



Associations of range use with individual behaviour, clinical welfare indicators, fear response and gastrointestinal characteristics of two laying hen hybrids

Kaitlin E. Wurtz^{a,*}, Fernanda M. Tahamtani^{a,1}, Leslie Foldager^{a,b}, Karen Thodberg^a, Anja B. Riber^a

^a Department of Animal and Veterinary Sciences, Aarhus University, Blichers Allé 20, P.O. Box 50, DK-8830 Tjele, Denmark

^b Bioinformatics Research Centre, Aarhus University, Universitetsbyen 81, DK-8000 Aarhus C, Denmark

ARTICLE INFO

Keywords:
Laying hen
Organic
Range use
Individual
Welfare
Behaviour

ABSTRACT

Access to an outdoor range provides laying hens with increased space and improved opportunities for performing natural behaviours. However, not all hens utilise the range to the same extent, and this may be associated with the welfare of the individual hen. The aim of this study was to assess if extent of range use was associated with several clinical welfare indicators, time budgets, measures of tonic immobility and gastrointestinal characteristics. One hundred twenty focal individuals (Bovans Brown, $n = 60$; Dekalb White, $n = 60$) randomly chosen from 12 pens of 100 hens (10 hens from each pen) were housed in accordance with the EU organic standards. Clinical welfare indicators including plumage condition, foot health, keel bone damage and body weight were assessed upon arrival at 18 weeks of age and again at 23, 28, 33 and 38 weeks of age. Over five three-day periods at corresponding weeks, hens received individual back marks for identification and behaviour was recorded by instantaneous scan sampling from 8:30–15:30 in the house and on the range. Behaviour recorded included comfort behaviours, drinking, dust bathing, eating, foraging, locomotion, nest use, pecking, perching, resting and standing. Frequency of passes through the pophole and duration of time outdoors were also determined through video observations. At 18 weeks of age a tonic immobility test was performed and number of inductions, latency to first head movement after induction and the duration of tonic immobility were recorded. At the end of the study (38 weeks of age), gastrointestinal tract morphology and contents were assessed. Individual range use varied, with hens differing in time spent on range, number of exits and consistency in these measures over time. Extent of range use was not associated with clinical welfare indicators nor fear levels as assessed by a tonic immobility test. Hens' time budgets differed depending on where they spent most of their day, with hens utilising the range to a greater extent generally being more active (duration on range: foraging: $P < 0.001$, standing: $P = 0.005$; number of exits: locomotion: $P < 0.001$). Hens that utilised the range to a greater extent likely consumed more forage, as evident by the greater weight of pasture found in their crop and gizzard ($P < 0.001$), contributing to more developed gastrointestinal organs including the crop (duration on range: $P = 0.004$), gizzard ($P < 0.001$), and proventriculus (duration on range: $P = 0.014$). To conclude, the fear level and clinical welfare condition of the hens were not associated with extent of range use, unlike the time budgets and gastrointestinal characteristics.

1. Introduction

Access to an outdoor range offers laying hens improved opportunities to perform natural behaviours and increases available foraging materials (Lay Jr et al., 2011). In addition, range use can effectively

reduce stocking density within the house, potentially contributing to improved environmental and social conditions. The extent to which a flock utilises the range has shown to be influenced by age, strain, rearing environment, weather conditions, and a number of other factors (Petersson et al., 2016; Wurtz et al., 2022). However, even within a single

* Corresponding author.

E-mail address: kwurtz@anivet.au.dk (K.E. Wurtz).

¹ Present address: Animalia AS, Norwegian Meat and Poultry Research Centre, Lørenveien 38, 0513 Oslo, Norway.

flock kept under the same conditions, variability between individuals' use of the range has been observed, with differences in how often they visit and how long hens remain on the range (Richards et al., 2011; Gebhardt-Henrich et al., 2014; Hartcher et al., 2016; Campbell et al., 2017b; Larsen et al., 2017; Larsen et al., 2018; Campbell et al., 2020). Research has suggested positive welfare benefits of range use at the flock level (Bestman and Wagenaar, 2003; Rodriguez-Aurrekoetxea and Estevez, 2016), but few have examined the association between range use and hen welfare at the level of the individual (e.g., (Rodriguez-Aurrekoetxea and Estevez, 2016; Larsen et al., 2018; Bari et al., 2020; Sibanda et al., 2020).

The increased choices that a range provides may impact an individual's condition. Conversely, a hen's condition may impact her choices (Daigle and Siegford, 2014). On the range hens have increased space and foraging materials which could contribute to reduced incidences of feather pecking and aggression, leading to improved plumage conditions and fewer comb wounds (Nicol et al., 2003; Schreiter et al., 2019). Foot health may be impacted by conditions within the house (e.g., litter moisture or house temperature) or on the range (e.g., standing water or faeces accumulation) (Wang et al., 1998). On the other hand, hens with foot disorders may be less motivated to explore the outdoors due to discomfort. Similarly, keel bone damage may impact a hens' mobility, and thus range access, and vice versa (Nasr et al., 2012; Rufener et al., 2019).

Within a flock, individual hens may vary in their daily behavioural time budgets, including use of the outdoor range (Hartcher et al., 2016; Campbell et al., 2017b; Larsen et al., 2017). These variations may be linked to underlying motivations or affective states which may be impacted by a multitude of factors (Mendl et al., 2010; Fureix and Meagher, 2015). Many studies have suggested a negative correlation between individual fear levels and ranging behaviour. For instance, indoor preferring hens have been shown to have elevated fear levels as assessed by open field tests (Campbell et al., 2016; Campbell et al., 2019), manual restraint tests (Campbell et al., 2016), novel object tests (Larsen et al., 2018), tonic immobility tests (Grigor et al., 1995; Hartcher et al., 2016) and physiological responses (Hernandez et al., 2014; Larsen et al., 2018). It is believed that individual fear levels may impact a hen's willingness to access range areas where they may be exposed to novel conditions or frightening stimuli such as predators and adverse weather conditions. Further, a hen's behavioural repertoire may differ depending on whether they are indoors or on the range where available resources may differ (Thuy Diep et al., 2018).

Differences in gastrointestinal characteristics have been noted in birds with different ranging profiles. Range preferring hens have been shown to be more efficient at utilizing feed than hens that prefer to stay indoors (Kolakshyapati et al., 2020). Birds that spend a greater amount of time on pasture are likely to be ingesting greater amounts of plant matter, insects, and grit stones (Marchewka et al., 2021). Increased forage consumption has been shown to lead to more developed crops as this organ is responsible for breaking down the plant material before it may advance further through the digestive system to the proventriculus and then the gizzard (Marchewka et al., 2021). The ingestion of grit and stones may also contribute to heavier gizzards in range-preferring hens (Singh et al., 2016; Bari et al., 2020). Birds with greater pasture intake have increased weights of other digestive organs including the spleen, liver, and pancreas (Iqbal et al., 2018; Bari et al., 2020). Extent of digestive organ development can impact hens' ability to digest nutrients and their susceptibility to disease (Yegani and Korver, 2008; Mateos et al., 2012), thus contributing to their welfare.

As welfare is an individual experience (Dawkins, 2004; Fraser, 2008), interpretation of welfare of animals housed in groups should utilise individual based indicators in addition to or in place of group level measures (Siegford et al., 2016). The aim of this study was to assess if extent of range use by two different hybrids was associated with various measures of welfare including several clinical indicators, time budgets, fear response, and gastrointestinal characteristics at the level of

the individual over time.

2. Materials and methods

2.1. Animals and housing

One thousand two hundred non-beak-trimmed layers from two commercial hybrid lines, Dekalb White (n = 600) and Bovans Brown (n = 600), were housed at the poultry experimental facilities at Aarhus University, Foulum, from 3 May to 27 September 2018. Hens from both hybrids were acquired from the same rearing company (TopÆg A/S, Viborg, Denmark) and were reared under the same organic conditions which consisted of multi-tier housing, provision of roughage, and outdoor access when weather permitted. Hens were housed in single hybrid flocks across twelve pens (100 hens/pen) according to organic housing standards (Landbrugsstyrelsen, 2018). From each pen, ten hens (n = 120) were selected at random as focal hens. These individuals received a numbered leg band for identification. Hens were granted four days to habituate before testing began. Popholes were opened the morning after their arrival. Additional details regarding the indoor housing conditions, range area and management practices can be found in Wurtz et al. (2022).

2.2. Data collection

The experiment was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study.

2.2.1. Welfare assessment

Clinical welfare indicators (Table S1) were assessed for all focal hens when the hens were 18 weeks of age, and again at 23, 28, 33 and 38 weeks of age. A trained observer caught each focal hen and scored the plumage condition of the wings and tail using the scale developed by Bilčík and Keeling (1999). Presence of keel bone fractures and deviations were examined by palpation and scored separately for three parts of the keel bone (anterior, medial and caudal portion). Feet condition was scored for presence or absence of bumblefoot and hyperkeratosis, and for severity of lesions, if present. Finally, hens were weighed and fitted with a back mark with a letter code for easy identification during behavioural observations. The back mark was attached the day prior to each three-day observation period and removed the day after.

2.2.2. Time budgets

The behaviour of focal hens was observed in five three-day periods (at 18, 23, 28, 33 and 38 weeks of age). The observational period began the day after clinical welfare indicators were assessed. Direct observations were done by instantaneous sampling and took place from 8:30–15:30. Six scans of the 10 focal hens in each pen and corresponding range areas were performed. In each scan, the observer first scanned all 12 outdoor range areas (pens 1–12), followed by the indoor pens following the same order. This was repeated six times in total. In each round of scanning, the observer noted the focal hen's location and behaviour, as defined in the ethogram (Table S2). During scans the observer was placed in the outdoor range next to the one under observation (or outside the range in the case of the first range but observing from the same distance as the other ranges) or, if indoor, outside the pen.

2.2.3. Tonic immobility

A tonic immobility (TI) test was performed by a single observer on all focal hens at 18 weeks of age, immediately prior to the welfare assessment. After catching the hen, the observer carried the hen to a testing area within the room and placed it on its back in a V-shaped cradle with the head hanging freely. TI was induced by placing one hand on the chest and one hand on the head of the hen for 12 s, at which point the

observer slowly removed their hands. If TI was not induced on the first attempt, the observer tried a maximum of 3 times. If TI was not induced by then, the hen was returned to its pen. Once a state of TI was induced, the observer stood nearby within sight of the bird but without holding direct eye contact. The number of inductions, latency to first head movement after induction (s) and the duration of TI (from immobility to when the hen was in an upright position; s) were measured. When the hen returned to an upright position or after a maximum of 10 min, the hen was returned to its home pen.

2.2.4. Gastrointestinal variables

At the end of the experimental period (38 weeks of age), the hens were euthanised and the entire digestive system (from the oesophagus to cloaca) was dissected following methods described in Lorenz et al. (2013). The crop, proventriculus and gizzard were cut away and frozen at -20°C until further examination. The segments of the intestines were identified and the length (cm) of the duodenum (from gizzard junction to the bile duct junction), the ileum (from the Meckel's diverticulum to the caeca junction), the colon (till the end of cloaca) and both caecum (averaged for analysis) was measured.

Prior to examination, the crop, proventriculus and gizzard were thawed overnight in a refrigerator. Before weighing the intact crop, proventriculus and gizzard, any fat was removed and weighed separately. The three parts were then opened with a scalpel and turned inside out, removing the content, and were weighed again. The total content of the crop was flushed and passed through three sieves with decreasing pore sizes (2.0 mm, 0.5 mm, 0.063 mm), whereby three fractions of content were obtained and weighed individually. The first fraction (collected using 2.0 mm sieve) contained feathers, pasture, seeds, insects, stones, eggshells and barley/wheat, and these categories were visually separated and weighed (g). This procedure was repeated for the gizzard. The proventriculus was mainly empty, and therefore only the intact weight was obtained for this organ.

2.2.5. Range use

To record the duration of time focal hens spent inside the house and out on the range, the area around the popholes were video recorded during the same three-day periods in which the direct behavioural observations were performed. The camera was placed inside at approximately 40 cm above the pophole. The time each focal hen moved back and forth between the inside room and the range area was recorded. Based on these recordings, the frequency of passes and the durations spent indoors and on the range were calculated. These results over the three-day periods were averaged per individual for each observational week for the analyses (i.e., response variable = daily average per week).

2.2.6. Mortality

In cases where a dead hen was found during the daily inspections, the pen number and date were recorded, and a post-mortem examination was performed to determine the cause of death.

2.3. Statistical analyses

Generalised linear mixed effects models included week, hybrid and range use (either duration of time on range or number of exits) as fixed effects and either pen and hen (or only pen when the response variable was measured at a single time point, e.g., tonic immobility and gastrointestinal measures) as random effects. Backwards stepwise reduction was utilised to reduce the models, and interaction of fixed effects were retained in the final models when significant. Binary responses (e.g., plumage condition, keel bone damage, bumblefoot, and hyperkeratosis) were examined by binomial models with logit link (mixed effects logistic regression), ordinal variables (e.g., feet wounds and number of inductions to induce tonic immobility) by multinomial cumulative logit (proportional-odds) models, counts of behavioural observations by Poisson models with log link and log(N observations) as offset, tonic

immobility variables (latency to first head movement and duration of tonic immobility) by Cox regression models and body weight by a normal linear mixed effects model. In some instances, due to lack of prevalence at earlier weeks, only the final two weeks were included in the analysis (e.g., caudal keel bone fractures and deviations, footpad lesions and bumblefoot).

For analyses of clinical welfare indicators, hens were split into either "low" or "high" categories for time spent on the range and number of exits per day, for each week independently, using rank-frequency distribution following a method similar to that described in Marchewka et al. (2020). In summary, birds were sorted based on range use (e.g., listed lowest to highest) and then segmented into two groups with equal rank intervals (e.g., if values ranged from 0 to 100, the first group would contain individuals with values from 0 to 50, and the second with values from 51 to 100). The nature of this method means that group sizes were not equal (Table S3).

The coefficient of variation (sample standard deviation divided by sample mean) for daily time on range and number of exits was calculated for each individual hen to assess individual consistency in range utilisation over time. The higher the coefficient of variation, the more variable the individual hen was in her range use.

Repeated measures correlation was performed using the R function `rmcorr()` to examine the common within-individual association between the two measures of range access (duration of time on range and number of exits) used in this study (Bakdash and Marusich, 2017). A more thorough explanation of the statistical methods can be found in the Supplementary Material and Methods.

Results are presented as slope and standard error, log odds ratio with 95 % confidence interval and standard error, or as hazard ratio and 95 % confidence interval when linear models, binomial or ordinal models, or cox models were fit, respectively. As the emphasis of this study was on the associations between range use and welfare at an individual level, effects of hybrid and age are not discussed in detail. For detailed results regarding hybrid differences in range use, please refer to Wurtz et al. (2022).

3. Results

3.1. Individual range use

Descriptive stats regarding daily time (hr) spent on the range and number of exits, averaged across the three observation days per observation week, are presented in Table 1. All but one individual accessed the range at least once throughout the study period. Fig. 1 displays the histograms of average duration and number of daily exits of individuals across the study period. The correlation between logged measures of time on range and logged number of exits at an individual level was positive and significant ($\rho = 0.50$, $p < 0.001$, 95 % CI [0.43,0.57]; Fig. 2). Coefficients of variation (a measure of individual consistency) ranged from 0.08 to 1.76 (mean = 0.42, median = 0.38, Q1 – Q2 = 0.26 – 0.49) for individual hens' number of exits over time and from 0.12 to 2.15 (mean = 0.53, median = 0.46, Q1 - Q3 = 0.32 – 0.60) for duration of time individual hens spent on the range (Fig. 3).

3.2. Clinical welfare indicators

The clinical welfare parameters of tail or wing plumage, keel bone fractures or deviations in any location, bumblefoot or hyperkeratosis were not found to be associated with time spent on the range nor number of exits to the range (Table 2). Body weight was similarly not found to be associated with either range use variable (duration on range: slope = 9.80, SE = 9.20, $t = 1.07$, $P = 0.29$; number of exits: slope = -4.63 , SE = 11.12, $t = -0.42$, $P = 0.68$). There was a tendency for the odds of being in a worse footpad lesion category to be higher for hens in the low ranging group based upon number of exits (log odds ratio = -1.01 , $z = -1.89$, $P = 0.06$, SE = 0.53, 95 % CI [$-0.04, 2.06$]), however, no

Table 1

Minimum, first quartile, median, mean, third quartile and maximum values for average daily duration on range (hr) and number of exits per individual hen for each observational age and across the whole study period.

Age (week)	Duration on range (hr)						Number of exits					
	Min.	Q1	Median	Mean	Q3	Max.	Min.	Q1	Median	Mean	Q3	Max.
18	0.00	1.09	2.14	2.64	3.64	9.18	0.0	9.7	14.2	15.6	21.0	46.7
23	0.00	2.55	5.24	4.96	7.12	11.77	0.0	17.3	25.0	25.7	34.1	60.7
28	0.00	2.13	4.58	4.80	7.24	11.32	0.0	13.2	19.0	19.7	26.0	53.0
33	0.00	3.16	7.18	6.32	8.98	11.84	1.0	12.6	18.0	19.5	22.7	106.3
38	0.00	2.18	4.51	4.27	6.25	9.80	2.3	10.0	13.3	14.7	18.0	53.7
All	0.00	1.94	4.40	4.59	6.99	11.84	0.0	11.7	17.0	19.0	24.7	106.3

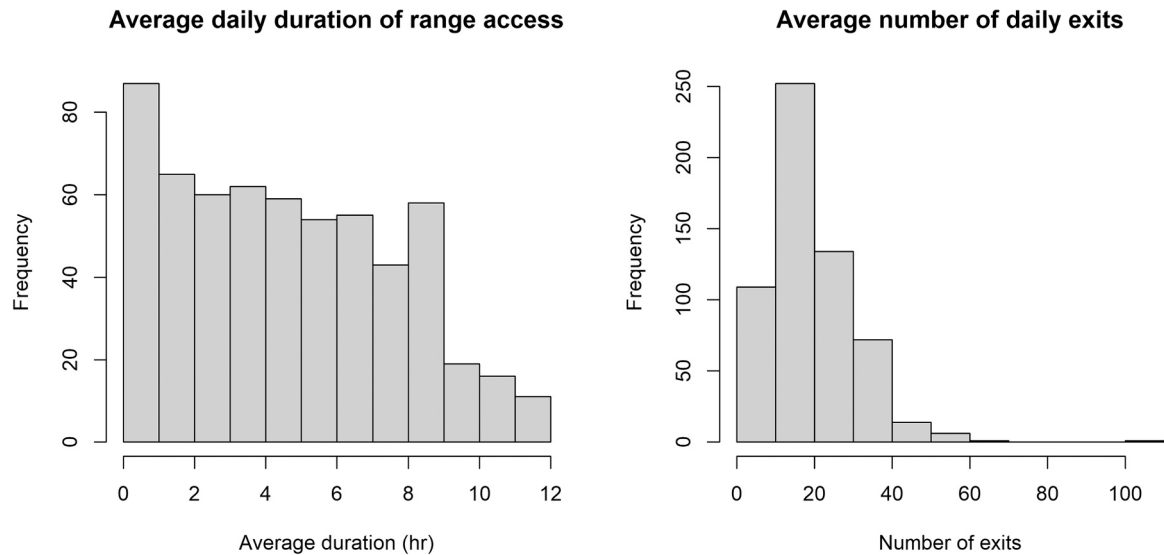


Fig. 1. Histogram of daily durations and number of exits per individual averaged across the 3 days of observation for each observational week.

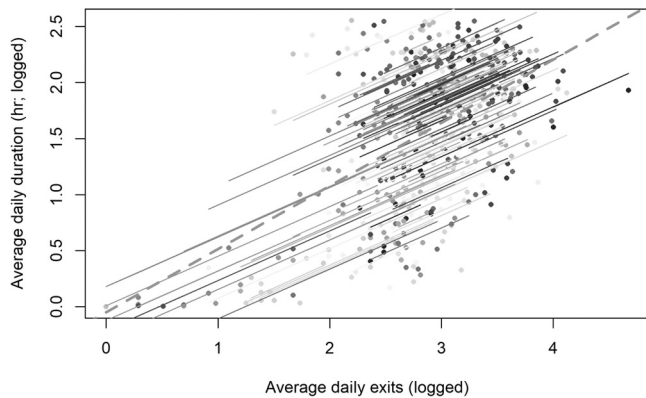


Fig. 2. Linear association between logged average time (min) on range and logged number of exits per day per individual (thin, solid lines) versus the linear global relationship (thick, grey dashed line).

significant relationship was found between footpad lesions and duration of time spent on the range. Prevalence of each clinical welfare indicator score for each range use category by week are presented in Fig. 4.

3.3. Time budgets

Time on the range was negatively associated with drinking (slope = -0.095 , SE = 0.024 , $z = -3.94$, $P < 0.001$), eating (slope = -0.036 , SE = 0.016 , $z = -2.241$, $P = 0.025$), nesting (slope = -0.063 , SE = 0.026 , $z = -2.385$, $P = 0.017$) and perching (slope = -0.165 , SE = 0.023 , $z = -7.249$, $P < 0.001$) and positively associated with foraging (slope =

0.127 , SE = 0.010 , $z = 12.877$, $P < 0.001$), dust bathing (slope = 0.050 , SE = 0.019 , $z = 2.667$, $P = 0.008$) and standing behaviour (slope = 0.037 , SE = 0.013 , $z = 2.794$, $P = 0.005$). Number of pophole exits was negatively associated with drinking (slope = -0.013 , SE = 0.007 , $z = -1.975$, $P = 0.048$), perching (slope = -0.016 , SE = 0.006 , $z = -2.651$, $P = 0.008$) and resting behaviour (slope = -0.009 , SE = 0.004 , $z = -2.321$, $P = 0.02$), and positively associated with eating (slope = 0.026 , SE = 0.011 , $z = 2.336$, $P = 0.019$) and locomotion (slope = 0.0199 , SE = 0.003 , $z = 6.132$, $P < 0.001$).

3.4. Tonic immobility

Tonic immobility was able to be induced in all focal birds, with 102 individuals requiring a single attempt, 17 birds requiring two attempts, and one individual requiring three attempts. The latency to first head movement ranged from 5 to 600 s (Q1 = 45.8, median = 83.5, mean = 126.0, Q3 = 151.0), with four individuals reaching the maximum duration of 600 s. Duration of tonic immobility ranged from 15 to 600 s (Q1 = 89.5, median = 178.5, mean = 253.8, Q3 = 374.0), with 17 individuals reaching the maximum duration of 600 s. Neither number of inductions (time on range: log odds ratio = -0.045 , SE = 0.047 , $z = -0.944$, $P = 0.345$; number of exits: log odds ratio = 0.006 , SE = 0.014 , $z = -0.468$, $P = 0.64$), latency to first head movements (time on range: hazard ratio = 1.03, 95 % CI[1.00,1.06], $P = 0.08$; number of exits: hazard ratio = 1.00, 95 % CI[0.99,1.01], $P = 0.50$) nor duration of TI (time on range: hazard ratio = 0.99, 95 % CI[0.96,1.02], $P = 0.50$; number of exits: hazard ratio = 1.00, 95 % CI[0.99,1.01], $P = 0.78$) were associated with duration of time spent on the range or number of exits.

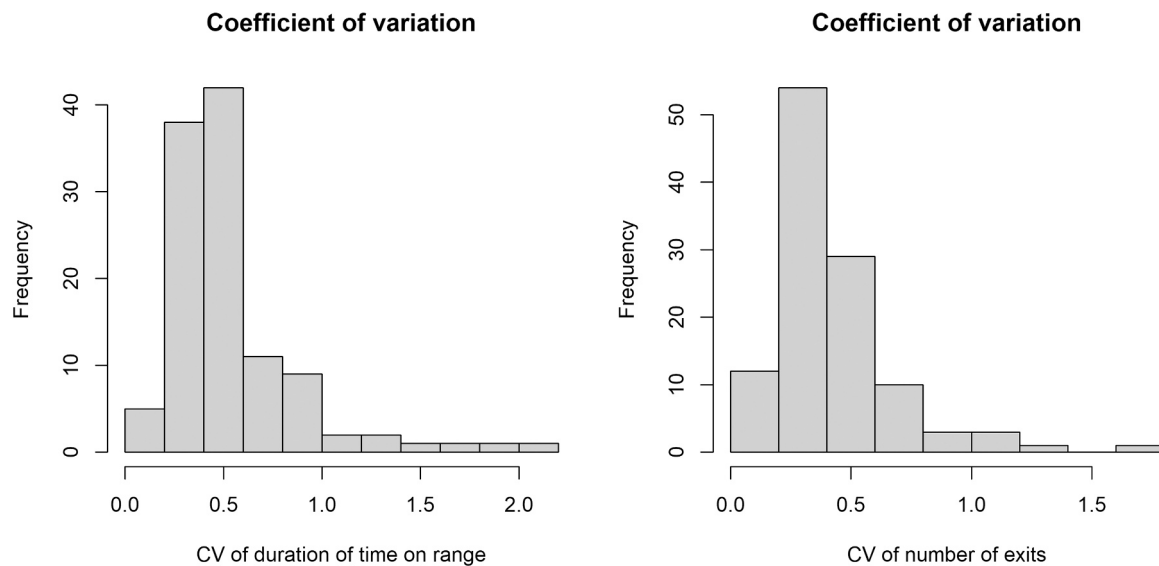


Fig. 3. Coefficient of variation for individual range access and time on range per day.

Table 2

Binomial logistic regression (plumage, keel damage, bumblefoot and hyperkeratosis) and proportional-odds logistic regression (feet wounds) model estimates for clinical welfare indicators for duration spent on range and number of exits. Estimates are reported as log odds ratios for high range users compared to low range users.

	Duration on range (hr)				Number of exits			
	Log odds ratio	Std Error	Z-value	P value	Log odds ratio	Std Error	Z-value	P value
Tail plumage	0.093	0.297	0.312	0.755	0.299	0.416	0.719	0.472
Wing plumage	-0.071	1.281	-0.056	0.956	2.734	3.020	0.905	0.365
Anterior keel fractures	-0.155	1.519	-0.102	0.919	1.230	1.985	0.620	0.535
Anterior keel deviations	-0.438	2.420	-0.181	0.856	0.130	3.974	0.033	0.974
Medial keel fractures	-0.222	1.778	-0.125	0.901	-1.426	-1.426	-0.336	0.737
Medial keel deviations	-0.896	2.629	-0.341	0.733	-0.796	4.643	-0.171	0.864
Caudal keel fractures	0.300	0.619	0.485	0.628	*	*	*	*
Caudal keel deviations	-0.620	0.932	-0.665	0.506	*	*	*	*
Feet wounds	0.323	0.317	1.019	0.308	<i>-1.010^a</i>	0.534	-1.891	0.059
Bumblefoot	0.140	2.884	0.049	0.961	*	*	*	*
Hyperkeratosis	0.275	2.190	0.126	0.900	-0.849	2.917	-0.291	0.771

*Model failed to converge for caudal keel bone fractures and deviations and bumblefoot when number of exits were the response variable.

^a Italics indicates a tendency ($P < 0.1$).

3.5. Gastrointestinal variables

The duration of time spent on the range was negatively associated with the length of the ileum (slope = -0.346 , SE = 0.138 , $t = -2.500$, $P = 0.013$) and positively associated with the length of the colon (slope = 0.082 , SE = 0.029 , $t = 2.826$, $P = 0.005$). Number of exits were positively associated with caeca length (slope = 0.013 , SE = 0.007 , $t = 2.00$, $P = 0.046$). There were no associations observed between the length of the duodenum with duration of time on the range or number of exits.

Time spent outside on the range was positively associated with crop empty weight ($P < 0.004$), crop fraction 1 weight ($P < 0.001$), pasture, insect and barley/wheat content in the crop ($P < 0.001$), gizzard (both full and empty) weight ($P < 0.001$), gizzard content weight (fraction 1: $P < 0.001$; fraction 3: $P = 0.011$), pasture and barley/wheat content in the gizzard ($P < 0.001$) and proventriculus empty weight ($P = 0.014$). Negative associations were found between time spent outside and seed content in the crop and gizzard ($P < 0.001$), proventriculus (full) weight and fat content of the gizzard and proventriculus ($P = 0.007$ and $P < 0.001$, respectively). Number of exits were positively associated with crop content (fractions 1 and 3) weight ($P < 0.001$), pasture and insect content of the crop ($P < 0.001$ and $P = 0.008$, respectively), gizzard (full and empty) weight ($P < 0.001$), pasture, stone and barley/wheat content in the gizzard ($P = 0.011$, $P = 0.022$ and $P = 0.018$) and

gizzard (fractions 1 and 3) weight ($P < 0.001$). A negative association was found between number of exits and feather content in the crop ($P = 0.045$), fat content of the gizzard ($P < 0.001$) and seed content in the gizzard ($P = 0.002$). See Table 3 for model estimates for each variable.

3.6. Mortality

One Dekalb White focal hen was found dead at week 32 of age and cause of death was deemed to be egg yolk peritonitis.

4. Discussion

The present study showed that the extent of range use was associated with behaviour and different gastrointestinal variables, but not with fearfulness as assessed by a TI test or a number of clinical welfare indicators. Our observed positive association between duration of time spent on the range and number of exits were in line with previous studies (Gebhardt-Henrich et al., 2014; Hartcher et al., 2016; Larsen et al., 2017). Upon visual inspection, our reported coefficient of variations appeared similar to those obtained in Larsen et al. (2017), with the bulk of individuals being fairly consistent (i.e., low coefficient of variation values) in their use of the range over time, and a small subset of the population appearing to vary much more in how they utilised the range

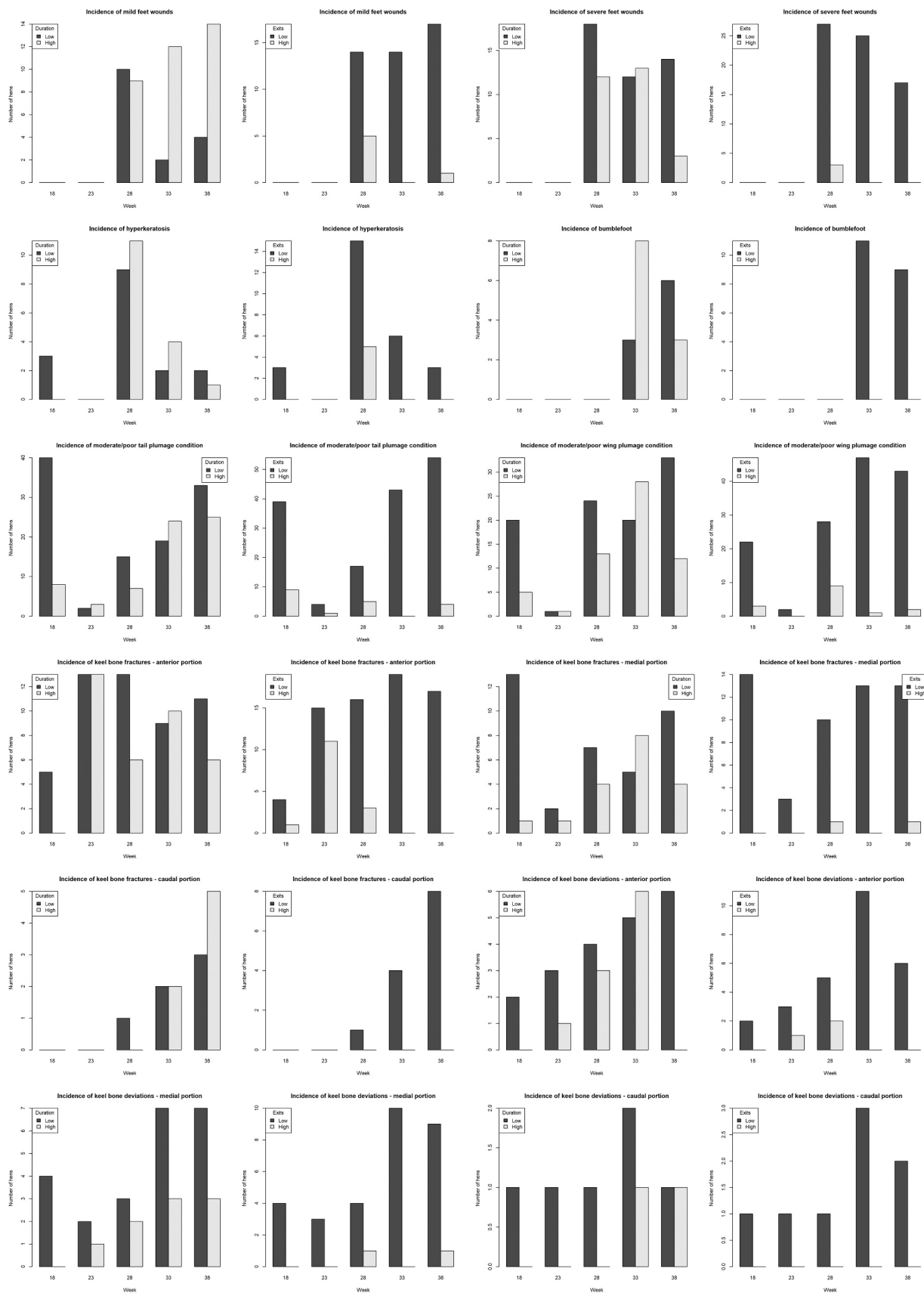


Fig. 4. Number of hens with mild and severe feet wounds, hyperkeratosis, bumblefoot, moderate/poor tail and wing plumage condition and fractures and deviations to the anterior, medial and caudal portions of the keel bone for low and high range use determined by duration of time spent on the range and number of exits to the range at 18, 23, 28, 33 and 38 weeks of age.

Table 3

Linear mixed effects model estimates for weight of crop, gizzard and proventriculus organs and crop and gizzard content for duration spent on range and number of exits.

	Duration on range (hr)				Number of exits			
	Slope	Std Error	Z/t value	P value	Slope	Std Error	Z/t value	P value
Crop (full)	-0.655	0.403	-1.626	0.105	0.026	0.107	0.240	0.810
Crop (empty)	0.068^a	0.023	2.922	0.004	<i>0.011</i>	0.006	1.797	0.073
Crop fat	-0.012	0.008	-1.515	0.130	-0.001	0.002	-0.261	0.795
Crop (fraction 1)	0.013	0.003	4.249	<0.001	0.003	0.001	3.544	<0.001
Crop (fraction 2)	0.002	0.002	0.739	0.460	0.001	0.001	1.341	0.181
Crop (fraction 3)	<i>0.004^b</i>	0.002	1.832	0.068	0.002	0.001	3.587	<0.001
Crop feather content	<i>0.066</i>	0.039	1.689	0.091	-0.017	0.009	-2.006	0.045
Crop pasture content	0.029	0.005	5.810	<0.001	0.007	0.001	5.543	<0.001
Crop seed content	-0.054	0.014	-3.788	<0.001	-0.006	0.004	-1.311	0.190
Crop insect content	0.188	0.025	7.566	<0.001	0.023	0.009	2.659	0.008
Crop stone content	<i>-0.014</i>	0.007	-1.831	0.068	0.001	0.002	0.716	0.474
Crop eggshell content	-0.079	0.049	-1.609	0.108	0.016	0.012	1.300	0.194
Crop barley/wheat content	0.012	0.003	3.606	<0.001	0.001	0.001	0.892	0.373
Gizzard (full)	0.949	0.137	6.937	<0.001	0.201	0.037	5.461	<0.001
Gizzard (empty)	0.631	0.070	9.005	<0.001	0.097	0.020	4.960	<0.001
Gizzard fat	-0.009	0.003	-2.730	0.007	-0.003	0.001	-3.493	0.001
Gizzard (fraction 1)	0.010	0.002	5.141	<0.001	0.002	0.001	3.342	0.001
Gizzard (fraction 2)	-0.036	0.035	-1.016	0.310	0.002	0.009	0.249	0.804
Gizzard (fraction 3)	0.004	0.001	2.569	0.011	0.002	0.000	4.458	<0.001
Gizzard pasture content	0.025	0.004	6.974	<0.001	0.003	0.001	2.535	0.012
Gizzard seed content	-0.081	0.017	-4.847	<0.001	-0.016	0.005	-3.135	0.002
Gizzard stone content	0.103	0.085	1.216	0.225	0.052	0.023	2.291	0.022
Gizzard barley/wheat content	0.016	0.004	4.405	<0.001	0.002	0.001	2.366	0.018
Proventriculus (full)	-0.027	0.013	-2.177	0.030	-0.001	0.003	-0.248	0.805
Proventriculus (empty)	0.022	0.009	2.472	0.014	0.003	0.002	1.224	0.221
Proventriculus fat	-0.053	0.012	-4.362	<0.001	-0.003	0.003	-1.057	0.291

^a Bold indicates significance ($P < 0.05$).

^b Italics indicates a tendency ($P < 0.1$).

(i.e., high coefficient of variation values). It is also important to note that the flock sizes in our experiment were significantly smaller than those typical of commercial conditions. There is literature available suggesting that hens kept in smaller flocks spend more time on the range and visit the range more frequently than those housed in larger flocks (Hegelund et al., 2005; Gebhardt-Henrich et al., 2014). This could have potentially reduced the amount of observed individual variation in range use if our conditions lead to increased range use by all individuals.

Our study found no associations between time spent on the range or number of exits with plumage condition, keel bone fractures or deviations, bumblefoot, hyperkeratosis or body weight, however, it is important to consider that the study ended at 38 weeks of age when the hens were relatively young and in overall good condition. Previous studies examining plumage condition and individual use of the range suggest that those that access the range more often and for greater durations of time had reduced feather damage (Mahboub et al., 2004; Chielo et al., 2016; Rodriguez-Aurrekoetxea and Estevez, 2016; Bari et al., 2020), though other studies did not observe this same association (Hartcher et al., 2016; Larsen et al., 2018). The lack of association found between plumage condition and range use in our study could be attributed to low levels of feather pecking and therefore limited plumage damage, or possibly due to the relatively young age that hens were when the study concluded, leaving limited time for accumulation of plumage damage. In an on-farm study examining the occurrence of a range of welfare indicators in different production systems, indoor housed hens were found to be more likely to have footpad lesions at 32 weeks than hens with access to an outdoor range (Riber and Hinrichsen, 2016). At the individual level, Rodriguez-Aurrekoetxea and Estevez (2016) found a similar pattern, with more frequent range use being associated with lower incidences of footpad dermatitis. However, no relationship between footpad lesions and time spent outdoors were found in our study, which was similar to findings by Larsen et al. (2018). Footpad lesions are believed to be painful, as demonstrated in studies on turkeys (Sinclair et al., 2015; Weber Wyneken et al., 2015) which could explain why number of exits to the outdoor range was found to be negatively

associated with footpad lesions and locomotion in our study. Occurrences of hyperkeratosis and bumblefoot were very low in our population, which could account for the failure to detect any associations with range use variables. No associations between duration of range use or number of exits were found with prevalence of keel bone fractures or deviations, though occurrences were relatively low in our population. Past studies have suggested that keel bone damage may impact hens' mobility (Nasr et al., 2012) or reduce pop-hole usage (Richards et al., 2012), though more recent research has found no such associations between high and low range use and keel bone damage (Larsen et al., 2018; Kolakshyapati et al., 2019; Bari et al., 2020). Further, body weight was not associated with range use suggesting that energy consumption was not impacted, indicating that those hens which utilised the range to a greater extent were able to compensate for energy lost due to increased locomotion and thermoregulation.

Hens that spent more time on the range were observed eating and drinking less, though this could in part be attributed to hens altering their feeding and drinking patterns (e.g., eating and drinking more before the popholes opened or after they closed) and thus not occurring during the observational period. In previous studies, broilers that had to travel longer distances between feed and water lines, or that had barriers placed in the environment, adapted to the inconvenience by increasing the duration of their feeding and drinking bouts (Bizeray et al., 2002; Ruiz-Feria et al., 2014). In our study, hens with increased range use were also observed utilising the nest boxes and perches less. This is logical as the feed pans, water lines, nest boxes and perches were located inside the house, and thus were only accessible to the hens when they were not on the range. We found that hens that ranged more performed more foraging and dust bathing behaviour. Campbell et al. (2017a) and Thuy Diep et al. (2018) similarly found that hens performed more foraging and dustbathing on the range and more resting behaviour indoors. The greater diversity of foraging materials outdoors likely encouraged more foraging behaviour than the litter indoors. Number of exits from the house was negatively associated with perching and resting, and positively associated with locomotion, thus suggesting that hens that moved

more between the house and the range were generally more active individuals.

No associations between number of inductions, latency to first head movement or duration of tonic immobility with range use variables were found in our study. The tonic immobility assessment was performed once at 18 weeks of age. Coping styles are thought to be invariable across time and context, though experience can shape future behaviour (Zidar et al., 2017). It is possible that hens that were initially fearful at the start of the study were able to habituate over time to the range area, though measures of fear were not associated with range use at any of time points assessed in our study. The provision of shelters in our experiment may also have provided enough security to encourage more fearful hens to exit the house. Links between tonic immobility test results and range use are inconsistent in the literature. For instance, Hartcher et al. (2016) found longer TI durations with low ranging hens while Campbell et al. (2016) found no differences between range use and TI durations. Larsen et al. (2018) found conflicting results depending on the flock assessed.

Contrary to our observed differences in ileum, colon and caeca lengths between hens with varying range use, a previous study did not find significant differences in intestinal lengths between high and low range users (Marchewka et al., 2021). Our results pertaining to digestive organ weights and content were in line with previous studies showing more developed (i.e., heavier) crop, gizzard and proventriculus in hens that spent more time on the range and that exited the house more often (Singh et al., 2016; Bari et al., 2020; Marchewka et al., 2021). This is likely a result of high ranging hens consuming more pasture and stone material, which was verified in our study with time spent outdoors being positively associated with amount of pasture and insects in the crop and more pasture and stones in the gizzard.

5. Conclusions

Individual range use varied with hens differing in time spent on range, number of exits and consistency in these measures over time. Extent of range use was not associated with clinical welfare indicators (except for a tendency for footpad lesions), nor fear levels as assessed by a tonic immobility test. Hens' time budgets differed depending on where they spent most of their time. Hens that utilised the range to a greater extent likely consumed more forage, contributing to the greater development of their gastrointestinal organs. The lack of observed associations between clinical welfare indicators and range use may be in part due to the relatively young age that hens were at the end of the study. The provision of environmental options may help to improve welfare at an individual level as opposed to providing environments that cater to the "average hen".

CRedit authorship contribution statement

Kaitlin E. Wurtz: Methodology, Formal analysis, Writing – original draft, Visualization. **Fernanda M. Tahamtani:** Conceptualization, Investigation, Writing – review & editing. **Leslie Foldager:** Statistical consulting, Writing – review & editing. **Karen Thodberg:** Data curation, Writing – review & editing. **Anja B. Riber:** Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was part of the project "FreeBirds: Optimising the use of

the free-range as the key to improve organic chicken production" under Coordination of European Transnational Research in Organic Food and Farming System Cofund (CORE Organic Cofund) by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727495. Funding for the Danish part was granted by Innovation Fund Denmark, grant number 7109-00005B. We would like to thank Samira Faryadi for collecting the data on content of the crop, proventriculus and gizzard.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105978.

References

- Bakdash, J.Z., Marusch, L.R., 2017. Repeated measures correlation. *Front. Psychol.* 8, 456.
- Bari, M.S., Laurenson, Y.C., Cohen-Barnhouse, A.M., Walkden-Brown, S.W., Campbell, D.L., 2020. Effects of outdoor ranging on external and internal health parameters for hens from different rearing enrichments. *PeerJ* 8, e8720.
- Bestman, M., Wagenaar, J.-P., 2003. Farm factors associated with feather pecking in organic laying hens. *Livest. Prod. Sci.* 80, 133–140.
- Bilčík, B., Keeling, L.J., 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *Br. Poult. Sci.* 40, 444–451. <https://doi.org/10.1080/00071669987188>.
- Bizeray, D., Estevez, I., Leterrier, C., Faure, J., 2002. Effects of increasing environmental complexity on the physical activity of broiler chickens. *Appl. Anim. Behav. Sci.* 79, 27–41.
- Campbell, D., Hinch, G., Downing, J.A., Lee, C., 2017a. Outdoor stocking density in free-range laying hens: effects on behaviour and welfare. *Animal* 11, 1036–1045.
- Campbell, D., Hinch, G., Dyall, T., Warin, L., Little, B., Lee, C., 2017b. Outdoor stocking density in free-range laying hens: radio-frequency identification of impacts on range use. *Animal* 11, 121–130.
- Campbell, D.L., Hinch, G.N., Downing, J.A., Lee, C., 2016. Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens. *Appl. Anim. Behav. Sci.* 185, 73–77.
- Campbell, D.L., Dickson, E.J., Lee, C., 2019. Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. *PeerJ* 7, e8122.
- Campbell, D.L., Dyall, T.R., Downing, J.A., Cohen-Barnhouse, A.M., Lee, C., 2020. Rearing enrichments affected ranging behavior in free-range laying hens. *Front. Vet. Sci.* 4, 46.
- Chielo, L.I., Pike, T., Cooper, J., 2016. Ranging behaviour of commercial free-range laying hens. *Animals* 6, 28.
- Daigle, C., Siegford, J., 2014. Welfare Quality® parameters do not always reflect hen behaviour across the lay cycle in non-cage laying hens. *Anim. Welf.* 23, 423–434.
- Dawkins, M.S., 2004. Using behaviour to assess animal welfare. *Anim. Welf.* 13, 3–7.
- Fraser, D., 2008. Understanding animal welfare. *Acta Vet. Scand.* 50, 1–7.
- Fureix, C., Meagher, R.K., 2015. What can inactivity (in its various forms) reveal about affective states in non-human animals? A review. *Appl. Anim. Behav. Sci.* 171, 8–24.
- Gebhardt-Henrich, S.G., Toscano, M.J., Fröhlich, E.K., 2014. Use of outdoor ranges by laying hens in different sized flocks. *Appl. Anim. Behav. Sci.* 155, 74–81.
- Grigor, P., Hughes, B., Appleby, M., 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., Hickey, K., Hemsworth, P., Cronin, G., Wilkinson, S., 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.
- Hegelund, L., Sørensen, J., Kjaer, J., Kristensen, I., 2005. Use of the range area in organic egg production systems: effect of climatic factors, flock size, age and artificial cover. *Br. Poult. Sci.* 46, 1–8.
- Hernandez, C.E., C. Lee, D. Ferguson, T. Dyall, S. Belson, J. Lea, and G. Hinch. 2014. Personality traits of high, low and non users of a free range area in laying hens. Proc. Moving on: Proceedings of the 48th Congress of the International Society for Applied Ethology (ISAE 2014).
- Iqbal, Z., Roberts, J., Perez-Maldonado, R., Goodarzi Boroojeni, F., Swick, R.A., Ruhnke, I., 2018. Pasture, multi-enzymes, benzoic acid and essential oils positively influence performance, intestinal organ weight and egg quality in free-range laying hens. *Br. Poult. Sci.* 59, 180–189.
- Kolakshyapati, M., Flavel, R., Sibanda, T., Schneider, D., Welch, M., Ruhnke, I., 2019. Various bone parameters are positively correlated with hen body weight while range access has no beneficial effect on tibia health of free-range layers. *Poult. Sci.* 98, 6241–6250.
- Kolakshyapati, M., Wu, S.-B., Sibanda, T.Z., Ramirez-Cuevas, S., Ruhnke, I., 2020. Body weight and range usage affect net energy utilisation in commercial free-range laying hens when evaluated in net energy chambers. *Anim. Nutr.* 6, 192–197.
- Landbrugsstyrelsen. 2018. Vejledning om økologisk jordbrugsproduktion [In Danish: Guidelines for organic farming]. L. Miljø- og Fødevarerministeriet ed. Landbrugsstyrelsen, (https://lbst.dk/fileadmin/user_upload/NaturErhverv/Filer/

- Indsatsomraader/Oekologi/Jordbrugsbedrifter/Vejledning_til_oekologisk_jordbrug_sproduktion/Oekologivejledning_marts_2018.pdf).
- Larsen, H., Cronin, G.M., Gebhardt-Henrich, S.G., Smith, C.L., Hemsworth, P.H., Rault, J.-L., 2017. Individual ranging behaviour patterns in commercial free-range layers as observed through RFID tracking. *Animals* 7, 21.
- Larsen, H., Hemsworth, P., Cronin, G., Gebhardt-Henrich, S., Smith, C., Rault, J.-L., 2018. Relationship between welfare and individual ranging behaviour in commercial free-range laying hens. *Animal* 12, 2356–2364.
- Lay Jr, D., Fulton, R., Hester, P., Karcher, D., Kjaer, J., Mench, J.A., Mullens, B., Newberry, R.C., Nicol, C.J., O'Sullivan, N.P., 2011. Hen welfare in different housing systems. *Poult. Sci.* 90, 278–294.
- Lorenz, C., Kany, T., Grashorn, M., 2013. Method to estimate feed intake from pasture in broilers and laying hens. *Arch. für Geflügelkd.* 77, 160–165.
- Mahboub, 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. *Br. Poult. Sci.* 45, 738–744.
- Marchewka, J., Sztandarski, P., Zdanowska-Sąsiadek, Ż., Damaziak, K., Wojciechowski, F., Riber, A.B., Gunnarsson, S., 2020. Associations between welfare and ranging profile in free-range commercial and heritage meat-purpose chickens (*Gallus gallus domesticus*). *Poult. Sci.* 99, 4141–4152.
- Marchewka, J., Sztandarski, P., Zdanowska-Sąsiadek, Ż., Adamek-Urbańska, D., Damaziak, K., Wojciechowski, F., Riber, A.B., Gunnarsson, S., 2021. Gastrointestinal tract morphometrics and content of commercial and indigenous chicken breeds with differing ranging profiles. *Animals* 11, 1881.
- Mateos, G., Jiménez-Moreno, E., Serrano, M., Lázaro, R., 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *J. Appl. Poult. Res.* 21, 156–174.
- Mendl, M., Burman, O.H., Paul, E.S., 2010. An integrative and functional framework for the study of animal emotion and mood. *Proc. R. Soc. B: Biol. Sci.* 277, 2895–2904.
- Nasr, M., Murrell, J., Wilkins, L., Nicol, C., 2012. The effect of keel fractures on egg-production parameters, mobility and behaviour in individual laying hens. *Anim. Welf. - UFAW J.* 21, 127.
- Nicol, C., Pötzsch, C., Lewis, K., Green, L., 2003. Matched concurrent case-control study of risk factors for feather pecking in hens on free-range commercial farms in the UK. *Br. Poult. Sci.* 44, 515–523.
- Pettersson, I., Freire, R., Nicol, C.J., 2016. Factors affecting ranging behaviour in commercial free-range hens. *Worlds Poult. Sci. J.* 72, 137–150.
- Riber, A., Hinrichsen, L., 2016. Feather eating and its associations with plumage damage and feathers on the floor in commercial farms of laying hens. *animal* 10, 1218–1224.
- Richards, G., Wilkins, L., Knowles, T., Booth, F., Toscano, M., Nicol, C., Brown, S., 2011. Continuous monitoring of pop hole usage by commercially housed free-range hens throughout the production cycle. *Vet. Rec.* 169, 338–338.
- Richards, G., Wilkins, L., Knowles, T., Booth, F., Toscano, M., Nicol, C., Brown, S., 2012. Pop hole use by hens with different keel fracture status monitored throughout the laying period. *Vet. Rec.* 170, 494–494.
- 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.* 95, 2503–2513.
- Rufener, C., Abreu, Y., Asher, L., Berezowski, J.A., Sousa, F.M., Stratmann, A., Toscano, M.J., 2019. Keel bone fractures are associated with individual mobility of laying hens in an aviary system. *Appl. Anim. Behav. Sci.* 217, 48–56.
- Ruiz-Feria, C., Arroyo-Villegas, J., Pro-Martinez, A., Bautista-Ortega, J., Cortes-Cuevas, A., Narciso-Gaytan, C., Hernandez-Cazares, A., Gallegos-Sanchez, J., 2014. Effects of distance and barriers between resources on bone and tendon strength and productive performance of broiler chickens. *Poult. Sci.* 93, 1608–1617.
- Schreiter, R., Damme, K., von Borell, E., Vogt, I., Klunker, M., Freick, M., 2019. Effects of litter and additional enrichment elements on the occurrence of feather pecking in pullets and laying hens—a focused review. *Vet. Med. Sci.* 5, 500–507.
- Sibanda, T.Z., O'Shea, C.J., de Souza Vilela, J., Kolakshyapati, M., Welch, M., Schneider, D., Courtice, J., Ruhnke, I., 2020. Managing free-range laying hens—part B: Early range users have more pathology findings at the end of lay but have a significantly higher chance of survival—An indicative study. *Animals* 10, 1911.
- Siegford, J.M., Berezowski, J., Biswas, S.K., Daigle, C.L., Gebhardt-Henrich, S.G., Hernandez, C.E., Thurner, S., Toscano, M.J., 2016. Assessing activity and location of individual laying hens in large groups using modern technology. *Animals* 6, 10.
- Sinclair, A., Weber Wyneken, C., Veldkamp, T., Vinco, L., Hocking, P., 2015. Behavioural assessment of pain in commercial turkeys (*Meleagris gallopavo*) with foot pad dermatitis. *Br. Poult. Sci.* 56, 511–521.
- Singh, M., A. Cowieson, C.E. Hernandez, G. Hinch, and C. Lee. 2016. Wanderers versus stay at home: who has the better guts? *Proc. 27th annual Australian poultry science symposium.*
- Thuy Diep, A., Larsen, H., Rault, J.L., 2018. Behavioural repertoire of free-range laying hens indoors and outdoors, and in relation to distance from the shed. *Aust. Vet. J.* 96, 127–131.
- Wang, G., Ekstrand, C., Svedberg, J., 1998. Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens. *Br. Poult. Sci.* 39, 191–197.
- Weber Wyneken, C., Sinclair, A., Veldkamp, T., Vinco, L., Hocking, P., 2015. Footpad dermatitis and pain assessment in turkey poults using analgesia and objective gait analysis. *Br. Poult. Sci.* 56, 522–530.
- Wurtz, K., Thodberg, K., Berenjian, A., Foldager, L., Tahamtani, F., Riber, A., 2022. Commercial layer hybrids kept under organic conditions: a comparison of range use, welfare, and egg production in two layer strains. *Poult. Sci.* 101, 102005.
- Yegani, M., Korver, D., 2008. Factors affecting intestinal health in poultry. *Poult. Sci.* 87, 2052–2063.
- Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O., Løvlie, H., 2017. A comparison of animal personality and coping styles in the red junglefowl. *Anim. Behav.* 130, 209–220.