintergardens (covered ranges) showing either 2 – 8 h per day (Thurner and Wendl, 2005) or on uncovered ranges showing between 3 h 25 min and 5 h 25 min per day outside (Thurner et al., 2010), although these times were highly dependent on weather and season. Hens in the lowest stocking density may have spent longer outdoors as they had the greatest amount of space available to them, not just on a per bird basis (5 m^2), but a larger-sized area overall (750 m^2 compared to 150 m^2 or 75 m^2) and, thus, the furthest available distance to travel within their range. Video observations did show hens used all areas of their ranges, including visiting the back portion of the largest-sized ranges. This contrasts with previous group-level direct observations in commercial flocks that showed hens preferred to remain in the areas close to the pop holes with almost no hens visiting the furthest 40% of the range (varying sized ranges observed, Hegelund et al., 2005). The smaller total area in the 20 000 hens/ha stocking densities may account for the higher number of shorter-duration visits shown by hens using these ranges.

The longer time spent on the range in the lowest stocking density may have been partially related to the ground coverage as vegetation did not disappear completely as within the ranges of the other two densities. Previous observations in free-range systems showed hens spent more time walking and foraging on grass over gravel (Gebhardt-Henrich et al., 2014), of which foraging in red junglefowl will take up 60% of their daylight time budget (Dawkins, 1989). However, differences in range use between the 10 000 hens/ha and 20 000 hens/ha densities which both lost all vegetation indicate ground coverage is not the only factor influencing range usage. Current range stocking density requirements within the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002) do stipulate range rotation is required as stocking densities increase over 1500 hens/ha. In this trial it was noted that as the ground cover depleted, the hens scratched out dust bathing pits in the dry dirt throughout all ranges. Further research could compare how behavioural time budgets of hens may change across the course of range ground-cover denudation.

Determining the space requirements and optimal stocking densities of hens is intertwined with flock size and enclosure size. Several previous studies documented an inverse relationship between range use and flock size, in flocks ranging from a few hundred to several thousand hens (e.g., Bubier and Bradshaw, 1998; Harlander-Matauschek et al., 2001; Gebhardt-Henrich et al., 2014), even with equal stocking densities (Whay et al., 2007). Thus, we may expect that a group of 150 hens stocked at 20 000 hens/ha would differ in range use from a group of 20 000 hens on a hectare. Studies with broilers have looked to isolate the different effects of flock size, group size and density on space utilisation, showing that nearest-neighbour distances were constrained by density but broilers adapted to increasing enclosure size by using all available area with no effects of group size (Leone and Estevez, 2008; Leone et al., 2010). Larger flock size in laying hens has been shown to reduce aggression, possibly via hens abandoning hierarchical formations (Nicol et al., 1999) but as yet there are no data on the social effects of range use, whether individual hens access the range in the same groups and how this social facilitation and/or range space utilisation outdoors may be impacted by flock size. It is also possible

that the use of all areas in the ranges in this study were influenced by other hens as barriers between ranges were transparent. Additionally, there are few data on the impact of enclosure shape on range use and whether different range shapes as per this study, including placement of vertical fences may modulate ranging behaviour (Rault et al., 2013).

1.5.2 Welfare Scoring

The visibly good condition of birds across all densities may have resulted from the management provided in the experimental setting and/or the small group sizes. Indoor stocking density was comparable to industry practice and resources were provided to either meet or exceed the model code of practice recommendations. Assessment occurred over the period of peak production when resource demand was likely to be at its highest, but greater differences in health status between the stocking densities may have become apparent towards the end of the lay cycle when hens are often in poorer condition (Nicol et al., 2006). Furthermore, greater differences may have also been seen if these densities were scaled up to commercial level. Birds would be part of a larger flock size that could limit range access (Pettersson et al., 2016) and hens would have a much larger area to traverse (e.g. 10 000 birds housed on 1 hectare compared to 150 birds stocked at 10 000 hens/ha on 150 m^2 of land in this study) that could further differentiate between individuals (Chielo et al., 2016). Additional measures such as comb colour (Whay et al., 2007), if included, may have shown more differences between treatment groups. However, there were some relationships between welfare variables and ranging behaviour. In particular toenail length, where at the end of the trial, hens that ranged longer had shorter toenails, likely resulting from scratching and walking in the dirt. On average, all birds had shorter toenails than those reported for hens housed in indoor aviary, enriched and conventional cages systems (Blatchford et al., 2015) indicating the benefits of ranging in keeping toenails from becoming overgrown.

1.5.4 Albumen Corticosterone

At 35 weeks, albumen corticosterone concentrations showed a non-linear relationship with outdoor stocking density as eggs from the 20 000 hens/ha density had the highest concentrations but eggs from the 10 000 hens/ha treatment showed the smallest mean change between 23 and 35 weeks. The hens in the lowest stocking density did not have the lowest albumen corticosterone values. The increase in albumen corticosterone concentrations with age was consistent with some previous flock-cycle patterns within multiple Australian commercial systems, although specific patterns did vary between individual farms (Downing, 2012). Studies of several hen strains within cages and floor-based systems also showed higher corticosterone concentrations at 22 weeks versus 42 weeks indicating increased stress with the onset of production but adaptation to environments over time (Singh et al., 2009). At 35 weeks, all corticosterone values in this study, except within pen replicate two of the 10 000 hens/ha density were above 1.5 ng/g which is higher than the average values shown in caged ISA Brown laying hens exposed to prolonged heat stress (Downing and Bryden, 2008). Additionally, albumen corticosterone is sensitive to short-term changes (Downing and Bryden, 2008; Singh et al., 2009), thus, there may have been unknown stressors present at the time of sampling that affected specific flocks, causing high corticosterone concentrations.

These results highlight the need for further sampling across more pen replicates over time to determine the welfare implications of specific corticosterone values and the adaptability of different hen groups to their environmental conditions.

1.5.5 Behavioural Observations

Behavioural observations of hens indoors and outdoors showed that hens preferred to dust bathe and forage outdoors but rest indoors, with the frequencies of all behaviours affected by stocking density to varying degrees. The positive relationship between outdoor resting and available outdoor space was in contrast to observations of hens housed indoors in perchery systems where the stocking density had no impact on resting time (Carmichael et al., 1999), although both the latter and the current study found low proportions of birds resting. More space may have permitted more resting as disturbances from other birds could be minimised, but more resting may have been observed outdoors in all groups if shelter had been provided on the range (Larsen et al., 2014). The similar proportions of hens observed dust bathing outdoors across all densities was comparable to observations made in aviary systems where there was no effect of stocking density on frequency of dust bathing (Zimmerman et al., 2006). However, the proportions of hens dust bathing indoors (litter was friable), and in total, were considerably less than the levels observed when hens were on friable litter in commercial aviaries (~15-20%, Odén et al., 2002) suggesting free-range birds may spend more of their daily time budget in other activities such as foraging. In the current study the overall proportions of hens foraging were much higher than previously reported for indoor percheries, but the proportions of indoor foraging were lower (5.8 – 9.1%: Carmichael et al., 1999; 5.9%: Channing et al., 2001), thus indicating more foraging opportunities were present outdoors. The birds from the lowest stocking density performed less foraging, similar to observations in percheries where foraging increased with increasing density (Carmichael et al., 1999). Observations in commercial systems also showed that foraging was performed more in the range areas furthest from the shed (Chielo et al., 2016) and thus, hens at the back of the larger-sized ranges may have been foraging more but in the current study these birds were not observed.

1.5.6 Behavioural Tests

Behavioural tests for fearfulness and coping style were applied to free-range laying hens categorised into groups based on differing range access preferences. The longer latencies to move and crossing of less squares in the OFT, identified the indoor-preferring hens as more fearful than birds categorised as outdoor-preferring hens. No significant differences in the duration of tonic immobility were observed between the different range access groups. There was a behavioural indication for indoor-preferring hens, based on fewer vocalisations in the MR test, to have a reactive coping style, but this was not accompanied by a higher corticosterone response. Across MR and OFT, the hens with moderate-outdoor range use had inconsistent responses when compared to the responses of the indoor-preferring or outdoor-preferring hens examined in the same test. In some behavioural measures the moderate-outdoor hens responded similarly to the indoor-preferring birds, and in some behavioural measures these birds matched responses of the outdoor-preferring birds.

The behavioural responses of the MR tests suggests the different range access groups of hens may have different coping styles with indoor-preferring hens being

more reactive (passive behavioural responses with fewer vocalisations made) compared to the proactive (more active behavioural and vocalisation responses) outdoor-preferring and moderate-outdoor hens. However, plasma corticosterone responses did not differentiate between ranging groups, thus the conclusions on coping style from this current study are limited. These results contrast with the significantly elevated plasma corticosterone concentrations in MR tests of indoor hens previously (Hernandez et al., 2014). This previous study had a larger sample of hens categorised exclusively as indoor hens (n = 20 cf. n = 8 in this study), and while these were compared to hens categorised as weak-outdoor (< 5 days on the range) range users and to outdoor hens, it was only the exclusively indoor hens that showed the elevated plasma corticosterone response in the MR test. If a reactive coping style is suggested to be more sensitive to environmental variation and thus coping better with change, we might predict these would be the birds visiting the range more frequently. Thus, further studies are needed, including additional physiological measures such as heart rate, to determine if hens that vary in their range use also vary in their coping strategies.

The elevated fear levels of indoor-preferring hens as shown by more freezing in the OFT suggests that the indoor environment is preferred as it may be perceived as a safer, protected and more environmentally consistent choice for these individuals. These results were also supported by previous OFT on indoor and outdoor-preferring birds showing similar findings (Hinch and Lee, 2014 unpublished data). However, we do not know if these hens experienced poorer welfare as a result. Fear is considered an indicator of poor welfare, but if indoor-preferring hens avoided fear of the outdoor environment by staying inside, and had all their ethological needs met via dust bathing and foraging on the floor litter for example, with continual access to food, water and perches, they may have had comparable welfare to the outdoor birds with the individual hens' environmental choice being an important welfare indicator (Nicol et al., 2009). Alternatively, indoor-preferring hens may have been motivated to access the outdoor resource (particularly for those birds that went outside on 2-10% of available days), but by being fearful, were thus 'restricted' to the indoor area and if this were the case then their welfare could be compromised. Some studies have shown correlations between range use and hen health and welfare (Bestman and Wagenaar, 2003; Rodriguez-Aurrekoetxea and Estevez, 2016) but further individual-based data are needed to determine if indoor-preferring hens in a free-range system experience poorer welfare than birds making use of the outdoor range.

1.5.7 Egg Production and Quality

Outdoor stocking density did not affect hen-day production, egg weight or egg deformation in these small, experimental free-range flocks. There were some effects of stocking density on egg quality measurements; hens from the highest stocking density had a higher percentage shell weight and hens from the lowest stocking density had the darkest yolk colour.

Hens in these flocks varied in their range use with hens in the lowest stocking density spending more time outside and hens in the highest density the least time outside. Free-range hens have been shown to have reduced egg weight in comparison to conventional caged hens (Samiullah et al., 2014) and free-range systems typically have lower production than caged systems (Miao et al., 2005). Egg

production is influenced by a multitude of variables such as disease, nutrition, stress, housing system and is an energy-costly activity; if more energy is spent roaming outdoors less energy may be used for production (Meng et al., 2015). Thus, we may have expected hens in the lower outdoor density to have lower production. Although not statistically different, the hens in the highest density did reach the highest percentage hen-day production, but all flocks of all densities surpassed the breed statistics of peaking at 95% production in alternative production systems (ISA, 2016). Furthermore, the average egg weight by 35 weeks of 67.07g also surpassed the breed standard of a 62.9g average egg weight at this age (ISA, 2016). Greater differentiation between densities may be found in commercial-scale flocks where there are larger total areas to roam and may also be greater competition for access to feeders. Hens in this study were in visibly good condition throughout the trial, production between densities may have been differentiated if there was an additive effect of poor health (e.g., disease outbreak) and outdoor stocking density.

In general, the changes in egg quality with age in this study were consistent with changes across the flock cycle in a commercial free-range flock within Australia housing Hy-Line Brown hens (Samiullah et al., 2014). The effects of stocking density on egg quality measures of percentage shell weight and yolk colour may all be related to possible dietary differences associated with different ranging times. The higher yolk colour in eggs from hens housed at the lowest density could also be attributed to greater consumption of vegetation, including the decrease in yolk colour with age as hens in all densities depleted the range coverage during initial ranging. Previous research has found higher yolk colour in hens from free-range versus caged systems (Senčić et al., 2006, Van Den Brand et al., 2004), and research on commercial free-range farms found inconsistencies in yolk colour across the flock cycle in comparison to caged birds (Samiullah et al., 2014), potentially due to variation in dietary intakes. Finally, hens at the highest density had the greatest percentage eggshell weight which could also be related to diet as a reduction in calcium and phosphorus may decrease eggshell percentage (Świątkiewicz et al., 2010). The relationship between range use, nutrition and egg quality, particularly at the individual level is an avenue for future research to understand the impacts of ranging on performance values.

Experiment 2: The Effect of Free-Choice Insect Feeding on Free-Range Flock Performance and Egg Quality

2.0 RECOMMENDATIONS

The aim of the present study was to determine for free-range laying hens the effect of BSF larvae choice feeding on flock performance and egg quality. The mature hens consumed an average of 14 g/hen/day of BSF after 6 or 12 weeks of availability (16% of diet). The availability of BSF had no effect on flock performance and egg quality of the free-range hens, confirming the nutritional value of this food source. Therefore, further studies on the use of BSF as a feed component on layer performance long term, on inclusion levels in the feed and on hen health and behaviour are warranted.

2.1 INTRODUCTION

Due to their nutritional, economic and ecological advantages, insects are considered to be a high quality alternative protein source in animal feed (Van Huis et al., 2014; Agence Nationale de la Recherche, 2016). Insects are a part of the natural diet of many domestic and non-domestic animals, including poultry (Sánchez-Muros et al., 2013; Agence Nationale de la Recherche, 2016). Despite the fact that the nutritional value of insects varies between the species, with the stage of their development, their feed and their growing habitat (Halloran and Vantomme, 2013), insects are known to have a relatively high protein and fat content (Agence Nationale de la Recherche, 2016). Micronutrients are also frequently found in insects and include copper, zinc, magnesium, manganese, phosphorus and selenium (Halloran and Vantomme, 2013; De Marco et al., 2015). Thus, insects can represent a valuable feed supplement to raw materials used in feed for monogastric animals. The nutritional value of Black Soldier Fly larva (*Hermetia illucens*) is presented in Table 2.1.1).

Table 2.1.1 The amount of the main chemical constituents in Black Soldier Fly larvae (*Hermetia illucens*) compared with soybean meal, wheat, lupine and sunflower meal.

Constituents (% in dry matter)	Black Soldier Fly larvae	Soybean meal	Wheat	Lupine	Sunflower meal
Crude protein	36.9 – 47.0	42.0 – 51.8	10.0 – 15.0	30.0 – 31.3	30.0 – 42.0
Ether extract	15.0 – 35.0	1.6 – 3.5	1.7 – 3.0	6.5	7.6
Crude fibre	6.7	6.5 – 7.0	2.8	NA	21.0
Ash	8.6 – 15.5	6.0 – 6.7	2.0	2.6	6.8 – 7.0
Calcium	5.00 – 7.56	0.20 – 0.39	0.05	0.21	0.43
Total phosphorus	0.90 – 1.5	0.60 – 0.69	0.30	0.30	1.00
References: Newton et al., 1977; Feillet, 2000; Courtney, 2002; St-Hilaire et al., 2007; Finke, 2008; Batal and Dale, 2011; Sánchez-Muros et al., 2013; Van Huis et al., 2014; De Marco et al., 2015; Food Standards Australia & New Zealand, 2015; Payne et al., 2015 ; USSEC, 2016					

Calcium and phosphorus are important in laying hen feed for egg production and quality. Table 2.1.1 shows that BSF larvae have a greater amount of calcium and total phosphorus, compared to soybean meal and wheat, and similar to sunflower meal. While the plant-based phosphorus is around 30% available, the phosphorus in insects is highly available, with for example an availability of 92% in face fly (*Musca autumnalis*) larvae (Finke, 2008).

The following table 2.1.2 presents the nutritional requirements of ISA Brown laying hens and compares these requirements to the nutrient composition of BSF larvae. Because of their high fat content, BSF larvae should not be included over 20% in the diet. This table underlines the feasibility of using BSF larvae to feed commercial laying hens as a complementary feed ingredient.

Table 2.1.2 The main nutritional requirements in ISA Brown laying hens compared to the nutrient composition of Black Soldier Fly (BSF) larvae.

Nutrients (% DM)	Amount in BSF larvae		Laying hens requirements		
	Whole larva	20% inclusion in layer diet	17-28 weeks	28-50 weeks	>50 weeks
Calcium	5.00 – 7.56	1.00 – 1.51	3.12 – 3.90	3.28 – 4.09	3.44 – 4.38
Phosphorus	0.90 – 1.5[1]	0.18 – 0.30	0.32 – 0.42[2]	0.30 – 0.40[2]	0.30 – 0.32[2]
Crude protein	36.9 – 47.0	7.38 – 9.40	18.2 – 20.0		17.4 – 19.2
Lysine	0.22 – 0.28	0.04 – 0.06	1.50		
Methionine	0.88 – 0.91	0.18	0.76		
Tryptophan	0.21	0.04	0.35		
Isoleucine	1.72 – 2.15	0.34 – 0.43	1.34		
Valine	2.20 – 3.45	0.44 – 0.69	1.44		
Threonine	1.52 – 1.55	0.30 – 0.31	1.10		

[1] Total phosphorus, considering its availability around 90% (Finke, 2008)
[2] Available phosphorus
References: Newton *et al*., 1977 ; St-Hilaire *et al*., 2007; Sánchez-Muros *et al*., 2013; Makkar *et al.*, 2014; De Marco *et al*., 2015; Payne *et al*., 2015 ; ISA, 2016a

2.1.1 Experimental Objectives

- The first objective: to determine the impact of choice feeding with BSF (*H. illucens*) on flock performance in free-range laying hens.

- The second objective: to determine the impact of choice feeding with BSF larvae (*H. illucens*) on egg quality in free-range laying hens.

2.2 MATERIALS AND METHODS

The research conducted was approved by the Animal Ethics Committee of the University of New England, Armidale, Australia (approval No AEC 15-120). Animals were housed in the University of New England facilities and treated in accordance with the Model Code of Practice for the Welfare of Animals, Australia (CSIRO, 2002). Housing conditions were according to the breeder's recommendations (ISA, 2016a).

2.2.1 Impact of Choice Feeding with BSF Larvae on Flock Performance

The objective of this study was to determine the impact of choice feeding with *H. illucens* on flock performance: egg production, egg weight, body weight, feed intake and FCR. For the purpose of this study, 160 ISA Brown laying hens were used. At the beginning of the trial, hens were 43 weeks of age. These hens were taken from the stocking density trial as hens that were known to visit the range on 100% of available ranging days. Each pen contained hens from each of the stocking densities to balance any effect of prior housing conditions. Hens were placed into eight different pens, each of them containing 20 individuals (Figure 2.2.1).

The hens of four pens were used as control animals (n=80), and the hens of another four pens were assigned the treatment group (n=80). Therefore, four replicates were performed. All hens were offered a typical Australian wheat-soy diet, formulated according to the breeders' standard recommendations (ISA, 2016a). The diet was mixed at the University of New England and offered *ad libitum* to all hens. Feeders were located in the indoor pens. The composition of this diet is given in Table 2.2.1.

Table 2.2.1 The chemical composition of the BSF larvae and the control diet (as analysed).

Ingredient	Percentage in the diet (%)
Rolled wheat	64.50
Soybean meal	21.83
Canola oil	2.02
Meat meal 59	1.00
Premix, including:	10.64
Limestone Fine Grit	*4.03*
Limestone	*6.06*
Salt	*0.23*
UNE Layer Premix[1]	*0.10*
Choline Chloride 60%	*0.04*
DL-Methionine	*0.16*
Xylanase	*0.01*
Phytase 5000 u	*0.01*
Total	**100.00**
[1] Including red and yellow egg color	

Table 2.2.2 The chemical composition of the control diet and the Black Soldier Fly (BSF) larvae offered *ad libitum* to the treatment group.

Constituents (% in dry matter)	Black Soldier Fly larvae	Control diet
Crude protein	35.0 – 35.3	16.4
Ether extract	38.0 – 40.5	2.76
Crude fibre	1.40	*NA*
Ash	10.0	*NA*
Calcium	2.65 – 3.87	4.10
Total phosphorus	0.60 – 0.82	
Available phosphorus	0.72	0.33

Table 2.2.3 The Amino Acid composition of the Black Soldier Fly (BSF) larvae offered *ad libitum* to the treatment group (as analysed).

Essential amino acids	Amount in BSF larvae (g/kg DM)	Non-essential amino acids	Amount in BSF larvae (g/kg DM)
Arginine	17.1	Alanine	NA
Histidine	8.80	Aspartic acid	NA
Isoleucine	14.9	Cysteine	3.80
Leucine	23.0	Glycine	NA
Lysine	**18.8**	Glutamic acid	NA
Methionine	**5.60**	Proline	NA
Phenylalanine	13.0	Serine	NA
Threonine	13.0	Tyrosine	16.5
Valine	21.4	Tryptophan	5.20

The BSF (Entofood SDN BHD, Rawang, Malaysia) were reared on brewery spend grain before being washed and dried at 90°C for 60 min. Whole dry BSF larvae were offered *ad libitum* on the range in bio-secure feeding stations (Feedomatic, Olba B.V, Coevorden, Netherlands). Hens of the control group also had access to feeding stations, which were not filled. Hens had access to the outdoor range and the feeding stations daily from 9am to 7pm. Hens were kept in indoor pens from 7pm to 9am. The illustration below shows the arrangements of hens in the different pens (Figure 2.2.1).

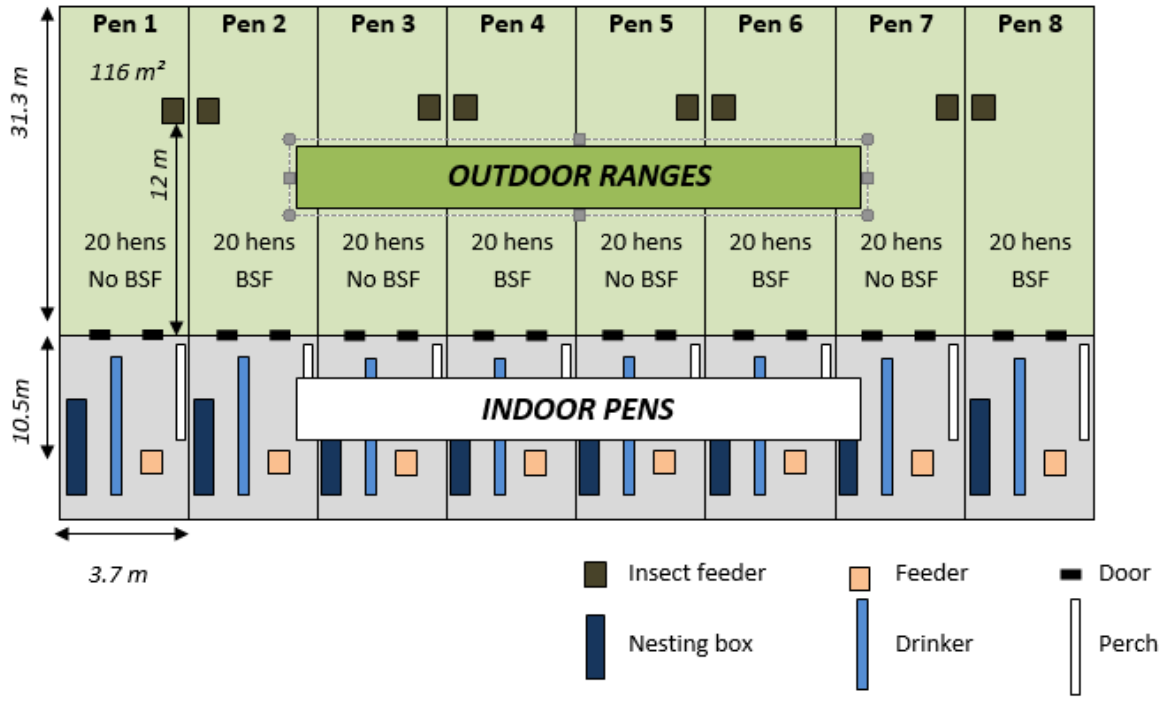

Figure 2.2.1 A diagram showing the arrangement of the pens, with "no BSF" for the control groups and "BSF" for the treatment groups receiving Black Soldier Fly (BSF) larvae on the range.

Hens were adapted to housing system and feed for a period of three weeks. Following the adaption period, egg production, egg weight, body weight, feed intake and FCR were recorded for four weeks (baseline). This period was followed by three weeks of transition, where hens of all groups were trained to use the bio-secure insect feeders, filled with the control diet. BSF were then offered to the hens of the treatment replicates in the bio-secure feeders on the range for the duration of six weeks (experimental period). Bio-secure feeders of hens assigned to the control group were also placed on the range, but left unfilled.

Health and welfare of the hens were checked twice daily (feed consumption, visual inspection of the environment sanitation level). Eggs were collected and individually weighed daily. The average daily egg weight and egg production per pen were calculated. Body weight of individual hens, feed intake per pen and BSF intake per pen were recorded weekly, egg mass and FCR calculated. Mean values per pen for egg production, egg weight, body weight, feed intake and FCR were used for analysis. Data were tested for normal distribution using the Shapiro-Wilk-Normality test and compared to each other using the ANOVA-test. All statistical analyses were performed using the software 'R' version 3.2.1 (R foundation statistical computing, Vienna, Austria). Statistically significant differences were set at $P < 0.05$.

2.2.2 Impact of Choice Feeding with BSF Larvae on Egg Quality

In order to determine the impact of choice feeding with *H. illucens* on egg quality, 70 ISA Brown laying hens (selected from the previous groups of hens) were assigned to

a control group (no BSF offered, n = 35), or a treatment group (BSF offered, n = 35), with each group housed in a separate pen. Hens that were previously insect-fed remained the treatment group, hens that were previously controls, remained controls. All hens were individually numbered and randomly assigned to groups of five within the treatment and control group. A total of 7 replicates per treatment could be used for statistical analysis.
All hens in their new pens (but identical set-up as previously) were then fed for the duration of six weeks. All hens had been trained to use bio-secure insect feeders for a three week period in the previous portion of this insect feeding experiment. BSF larvae were then offered to hens of the treatment group on the range for the total duration of 12 weeks (6 weeks from the previous portion of the experiment and 6 weeks for the current portion of the experiment). Per 20 hens, one insect feeder was available. Hens had access to the range and the insects between 9 am – 7 pm daily.

Body weight of the individual hens was recorded at the beginning and the end of the experiment. Eggs from individual hens were collected on two consecutive days prior to hen sampling. Internal and external egg quality were evaluated on the day of collection and averaged across the two days: egg weight, shell colour by reflectivity, egg shell breaking strength by quasi-static compression, shell deformation to breaking point, albumen height, Haugh Unit and yolk colour (egg quality equipment, Technical Services and Supplies, Dunnington, York, UK). Egg yolk colour was measured on a range from 1 to 15, from the brightest to the darkest shade. The shell was weighed and its thickness measured (Mitutoyo Dial Comparator gauge Model 2109-10, Kawasaki, Japan) (Roberts *et al*., 2013). The Haugh expresses the protein quality (Haugh, 1937; Bhale *et al.*, 2003).

- $Haugh\ Unit = 100 \log (H - 1.7W^{0.37} + 7.6)$

Where H = Albumen height (mm), W = weight of egg when tested (g). The ranking for Haugh Unit was as following: AA = 72–130, A = 60–71, B = 31–59, C < 30 (Bhale *et al*., 2003). A higher the score is associated with a better albumen quality.

The effects of choice feeding with the BSF on egg quality and nutrient digestibility was investigated using the mean values of 7 replicates per group, tested for normal distribution using the Shapiro-Wilk-Normality test and compared to each other using the t-test when normally distributed, or Wilcoxon-Mann-Whitney test when not. All statistical analyses were performed using the software ’R’ version 3.2.1 (R foundation statistical computing, Vienna, Austria). Statistically significant differences were set at $P < 0.05$.

2.3 RESULTS

2.3.1 Impact of Choice Feeding with BSF Larvae on Flock Performance

During the six weeks duration of the treatment period, hens of the treatment group consumed on average 15 ±1.7 g BSF/hen/day. Flock performances of control group *vs*. BSF group at the end of the experiment are presented in Table 2.3.1. There was no significant difference ($P > 0.05$) between the control and the treatment group for the egg production, egg weight, feed intake, FCR and body weight. The egg production, egg weight, egg mass, total feed intake (including BSF in the treatment group) and body weight were higher ($P < 0.05$) during the baseline period than after six weeks of treatment. There was also no impact ($P > 0.05$) of the treatment or the period on the FCR.

Table 2.3.1 Effect of choice feeding with Black Soldier Fly dry larvae (*Hermetia illucens*) for the duration of six weeks on the flock performances of 160 ISA Brown free range laying hens.

Parameters	Treatment	Week		p-value		
		Baseline	Week 6	Treatment	Week	Treatment*Week
Egg production (%)	Control group[1]	96.15[a] ± 1.13	82.82[b] ± 4.24	0.348	0.002**	0.144
	Treatment group[1]	94.72[a] ± 1.79	89.02[b] ± 1.20			
Egg weight (g)	Control group	68.28[a] ± 0.62	66.88[b] ± 0.75	0.390	0.042*	0.705
	Treatment group	68.54[a] ± 0.21	67.55[b] ± 0.34			
Egg mass (g)	Control group	65.66[a] ± 1.27	55.48[b] ± 3.47	0.352	0.003**	0.208
	Treatment group	64.93[a] ± 1.35	60.14[b] ± 0.99			
Feed intake (g/day/hen)	Control group	108.41[a] ± 2.39	94.65[b] ± 6.98	0.831	0.017*	0.662
	Treatment group	112.36[a] ± 2.30	93.29[b] ± 7.93			
Feed conversion ratio (kg egg/kg feed)	Control group	1.66 ± 0.07	1.70 ± 0.07	0.676	0.478	0.220
	Treatment group	1.73 ± 0.03	1.55 ± 0.15			
Body weight (kg/hen)	Control group	2.06[a] ± 0.02	2.03[b] ± 0.02	0.062	0.030*	0.540
	Treatment group	2.11[a] ± 0.01	2.05[b] ± 0.01			

[1]Mean values ± standard error of the mean of 80 hens, 4 replicates per treatment.

2.3.2 Impact of Choice Feeding with BSF Larvae on Egg Quality

For the 12 weeks of experiment, the average BSF intake in the treatment group was 13±1.5g BSF/hen/day. Eggs produced from hens in the treatment group were significantly lighter ($P < 0.05$) and the shell thickness was significantly lower ($P < 0.05$) compared to eggs from hens in the control group. There was no significant difference ($P > 0.05$) in body weight between the two groups. Egg yolk colour was significantly brighter ($P < 0.05$), with a lower yolk colour number, in eggs from hens fed with BSF larvae. There was no significant effect ($P > 0.05$) of BSF choice feeding on the shell quality for these parameters: breaking strength, deformation, thickness and reflectivity. The albumen height and Haugh Unit were not affected by the treatment ($P > 0.05$). Table 2.3.2 presents details from the results.

Table 2.3.2 Effect of choice feeding with BSF dry larvae (*Hermetia illucens*) for the duration of 12 weeks on the internal and external egg quality of 70 ISA Brown free range laying hens.

Parameter	Control group[1]	Treatment group[1]	P-value
Body weight (kg)	2.08± 0.02	2.07± 0.02	0.654
Egg weight (g)	71.7 ± 0.97[a]	67.3± 0.57[b]	0.003**
Shell weight (g)	6.99± 0.06[a]	6.55 ± 0.11[b]	0.004 **
Shell reflectivity (%)	24.9± 0.84	24.4± 0.67	0.643
Shell thickness (mm)	0.46± 0.004	0.45± 0.01	0.089
Shell deformation (mm)	0.29± 0.01	0.28± 0.004	0.491
Breaking strength (N)	46.0± 1.15	47.3± 1.42	0.506
Albumen height (mm)	9.18± 0.28	8.91± 0.21	0.468
Haugh Unit	92.7 ±1.68	92.1 ± 1.52	0.798
Yolk score	11.7± 0.13[a]	10.3± 0.26[b]	<0.001 ***

[1]Mean values ± standard error of the mean of 35 hens, 7 replicates per treatment.

2.4 DISCUSSION AND IMPLICATIONS

2.4.1 Impact of Choice Feeding with BSF Larvae on Flock Performance

On average, laying hens consumed 15 g of BSF /hen/day, which represents 16% of the total feed intake. This intake suggests the insects were palatable. *H. illucens* had already been included at 25% in broiler diets (De Marco et al., 2015). However, the total feed intake (control diet and larvae) was much lower in both groups after six weeks of treatment than at the beginning of the experiment (108 *vs*. 94g/hen/day for the control group; 112 *vs*. 93g/hen/day for the treatment group; Table 14), even though it is supposed to remain constant with increasing age of hens (ISA, 2016a). In addition, the total feed intake during the experiment was lower than indicated by the breeder standard (122g/hen/day; ISA, 2016 a) although the standards are generated on hens kept at optimised housing conditions with controlled environmental temperature and humidity while in contrast, hens in this experiment were exposed to Australian summer temperatures.

Laying hens are known to reduce their feed intake with increasing temperatures, especially over 27°C (Talukder et al., 2010)and ambient temperatures during the study period Nov-Dec) were between 25 and 35°C and in February (end of the experiment for objective one) between 34 and 38°C) and the observed intake results are consistent with those reported by Talukder et al. (2010).

The observed egg production also corresponds to levels reported for this strain of bird (ISA, 2016a). Egg weight decreased in both experimental groups between the beginning and the end of the trial but was expected to increase slightly (ISA, 2016 a). This could be due to the loss of body weight in both groups (2.06 to 2.03kg in the control group and from 2.11 to 2.05kg in the treatment group). The significant reduction in feed intake probably due to the heat is the likely cause of the weight loss and may be the reason for no significant effect of BSF feeding on egg weight, even when a high proportion of lipids in the larvae (40% in DM) should have had a positive effect. It can also be argued that when choice-fed, hens choose their feed to meet their nutritional requirements (Wilkinson et al., 2011). And that hens “balanced” their intake of insect and control diets such that egg characteristics remained the same.

Because egg production and egg weight decreased with time, egg mass was also lower at the end than at the beginning of the trial. The observed values were in the range of the expected egg mass for 55 week old free range hens, and a decrease in egg mass is expected with the age (ISA, 2016a).

The decrease in egg mass combined with a decrease in feed intake lead to a constant FCR during the experiment. However, FCR was much lower than expected (1.6 *vs*. 2.2 kg egg/kg feed (ISA, 2016a).) because of the low feed intake. However the lack of treatment difference is consistent with the effect of insect feeding (*M. domestica* and *T. molitor*) on FCR in broilers (Ocio and Viñaras, 1979; Awoniyi et al., 2003; Dordevic et al., 2008).

2.4.2. Impact of Choice Feeding with BSF Larvae on Egg Quality

The observed egg weight for the control (71.7 g) and treatment groups (67.3 g) at 61 weeks of age are in the range of expected (Roberts and Ball, 2003; ISA, 2016a). The

egg weight in the control group was significantly higher than in the treatment group and it is not apparent why this should be given similar intake levels.

Apart from the egg weight, external egg quality was generally not affected by the insect intake but, shell weight was significantly lower in the insect supplemented group while shell thickness, deformation and breaking strength were not affected. The observed values for the egg shell quality correspond to the values found in the literature for 60 week-old hens (Roberts and Ball, 2003) and it appears that in general egg quality is not impacted by the insect supplement. The quality of the albumen (albumen height and Haugh Unit) was also not significantly affected by insect provision. The nutritional composition of the BSF larvae offered to the treatment group, was of similar protein content compared to soybean meal, which was a major ingredient in the control diet. Therefore, a substitution of soybean meal by BSF larvae offered the same amount of proteins to the hens, and it is not surprising that the albumen quality remained constant. The observed values for the Haugh Unit correspond to the values found in the literature for 60 week-old hens (Roberts and Ball, 2003).

Yolk colour was significantly lower in the insect supplemented group but as artificial carotenoids were added to the control diet the total intake of pigments was lower for the hens that consumed insects. If insect feeding gets more common in layer diets, it may be useful to study the internal and external egg quality from the consumer point of view, to attest if these brighter egg yolks are accepted.

Experiment 3: Early Enrichment in Free-Range Laying Hens: Effects on Ranging Behaviour, Welfare and Response to Stressors

3.0 RECOMMENDATIONS

The aim of this study was to determine if enrichments provided during early stages of development could have impacts on ranging, health and welfare of adult birds. The enrichments applied in the experimental setting were comprehensive and had some positive impacts on hen behaviour during adulthood. Further study would now look into practical enrichments that could be applied in industry and optimal periods of exposure.

3.1 INTRODUCTION

Pullets for free-range systems are typically reared in controlled indoor environments - litter or aviary systems, and transferred to the layer house at 15 weeks. Pop holes for range access in the laying facility are then usually first opened between 21 and 26 weeks of age after which birds get daily access to the range for the duration of the flock cycle. Thus for the first period of their lives, birds are kept in controlled, indoor conditions following which they are suddenly provided the option of accessing an environment that exposes them to variable weather, unpredictable external noise and stimuli, natural light and predators. This new environment can be stressful to the laying hens (Gilani et al., 2014; Richards et al., 2011) and hens may not initially make full use of the range area (see experiment 1), even though this is perceived to be a highly valued resource. Additionally, initial range-access timing is coupled with the physiological stress of coming into full laying production.

Several studies have highlighted the importance of early rearing environments in optimising hens' adjustment to alternative layer housing systems (Janczak and Riber, 2015). Early rearing conditions can also impact the health and welfare of birds as adults (Janczak and Riber, 2015) where chicks may be particularly susceptible during sensitive periods of development. But there is very little research regarding optimal rearing environments for free-range laying hens to best prepare them for outdoor access. Some early studies have shown that laying hens provided outdoor experience (daily 30 min or 60 min forced outdoor exposure) and regularly handled between 12 and 20 weeks of age were faster to emerge from a familiar box into the outdoor paddock and dispersed further than birds with either no experience of the outdoor paddock or had only been regularly handled (Grigor et al., 1995). A growing collection of studies have documented that less fearful birds as measured in behavioural tests, also spend more time out on the range (see Experiment 1 in this report; Grigor et al., 1995; Hartcher et al., 2016). Thus early range access may reduce fearfulness and improve range use for free-range hens, but this is generally not a practical option for current pullet rearing facilities.

3.1.2 Experimental Objectives:

- The first objective: to determine if indoor enrichments could be applied for a short period in early chick development that would have impact on range use in adulthood.

- The second objective: to determine if the enrichments affected hen welfare during adulthood.

- The third objective: to determine if the early enrichments enabled the birds better adapt to stressor events experienced during adulthood.

-

3.2 MATERIALS AND METHODS

All research was approved by the University of New England Animal Ethics Committee (AEC 15-119) prior to the start of data collection.

3.2.1 Chick Housing and Early Enrichment

Three hundred day-old Hy-Line® Brown chicks (infra-red beak-trimmed) were obtained (November 2015) from a commercial supplier (flown to the research location) and randomly allocated into two separate rooms (4.5 m L x 3 m W) at the University of New England Animal House facilities where they were housed until 12 weeks of age. Both rooms were heated following the Hy-Line® Brown rearing management guide with hours of lighting adjusted during rearing as per the recommended guidelines (Hy-Line® International, 2014). Birds were provided ad libitum commercial mash formulated for specific growth stages, access to water nipples (10 birds/nipple) with wood shavings as a floor substrate. One perch rack (1.6 m H x 2.2 m W with 6 perch bars) per room was added at 4 weeks of age.

Birds in one room (standard non-enriched conditions) had no additional interventions with personnel entering the room typically once per day after week one (all birds were checked multiple times/day during the first week). Birds in the second room (enriched treatment) were subjected to a novel, changing, unpredictable environment from 4 – 21 days of age. Across the enrichment period a variety of stimulation was provided including patterned wallpaper, cinder blocks, large sealed plastic tubs, novel objects attached to feeders and water nipples, coloured flashing lights and auditory playbacks that included sounds of doors opening, moving vehicles, weather, voices, machinery). Enrichments were changed out on a daily basis or shifted location within the pen, with coloured lights and auditory playbacks on a random schedule. After 3 weeks of age, all additional enrichments were removed and birds in both rooms were housed in the same conditions henceforth.

3.2.2 Pullet and Layer Housing

Following the behavioural tests, at 12 weeks of age all birds were moved to the University of New England's Laureldale experimental free-range facility located in Armidale, Australia and distributed between 6 indoor pens (3 enriched rearing treatment, 3 non-enriched rearing treatment 46-50 birds per pen). Birds that had been testing in behavioural tests were divided in no specific order across pens within

treatments, similarly with birds that had not been tested so all pens contained tested and untested birds, equating to differences of up to 4 birds between some pens. All pens had equal food, water, perch and nestbox resources that exceeded the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002; Figure 1). Indoor stocking density was approximately three birds per m^2 with rice hulls provided as a litter substrate. Birds were fed *ad libitum* commercial mashes formulated for pullet followed by layer life stages.

The shed was fan-ventilated but not temperature or humidity controlled. Incandescent lighting gradually increased to 16 h of light by 30 weeks of age (lights on at 0400, lights off at 2000 h). The lux (Lutron Light Meter, LX-112850, Lutron Electronic Enterprise CO., Ltd. Taipei, Taiwan) inside the pen with the pop holes closed, measured at bird height in three locations within the pen (front, middle and back), ranged from 4 to 21 lux. This range increased to 5 to 35 lux when the pop holes were opened as measured on one sunny day.

3.2.3 Radio-Frequency Identification of Range Use

The 6 indoor pens were associated with separate designated fenced (2 m high to prevent birds flying over) straight outdoor runs (3.7 m W x 31 m L) which were initially 80-90% covered (prior to bird access) with a variety of grass and weeds (birds could not hide in the vegetation) but no additional trees or structures (Figure 3.2.2). Based on visual estimates from weekly range photos of brown vs green area, by week 8 following first range access (birds were 29 weeks of age) the vegetation coverage changed to approximately 5% and stayed at this level for the remainder of the trial. Outdoor stocking density at maximum occupancy was approximately 4200 hens per hectare. Shade cloth (Universal Shade Cloth, 90% UV block grade, Shade Australia, Ingleburn, NSW, Australia) was placed doubled over at a height of 0.9 m along the fences to minimise visual contact between birds although auditory contact was still possible. The pop holes containing two radio-frequency identification (RFID) passageways (Figure 3.2.1) that provided range access were first opened at 21 weeks of age (April 2016) with subsequent daily access from 0900 – 1630 h across 21 weeks over autumn and winter where days were typically dry and mild (average outdoor temperatures during range access hours were 15.57°C ± SD 5.79; range: 1.07 to 29.45°C; corresponding indoor temperatures across the range access weeks at all times of day were 10.43°C ± SD 4.75; range: -2.45 to 25.09°C). Birds were not forced onto the range as measuring natural range usage was the objective of this research. Hens were trained to return inside each afternoon using a poultry grain mix and held inside at all other times.

Prior to pop hole opening, all birds (at 20 weeks of age) were fitted with a microchip leg band. Each RFID system consisted of two passageways (36 cm H x 18 cm W) situated within the pop holes (Figure 3.2.1).

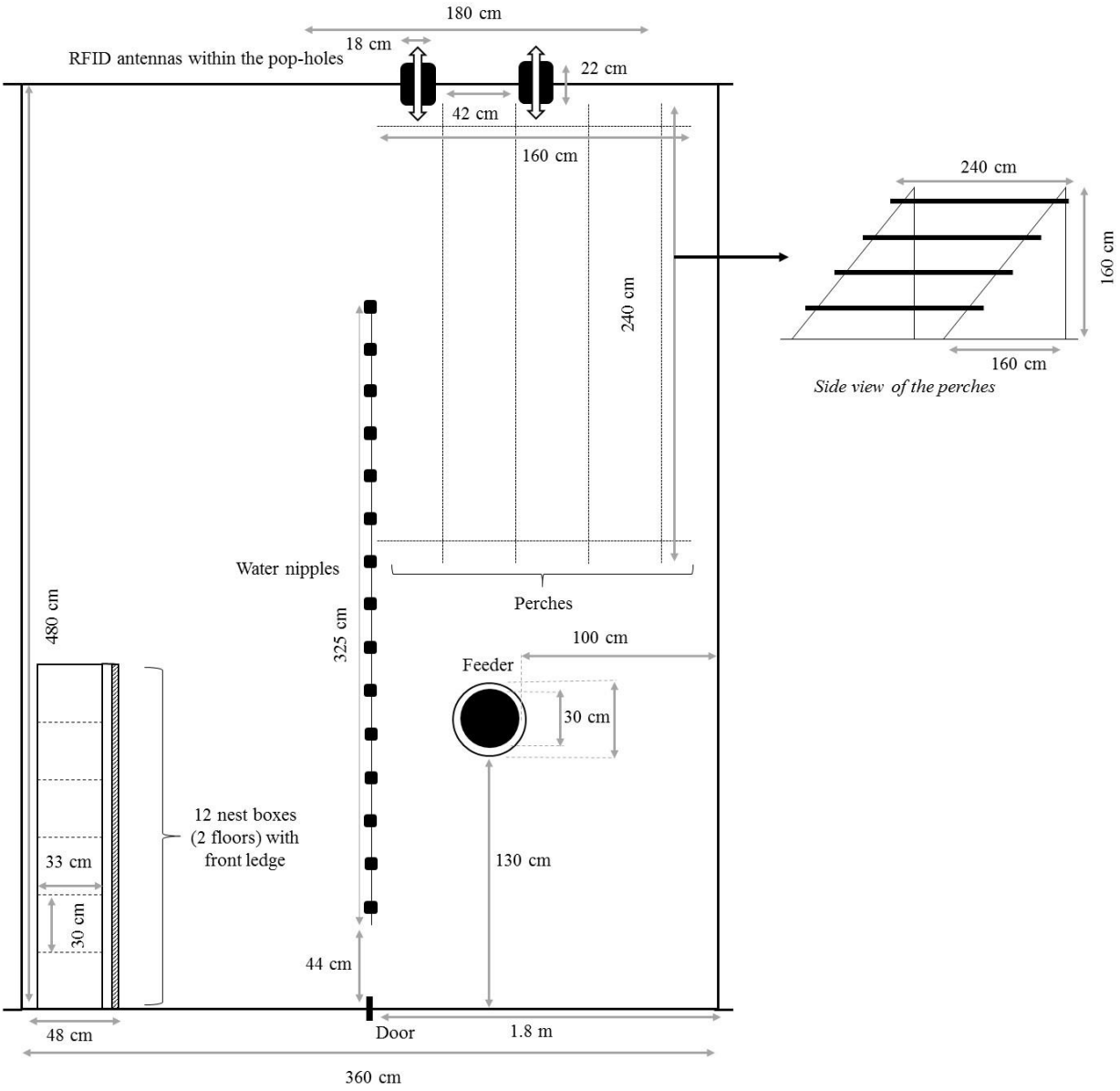

Figure 3.2.1 A top-down schematic of the hens' indoor pen set-up showing location of the range pop holes (including radio-frequency identification antennas), perches (side view included), nest boxes, feed and water. Each indoor pen had identical resources and configuration.

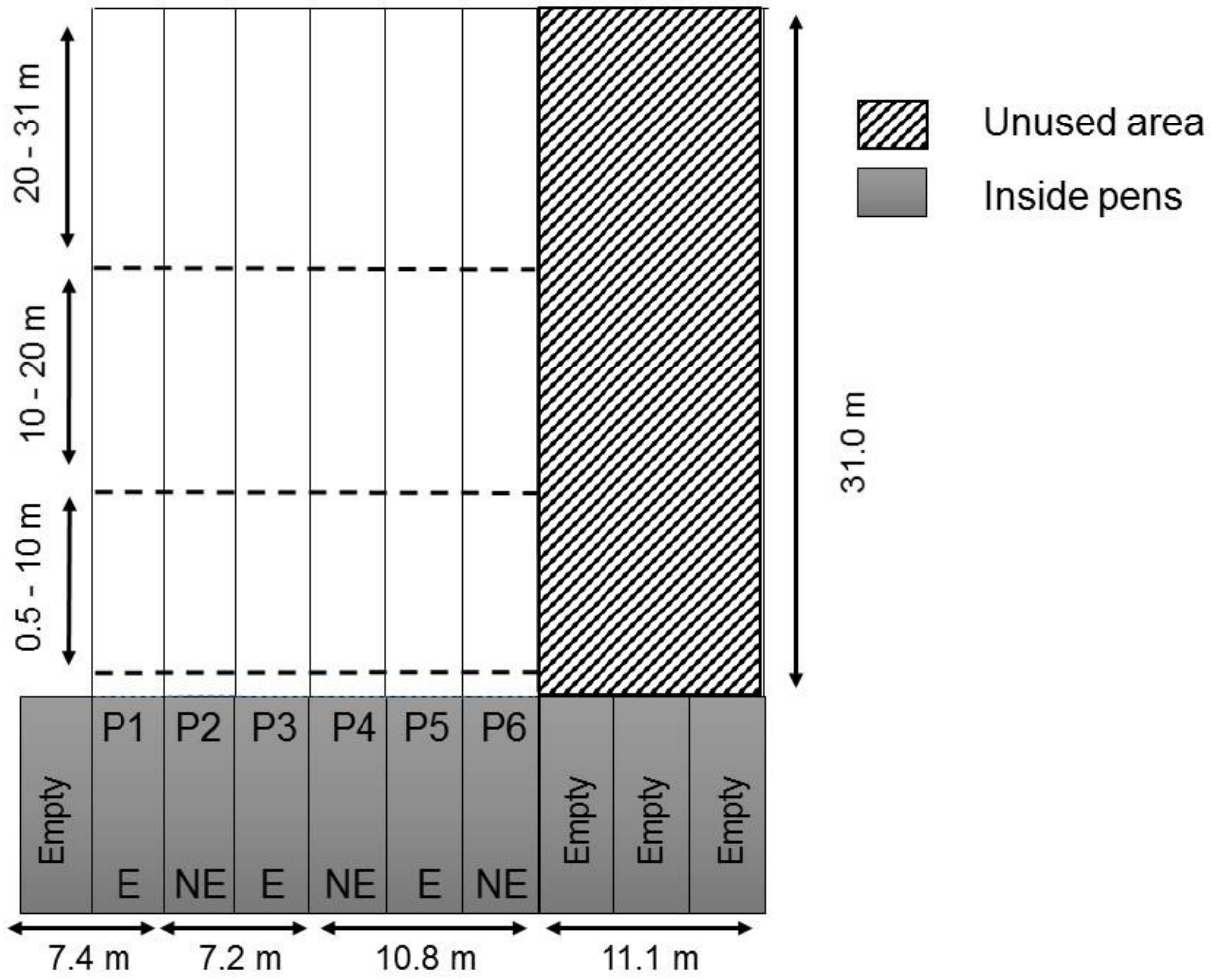

Figure 3.2.2 The six indoor pens and their designated outdoor range areas for the three replicates of each rearing treatment (E = enriched, NE = non-enriched). The range delimitations used during behavioural observations are indicated with dashed lines.

3.2.4 Video Recording and Decoding

Six Hikvision (DS-2CD2T42WD-14 Outdoor EXIR Bullet; iCam Security, Forest Lake, QLD, Australia) cameras were used to record all outdoor range areas (excluding an approximately 0.5 m distance section directly in front of the pop holes that the cameras could not view). To assess acclimation to the range area, the resulting videos were used to count the numbers of birds outside during the first 3 weeks of range access (21 to 23 weeks of age) and the distance these birds were from the pop holes (0.5 - 10 m, 10 - 20m, 20 - 31m). Counts of all birds on the range at the 3 distances were made 10 mins after pop holes first opened, then every 20 mins until pop holes closed for 21 days. The videos were also decoded to document natural disturbance behaviours of birds on the range (possibly due to sounds, weather, overhead birds etc), defined as birds suddenly running towards the pop holes. Videos of all range areas were observed from pop hole opening until pop hole closing across weeks 2 and 3 (birds 22, 23 weeks of age) following first pop hole

opening (1 week permitted for birds to acclimate to range access and ensure birds were using the range area) and across weeks 12 and 13 (birds 33, 34 weeks of age) following first pop hole opening (when birds were hypothesised to be accustomed to the variable environmental stimuli outdoors). The occurrence of daily disturbance behaviours within each range area during the observation weeks and the percentage of birds currently on the range that were disturbed during each occurrence were documented. All hen counts on the range and observations of disturbance behaviours were completed by a single observer that was blind to the rearing treatment of the birds, intra-observer reliability as measured by Cohen's Kappa coefficient was $\kappa = 0.86 – 0.97$).

3.2.5 Welfare Scoring

Prior to pop hole first opening (20 weeks of age), and then following pop hole opening at 26, 32 and 37 weeks of age all birds were weighed (BAT1, VEIT Electronics, Moravany, Czech Republic) and using a modified version of the Welfare Quality® scoring protocol (Welfare Quality®, 2009), basic health and welfare measures were taken on all individuals on the same day. The length of the middle toenail of the right foot was measured to the nearest mm with a seamstress tape measure fitted to the toenail curve and feet were checked for broken, missing or injured toes (of which only 2 NE birds at 37 weeks had a broken toe). Footpad dermatitis was determined using a scale where 1 = footpad dermatitis on one or both feet with no swelling, 2 = dermatitis on one or both feet with moderate swelling, 3 = footpad dermatitis on one or both feet with dorsally visible swelling but only 2 birds (one E, one NE) were observed at category 3 during the 37 weeks sampling age point. Birds were manually palpated for keel damage with the scoring being N for no damage and Y for any damage (deviations or indication of fracture) and at 26 weeks of age onwards an additional category (Y*) was added to classify comparatively more severe keel damage. Birds were visually assessed for comb discolouration, comb wounds, skin pecking wounds, presence of external parasites, feather damage and feather loss but no evidence of these conditions were observed (except for two EE birds at 32 weeks and one EE bird at 37 weeks with feather loss on underside). The same experimenter (who was aware of the rearing treatment groups) did all visual health scoring and keel palpations. At the 26, 32 and 37 weeks sampling age points, images were taken of all bird combs and were later measured using (AxioVision 4.8, Carl Zeiss Microscopy GmbH, Jena, Germany) by a single experimenter unaware of the treatment rearing groups. Finally, at the 20 week and 37 week age point all bird beaks (top and bottom) were also measured using electronic callipers by a separate experimenter unaware of the rearing treatment groups.

3.2.6 Albumen Corticosterone

At 20, 26, 32 and 38 weeks of age, 50 eggs from each pen across 2 days (25 eggs per pen per day) were sampled for assessment of albumen corticosterone concentrations. On the day of collection, all eggs were weighed, cracked open, the albumen was separated out into a 50ml tube, weighed and then stored at -20° C until processing via radioimmunoassay following procedures reported by Downing and Bryden (2008). All albumen corticosterone analyses were conducted blind to rearing treatment.

3.2.7 Stressor Events and Measurements

At 39 weeks of age, all birds were locked inside for 2 days to simulate a potentially stressful event. All daily morning husbandry activities remained unchanged across the 2 days but pop holes were not opened as per usual practice. Following this, pop holes were opened again for 1 week with range access again from 0900 until 1630. At 40 weeks of age, a second potential stressor event was initiated and the available range area outside was shrunk to 20% of its original size. Birds were still provided daily access for the same time but with reduced available ranging area. RFID recording continued in the week following bird lock-in and in the 2 weeks following range shrinkage. Approximately 45 eggs per pen (all eggs laid in a single day) were sampled on day 2 of bird lock-in and both day 2 and day 14 following range shrinkage to assess albumen corticosterone concentrations in response to the stressor events. Egg processing occurred as stated previously with corticosterone analyses conducted blind to bird rearing treatment.

3.3 DATA AND STATISTICAL ANALYSES

All analyses were conducted in JMP®12.1.0 (SAS Institute, Cary, NC, USA) with α set at 0.05. Where significant differences were present, post-hoc comparisons were made using Student's t-tests with Bonferroni correction applied for multiple post-hoc testing. Data transformations were applied where stated but the LSM ± SEM of the raw values are presented in the results as there was virtually no difference between the raw and back-transformed means.

3.3.1 Video Counts and Disturbance Behaviours

Counts of the numbers of hens on the range at the three different distances from the pop holes were averaged across each observation day for each pen. Data were grouped into 3 week periods (21, 22 and 23 weeks of age) corresponding to the first 3 weeks of range access (n = 378, 3 distances x 21 days x 6 pens). Using General Linear Models with repeated measures the effect of pen within rearing treatment, rearing treatment, distance and week of age were compared including all interactions, with non-significant interaction terms removed from the final model.

Counts of the number of disturbance occurrences on the range within each pen of birds were summed across each observation day and all proportions of birds disturbed during each occurrence were averaged across each observation day. All count and proportional data were then divided into the 'initial' range access period (22, 23 weeks of age, n = 84, 14 days per 6 pens) and 'acclimated' range access period (33, 34 weeks of age, n = 84, 14 days per 6 pens) Count data were square-root transformed and proportional data were logit transformed (Warton and Hui, 2011) with all data analysed using General Linear Models that included repeated measures. The effects of pen nested within rearing treatment, rearing treatment and range access period were included in the final model and all interactions.

3.3.2 RFID Data

For each individual hen, data were collated on daily hours outside, daily number of visits to the range, the minimum time (hours) and maximum time (hours) spent outside during a single visit. The percentage of available days that each hen visited the range were calculated across three divisions of the ranging period to assess change over time (21-26 weeks, 27-32 weeks, 33-37 weeks of age). All daily data

were compiled from hen ages 21 – 36 weeks (pop hole first opening until prior to first stressor) for each individual hen (n = 109 recording days per hen). The proportional data of hens using available ranging days were arc-sine transformed prior to analysis but the raw percentage data are presented in the results as there was virtually no difference between the raw and back-transformed means. The count data of hens daily visits were square-root transformed but the raw values are presented in the results as there was virtually no differences between the raw and back-transformed values. GLM's with repeated measures were applied to assess the effect of pen nested within rearing treatment, rearing treatment, week of age and the interactions.

3.3.3 Welfare Scoring

Data from the health and welfare measures were compiled separately for pen within each rearing treatment for each sampling age point. Toenail length and body weight were compiled for each individual bird for each sampling age week. The differences in beak length between the 20 weeks and 37 weeks of age measurements were calculated separately for the top and bottom parts of the beak for each individual bird. The effects of rearing treatment, pen nested within rearing treatment and sampling age week (except for beak measurements) including all interactions were compared using GLM's with repeated measures. Non-significant interaction terms were removed from the final model and where significant differences were present, post-hoc comparisons were made using Student's t-tests. Body weight uniformity for each pen of birds was calculated using the online Hy-Line Brown uniformity calculator (available at: http://www.hyline.com/aspx/redbook/redbook.aspx?s=2&p=43, accessed November 16, 2016). The relationships between average hours outside across the entire trial period (prior to stressors) and the difference in top and bottom beak length were compared using Pearson's Product Moment Correlations for each separate pen of birds. Comparisons were also made within each pen of birds between average hours outside and body weight and average hours outside and toenail length. Pearson's Product Moment Correlations were applied to body weight or toenail length data at each sampling age (26, 32, 37 weeks) and the average hours outside for the ranging weeks prior to welfare sampling (i.e. ranging age weeks 21-26, 27-32, 33-37). The number of birds with keel damage (including severe damage) within each treatment group were compared separately for each age point using Pearson's chi-squared tests. The numbers of birds with severe keel damage were too small for accurate statistical tests to be applied and thus all keel damage was included together in analyses. However, the percentages of birds with both types of damage are presented separately in the results.

3.3.4 Albumen Corticosterone and Stressor Events

All albumen corticosterone concentrations from individual eggs were compiled for samples taken at 20, 26, 32, 38 weeks of age, following being locked inside, immediately following range shrinkage and 2 weeks following range shrinkage from hens within each pen from the two rearing treatments. GLM's with repeated measures were used to compare the effect of rearing treatment, pen nested within rearing treatment, hen age/stressor events and all interactions.

To compare the effects of range shrinkage on range usage, 15 days of ranging immediately prior to the lock in event were selected as 'normal' ranging days in

closest proximity to the 15 days of ranging following range shrinkage. There were 7 days of ranging following lock in that were not included in the comparisons as they were directly following a potentially stressful event. Daily RFID data were compiled for each individual hen including the hours outside, number of visits, minimum and maximum time per visit. Count data were square-root transformed. Differences between pens within rearing treatments and rearing treatments were first assessed for the period prior to range shrinkage. The difference between these measures (prior to and after shrinkage) was then calculated (positive values indicate more time ranging prior to range shrinkage) and the effects of pen within rearing treatment and rearing treatment were compared on all measures using GLM's.

3.5 RESULTS

3.5.1 Video Counts and Disturbance Behaviours

There was no effect of rearing treatment ($F_{(1,364)} = 0.32$, $P = 0.57$) on the numbers of hens using the range simultaneously during the first 3 weeks of range access and no interactions with week of age or distance from pop holes (all $P > 0.70$). There were however differences between pens within treatment groups ($F_{(4,364)} = 36.70$, $P < 0.0001$). There was an effect of week of age ($F_{(2,364)} = 168.97$, $P < 0.0001$) with greater numbers of hens accessing the range each subsequent week (Figure 3.5.1). Also an effect of distance from the pop holes ($F_{(2,364)} = 151.10$, $P < 0.0001$) with the most hens seen close to the pop holes and the least hens in the middle portion of the range (Figure 3.5.1). Finally, there was also an interaction between week of age and distance from the pop holes ($F_{(4,364)} = 34.06$, $P < 0.0001$, Figure 3.5.1).

There was no effect of rearing treatment on the daily frequency of behavioural disturbance occurrences out on the range ($F_{(1,156)} = 2.86$, $P = 0.09$) and no effect of pen within treatment groups ($F_{(4,156)} = 1.66$, $P = 0.16$). There was however, an effect of range access period ($F_{(1,156)} = 105.99$, $P < 0.0001$) with fewer disturbances observed when hens had already spent 11 weeks on the range (LSM ± SEM enriched initial range access period: 13.71 ± 0.79, non-enriched initial range access period: 12.12 ± 0.79, enriched acclimated range access period: 5.29 ± 0.79, non-enriched acclimated range access period: 4.38 ± 0.79). There were no interactions between treatment and range access period ($F_{(1,156)} = 0.0005$, $P = 0.98$) and between pen within treatment and range access period ($F_{(4,156)} = 1.45$, $P = 0.22$).

There was no effect of rearing treatment on the average proportion of birds that were observed running during each disturbance ($F_{(1,156)} = 1.14$, $P = 0.29$) and only a trend for an effect of range access period ($F_{(1,156)} = 3.22$, $P = 0.07$, LSM ± SEM enriched initial range access period: 65.25 ± 1.47 %, non-enriched initial range access period: 65.79 ± 1.47%, enriched acclimated range access period: 61.29 ± 1.47%, non-enriched acclimated range access period: 63.65 ± 1.47%), with no interaction between rearing treatment and range access period ($F_{(1,156)} = 0.48$, $P = 0.49$). However, there were differences between pens within rearing treatment groups ($F_{(4,156)} = 0.04$, $P < 0.0001$) and an interaction between pens within rearing treatment and range access period ($F_{(4,156)} = 2.69$, $P = 0.03$).

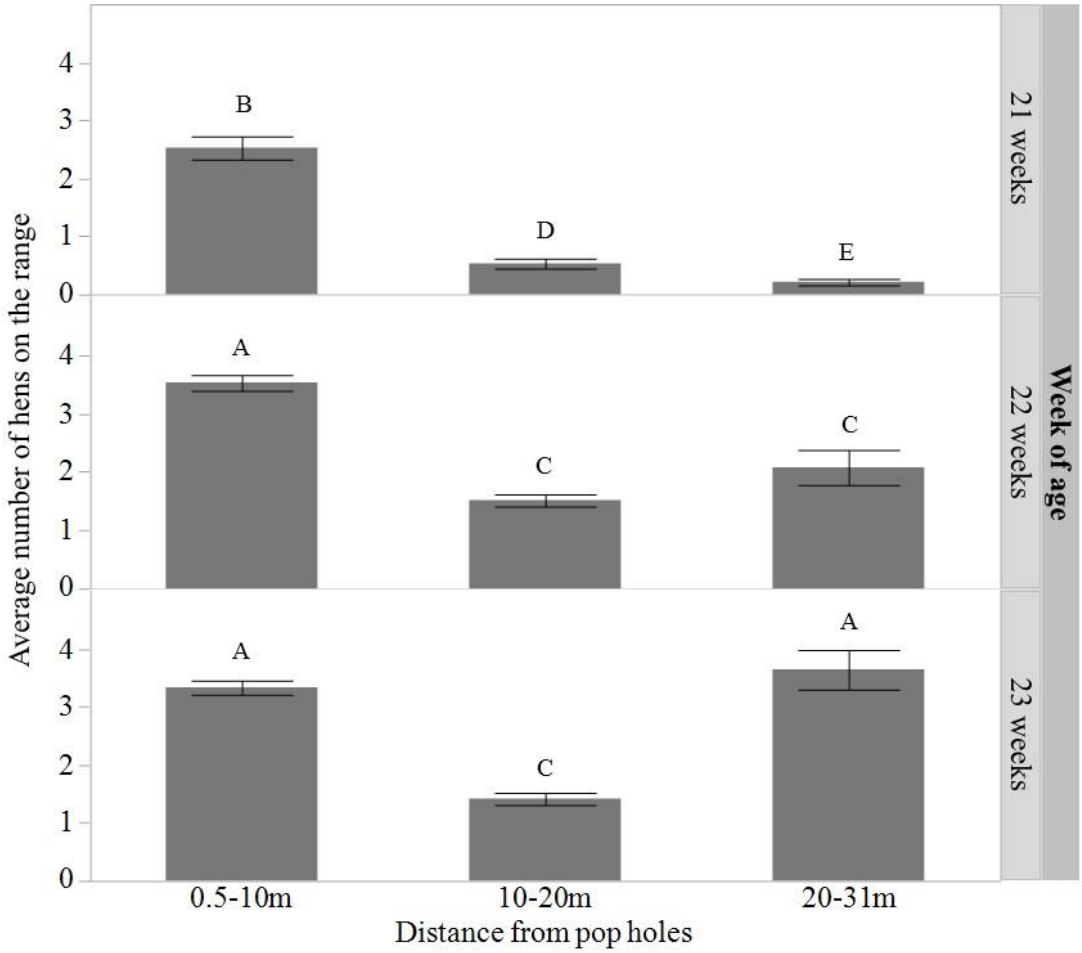

Figure 3.5.1 The mean ± SEM numbers of hens on the range simultaneously at three separate weeks of age following first pop hole opening (21 weeks of age) located at three distances from the pop holes (0.5-10m, 10-20m, 20-31m). Dissimilar letters indicate significant differences between weeks of age across distance from the pop holes.

3.5.2 RFID Tracking of Range Use

There was an effect of rearing treatment on the average number of hours outside daily ($F_{(1,29626)}$ = 41.88, P < 0.0001) with E hens spending less time outside on average (LSM ± SEM E: 4.18 ± 0.01 hours, NE: 4.30 ± 0.01 hours). However, there was also an effect of pen nested within rearing treatment ($F_{(4,29626)}$ = 540.90, P < 0.0001), an effect of week of age ($F_{(15,29626)}$ = 310.48, P < 0.0001) and an interaction between both rearing treatment and week of age ($F_{(15,29626)}$ = 4.94, P < 0.0001) and pen nested with rearing treatment and week of age ($F_{(60,29626)}$ = 14.57, P < 0.0001). Hens generally used the range more as the trial progressed with some E pens spending more time outside than NE pens on some weeks and vice versa (Figure 3.5.2, Table 3.5.1).

There was also an effect of rearing treatment on the average daily visits outside ($F_{(1,29626)}$ = 19.25, P < 0.0001) with E birds showing more visits to the range than NE birds (E: 13.74 ± 0.06, NE: 13.63 ± 0.06). However, there was also an effect of pen

within rearing treatment ($F_{(4,29626)}$ = 300.01, P < 0.0001), an effect of week of age ($F_{(15,29626)}$ = 147.20, P < 0.0001), and an interaction between both rearing treatment and week of age ($F_{(15,29626)}$ = 13.58, P < 0.0001) and pen nested within rearing treatment and week of age ($F_{(60,29626)}$ = 19.33, P < 0.0001). Hens generally showed more visits as the trial progressed but the differences between treatment groups were not consistent across age weeks (Table 3.5.1, Figure 3.5.3).

There was no effect of rearing treatment on the daily minimum time per individual visit ($F_{(1,28056)}$ = 0.01, P = 0.92) but there were differences between pens within rearing treatment groups ($F_{(4,28056)}$ = 76.11, P < 0.0001, Table 3.5.1). There was also an effect of week of age ($F_{(15,28056)}$ = 7.30, P < 0.0001) and interactions between rearing treatment and week of age ($F_{(15,28056)}$ = 2.53, P = 0.0009) and pen nested within rearing treatment and week of age ($F_{(60,28056)}$ = 4.07, P < 0.0001, Figure 3.5.4).

There was an effect of rearing treatment on the daily maximum time per individual visit ($F_{(1,28056)}$ = 5.84, P = 0.02) with the E hens showing a shorter maximum visit time (LSM ± SEM E: 1.04 ± 0.005, NE: 1.06 ± 0.005). There was also an effect of pen nested within rearing treatment group ($F_{(4,28056)}$ = 475.21, P < 0.0001, Table 3.5.1), an effect of week of age ($F_{(15,28056)}$ = 52.45, P < 0.0001) and interactions between rearing treatment group and week of age ($F_{(15,28056)}$ = 3.94, P < 0.0001) and pen within rearing treatment group and week of age ($F_{(60,28056)}$ = 10.14, P < 0.0001, Figure 3.5.5).

Finally there was an effect of rearing treatment on the percentage of available days that individual hens accessed the range ($F_{(1,837)}$ = 4.73, P = 0.03) with E hens accessing the range on more available days than NE hens (Figure 3.5.6). There was also an effect of pen within rearing treatment ($F_{(4,837)}$ = 7.89, P < 0.0001), an effect of weeks of age ($F_{(2,837)}$ = 85.76, P < 0.0001) with most hens using the range on all available days as the trial progressed (Figure 3.5.6) and an interaction between pen within rearing treatment and weeks of age ($F_{(8,837)}$ = 3.53, P = 0.005). There was no interaction between rearing treatment and weeks of age (P = 0.67) and thus this interaction was removed from the final model.

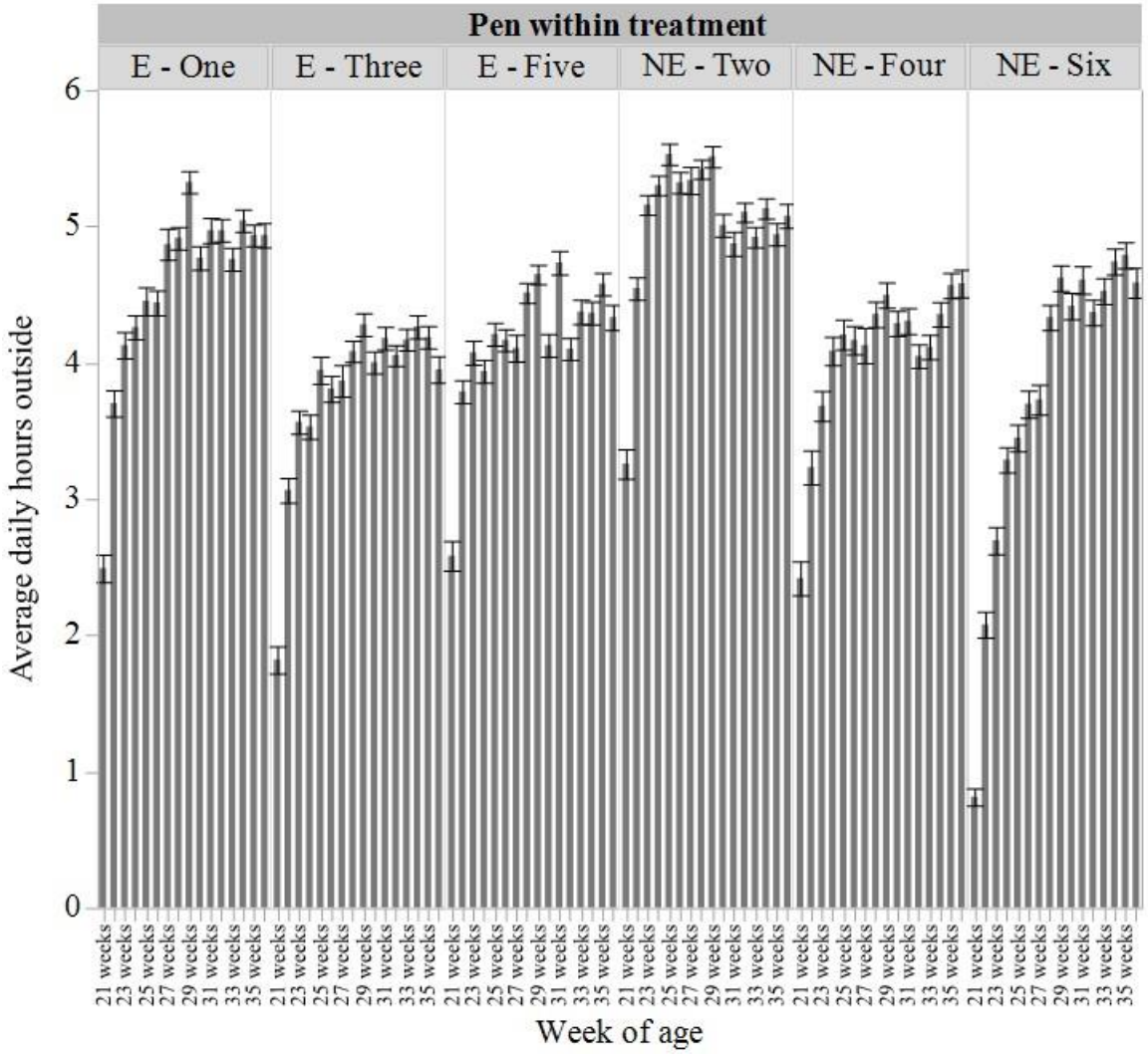

Figure 3.5.2 The mean ± SEM daily hours outside across 21 to 36 weeks of age for individual hens housed in 6 pens within 2 rearing treatments (E = enriched, NE = non-enriched).

	Enriched			Non-enriched		
	Pen 1	Pen 3	Pen 5	Pen 2	Pen 4	Pen 6
Hours outside	4.56 ± 0.02^{B}	3.80 ± 0.02^{D}	4.17 ± 0.02^{C}	5.03 ± 0.02^{A}	4.07 ± 0.02^{C}	3.80 ± 0.02^{D}
# visits	12.46 ± 0.10^{C,D}	11.76 ± 0.10^{D}	17.01 ± 0.11^{A}	13.10 ± 0.10^{C}	13.24 ± 0.10^{C}	14.53 ± 0.10^{B}
Min hr/visit	0.08 ± 0.003^{A}	0.07 ± 0.003^{A}	0.04 ± 0.003^{A,B}	0.09 ± 0.003^{A,B}	0.07 ± 0.003^{B,C}	0.03 ± 0.003^{C}
Max hr/visit	1.17 ± 0.009^{A,B}	1.09 ± 0.009^{A}	0.86 ± 0.009^{B,C}	1.26 ± 0.009^{C}	1.07 ± 0.009^{D}	0.84 ± 0.009^{D}
% days	94.88 ± 1.34^{A,B,C}	95.67 ± 1.34^{A,B}	97.98 ± 1.41^{A}	99.01 ± 1.35^{A}	90.27 ± 1.32^{C}	91.96 ± 1.21^{B,C}

Table 3.5.1 The LSM ± SEM daily hours outside, daily number of visits outside, minimum time (hours) per visit, maximum time (hours) per visit and percentage of available days the range was accessed for individuals hens from each pen (1 to 6) within each rearing treatment (enriched, non-enriched). Dissimilar connecting letters indicate significant differences between pens across both rearing treatments.

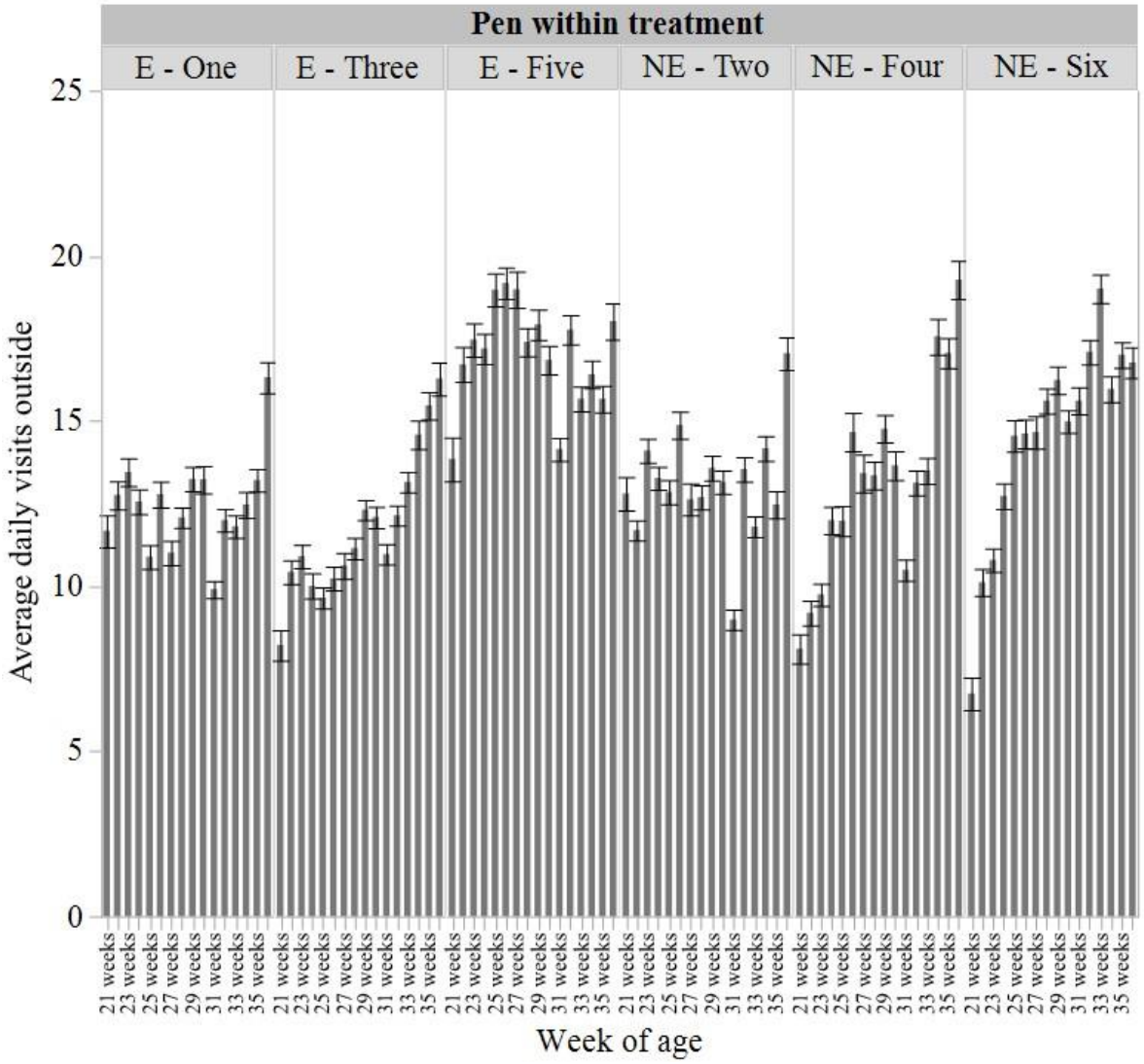

Figure 3.5.3 The mean ± SEM daily visits outside across 21 to 36 weeks of age for individual hens housed in 6 pens within 2 rearing treatments (E = enriched, NE = non-enriched).

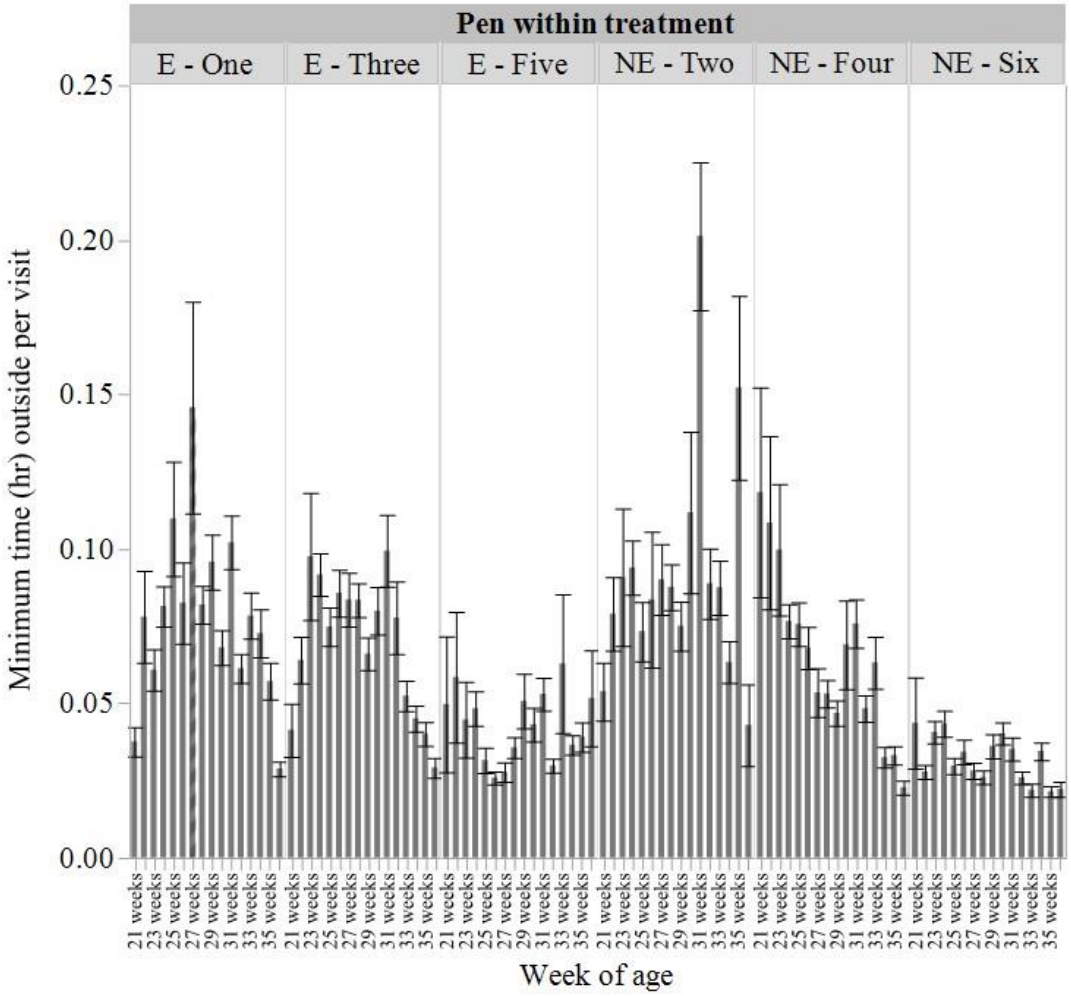

Figure 3.5.4 The mean ± SEM daily minimum time (hours) per visit outside across 21 to 36 weeks of age for individual hens housed in 6 pens within 2 rearing treatments (E = enriched, NE = non-enriched).

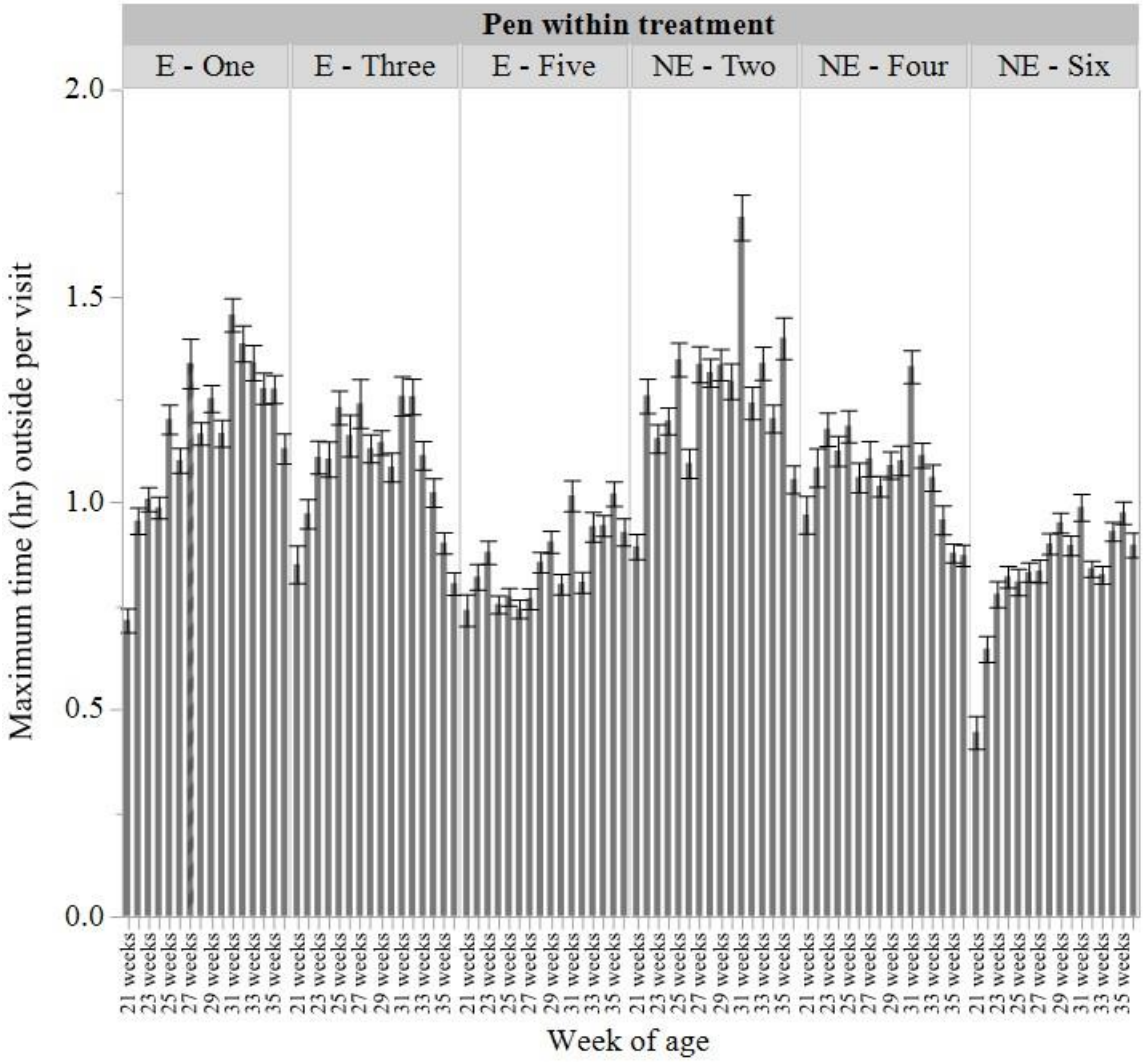

Figure 3.5.5 The mean ± SEM daily maximum time (hours) per visit outside across 21 to 36 weeks of age for individual hens housed in 6 pens within 2 rearing treatments (E = enriched, NE = non-enriched).

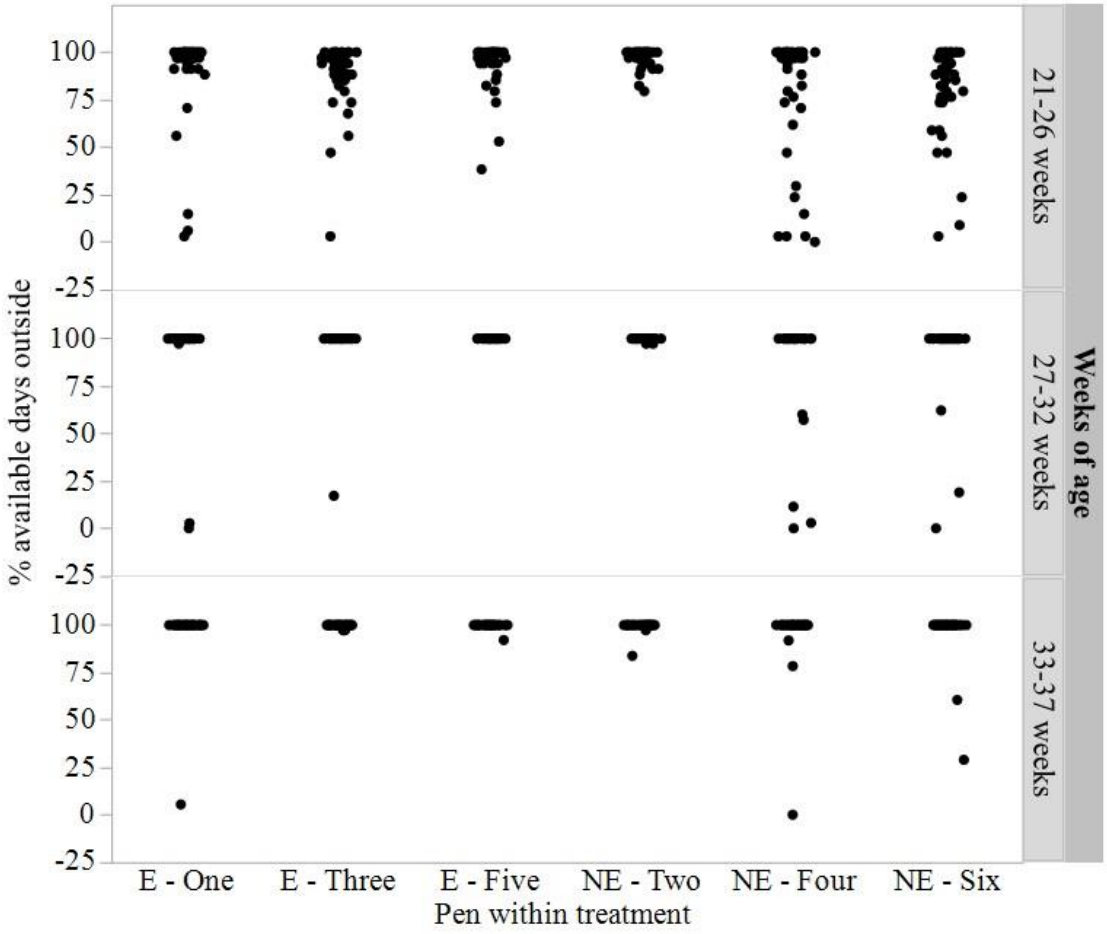

Figure 3.5.6 The mean ± SEM percentage of available days individuals hens visited the range across three age periods (21 to 26 weeks, 27-32 weeks, and 33-37 weeks of age) housed in 6 pens within 2 rearing treatments (E = enriched, NE = non-enriched).

3.5.3 Welfare Scoring

Only 2 birds died due to unknown causes across the trial period and thus were removed from all final datasets, all other birds visibly appeared to be in good health.

There was an effect of rearing treatment on average body weight ($F_{(1,1132)} = 4.0$, $P = 0.04$) with enriched-reared birds heavier than non-enriched-reared birds (LSM ± SEM body weight E: 1.88 ± 0.006 kg, NE: 1.87 ± 0.005 kg). There were also differences between pens within rearing treatment groups ($F_{(4,1132)} = 6.10$, $P < 0.0001$), and corresponding pen differences in body weight uniformity across sampling ages where 80% pen uniformity is the Hy-Line® management recommendation (Table 3.5.2). There was an effect of sampling age ($F_{(3,1132)} = 183.60$, $P < 0.0001$) with birds increasing in weight with age, as expected (LSM ± SEM body weight, 20 weeks 1.73 ± 0.008 kg, 26 weeks: 1.86 ± 0.008 kg, 32 weeks: 1.93 ± 0.008 kg, 37 weeks: 1.97 ± 0.007 kg). There was no interaction between sampling age and rearing treatment ($P = 0.79$), or pen nested within rearing treatment and sampling age ($P = 0.40$) and thus these interactions were removed from the final model. There were no correlations between average hours outside and body weight within each pen at the 27 week sampling age (all $r = -0.22 – 0.008$, $P \geq$

0.13) or the 37 week sampling age (all r = -0.20 – 0.18, $P \geq 0.17$) but there was one pen (NE) with a negative relationship (r = -0.30, P = 0.04) between body weight and average hours outside at the 32 weeks sampling age indicating hens were lighter if they spent more time outside (all other r = -0.22 – 0.14, $P \geq 0.14$). There were no correlations between average number of daily visits and body weight at the 27 week sampling point except for pen 3 (E) that showed a negative correlation (r = -0.39, P = 0.007) indicating those hens with a lower body showed more visits outside, possibly due to needing to regularly visit the feeder indoors (all other r = -0.22 – 0.13, $P \geq$ 0.14). There were negative correlations between average number of daily visits and body weight for pen 3 (E) (r = -0.39, P = 0.006) and pen 4 (NE) (r = -0.30, P = 0.04) only at the 32 week sampling point (all other r = -0.15 – 0.20, $P \geq 0.21$). At the 37 week sampling age, only pen 3 (E) again showed a negative correlation (r = -0.38, P = 0.008) between average daily visits and body weight (all other r = -0.21 – 0.02, $P \geq$ 0.17). Pen 3 was also the pen with the lowest bodyweight uniformity, below management recommendations, throughout the trial period (Table 3.5.2).

There was no effect of rearing treatment on average toenail length ($F_{(1,1120)}$ = 0.001, P = 0.99) but there were differences between pens within rearing treatments ($F_{(4,1120)}$ = 3.94, P = 0.004). There was also an effect of sampling age with toenails the longest length prior to release onto the range (LSM ± SEM toenail length, 20 weeks: 1.40 ± 0.006 cm, 26 weeks: 1.37 ± 0.006 cm, 32 weeks: 1.36 ± 0.006 cm, 37 weeks: 1.35 ± 0.006 cm). There was a trend for an interaction between pen nested within treatment and age point ($F_{(12,1120)}$ = 1.75, P = 0.05) but there was no interaction between sampling age and rearing treatment (P = 56) and thus this interaction was removed from the final model. There were no relationships between toenail length and average hours outside within pens at the 26 weeks of age sampling point except for pen 6 (NE) which showed a positive relationship (r = 0.38, P = 0.007) indicating longer toenails on those hens spending more time outside, possibly due to the grass coverage on the range being a softer surface with less abrasions to the nails (all other r = -0.02 – 0.26, $P \geq 0.07$). There was no correlation between toenail length and average hours outside at the 32 weeks sampling point (all r = -0.006 – 0.19, $P \geq$ 0.19) or 37 week sampling point (all r = -0.15 – 0.28, $P \geq 0.05$).

There was no effect of rearing treatment on the difference in beak length between measurements taken at 20 and 37 weeks of age (top beak: $F_{(1,279)}$ = 0.28, P = 0.60, bottom beak: ($F_{(1,279)}$ = 0.26, P = 0.61). There was also no effect of pen nested within rearing treatment (top beak: $F_{(4,279)}$ = 1.28, P = 0.28, bottom beak: $F_{(4,279)}$ = 1.04, P = 0.39). Across all birds, on average both beak parts were shorter (negative difference) at the end of the trial period (LSM ± SEM beak length, top beak: -0.09 ± 0.04 mm, bottom beak: -0.09 ± 0.04 mm). But only pen 4 (NE) showed a negative relationship between average hours outside and the difference in beak length (r = -0.32, P = 0.03) indicating more hours outside reduced beak length, possibly through foraging in the dirt. All other correlations within pens between average hours outside and the difference in beak length were non-significant (top beak: r = -0.22 – 0.15, $P \geq 0.13$, bottom beak: r = -0.08 – 0.14, $P \geq 0.35$).

There was no effect of rearing treatment on the incidence of keel damage as 26 weeks of age (χ^2 1, N = 286) = 1.09, P = 0.30), 32 weeks of age (χ^2 1, N = 286) = 1.70, P = 0.19), or 37 weeks of age (χ^2 1, N = 286) = 0.005, P = 0.94). There was no

keel damage detected at 20 weeks of age and across all hens, frequencies of keel damage increased as the hens aged (26 weeks: Y = 9.44%, Y* = 0.35%, 32 weeks: Y = 15.73%, Y* = 2.10%, 37 weeks: 18.53%, Y* = 2.80%).

	Enriched			**Non-enriched**		
Hen age	Pen 1	Pen 3	Pen 5	Pen 2	Pen 4	Pen 6
20 weeks	90 ± 5.7	77 ± 7.3	86 ± 7.1	91 ± 6.3	92 ± 5.7	82 ± 7.5
26 weeks	92 ± 5.6	77 ± 7.4	88 ± 6.3	94 ± 5.8	88 ± 6.5	86 ± 7.5
32 weeks	94 ± 5.3	79 ± 8.0	86 ± 6.6	91 ± 6.1	86 ± 7.0	82 ± 7.8
37 weeks	90 ± 6.1	75 ± 10.7	91 ± 6.7	89 ± 6.4	84 ± 7.1	82 ± 7.9

Table 3.5.2 The percentage body weight uniformity ± coefficient of variation of each pen of birds (1-6) within each rearing treatment group (enriched, non-enriched) for all measured age points (20, 26, 32, 37 weeks of age).

3.5.5 Albumen Corticosterone and Stressor Events

There was a marginal effect of rearing treatment on albumen corticosterone concentrations ($F_{(1,1928)}$ = 3.74, P = 0.05 with E birds showing eggs with lower albumen corticosterone than NE birds (LSM ± SEM E: 3.31 ± 0.02 ng/g, NE: 3.38 ± 0.02 ng/g), including significant differences between pens within rearing treatments ($F_{(4,1928)}$ = 4.58, P = 0.001). There was an effect of hen age and stressor events ($F_{(6,1928)}$ = 787.70, P < 0.0001) with the lowest concentrations at 20 and 26 weeks with a steady increase for every sampling age week/event following and the highest concentrations at 40 weeks following 2 weeks of range shrinkage (Figure 3.5.7). There was also an interaction between rearing treatment and hen age/stressor events ($F_{(6,1928)}$ = 9.72, P < 0.0001) with eggs from E hens showing higher albumen corticosterone concentrations than eggs from NE hens at 38 weeks (immediately prior to being locked inside), but this pattern was reversed following being locked inside, and 2 weeks after the range had been shrunk (Figure 3.5.7). Finally, there was also an interaction between pen nested within rearing treatment and hen age/stressor events ($F_{(24,1928)}$ = 9.72, P < 0.0001).

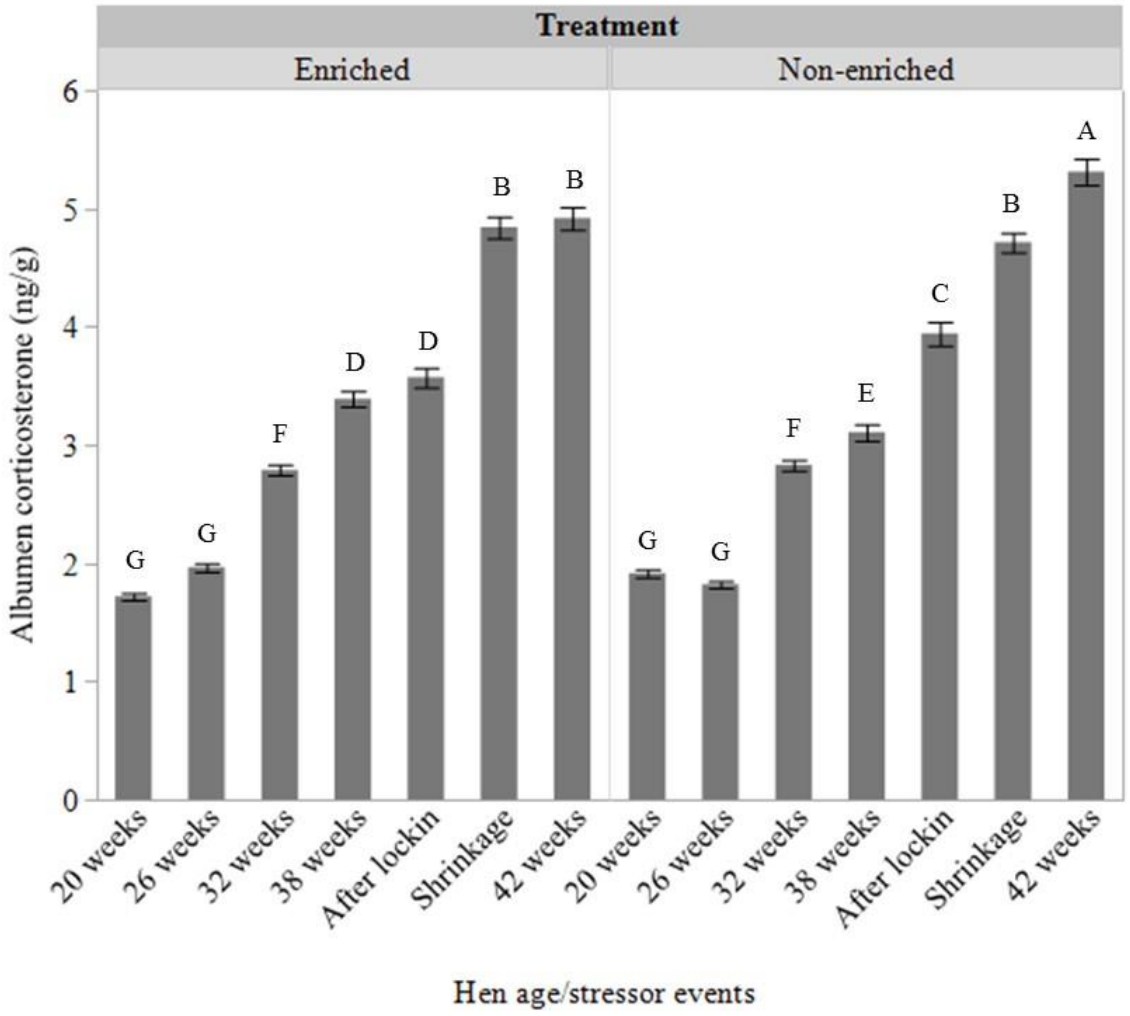

Figure 3.5.7 The albumen corticosterone concentrations (ng/g) of eggs from hens from two rearing treatments (enriched, non-enriched) across 20, 26, 32, 38 weeks of age, following being locked inside for 2 days (after lockin), then following range shrinkage to 20% original size (shrinkage) and at 42 weeks of age (2 weeks following range shrinkage). Dissimilar letters indicate significant differences between hen ages/stressor events across both rearing treatments.

3.5.6 Stressor Events and Range Use

During the 15 days selected prior to range shrinkage there was no effect of rearing treatment on the daily hours outside ($F_{(1,277)}$ = 2.18, P = 0.14) but there were differences between pens within treatment groups ($F_{(4,277)}$ = 3.73, P = 0.006). There was also no effect of rearing treatment on the daily visits to the range ($F_{(1,277)}$ = 0.49, P = 0.48), but differences were present between pens within treatment groups ($F_{(4,277)}$ = 4.47, P = 0.002, Table 3.5.3). Similarly, there was no effect of rearing treatment on the maximum time per visit ($F_{(1,277)}$ = 0.46, P = 0.50), but differences present between pens ($F_{(4,277)}$ = 19.16, P < 0.0001, Table 3.5.3).

Following range shrinkage, the difference between ranging behaviour prior and after shrinkage showed no effect of rearing treatment on the difference in hours outside per day ($F_{(1,277)}$ = 2.29, P = 0.13) no differences between pens within treatment

groups ($F_{(4,277)}$ = 0.84, P = 0.50). All pens showed an average positive difference indicating more time was spent ranging prior to the range shrinkage (Table 3.5.4). In contrast, there was an effect of rearing treatment on the difference in daily visits ($F_{(1,277)}$ = 5.87, P = 0.02) with E hens showing a greater increase in the number of visits compared to NE hens (LSM ± SEM E: -1.6 ± 0.39, NE: -0.30 ± 0.38). There were also differences between pens within treatment groups ($F_{(4,277)}$ = 8.31, P < 0.0001, Table 3.5.4). Finally, there was an effect of rearing treatment on the difference in maximum visit time per individual visit ($F_{(1,277)}$ = 27.16, P < 0.0001) with E hens showing shorter maximum visit times after shrinkage and NE hens also showing shorter maximum visit times following shrinkage but to a lesser extent (LSM ± SEM E: 0.24 ± 0.02, NE: 0.06 ± 0.02). There were also differences between pens within rearing treatments ($F_{(4,277)}$ = 10.41, P < 0.0001, Table 3.5.4).

	Enriched			**Non-enriched**		
RFID	Pen 1	Pen 3	Pen 5	Pen 2	Pen 4	Pen 6
Hours outside	4.97 ± 0.19^{A}	4.01 ± 0.18^{C}	4.34 ± 0.19^{B,C}	4.82 ± 0.19^{A,B}	4.58 ± 1.84^{A,B}	4.59 ± 0.18^{A,B}
# visits	13.86 ± 0.81^{B,C}	15.42 ± 0.81^{A,B,C}	15.69 ± 0.86^{A,B,C}	12.79 ± 0.82^{C}	17.32 ± 0.81^{A}	16.67 ± 0.80^{A,B}
Max hr/visit	1.28 ± 0.05^{A}	0.85 ± 0.05^{A}	0.98 ± 0.05^{B}	1.35 ± 0.05^{B}	0.92 ± 0.05^{B}	0.93 ± 0.05^{B}

Table 3.5.3 The LSM ± SEM daily hours outside, daily number of visits outside, maximum time (hours) per visit for individuals hens from each pen (1 to 6) within each rearing treatment (enriched, non-enriched) as measured across 15 days prior to the range being reduced in size. Dissimilar connecting letters indicate significant differences between pens across both rearing treatments.

	Enriched			**Non-enriched**		
RFID difference	Pen 1	Pen 3	Pen 5	Pen 2	Pen 4	Pen 6
Hours outside	0.64 ± 0.11	0.73 ± 0.11	0.76 ± 0.11	0.44 ± 0.11	0.69 ± 0.11	0.59 ± 0.11
# visits	-2.76 ± 0.67^{C}	0.13 ± 0.66^{A,B}	-2.26 ± 0.70^{B,C}	0.60 ± 0.67^{A}	1.31 ± 0.66^{A}	-2.82 ± 0.65^{C}
Max hr/visit	0.32 ± 0.04^{A}	0.14 ± 0.04^{B,C}	0.27 ± 0.04^{A,B}	-0.10 ± 0.04^{D}	0.06 ± 0.04^{C}	0.23 ± 0.04^{A,B}

Table 3.5.4 The LSM ± SEM difference in daily hours outside, daily number of visits outside, maximum time (hours) per visit for individuals hens from each pen (1 to 6) within each rearing treatment (enriched, non-enriched) as measured across 15 days prior to the range being reduced in size and 15 days immediately following the range reduction. Dissimilar connecting letters indicate significant differences between pens across both rearing treatments.

3.4 DISCUSSION AND IMPLICATIONS

This current study looked at the effects of enriching the chicks' environment on range use, welfare and response to stressors in adult free-range laying hens. Video observations at the flock level showed birds increased range use over the first 3 weeks, both venturing further onto the range and in higher numbers, but E birds did not differ from the NE birds. Additionally, video observations of natural disturbance behaviours on the range showed birds were disturbed by unknown environmental stimuli more often in the early weeks following first pop hole open than after several weeks of ranging, but again, no differences were observed between E and NE birds. Conversely, individual tracking using RFID technology showed that overall, the E birds spent less time on the range, with more visits of shorter duration than the NE birds. However, range use did vary across the weeks and the E birds sometimes spent more time outside. The E birds also spent more available days ranging but by 27 weeks of age, the majority of birds (E and NE) were using the range daily. On average the enriched birds showed slightly higher body weight but there were no other impacts of early rearing treatments on basic health measures. There were some relationships between health measures and range use but with high inter-pen variability within treatment groups. From 37 to 42 weeks of age all birds were exposed to two stressful events, being locked inside for 2 days followed by 80% reduction in range size. Over the experimental period, E birds showed marginally lower albumen corticosterone than NE birds, but significantly lower concentrations following the stressor events. All birds adjusted their ranging behaviour during the range shrinkage period compared to prior range use patterns but the E birds showed greater changes, coupled with lower albumen corticosterone concentrations, potentially indicating greater behavioural flexibility and adaptability. For the majority of measures there were differences between the pen replicates within rearing treatments and thus the influence of social dynamics and flock uniformity needs to be considered for optimal flock management.

The enrichments in the chicks' environment did impact ranging behaviour but not in all measures; specifically no effects of rearing were found during the first few weeks of range acclimation and the range disturbance behaviours, contrary to what was predicted. A recent study that provided early enrichment (hay bale, white strings, grain and mealworms) to slow-growing free-range broilers found no effect of enrichment on fearfulness in the tonic immobility test or in their use of the range but the enriched birds did spent less time standing than the non-enriched birds and more time sitting and lying (Stadig et al., 2016). Thus the E and NE birds may have differed in the behavioural time budgets outdoors, an avenue for future observations.

Across the experimental period the range use of treatment groups varied across the weeks, but on average, early enrichments reduced range use in comparison to NE hens, the contrary of what was predicted. It was hypothesised that if chicks were exposed to a more variable developmental environment they would spend more time in the variable unpredictable outdoor environment as adults. Enriched birds did use the range for a higher percentage of the available ranging days but overall spent less time outdoors with shorter individual visits. The enrichments may have enhanced the chickens' behavioural plasticity, which is the ability for the individual bird to flexibly

adjust their behaviour in response to stimuli and resources (Mason et al., 2013). Free-range birds have the choice of whether to venture outside or not. The outside environment may be more stimulating and rewarding, but typically feed and water are located indoors (as per this current study). The ability for birds to be more flexible in their behavioural patterns and have greater control over their behaviour with more frequent visits indoors could enable better nutrition and hydration (Singh and Cowieson, 2013). This is also supported by the slightly higher body weight in the E birds. Early research shows chicks exposed to the range perform better in maze tasks with more exploratory behaviour and faster learning (Krause et al., 2006). Studies with caged birds (parrots and songbirds) show enrichments reduce stereotypic behaviour (e.g., Garner et al., 2003, Meehan et al., 2004) where stereotypies also correlate with general behavioural disinhibition or lack of behavioural control (Garner et al., 2003). Further research could thus experimentally test measures of behavioural control such as perseveration (Kjaer et al., 2015) in laying hens and look at both impacts of enrichments and correlations with ranging behaviour.

Albumen corticosterone concentrations increased for all birds across the trial period. In a recent study across 12 Australian commercial farms (free-range, barn and conventional), the typical flock-cycle pattern of albumen corticosterone concentrations was a peak around 32 weeks followed by a decrease to steady levels until end of lay (Downing, 2012). This suggests the continuing increase in albumen corticosterone in this study was a response to the stressors and not just age-related. Additionally, the concentrations following the stressor events surpassed previous reported concentrations following handling or heat stress in ISA Brown hens (Downing and Bryden, 2008). The non-enriched birds showed the greatest change in albumen corticosterone following being locked inside, and continued elevation following 2 weeks of reduced range size in comparison to the enriched birds. This was coupled with the NE birds showing fewer changes in ranging behaviour in the 2 weeks following range shrinkage. This suggests the E birds, as predicted, were better able to cope and adapt to stressful events in their environment, similar to some previous findings (Altan et al., 2013). The mechanisms for this may be via changes in brain structure (e.g. Freire and Cheng, 2004) or differential brain gene expression patterns (e.g. Elfwing et al., 2015). Interestingly, in the 2 weeks of ranging prior to the stressor events, there was no effect of rearing treatment on any ranging measures indicating all birds eventually similarly adapted to their typical ranging environment (see more ranging results in Campbell et al., 2017a). However, reducing the range-size differentiated the E and NE birds demonstrating long-term effects of the rearing treatments were still present.

There was inter-pen variation in the majority of health, welfare and behavioural measures. With the advent of alternative laying hen production systems, hens are typically kept in larger group sizes than conventional cages of fewer than 10 hens. Welfare and productivity is also typically poorer in alternative housing with high levels of mortality (Weeks et al., 2016). Alternative housing such as free-range, provides hens greater environmental complexity to cater to individual needs and preferences, and environmental choice which may itself improve welfare (Nicol et al., 2009). However, alternative housing may also facilitate greater individual differences leading to poorer flock uniformity and allow influence of flock dynamics. In these group sizes of approximately 50 hens, it is possible dominance hierarchies were

formed (De'Eath and Keeling, 2003), differentiating individuals and creating flock-specific dynamics. Additionally, research shows feather pecking behaviour, for example, may be socially transmitted (e.g., Zeltner et al., 2000; McAdie and Keeling, 2002), and fear levels of a few individuals can increase the stress levels of the laying hen group (de Haas et al., 2012). Ranging behaviour and associated health impacts may also be socially facilitated but further research would be needed to verify this. Thus, while alternative housing may provide environments for individual needs, the role of flock dynamics and how to best manage within-bird interactions in large group sizes needs to be better understood.

The chicks were exposed to what could be perceived as 'excessive' environmental stimuli but it is possible there may have been a more optimal developmental window, particularly for certain stimuli. Thus future research should determine the specific time periods during chick or pullet development in which visual, spatial or auditory stimuli could be applied for maximal impact. If optimal periods are identified, then fewer enrichments may need to be applied, thus enabling practical enrichments for use in commercial settings.

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