Final Report

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Effects of rearing environments on ranging, adaptation to stressors and health in free-range laying

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Project Summary

Project Title	*Effects of rearing environments on ranging, adaptation to stressors and health in free-range laying*
Project No.	2017-20
Date	Start: 30/04/2017 End: 23/03/2020
Project Leader(s)	Dana Campbell
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Project Aim	To determine effective enrichments during the rearing period for free-range hens that improve their behaviour, welfare, production, health, and response to environmental stressors.
Background	Rearing environments have impact on adult hen adaptation to and performance in the laying environment. Limited data are available on enrichments during pullet rearing for free-range laying hens and the effects they have on adult hen behaviour, welfare, and production. Ranging may improve hen welfare, but there are limited individual-based data. This study will determine the impacts of different types of enrichments during rearing for improving range usage, health, welfare, production and adaptation to stressors during the lay cycle. Additionally, the relationship between ranging and multiple health and welfare indicators across the lay cycle, including post-mortems will be assessed. These data will inform industry on strategies to implement during pullet rearing and the impacts of ranging on hen health.
Research Outcome	This research demonstrated the impacts of two types of enrichment strategies across the 16-week pullet rearing period on: ranging behaviour, production, response to an implemented stressor, and hen welfare. This research also demonstrated relationships between individual range usage patterns and multiple health and welfare measures.
Impacts and Outcomes	This research provides evidence for producers on the importance of optimising rearing environments for free-range hens and managing ranging patterns in adult hens.
Publications	5 manuscripts are published, 1 manuscript is submitted, 4 manuscripts are in preparation. 4 conference presentations have been delivered.

Executive Summary

In Australia, free-range layer pullets are typically reared indoors, but adult layers go outdoors, this mismatch might reduce adaptation in laying environments. Enrichments during rearing may optimise pullet development and subsequent welfare as adult free-range hens. In the outdoor environment, hens may have greater opportunities for exercise and natural behaviours which might contribute to improved health and welfare. But the outdoor environment may also result in greater exposure to parasites and pathogens. Individual variation in range use may thus dictate individual health and welfare. This study was conducted to evaluate whether adult hens varied in behaviour, welfare, production, and response to stressors due to rearing enrichments, and their external and internal health following variation in range use. A total of 1386 Hy-Line Brown® chicks were reared indoors across 16 weeks with 3 enrichment treatments including a control group with standard housing conditions, a novelty group providing novel objects that changed weekly, and a structural group with custom-designed structures to increase spatial navigation and perching. At 16 weeks of age the pullets were moved to a free-range system and housed in 9 identical pens within their rearing treatments. All hens were leg-banded with microchips and daily ranging was assessed from 25 to 64 weeks via radio-frequency identification technology. Daily production and laying locations were recorded from 18 to 64 weeks of age and all hens were assessed periodically for individual external welfare. At 40-42 weeks of age the range area was shrunk to induce stress and concentrations of albumen corticosterone were measured. At 64-65 weeks of age, 307 hens were selected based on their range use patterns across 56 days up to 64 weeks: indoor (no ranging), low outdoor (1.4.h or less daily), and high outdoor (5.2 – 9 h daily). The external and internal health and welfare parameters were evaluated via external assessment of body weight, plumage, toenails, pecking wounds, illness, and post-mortem assessment of internal organs and keel bones including whole-body CT scanning for body composition. The results showed that structural hens spent the longest time ranging and showed the greatest change in behaviour during the stressor period accompanied by a decrease in albumen corticosterone when other treatment groups showed an increase. Novelty hens used the large, elevated nest boxes the most but there were no differences in egg production between rearing treatments. The control hens had the lowest plumage coverage at the later ages. The high outdoor rangers had fewer comb wounds than the indoor hens, the shortest toenails, and the most feather coverage, but lower body weight than the indoor hens. High outdoor ranging decreased both body fat and muscle. Overall, rearing enrichment had long-lasting effects on hen behaviour and their responses to stressful changes in their environment. There were also effects on hen health and welfare at the later stages of the production cycle but subsequent range use patterns had the greatest impact. Providing enrichments during rearing and increasing range usage in adult hens may reduce feather pecking in flocks. It is recommended that producers be aware of how their pullets are reared and implement enrichment strategies if possible, such as providing perching structures. Further validation of these findings in commercial settings is warranted.

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Introduction

In many countries, the laying hen industry is making a transition away from conventional caged housing towards alternative systems that provide hens with more resources and space to accommodate their behavioural needs. Within Australia, free-range systems are increasingly prevalent as consumers believe these systems provide better hen welfare (Carey et al., 2017) and eggs are healthier and tastier (Bray and Ankeny, 2017). However, free-range systems provide hens a choice to range or remain indoors, and in some instances the use of the outdoor range can be low (Chielo et al., 2016). This potentially limits the benefits of this system and/or could reduce consumer satisfaction. There is also high individual variation in range use patterns. There is some evidence that higher use of the range area will improve plumage condition, footpad condition, and reduce toenail length (Rodriguez-Aurrekoetxea and Estevez, 2016: Campbell et al., 2017a). Ranging hens may have improved digestive and gut function over non-ranging hens as they ingest stones and grit that eventually contribute to heavier gizzard (Singh et al., 2016). Overall, there is minimal information on both external and internal health and welfare parameters of individual free-range hens that vary in their range use patterns.

For hens that are not reared with outdoor access, there is often a long period (weeks) for hens to become accustomed to the range area following first pop-hole opening. It may be stressful to enter the outdoor environment following 16 plus weeks of being inside (first pop-hole opening age varies between commercial producers). Some hens even choose to never exit to the range and these hens have been identified as more fearful than frequent range users (Campbell et al., 2016a; Hartcher et al., 2016). Variation in the free-range environment may also cause stress and requires these hens to be adaptable to change. Environmental stressors such as heat can reduce egg production and affect egg quality (Lin et al., 2004) and free-range systems in particular may be stressful for birds as the environments are unpredictable and less controlled, with multiple disease, nutritional, physical and behavioural challenges presented to the birds. This stress can likely impact the production and quality of eggs produced within a free-range system.

Rearing environments for pullets are important for optimal development, adaptability, and performance as adult hens (Janczak and Riber, 2015) with studies showing that hens will better adapt to the layer system if they are reared in a similar manner. In Australia, pullets destined for free-range systems are typically reared inside due to vaccination schedules and health risks associated with outdoor access, and the logistics of current shed designs which do not have outdoor ranges. Thus, pullets entering free-range systems may be at a disadvantage which could impact their range use, health, and welfare as adults. In the absence of feasible outdoor access options, enrichments in the rearing sheds could better prepare pullets for free-range housing. Enrichments may improve behaviour, welfare, adaptability, and production.

Objectives

The original objectives are listed out below, all objectives were met by the experiment and are presented in more detail in subsequent sections.

The **first objective** for this research was to: ‘Write a literature review on impacts of enrichments applied during laying hen rearing in all housing systems, including timing of these enrichments and a discussion of critical periods of physiological and neurophysiological chicken development'.

The **second objective** was to: 'Evaluate individual range usage of hens with or without exposure to an enriched rearing environment'.

The **third objective** was to: 'Evaluate flock production across the lay cycle with comparisons between hens with or without exposure to an enriched rearing environment'.

The **fourth objective** was to: 'Evaluate stress responses and adaptation of hens to stressor events with comparisons between hens with or without exposure to an enriched rearing environment'.

Finally, the **fifth objective** was to: 'Evaluate individual hens varying in range usage for multiple health and welfare indicators throughout the lay cycle, including post-mortem examination at conclusion of lay'.

Methodology

Objective 1: Literature review – published as *Campbell et al., 2019. Poultry Science.*

Summary: Globally, laying hen production systems are a focus of concern for animal welfare. Recently, the impacts of rearing environments have attracted attention, particularly with the trend towards more complex production systems including aviaries, furnished cages, barn, and free-range. Enriching the rearing environments with physical, sensory, and stimulatory additions can optimise the bird's development but commercial-scale research is limited. In this review, 'enrichment' is defined as anything additional added to the bird's environment including structurally complex rearing systems. The impacts of enrichments on visual development, neurobehavioral development, auditory stimulation, skeletal development, immune function, behavioural development of fear and pecking, and specifically pullets destined for free-range systems are summarised and areas for future research identified. Visual enrichment and auditory stimulation may enhance neural development, but specific mechanisms of impact and suitable commercial enrichments still need elucidating. Enrichments that target left/right brain hemispheres/behavioural traits may prepare birds for specific types of adult housing environments (caged, indoor, outdoor). Similarly, structural enrichments are needed to optimise skeletal development depending on the adult layer system, but specific physiological processes resulting from different types of exercise are poorly understood. Stimulating appropriate pecking behaviour from hatch is critical but producers will need to adapt to different flock preferences to provide enrichments that are utilised by each rearing group. Enrichments have potential to enhance immune function through the application of mild stressors that promote adaptability, and this same principle applies to free-range pullets destined for variable outdoor environments. Complex rearing systems may have multiple benefits, including reducing fear, that improve the transition to the layer facility. Overall, there is a need to commercially validate positive impacts of cost-effective enrichments on bird behaviour and physiology.

Environmental enrichment is a potential method for improving bird development. A frequently used definition of enrichment stated by Newberry (1995) is ‘improvement in the biological functioning of captive animals resulting from modifications to their environment’. This definition highlights the important distinction between environmental enrichment that has a demonstrable impact on the animals versus environmental change - modifications that lead to no quantifiable improvements but are anthropomorphically included (Newberry, 1995). Enrichments should have impact by increasing the performance of natural behaviour, reducing the incidences of abnormal and damaging behaviour, reducing negative emotional states, improving physical health and improving the use of the provided environmental resources (Newberry, 1995). To further build on this, and important for commercial poultry production, enrichments must also be economically and practically feasible. This includes having no adverse impacts on the animals, such as increasing rates of injury, posing hygiene risks (Van de Weerd and Day, 2009) or reducing consumption of formulated feed (e.g. a consumable pecking toy is eaten instead) which is needed to maintain high levels of production. Some types of ‘enrichment’ for layers may be considered as a basic necessity, such as litter to forage in, perches to roost on, and nest boxes to lay in. But these are not always present in all housing systems, and the conventional system for laying hens is a cage with no additional provisions. Thus, in comparison to a simple cage environment, even provisions of basic items could be considered as ‘enrichments’ in poultry production. Newberry (1995), also highlighted the importance of experiences during rearing periods, where modifications of certain behavioural traits may be more difficult, and enrichments less relevant, if applied after the ontogenetic periods in which certain behaviours may develop and mature (Gunnarsson et al., 2000; Johnsen et al., 1998; Rogers, 1993). Similarly, physiological benefits such as improved musculoskeletal strength are likely to be greater when environmental modifications are applied during growth and physical development.

Laying hens are descendants of the red junglefowl, and although the domestication process has both differentiated the modern laying hen from its ancestors and differentiated between strains of modern layers, the basic biology and behaviour of the fowl remains similar. Behaviourally, the needs, priorities, and preferences of the modern hen are to perch, nest, forage, and dust bathe (reviewed in Weeks and Nicol, 2006). Time engaged in some behaviours within commercial strains, such as foraging (Campbell et al., 2017b), are different to what has been documented in semi-wild populations of junglefowl, potentially resulting from different energetic investments by the high-producing modern layer (Dawkins, 1989; Schütz and Jensen, 2001). High levels of damaging behaviours such as feather pecking and cannibalism are also seen in alternative housing systems (Fossum et al., 2009; Singh et al., 2017; Weeks et al., 2016). This suggests the current environments are not meeting the needs of the birds, exacerbating the prevalence of undesirable behaviour. Similarly, the high rates of injuries such as keel fractures suggest that the modern hen is not physically suited to structurally complex housing, or that the artificial environment is not suitably designed based on the modern hens locomotor and flying skills (Campbell et al., 2016b; Wilkins et al., 2011), and that hens are potentially inadequately reared for such environments.

Currently pullets are reared in all types of housing systems including conventional cages, furnished cages, aviaries, and floor-based systems. The latter two systems may include covered or uncovered outdoor access and floor-based system may use litter, slats, and perches in varying configurations. Typically, day-old chicks are transferred from the hatchery to a rearing farm, and then transferred around 16 weeks to a similar variety of layer housing systems. Generally, producers will try to match type of rearing facility with the type of layer facility (Janczak and Riber, 2015) but this is not always possible. Complex rearing systems are commercially available which are designed to stimulate navigation and locomotor skills of the birds, and thereby improve muscle and bone strength of pullets by having different levels of height in the rearing system. However, cost of these systems may restrict their wide use and simple cost-effective enrichments could be particularly valuable to smaller-scale producers. Several countries have banned the use of conventional cages (e.g., Switzerland from 1992, Austria from 2009, European Union from 2012), and producers are phasing out cages to meet consumer demands for perceived more welfare-friendly eggs. Thus, there is an increasing use of alternative rearing and layer systems that both allow and require more from the birds in terms of physical effort and/or behavioural capabilities than a conventional caged system. Improving animal welfare is a key focus for both quality of life for the birds and for improvements in their health and productivity. Enrichments during rearing offer the potential to enhance bird development to suit alternative systems. Some current welfare standards such as the Australian and UK RSPCA pullet rearing standards (RSPCA Australia 2015; RSPCA UK 2016) require pecking enrichments, perches and litter or accessible ground area and many producers may already implement enrichments that they have found to be or are believed to be beneficial to the birds. A critical evaluation of tested enrichments within the literature and identification of gaps in knowledge for future research is necessary to identify enrichment schemes which have quantifiable impact on bird behaviour and health for improved welfare.

This review focussed on the sensory, physical, and behavioural development of laying hen pullets and how enrichments can impact in these areas of biology. Enrichments are of value for improving the health, behaviour and welfare of layer pullets. However, numerous areas where future research is needed were identified. The following provides a summary of the main findings and need for further study.

- Vision is a critical sense for chickens and appropriate visual development depends on lighting environments. Chicks will attend to multiple visual parameters such as colour, and patterns and prefer moving over static images. However, static patterns and colours could be a simple way of providing visual stimulation where moving images are not feasible. Different structural-shaped areas may stimulate neural development but the precise visual enrichments for optimal brain growth still requires further research. Robotic birds which provide both visual and potentially auditory stimulation are an avenue for future study.
- Chickens have high cognitive capabilities with lateralised brain development. There are defined critical periods of brain development within the first 2 weeks of life. Enrichment strategies that target specific hemispheres/behavioural traits could prepare birds to be more suited to different types of adult housing environments (caged, indoor,

outdoor). Birds that are more competent in spatial navigation will likely be more confident within complex housing systems (e.g. tiered aviaries). The role of light during incubation, subsequent brain lateralisation, and impacts on bird behaviour needs to be studied further.

- Chicks hatch with full auditory capabilities but are limited in species-specific development by the absence of the mother hen and loud surrounding noises of rearing facilities. Sound playbacks such as via radio may have positive impacts but the mechanisms by which auditory enrichment operates (masking other noises, habituating birds to noise, neurological development) requires clarification and the impacts of maternal vocalisations needs further study.
- Structural enrichments such as perches or elevated tiers are necessary for optimal skeletal development, but provision of such physical enrichments needs to be made in a way to avoid bird injury (e.g. soft perch material, ramps). Different types of exercise (e.g. running, jumping, flying) can impact bones in different ways, but more research across the developmental period is needed to identify potential critical periods of bone growth. The type of structural enrichments provided will depend on the layer housing system birds are destined for. Aviary housing generally permits flight, thus birds will need opportunities to develop their wing bones during the rearing period.
- Enriched housing environments can have positive impacts on immunocompetence but the precise mechanisms behind these impacts are currently poorly understood due to limited research in this area. Further investigation into the potential for enhancing immune responses through application of mild stressors such as novel objects is warranted. Overall, catering to the birds behavioural needs (dust bathing, perching, foraging) will improve well-being and will likely result in a bird that is able to respond better to infection.
- Reduction in fear through habituation to novelty/environmental change will have positive impacts on bird behaviour and adaptation to the layer facility. Complex rearing systems may also act to reduce fear but additional commercial studies are warranted.
- The environment in the first 2-4 weeks of development can have long-lasting impacts, thus provision of pecking enrichments either in addition to litter, or particularly where litter is not a practical option is recommended. Provision of pecking stimulation will reduce the development of gentle and severe feather pecking behaviour. Producers may need to adapt to different flock preferences to provide enrichments that will be used by each current rearing group. Consideration of the layer housing environment needs to be made to ensure birds that are provided pecking enrichment during rearing are also supplied pecking enrichments during lay to avoid frustration and feather pecking onset.
- Pullets destined for free-range systems may require different/greater enrichment effort given the disparity between their rearing and layer housing environment. More research is needed to identify the best practice methods where outdoor access during rearing is not feasible.
- The current adult housing environment is still critical for optimal flock behaviour, health and welfare and attention needs to be paid to enrichments during the layer phase too.

2.0 General Methodology for Experimental Trials

2.1.1 Ethical statement

All research was approved by the University of New England Animal Ethics Committee (AEC17-092).

2.1.2 Animals and pullet housing

The study was conducted in the Kirby Poultry facility and the Laureldale free-range facility of the University of New England, Armidale, Australia. A total of 1700 Hy-Line® Brown day-old layer chicks were housed within 9 floor pens (6.2 m L x 3.2 m W) across three separate rooms. Each pen contained rice hulls as a litter substrate, water nipples, and round feeders provided in accordance with the current Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002). Water and commercially mixed mash formulated for specific growth stages was provided *ad libitum*. The pullets were reared for 16 weeks at this facility and exposed to three different rearing treatments with a treatment replicate within each room, balanced for location. The enrichment treatments were: 1) a control treatment with no additional enrichments, 2) a novelty treatment (various novel objects were added/removed approximately weekly from four days of age including balls, bottles, brooms, buckets, disks, ropes, chain, cinder blocks, containers, dog toys, milk jugs, plastic kids toys, pipes, strings) and 3) a structural treatment. This structural treatment included four custom-designed perching apparatuses constructed of metal, painted black and coated in a non-slip covering (L, W, H = 0.60 m). They had two solid sides and one open-framed side forming an H-shaped structure that could be placed in different orientations. Space was available underneath the structures for equal floor density in all treatments. Shade cloth was hung on the wire pen dividers to visually isolate birds and enrichments. At 16 weeks of age bird density was approximately 15 kg/m^2 with a total of 174-190 birds per pen (pen variation resulted from both chick mortality and some placement error). The rooms were mechanically ventilated with heating as needed but no cooling system. Chicks and pullets were vaccinated to meet both regulatory requirements and standard recommendations for the region (Newcastle disease, Marek's disease, fowl pox, fowl cholera, egg drop syndrome, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, infectious bronchitis, infectious larngotracheitis, and avian encephalomyelitis).

2.1.3 Layer housing

At the end of rearing, 16-week old pullets were transferred to the Laureldale free-range facility and remixed within pen replicates of the rearing treatments across 9 pens within a single shed (3 pen replicates per rearing treatment). The indoor pens were of the same configuration (Figure 1) and visually isolated via shade cloth. Each pen contained nest boxes, perches, feeders, and water nipples to fulfil the requirements of the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002). The shed was fan-ventilated with no temperature or humidity control.

The 9 indoor pens were each connected to an outdoor range area (Figure 1) accessible via two pop-hole openings (18 cm W x 36 cm H) and visually isolated from each other via shade cloth on the wire fences. Automatic pop-holes were first opened at 25 weeks of age (May

2018) allowing daily access to the hens for most of the daytime. The pop-holes opened at 9:15 am and closed after sunset daily. This equated to approximately 9 hours of available ranging time across winter followed by approximately 11 hours of available ranging time after daylight saving time started (October 2018).

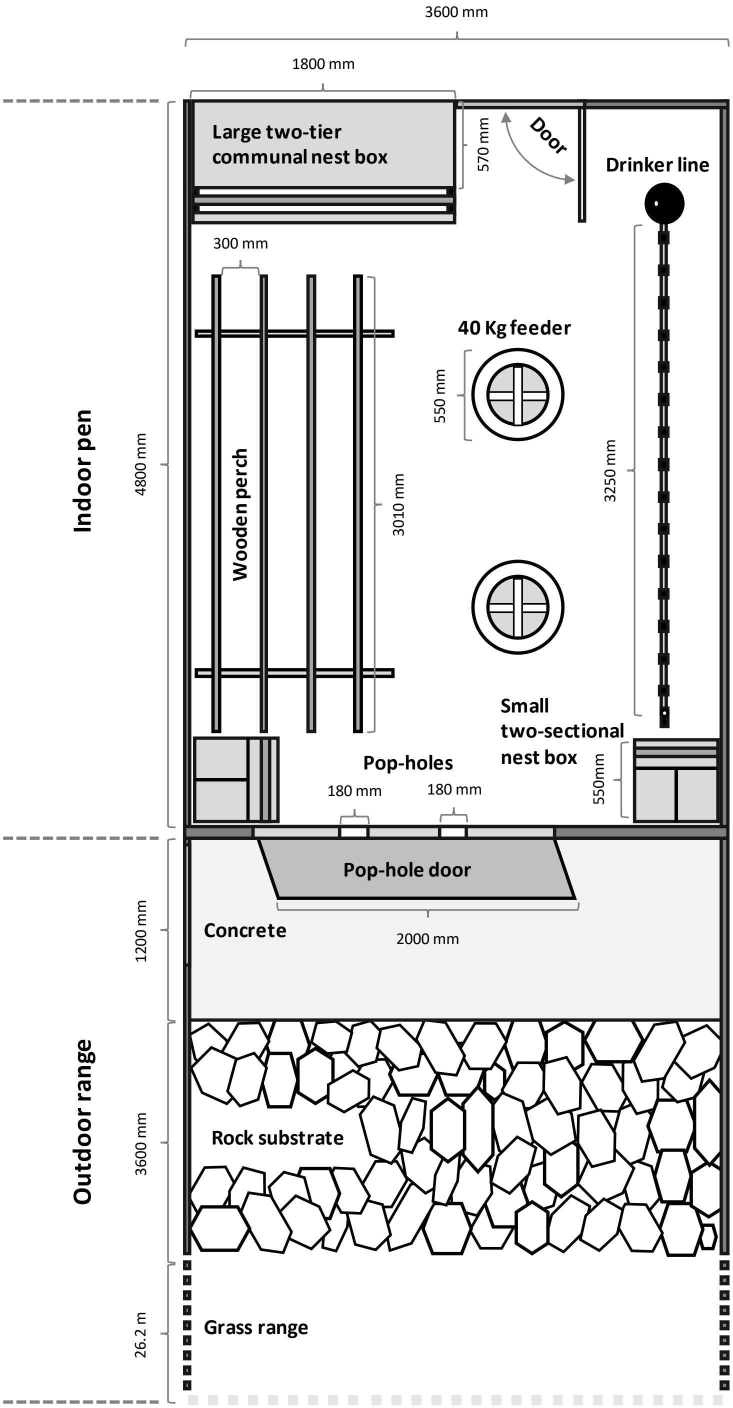

Figure 1: Top-down view of the indoor pen and outdoor range showing placement and dimensions of the indoor perch, nest box, water, and feed resources, the range access pop-holes, and different range substrates. Each of the 9 pens had identical indoor configuration except for 3 pens which had a radio-frequency identification box in the front right corner that the small nest box sat upon (the small nest boxes were elevated by cinder blocks in the remaining pens).

2.2 Objective 2: Range use – submitted as *Campbell et al., 2020. Frontiers in Veterinary Science.*

2.3.1 Radio-frequency identification (RFID) system and data

Before transfer to the laying facility, all hens were banded with microchips (Trovan® Unique ID 100 (FDX-A operating frequency 128 kHz) glued into adjustable leg bands (Roxan Developments Ltd, Selkirk, Scotland). Radio-frequency identification (RFID) systems were set up in the indoor pens. The RFID system recorded the date and time of each banded bird passing through the pop-hole 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 64 weeks of age.

Daily RFID data of individual hens from 25 until 64 weeks of age (272 days) were grouped into six time periods comprising: 25-27 weeks, 27-31 weeks, 31-38 weeks, 38-44 weeks, 47-54 weeks, and 55-64 weeks of age. Due to technical malfunction, unforeseen circumstances, and experimental interventions (e.g. weighing days and a stressor period as part of a separate dataset), some days of data were excluded resulting in a total of 232 days analysed across the 272-day recording period. Once grouped, the data were 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. The same program summarised the daily data to provide an average of hours outside, number of visits outside, maximum individual visit time, and total percentage of available days accessed per individual hen.

2.3.2 Albumen sampling

At 24 weeks of age, 4 days before the hens were provided outdoor access for the first time, a total of 50 eggs from each pen were randomly selected in the morning across all laying locations (floor, small, and large nest boxes). Substantially dirty eggs were not included. The same number of eggs was collected again 7 days following initial range access. On the day of collection, all eggs were weighed, broken open, the albumen was separated from the yolk then weighed and stored at -20° C until processing via radioimmunoassay following procedures reported in Downing and Bryden (2008). All albumen corticosterone analyses were conducted blind to rearing treatment.

2.4 Results

2.4.1 Albumen corticosterone

There was a significant interaction between rearing treatment and range access on the concentrations of albumen corticosterone ($F_{(2,889)} = 3.27$, $P = 0.04$) with the structural hens showing a higher corticosterone concentration prior to range access, but all treatment groups had similar elevated concentrations following first range access (Figure 3). There was also a significant effect of range access with hens across all treatments showing elevated concentrations of albumen corticosterone 1 week following first range access ($F_{(1,889)} = 121.68$, $P < 0.0001$, Figure 2).

2.4.2 Ranging behaviour

There was no effect of rearing treatment on the daily hours outside across the first two weeks of range access ($F_{(2,633.5)} = 1.10$, $P = 0.33$), but there were significant effects of rearing treatment for each age period for the remainder of the flock cycle ($F_{(2,1356\text{-}1381)} = 4.53\text{-}20.10$, $P \leq 0.01$) with the hens from the structural rearing treatment spending the most time outside at each age period (Figure 3). There was no significant effect of rearing treatment on the number of daily visits to the range at 25-27, and 27-31 weeks of age ($F_{(2,1340\text{-}1381)} = 1.32\text{-}1.97$, $P \geq 0.27$). For the remaining time periods, there was a significant effect of rearing treatment ($F_{(2,1356\text{-}1379)} = 10.06\text{-}16.70$, $P < 0.0001$) with the novelty hens showing the fewest visits (Figure 4). There were no significant effects of rearing treatment on the maximum visit duration at ages 31-38 weeks and 38-44 weeks ($F_{(2,1227)} = 2.10 – 2.56$, $P \geq 0.08$, Figure 5) but there were significant effects of rearing treatment for the four other age periods ($F_{(2, 754\text{-}1258)} = 3.89 - 27.70$ $P \leq 0.02$) with typically the enriched hens (novelty and structural) both showing longer maximum visit times than the control hens (Figure 5).

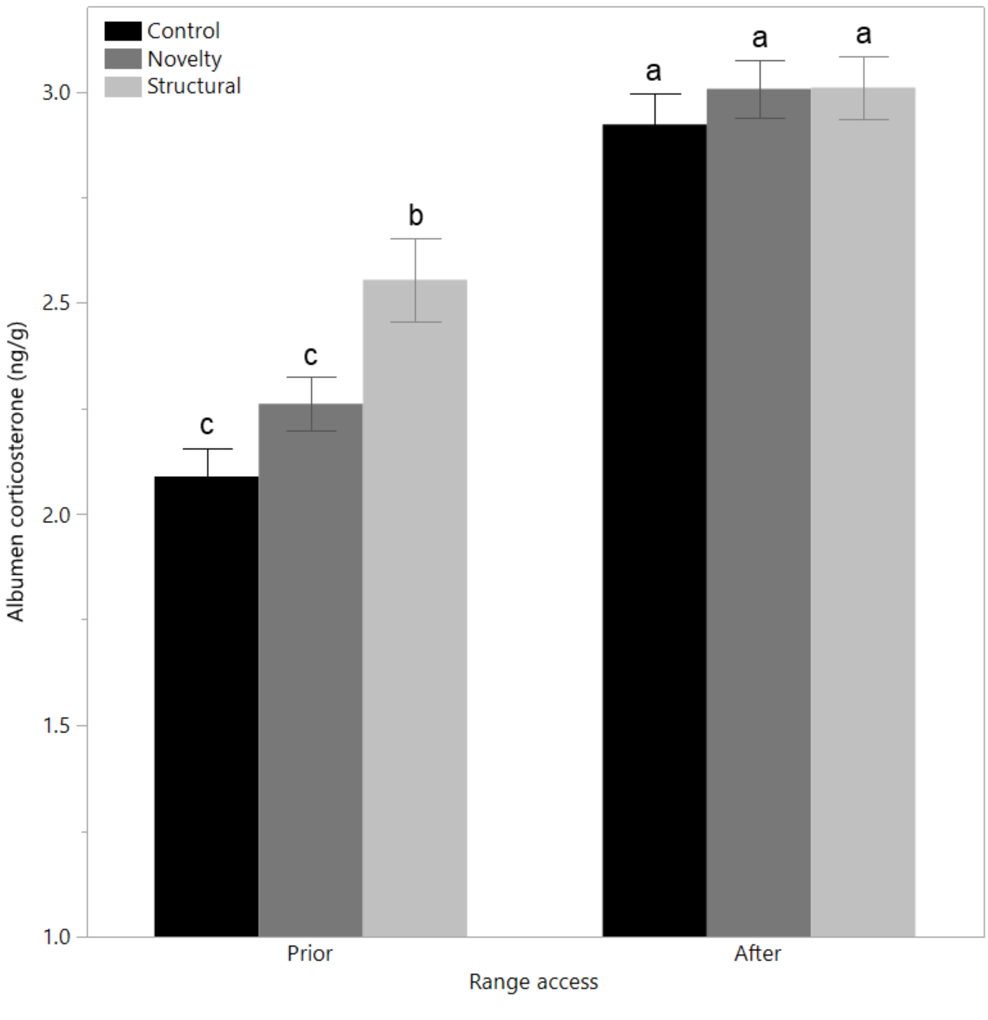

Figure 2: The mean (±SEM) corticosterone concentrations (ng/g) of egg albumen from hens exposed to three rearing treatments (control, novelty, structural) sampled 4 days prior to and 7 days after first range access. [a,b,c] Dissimilar superscript letters indicate significant differences across rearing treatments and range access.

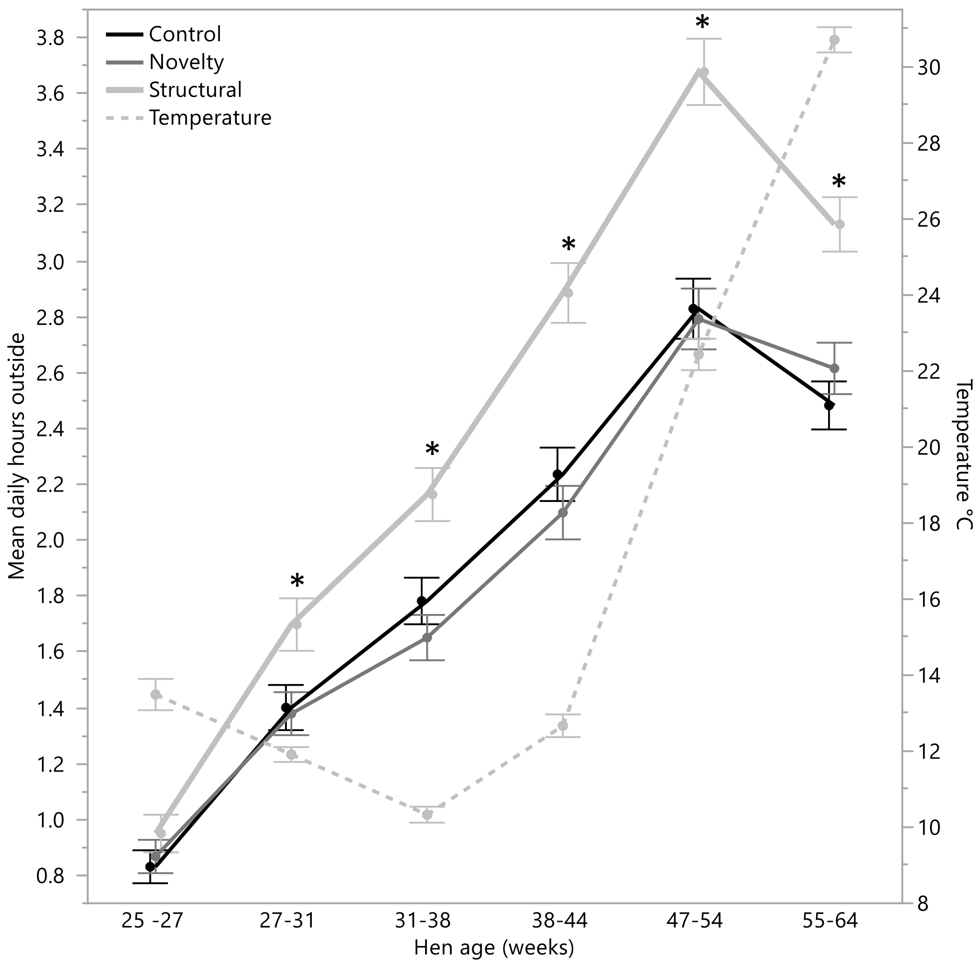

Figure 3: The mean (±SEM) daily hours spent outside on the range for hens from three rearing treatments (control, novelty, structural) across hen age periods. The mean daily temperature during ranging hours is also plotted. Asterisks indicate the structural hens differed significantly from the control and novelty hens across 5 of the 6 age periods.

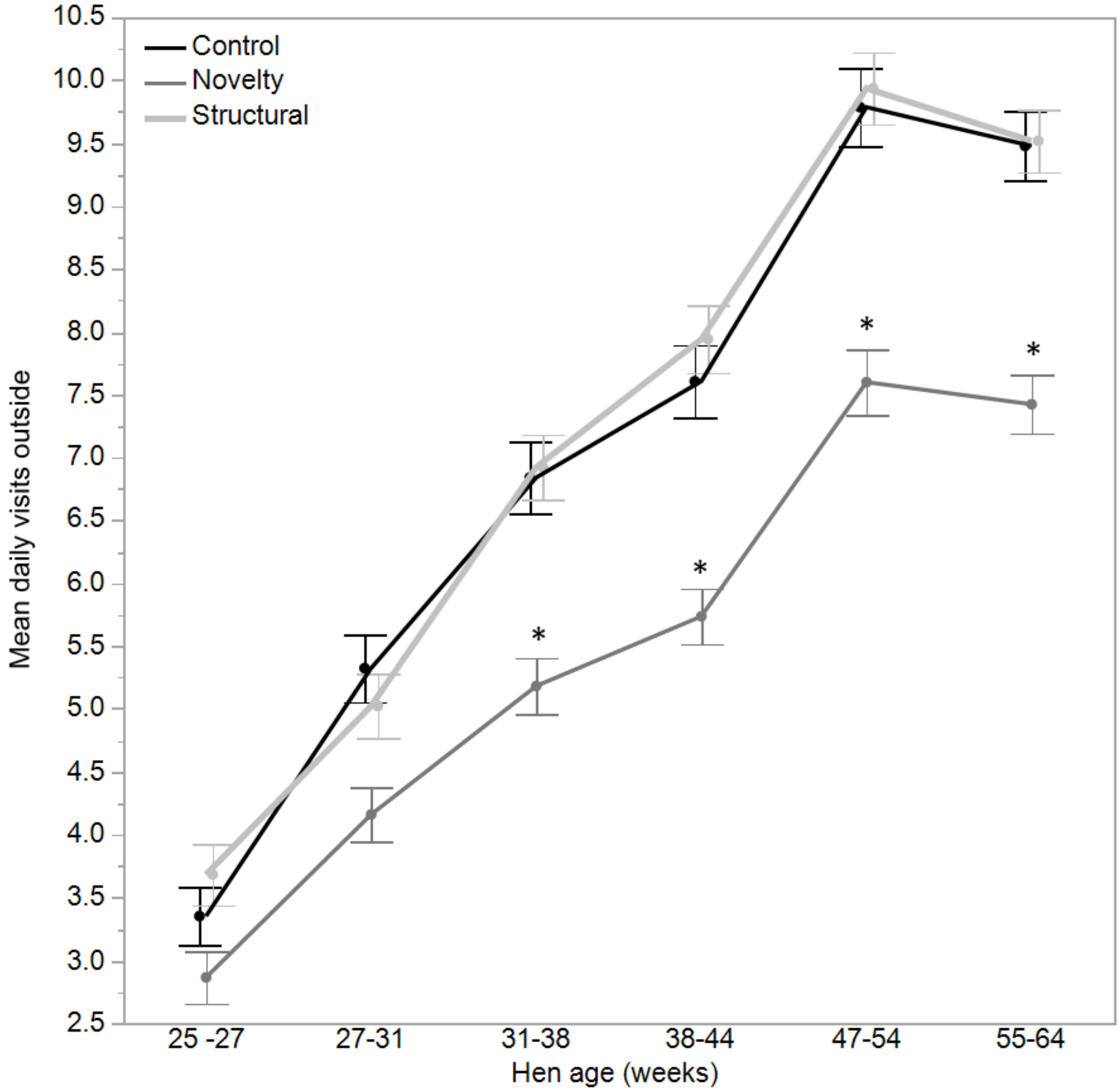

Figure 4: The mean (±SEM) daily visits to the outside range for hens from three rearing treatments (control, novelty, structural) across the periods of hen age. Asterisks indicate the novelty hens differed significantly from the control and structural hens across 4 of the 6 age periods.

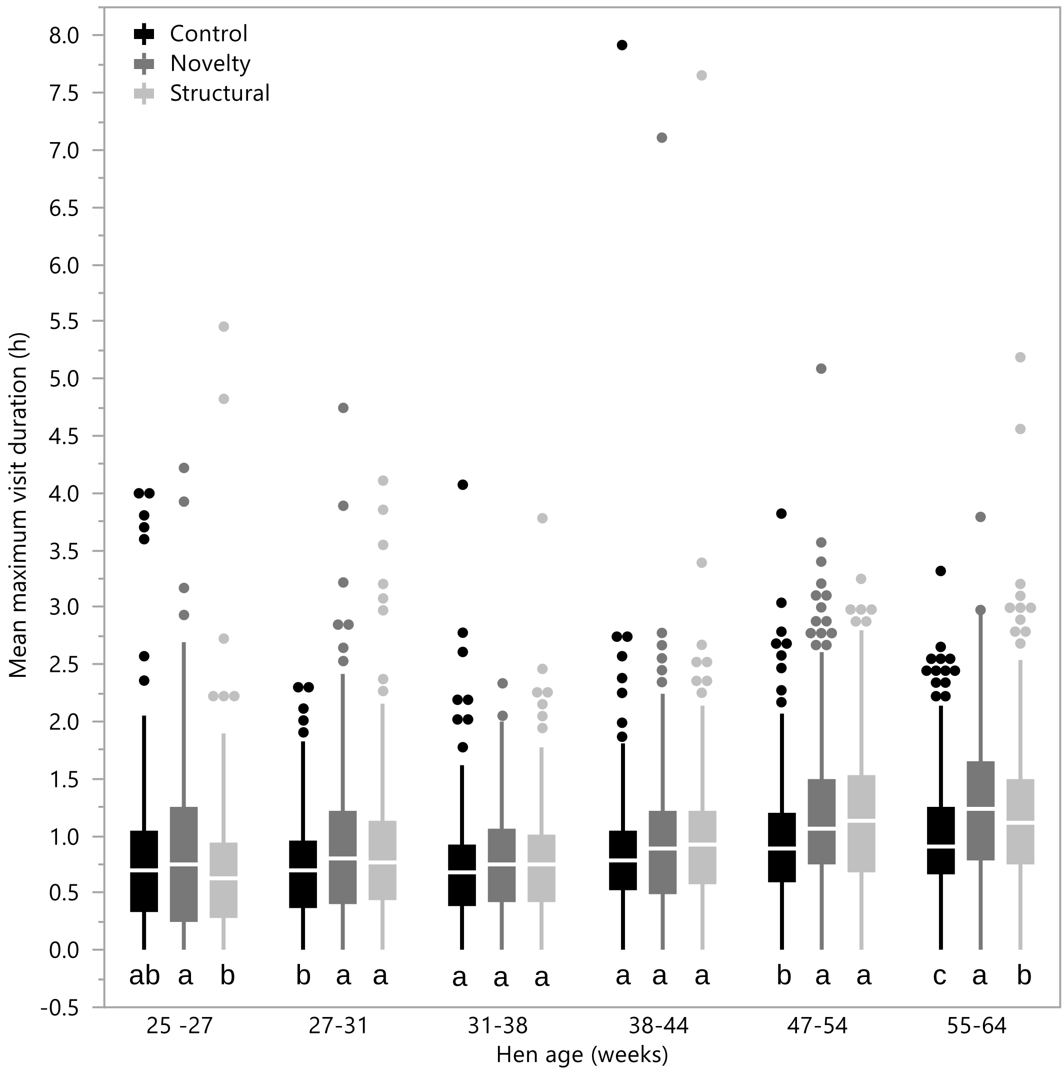

Figure 5: The mean maximum daily visit time outside for hens from three rearing treatments (control, novelty, structural) across the periods of hen age (weeks). Box ends represent the 1st and 3rd quartile with whiskers extending to data within 1.5 x the interquartile range or upper and lower data points (excluding outliers) if the data do not reach the computed ranges. Isolated data points indicate outliers. [abc] Dissimilar superscript letters indicate significant post-hoc differences between rearing treatments for each age period.

3.0 Objective 3: Egg production – published as *Bari et al., 2020. Animal*

3.1 Hen-day production

From 18 to 64 weeks of age, daily egg production for each of the nine pens was recorded after manual collection each morning. Hen-day production was calculated taking into account any mortality across the lay cycle. The location the birds laid in was also recorded. The locations were initially floor-laid eggs and large nest box only. Several common practices of encouraging pullets to lay in the nest boxes were implemented as the pullets came into lay. As the first eggs were laid on the floor behaviour modification took place with 5 randomly selected birds from each pen placed into the large nest box each morning for 10 days starting at 18 weeks of age. At 20 weeks, to encourage large nest box usage, dummy eggs were zip-tied into each nest box and personnel arrived at lights on and walked through all pens multiple times across approximately 1.5 hours (for 5 days) placing 5 birds into the large nest boxes for each rotation. Finally, at 25 weeks of age, additional nesting areas, the two small nest boxes, were added to each pen. From 26 weeks of age onwards, the location laid was recorded as floor, small nest box, and large nest box. Eggs were rarely found on the outdoor range (110 across the trial duration) and if this occurred, they were added to the floor egg total.

The average weight of all eggs per pen laid on a single day was measured weekly. The eggs were also visually scored for external shell quality as 'normal' or 'abnormal' which included: pimpled, misshapen, rough-shelled, soft-shelled, elongated, or visibly large or small (generally double yolk or no yolk eggs). These large and small eggs were those that were distinctly separate from the average egg size and were not included in any egg weight measurements (nor were soft-shelled, misshapen, or elongated eggs).

3.1.2 Egg quality

The external and internal quality parameters of individual eggs produced from the laying hens of each pen were evaluated at 44, 52, 60, and 64 weeks of age. A total of 270 eggs (30 from each pen) were collected at each of the age points and individually assessed for egg weight, shell reflectivity, breaking strength by quasi-static compression, shell deformation to breaking point, albumen height, Haugh unit, yolk colour score, shell weight and shell thickness (egg quality equipment, Technical Services and Supplies (TSS), Dunnington, York, UK). The yolk colour was measured digitally as corresponding to the DSM YolkFan (TSS equipment). Eggs were randomly selected on the day of collection but included eggs from all laying locations in the pen. Eggs that were substantially dirty were not included. The eggs were unwashed but free of litter debris for all measures. Following the internal quality measurements, the empty shells were then washed and left to dry for 24 h. Thickness of the dried shells was measured at the eggshell equator in three places using a custom-made gauge based on a Mitutoyo Dial Comparator gauge (Model 2109–10). All the measurements of eggs (except for eggshell thickness) were made on the day of collection by personnel blind to the rearing treatment of the birds.

3.2 Results

3.2.1 Hen-day production

There was a significant interaction between rearing treatment and egg-laying location from weeks 18-25 ($F_{(2,54)} = 81.84$, $P < 0.0001$) with the novelty hens laying the most eggs and the control hens the fewest eggs in the nest box (Figure 6). There was a significant interaction between hen age and laying location ($F_{(3,54)} = 55.04$, $P < 0.0001$) with the fewest eggs laid in the nest during weeks 18-19 and the most during weeks 22-25. As expected, the overall number of eggs laid increased with age ($F_{(3,54)} = 753.81$, $P < 0.0001$). There was no overall effect of rearing treatment ($F_{(2,6)} = 3.71$, $P = 0.09$). There was no interaction between hen age and rearing treatment ($P = 0.64$), or hen age, rearing treatment, and egg laying location ($P = 0.65$) so these were removed from the final model.

For hen-day production data from weeks 26-64, there was a significant interaction between rearing treatment and egg laying location ($F_{(4,228)} = 114.87$, $P < 0.0001$, Figure 7) with the novelty hens laying more eggs in the large nest boxes and fewer eggs on the floor than both the control and structural hens (Figure 7). Overall, hens from all groups used the small nest boxes the most ($F_{(2,228)} = 758.76$, $P < 0.0001$, Figure 3). There was also a significant interaction between hen age and egg laying location ($F_{(18,228)} = 9.44$, $P < 0.0001$) with use of the large nest box steadily declining across age compared to more consistent use of the small nest box. As expected, laying decreased towards the end of the production cycle ($F_{(9,228)} = 12.36$, $P < 0.0001$) with all groups reaching peak production in the period of 26-29 weeks of age (mean ± SEM, 92.96 ± 0.92%). There were no significant interactions between hen age and rearing treatment ($P = 0.98$), or hen age, rearing treatment, and egg laying location ($P = 0.98$), so these were removed from the final model.

There was no effect of rearing treatment on the average weekly egg weights ($F_{(2,6)} = 0.20$, $P = 0.82$) but egg weight did increase in size with age as expected followed by a decline towards the end of the production cycle ($F_{(8,379)} = 376.11$, $P < 0.0001$, Table 1). There was also no significant effect of rearing treatment on the proportion of abnormal eggs ($F_{(2,6.05)} = 2.22$, $P < 0.19$) but there was an increase with hen age across all treatments ($F_{(8,351.3)} = 57.06$, $P < 0.0001$, Table 1). There were no significant interactions between rearing treatment and hen age for egg weight ($P = 0.99$) or the proportion of abnormal eggs ($P = 0.87$) and these were removed from the final models.

3.2.2 Egg quality

There was a significant interaction between hen age and rearing treatment for egg shell reflectivity ($F_{(6,\ 1062)} = 2.50$, $P < 0.02$, Figure 8). The eggs from control hens had a comparatively greater decrease in shell colour at 60 and 64 weeks of age than the eggs from the novelty and structural groups (Figure 8). All eggs had darker shells at 52 weeks of age ($F_{(3,1062)} = 23.10$, $P < 0.0001$), Figure 8). There was also a significant interaction between hen age and rearing treatment for yolk colour score ($F_{(6,1062)} = 4.90$, $P < 0.0001$) where the eggs from control hens showed a decrease in colour score (paler yolks) after 44 weeks of age (Figure 9). All eggs had the palest yolk colour score at 60 weeks of age ($F_{(6,1062)} = 4.90$, $P < 0.0001$, Figure 9).

There was no effect of rearing treatment on egg weight, albumen height, Haugh unit, breaking strength, shell deformation, shell weight, or shell thickness ($P \geq 0.21$, Table 2). However, there were significant effects of age on all those egg quality parameters with all measurements decreasing across hen age (all $P < 0.0001$, Table 2). There were no interactions between hen age and rearing treatment for any of these egg quality parameters (all $P \geq 0.06$) and these was removed from the final models.

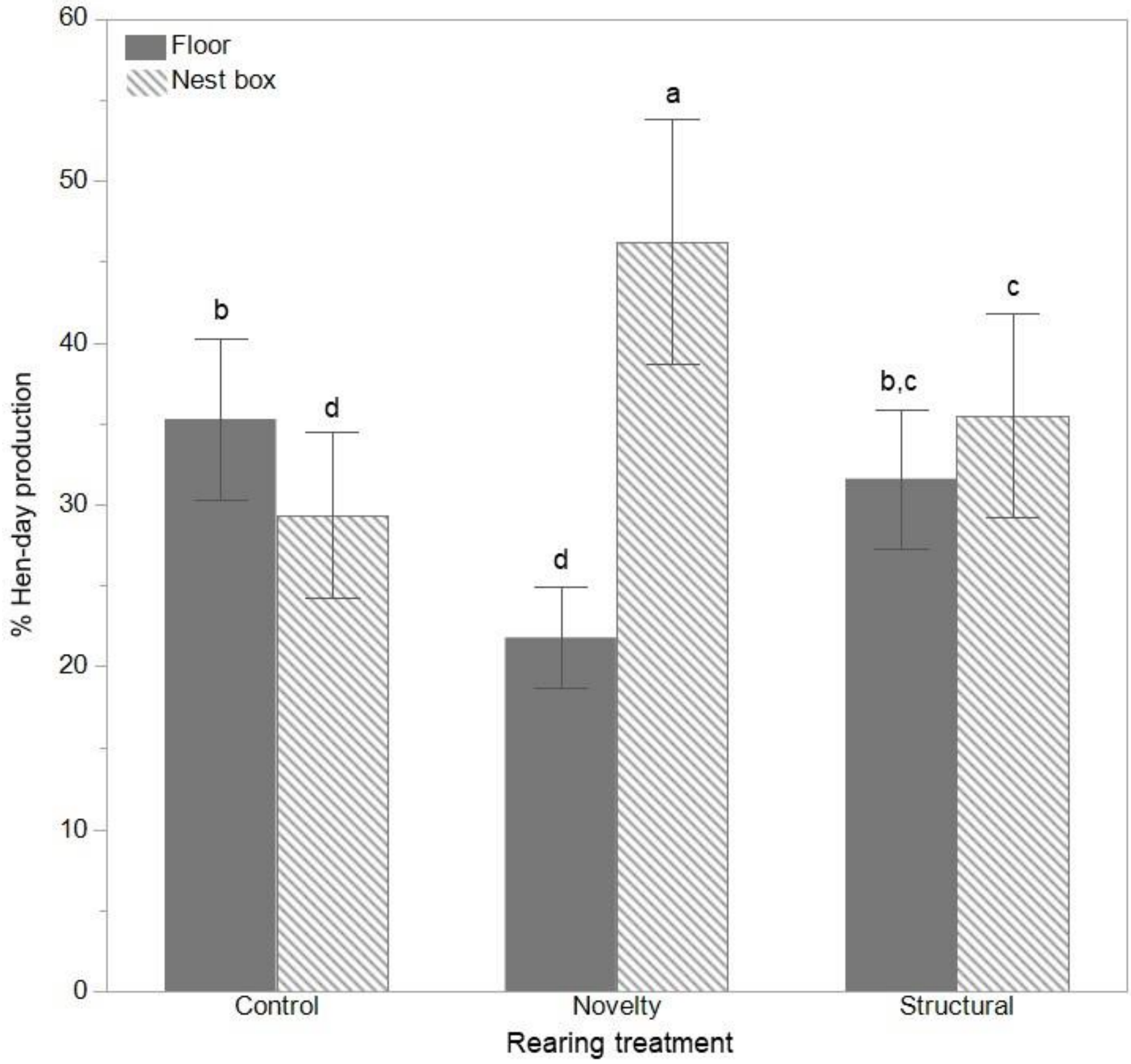

Figure 6: The mean ± standard error of the mean (SEM) of percentage hen-day production data from 18-25 weeks of age within two different laying locations (floor, nest box) by hens from three different rearing treatments (control, novelty, structural). [a-d] Dissimilar superscript letters indicate significant differences between laying location and rearing treatment ($P < 0.008$). Raw data are presented with analyses conducted on transformed data.

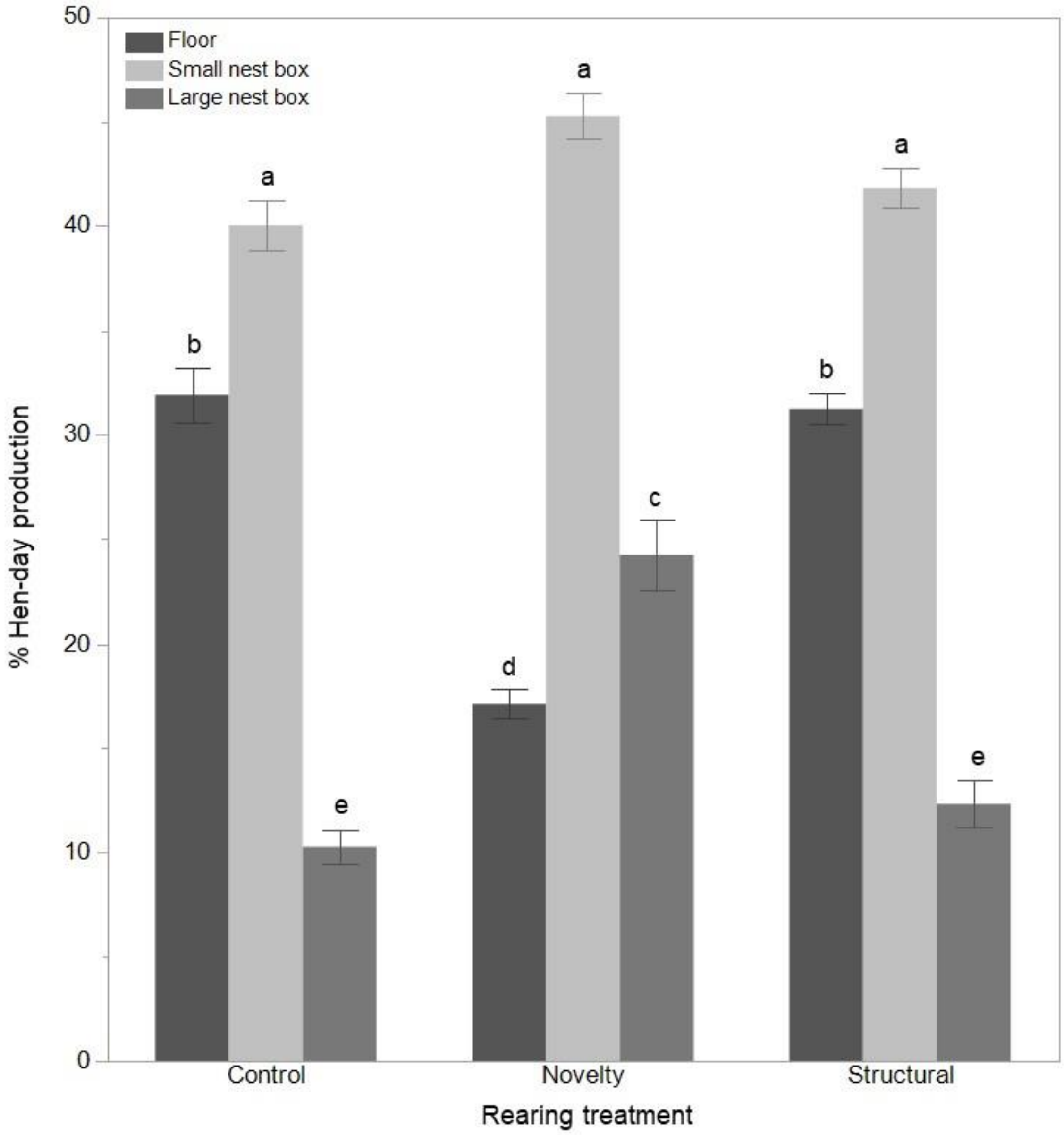

Figure 7: The mean ± SEM of percentage hen-day production data from 26-64 weeks of age within three different laying locations (floor, small nest box, large nest box) by hens from three different rearing treatments (control, novelty, structural). Raw data are presented with analyses conducted on transformed data.

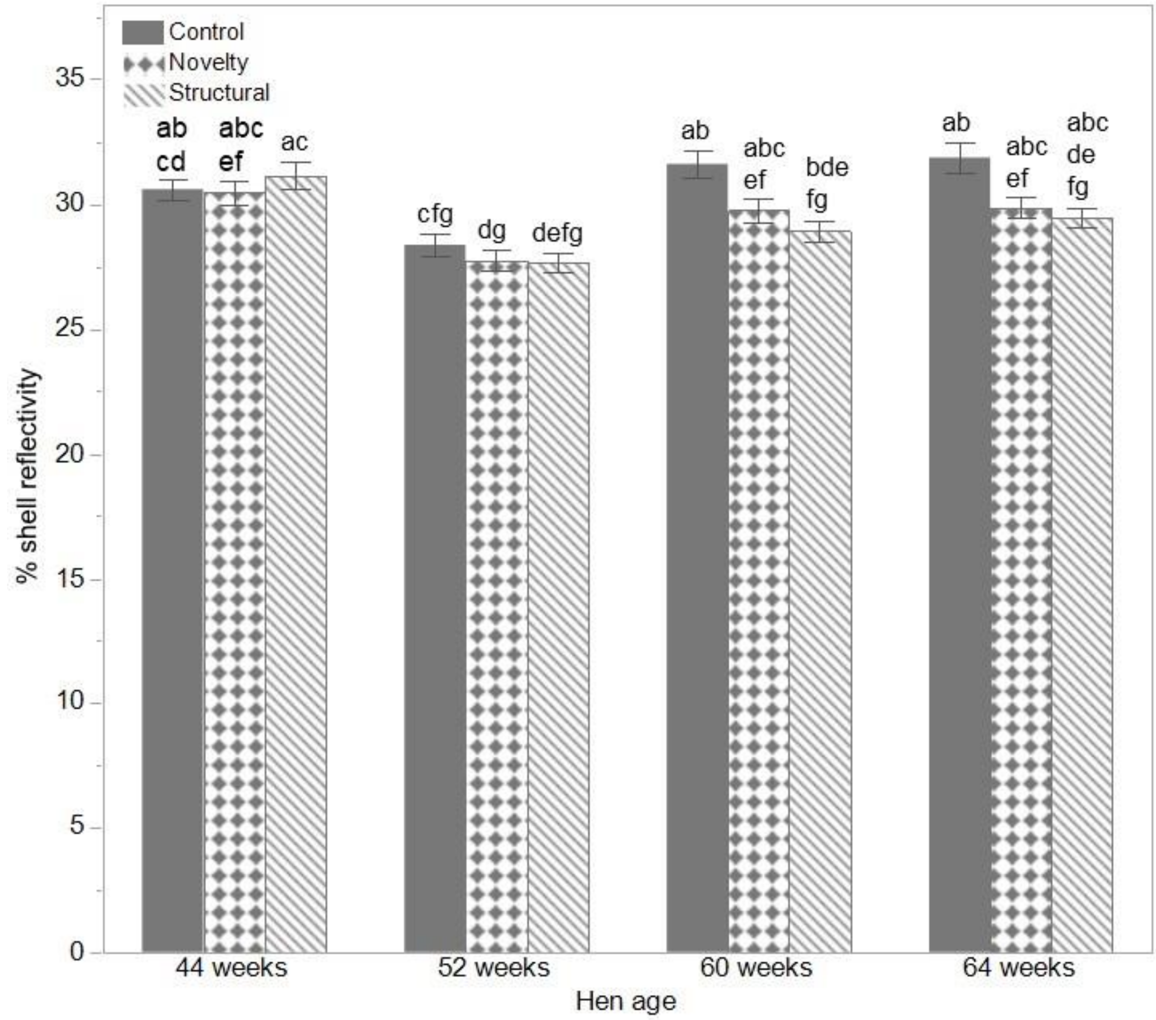

Figure 8: The mean ± SEM of percentages of egg shell reflectivity of hen eggs from different rearing treatments (control, novelty, structural) at different age points (44, 52, 60, and 64 weeks of age). [a-g] Dissimilar superscript letters indicate significant differences between rearing treatments and hen age (P < 0.006). Raw data are presented with the analysis conducted on transformed data.

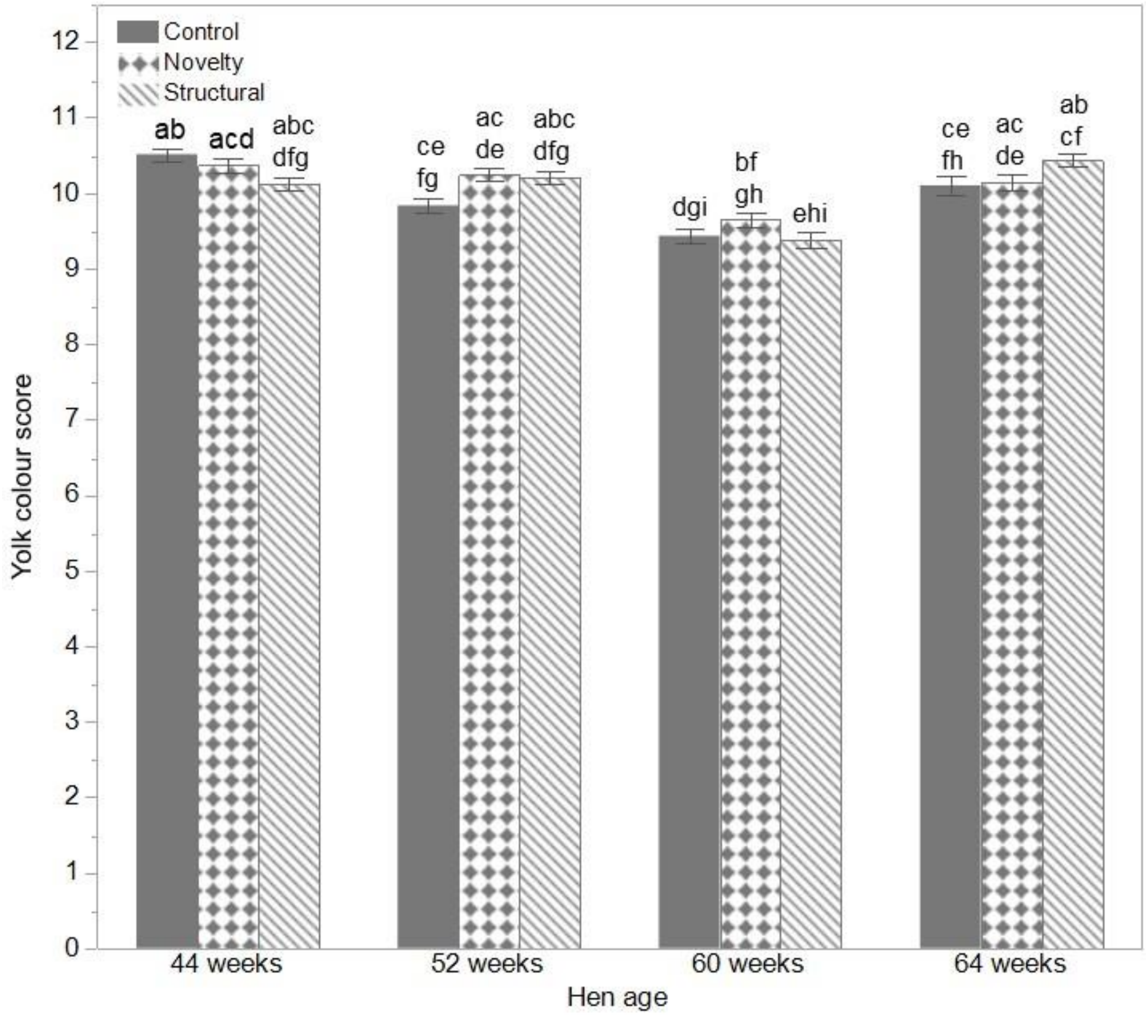

Figure 9: The mean ± SEM of the yolk colour score of hen eggs from different rearing treatments (control, novelty, structural) at different age points (44, 52, 60, and 64 weeks of age). [a-i] Dissimilar superscript letters indicate significant differences between rearing treatments and hen age ($P < 0.006$). Raw data are presented with the analysis conducted on transformed data.

Table 1: The least squares means ± standard error of the mean for egg weights and percentage of abnormal eggs (i.e. elongated, misshapen, pimpled, rough-shelled, extra small or extra-large) across monthly age intervals and for the three rearing treatments (control, novelty, structural)[1].

Variables	Category	Egg weight (g)	% Abnormal eggs[2]
Hen age (weeks)	21 – 24	52.97 ± 0.33^{g}	1.26 ± 0.21^{d}
	25 – 29	58.37 ± 0.19^{f}	1.53 ± 0.19^{d}
	30 – 34	60.23 ± 0.11^{e}	$1.95 \pm 0.23^{c,d}$
	35 - 39	60.62 ± 0.10^{e}	$2.89 \pm 0.27^{b,c}$
	40 - 44	$61.70 \pm 0.10^{c,d}$	3.55 ± 0.23^{b}
	45 – 49	$62.12 \pm 0.18^{b,c}$	3.42 ± 0.23^{b}
	50 - 54	$62.50 \pm 0.08^{a,b}$	6.09 ± 0.37^{a}
	55 - 59	62.88 ± 0.09^{a}	7.76 ± 0.40^{a}
	60 - 64	61.25 ± 0.13^{d}	8.25 ± 0.52^{a}
Rearing treatments	Control	60.55 ± 0.26	3.87 ± 0.27
	Novelty	60.36 ± 0.25	4.80 ± 0.31
	Structural	60.47 ± 0.24	3.76 ± 0.26

[1a-g]Different superscript letters indicate significant differences between egg weights or abnormal eggs across hen age ($P < 0.005$).

[2]Raw data are presented with analyses conducted on transformed data where applicable.

Table 2: Variation in the quality of eggs based on different hen ages and enriched rearing treatments (control, novelty, structural).[1,2,3]

Variables	Category	Egg weight (g)	Albumen height	Haugh unit	Breaking strength (N)	Shell deformation (μm)	Shell weight (g)	Shell thickness (μm)
Hen age	44 weeks	62.69 ± 0.27^{a}	9.96 ± 0.10^{a}	98.10 ± 0.51^{a}	46.85 ± 0.46^{a}	0.29 ± 0.004^{a}	6.13 ± 0.03^{a}	0.43 ± 0.002^{a}
	52 weeks	$63.27 \pm 0.27^{a,b}$	9.90 ± 0.10^{a}	97.67 ± 0.51^{a}	42.93 ± 0.46^{b}	0.27 ± 0.004^{b}	6.02 ± 0.03^{b}	0.42 ± 0.002^{b}
	60 weeks	62.42 ± 0.27^{b}	8.61 ± 0.10^{b}	91.36 ± 0.51^{b}	41.39 ± 0.46^{c}	0.26 ± 0.004^{c}	5.83 ± 0.03^{c}	0.41 ± 0.002^{c}
	64 weeks	61.43 ± 0.27^{c}	8.88 ± 0.10^{b}	92.96 ± 0.51^{b}	41.17 ± 0.46^{c}	0.26 ± 0.004^{c}	5.81 ± 0.03^{c}	0.41 ± 0.002^{c}
	Stats, *P* – value	$F_{(3,1068)} = 8.09$, < 0.0001	$F_{(3,1068)} = 53.24$, < 0.0001	$F_{(3,1068)} = 44.25$, < 0.0001	$F_{(3,1068)} = 32.37$, < 0.0001	$F_{(3,1068)} = 16.44$, < 0.0001	$F_{(3,1068)} = 28.48$, < 0.0001	$F_{(3,1068)} = 51.78$, < 0.0001
Rearing treatments	Control	62.90 ± 0.23	9.19 ± 0.08	94.16 ± 0.44	42.51 ± 0.40	0.27 ± 0.003	5.95 ± 0.03	0.41 ± 0.001
	Novelty	62.21 ± 0.23	9.34 ± 0.08	95.02 ± 0.44	43.41 ± 0.40	0.27 ± 0.003	5.96 ± 0.03	0.42 ± 0.001
	Structural	62.24 ± 0.23	9.48 ± 0.08	95.90 ± 0.44	43.33 ± 0.40	0.27 ± 0.003	5.94 ± 0.03	0.42 ± 0.001
	Stats, *P* – value	$F_{(2,6)} = 2.56$, 0.60	$F_{(2,6)} = 0.68$, 0.54	$F_{(2,6)} = 1.01$, 0.42	$F_{(2,6)} = 0.84$, 0.48	$F_{(2,6)} = 0.45$, 0.66	$F_{(2,6)} = 0.11$, 0.90	$F_{(2,6)} = 2.04$, 0.21

[1] Values are presented as least squares means ± standard error of the mean

[2] Different superscripts within the values of the same column of each variable differ significantly ($P \leq 0.05$).

[3] Analyses were conducted on transformed data where applicable with the raw values tabled.

4.0 Objective 4: Response to a stressor – in preparation as *Bari et al., 2020. Frontiers in Veterinary Science.*

4.1 Implemented stressor

The implemented environmental stressor for this trial was a reduction in available range area, similar to what has been applied in a previous study (Campbell et al., 2018). The total outdoor area for each pen was reduced using shade cloth to approximately 20% of its original size (from 31 m L to 6 m L). The range area was reduced for 11 days from 44 to 45 weeks of age with egg measurements (see following sections on egg quality and albumen corticosterone) taken prior to the range shrinkage, the first days of shrinkage (immediate stress) and at the end of the stressor period (prolonged stress).

4.1.2 Albumen corticosterone

A total of 50 eggs from each of the 9 pens were sampled at 3 stages on the same days as the egg quality measurements, day 4 prior to range shrinkage, and days 3 and day 11 following shrinkage for the evaluation of the concentrations of albumen corticosterone. Eggs were collected from all laying locations but excessively dirty eggs were excluded. On the day of collection, the eggs were opened individually by breaking its shell, the yolk was separated out and then the albumen was weighed and stored at – 20˚C till assessment using radioimmunoassay as per the protocol reported by Downing and Bryden (2008). All the egg corticosterone samples were analysed blindly to the enriched rearing treatments and implemented stressor.

4.1.3 RFID data

To assess the effect of the shrinkage of ranging area on ranging behaviour (time on the range and number of visits) the individual-hen data 10 days before the stressor was applied and 10 days during the stressor were compiled.

4.2 Results

4.2.1 Ranging behaviour

The average number of visits outside was significantly increased (negative difference values) due to stress and varied between rearing treatments with a lower increase in the number of visits for the structural group of hens ($F_{(2,\ 1300)} = 3.51$, $P = 0.03$) (Figure 10). Due to the implementation of stressors the hens' ranging time (hrs) outside decreased (positive difference values) but did not differ significantly between the rearing treatments ($P > 0.05$).

4.2.2 Albumen corticosterone

There was a significant interaction between rearing treatment and the implemented stressors periods ($F_{(4,1314)} = 90.30$, $P < 0.0001$). The control and novelty hens showed an increase in albumen corticosterone concentrations from baseline immediately following the shrinkage of the range area (implemented stressor) whereas the structural hens showed a decrease in concentrations (Figure 11).

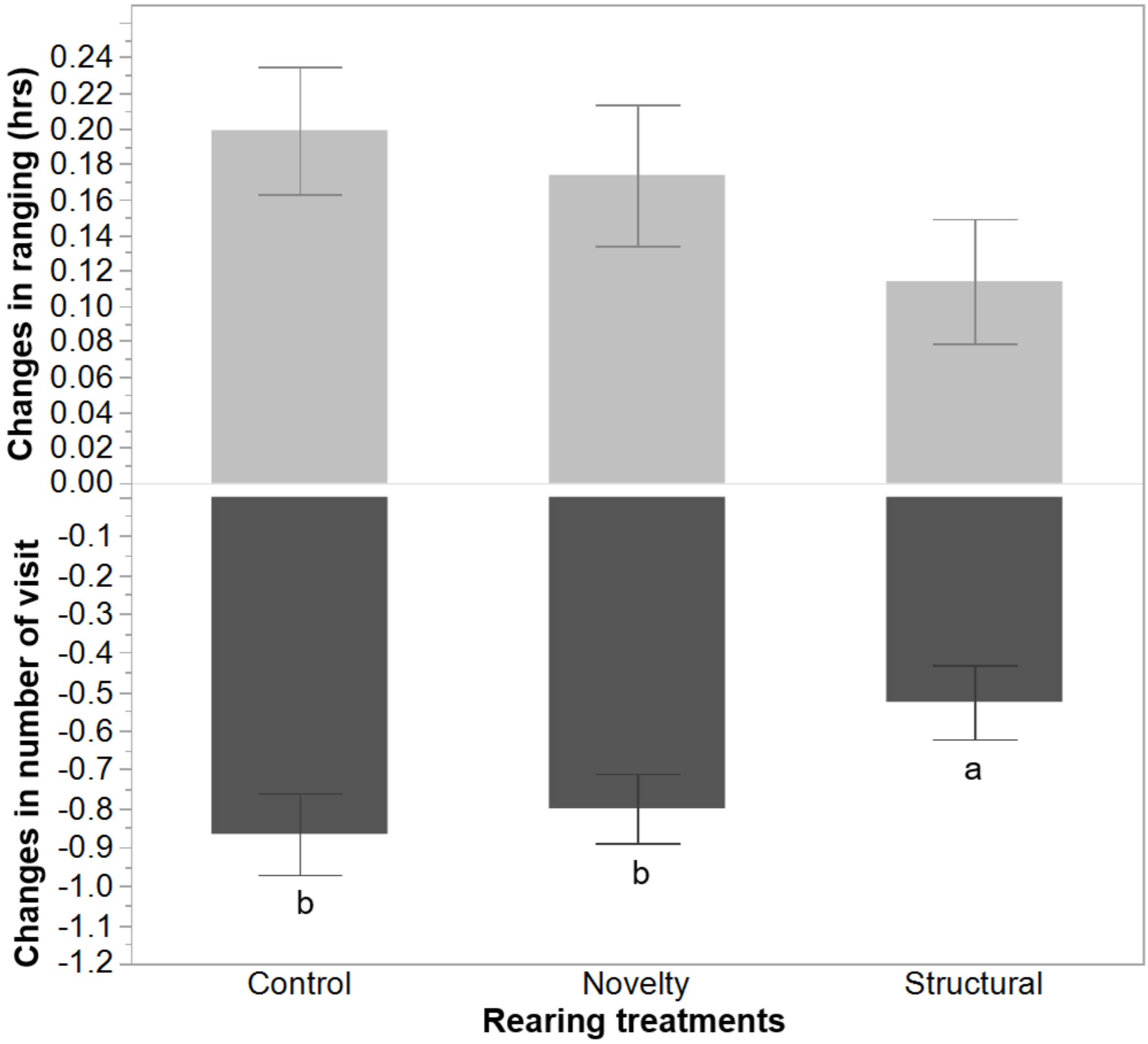

Figure 10: The mean ± SEM of the changes in ranging time (hrs) and number of visits due to the applied stress on free-range hens from different rearing treatments (control, novelty, structural). [a-b] Dissimilar superscript letters indicate significant differences between the change in the number of visits across different rearing treatments ($P < 0.05$).

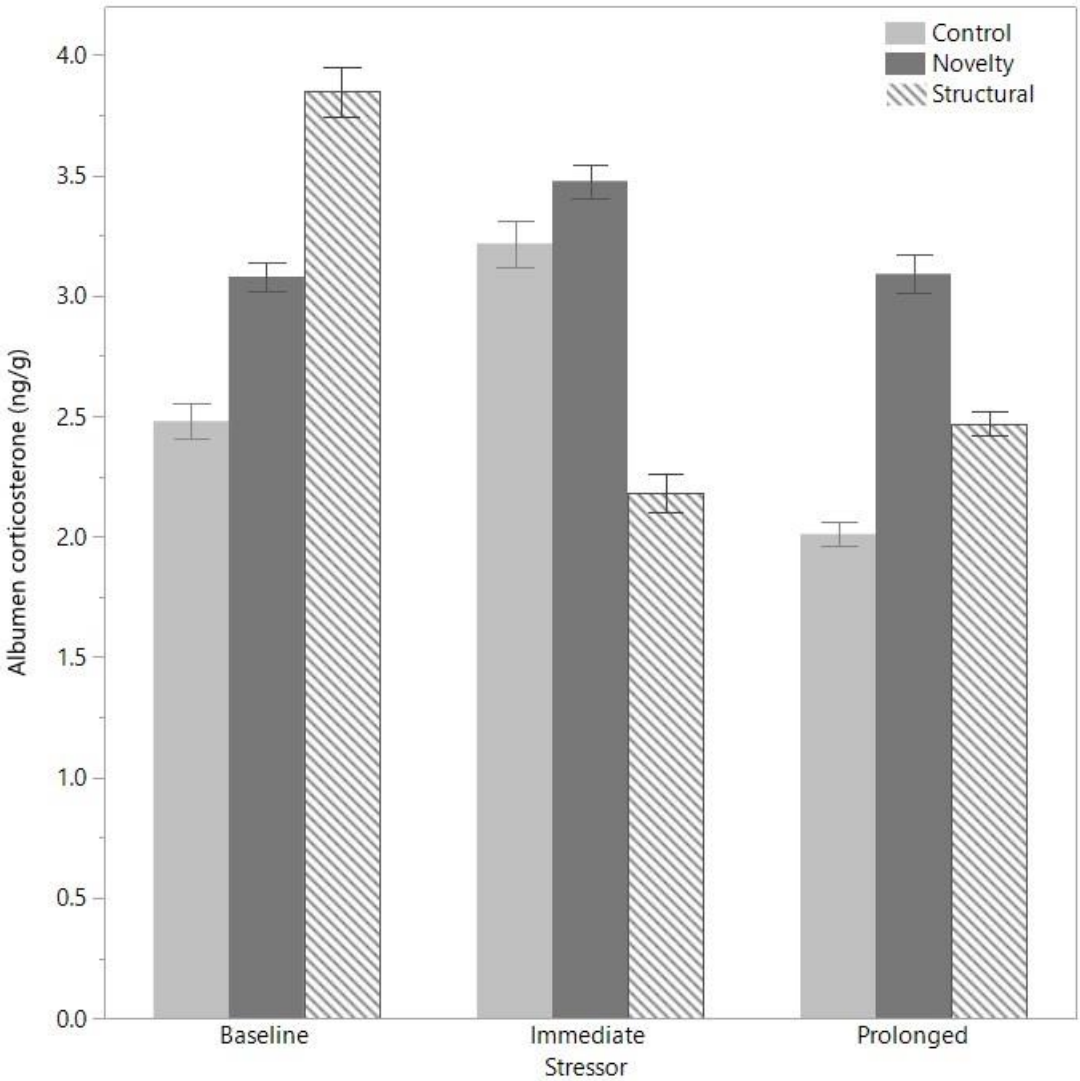

Figure 11: The mean (± SEM) albumen corticosterone concentrations (ng/g) of eggs from hens from different rearing treatments (control, novelty, structural) across an implemented stressor period.

5.0 Objective 5: Welfare measures – published as *Bari et al., 2020. PeerJ* and *Bari et al. 2020 (in preparation)*

5.1 Individual welfare assessment

The welfare assessment of all hens was done individually at 5 age points including 27 weeks, 33 weeks, 43 weeks, 56 weeks and 64 weeks of age. All the hens were weighed individually using electronic hanging scales (BAT1; VEIT Electronics, Moravany, Czech Republic). The external welfare parameters of 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 where a score of 4 indicated minimal feather damage, and a score of 1 indicated no plumage, just bare skin. The back of the neck was scored separately from the front of the neck and 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 damage. 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 or dermatitis (an additional category to the Tauson et al. 2005 system) and a score 1 for swollen, infected bumblefoot. The exact number of fresh or healing comb wounds were also counted and toenail length was measured in mm using a seamstress tape measure. Beaks were scored as 0, 1, or 2 indicating no, mild or moderate damage respectively. The birds were also examined for any other external signs of injury or illness such as swollen abdomen, enlarged crop, and prolapse.

The mortality of the hens was also counted throughout the flock cycle. Hen mortality was recorded if a hen died, was euthanised, or rehomed if severely feather-pecked. A total of 28 hens were recorded throughout the cycle as the mortality of which 10 were from the control group, 9 from the novelty group and 9 from the structural groups of rearing treatments.

5.1.2 Post-mortem health examination

A group of 307 hens were selected across all rearing treatments that were categorised as 'indoor' – accessed the outdoors on one or zero of 54 ranging days at the end of the trial, '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. Post-mortem examinations were carried out across two days at 65 weeks of age at a post-mortem facility. The selected hens of a single pen were transported to the post-mortem lab up to 2 hours prior to the dissection using plastic carrier crates. Immediately after death via CO_2 administration and cessation of all hen movement, the birds were opened by a veterinarian to examine the health condition of the hens (presence/absence of diseases) by inspecting the visceral organs for any abnormalities including haemorrhage, tumours, caseous necrosis and/or other exudates, the respiratory system (nares and trachea) for any haemorrhage, inflammation or exudates and the reproductive system for signs of salpingitis, being egg bound, or other abnormalities. Whether the hen was in production or not was determined by examination of the ovary and presence of active or regressing follicles. The spleen, gizzard, and right adrenal gland were removed and weighed (to the nearest mg) from each of the selected birds. The gizzard was emptied prior to weighing with surrounding connective tissue and fat removed. The jejunum, duodenum and ileum were opened longitudinally and the number of *Ascaridia galli* worms in each bird recorded.

5.1.3 Assessment of the keel bone

Following post-mortem examination, the keel bone was excised from each hen to assess deformities and damage. All keel bones were stored at -20°C until thawed for processing. For processing, the fleshes on the keels were removed using a knife and scissors. The defects on the keels were examined by two experimenters using a visual scoring system for bending of the spine that was classified as low, moderate, or high based on the extent (Figure 12). The two observers scored each bone independently and then immediately confirmed scores to ensure agreement for each bone. The dorsal surface of the tip of the keels were also observed for the presence of calluses to indicate healed fractures and the number of calluses were counted and recorded by two observers in agreement (Figure 13). No fresh pre-mortem fractures were observed.

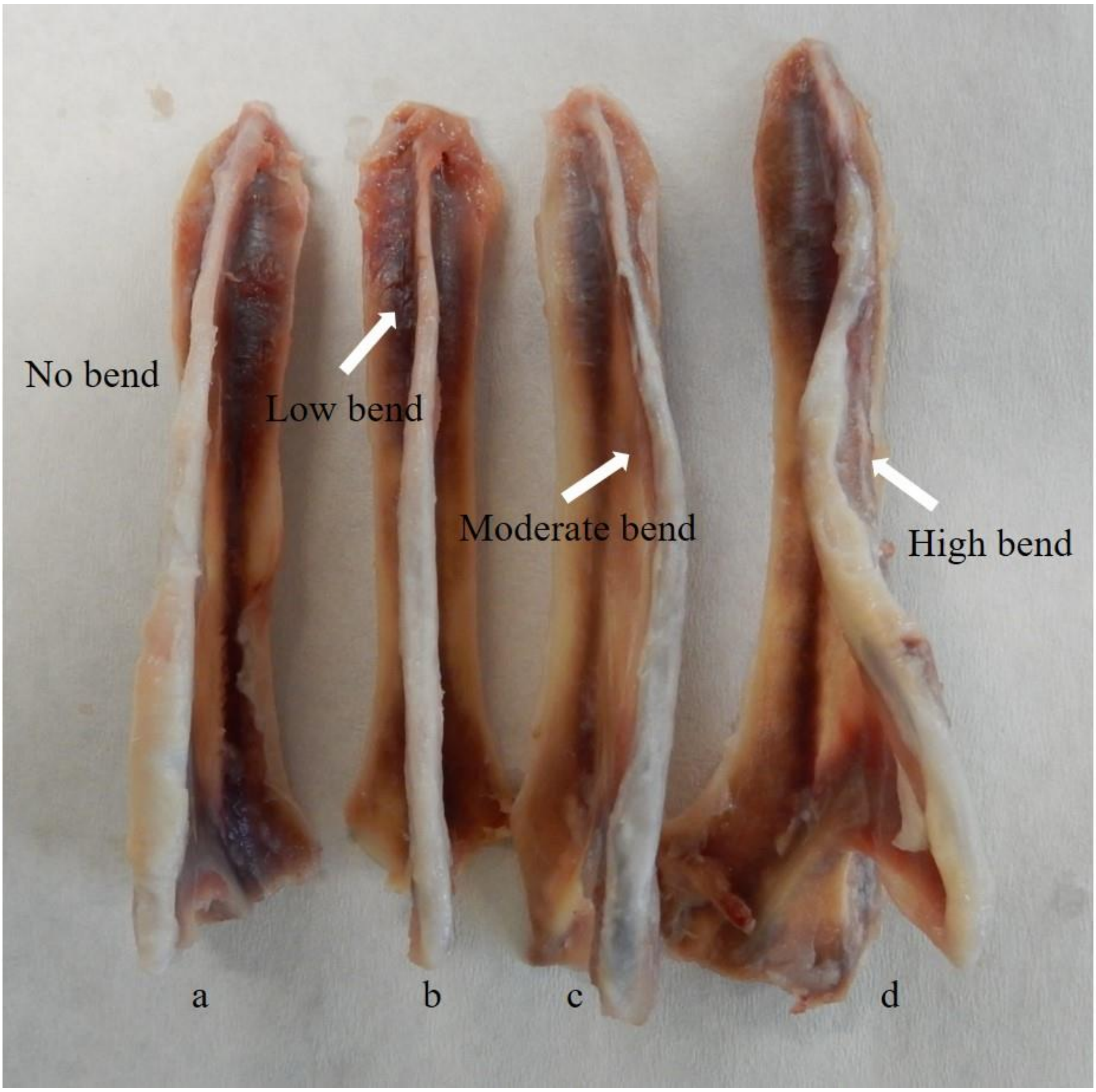

Figure 12: Different types of spine bending of keel bones. Keel (A) indicates a spine with no bending, (B) low bending, (C) moderate bending and (D) indicates high bending in the spine.

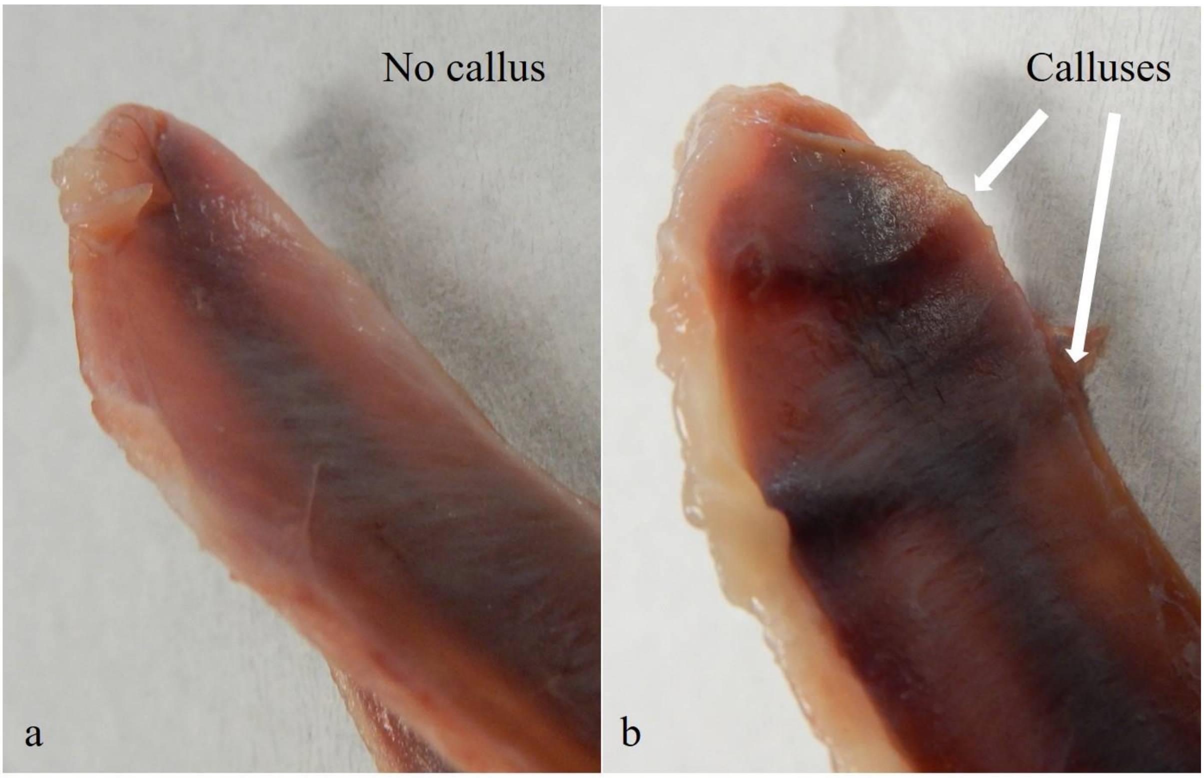

Figure 13: Calluses on the dorsal surface of keel bones. (A) indicates a keel surface with no calluses, but (B) shows two calluses.

5.4 Results

5.4.1 Individual welfare

There was a significant interaction between hen age and rearing treatments for live weight of free-range hens ($P < 0.003$) with the control hens dropping more in body weight towards the end of the production cycle (Figure 13). There were significantly fewer comb wounds in the novelty group than the other two groups ($F_{(2, 1378)} = 6.87$, $P = 0.001$) and the number of comb wounds significantly decreased ($F_{4, 5489} = 176.53$, $P < 0.0001$) with increasing hen age from 33 weeks onwards (Figure 14). Both the novelty and structural hens had better plumage than the control hens ($F_{(2, 1373)} = 99.69$, $P < 0.0001$, Figure 15). Hen age significantly affected ($F_{(4, 5479)} = 964.12$, $P < 0.0001$) the plumage condition score of layers in a decreasing trend throughout the laying cycle (Figure 15). The structural hens had shorter toenails ($F_{(2, 1389)} = 6.4$, $P = 0.002$) compared to the novelty hens (Figure 16). The toenail length decreased ($F_{(4, 5482)} = 391.77$, $P < 0.0001$) as the age advanced up to 56 weeks age point, then it increased again by 64 weeks of age (Figure 16).

5.4.2 Post-mortem assessments

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

Post-mortem examination also revealed all hens under study except one, were in production. There were no disease lesions observed on the respiratory system of the hens. One bird had a fatty liver, one peritonitis and one had keel adhesion. A cystic right oviduct was also present in 0.04% of hens.

5.4.3 Keel bone damage

The free-range hens from different rearing treatments and ranging patterns showed no significant differences in the overall presence of keel bone defects, presence of spine bending, spine bending types, and the presence of callus formation on the dorsal surface of keel tips (all $P \geq$ 0.19, Table 4). The rearing treatments ($F_{(2, 295)}$ = 0.40, P = 0.67), ranging patterns ($F_{(2, 295)}$ = 1.31, P = 0.27) and their interaction (P = 0.55) had no effect on the number of calluses on the tip of the dorsal surface of the keels.

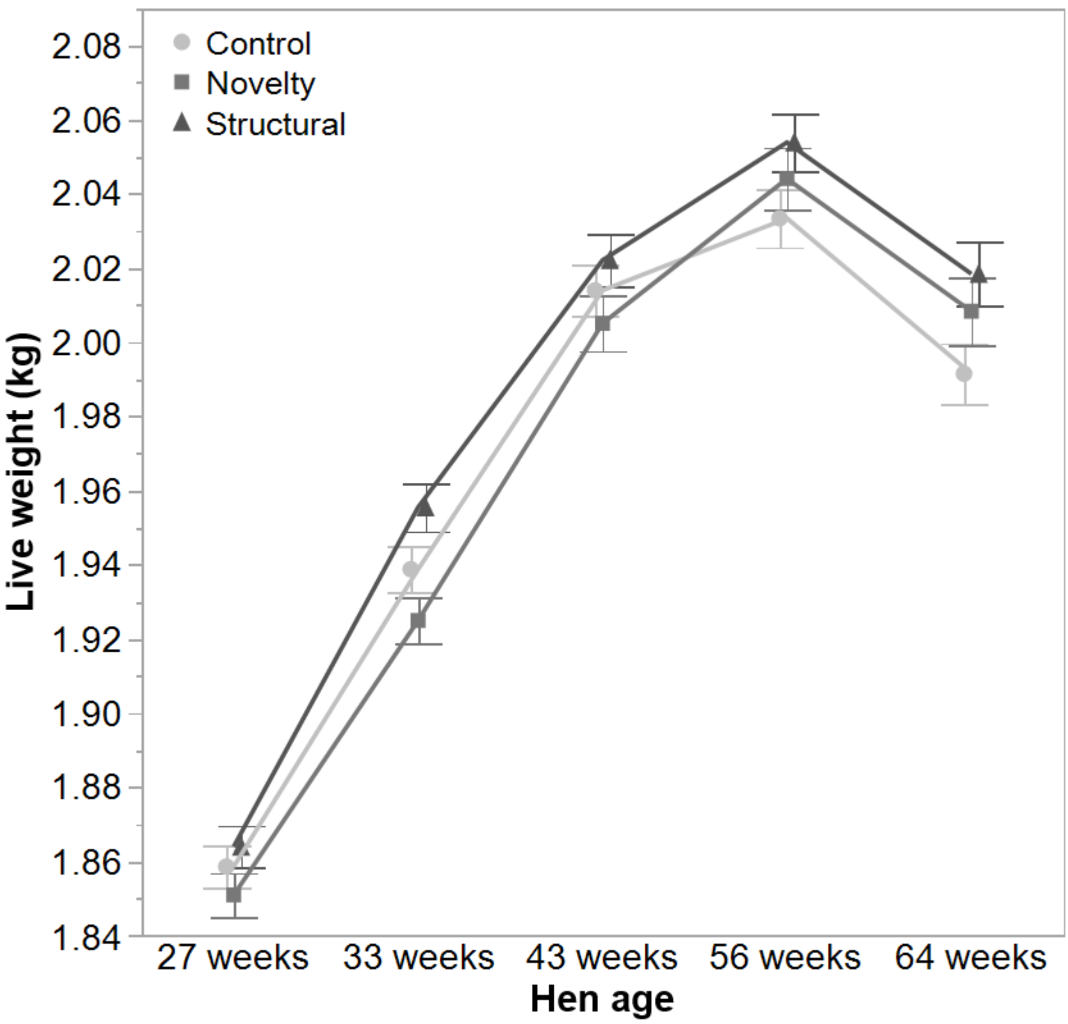

Figure 13: The LSM ± SEM of live weight (kg) of hens from different rearing treatments (control, novelty, structural) at different age points (27 weeks, 33 weeks, 43 weeks, 56 weeks, 64 weeks) in their laying cycle.

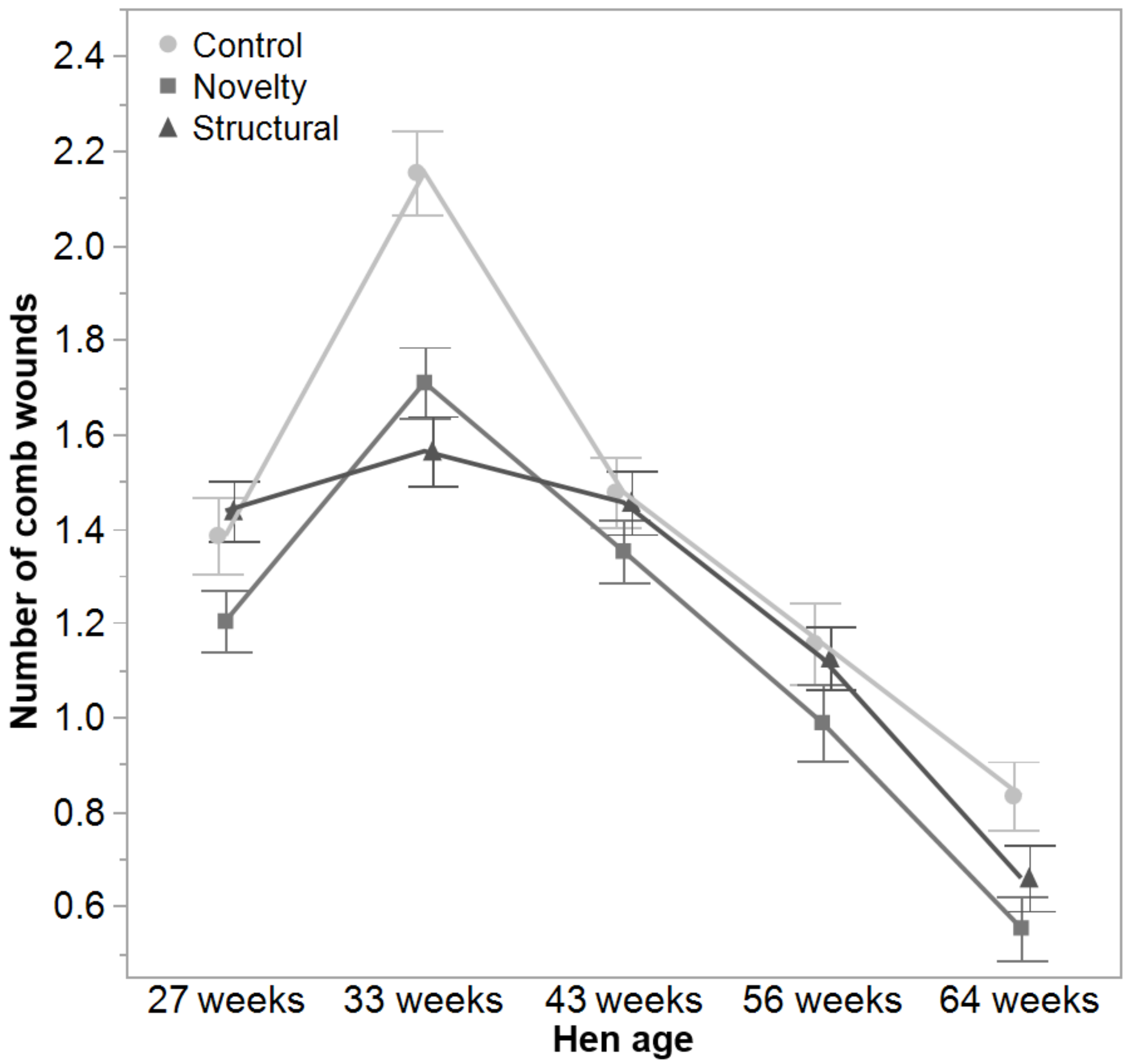

Figure 14: The LSM ± SEM of the number of comb wounds in hens from different rearing treatments (control, novelty, structural) at different age points (27 weeks, 33 weeks, 43 weeks, 56 weeks, 64 weeks) in their laying cycle. Raw data are presented with analysis conducted on transformed data.

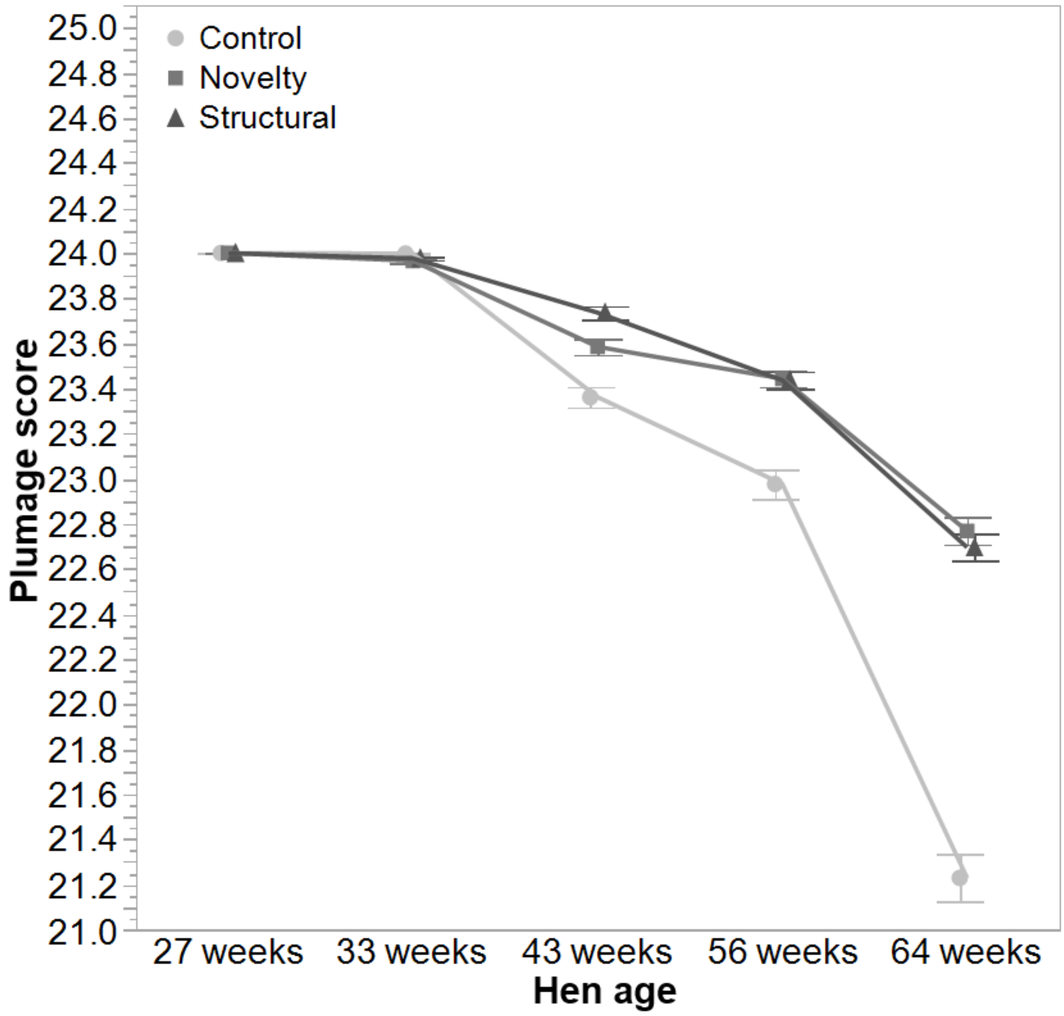

Figure 15: The LSM ± SEM of plumage score of hens from different rearing treatments (control, novelty, structural) at different age points (27 weeks, 33 weeks, 43 weeks, 56 weeks, 64 weeks) in their laying cycle. Raw data are presented with analysis conducted on transformed data.

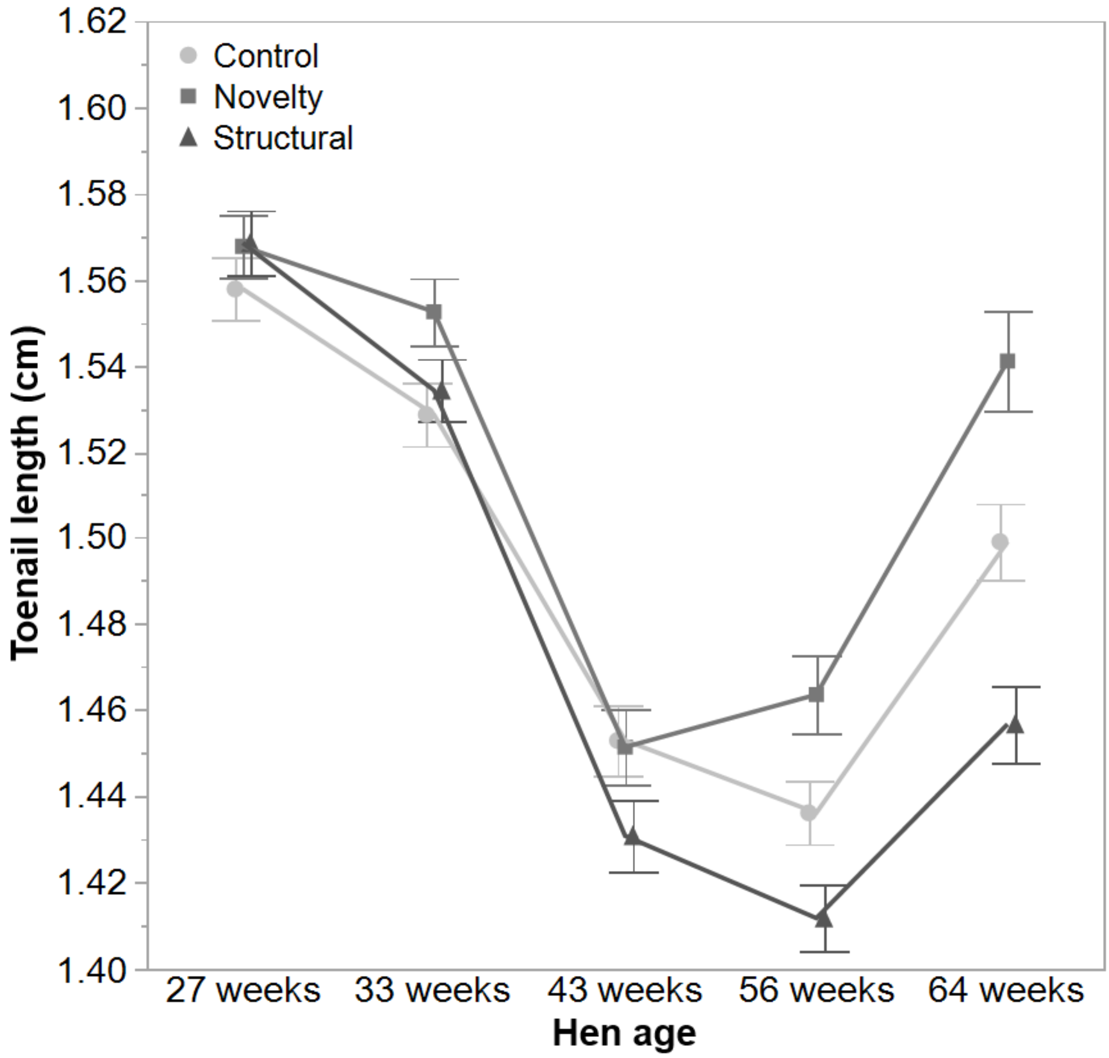

Figure 16: The LSM ± SEM of toenail length (cm) of hens from different rearing treatments (control, novelty, structural) at different age points (27 weeks, 33 weeks, 43 weeks, 56 weeks, 64 weeks) in their laying cycle. Raw data are presented with analysis conducted on transformed data.

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

Table 3: 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 *A. galli* 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).

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*			χ^2 = 1.69, 2, 0.43	χ^2 = 0.06, 2, 0.97	χ^2 = 2.79, 2, 0.84			χ^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*			χ^2 = 0.15, 2, 0.93	χ^2 = 0.52, 2, 0.77	χ^2 = 2.60, 2, 0.86			χ^2 = 3.29, 2, 0.19	$F_{(2, 295)}$ = 1.31, 0.27

Table 4: 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.

6.0 Discussion of Results

Full discussions of individual objectives are provided in the respective publications as listed.

The rearing enrichments provided had long-lasting impacts on hen behaviour, welfare, and response to stressors. Some of these impacts were similar between both enrichment treatments relative to the control hens, other impacts were distinct between different enrichments and the control hens.

To first consider the impacts on hen ranging behaviour, the two types of enrichments had disparate, yet sustained impacts on ranging. The increase in ranging hours by the structural hens in the current study may have been due to improvements in their spatial navigation abilities. Previous research has shown some effects of elevated structures during rearing on the speed of completing cognitive tasks in chicks (Norman et al., 2018) or spatial jumping tasks in pullets (Gunnarsson et al., 2000). Laying hens reared in aviaries also showed improved three-dimensional use of their new pens when transferred to the laying facility compared with hens reared in cages, although these differences were not sustained past the first 4 weeks following transfer to the laying facility (Brantsæter et al., 2016). Chicks with exposure to occlusion barriers within the first two weeks of development showed some modification of their spatial behaviour compared with control chicks receiving no occlusion experience (Freire et al., 2004). The structural groups had experience with large opaque barriers throughout rearing, although some of the initial objects in the novelty group (cinder blocks, buckets) may have also functioned as occlusion barriers to the small chicks. The structural hens may have felt more competent in moving between the indoor and outdoor areas, thus increasing the overall amount of time they spent outdoors. The structural hens also showed the greatest change in ranging behaviour during the stressor period and actually showed a decrease in albumen corticosterone concentrations following the implemented stressor of range shrinkage. Comparatively the control and novelty hens showed an increase immediately following the stressor.

The novelty hens laid more eggs in the large nest boxes that were elevated off the ground. This contrasted with the predictions of the structural birds showing the greatest use of the elevated nest box due to their experience with jumping onto perching structures during rearing. This has been found in previous studies where perches provided during the early stages of rearing (4 weeks onwards) resulted in fewer eggs on the floor compared with perch access at 8 weeks of age or later (Appleby et al., 1988). Anecdotally pullets were observed to use the structures during rearing (from 2 weeks of age) and the structure height was equivalent to the tiers of the large nest box (distance off the ground and between tiers). However, there might not have enough space for all the birds to gain sufficient experience, or perching did not occur early enough. Some of the enrichments in the novelty treatment (e.g. overturned containers, bricks) allowed the chicks to jump small heights within the first 2 weeks. Alternatively, it might have been that the novelty birds became more accustomed to new environments during rearing and were thus more willing to utilise the large enclosed space which may have been more limiting than the elevation of the nests. In support of this statement, previous studies have revealed that exposure to a more complex environment in rearing reduced fearfulness in hens and also increased their use of different areas of an aviary system when first transferred to the layer house (Brantsaeter et al., 2016).

Hens reared with enrichments had better plumage indicating a persistent effect of rearing conditions through until later in the production cycle. Multiple studies have documented the positive long-term effects of providing substrates during rearing where opportunities to forage and dust bathe are suggested to prevent the development of feather pecking (reviewed in Rodenburg et al., 2013) although substrate availability during the laying period is also critical (Rodenburg et al., 2013). All birds in this study were both reared and then housed with access to a floor litter substrate but the additional pen enrichments still had positive impacts. The increased complexity of the enriched pens may have improved behavioural development or provided more opportunities for the pullets to regulate their social interactions by having elevated escape areas. Pullets did not show feather damage at the end of rearing (see Campbell et al., 2020 *Animals*) but it is unclear whether pecking behaviour in the control hens resulted from behavioural patterns established during rearing, and/or if the adult control hens were more susceptible to environmental stress in the free-range setting which triggered the development of the negative pecking behaviour.

The hens that ranged the most showed the best plumage coverage which provides further support to previous research. Several studies have demonstrated better plumage in individuals, or flocks that show more frequent use of the outdoor range (e.g., De Koning et al., 2018; Rodriguez-Aurrekoetxea & Estevez, 2016), or who range farthest when outdoors (Chielo et al., 2016). It might be that hens outdoors are able to or are motivated to engage in more foraging compared with hens indoors where a lack of foraging is often redirected to feather pecking, causing plumage damage (Rodenburg et al., 2013). This result contrasts with that of Larsen et al. (2018) who found no association between outdoor ranging and plumage condition of Hy-Line® Brown hens in commercial Australian conditions. It is also possible that better plumage coverage led to more ranging if hens were able to thermoregulate more effectively and/or avoid sun exposure on bare skin.

Hens that ranged the most showed the lowest body weight, specifically, lower body fat and muscle, but not lower skeletal mass. The high outdoor hens showed an average body weight (1.95 kg) lower than the indoor hens but were within the limit of the expected body weight by breed standards (1.90 – 2.02 kg). Previous research has found some evidence of a similar negative relationship between body weight and range use, but not at all measured age points (Campbell et al., 2017a). This negative relationship might be due to the ingestion of vegetation, insects or grit during ranging and thus consumption of less formulated food (Singh & Cowieson, 2013). This would also correspond with the higher empty gizzard weight observed in the ranging hens, similar to findings of larger gizzards in free-range versus caged hens (Yang et al., 2014). The reduction in body weight might also be a result of greater energy utilisation during locomotion, although greater exercise opportunities have previously been shown to increase bone and muscle development (Regmi et al., 2016) which was not found in the outdoor hens. Other measured skeletal properties rather than overall mass may have revealed differences between ranging groups although recent work showed no effect of ranging on multiple tibial measurements across hens from a commercial aviary-free-range system

(Kolakshyapati et al., 2019). Furthermore, there was no effect of either rearing enrichments or ranging patterns on keel damage of free-range hens. This coincides with observations on commercial farms that assessed damage via both dissection (Kolakshyapati et al., 2019) and palpation (Larsen et al., 2018) and found no association between individual ranging and keel damage.

The study showed that rearing enrichments had long-term effects on adult free-range hens, particularly in reducing the degree of plumage damage (novelty and structural) and increasing use of nest boxes (novelty). However, subsequent individual ranging patterns by the hens had a stronger influence on their health and welfare with high outdoor use resulting in better plumage, fewer comb wounds, shorter nail length, higher spleen and gizzard weight, but lower body weight, fat and muscle. Rearing enrichments are thus recommended for long-term positive effects on hen welfare, but management of range access may have the strongest impact on bird welfare. This study was conducted in an experimental setting with small flock sizes. Large commercial groups of layers may interact with enrichments in different ways if competition for access is greater and they are likely to be exposed to more pathogens where outdoor access may have different effects on hen susceptibility. Similar long-term studies on commercial free-range farms would confirm the benefits and consequences of different ranging patterns and long-term impacts of rearing enrichments.

7.0 Implications

This project demonstrates that rearing is a critical period for pullet development and that conditions during this time will have long-term impacts on the adult hens. This period will be easier to manage for those producers that rear their own pullets but will be challenging for those that purchase pullets at 16 weeks from a contracted rearer. It will be important to request information on the conditions that the pullets were reared in so there can be a better understanding of what they were exposed to or not during the developmental period. There will be a financial investment to providing enrichments during rearing, this may be achieved through construction of perches in floor barns or use of complex rearing systems designed by specialised poultry housing companies. Careful management of range access will likely result in individual welfare impacts. If more hens can range, then problems of feather pecking may be reduced.

The information from this project is being published for peer-review. There are several papers that are already available online and most of these have been made open access. Presentations have been given at multiple national conferences/meetings. Once full analysis and peer-review of all papers resulting from this project have been completed, an online summary article written for a lay audience is recommended. This would provide an overview of the project and main findings for producers and consumers.

8.0 Recommendations

It is recommended that poultry producers provide enrichments during the rearing period for their pullets. This is suggested to be in the form of stable structures that the hens have to perch/jump on to increase their ability to navigate within their environment. It is also recommended for producers to encourage range use to minimise feather pecking damage. However, these were the results from one flock of birds in an experimental setting, further validation of these findings by repeating the experiment or assessing birds on commercial farms is recommended.

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10.0 Media and Publications

10.1 Peer-reviewed publications

Bari, M.S., Laurenson, Y.C.S.M., Cohen-Barnhouse, A.M., Walkden-Brown, S.W., and Campbell, D.L.M. (2020). Effects of outdoor ranging on external and internal health parameters for hens from different rearing enrichments. *PeerJ* 8:e8720. http://doi.org/10.7717/peerj.8720

Bari, M.S., Cohen-Barnhouse, A.M., and Campbell, D.L.M. (2020). Early rearing enrichments influenced nest use and egg quality in free-range laying hens. *Animal*, in press. doi:10.1017/S1751731119003094

Campbell, D.L.M., Dyall, T.R., Downing, J.A., Cohen-Barnhouse, A.M., and Lee, C. (2020). Rearing enrichments affected range use in free-range layers. *Frontiers in Veterinary Science*, submitted (Feb 2020).

Campbell, D.L.M., de Haas, E.N., and Lee, C. (2019). A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. *Poultry Science*, 98, 9-28.

Campbell, D.L.M., Dickson, E.J., and Lee, C. (2019). Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. *PeerJ*, 7:e8122 http://doi.org/10.7717/peerj.8122

Campbell, D.L.M., Gerber, P.F., Downing, J.A., and Lee, C. (2020). Minimal effects of rearing enrichments on pullet behaviour and welfare. *Animals*, 10, 314; doi:10.3390/ani10020314

10.2 Conference presentations

Bari, M.S., Laurenson, Y.C.S.M., Cohen-Barnhouse, A.M., Walkden-Brown, S.W., and Campbell, D.L.M. (2020). Consequences of outdoor ranging on external and internal health parameters of hens from different rearing enrichments. Proceedings of the Australian Poultry Science Symposium, Feb 16-19, Sydney, Australia.

Campbell, D.L.M., Dyall, T.R., Cohen-Barnhouse, A.M., and Lee, C. (2020). Impacts of rearing enrichments on ranging in free-range laying hens. Proceedings of the Australian Poultry Science Symposium, Feb 16-19, Sydney, Australia.

Campbell, D.L.M., Bari, M.S., Laurenson, Y., Dyall, T.R., Gerber, P.F., and Lee, C. (2019). Impact of rearing enrichments and range use on hen welfare. AVPA Scientific Meeting, 14-15 November, Adelaide, Australia.

Bari, S., and Campbell, D.L.M. (2019). Outdoor ranging but not rearing enrichments reduced fearfulness of adult free-range hens. Proceedings of the International Society of Applied Ethology, Nov 21-22, Wellington, NZ.

11.0 Intellectual Property Arising

N/A

12.0 References

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