Effects of outdoor ranging on external and internal health parameters for hens from different rearing enrichments

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Abstract

42   In Australia, free-range layer pullets are typically reared indoors, but adult layers go outdoors,
43   this mismatch might reduce adaptation in laying environments. Enrichments during rearing may
44   optimise pullet development and subsequent welfare as adult free-range hens. In the outdoor
45   environment, hens may have greater opportunities for exercise and natural behaviours which
46   might contribute to improved health and welfare. But the outdoor environment may also result in
47   potential exposure to parasites and pathogens. Individual variation in range use may thus dictate
48   individual health and welfare. This study was conducted to evaluate whether adult hens varied in
49   their external and internal health due to rearing enrichments and following variation in range use.
50   A total of 1386 Hy-Line Brown® chicks were reared indoors across 16 weeks with 3 enrichment
51   treatments including a control group with standard housing conditions, a novelty group providing
52   novel objects that changed weekly, and a structural group with custom-designed structures to
53   increase spatial navigation and perching. At 16 weeks of age the pullets were moved to a free-
54   range system and housed in 9 identical pens within their rearing treatments. All hens were leg-
55   banded with microchips and daily ranging was assessed from 25 to 64 weeks via radio-frequency
56   identification technology. At 64-65 weeks of age, 307 hens were selected based on their range
57   use patterns across 54 days up to 64 weeks: indoor (no ranging), low outdoor (1.4 h or less
58   daily), and high outdoor (5.2-9 h daily). The external and internal health and welfare parameters
59   were evaluated via external assessment of body weight, plumage, toenails, pecking wounds,
60   illness, and post-mortem assessment of internal organs and keel bones including whole-body CT
61   scanning for body composition. The control hens had the lowest feather coverage ($P < 0.0001$)
62   and a higher number of comb wounds ($P = 0.03$) than the novelty hens. The high outdoor rangers
63   had fewer comb wounds than the indoor hens ($P = 0.04$), the shortest toenails ($P < 0.0001$) and
64   the most feather coverage ($P < 0.0001$), but lower body weight ($P < 0.0001$) than the indoor
65   hens. High outdoor ranging decreased both body fat and muscle (both $P < 0.0001$). The novelty
66   group had lower spleen weights than the control hens ($P = 0.01$) but neither group differed from
67   the structural hens. The high outdoor hens showed the highest spleen ($P = 0.01$) and empty
68   gizzard weights ($P = 0.04$). Both the rearing enrichments and ranging had no effect on keel bone
69   damage (all $P \geq 0.19$). There were no significant interactions between rearing treatments and
70   ranging patterns for any of the health and welfare parameters measured in this study ($P \geq 0.07$).
71   Overall, rearing enrichments had some effects on hen health and welfare at the later stages of the
72   production cycle but subsequent range use patterns had the greatest impact.

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Introduction

76   Free-range egg production is prevalent within Australia as consumers perceive free-range
77   eggs to be healthier and tastier than caged eggs (Bray & Ankeny, 2017). The free-range system is
78   also perceived to improve hen welfare (Pettersson et al., 2016a) as hens have the choice to move

79   freely outdoors, are exposed to daylight, and have greater opportunities for exercise and natural
80   behaviors which might contribute to improved health and welfare. However, free-range systems
81   can comparatively also bring increased risk of disease (Fossum et al., 2009), heat stress (Singh et
82   al., 2017), predation (Bestman & Wagenaar, 2014), parasites (Permin et al., 1999), vent-pecking
83   (Bestman & Wagenaar, 2014), and mortality (Bestman & Wagenaar, 2014; Singh et al., 2017;
84   Richards et al. 2012). Furthermore, it is well documented both within Australia and
85   internationally that range use varies by individual choice with some hens ranging daily while
86   others do not range at all (Larsen et al., 2017; Pettersson et al., 2016b). This variation could
87   result in large differences in activity and diet between hens which may impact health and welfare
88   such as influencing body composition by lowering body fat accumulation (Crespo & Esteve-
89   Garcia, 2001; Renema et al., 1999; Sun et al., 2006) and strengthening the bones (Regmi et al.,
90   2016) and muscles (Casey-Trott et al., 2017a). Although recent work with commercial free-
91   range layers showed no relationship between range use and tibial bone strength (Kolakshyapati
92   et al., 2019).
93   Range use has been shown to impact external welfare parameters of hens. Individually
94   tracked free-range hens that use the outdoor area more, show less feather damage than hens that
95   prefer to spend time indoors (Mahboub et al., 2004; Rodriguez-Aurrekoetxea & Estevez, 2016).
96   Similarly, opportunistic scoring of hens on the range has shown better plumage condition
97   compared with hens scored in the shed (De Koning et al., 2018) and better plumage in hens that
98   ranged farther (Chielo et al., 2016). Outdoor ranging hens or hens that have access to a range
99   area show comparatively reduced footpad dermatitis (Heerkens et al., 2016; Rodriguez-
100 Aurrekoetxea & Estevez, 2016) and range use keeps toenails shortened (Campbell et al., 2017;
101 Yilmaz Dikmen et al., 2016). However, not all studies demonstrate strong relationships between
102 individual ranging and welfare parameters. Larsen et al. (2018) found no association between
103 range access and comb colour, beak, footpad, plumage, or keel bone condition, although hens
104 that ranged further had better beak condition and darker comb colour. Ranging is related to hen
105 body weight, but the direction of the relationship varies between studies rendering this
106 relationship currently equivocal (Campbell et al., 2016; Hartcher et al., 2016; Singh et al., 2016).
107   Range use can also impact internal health and welfare parameters but data on individual
108 patterns are currently limited. Ranging hens may have improved digestive and gut function over
109 non-ranging hens as they ingest stones and grit that eventually contribute to heavier gizzards
110 (Singh et al., 2016). Keel bone damage can reduce pop-hole usage, but the causal relationship is
111 unclear (Richards et al. 2012). Furthermore, Kolakshyapati et al. (2019) recently showed no
112 relationship between high and low range use and keel bone damage. Ranging hens might be
113 susceptible to internal parasite infections such as *Ascaridia galli* that are present in soil
114 (Kaufmann et al., 2011; Permin et al., 1999) as well as the range being contaminated by previous
115 batches of hens (Höglund & Jansson, 2011). But some research shows a reduction in flock level
116 parasitic infections with increased range use (Sherwin et al., 2013) as the outdoor hens excrete
117 more in the range and less indoors thus lowering the density of faeces and possibility of
118 reinfection (Sherwin et al., 2013). Necropsies of hens from varying housing systems in Sweden

119   showed a higher occurrence of viral and bacterial infections and diseases in free-range and floor-
120   based systems (Fossum et al., 2009). Overall, there is minimal information on both external and
121   internal health and welfare parameters of individual free-range hens that vary in their range use
122   patterns.
123   For optimising the health and welfare of adult hens, it has been suggested to provide
124   similar environments for both the rearing and layer housing (Janczak & Riber, 2015). In
125   Australian free-range systems (and elsewhere), the pullet rearing and adult housing environments
126   are dissimilar. Adult hens range outdoors but the pullets are reared indoors, which could result in
127   poorer adaptation of adults to the free-range environment. Although it is typically not feasible to
128   provide outdoor access to pullets, rearing enrichments may be a method of improving the pullet's
129   developmental environment (Campbell et al., 2019). Regularly changing novel objects might
130   simulate the frequently changing and unpredictable free-range environment and improve hen's
131   adaptation to stressful change as adults (Campbell et al., 2018a). Enriching with structures to
132   enhance perching and encourage spatial navigation may increase physical development and
133   spatial awareness (Gunnarsson et al., 2000) which could benefit ranging hens. Management
134   approaches that include rearing enrichments in their pullet stages may improve adaptability,
135   reduce stress, and improve hen immunity (Arbona et al., 2011; Moe et al., 2010), thus reducing
136   disease prevalence or infections in free-range hens.
137   The current study was conducted with the aim to evaluate the effect of individual ranging
138   patterns on health parameters through post-mortem examination of free-range layers from
139   different rearing enrichments. We predicted improved health and welfare of enriched over
140   control hens and both benefits and consequences of ranging.
141

Materials & Methods
Ethical statement
144   All research was approved by the University of New England Animal Ethics Committee
145   (AEC17-092).
Animals and Housing
147   A total of 1386 Hy-Line® Brown layers were used for this study. The chicks and pullets were
148   reared indoors at the Kirby poultry facility and the adults were housed in the Laureldale free-
149   range facility at the University of New England, Armidale, NSW, Australia. Day-old chicks
150   (including additional chicks delivered by the hatchery but not transferred to the laying facility)
151   were reared across 16 weeks within 9 pens (6.2 m L x 3.2 m W) distributed across three separate
152   rooms. The pullets were exposed to 3 separate rearing enrichment treatments. These included a
153   control group having no extra materials over the pen standard of rice hulls as floor litter, a
154   novelty group where novel objects were added and changed at weekly intervals (e.g. balls,
155   bottles, bricks, brooms, brushes, buckets, containers, pet toys, plastic pipes, strings) and a
156   structural group where four custom-designed H-shaped perching structures (L, W, H = 0.60 m)
157   with two solid panels and one open-framed side were provided. Each of the rooms had one pen
158   replicate per treatment, balanced for location within rooms. Shade cloth hung on the wire pen

159 dividers visually isolating birds from each other. At 16 weeks of age, bird density was
160 approximately 15 kg/m² or 9 pullets/m² (average 174 -190 pullets/pen). Round feeders provided
161 *ad libitum* access to commercially-formulated mash appropriate for the developmental stage and
162 nipples supplied *ad libitum* water access. These resources were provided as per the current
163 Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary
164 Industries Standing Committee, 2002). Artificial lighting and temperature schedules followed the
165 recommended Hy-Line® Brown alternative management guidelines (Hy-Line Brown
166 management guide, 2016) but the LED lighting was maintained at 100 lux as the pullets were
167 destined for outdoor access (no natural light was present during rearing). Mechanical ventilation
168 with heating operated as needed but no cooling system was present. Chicks were infra-red beak-
169 trimmed at the hatchery with a vaccination schedule as per regulatory requirements and standard
170 recommendations including vaccination against Newcastle disease, Marek's disease, fowl pox,
171 fowl cholera, egg drop syndrome, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, infectious
172 bronchitis, infectious laryngotracheitis, and avian encephalomyelitis.
173   At 16 weeks, 1386 pullets were transferred to the Laureldale free-range facility and
174 remixed within pen replicates (extra delivered chicks that were grown out were rehomed). The
175 hens were housed within their rearing treatments across 9 pens located in a single shed (n = 154
176 hens/pen, indoor density approximately 9 hens/m²). The indoor pens were visually separated via
177 shade cloth and included nest boxes (2 small and 1 large nest box), perches, round hanging
178 feeders and water nipples to fulfil the requirements of the Australian Model Code of Practice for
179 the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002).
180 Rice hulls were used as floor litter material with one complete litter replacement mid-way
181 through the flock cycle. The LED lighting schedule gradually increased to 16 hours light and 8
182 hours dark by 30 weeks of age with an average pen intensity of 10.0 (± 0.84 SE) lux (Lutron
183 Light Meter, LX-112850; Lutron Electronic Enterprise CO., Ltd, Taipei, Taiwan) as measured at
184 birds' eye height from 3 pen locations (front, middle, back) when the pop-holes were closed.
185 This lux was the highest that could be achieved with the shed lighting system. The shed was fan-
186 ventilated with no temperature or humidity control.
187 Each of the 9 pens was connected to an outdoor range area (31 m L x 3.6 m W for each pen,
188 density approximately 1.4 hens/m²) which was accessed via two pop-hole openings (18 cm W x
189 36 cm H). The range area immediately after the pop-holes was 1.1 m of concrete path, then 1.6 m
190 of river rock followed by a grassed area with no additional trees or shelter. The grassed area
191 became denuded following both hen access and the winter season. Each range was visually
192 divided by shade cloth hung along the wire fences. Hens were provided access to the outdoor
193 area from 25 weeks of age (May 2018) for most of the day time via automatic opening and
194 closing of the pop-holes. The pop-holes opened at 9:15 am and closed after sunset daily. This
195 equated to approximately 9 hours of available ranging time across winter followed by
196 approximately 11 hours of available ranging time after daylight saving time started (October
197 2018).
198

Radio-frequency identification (RFID) system

All birds were banded with microchips (Trovan® Unique ID 100 (FDX-A operating frequency 128 kHz) glued into adjustable leg bands (Roxan Developments Ltd, Selkirk, Scotland) to track their movement in and out of the range pop-holes via radio-frequency identification (RFID) systems. The RFID systems were designed and supported by Microchips Australia Pty Ltd (Keysborough, VIC, Australia) with equipment developed and manufactured by Dorset Identification B.V. (Aalten, the Netherlands) using Trovan® technology. For a schematic of the RFID system, see (Campbell et al., 2018b). The system recorded the date and time of each tagged bird passing through and in which direction (onto the range, or into the pen) with a precision of 0.024 s (maximum detection velocity 9.3 m/s). Individual ranging data were collected daily from 25 until 65 weeks of age.

RFID data and selection of hens

RFID data from 56 until 64 weeks of age (54 days of data) were initially run through a custom-designed software program written in the 'Delphi' language (Bryce Little, CSIRO, Agriculture and Food, St Lucia, QLD, Australia) that filtered out any unpaired or 'false' readings that may occur if, for example, a hen sits inside the pop hole but does not complete a full transition onto the range or back into the pen. Once screened in this way, the data were used to select a sample of birds from each pen to conduct post-mortem examinations on. A total of 307 hens were selected across all of the 9 pens at 64 weeks of age. The selected hens were categorized as 'indoor' – accessed the outdoors on one or zero of the 54 days, 'low outdoor – accessed the range on 53 or 54 of the 54 days but only for 1 h 24 mins or less, and 'high outdoor' – accessed the range for 54 of 54 days for 5 h 12 mins to 9 h. Based on these criteria, a total of 95 indoor, 109 low outdoor and 104 high hens outdoor were selected from the flock. Maximum ranging times differed between pens, thus the 'high outdoor' birds were selected as the highest for their pen, resulting in the variation in hours. Where possible, hen selection was balanced across pens within treatments, but some pen replicates did show higher numbers of the extreme ranging patterns than others.

Pre-mortem welfare assessment

All the selected hens were weighed 4 to 5 days before dissection using electronic hanging scales (BAT1; VEIT Electronics, Moravany, Czech Republic). The external health and welfare parameters including feather loss at different body parts (neck, chest, back, wing, vent, tail) and footpad lesions were assessed using the scoring system described by Tauson et al. (2005). In this scoring system, 4 scores were available for feather coverage with a score 4 indicating minimal feather damage, whereas a score 1 indicated bare skin. The back of the neck was scored separately from the front of the neck and was not included in the analyses as the majority of damage on the neck front was believed to have resulted from rubbing on the feeder rims rather than pecking. A maximum score of 24 could be obtained for feather condition across 6 body parts. Footpad lesions were scored as a 4 for a normal footpad with no lesions (an additional

239   category to the Tauson et al. (2005) system) and a score 1 for swollen, infected bumble foot. The
240   exact number of fresh or healing comb wounds were counted, and toenail length was measured
241   in mm using a seamstress tape measure. Beaks were scored as 0, 1, or 2 indicating no, mild, or
242   moderate damage respectively. The birds were also examined for any other external signs of
243   injury or illness, such as body wounds, bleeding, abnormal respiratory sound etc., but none were
244   observed in these hens. All selected hens were tagged with an additional leg band for
245   identification in their home pen to enable later capture.
246

247   **Post-mortem health examination**
248   Post-mortem examinations of the selected 307 hens were carried out across two days at 65 weeks
249   of age at a separate post-mortem facility located 5.5km from the free-range facility. The selected
250   hens of a single pen were transported to the post-mortem lab up to 2 hours prior to the dissection
251   using plastic carrier crates. Hens were killed using CO_2 to maintain hen physical structure for
252   later scanning. Immediately after the CO_2 administration and cessation of all hen movement, the
253   birds were opened by a veterinarian to examine the health condition of the hens
254   (presence/absence of diseases) by inspecting the visceral organs for any abnormalities including
255   haemorrhage, tumours, caseous necrosis and/or other exudates, the respiratory system (nares and
256   trachea) for any haemorrhage, inflammation or exudates and the reproductive system for signs of
257   salpingitis, being egg bound, or other abnormalities. Whether the hen was in production or not
258   was determined by examination of the ovary and presence of active or regressing follicles. The
259   spleen, gizzard, and right adrenal gland were removed and weighed (to the nearest mg) from
260   each of the selected birds. The gizzard was emptied prior to weighing with surrounding
261   connective tissue and fat removed. The jejunum, duodenum and ileum were opened
262   longitudinally and the number of *Ascaridia galli* worms in each bird recorded. This is one of the
263   most prevalent nematode parasites present within Australian commercial free-range systems
264   (Dao et al., 2018). The carcass weight of each bird was taken after post-mortem examination
265   (minus the removed organs and small intestines) prior to the Computed Tomography (CT)
266   scanning.
267

268   **CT scanning and image analysis**
269   Following post-mortem examination, all birds were scanned using a HiSpeed QX/I (2003) CT
270   scanner (GE Medical Systems). Scans were performed using a voltage of 100kV, a current of
271   80mA, a pitch of 0.75:1, a field of view of 500 x 500mm, a thickness of 2.5mm, and a spacing of
272   3.75mm per rotation. Resultant images had a pixel matrix of 512 x 512, and hence a pixel
273   resolution of $2.384mm^3$.
274   CT image stacks of each individual hen were analyzed using ImageJ (Rasband W.S.,
275   ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/,
276   1887-2018) to generate pixel frequency plots against greyscale pixel values (0-255). Greyscale
277   value density was defined using a linear regression parameterized to known carcass weights (g)

278 recorded immediately prior to scanning. As such, the linear regression between density (ρ,

279 μg/mm^3) and greyscale value (x) was given as:

280

281 $\rho = 10.19(\pm 0.4546\sigma) \cdot x$ [Eq. 1]

282

283 where σ is a standard deviation.

284 The weight of individual chicken (w, g) was therefore given as:

285

286 $w = \sum_{x=0}^{255} y \cdot \alpha \cdot \rho \cdot 1000000$ [Eq. 2]

287

288 where y is the frequency of each greyscale value; α is the pixel resolution (2.384mm^3); and ρ is

289 density (μg/mm^3, Eq. 1).

290   Body composition of each chicken was estimated assuming that the density of raw

291 chicken fat was ≤865.64μg/mm^3 (https://www.aqua-calc.com/page/density-

292 table/substance/chicken-coma-and-blank-broilers-blank-or-blank-fryers-coma-and-blank-

293 separable-blank-fat-coma-and-blank-raw), and that bone was identified as having a greyscale

294 value of 255 (2598.45μg ±0.4546σ), with the remaining mass being attributed to muscle. Some

295 hens contained an egg which was not removed prior to scanning and was classified the same as

296 bone in the images. All hens with an egg present (1/0) were later identified (n = 87) and removed

297 from the analysis.

298

299 **Assessment of the keel bone**

300 Following CT scanning, the keel bone was excised from each hen to assess deformities and

301 damage. All keel bones were stored at -20°C until thawed for processing. For processing, the

302 fleshes on the keels were removed using a knife and scissors. The defects on the keels were

303 examined by two experimenters using a visual scoring system for bending of the spine that was

304 classified as low, moderate, or high based on the extent (Figure 1). The two observers scored

305 each bone independently and then immediately confirmed scores to ensure agreement for each

306 bone. The dorsal surface of the tip of the keels were also observed for the presence of calluses to

307 indicate healed fractures and the number of calluses were counted and recorded by two observers

308 in agreement (Figure 2). No fresh pre-mortem fractures were observed.

309

310 **Data and statistical analyses**

311 Statistical analyses were conducted in JMP® 14.0 (SAS Institute, Cary, NC, USA) with α set at

312 0.05. Data were transformed where needed but the raw values are presented in the tables and

313 graphs. Non-significant interactions were removed from the final models and post-hoc Student's

314 t-tests were applied to the least-squares means where significant differences were present. For

315 the pre-mortem welfare assessment data, the per hen values (n = 307 hens) for the total feather

316 score (up to 24) and the number of comb wounds were square-root transformed. The body

317 weight and toenail length measurements per bird were compiled. General Linear Mixed Models

318   (GLMM) were fitted to each welfare variable with rearing enrichment treatments and ranging
319   patterns as fixed effects including their interaction and bird ID nested within pen, rearing
320   treatment, and ranging as a random effect. Restricted maximum likelihood estimation methods
321   were applied.
322   The feather scores for each body part and footpad damage scores were compiled per
323   individual hen and the effects of rearing treatment, ranging, and their interaction were tested
324   using ordinal logistic regressions.
325   The body composition data (muscle, fat, bone in grams) from the CT scans (n = 307), post-
326   mortem parameters including organ weights (liver, spleen, right adrenal gland, and empty
327   gizzard proportional to live body weight), and worm counts were compiled per individual bird (n
328   = 306 hens, data from 1 bird were missing). The relative organ weight proportional data were
329   logit-transformed for analysis but expressed as percentages in the table. The worm count data
330   were square-root transformed. GLMMs were fitted to test the fixed effects of rearing treatment
331   and ranging groups including their interaction, with bird ID nested within pen, rearing treatment,
332   and ranging as a random effect. The bone composition data of hens with an egg present (n = 87)
333   were excluded from the analysis and data from 220 hens only were used.
334   Although a total of 307 birds were dissected, only 300 keels were assessed due to
335   labelling issues with 7 keels. The qualitative data for the keel bones was compiled per individual
336   bird (n = 300) including the overall presence of damage to the keel bone (yes/no), the type of
337   keel spine bending, the presence (yes/no) and the number of calluses on the tips of the keels.
338   Pearson's Chi-square tests for differences between rearing treatments and ranging patterns were
339   conducted separately for each variable of each set of data. When conducting the Chi-square test
340   with one treatment, the other treatment effect was blocked for each of the variables. The counts
341   of calluses were square-root transformed and analysed using a GLMM to test the effects of
342   rearing treatment and ranging groups including their interaction, with bird ID nested within pen,
343   rearing treatment, and ranging as a random effect.
344

Results

Pre-mortem welfare assessment

347   Enriched rearing treatments did not affect the live weight of hens ($F_{2, 302} = 0.11$, $P = 0.90$) but the
348   high outdoor hens had the lowest body weight ($F_{(2, 302)} = 10.90$, $P < 0.0001$, Table 1). There was
349   no interaction between rearing treatment and ranging ($P = 0.32$). The total feather score of hens
350   was the lowest in the control rearing treatment ($F_{(2, 302)} = 31.41$, $P < 0.0001$) indicating the most
351   feather loss in these birds compared with the enriched hens (Table 1). The total feather score was
352   highest in the high outdoor hens ($F_{(2, 302)} = 18.06$, $P < 0.0001$) compared with low outdoor and
353   indoor hens indicating the least feather loss in these birds (Table 1). There was no interaction
354   between rearing treatment and ranging ($P = 0.11$). The control hens had more comb wounds than
355   the novelty hens but neither group differed from the structural group ($F_{(2, 302)} = 3.63$, $P = 0.03$,
356   Table 1). The high outdoor hens had fewer comb wounds compared with the indoor hens but
357   neither ranging group differed from the low outdoor hens ($F_{(2, 302)} = 3.08$, $P = 0.04$, Table 1).

358   There was no interaction between rearing treatment and ranging ($P = 0.44$). Rearing treatment
359   did not affect toenail length ($F_{(2, 302)} = 2.19$, $P = 0.11$), but the indoor hens had the longest nails
360   and the high outdoor hens had the shortest ($F_{(2, 302)} = 86.84$, $P < 0.0001$, Table 1). There was no
361   interaction between rearing treatment and ranging patterns on any of the pre-mortem variables (P
362   ≥ 0.11).
363   A higher percentage (95 %) of hens from the structural group showed feet with no
364   damage (score 4) compared with the control (93 %) and novelty groups (93 %). A higher
365   percentage of hens (97 %) from the low outdoor ranging groups showed no footpad damage
366   (score 4) compared with the high outdoor (88 %) and indoor hens (95 %). Only 0.92 % of hens
367   from the novelty group and 0.96 % hens from the high outdoor ranging group showed the worst
368   footpad condition (score 1). Ordinal logistic regression analysis showed ranging patterns
369   significantly affected the footpad damage of hens ($\chi2 = 7.07$, df = 2, $P = 0.03$) with high ranging
370   increasing damage but the rearing treatments did not ($\chi2 = 0.86$, df = 2, $P = 0.65$). There was no
371   interaction between rearing treatment and ranging patterns ($P = 0.14$).
372   For other health issues examined pre-mortem, 0.01 % of hens showed a prolapse. Most of
373   the hens (91.86 %) had no defects on the beaks with a scoring of '0', but 0.06 % and 0.03% of
374   hens had beaks with a score of '1' for mild and a score of '2' for moderate defects, respectively.
375

376   **Feather scores of individual hen body parts**
377   Analyses of the feather scores of separate hen body parts showed rearing treatments had
378   significant effects on plumage damage to the back of the neck, chest, and back with control hens
379   showing the poorest feather coverage (Table 2). Ranging patterns had significant effects on
380   plumage damage to the chest with high outdoor hens showing the greatest plumage coverage
381   (Table 2). There were no significant interactions between rearing treatments and ranging groups
382   (all $P \geq 0.07$).
383

384   **Post-mortem parameters**
385   Rearing treatments affected the relative weight of the spleen ($F_{(2, 301)} = 4.82$, $P = 0.01$) with the
386   spleens from the novelty hens having lower weight than the spleens from the control hens but
387   neither group differed from the structural group (Table 3). The high outdoor birds' relative
388   spleen weight was the highest ($F_{(2, 301)} = 4.44$, $P = 0.01$). The high outdoor hens had higher
389   empty gizzard weights than the indoor hens ($F_{(2, 301)} = 3.22$, $P = 0.04$) but there was no effect of
390   rearing treatment ($F_{(2, 301)} = 0.85$, $P = 0.43$). Both the rearing treatments and ranging had no
391   significant effects on the relative liver and adrenal weights (Table 3). There was no overall
392   significant effect of rearing treatments on the number of worms in the GI tract ($F_{(2, 301)} = 2.30$, P
393    $= 0.10$), but post-hoc tests (which are more focussed to differentiate the clear visual differences
394   in means, (Hsu 1996) showed the novelty hens had more worms than the control hens and
395   neither group differed from the structural hens (Table 3). There was no effect of ranging group
396   on the number of worms ($F_{(2, 301)} = 1.15$, $P = 0.32$). There were no significant interactions
397   between rearing treatment and ranging for any of the measured variables (all $P \geq 0.21$).

398   Post-mortem examination also revealed all hens under study except one, were in
399   production. There were no disease lesions observed on the respiratory system of the hens. One
400   bird had a fatty liver, one peritonitis and one had keel adhesion. A cystic right oviduct was also
401   present in 0.04% of hens.
402
403   **Body composition**
404   There was a trend for an effect of rearing treatment on body fat ($F_{(2, 302)} = 2.80$, $P = 0.06$) with
405   the novelty group showing lower body fat (LSM ± SEM = 122.39 ± 1.78) than the control group
406   (LSM ± SEM = 128.15 ± 1.91) but neither differed from the structural group (LSM ± SEM =
407   126.99 ± 1.83). Ranging had a significant effect on body fat ($F_{(2, 302)} = 19.70$, $P < 0.0001$) and
408   muscle ($F_{(2, 302)} = 11.49$, $P < 0.0001$) with the high outdoor birds showing the lowest amount of
409   fat and muscle (Figure 3). There was no effect of rearing treatment on muscle mass ($F_{(2, 302)} =$
410   0.25, $P = 0.78$). There was a trend for an effect of rearing treatments ($F_{(2, 215)} = 2.84$, $P = 0.06$) on
411   bone mass with the post-hoc tests showing the novelty hens had higher bone mass than the
412   control group but the structural group did not differ significantly from either (Figure 4). The
413   ranging patterns had no significant effect ($F_{2, 215} = 1.95$, $P = 0.14$) on bone mass of the hens.
414   There were also no significant interactions of rearing treatments and ranging on any measured
415   variable (all $P \geq 0.16$).
416
417   **Keel bone damage**
418   The free-range hens from different rearing treatments and ranging patterns showed no significant
419   differences in the overall presence of keel bone defects, presence of spine bending, spine bending
420   types, and the presence of callus formation on the dorsal surface of keel tips (all $P \geq 0.19$, Table
421   4). The rearing treatments ($F_{(2, 295)} = 0.40$, $P = 0.67$), ranging patterns ($F_{(2, 295)} = 1.31$, $P = 0.27$)
422   and their interaction ($P = 0.55$) had no effect on the number of calluses on the tip of the dorsal
423   surface of the keels.
424

Discussion

426   This study assessed the effects of different rearing enrichments and outdoor ranging patterns on
427   the external and internal health and welfare of free-range hens at 64 to 65 weeks of age. Hens
428   enriched during rearing had better feather coverage than control hens and the hens enriched with
429   novel objects had fewer comb wounds than the control hens. The novelty hens also had lower
430   spleen weights than the control birds. Range access had multiple clear effects with the hens that
431   ranged the most showing lower total body weight, lower fat and muscle content, better plumage,
432   shorter toenails, the highest spleen weight and fewer comb wounds than the indoor birds.
433   Rearing or ranging did not affect the prevalence and degree of keel bone damage, but most
434   sampled birds showed some type of damage. Overall, enriched rearing environments still had
435   some effects on hens at the later stages of the production cycle but subsequent range use patterns
436   had the greatest impact.

437   Hens reared with enrichments had better plumage indicating a persistent effect of rearing
438 conditions through until later in the production cycle. Multiple studies have documented the
439 positive long-term effects of providing substrates during rearing where opportunities to forage
440 and dust bathe are suggested to prevent the development of feather pecking (reviewed in
441 Rodenburg et al., 2013; Van de Weerd & Elson, 2006) although substrate availability during the
442 laying period is also critical (Rodenburg et al., 2013). All birds in this study were both reared and
443 then housed with access to a floor litter substrate but the additional pen enrichments still had
444 positive impacts. Huber-Eicher & Audigé (1999) did find that access to perches during rearing
445 significantly reduced the risk of feather pecking. The structural rearing group had access to
446 elevated perches for 16 weeks, and some of the objects initially placed in the novelty pens did
447 allow chicks to perch (e.g. bricks, containers) with later objects provided to allow
448 pecking/exploration (e.g. strings, pet toys). The increased complexity of the enriched pens may
449 have improved behavioural development or provided more opportunities for the pullets to
450 regulate their social interactions by having elevated escape areas. These positive effects of
451 enrichments contrast with the findings by Hartcher et al. (2015) who reported that rearing
452 enrichments of polypropylene pecking strings, whole oats and increased litter depth from 12 days
453 of age had no effect on the plumage of adult free-range hens at 43 weeks of age. These
454 discrepancies between results might have occurred due to the variation in novel objects of the
455 different studies or age at first provision. Pullets did not show feather damage at the end of
456 rearing (unpublished data) but it is unclear whether pecking behaviour in the control hens
457 resulted from behavioural patterns established during rearing, and/or if the adult control hens
458 were more susceptible to environmental stress in the free-range setting which triggered the
459 development of the negative pecking behaviour.
460   The novelty hens had lower relative spleen weights than the control hens but showed a
461 higher worm count. Differences in spleen size can be related to parasite burden (John, 1995)
462 where a greater burden increases spleen size. Spleen size can also reduce with stress where
463 Odihambo Mumma et al. (2006) found that an adrenocorticotropin (ACTH) treatment to
464 experimentally increase stress in layers reduced relative spleen weight compared with control
465 hens. Thus, it is unclear the causes for the differences in spleen size in the current study.
466 Additionally, only adult *A. galli* worms (no other infection life stages or other parasites) were
467 counted in the current study and other measured organs were not affected by rearing treatments.
468 Therefore, further study would be needed to confirm any relationship between rearing
469 enrichments, stress, infections and spleen size in adult hens.
470   The outdoor hens had relatively heavier spleens. This heavier weight could be a result of
471 reduced stress through outdoor access and potential behavioural freedom. However,
472 experimental exposure to a lipopolysaccharide stressor from *E. coli* increased relative spleen
473 weight and thus the outdoor hens may have had higher spleen weights through exposure to more
474 pathogens (Shini et al., 2009). Increased spleen weight is a very general response to many types
475 of infections although other factors might be involved (Smith & Hunt, 2004) such as seasonal
476 influence (John, 1994). Outdoor hens were previously reported to be more stressed as indicated

477   by the heterophil/lymphocyte ratio (Mahboub et al., 2004). However, variation in spleen weight
478   might be dependent on type of stressors and further research is needed to confirm any
479   relationship between ranging and spleens. There was no significant difference in the *A. galli*
480   parasite burden between indoor and outdoor hens which is supported by recent findings by
481   (Bestman et al., 2019) and previous research showing high parasite loads for hens housed in litter
482   systems (Permin et al., 1999) suggesting all hens in the free-range system can become infected.
483   However, further studies, particularly on commercial farms, are still required to confirm the
484   individual-level relationship between parasitic burden and ranging patterns.
485   The hens that ranged the most showed the best plumage coverage which provides further
486   support to previous research. Several studies have demonstrated better plumage in individuals, or
487   flocks that show more frequent use of the outdoor range (De Koning et al., 2018; Lambton et al.,
488   2010; Mahboub et al., 2004; Rodriguez-Aurrekoetxea & Estevez, 2016), or who range farthest
489   when outdoors (Chielo et al., 2016). It might be that hens outdoors are able to or are motivated to
490   engage in more foraging compared with hens indoors (Campbell et al., 2017) where a lack of
491   foraging is often redirected to feather pecking, causing plumage damage (Bestman et al., 2009;
492   Gilani et al., 2013; Rodenburg et al., 2013). This result contrasts with that of Larsen et al. (2018
493   who found no association between outdoor ranging and plumage condition of Hy-Line® Brown
494   hens in commercial Australian conditions. It is also possible that better plumage coverage led to
495   more ranging if hens were able to thermoregulate more effectively and/or avoid sun exposure on
496   bare skin.
497   The outdoor rangers also had shorter toenails which confirms a previous finding at the
498   same experimental research facility (Campbell et al., 2017) as well as research comparing
499   between hens from free-range or caged systems (Yilmaz Dikmen et al., 2016). This is probably
500   due to increased walking and scratching outside which allows hens to appropriately manage
501   growing nail length.
502   Hens that ranged the most showed the lowest body weight, specifically, lower body fat
503   and muscle, but not lower skeletal mass. The high outdoor hens showed an average body weight
504   (1.95 kg) lower than the indoor hens but were within the limit of the expected body weight by
505   breed standards (1.90 – 2.02 kg; Hy-Line Brown management guide, 2016). Previous research
506   has found some evidence of a similar negative relationship between body weight and range use,
507   but not at all measured age points (Campbell et al., 2017) and some authors have found the
508   opposite relationship (Singh et al., 2016). This negative relationship might be due to the
509   ingestion of vegetation, insects or grit during ranging and thus consumption of less formulated
510   food (Singh & Cowieson, 2013). This would also correspond with the higher empty gizzard
511   weight observed in the ranging hens, similar to findings of larger gizzards in free-range versus
512   caged hens (Yang et al., 2014). The reduction in body weight might also be a result of greater
513   energy utilisation during locomotion, although greater exercise opportunities have previously
514   been shown to increase bone and muscle development (Casey-Trott et al., 2017a; Regmi et al.,
515   2016) which was not found in the outdoor hens. Other measured skeletal properties rather than
516   overall mass may have revealed differences between ranging groups although recent work

517   showed no effect of ranging on multiple tibial measurements across hens from a commercial
518   aviary-free-range system (Kolakshyapati et al., 2019). There is currently limited knowledge in
519   the comparative activity levels of hens that remain indoors, and this warrants further
520   investigation.
521   The present study suggested no effect of rearing enrichments and ranging patterns on keel
522   damage of free-range hens. This coincides with observations on commercial farms that assessed
523   damage via both dissection (Kolakshyapati et al., 2019) and palpation (Larsen et al., 2018) and
524   found no association between individual ranging and keel damage. It was expected that rearing
525   with structural enrichments may have reduced the later occurrence of fractures (Casey-Trott et
526   al., 2017b), but this was not supported by the current study. The actual relationship between
527   range use and keel damage might be inconclusive because painful keel fractures might prevent
528   birds passing through the pop holes (Richards et al., 2012) and ultimately reduces range access.
529   The novelty rearing treatment increased bone mass relative to the control birds which may have
530   been a result of novel objects that allowed perching in the first 2 weeks of life (e.g. overturned
531   containers). The birds in the structural treatment were first observed starting to perch at 2 weeks
532   of age onwards. Further specific bone measures would confirm any effects of the rearing
533   treatments on skeletal development.
534
535   **Conclusion**
536   The study showed that rearing enrichments had long-term effects on adult free-range hens,
537   particularly in reducing the degree of plumage damage. However, subsequent individual ranging
538   patterns by the hens had a stronger influence on their health and welfare with high outdoor use
539   resulting in better plumage, fewer comb wounds, shorter nail length, higher spleen and gizzard
540   weight, but lower body weight, fat and muscle. Rearing enrichments are thus recommended for
541   long-term positive effects on hen welfare, but management of range access may have the
542   strongest impact on bird welfare. This study was conducted in an experimental setting with small
543   flock sizes and low incidence of infection. Large commercial groups of layers are likely to be
544   exposed to more pathogens where outdoor access may have different effects on hen
545   susceptibility. Similar long-term studies on commercial free-range farms would confirm the
546   benefits and consequences of different ranging patterns.
547

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553

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Figure 1

Different types of spine bending of keel bones

Keel (a) indicates a spine with no bending, (b) low bending, (c) moderate bending and (d) indicates high bending in the spine. The white arrows indicate the specific part of bending on the spine.

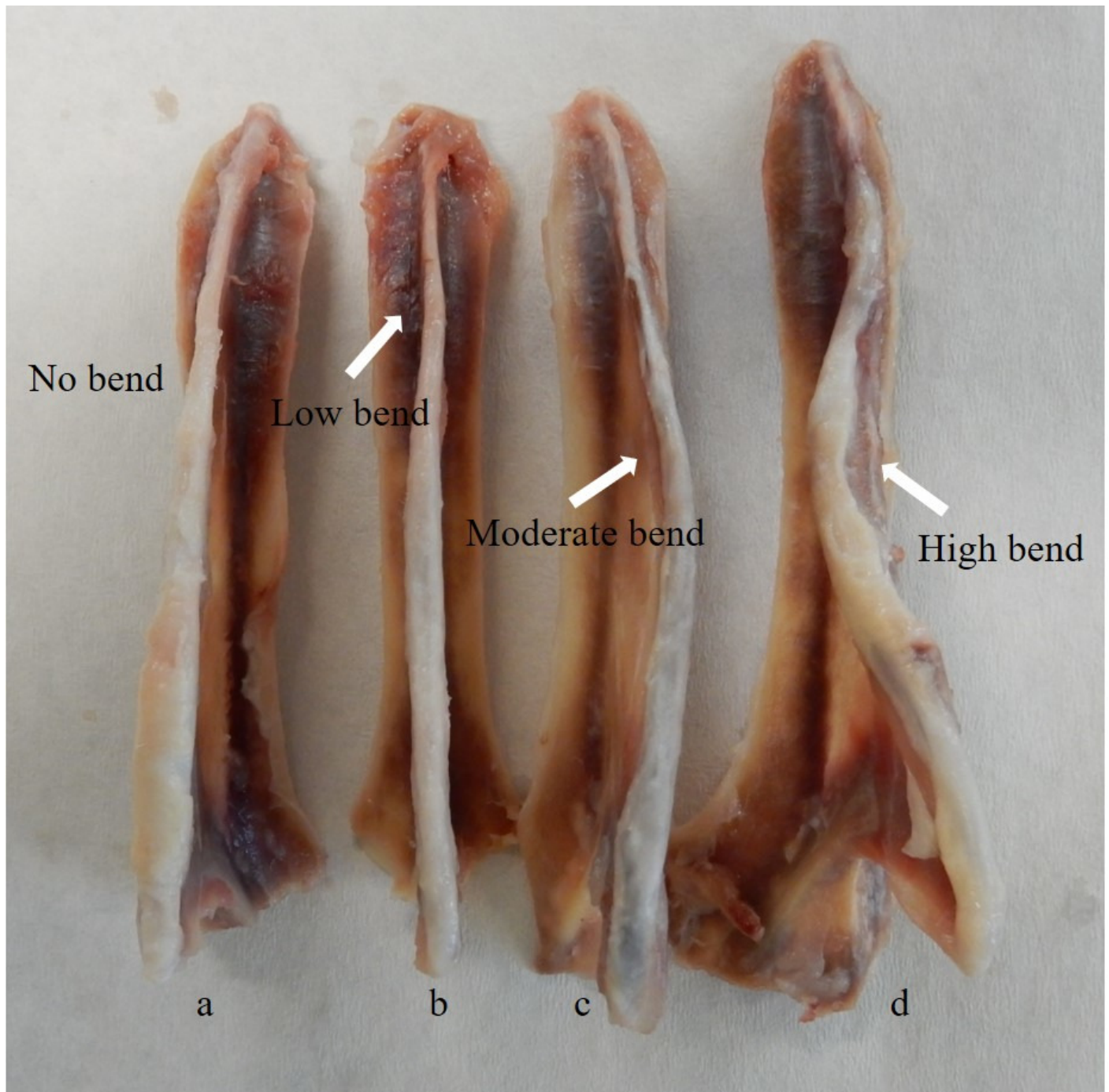



Figure 2

Calluses on the dorsal surface of keel bones.

(a) indicates a keel surface with no calluses, but (b) shows two calluses as indicated by the white arrows.

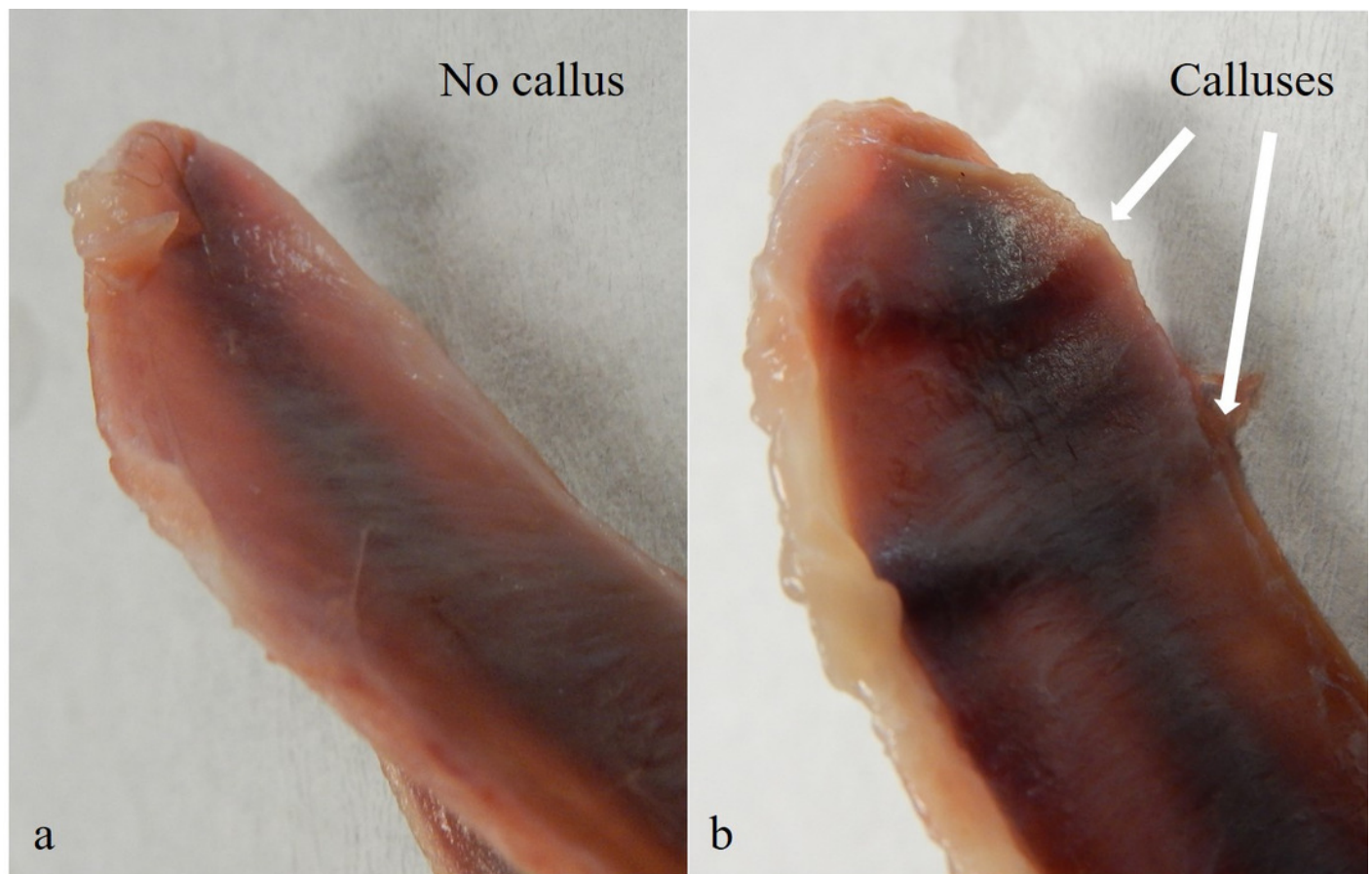



Figure 3

The relative CT-scanned body composition of hens from different range use patterns.

The least squares means ± standard error of the mean of fat (A) and muscle (B) are presented from hens at 65 weeks of age that did not range (indoor), or ranged daily for low or high amounts of time. [a,b]Dissimilar superscript letters indicate significant differences between ranging patterns (P < 0.0001).

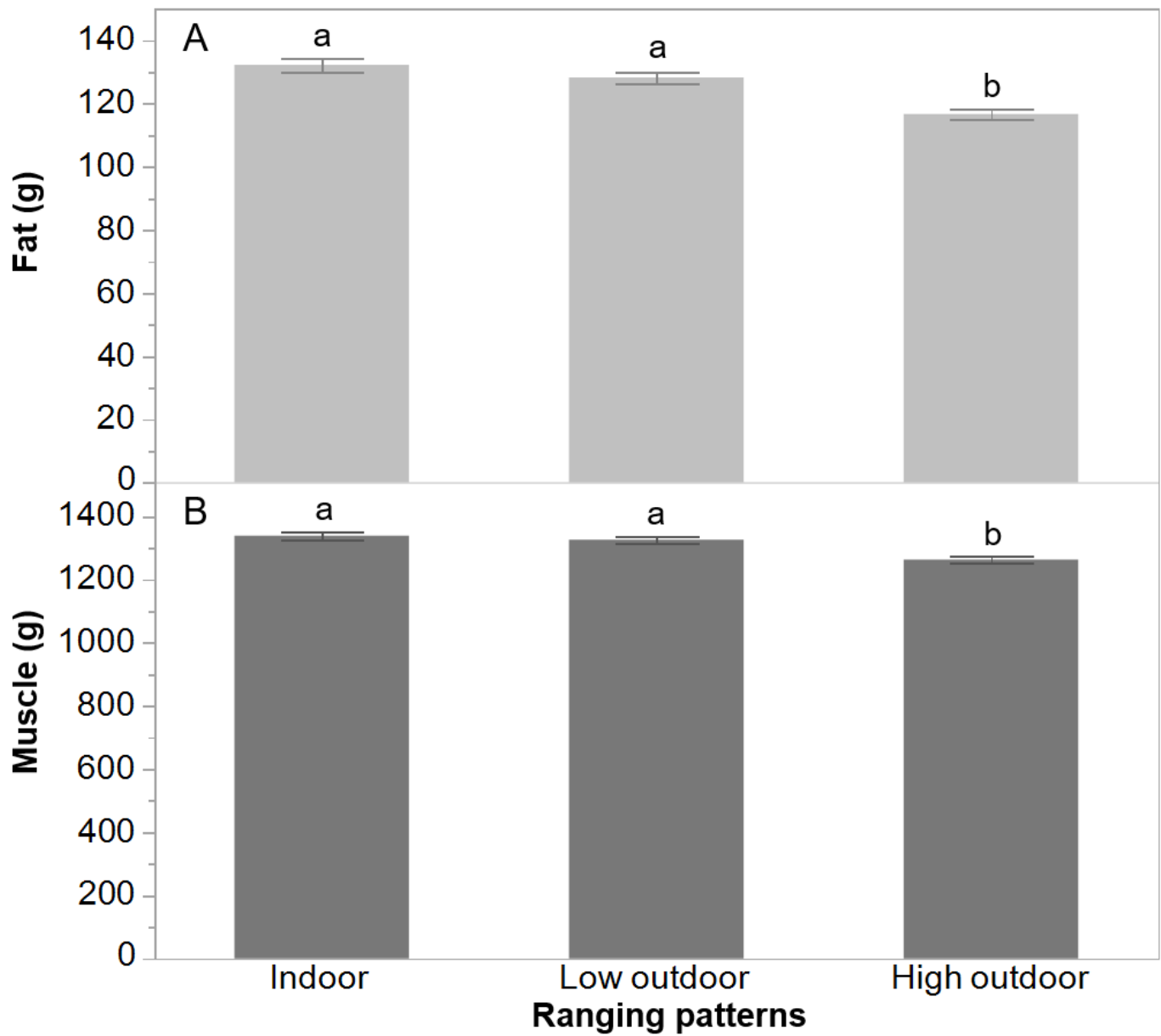
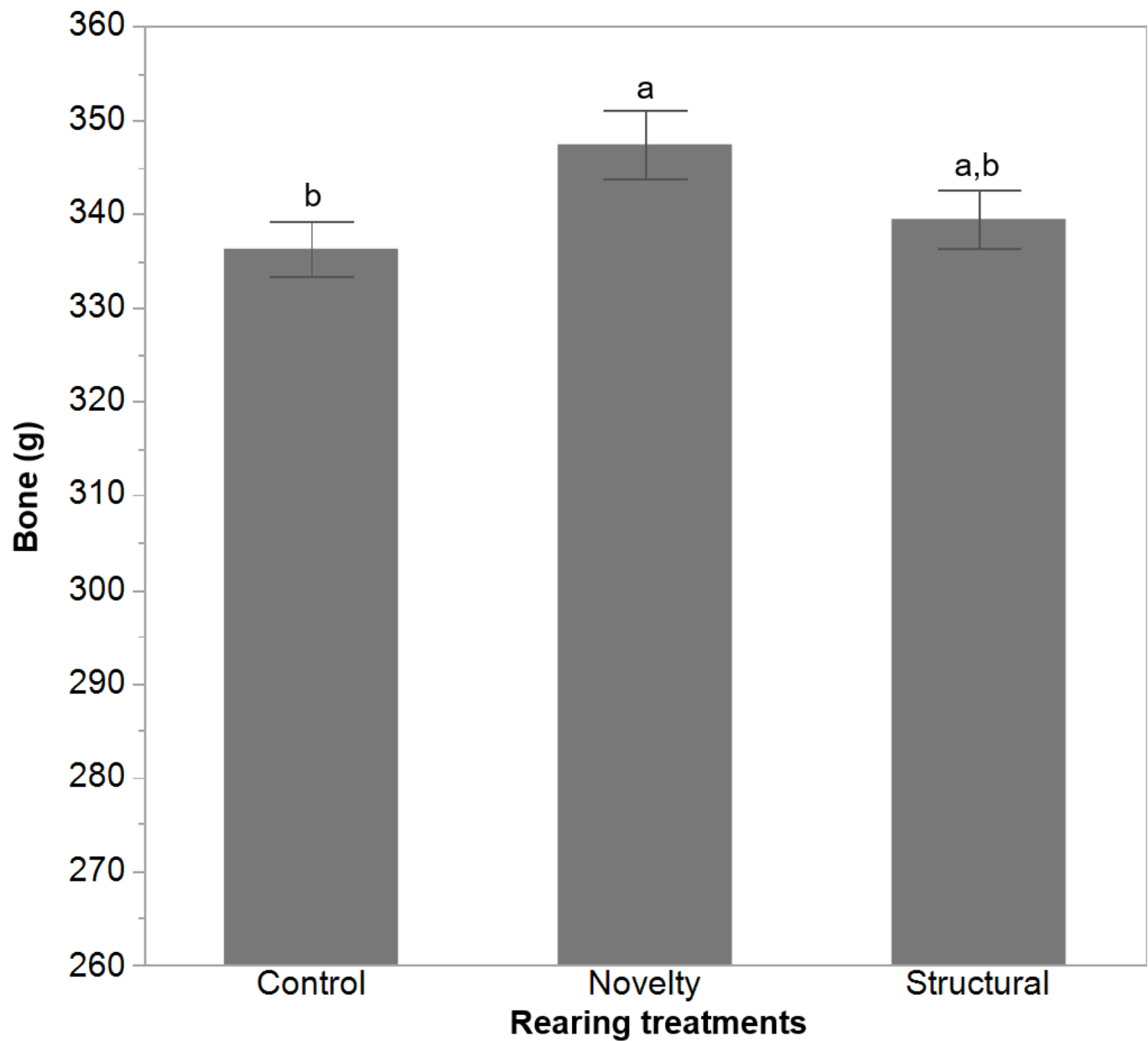



Figure 4

CT-scanned bone mass of hens from different rearing treatments.

The least squares means ± standard error of the means are presented for free-range hens from control, novelty or structural rearing treatments at 65 weeks of age. [a,b]Dissimilar superscript letters indicate significant differences between rearing treatments as identified by a post-hoc Student's t-test. Only the data from the hens that had no eggs present during scanning were considered (n = 220).

The welfare parameters of free-range hens at 64 weeks of age.

The least squares means ± standard error of the mean are presented for hens from different rearing treatments (control, novelty, structural) and ranging patterns (indoor, low outdoor, high outdoor). [a,b]Dissimilar superscript letters indicate significant differences between rearing enrichments or ranging patterns ($P < 0.05$). Raw values are presented with the analyses conducted on transformed data.

Variable	Category	Live weight (kg)	Feather score (out of 24)	Number of comb wounds	Nail length (cm)
Rearing enrichments	Control	2.01 ± 0.02	21.31 ± 0.15[b]	0.91 ± 0.13[a]	1.54 ± 0.02
	Novelty	2.01 ± 0.02	22.83 ± 0.14[a]	0.48 ± 0.12[b]	1.58 ± 0.02
	Structural	2.02 ± 0.02	22.57 ± 0.14[a]	0.68 ± 0.13[a,b]	1.53 ± 0.02
	P – value	0.90	< 0.0001	0.03	0.12
Ranging	Indoor	2.05 ± 0.02[a]	21.78 ± 0.15[b]	0.79 ± 0.13[a]	1.67 ± 0.02[a]
	Low outdoor	2.04 ± 0.02[a]	21.99 ± 0.14[b]	0.82 ± 0.12[a,b]	1.61 ± 0.02[b]
	High outdoor	1.95 ± 0.02[b]	22.94 ± 0.14[a]	0.47 ± 0.12[b]	1.37 ± 0.02[c]
	P – value	< 0.0001	< 0.0001	0.04	< 0.0001

The feather scores on different body parts for free-range hens.

The number and percentages of sampled hens within each group for each feather score category of six body parts (back of the neck, chest, back, wing, tail, vent) of free-range hens from different rearing treatments (control, novelty, structural) and ranging patterns (indoor, low outdoor, high outdoor) at 64 weeks of age. A score of 4 indicates the most feather coverage and a score of 1 the least, based on the scoring system of Tauson et al. (2005).

N = 95 for control, N = 109 novelty, N = 103 Structural, N = 94 indoor, N = 109 low outdoor and N = 104 for high outdoor groups. A '–' is given when no birds within any treatment group had that score.

* Chi-square tests were not performed due to insufficient data within each scoring group.

Feather location	Variable	Category	Damage score n (%)				χ^2, df, P
			1	**2**	**3**	**4**	
Neck (back only)	Rearing enrichments	Control	-	14 (14.74)	6 (6.32)	75 (78.94)	26.50, 2, < 0.0001
		Novelty	-	0 (0)	6 (5.50)	103 (94.5)	
		Structural	-	2 (1.94)	0 (0)	101 (98.06)	
	Ranging	Indoor	-	8 (8.52)	9 (9.57)	77 (81.91)	*
		Low outdoor	-	8 (7.34)	2 (1.83)	99 (90.83)	
		High outdoor	-	0 (0)	1 (0.96)	103 (99.04)	
Chest	Rearing enrichments	Control	-	49 (51.58)	13 (13.68)	33 (34.74)	6.88, 2, 0.03
		Novelty	-	36 (33.03)	23 (21.1)	50 (45.87)	
		Structural	-	37 (35.92)	26 (25.25)	40 (38.83)	
	Ranging	Indoor	-	45 (47.87)	20 (21.28)	29 (30.85)	33.26, 2, < 0.0001
		Low outdoor	-	58 (53.21)	18 (16.51)	33 (30.28)	
		High outdoor	-	19 (18.27)	24 (23.08)	61 (58.65)	
Back	Rearing enrichments	Control	-	7 (7.37)	27 (28.42)	61 (64.21)	55.43, 2, < 0.0001
		Novelty	-	0 (0)	1 (0.92)	108 (99.08)	
		Structural	-	1 (0.97)	9 (8.74)	93 (90.29)	
	Ranging	Indoor	-	2 (2.13)	11 (11.70)	81 (86.17)	*
		Low outdoor	-	2 (1.83)	17 (15.6)	90 (82.57)	
		High outdoor	-	4 (3.85)	9 (8.65)	91 (87.5)	
Wing	Rearing enrichments	Control	-	-	14 (14.74)	81 (85.26)	0.42, 2, 0.81
		Novelty	-	-	14 (12.84)	95 (87.16)	
		Structural	-	-	12 (11.65)	91 (88.35)	
	Ranging	Indoor	-	-	14 (14.89)	80 (85.11)	2.84, 2, 0.24
		Low outdoor	-	-	17 (15.6)	92 (84.4)	
		High outdoor	-	-	9 (8.65)	95 (91.35)	
Tail	Rearing enrichments	Control	-	-	19 (20.0)	76 (80.0)	4.70, 2, 0.10
		Novelty	-	-	11 (10.09)	98 (89.91)	
		Structural	-	-	13 (12.62)	90 (87.38)	
	Ranging	Indoor	-	-	19 (20.21)	75 (79.79)	5.13, 2, 0.08
		Low outdoor	-	-	14 (12.84)	95 (87.16)	
		High outdoor	-	-	10 (9.62)	94 (90.38)	
Vent	Rearing enrichments	Control	1 (1.06)	13 (13.68)	5 (5.26)	76 (80.0)	*
		Novelty	0 (0)	1 (0.92)	0 (0)	108 (99.08)	
		Structural	0 (0)	0 (0)	5 (4.85)	98 (95.15)	
	Ranging	Indoor	0 (0)	6 (6.38)	7 (7.45)	81 (86.17)	*
		Low outdoor	1 (0.92)	4 (3.67)	2 (1.83)	102 (93.58)	
		High outdoor	0 (0)	4 (3.85)	1 (0.96)	99 (95.19)	

The relative organ weights and worm counts of free-range hens.

The least squares means ± standard error of the mean of the percentages of relative organ weights and worm counts of free-range hens at 65 weeks of age from different rearing treatments (control, novelty, structural) and ranging patterns (indoor, low outdoor, high outdoor). [a,b]Dissimilar superscript letters indicate significant differences between rearing enrichments or ranging patterns ($P < 0.05$). Raw values are presented with the analyses conducted on transformed data.

Variable	Category	Liver weight (%)	Spleen weight (%)	Adrenal weight (%)	Gizzard weight (%)	Worms in GI tract (N)
Rearing enrichments	Control	2.59 ± 0.04	0.097 ± 0.002^a	0.003 ± 0.01	1.72 ± 0.03	4.52 ± 0.84
	Novelty	2.60 ± 0.04	0.089 ± 0.002^b	0.004 ± 0.01	1.67 ± 0.02	7.03 ± 0.79
	Structural	2.51 ± 0.04	0.092 ± 0.002^{ab}	0.025 ± 0.01	1.69 ± 0.03	5.66 ± 0.80
	Test statistics	$F_{(2,301)} = 2.23, P = 0.11$	$F_{(2,301)} = 4.82, P = 0.01$	$F_{(2,301)} = 1.82, P = 0.16$	$F_{(2,301)} = 0.85, P = 0.43$	$F_{(2,301)} = 2.30, P = 0.10$
Ranging	Indoor	2.58 ± 0.04	0.091 ± 0.002^b	0.004 ± 0.01	1.65 ± 0.03^b	4.88 ± 0.85
	Low outdoor	2.53 ± 0.04	0.089 ± 0.002^b	0.024 ± 0.01	1.69 ± 0.02^{ab}	5.59 ± 0.78
	High outdoor	2.60 ± 0.04	0.097 ± 0.002^a	0.003 ± 0.01	1.74 ± 0.03^a	6.74 ± 0.80
	Test statistics	$F_{(2, 301)} = 1.29, P = 0.28$	$F_{(2, 301)} = 4.44, P = 0.01$	$F_{(2, 301)} = 0.57, P = 0.57$	$F_{(2, 301)} = 3.22, P = 0.04$	$F_{(2, 301)} = 1.15, P = 0.32$

Keel bone defects of free-range hens.

The number and percentages of keel bone damage of free-range hens at 65 weeks of age from different rearing treatments (control, novelty, structural) and ranging patterns (indoor, low outdoor, high outdoor). Values of each category of damages are presented as n (%) but the number of calluses on tips of keels are expressed as least squares means ± standard error of mean (LSM ± SEM). For the number of calluses on tips, raw values are presented with the analyses conducted on transformed data.

Treatment	Category	N	Damages n (%)	Spine bending n (%)	Spine bending types n (%)			Callus formation n (%)	Number of calluses (LSM ± SEM)
					Low	Moderate	High		
Rearing enrichments	Control	91	71 (78.02)	57 (62.64)	46 (50.55)	7 (7.69)	4 (4.40)	40 (43.96)	0.71 ± 0.11
	Novelty	106	82 (77.36)	68 (64.15)	51 (48.11)	7 (6.60)	10 (9.43)	42 (39.62)	0.70 ± 0.10
	Structural	103	73 (70.87)	66 (64.08)	53 (51.46)	8 (7.77)	5 (4.85)	39 (37.86)	0.60 ± 0.10
Test statistics, df, P			$\chi^2 = 1.69, 2, 0.43$	$\chi^2 = 0.06, 2, 0.97$	$\chi^2 = 2.79, 2, 0.84$			$\chi^2 = 0.78, 2, 0.68$	$F_{(2, 295)} = 0.40, 0.67$
Ranging patterns	Indoor	92	68 (73.91)	57 (61.96)	42 (45.65)	9 (9.78)	6 (6.52)	39 (42.39)	0.72 ± 0.10
	Low outdoor	107	81 (75.70)	71 (66.36)	57 (53.27)	8 (7.48)	6 (5.61)	36 (33.64)	0.56 ± 0.10
	High outdoor	101	77 (76.24)	63 (62.38)	51 (50.50)	5 (4.95)	7 (6.93)	46 (45.54)	0.73 ± 0.10
Test statistics, df, P			$\chi^2 = 0.15, 2, 0.93$	$\chi^2 = 0.52, 2, 0.77$	$\chi^2 = 2.60, 2, 0.86$			$\chi^2 = 3.29, 2, 0.19$	$F_{(2, 295)} = 1.31, 0.27$