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Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens

Dana L. M. Campbell[1,2*], Geoff N. Hinch[1], Jeff A. Downing[3] and Caroline Lee[2]

[1] *School of Environmental and Rural Science, University of New England, Armidale, NSW, 2351, Australia*

[2] *Agriculture and Food, CSIRO, Armidale, NSW, 2350, Australia*

[3] *School of Life and Environmental Science, Faculty of Veterinary Science, University of Sydney, Sydney, NSW, 2006, Australia*

[*]Corresponding author: D. L. M. Campbell, The Woolshed, Department of Animal Science, University of New England, Armidale, NSW 2351. Phone: +61 2 6773 5124. Email: danaleigh.m.campbell@gmail.com

ABSTRACT

Free-range systems are increasing in popularity as outdoor access is perceived to improve hen welfare, but radio-frequency identification tracking of individuals shows not all hens access the range daily with some hens never going outside. The individual variation in range use may be correlated with individual differences in fear and coping styles. In this study, tests of tonic immobility, manual restraint (coupled with plasma corticosterone responses) and open field were applied to ISA Brown hens (*Gallus gallus domesticus*: n = 104) at 37-39 weeks of age. Focal hens were selected as categorised into three different range access groups based on the percentage of available days across the trial period that they went outdoors: indoor-preferring (accessed the range on 0 – 10% of available days), moderate-outdoor-preferring (accessed the range on 30-60% of available days), and outdoor-preferring (accessed the range on 100% of available days). The indoor-preferring hens produced fewer vocalisations during manual restraint compared to the moderate-outdoor and outdoor-preferring hens ($P = 0.004$) and tended to be slower to vocalise ($P = 0.08$) but no differences were found in latency to first struggle ($P = 0.55$), number of struggles ($P = 0.44$) or plasma corticosterone responses ($P = 0.82$). During the open field tests, the indoor-preferring and moderate-outdoor hens showed longer latencies to move and fewer squares were crossed compared to outdoor-preferring hens ($P \leq 0.009$) but no differences were found in latency to first vocalise ($P = 0.72$) and total number of vocalisations ($P = 0.91$). There were no differences between ranging groups in duration of tonic immobility ($P = 0.14$). These results show indoor-preferring hens have elevated fear levels, and a possible behavioural indication of a different coping style compared to outdoor-preferring hens, but the inconsistent results within the moderate-outdoor range access group warrant further investigation.

Key words: tonic immobility, open field, manual restraint, free-range, laying hen, fear

1. Introduction

Free-range laying hen (*Gallus gallus domesticus*) production systems with outdoor area provide hens a choice to access more space, fresh air and to exhibit behaviour such as dust bathing, foraging and sun bathing in relatively more natural surroundings, and are thus believed to improve hen welfare in comparison to caged or other indoor systems (Fanatico, 2006; Knierim, 2006; Schröder and McEachern, 2004). Consequently, these systems are increasing in popularity within Australia and elsewhere (Rault et al., 2013; Richards et al., 2011). However, there are an increasing number of studies using radio-frequency identification (RFID) tracking of individual hens in both experimental and commercial flocks showing that not all hens choose to access the range on a daily basis, with some hens exclusively staying indoors (Campbell et al., 2016; Gebhardt-Henrich et al., 2014; Richards et al., 2011).

Range use can be influenced by a number of variables such as age of hens, season, climate, artificial or natural cover, artificial structures, flock size or pop hole availability (Pettersson et al., 2016). However, there are 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. Venturing outdoors may provide some welfare benefits (Rodriguez-Aurrekoetxea and Estevez, 2016; Mahboub et al., 2004; Bestman and Wagenaar, 2003) but the outdoor range is exposed to highly variable environmental conditions (i.e., sun, rain, snow, wind, storms) compared to climate-controlled indoor housing (Gilani et al., 2014; Richards et al., 2011), and also poses an increased risk of predation (Bestman and Ouwejan, 2016). Thus, individual hens may be fearful of entering the outdoor range, and less able to cope with the possible stress of environmental change. Consequently, individual differences in behavioural traits of hens, specifically fearfulness, response to stress and coping styles, as detected by laboratory-based behavioural tests, may correlate with the level of range use.

Fear is generally considered to be associated with a negative welfare state (Jones, 1996), and various behavioural tests demonstrate individual hens differ in fear responses resulting from, for example, genotype and early rearing environments (Jones, 1996). Tonic immobility (TI) is a well-validated and a widely-used test of fear in poultry. The test involves inducing an immobile state using a brief period of restraint by placing the bird on its back and determining the time taken for the bird to ‘right’ itself. The more fearful individuals are, the longer they take to resume an upright position (Forkman et al., 2007; Jones, 1996). Another common test of fear and/or social motivation in poultry is the open field/arena test (OFT) (Forkman et al., 2007), where hens isolated in a novel test arena exhibit behaviours thought to be representative of the internal conflict between minimising detection by predators (e.g., freezing behaviour) and the need to re-group with conspecifics (e.g., vocalisations and escape attempts; Gallup and

Suarez, 1980). Finally, the escape attempts and vocalisations produced during prolonged manual restraint (MR) (e.g., 5 minutes) of an individual hen, coupled with physiological measures (e.g., corticosterone, heart rate) can be indicative of either a proactive (active) or reactive (passive) coping style (Koolhaas et al., 1999; Korte et al., 1999; Korte et al., 1997; de Haas et al., 2012). A bird that is classed as having a more proactive coping style will generally be behaviourally more active, but have low corticosterone responses while reactive birds typically display more passive behaviours, but higher corticosterone responses (Cockrem, 2007). However, birds with proactive coping styles are predicted to be more rigid in their behaviours and, thus, less sensitive and flexible to environmental variation suggesting no optimal behavioural repertoire to suit all situations (Cockrem, 2007).

Early research with Hi-sex laying hens, found that those birds observed spending more than half of their time outdoors had shorter durations of TI compared to those birds never observed to go outdoors when tested at 60 weeks of age (Grigor et al., 1995). A more recent study tested ISA Brown hens at 29 weeks of age and also found a negative correlation between duration of TI and extent of range use (Hartcher et al., 2016). Finally, previous studies at the current facility using 32 week old ISA Brown hens, found that birds that never visited the outdoor range had higher blood plasma corticosterone responses following MR compared to both hens having low range use (< 5 days range use) or those having high range use (daily range use; Hernandez et al., 2014). But no significant relationships were found between responses in TI and OFT and range use patterns (Hernandez et al., 2014).

The objective of the study was to use multiple tests of fear and coping style to determine if ISA Brown hens that differed in their range access habits could be consistently categorised as showing different personality traits to aid understanding of individual ranging variation. Using TI, MR and OFT, the hens preferring to stay indoors were predicted to show longer durations of TI, longer latencies to respond during MR with fewer vocalisations, fewer struggles and a higher plasma corticosterone response and fewer vocalisations and less movement with longer latencies to respond during the OFT compared to hens showing greater preference for the outdoor range.

2. Materials and methods

2.1 Ethical statement

All research was approved by the University of New England Animal Ethics Committee (AEC 14-100) and complied with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

2.2 Animals and housing

Nine hundred ISA Brown pullets (*Gallus gallus domesticus*) were obtained from a commercial supplier at 16 weeks of age, and placed into the University of New England's Laureldale experimental free-range facility, located in Armidale, NSW, Australia. Birds had been laser beak-trimmed at 1 day old with a second hot-blade trim at 11 weeks of age. Focal test subjects (n = 104) were selected from this total group of 900 hens based on their ranging behaviour, as described in the following section. All hens (including focal test subjects) were evenly distributed between 6 indoor pens (150 birds/pen) and housed at an indoor stocking density of nine birds/m^2 with feed, water, perch and nestbox resources either set to or exceeding the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002). As part of a larger project on the impacts of outdoor stocking density (see Campbell et al., 2016) each pen had an associated outdoor range of varying size to simulate three different outdoor stocking densities (at maximum bird occupancy) with two replicates per density treatment: 2000 hens per hectare (ha) (5 m^2/hen), 10000 hens/ha (1 m^2/hen), and 20000 hens/ha (0.5 m^2/hen). For further details on the experimental facility and housing conditions see Campbell et al. (2016). The pop holes providing range access were first opened at 21 weeks of age with subsequent daily access from 09:00 – 16:30 h until 36 weeks of age. Seventy-five randomly-selected birds in each pen (50%) were fitted with an adjustable leg band (Roxan Developments Ltd, Selkirk, Scotland) containing an RFID microchip (Trovan® Unique ID 100 (FDX-A): operating frequency 128 kHz; Microchips Australia Ptd Ltd, Keysborough, VIC, Australia) and individual daily range usage was tracked using RFID technology using three RFID systems purchased from Microchips Australia Ptd Ltd with equipment developed and built by Dorset Identification B.V. (Aalten, The Netherlands) using Trovan® technology. For additional details on the RFID system set-up see Campbell et al. (2016). As only three RFID systems were available, one replicate from each stocking density was recorded for approximately 2 weeks, and then the second replicate was recorded for approximately 2 weeks, with alternating recording for 15 weeks of range access.

2.3 Test birds

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.

2.4 Behavioural tests – general protocol

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.

2.4.1 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.

2.4.2 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).

2.4.4 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. Intra-rate reliability as measured by Cohen's Kappa coefficient (McHugh, 2012) was $\kappa = 0.89 – 0.92$).

2.5 Data and statistical analyses

There were no effects of stocking density within the three range access groups for all measured behavioural and physiological variables (Kruskal-Wallis tests for non-parametric data, all $P \geq 0.06$) except for one difference in the latency to first vocalise during MR within the moderate-outdoor group ($P = 0.03$), thus birds within range access groups were combined across stocking density treatments. Data from individual birds within each range access group (indoor-preferring, moderate-outdoor, outdoor-preferring) were compiled for each of the three separate behavioural tests (TI, MR and OFT) with individual hen as the unit of analysis. Shapiro-Wilk tests were applied to determine the data were from a non-parametric distribution and thus Kruskal-Wallis tests were used to compare values between the three range access groups. Comparisons were made for the number of attempts to induce and total duration of induced TI, the latency to first struggle, number of struggles, latency to first vocalise, number of vocalisations made during MR and 20-min plasma corticosterone response and the latency to first move, number of squares crossed, latency to first vocalise and total number of vocalisations made during the OFT. Where significant differences were present, non-parametric Wilcoxon each-pair post-hoc tests were applied. All analyses were conducted in JMP 12.1.0 (SAS Institute, Cary, NC, USA) with significance set at $P < 0.05$.

3. Results

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). 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). 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) 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). 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). But there were significant differences in the latency to first movement (H = 9.65, DF = 2, P = 0.008, Table 1) and total number of squares crossed (H = 9.48, DF = 2, P = 0.009, Table 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.

4. Discussion

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 moderate-outdoor group of hens contrasted with the outdoor-preferring or indoor-preferring hens as they had inconsistent range access patterns. Initially these birds did not use the range at all, typically for several weeks, but then began to use the range daily. Their inconsistent responses across the tests compared to the more consistent responses seen in indoor-preferring or outdoor-preferring range access groups, suggests they may warrant further study. It could be that their choice to eventually go outside correlates with social dynamics, individual behavioural needs (e.g., desire to forage outside) or age. Additional behavioural tests such as a novel object, emergence test, or voluntary approach to humans may help to distinguish further differences between moderate-outdoor ranging hens to ascertain causes of their acclimation period to ranging. Furthermore, observations of their time budgets could determine how their daily behavioural expression changes across successive weeks.

The duration of TI did not differentiate between focal hens in different range access groups, although this may not be surprising given previous reports which are inconsistent in their efforts to correlate TI with ranging behaviour. Some reports have found a negative correlation with range use (Hartcher et al., 2016; Grigor et al., 1995) while Hernandez et al., (2014) found no such correlation with range use. Furthermore, previous studies have found no correlation within individuals between duration of TI and behavioural responses during OFT, which did differentiate ranging groups in this study (Heiblum et al., 1998; Jones et al., 1995), thus highlighting the inconsistencies of individual responses between multiple behavioural tests. It is possible that different methodologies such as testing outside of the home pen (Bilcík, 1998), testing in isolation from conspecifics, the presence of the experimenter and/or the lack of eye contact made by the experimenter may all be important to bird responses (Forkman et al., 2007).

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. Previous research on coping styles within laying hens is related to different lines of hens characterised by low or high levels of feather-pecking or mortality. The low feather pecking hens are shown to have higher plasma corticosterone concentrations while resting and during MR, higher parasympathetic activity and fewer struggles during crush cage tests than hens from high feather pecking lines (Korte et al., 1997; Korte et al., 1999), all suggesting a reactive coping style in the low feather pecking line. However, further applications of MR with hens from low mortality and control lines show inconsistent results both between studies and between behavioural and physiological measures during MR (de Haas et al., 2012; Rodenburg et al., 2009). Overall the plasma corticosterone concentrations in this study were similar to previous reports for similar aged ISA Brown free-range hens (Hernandez et al., 2014) but were much lower than reported concentrations induced by intensive handling stress in older, cage-housed ISA Brown hens (Downing and Bryden, 2008). It is possible the birds in this study were more accustomed to handling resulting from five weighing and health scoring sessions (each bird individually handled) that occurred periodically from 16 weeks up to behavioural testing as part of a separate dataset (Campbell et al., unpublished data). 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 (Rodriguez-Aurrekoetxea and Estevez, 2016; Bestman and Wagenaar, 2003) 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.

5. Conclusions

Overall, individual hens that differed in their patterns of range use also differed in some behavioural responses when subjected to a MR test and an OFT. No differences in TI were observed. Further studies are needed to determine if environmental preferences within the free-range system affect individual hen welfare and to identify what behavioural repertoire is best suited to a free-range environment.

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References

Bestman, M.W.P., Wagenaar, J.P., 2003. Farm level factors associated with feather pecking in organic laying hens. Livest. Prod. Sci. 80, 133-140.

Bestman, M., Ouwejan, J., 2016. Predation of free-range laying hens. Proc. Intl. Soc. Appl. Ethol. 50: 283 (Abstr.).

Bilcík, B., Keeling, L.J., Newberry, R.C., 1998. Effect of group size on tonic immobility in laying hens. Behav. Proc. 42, 53-59.

Campbell, D.L.M., Hinch, G.N., Dyall, T., Warin, L., Little, B., Lee, C., 2016. Outdoor stocking density in free-range laying hens: radio-frequency identification of impacts on range use. Animal, 22, DOI: 10.1017/S1751731116001154.

Cockrem, J.F., 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148, S169-S178.

de Haas, E.N., Kops, M. S., Bolhuis, J.E., Groothuis, T.G.G., Ellen, E.D., Rodenburg, T. B., 2012. The relation between fearfulness in young and stress-response in adult laying hens, on individual and group level. Physiol. Behav. 107, 433-439.

Downing, J.A, Bryden, W.L., 2008. Determination of corticosterone concentrations in egg albumen: A non-invasive indicator of stress in laying hens. Physiol. Behav. 95, 381-387.

Fanatico, A., 2006. Alternative poultry production systems and outdoor access. ATTRA Publication #IP300.

Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340-374.

Gallup, G.G., Suarez, S.D., 1980. An ethological analysis of open-field behaviour in chickens. Anim. Behav. 28, 368-378.

Gebhardt-Henrich, S.G., Toscano, M.J., Fröhlich, E.K.F., 2014. Use of outdoor ranges by laying hens in different sized flocks. Appl. Anim. Behav. Sci. 155, 74-81.

Gilani, A.-M., Knowles, T.G., Nicol, C.J., 2014. Factors affecting ranging behaviour in young and adult laying hens. Br. Poult. Sci. 55:127-135.

Grigor, P.N., Hughes, B.O., Appleby, M.C., 1995. Effects of regular handling and exposure to an outside area on subsequent fearfulness and dispersal in domestic hens. Appl. Anim. Behav. Sci. 44, 47-55.

Hartcher, K.M., Hickey, K.A., Hemsworth, P.H., Cronin, G.M., Wilkinson, S.J., Singh, M., 2016. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens. Animal, 10, 847-853.

Heiblum, R., Aizenstein, O., Gvaryahu, G., Voet, H., Robinzon, B., Snapir, N., 1998. Tonic immobility and open field responses in domestic fowl chicks during the first week of life. Appl. Anim. Behav. Sci. 60, 347-357.

Hernandez, C.E., Lee, C., Ferguson, D., Dyall, T., Belson, S., Lea, J., Hinch, G., 2014. Personality traits of high, low and non-users of a free range area in laying hens. Proc. Intl. Soc. Appl. Ethol. 48: 89 (Abstr.).

Hinch, G.N., Lee, C. 2014. New approaches to assess welfare in free range laying hens. Poultry CRC Ltd Final Report 1.5.2. Unpublished data.

Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications and imperatives. Worlds Poult. Sci. J. 52, 131-174.

Jones, R.B., Blokhuis, H.J., Beuving, G., 1995. Open-field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. Brit. Poult. Sci. 36, 525-530.

Knierim, U., 2006. Animal welfare aspects of outdoor runs for laying hens: A review. NJAS – Wagen. J. Life Sci. 54, 133-145.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925-935.

Korte, S.M., Beuving, G., Ruesink, W., Blokhuis, H.J., 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiol. Behav. 62, 437-441.

Korte, S.M., Ruesink, W., Blokhuis, H.J., 1999. Heart rate variability during manual restraint in chicks from high- and low-feather pecking lines of laying hens. Physiol. Behav. 65, 649-652.

Mahboub, H.D.H., Müller, J., von Borell, E., 2004. Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. Brit. Poult. Sci. 45, 738-744.

McHugh, M.L., 2012. Interrater reliability: the kappa statistic. Biochem. Med. 22, 276-282.

National Health and Medical Research Council, 2013. Australian code of practice for the care and use of animals for scientific purposes. 8th ed. Commonwealth Government of Australia, Canberra, ACT.

Nicol, C.J., Caplen, G., Edgar, J., Browne, W.J., 2009. Associations between welfare indicators and environmental choice in laying hens. Appl. Anim. Behav. Sci. 78, 413-424.

Pettersson, I.C., Freire, R., Nicol, C.J., 2016. Factors affecting ranging behaviour in commercial free-range hens. Worlds Poult. Sci. J. 72, 137-149.

Rault, J.L., van de Wouw, A., Hemsworth, P.H., 2013. Fly the coop! Vertical structures influence the distribution and behaviour of laying hens in an outdoor range. Aust. Vet. J. 91, 423-426.

Richards, G.J., Wilkins, L.J., Knowles, T.G., Booth, F., Toscano, M.J., Nicol, C.J., Brown, S.N., 2011. Continuous monitoring of pop hole usage by commercially housed free-range hens throughout the production cycle. Vet. Rec. 169, 338.

Rodenburg, T.B., Bolhuis, J.E., Koopmanschap, R.E., Ellen, E.D., Decuypere, E., 2009. Maternal care and selection for low mortality affect post-stress corticosterone and peripheral serotonin in laying hens. Physiol. Behav. 98, 519-523.

Rodriguez-Aurrekoetxea, A., Estevez, I., 2016. Use of space and its impact on the welfare of laying hens in a commercial free-range system. Poult. Sci. doi: 10.3382/ps/pew238.

Schröder, M.J.A., McEachern, M.G., 2004. Consumer value conflicts surrounding ethical food purchase decisions: a focus on animal welfare. Int. J. Consum. Stud. 28, 168-177.

TABLE CAPTIONS

Table 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 $P \leq 0.02$.

Table 1

		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*