Nutritional management of free range laying hens

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Foreword

Free range laying hens can be provided with the opportunity to access various structural areas including open floor space, feed areas, water lines, nest boxes, perches, aviary tiers, winter gardens and ranges. Different individual location preferences can lead to the development of hen subpopulations that are characterised by various performance, health, and welfare parameters. Understanding the complexity of hen movement and hen interactions within their environment provides an opportunity to limit the disadvantages that are associated with housing in non-caged husbandry systems and aids in decision-making for farm staff, managers, and equipment designers.

This work highlights the dynamics of hen movement in free range systems, and its impact on hen performance and egg quality. Integrating knowledge about flock subpopulations into modern flock management will not only ensure that elite hens are able to use their full genetic potential, but will also help to improve the care of under-performing hens, allowing a more ethical, sustainable and welfare friendly egg production.

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This report is an addition to Australian Eggs Limited's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Mr Sibanda is a Higher Degree Research student at the University of New England. After he graduated from the Lupane State University (Zimbabwe) in Animal and Agricultural Science, he worked for the Canadian Foodgrains Bank and the Ministry of Agriculture, and the Department of Agricultural Research and Extension Services (Zimbabwe) as a research assistant. His understanding and experience of research, agriculture and international community development has allowed Mr Sibanda to make a significant contribution to the project 'Nutritional management of free range laying hens'.

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Dr Ruhnke is a Senior Lecturer at the University of New England, Australia, and is passionate about managing laying hens for improved health and performance. Dr Ruhnke graduated in Veterinary Medicine from the Freie Universität Berlin (Germany), and then worked at the Gastrointestinal Laboratory at Texas A&M University (USA) and at the Institute of Animal Nutrition in Berlin. She pursued her Dr.med.vet thesis on companion animal gut health, and her PhD about the impact of feed technologies and feed particle size on broiler chickens and laying hens. Dr Ruhnke is a certified veterinarian specialising in Animal Nutrition and Dietetics, and a Resident of the European Society of Veterinarian Comparative Nutrition. Dr Ruhnke is known for her applied research on commercial egg farms.

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Abbreviations

ANOM	Analysis of means
BV/TV	Trabecular bone volume fraction
Conn.Dens	Connectivity density
cm	Centimetre
CRC	Cooperative Research Centre
CT	Computed tomography
CV	Coefficient of variation
dB	Decibel
g	Gram
GHz	Gigahertz
ha	Hectare
HSD	Honest Significant Difference
HU	Haugh Unit
kg	Kilogram
kV	Kilovolt
KHz	Kilohertz
LDL	Lower decision line
m	Metre
mA	Milliampere
ME	Metabolisable Energy
Met+Cys	Methionine + Cysteine (also M+C)
MHZ	Megahertz
mm	Millimetre
ms	Millisecond
µm	Micrometre
N	Newton
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
REML	Restricted estimate of maximum likelihood
RFID	Radio frequency identification
RSSI	Received signal strength indicator
SD	Standard deviation
SLD	Spotty liver disease
SEM	Standard error of the mean
SMI	Structure model index
Tb.Th	Trabecular thickness
T.Dig.	Total digestible
t-(SNE)	T-distributed Stochastic Neighbour Embedding
UDL	Upper decision line
UHF RFID	Ultra High Frequency RFID

Executive Summary

This project investigated the impact of flock dynamics and flock subpopulations on nutrient requirements, and determined how alterations in feed formulation and feeding management might improve overall flock performance. While every farm has its own individual set up (flock size, hen house design, feed source), the research approach was based on the behaviour of commercial laying hens and is therefore applicable to all commercial free range egg producers. The objectives of the project were to:

a) characterise subpopulations of free range laying hens
b) determine the dynamics of free range subpopulations
c) develop and validate feeding strategies for subpopulations of free range laying hens.

The research was conducted at a commercial farm over a three-year period. In order to quantify individual hen usage of the range and the aviary system, a Radio Frequency Identification (RFID) system was custom built by the University of New England. The validation of the RFID system demonstrates the suitability, reliability and accuracy of this method, allowing for commercial flock observation and big data collection.

Hens that spend the majority of their available days in the shed (stayers), hens that spend some time in the shed and some time on the range (roamers), and hens that prefer to spend the majority of their available days on the range (rangers) were characterised in respect of their use of resources (feeder lines, nest boxes, and range), as well as their laying performance and egg quality. The use of the range was significantly correlated with the use of the aviary system, stayers preferred to use the upper tiers of the aviary system, while rangers accessed predominantly the lower tiers. The development of stayer and ranger subpopulations resulted in an uneven use of resources and the dynamics of these distinct subpopulations can predict range use. The time spent on the range and the accessing of the lower or upper feed chains was also significantly correlated to hen body weight, at 16 and 22 weeks of age. Flock uniformity varied between flocks but it was not associated with range or aviary system usage.

Stayers, roamers and rangers differed significantly in egg laying performance. At 22 weeks of age, the subpopulation of hens that ranged frequently was at 88.0% hen-day production, but hens that stayed in the shed laid significantly less eggs (78.2% hen-day production; $P < 0.05$). It was not until 52 weeks of age that the hens that preferred to stay in the shed performed as well as the range users. In this research, hens that stayed in the shed contributed 20% of the overall flock population, therefore representing a significant loss for the egg producer allowing for serious reconsideration of current management and feed practices. By contrast, the performance of hens that accessed the range frequently exceeded the expected performance of the breed standard. Egg quality, however, differed only in rare occasions between the subpopulations and was therefore seen as of less concern.

The range use of the rangers was consistent. While 33% of the stayers became rangers over time, the egg production of the stayers increased as well until the stayers outperformed rangers at 62 and 72 weeks of age. These observations about laying persistency are especially relevant when considering the use of hens for an extended laying period, for example until 100 weeks of age. The lack of difference in egg quality between stayers and rangers indicates that there would be limited disadvantage in housing rangers only.

There was a significant difference between stayers, roamers and rangers in their welfare and health status. Overall, the rangers had a better feather cover compared to the stayers ($P = 0.0001$) while stayers had a poor fatty liver score ($P = 0.0026$).

When investigating strategies to increase the laying performance of rangers in more detail, feeding a diet of higher metabolisable energy (+10%) and elevated amino acid concentration (up to 10%) resulted in significantly higher laying performance compared to hens that were fed a conventional diet. There was no significant benefit for providing a feeding station on the range.

In conclusion, subpopulations of free range laying hens require individual nutrient support to achieve outstanding performance and health status. Performance-based feeding would allow for an efficient and responsible use of resources. Other potential solutions to prevent and manage flock subpopulations may include modifications of the hen house and the aviary system. Also, applied solutions to identify and prevent flock subpopulations during pullet rearing are highly warranted, and investigation of hen mortalities could be very beneficial to prevent incidences in the future.

Overall Conclusions

Understanding movement of individual hens as well as flock subpopulations is critical to monitor and improve hen health, welfare and productivity. This is especially true when operating large farm systems where small variations in performance parameters result in large variations of profit.

The characterisation of subpopulations within one flock using modern technology allows for identification of hen clusters and classification into various subpopulations according to their performance, health status, and metabolic energy requirements. Using this information routinely to manage flocks for commercial egg production allows for the achievement of outstanding performance of all flock subpopulations, while at the same time providing for the efficient and responsible use of feed resources. Furthermore, using big data and computer learning can be a powerful tool to aim in evidence-based decision-making regarding housing design and management practices to achieve the desired performance and welfare outcomes.

1 Literature review

1.1 The consequences of range usage subpopulations on feed intake and egg production

In Australia, commercial brown laying hens with a genetic background for intensive in-house cage production are most commonly used. Therefore, genetic selection criteria in the past focused predominantly on productivity and improved feed conversion ratio rather than other traits, such as sociability, feeding and foraging, or ranging behaviour. Furthermore, current feed recommendations were developed based on housing in a climate-controlled environment with limited hen movement. Those given circumstances may play a significant contribution to the reduced performance in free range birds compared to hens housed in conventional cages (Aerni et al. 2005; Durali et al. 2012; Eits et al. 2005; Ferrante et al. 2009; Van Horne 1996). Additionally, free range hens have been observed to exhibit reduced body weight and reduced egg laying performance, whilst mortality can be as high as 40% within a flock, in comparison with conventional systems (Bilcik & Keeling 1999; Glatz et al. 2005; Lay et al. 2011; Sommer & Vasicek 2000; Ruhnke et al. 2015b). These findings are in agreement with the results obtained from a recent survey conducted in Australia where the average hen body weight per flock ranged from 1.42 to 2.1 kg and uniformity of the flock from 83-96% (Poultry CRC report 2015). While many researchers found significantly more disadvantages associated with non-cage husbandry systems, others have clearly demonstrated that hens housed in barn, free range or organic systems can exceed caged production or breeder guidelines (Clerici et al. 2006). Therefore, the impact of hen management and stockmanship skills on hen performance, health and farm economics cannot be underestimated (Blokhuis et al. 2007).

The poultry industry is still one of the few food producing sectors where animals are fed on a group ration based on the average flock performance, rather than taking individual animal requirements based on performance or behaviour into account. This is especially challenging in free range laying hens where the availability of a range area can result in the development of various subpopulations, which may potentially impact feed intake, body weight uniformity and egg production. For example, it has been shown that while some hens prefer to use the range area frequently, others choose to spend the majority of their life span in the sheltered shed (Gebhardt-Henrich et al. 2014b; Hartcher et al. 2016). The overall range usage depends on various factors such as flock size, number of pop holes, shelter on the range, weather conditions, age and experience of the flock (Glatz et al. 2010; Hegelund et al. 2005; Nicol et al. 2003). In commercial flocks, the range may be utilised by 5-95% of the flock (Bubier 1998; Hinch & Lee 2014; Hegelund et al. 2005). However, in a given shed and range design, various subpopulations have been classified: the hens that never leave the barn ('stayers'); the ones that access the range frequently ('roamers'); and the ones that spend the majority of their time on the range ('rangers') (Hinch & Lee 2014). These subpopulations have not been fully characterised to date, but different nutrient requirements may be a cause for consideration.

The likelihood of increased distances between the hen and the feed resources available in the shed increases with the size of the available range area and may compromise frequent feeder access. The energy requirements of birds that range frequently are higher compared to hens that prefer to stay in the shed due to the increased metabolic activity required for locomotion and thermoregulation. The additional metabolic energy requirement for maintenance has been estimated to be 10% (floor-housed) or 15% (free range) higher compared to hens housed in cages (Aerni et al. 2005; GfE 1999; Tiller 2001). Not only is additional energy for increased metabolic activity required, but exposure to the changing climate has to be taken into account. Active cooling or warming of the body is energy consuming (Arad & Marder 1982; Gonyou & Morrison 1983; Roland et al. 1996). The thermo-neutral temperature of a commercial brown laying hen is confined by the upper and lower ideal temperature, 25°C and 10°C (Es et al. 1973), with an ideal humidity of at least 40% (Hy-line International 2014; Institut de Sélection Animale BV 2014). Australian weather conditions can differ significantly from those ideal conditions. Consequently, hens on the range may be in the need of more energy, but reduced feed intake increases the likelihood of reduced body condition and death due to energy deficiency.

Reduced body condition can result in many of the challenges that free range hens are facing. Diets have a significant impact on susceptibility to infectious diseases, and subsequently the health status of a hen (Daghir 1995; Klasing, 1998). Undernourished hens or hens with an imbalanced nutrient supply are more likely to develop infectious diseases (Gross 1992). The level of nutrients such as vitamins A, D and E, polyunsaturated fatty acids, linoleic acid, iron, biotin, lectins, the overall protein, fibre and energy content, and the types of ingredients are of critical importance (Fritsche et al. 1991; Riddel & Kong 1992). Nutritional intervention is highly warranted when specific challenges, such as heat stress, have an impact on the health status of laying hens (Lin et al. 2006; Bollengier-Lee et al. 1998). In fact, the diet and the ability of efficient feed utilisation can influence the stress level of the hen, as well as hen behaviour, reflected in frustration and aggression (Braastad & Katle 1989). Furthermore, the impact of feed quality, the nutritional status of the hen, and hen body weight affect internal and external egg quality (Leeson & Summers 2009; Roberts 2004; Sahin et al. 2002). Subsequently, in order to maintain animal health and productivity it is crucial to measure, control and modify the nutrient intake of commercial free range laying hens.

The nutritional value of pasture is minor. Research has shown that birds given access to pasture may, in part, compensate for small deficiencies in methionine through pasture access (Moritz et al. 2005). However, the predominant polymer of grass is cellulose, which contributes 48% to the total crude fibre fraction (Bach-Knudsen 1997). Chickens have a very limited ability to access fibre as a nutrient source (Choct et al. 1996; Fengler & Marquardt 1988; Kocher et al. 2000, Walker & Gordon 2003). Constant access to pasture can result in excessive fodder intake, reducing the intake of a balanced feed, leading to undernourishment in energy and essential nutrients such as amino acids (Ruhnke et al. 2015b). While the benefit of the range from the perspective of the hen can be questioned, the uneven flock distribution within the shed and range area is an additional challenge to the egg producer.

1.2 Feed management practices in free range systems

Several feeding strategies have been used in commercial free range enterprises. However, since one diet is fed to the entire flock, the hens that stay in the barn ('stayers') may be oversupplied, which can lead to animal fattening and is not ideal from an economic point of view. Choice feeding is another alternative feeding strategy that can be implemented. Research has shown that birds are able to balance between their nutrient requirements and feed intake if they are given an appropriate choice (Summers & Leeson 1978; Emmans 1977). However, in order to ensure the uptake of a balanced diet, certain rules have to be taken into account such as considering the colour, taste and position of the feeder, as well as a limited number of feed choices (Pousga et al. 2005). Furthermore, the concept of choice feeding has been proven under standardised research conditions but has not been validated for large commercial flocks.

On-range feeding is another alternative feeding strategy commonly used. In Australia, up to 47.5% of free range egg producers provide feed on the range. While this strategy may be beneficial for the hens on the range, the biosecurity risk associated with this practice cannot be overestimated. Feed and water on the range frequently attract wild birds, which are potential vectors for disease, and may also attract rodents. Recent data suggest the presence of endemic Low Pathogenic Avian Influenza H5 and H7 in Australian wild birds, which has the potential to be introduced to commercial poultry and mutate into High Pathogenic Avian Influenza (Grillo 2015; Feare 2010).

1.3 Technological advances to improve the nutritional management of free range laying hens

While automated feeding systems based on daily animal performance are successfully used in the cattle, dairy and pig industries, the management of poultry is still performed using flock average values (Rossing 1976; Perez-Munoz et al. 1998; Trevarthen & Michael 2007; Voulodimos et al. 2010). The feasibility of adapting various technological solutions for poultry has been shown in research facilities, but also under semi-commercial and commercial conditions (Singh & Cowieson 2013; Gebhardt-Henrich et al. 2014a; Larsen et al. 2017). Radio Frequency Identification (RFID) is the most commonly used method for poultry monitoring, and demonstrated its value especially when determining the range usage of broiler and layer flocks (Gebhardt-Henrich et al. 2014b; Campbell et al. 2017). By matching individual hen movement with nest box access, RFID systems have also demonstrated their value in recording individual hen performance (Marx et al. 2002; Thurner et al. 2006; Icken et al. 2008; 2013). The RFID tags can be attached to the birds using leg bands, wing tags, neck tags, or intradermal microchip injection (Dennis et al. 2008; Icken et al. 2008; Durali et al. 2014; Zaninelli et al. 2016). The fact that the identification method itself can have a negative effect on the animal's health and welfare status should be considered when selecting the ideal tracing method and when handling the birds. For example, the body weight of laying hens was significantly reduced when hens were equipped with leg bands, possibly due to a disadvantaged access to resources, decreased appetite/feed intake caused by handling stress, or increased stress metabolism (Dennis et al. 2008).

When using High or Low Frequency RFID responders, the limitations of the system need to be taken into account. These may include: human error in applying the transponder, physical loss of tags, malfunctioning transponders, and system failures due to adverse events (power failure, adverse weather conditions, software recording errors, incorrect time stamp recordings or other factors) (Wisanmongkol & Pongpaibool 2009; Gebhardt-Henrich et al. 2014a; Sales et al. 2015; Larsen et al. 2017). Optimal combination of the technical specifications for a defined application (e.g. feeding station, nest box, slaughter line) must always be evaluated within the context of application-oriented experiments, and improved equipment for increased accuracy and timely data transfer is under continuous development (Zaninelli et al. 2015; 2016). Allowing the accumulation of big data for data mining, clustering, and machine learning has great potential not only for real-time data and flock management but can be extended to large-scale poultry disease warnings and poultry risk classifications (Feiyang et al. 2016).

The combination of RFID with automated weighing systems has also been used to determine poultry behaviour including movement speed, resting time, and the ability to feed, allowing for categorising birds as being sick or physiologically active ('normal') (Feiyang et al. 2016). Combining RFID with time-of-flight of light-based 3D vision cameras using image processing techniques on top-view images of hens performing locomotion, perching, feeding, drinking, and nesting activities, allowed for the determination of the impact of the physical environment (space allocation) on bird behaviours, with a 95% agreement between the hen movement as determined by manual observation and by the technology used in the investigation (Nakarmi et al. 2014).

By classifying occupied areas, activity indices, the total number of birds presented in a specific area, and performing a specified activity such as eating, computer algorithms can calculate and observe the activity in the shed (Neves et al. 2015). Similarly, Optical Flow Image Analysis has been used to detect and monitor chicken behaviour as an indicator of welfare status (McCarthy 2019). These systems enable researchers to assess the impact of housing and/or management factors on poultry behaviour, and some of them are under development to be tested in commercial poultry facilities.

The use of technology has been extended beyond passive observation and allows for improved management regimes. For example, detection sensors based on Infrared Technology and Image Pattern Recognition are used to control hen movement and restrict their access to the shed/certain shed areas (Zaninelli et al. 2017). Zuidhof et al. (2017) developed a precision feeding station to increase the flock uniformity of breeding stock. This reduced the coefficient of variation (CV) of a target body weight to decrease below 2%. Such innovations can therefore increase the welfare of birds significantly as traditional methods of controlling weight can result in expression of hunger, frustration, and distress (Mench 2002).

If the skillset and knowledge about flock management is extended to a substantial understanding of the differences and needs of various flock subpopulations, the use of non-cage systems will become more sustainable and profitable, while improving hen health and welfare.

2 Objectives of this research

This project investigated the impact of flock dynamics on hen performance and determined how alterations in feed formulation and feeding management might improve overall egg production. While every farm has its own individual set up (flock size, hen house design, feed source), this research approach was based on the behaviour of commercial laying hens and is therefore applicable to all commercial free range egg producers. The objectives of the project were to:

A Characterise subpopulations of free range laying hens

Characterisation of free range flock subpopulations allows the identification of the special nutrient requirements of hens that prefer to stay in the shed ('stayers'), and hens that prefer to spend their time on the range ('rangers'). The following key questions were investigated:

- Are stayers or rangers underproductive?
- Are stayers or rangers undernourished?
- What are typical behaviour patterns of stayers and rangers (e.g. what is the load on resources, how often do they access feed, water and nesting boxes)?

B Determine the dynamics of free range subpopulations

Understanding flock dynamics will allow the industry to address management strategies according to the need of the flock. The following key questions were investigated:

- Is it possible to create uniform flocks composed of stayers and rangers?
- Is it possible to identify the subpopulations and manage them differently, or is the diversity of a flock a given, and has to be accepted?

C Develop and validate feeding strategies for subpopulations of free range layers

Developing and validating various feeding strategies may improve hen health and welfare, and reduce egg production costs.

3 Materials and methods: validation of a (UHF) RFID system for location tracking on an aviary system

3.1 Introduction

Radio Frequency Identification (RFID) systems are an automatic identification technology that is commonly used in monitoring of commercial livestock. From extensive sheep and cattle production systems to intensive housed animal production systems, the need to monitor livestock in real-time is increasing due to welfare, production and economic concerns. RFID technology has been used for animal behaviour and welfare research, to monitor and to obtain feedback on animal location and resource utilisation. For instance, RFID tracking systems have been proven effective for monitoring nesting behaviour, animal feeding and/or drinking behaviour (Fuller 2006; Brown-Brandl & Eigenberg 2011; Tu et al. 2011) and environmental preferences (Sales et al. 2015).

In laying hens behavioural research accelerometers, and RFID-based sensors in combination with machine learning, are used to assess the activity, the location of individual laying hens, and the measurement of individual performance of laying hens (Siegford et al. 2016; Chien & Chen 2018, Zaninelli et al. 2017). The RFID system is composed of the transponder or tag, the antenna and the reader. For RFID systems, a transponder with a unique number is interrogated by the antenna connected to a reader. The unique number on the antenna allows each hen to be individually identified. Hen movement information is extrapolated by linking the time stamp data from individual hens provided by the transponder, antennae and reader. Unquestionably, the precision of the RFID systems depends on several factors such as the number of antennae installed, the environmental conditions around the antennae, tag geometry and data management. Due to these challenges most RFID systems have been developed, basically, to perform research activities. In commercial farm settings, RFID systems would be inadequate because of the extra cost, as each hen has to be tagged. There is also some system complexity involved due to tag losses and complex algorithms required to process the data, and the requisite management procedures that all result in it being expensive, not easy and time consuming.

RFID technology can be classified into three systems, namely low frequency (30 KHz–300 KHz), high frequency (3 MHz–30 MHz) and the ultra-high 300 MHz–3 GHz system. Several low frequency systems have to be used for the location tracking of pigeons, broilers and layers, but their precision is open to question as they can only interrogate one transponder at a time and they have poor capability in capturing running hens. Furthermore, the system needs more than one antenna to cover a large pop hole or large distance. By contrast, ultrahigh RFID (UHF-RFID) has been used in the development of a monitoring system for laying hens in commercial organic egg farms. UHF-RFID systems are more advantageous because they allow multi-transponders to be interrogated by the antenna and they are very precise if fitted with an anti-transponder collision system. The UHF system is, however, more sensitive to metal, liquids and electromagnetic signal interference.

The aim of this study was to validate a high frequency UHF-RFID system to be used in a large scale commercial free range egg production facility. The system was designed to detect when the animals were moving from one area of the facility to another, in an effort to calculate the time spent in each area. The installation was performed as part of the initial shed construction and was completely incorporated into free standing aviary systems. Hens had access to two free standing aviary systems, one being located close to the pop holes, and the other one along the centre of the shed. For testing and experimental purposes, the validation area was separated into five pens with commercially available section partitions preventing hen movement from one pen to another.

3.2 Materials and methods

3.2.1 Experimental pens

Three commercial free range layer sheds were the subject of this study. Each flock was placed in an identical hen house, and in each of the sheds there were five identical experimental pens as illustrated in Figure 3-1. Each shed housed 40,000 hens in total, of which 3125 hens were randomly selected and placed at 16 weeks of age to allow for acclimatising to the environmental conditions until 18 weeks of age. From 18–22 weeks of age, the hen movement was then traced and recorded throughout the aviary system.

Custom-built HF-RFID antennae manufactured at the Science Engineering Workshop of the University of New England were placed along a three-tier aviary systems, as illustrated in Figures 3-2 and 3-3. Two antennae (A1 and A2) were placed 30 cm apart along the inner and outer sides of the pop hole areas for their entire length (3.6 m) to indicate the direction of hen movement in and out of the shed, and to distinguish between hens sitting on the antennae and hens moving across the antennae. Antennae were also placed at 15 cm distances along the right and left side of each of the three feed chains on the upper tier (A4–A15) and this was referred to as the 'upper feeder tier'. Placement of antennae along the entrances to the nest boxes that were located on the middle tier (A16–A19) detected the 'nest box' location. Placement of antennae at 15 cm distances along the right and left side of each of the two feed chains on the lower tier (A20–A27) allowed for hen detection at the 'lower feeder tier'.

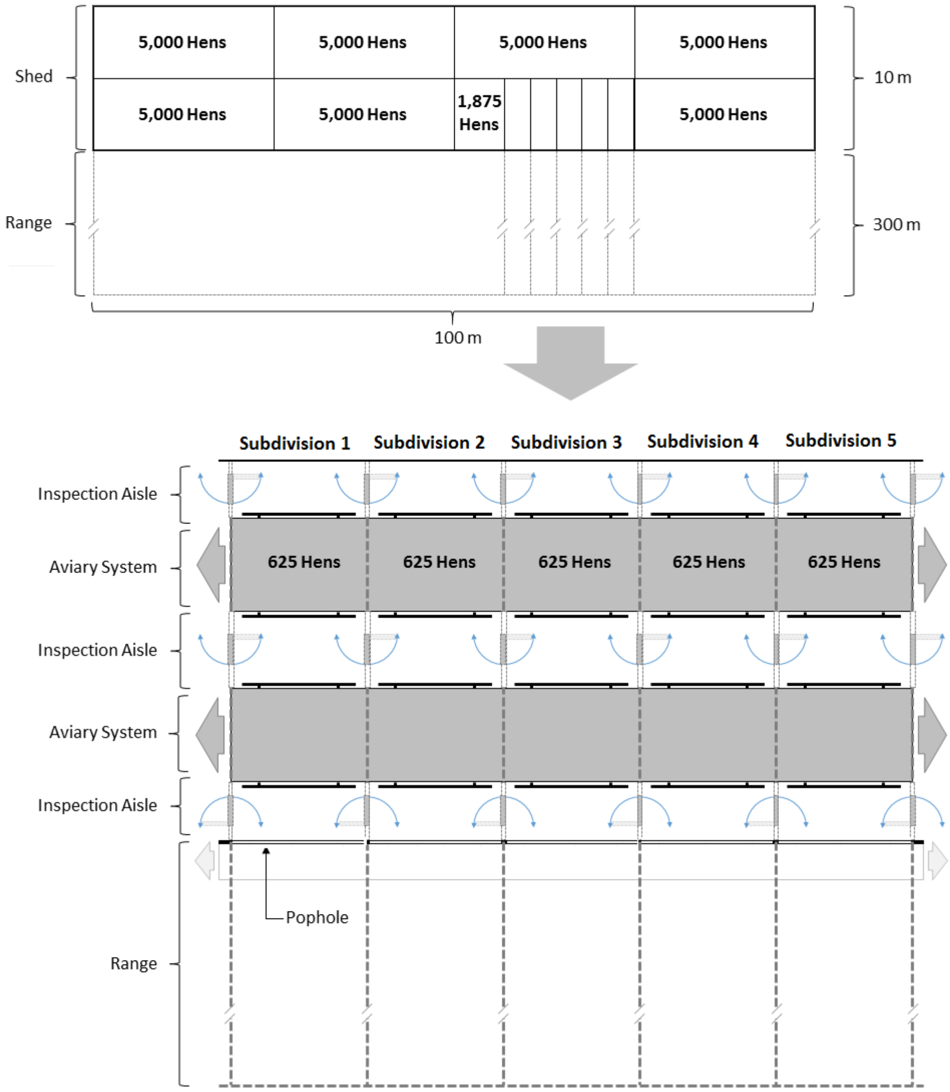

Figure 3-1 Schematic diagram of the subdivision housing the study population in each experimental shed

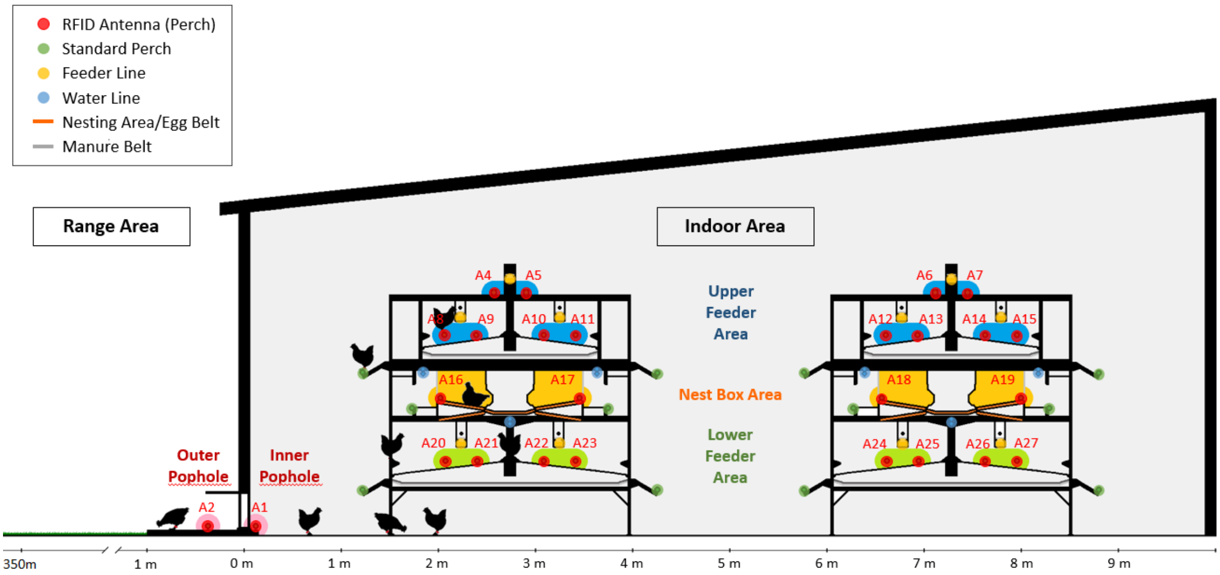

Figure 3-2 Cross-sectional view of the experimental shed showing details of the two aviary systems used within each shed

3.2.2 RFID hardware

Several antennae were positioned on the feeders, nest boxes and on the range. The time stamp data provided by the transponder, the antenna and the reader were linked to provide the locomotory movement of each hen (Figure 3-3).

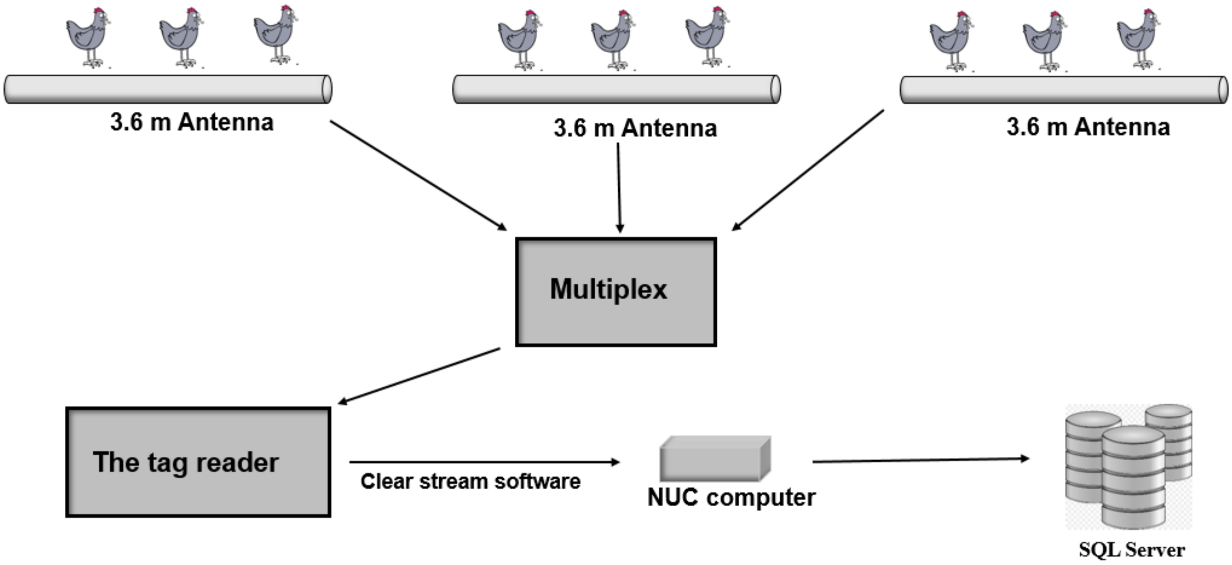

Figure 3-3 ***Schematic diagram of the laying hen position tracking system***

Speedway R420 RFID tag readers (Impinj, Inc. – Seattle, WA, USA), Monza R6 UHF-RFID Tags, (Impinj, Inc.) and Clear Stream RFID software developed by Portable Technology Solutions (Calverton, New York, USA) were used to identify and track the individual hens. Each experimental hen was equipped with an RFID leg band displaying a unique RFID number (Monza R6 Tag Chip, Impinj, Inc.) with a frequency of 915 MHz. The leg bands had an outer diameter of 30.0 mm, a thickness of 5 mm and an inner diameter of 20 mm (Figure 3-4). The weight of the leg band was 5.05 ± 0.025 g. In order to measure repeatability, five hens of each pen were equipped with an additional RFID leg band, attached to their other leg. The tubular antenna was 360 cm in length and 3.2 cm in diameter.

Figure 3-4 Individual High-Frequency RFID leg band attached to a hen's leg

3.2.3 Data handling

ClearStream software (Portable Technology Solutions – Calverton, New York, USA) was programmed to log all detections to a local MySQL database, and data were then securely transferred off-site to a PostgreSQL 9.6 database on a server located at the University of New England. Approximately 1.9 million rows of data (150 MB) were generated daily from each shed during the trial period. The RPostgreSQL (Conway et al. 2013) package in R version 3.5.0 (R Core Team 2018) was used to interface with the raw PostgreSQL database. An in-house R script was written to arrange and categorise the relevant hen activities. The R packages 'Lubridate' (Grolemund & Wickham 2011) and 'doParallel' (Calaway et al. 2015) were used to handle data transformations. Another R script was used to summarise the number of visits and time spent in the five different areas each day. Figures including regression graphs were produced using JMP statistical software (version 14 – SAS Institute Inc., Cary, NC, 1989-2019) and RStudio (R Core Team 2018).

3.2.4 Laboratory validation of the RFID system parameters

In order to quantify the accuracy of the measurements of the RFID system, the detection range and signal strength was determined by the Science Engineering Workshop at the University of New England. The antennae were tested for signal strength with Received Signal Strength Indicator (RSSI) values at varying vertical and horizontal distances (25, 55, 85, 120, 155, 200, 300, 400 and 500 mm) from the reader, as well as laterally along the antennae at 5 cm intervals. A total of 60 randomly selected RFID leg bands were tested to determine the inter-leg band variability, resulting in a total of 600 measurements being recorded at the signal strength of 900 MHz frequency.

A map of the measured signal propagation was prepared, and the theoretical signal propagation was plotted as a heat map. Leg bands were orientated as though they were on a chicken's leg, by hanging them on a horizontal plane at all measurement locations. The effect of the detection time was included in the analytical model to detect any temporal patterns indicating the reliability of the antennae. Constant voltage supply was ensured by attaching the test antenna to a desktop power supply. All other objects and human participants remained stationary and at distances greater than 2 m during the measurements, and the only immovable object within 2 m of the antenna was the concrete floor of the building at a distance of 2 cm. The data recorded were analysed using R statistical software and a general linear model was constructed.

3.2.5 On-site validation of the RFID system parameters

Furthermore, a total of 50 randomly selected hens (five hens/pen) were equipped with two tags each (one on the left leg, one on the right leg) and observed for the duration of 30 days, being exposed to 27 antennae each. The data from the tags were fitted into a general linear model using JMP version 14 (version 14 – SAS Institute Inc., Cary, NC, 1989-2019).

3.3 Results

The results of experiment one are presented in Figures 3-5 and 3-6. The highest signal strength of -37.4 dB was recorded at a distance of 25 mm from the antenna. The signal strength decreased with increasing distance from the antenna. At a distance of 500 mm, no significant signal strength could be recorded at either side of the antenna (P > 0.05).

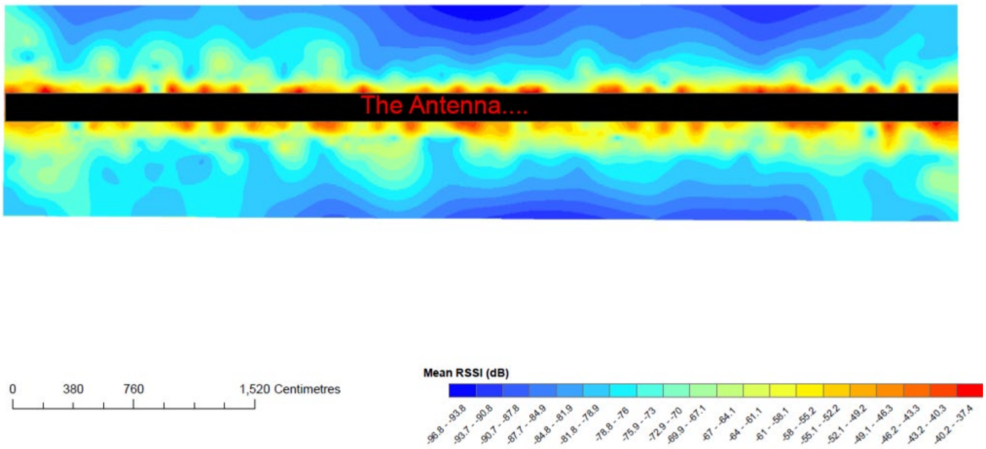

Figure 3-5 Average signal strength (RSSI)/second of the tested tags in relation to the distance from the antenna

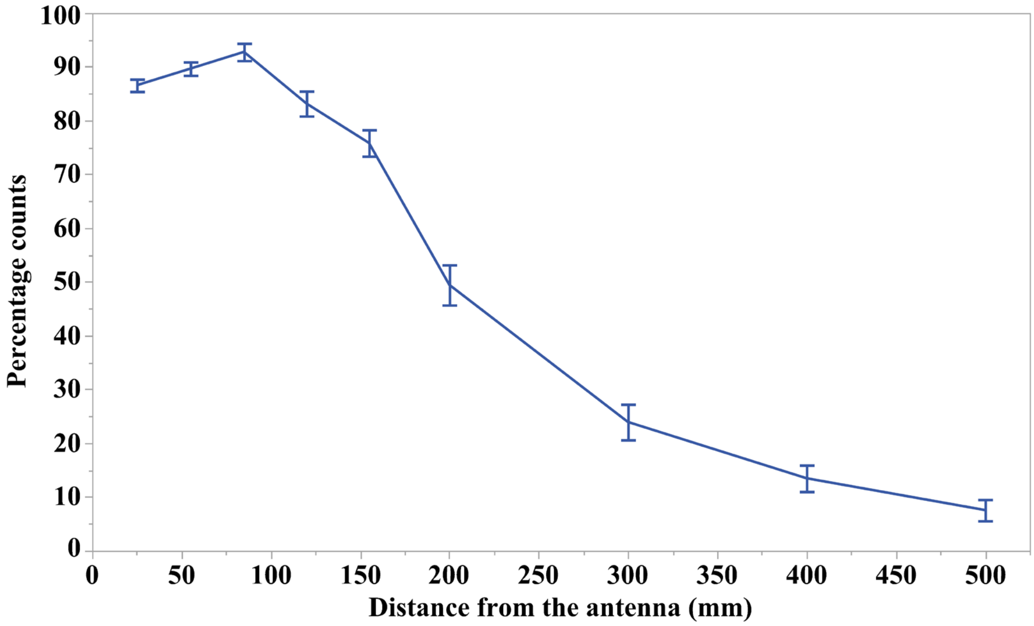

Figure 3-6 The tag detection frequency/second for all of the 60 tags in relation to the distance from the antenna

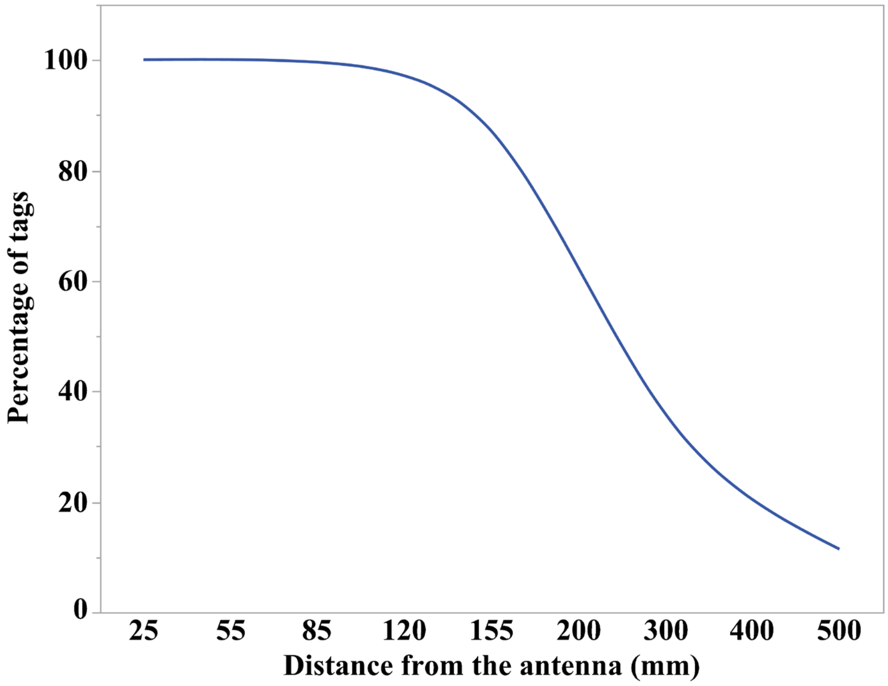

Figure 3-7 The proportion of the 120 tags detected by the antenna in relation to the distance from the antenna

One hundred percent (100%) of RFID tags were detected by the antenna at a distance of 25 mm (Figure 3-7). The detection frequency was at least 1/second up to a distance of 500 mm (Figure 3-5), and the average reading/second decreased with increasing tag distance from the antenna (Figure 3-6). The effective detection range was 200 mm (Figures 3-5 and 3-6).

Investigating the 18 hens equipped with two (2) RFID tags (one on the left leg, one on the right leg) for the duration of 30 days resulted in a total of 191,871 recorded events to be analysed. The results of the regression analysis detecting two (2) tags attached to the same hen (on the left and right leg, respectively) is shown in Figures 3-8 and 3-9. Each plotted dot represents a tag. The different colours represent different hens. The red line represents the regression line of best fit. There was a highly significant relationship between the two (2) tags ($R^2 = 0.66$; P = 0.0001). The difference of the number of detection events was most likely due to the fact that both legs of a hen are rarely at exactly the same distance from the antenna, the orientation of the RFID microchip within the leg band could have been alternated due to rotation of the leg band (and therefore being closer or more distant to the antenna), or destructive signal interference due to the metal construction of the aviary system.

The relationship in the time spent on different antennae between the two tags on each hen is represented in Figure 3-7. There was a significant relationship between the two (2) tags ($R^2 = 0.53$; P = 0.0001). The total duration of all tag detections was 599.69 hours.

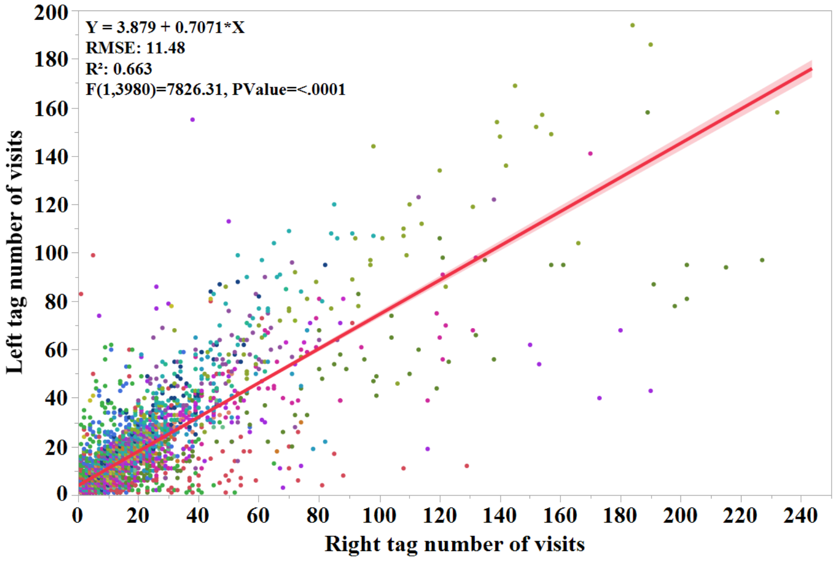

Figure 3-8 Regression model comparing the number of tag detections attached to the right and left leg of laying hens

Each plotted dot represents a tag.
The different colours represent different hens.
The red line represents the line of best fit.

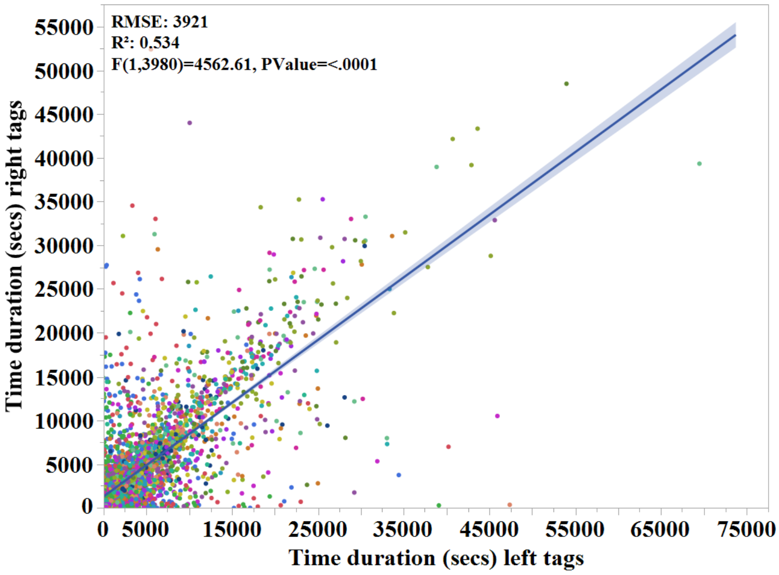

Figure 3-9 Regression model demonstrating the total time that RFID tags attached to the right and left leg of laying hens were detected at various RFID antennae

A total of 18 hens were equipped with two (2) tags each and observed for the duration of 30 days being exposed to 27 antennae; this resulted in a total of 191,871 recorded events to be analysed.
Each plotted dot represents each tag.
The different colours represent each hen.
The blue line represents the line of best fit.

3.4 Discussion and conclusion

RFID technology is commonly used in precision farming and supply chain management. RFID systems have also proven to be a useful tool to track the behaviour of poultry (Sales et al. 2015). In order to validate the custom-built RFID system placed in a multi-tier aviary shed, we successfully measured the sensitivity and specificity, false positive and false negative reads under laboratory conditions, and the accuracy of RFID tag detections. The effective detection range in this study was estimated to be at a 20 cm distance, which is less than the 29 cm reported by Sales et al. (2015) who also developed an RFID system for poultry. Given the fact that the length of a hen is 30-45 cm, and in order for a hen to eat comfortably from a feed trough, the trough should be at least 40 cm in height, resulting in the hen’s legs being at a distance of 15 cm. Therefore a 15 cm signal strength can be considered ideal for the purpose of detecting hens eating from a feed trough. Validation of the RFID system for the tag registrations assessment showed that the correlation coefficient between the two (2) tags was 0.66, reflecting an acceptable agreement between the two (2) tag detection. This is especially true when considering that both legs of one (1) hen are usually at a different distance from the antenna, and leg band rotation allows the RFID chip to be at various orientations. In most of the studies involving the use of RFID, it is surprising that few of the systems used a data management system. In this study, a data management system was used to handle the enormous amount of data produced by RFID.

In conclusion, the RFID system used in this experiment was able to reliably identify individual hens, determine the total time that hens spend at each antenna, and record the number of visits at each aviary section as well as on the range. The detection error rate can be considered as acceptable. Future applications of the system may include real-time analysis, allowing farmers to make prompt management decisions and adjust management practices with immediate effect.

4 Objective A: Characterising subpopulations of free range laying hens

4.1 Identifying typical behaviour patterns of free range flocks including the load on resources

4.1.1 Summary

The objective of this study was to investigate the impact of using a multi-tier aviary system and the range on flock uniformity in free range laying hens, and to investigate whether the extent of range use or flock uniformity can be predicted from the usage of different levels of the aviary system. A total of 15,625 Lohmann Brown hens from five commercial free range flocks housed in identical sheds on the same farm were individually weighed at 16 weeks of age, and allocated to five subdivisions within each shed. Hen movement on the multi-tier aviary system and on the range was individually monitored using the radio frequency identification (RFID) system described in Chapter 3. All hens had access to the range from 18 to 22 weeks of age and were exposed to the same management conditions. Whilst only one flock significantly changed its flock uniformity with time, flocks differed from each other in flock uniformity and body weight (P = 0.001).

Hens spent most of their available time on the lower feeder tier (7.29 ± 0.029 hours/hen/day) and on the upper feeder tier (4.29 ± 0.024 hours/hen/day) while the least amount of time was spent on the range and in the nest boxes, (0.93 ± 0.005 hours/hen/day and 1.48 ± 0.007 hours) respectively (P = 0.001). Range use was negatively correlated (r = -0.30) to the time spent on the upper feeder tier and positively correlated (r = 0.46) to the time spent on the lower feeder tier (P = 0.001). Bivariate analysis revealed that range usage, upper feeder usage and lower feeder usage had a significant curvilinear association. In conclusion, this study showed that range use is influenced by the time that hens spent on the different tiers of the aviary system. Flock uniformity varied between flocks, but it was not associated with range and aviary system usage when hens were 18-22 weeks of age.

4.1.2 Introduction

Multi-tier aviaries are one of the most common indoor structures used in non-cage egg production systems, e.g. free range and barn housing (Xin et al. 2012). Producers are motivated to use multi-tier aviaries as a form of environmental enrichment, to increase the number of hens per land unit area, to improve hen welfare, to meet legislative and accreditation requirements, and to satisfy consumer perceptions (Campbell et al. 2016a; Heerkens 2015). In a multi-tier aviary system hens have access to the open litter floor, nest boxes, feeders, drinkers, and perches (Colson et al. 2007). Furthermore, aviaries allow hens to express natural behaviour including foraging, dustbathing, scratching, locomotion, and roosting, which is known to improve welfare and musculoskeletal health (Nicol et al. 2009; Knierim 2006; Rodenburg et al. 2008; Hartcher & Jones 2017). While free range access can improve welfare parameters for the reasons mentioned above, poor flock uniformity and high mortality rates are also more prevalent in non-caged housing systems, with feather pecking, grass impaction, access to a wider range of pathogens, and cannibalism as the major contributing factors (Keeling 1994; Iqbal et al. 2018). Understanding the use of shed furniture and the range might provide solutions to manage these challenges (Rault & Taylor 2017).

To our knowledge, the association between multi-tier aviary system usage and range use of free range laying hens has not been studied to date. While the space usage of the multi-tier aviary system in combination with the outdoor access of free range laying hens is poorly understood, it is well known that individual hens, even within the same flock, of the same genetic strain, experiencing the same farm management system and weather conditions, have individual different preferences with regards to their location within the shed and on the range (Richards et al. 2011; Chielo et al. 2016). The formation of flock subpopulations due to individual hen movement preferences may challenge optimum shed design and flock management, resulting in reduced flock uniformity. Flock uniformity is considered a key component of paramount importance to manage hen welfare, health and production. The physical location of the hen can have a direct impact on feed intake and laying performance. For example, hens that visit the range more frequently may display increased locomotion and thus use more energy on movement and thermoregulation, while spending less time on the feeders for nutrient uptake. Understanding the relationship between time spent in the aviary system and on the range is therefore of paramount importance to the management of commercial free range laying hens, improved hen welfare, and ideal shed furniture design.

The aim of this study was: (1) to describe the variation of key parameters (body weight, body weight gain, and flock uniformity) in commercial free range flocks; (2) to quantify the impact of the aviary system usage on flock uniformity and range use in free range laying hens; and (3) to predict range use and flock uniformity based on individual hen use of the aviary system.

4.1.3 Materials and methods

4.1.3.1 Housing conditions

Five commercial free range layer flocks (Flocks A-E) at 16 to 22 weeks of age were subjected to this study. Flocks were placed sequentially with the first and last flock separated by a period of 18 months. Each flock was housed in identical sheds equipped with two 3-tier aviaries using chain feeding systems (NATURA Step – Big Dutchman, Michigan, USA; Figure 3-2). Each shed housed a total of 40,000 hens. Within each flock a subpopulation of 3,125 hens was placed in five identical subdivisions with approximately 625 chickens allowing equal access to all features of the shed as well as access to the whole width of the range (Figure 3-1). Each replicate had an indoor stocking density of 9 hens/m^2 and an outdoor stocking density of 1,500 hens/ha, identical to the stocking density of the remaining 36,875 hens in the shed.

4.1.3.2 RFID monitoring of range and aviary system usage

An in-house custom build RFID system (designed and constructed by the Science and Engineering workshop at the University of New England, Armidale, NSW Australia) was used to monitor the amount of time that individual hens spent in each area in the aviary system and on the range (for further details please see Chapter 3). Briefly, Speedway R420 RFID tag readers (Impinj, Inc. – Seattle, WA, USA), Monza R6 UHF-RFID Tags, (Impinj, Inc.) and ClearStream RFID software developed by Portable Technology Solutions (Calverton, New York, USA) were used to identify and track the individual hens.

In each of the subdivisions, radio frequency antennae were placed in the three tier aviary systems, as illustrated in Figure 3-2. Two antennae (A1 and A2) were placed 30 cm apart along the inner and outer sides of the pop hole areas for its entire length (3.6 m) to indicate the direction of hen movement in and out of the shed and to distinguish between hens sitting on the antennae and hens moving across the antennae. Antennae were also placed at a 15 cm distance along the right and left side of each of the three feed chains on the upper tier (A4–A15) and this was referred to as the ‘upper feeder tier’. Placement of antennae along the entrances to the nest boxes, which were located on the middle tier (A16–A19), detected the ‘nest box’ location. Placement of antennae at a 15 cm distance along the right and left side of each of the two feed chains on the lower tier (A20-A27) allowed for hen detection at the ‘lower feeder tier’. At the time of hen placement at 16 weeks of age, each hen was equipped with an RFID leg band displaying a unique RFID number (Monza R6 Tag Chip – Impinj, Inc.). Hens were acclimatised to the environmental conditions until 18 weeks of age then their movement throughout the aviary system and on the range was recorded from 18 to 22 weeks of age. The pop holes were open from 9am to 8pm daily during the period of data collection.

4.1.3.3 Flock uniformity

Individual body weight of all 15,625 hens was measured at 16 and 22 weeks of age using poultry weighing scales (BAT 1 – VEIT Electronics, Moravany, Czech Republic) with a precision of 0.001 kg. Uniformity of body weight was calculated within each 625 hen subdivisions as the proportion of hens whose individual weight was within 10% above and below the group mean (Hudson et al. 2001).

$$\text{Flock uniformity} = \frac{\text{number of hens within} \pm 10\% \text{ mean body weight}}{\text{total number of hens in a flock}} \times 100\%$$

4.1.3.4 Data analysis

While 15,625 hens were initially placed, a total of 12,445 hens were used for statistical analysis due to lost and malfunctioning RFID tags, as well as hen mortality. Unless identified otherwise, data were analysed using JMP Statistics software (version 14 – SAS Institute Inc., Cary, NC, 1989-2019). Data from three types of analysis are presented.

Firstly, summary statistics on all hens and flocks are provided without formal statistical analysis to provide indications of distributions and variance for different measures. The individual hen (n = 12,445) was used to report these flock descriptives.

Secondly, formal statistical analysis to test for differences between flocks was undertaken with the subdivision (pen) as the experimental unit, and allowing for evaluation of five replicates per shed (total n = 25). The effects of flock and hen age on body weight and flock uniformity per flock were analysed using a mixed restricted estimate of maximum likelihood (REML) model with subdivision (replicate pen) as a random factor, and flock, hen age and their interactions as fixed effects. Flock differences in the number of visits per hen per day, or time spent per hen per day at the different parts of the aviary system and the range were tested in a simple linear model fitting the effect of flock alone. Following a significant main effect or interaction, Tukey's HSD test was used to determine the significance of differences between means within that effect.

The third series of analyses investigated associations between measured variables in individual hens with the individual hen as the experimental unit (n = 12,445). A Spearman correlation matrix was created using R 3.5.0 (R Core Team 2018, 'Performance Analytics' package; Peterson et al. 2018). Following this, associations between selected pairs of variables were tested within flocks and overall using bivariate regression models with linear and curvilinear curve fitting. Finally, a multiple regression model was constructed using individual hen variables to predict the time at the range with backward elimination used to construct an optimal regression equation.

4.1.4 Results

4.1.4.1 Descriptive statistics

Flock descriptive statistics for body weight, body weight gain and uniformity are presented in Table 4-1. Time spent and number of visits at the different areas of the aviary and range are presented in Figure 4-1 and 4-2 respectively.

Table 4-1 Descriptive summary for each of the five flocks investigated providing details of the body weight and flock uniformity for individual hens at 16–22 weeks of age (total n = 12,445)

Parameters	Body weight (kg) [1]		Body weight gain (kg) [1]	Flock uniformity (%)[1]		Number of hens (n)
Age of hens	**Week 16**	**Week 22**	**Week 16–22**	**Week 16**	**Week 22**	
Flock A	1.36 ± 0.002	1.75 ± 0.003	0.39 ± 0.002	88.4 ± 0.53	87.2 ± 1.10	2,435
Flock B	1.26 ± 0.002	1.74 ± 0.003	0.48 ± 0.002	81.0 ± 1.06	83.4 ± 1.28	2,241
Flock C	1.27 ± 0.002	1.74 ± 0.002	0.47 ± 0.002	81.3 ± 1.68	83.3 ± 0.68	2,646
Flock D	1.35 ± 0.002	1.76 ± 0.003	0.41 ± 0.003	81.6 ± 0.90	78.9 ± 3.15	2,659
Flock E	1.34 ± 0.002	1.79 ± 0.003	0.45 ± 0.002	84.4 ± 1.06	83.7 ± 0.25	2,464
Pooled	1.32 ± 0.001	1.76 ± 0.001	0.44 ± 0.007	83.4 ± 1.03	83.3 ± 0.53	12,445

[1] The numbers are presented as mean ± SEM.

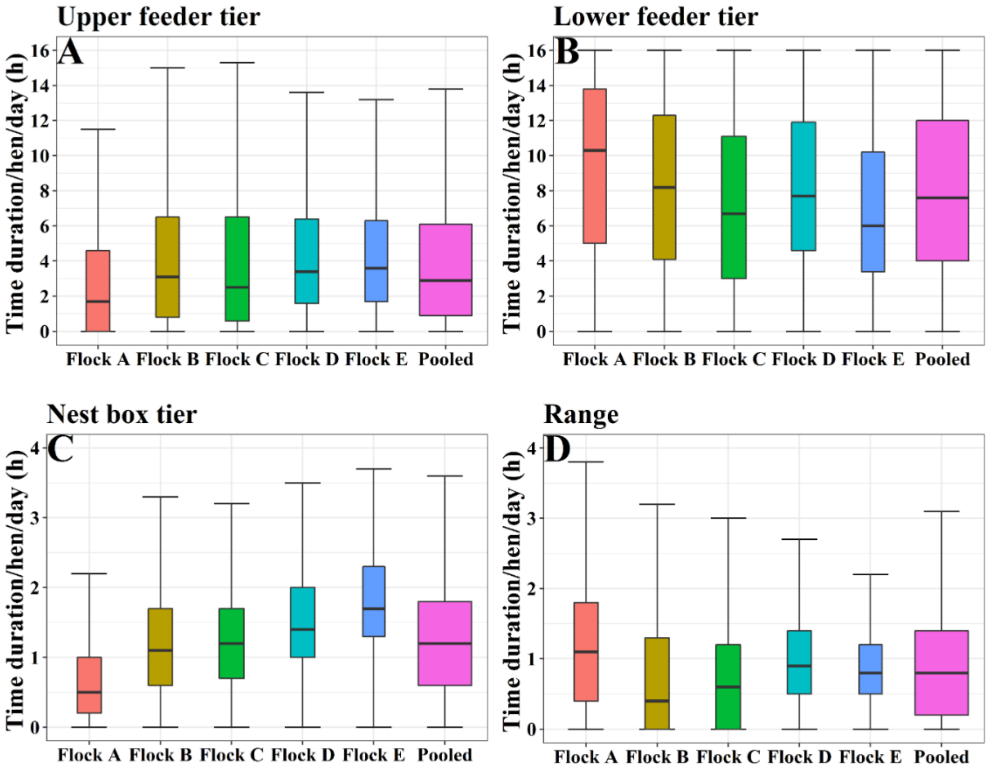

Figure 4-1 Box and whisker plots of the time that individual hens spent per day in each of the three tiers of the aviary system and on the range (n = 12,445)

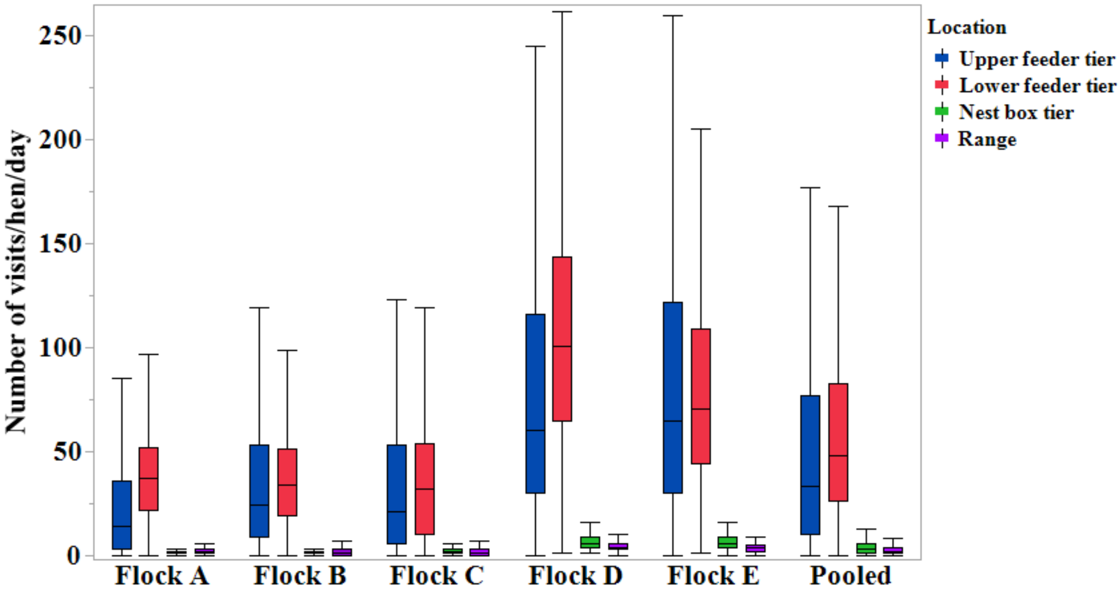

Figure 4-2 Box and whisker plot of the number of individual hen visits per day at each of the three tiers of the aviary system and on the range from 18–22 weeks of age (n = 12,445)

4.1.4.2 Body weight and flock uniformity

Analysis of body weight revealed significant effects of flock ($P < 0.001$) and age ($P < 0.001$) with significant interaction between these effects ($P < 0.001$, Figure 4-3). Analysis of flock uniformity also revealed highly significant effects of flock ($P < 0.001$), age ($P < 0.001$) and flock x age ($P = 0.140$, Figure 4-3) with body weight fitted as a covariate, also having a highly significant positive effect ($P < 0.001$). Body weight and flock uniformity at week 16 were significantly higher in hens of Flock A compared to those in all other flocks ($P = 0.001$, Figure 4-3a). Hens of Flock E had the highest average body weight at 22 weeks of age compared to all other flocks. There was no significant change in flock uniformity between 16 and 22 weeks of age in Flock A, B, D and E while it increased significantly by 7% in Flock C. Hens of Flock B had the lowest body weight at week 16 and the highest body weight gain ($P = 0.001$).

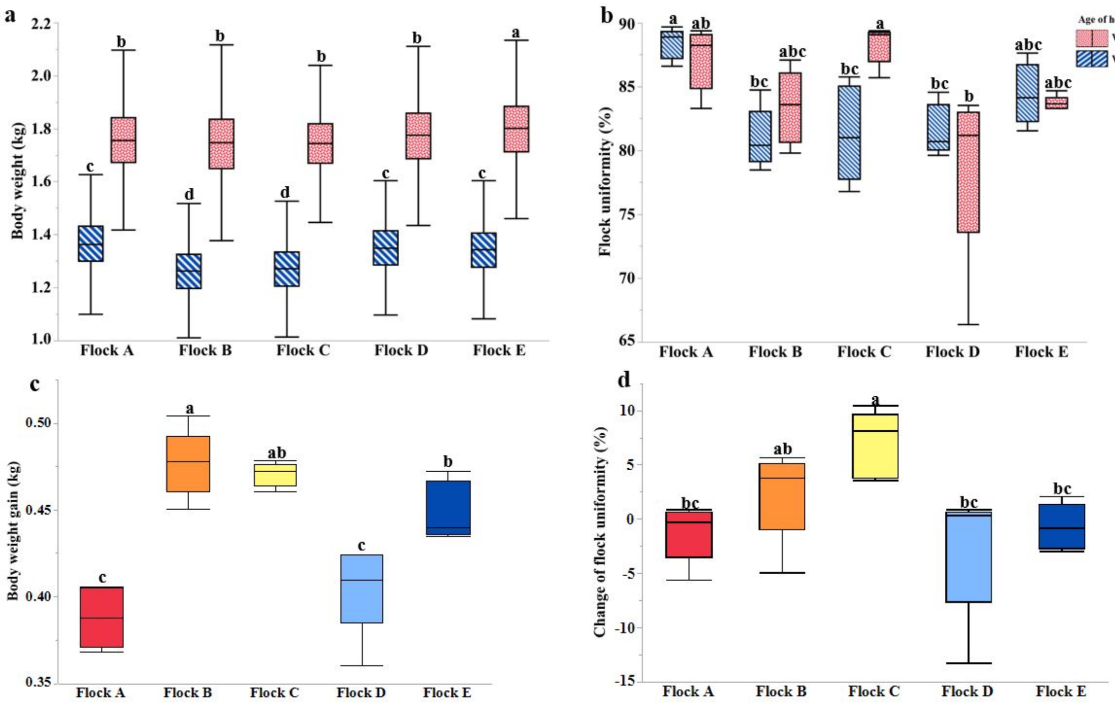

Figure 4-3 Comparisons of the body weight variables among the 5 Lohmann Brown flocks at 16 and 22 weeks of age

The letters a, b, c, d in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

4.1.4.3 Time spent at various areas of the aviary system and on the range

Hens spent an average of 7.92 ± 0.029 hours/hen/day at the lower feeder tier, followed by the upper feeder tier (4.29 ± 0.024 hours/hen/day) while the hens spent the least time at the nest box (1.48 ± 0.007 hours/hen/day) and on the range (0.93 ± 0.005 hours/hen/day). Analysis of the time duration revealed a significant effect of location (P < 0.0001) with significant interaction between location and flock (P < 0.0001). As shown in Figure 4-4, the hens in Flock A spent significantly more time in the lower feeder tier (P = 0.001) and on the range (P = 0.001) compared to hens in all other flocks (Figure 4-4B). On the other hand, the hens in Flock E spent significantly more time at the nest box (P = 0.001). Hens in all of the flocks spent a similar amount of time on the upper feeder tier (P = 0.256) (Figure 4-4A).

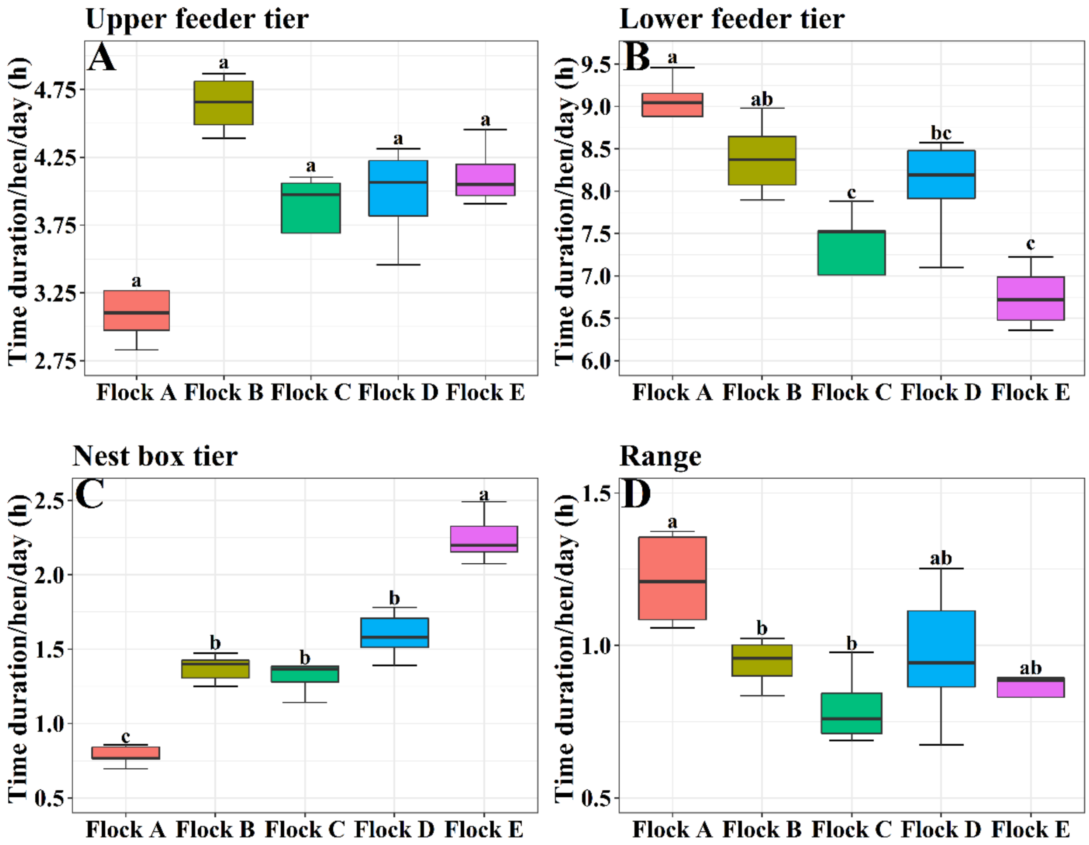

Figure 4-4 Comparisons of the time hens spent at the different tier levels of the aviary system and on the range during 18–22 weeks of age

The letters a, b, c, d in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

4.1.4.4 Number of hen visits to the aviary tiers and the range

Significant effects of flock (P < 0.001) and location (P < 0.001) and an interaction between these effects (P < 0.001) were evident on the number of visits/hen/day. The most visited tiers were the lower and upper feeder tiers with 62.7 ± 0.36 and 62.4 ± 0.63 visits/hen/day while the range and the nest box were the least visited areas with 4.1 ± 0.03 visits/hen/day and 2.7 ± 0.02 visits/hen/day, respectively. Figure 4-5 shows that the average number of hen visits to the lower and upper feeder tiers, the nest box and the range was highest in Flock D and E compared to all other flocks (P = 0.001). Flock E had the highest nest box visits compared to all other flocks (P = 0.001, Figure 4-5C).

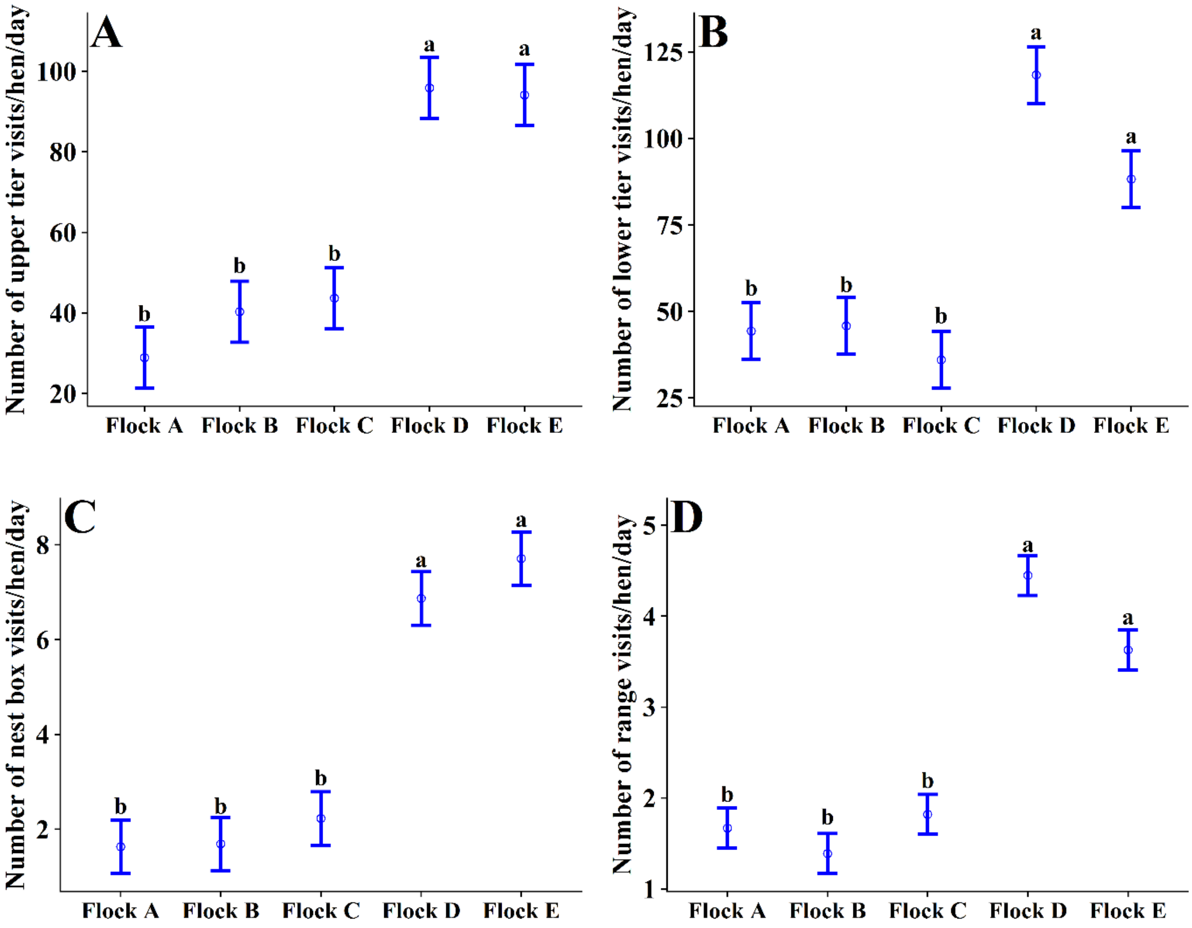

Figure 4-5 Statistical analysis representing the means of the five investigated flocks at the different tier levels of the aviary system and on the range during 16–22 weeks of age (n = 5)

The letters a, b, c, d in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

4.1.4.5 Correlation of the time spent in the different observation areas

Correlation plots, distribution histograms and Spearman correlation coefficients between individual body weights, body weight gain, time spent on the aviary system, and time spent on the range are shown in Figure 4-6.

The time hens spent on the upper feeder tier was negatively correlated with the time spent on the lower feeder tier (r = -0.75, P = 0.001). There was a moderate negative relationship between the time spent by the hens on the range and the time the hens spend on the areas in proximity to the upper feeder tier (r = -0.46, P = 0.001). A weaker positive association between the time spent on the range and the time spent on the lower feeder tier was also observed (r = 0.30, P = 0.001).

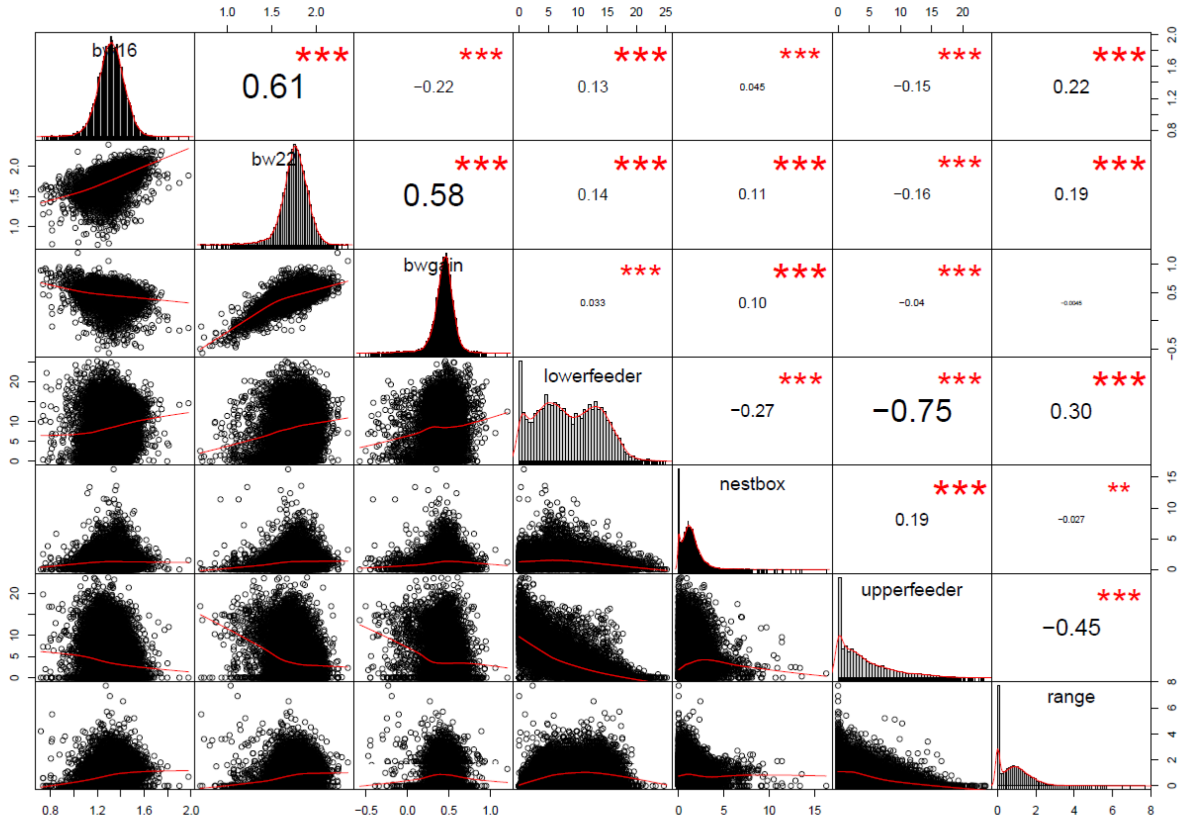

Figure 4-6 Correlation plots, distribution histograms and Spearman correlation coefficients for the time hens spent in the different observation areas and their relationship to body weight parameters at 18–22 weeks of age

The diagonal histogram matrix illustrates the distribution of each variable while the lower triangular part of the matrix indicates the bivariate scatterplots with red smooth fitted splines. In the diagonal histogram matrix the abbreviation bw16, bw22 and bwgain represent the body weight of each hen at 16 and 22 weeks of age and body weight gain respectively. The time spent at the lower feeder, upper feeder, nest box tiers and at the range are represented by lowerfeeder, nestbox, upperfeeder and range, respectively. The upper triangular part of the matrix provides data regarding the Spearman correlation coefficients and the respective levels of significance. The numbers on the x and y axis represent the time duration in each area (h) and the body weight (kg). The font size of each correlation coefficient (r) is proportionate to the significance value while the asterisks (***, **, *) and indicates statistical significance below 0.001, 0.01 and 0.05, respectively. The lower triangular part of the matrix provides scatterplots of all hens in the 5 flocks between all variables.

4.1.4.6 Predicting individual range use by the time spent on the different aviary system tiers

Significant positive linear and curvilinear (2nd order polynomial) relationships between the lower feeder tier usage and range usage were detected in all flocks with $R^2 = 0.122$ ($P < 0.001$) and $R^2 = 0.171$ ($P < 0.001$) respectively for the pooled data (Figure 4-7). In all cases the curvilinear fit was a better fit than the linear, indicating broadly positive association for low to moderate values of time at the lower feeder, but negative association at high values for time at the lower feeder. The R^2 values for the flocks ranged from 0.118 to 0.235 in the curvilinear prediction for time spent on the range (Figure 4-7).

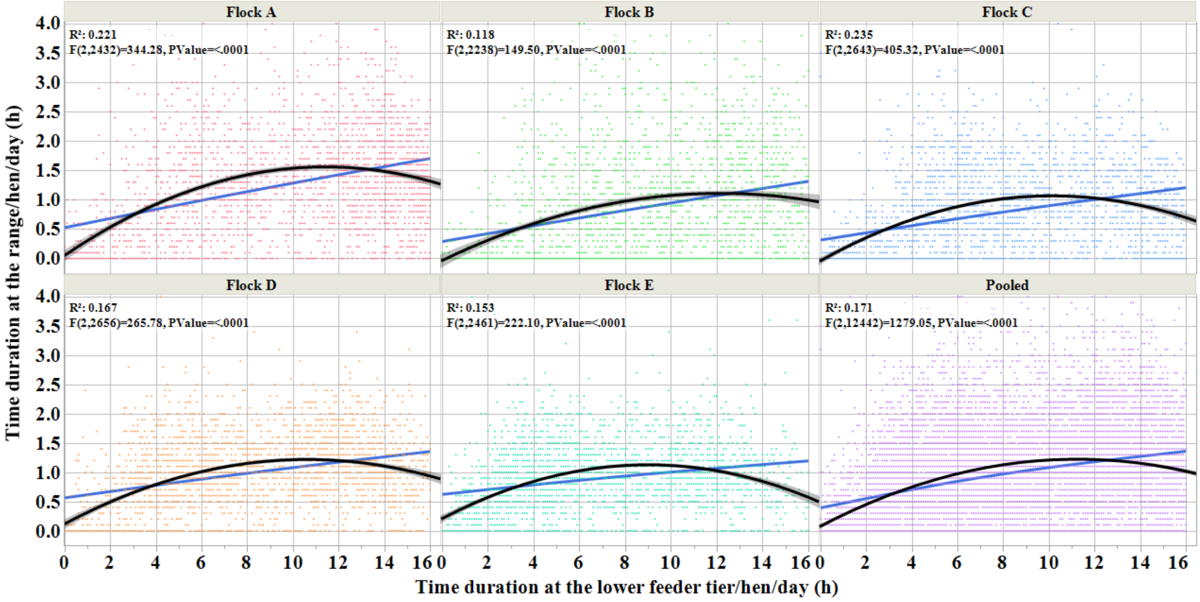

Figure 4-7 Association between range usage and lower feeder tier usage for each of the five investigated flocks (n = 12,442)

The linear and the curvilinear fit are presented in Figure 4-8, for the upper feeder tier. A significant negative relationship between the time spent at the upper feeder tier and the time spent on the range was detected in all flocks ($R^2 = 0.26$, $P < 0.001$ for overall data, R^2 ranged from 0.232 to 0.308 in individual flocks).

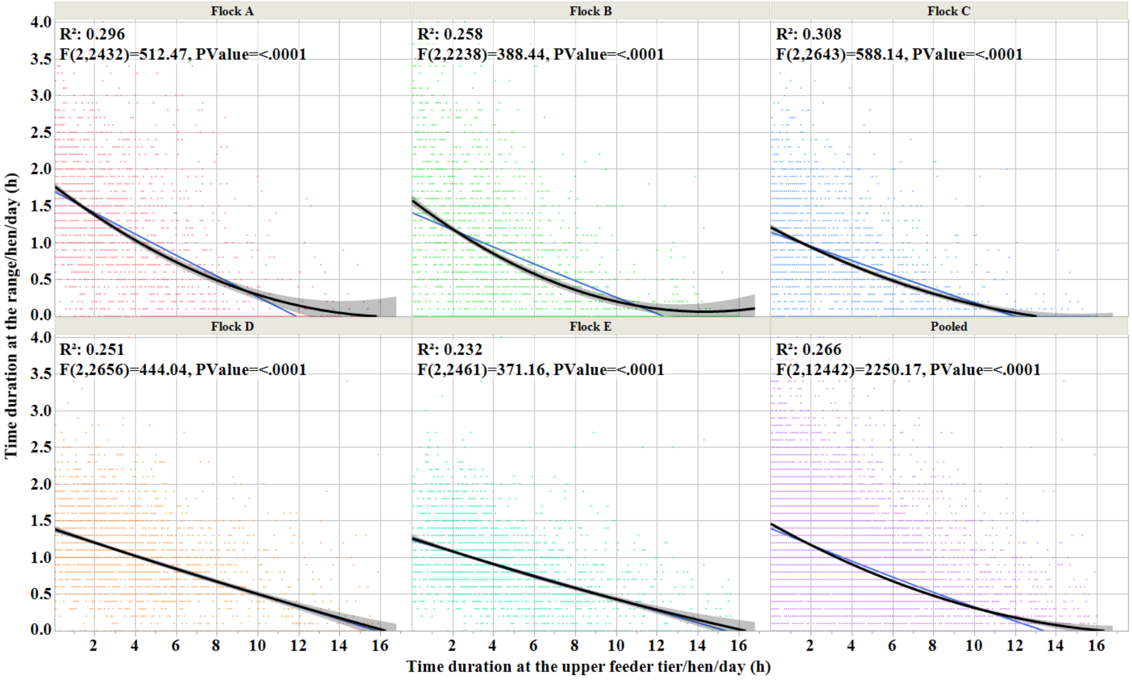

Figure 4-8 Fit plots for each of the five flocks and the pooled data from all *of* the flocks to predict range use based on the time *that* the hens spent on the upper feeder

Using a combination of the time spent in different tiers of the aviary system did not improve the goodness of fit of the prediction equations for time spent on the range above the quadratic prediction equations based on time spent at the upper feeder tier, and as such could not be recommended for prediction (Table 4-2).

Table 4-2 Multiple regression estimates to predict the time hens spent on the range

	Flock A	Flock B	Flock C	Flock D	Flock E	Pooled
Intercept	1.749 ± 0.229 ***	1.092 ± 0.212***	0.466 ± 0.170**	1.260 ± 0.164***	1.03 ± 0.167***	0.752 ± 0.084***
Time at lower feeder (h)	-0.075 ± 0.005***	-0.043 ± 0.004***	-0.030 ± 0.003***	-0.005 ± 0.003	0.015 ± 0.003***	-0.025 ± 0.002***
Time at nest box (h)	-0.187 ± 0.021***	-0.091 ± 0.017***	-0.047 ± 0.012***	-0.067 ± 0.013***	-0.008 ± 0.007***	-0.065 ± 0.005***
Time at upper feeder (h)	-0.199 ± 0.008***	-0.113 ± 0.005***	-0.100 ± 0.004***	-0.084 ± 0.003***	-0.088 ± 0.004***	-0.104 ± 0.003***
Body weight (kg) at 16 weeks of age	-0.622 ± 0.177***	1.110 ± 0.181***	0.302 ± 0.131*	-0.175 ± 0.127	0.216 ± 0.131	0.907 ± 0.063
Body weight (kg) at 22 weeks of age	-0.028 ± 0.127	-0.372 ± 0.126**	-0.332 ± 0.104***	-0.002 ± 0.083	-0.030 ± 0.091	-0.173 ± 0.048
R^2	0.259	0.214	0.282	0.237	0.228	0.238
Adjusted R^2	0.258	0.212	0.281	0.236	0.227	0.238
Degrees of Freedom	2761	2764	2867	2773	2521	12,445

Statistical significance at 0.05%, 0.01% and 0.001% levels are denoted respectively by '*', '**', '***'.

4.1.5 Discussion and conclusion

4.1.5.1 Body weight and flock uniformity

Flock uniformity varied significantly between the flocks and hen age, and there was no relationship between body weight, flock uniformity and time spent on the different aviary tiers and range (Figure 4-6). This suggests that the difference in body weight and flock uniformity among the flocks was caused by confounding factors such as genetics, weather, pasture intake and/or rearing conditions. Reduced body weight gain and flock uniformity in Flocks A, D and E may be due to increased energy expenditure on egg production, thermoregulation, reduced feed intake or increased pasture intake.

Metabolic energy is not only required for hen movement, but also for thermoregulation as the hens are exposed to the inherently varying weather conditions on the range (Nyoni et al. 2018). The outdoor weather conditions can differ substantially from the thermo-neutral temperature requirement for laying hens of 10–18°C, and may cause reduction in feed intake and heat stress (Charles & Walker 2002). However, it would be possible that hens in Flocks A, D and E used more energy for physical activities at the expense of egg production, which would require further investigation.

Moreover, a variation of energy and essential nutrient uptake due to uncontrolled pasture intake of individual ranging hens may have also caused the observed variation in body weight gain and flock uniformity (Iqbal et al. 2018). Depending on the development of the gastrointestinal tract and the quantity of fibre accessed during early life, pasture intake may improve weight gain, feed conversion ratio and ileal digestibility of nutrients (Walker & Gordon 2018). However, uncontrolled pasture intake has been shown to decrease body weight and increase mortality due to grass impaction (Ruhnke et al. 2015b).

The time hens spent on the range varied between flocks, with the longest average time on the range found in Flock A, which was not significantly different from Flock D and E, and the shortest in Flocks B, C and E (Figure 4-4). A variation in range usage observed in different flocks was indicated already by differences found in previous studies, where 6.4–80% of hens range at a certain time (Petterson et al. 2016). Although 78.4% of hens visited the range at least once during the observation period, the hens spent on average 0.7–1.2 hours/hen/day on the range. This is comparable with research performed by other investigators where 95% of the observed hens spent 5.0–6.1 hours/hen/day on the range, and 51.5 min per visit (Hartcher et al. 2015). The reasons for these longer flock ranging times may include the relatively large flock size, differences in shade type and quantity provided on the range, the breed of hens, and the relatively young age of the hens used in this study (Hegelund et al. 2005).

Hen locomotion and dispersal are highly influenced by the distribution of food and water. In all flocks, hens spent significantly more time (7.9 ± 0.04 and 4.2 ± 0.03 hours/hen/day) at the lower and upper feeder tiers compared to the range and nest box. This uneven spatial distribution is similar to previous research, where at a given time period 46% of the observation hens were located at a slatted floor area, and 8.85% of hens in the nest boxes (Mench & Keeling 2001; Carmichael et al. 1999). The frequent use of feed chains reduces the likelihood for competition, allowing hens to access the feed source throughout the day (Carmichael et al. 1999). In addition, hens are known to use all available space unevenly, potentially forming clusters, leading to smothering if not managed appropriately (Estevez et al. 1997; Arnould et al. 2001).

There was no significant difference in the time spent on the upper feeder tiers between all flocks. This can be explained by the fact that there is no need for hens to defend food resources in an *ad libitum* feeding system (Estevez et al. 1997). Furthermore, the provision of five feed chains along the entire length of the aviary system may have reduced the competition for food and water as seen in other study facilities (Arnould et al. 2001). Social interactions on the three-tier aviary system might influence how long the hen stays at the nest site and on the upper feeder tier.

It is also important to mention that other factors not measured in this study may have contributed to the individual preference such as hen house climate, early life experience, and potential pathological conditions of the hen. Using the nest box and the upper feeder tier during the day might also be considered as a safer environment to prevent an attack by dominant hens of socially low-ranked hens or lighter hens.

Although Flocks D and E spent comparable time at the observational areas to all other flocks, the flocks had significantly a higher number of visits compared to all other flocks. This is an indication that the hens were more motivated to explore their environment at an early age. The reason for the high level of activity in Flocks D and E might be the difference in the age of parent stock and/or early habituation to the aviary system.

4.1.5.2 Correlation of the time spent on the range and usage of the aviary system

The results of our experiment suggest that the time hens spent on the range is associated with the time spent on the different tiers of the aviary system, as shown by the evident relationships in all the five flocks (Figure 4-6). In a review summarising the range use of broiler and laying hen trials, 5.1% to 80% of birds accessed the range at a certain time (Petterson et al. 2016). In none of these range use studies, did the authors investigate correlation of the hen house furniture and range use. The strong negative correlation between the time spent at the lower feeder tier and the time spent at the upper feeder tier suggests that individual hen prefers certain locations. The difference of individual hen preferences may be attributed to social dynamics, fear, and coping styles (Campbell et al. 2016b). In large flocks, the social dynamics can be determined by the comb size and body weight (D`Eath & Keeling 2003). Evaluation of these parameters might be helpful to investigate the impact of social ranking on hen usage of the aviary system in the future. It would be important to know if hens on the top tiers need special attention, especially when managing flocks to prevent unwanted behaviour such as severe feather pecking, or reducing the number of misplaced eggs.

The moderate negative relationship between the time spent on the range and the time spent on the upper feeder tier suggests that individual hens that prefer to use the upper feeder are unlikely to access the range. This is of importance when managing a flock that is encouraged to access the range.

4.1.5.3 Predicting time on the range and aviary usage

As already highlighted above, the time that individual hens spent in different tiers of the aviary system is associated with their range use. The predictive curvilinear relationship between aviary usage and ranging was evident in individual hens. Range use was positively associated with lower feeder usage, which might be an indication that hens had access to the feed before or after they used the range.

The negative association between use of the range and the upper tier emphasises that hens on the upper tier might need to be managed separately to allow for better range usage. For example, farmers may install ramps that provide for an easy movement of hens from the upper and lower feeders, to encourage hens to use the aviary system in equal proportion. Ramps reduce collisions, falls and keel bone fractures, and support movement in the aviaries (Stratmann et al. 2015; Petterson et al. 2017). The time spent at the nest box was of minor relevance in predicting both the time spent on the range and flock uniformity. As opposed to farmers' perception that free range hens may misplace eggs on the range frequently, this study provides evidence that use of the nest box is comparable by hens that range and hens that prefer not to range.

Addition of body weight as a factor in the multiple regression analyses conducted to examine the relationship between range use and aviary usage (Table 4-2) did not improve the goodness of fit when compared with the bivariate regression models. It will be interesting to add other confounding factors such as temperature variation in the future to achieve more robust prediction equations.

In conclusion, the main finding from the present exploratory study was that range use is negatively associated with the time that individual hens spent on the upper feeder, and is positively associated with the time that hens spent on the lower feeder tiers of the aviary system. Further research is required to investigate the factors contributing to the usage of the various areas of the aviary system, as well as the consequences of hens using some areas only.

4.2 Determining performance parameters of hen subpopulations and the load on resources

4.2.1 Summary

This study was conducted to evaluate the effects of different range use on egg production. At 22 weeks of age, hens were separated according to their ranging behaviour. The stayers group spent less than 20% of their time on the range, the roamers spent 21% to 42% of their time on the range, the rangers spent more than 42% of their available time on the range. Parameters evaluated included egg production, quality and egg grade at 22, 32, 42, 52, 62 and 72 weeks of age. Hens that spent more time on the range (‘rangers’), came into lay earlier compared to hens that preferred to stay in the shed (‘stayers’). For example, at 22 weeks of age, rangers enjoyed a laying rate of 88.0 ± 1.1%, while stayers performed at 78.2 ± 1.9%. Stayers did not achieve the performance of rangers until they were older than 52 weeks of age.

4.2.2 Introduction

In free range laying hen behaviour range usage depends on flock size, the number of pop holes, shelter on the range, weather conditions, age and experience of the flock (Petterson et al. 2016). The freedom of choice results in the development of several subpopulations within one flock. Previous studies revealed that a certain percentage of birds never leave the hen house, while others spend the majority of time on the range (Gebhardt-Henrich et al. 2014a; Gilani et al. 2014). The consequences of these subpopulations include reduced flock uniformity, sup-optimal flock nutrition, and subsequently sub-optimal flock production (Coletta et al. 2012; Fanatico 2006). However, the direct impact of the flock subpopulations on egg production and egg quality has not been investigated to date, whereas the quantity of production loss and production cost has not been allocated to a specific subpopulation. This prevents the industry from developing strategies to support these underperforming subpopulations. The aim of this study was to investigate the effects of different range use on egg production, including egg quality.

4.2.3 Materials and methods

4.2.3.1 Animal housing and range use

Five (5) commercial free range laying flocks were subject to this research. In each shed, 3,125 Lohmann Brown hens were housed amongst their 36,875 flock companions. These hens were individually monitored using the radio frequency identification (RFID) system described in Chapter 3 and classified according to their daily range usage from 18 to 21 weeks of age. At 22 weeks of age, the low, medium, and top percentages of range users were categorised into three (3) groups and physically rearranged into pens, whereas all stayers were allocated to one pen, all roamers were allocated to a different pen, and all rangers were allocated to a third pen. Each pen housed 625 hens, allowing for a comparable stocking density of 9 hens/m^2. The individual range usage of all hens was continuously monitored until hens were 72 weeks of age.

4.2.3.2 Egg quality and laying performance

Egg production per pen (stayers, roamers, rangers) was determined at 22, 32, 42, 52, 62 and 72 weeks of age. During these collection weeks, all eggs laid were collected for the duration of seven (7) consecutive days and subjected to on-farm grading. During egg collection, the eggs collected on the system, on the floor, and waste eggs were recorded for each treatment group in each flock.

Egg quality was measured in five (5) eggs/pen/day, with eggs being randomly selected from each pen for the duration of five (5) consecutive days when hens were 22, 32, 42, 52, 62 and 72 weeks of age (Figure 4-9). All eggs were individually weighed, and the external and internal quality was determined by using an egg multi-tester instrument (DET6000 – Nabel, Kyoto, Japan). Shell colour was measured by using a QCR shell colour reflectometer (Technical Services and Supplies (TSS), U.K.). All of the eggs were then broken, and their contents were removed. Yolk colour was measured using a TSS automatic yolk Colorimeter measuring the wavelength of light reflected from yolk. Albumen height (±0.1 mm) was measured using an electronic height gauge (Nabel DET6000). Haugh units were calculated on the input of egg weight and albumen height by the egg tester Nabel DET6000 as indicated by the formula:

$$HU = 100 \times \log (H - 1.7\ 0.37 + 7.6)$$

Where HU: Haugh unit

H: Albumen height

Figure 4-9 Manual egg collection using colour coded trays to determine the egg laying performance and egg quality for each of the ranging groups (stayers, roamers, rangers)

4.2.3.3 Feather cover

The feather score was assessed using a 4-point based system for each body region (neck, chest, wing, back, and vent/cloaca) following (Tauson et al. 2005). The same investigator performed all observations of all hens.

4.2.3.4 Keel bone

Hens were humanely sacrificed, and the skin integrity ruptured, exposing the keel bone and abdominal organs. The keel bone health of the laying hens was categorised based on the severity of keel bone deformities on a scale of 0 to 2, where the score 0 indicated no damage, score 1 indicated a single fracture, and 2 indicated severe or multiple fractures (Figure 4-10). The keel bone damage was inspected by visual and palpatory means, by the same investigator allowing the detection of dorsal and ventral fractures.

Figure 4-10 Examples of keel bone damage observed in free range laying hens

The keel bone at the far left would be classified as 0 (no damage).
The two keel bones in the middle would be classified as 1 (single fracture).
The keel bone to the right would be classified as 2 (severe or multiple fractures).
Keel bone damage was evaluated by inspection as well as palpation of the carcass, allowing the detection of dorsal fractures.

4.2.3.5 Health status of the liver

To assess the health status of the liver, the presence, absence and severity of fatty liver were categorised using a score of 0 (normal liver), 1 (mild fatty liver), and 2 (severe fatty liver; Figure 4-11A). The presence or absence of spotty liver was noted on a score of 0 (spots absent) or 1 (spots present), whereas pathognomonic spotty liver lesions were identified (Figure 4-11B). A few representative livers were analysed via qPCR, resulting in confirmed infection of *Campylobacter hepaticus*, the known pathogen responsible for Spotty Liver Disease.

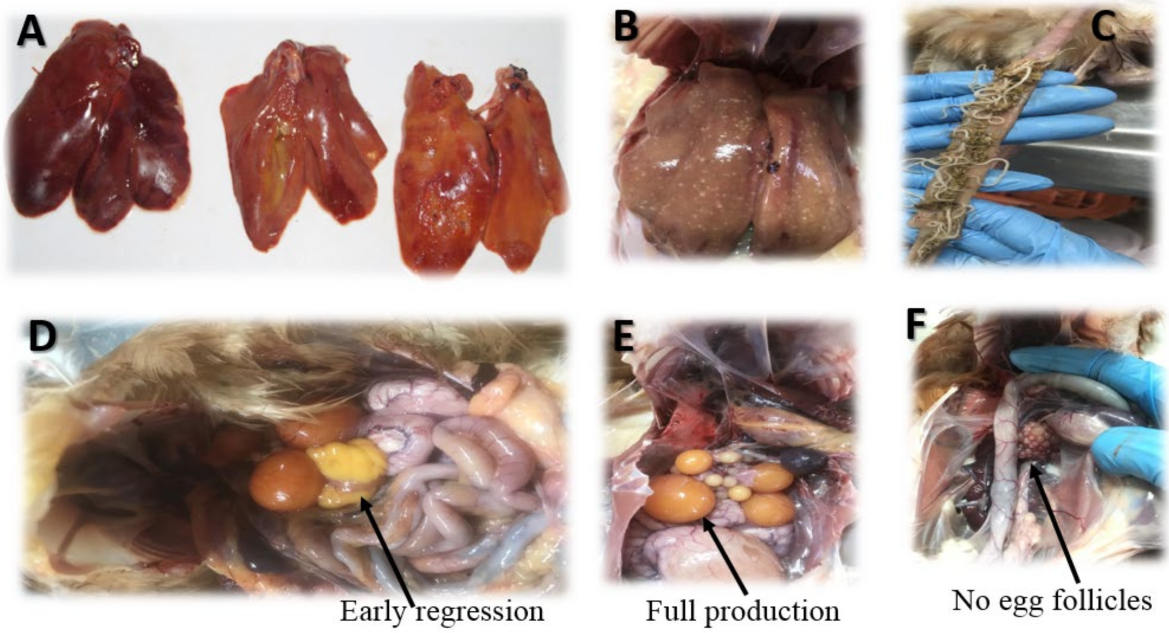

Figure 4-11 Examples of necropsy results observed for free range laying hens subject to this research study

A No, mild, and severe fatty livers.
B Spots on the liver.
C Nematodes (*A. galli*).
D–F Egg follicles.

4.2.3.6 Prevalence of gastrointestinal parasites

To investigate the prevalence of gastrointestinal parasites, the jejunum was extracted from the abdominal cavity, longitudinally opened using a pair of scissors and visually inspected. The presence or absence of cestodes and *Ascaridia galli* (*A. galli*) was recorded for each individual hen (Figure 4-11C).

4.2.3.7 Egg follicles

To assess the stage of egg production, the presence of egg follicles was scored visually using a 4-point scoring system where 1 indicated no active follicles, 2 indicated the presence of follicles in late regression, 3 indicated the presence of follicles in early regression, and 4 indicated full egg production (Figure 4-11D–F).

4.2.4 Statistical analyses

All the data were analysed using JMP Statistics software (version 14 – SAS Institute Inc., Cary, NC, 1989-2019). Boxplots were created to show the difference between hens – stayers, roamers and rangers of 18–21 weeks of age (Figure 4-13). Range use time was calculated into four production periods, namely: the pre-laying period (18–22 weeks); peak laying period (23–33 weeks); late laying period (34–55 weeks); and end of laying period (56–74 weeks).

The average result of the five (5) eggs/day was used for statistical analysis. The data on range use, laying performance, and egg quality were analysed by ANOVA with a completely randomised design by JMP version 14. The biplot graphs were produced using JMP version 14.

To determine the effect of range use, subpopulations, flocks and their interactions on keel bone damage, fatty liver score, egg follicle score and feather scores, a nominal logistic regression model was used. For fatty liver and spots on the liver scores, an additional analysis was carried out to compare the overall population mean using Analysis of Means – Transformed Ranks (ANOM). ANOM graphically tests the equality of means on count data and allows multiple comparison of the subgroups to the overall population mean. To further determine the differences on the mean scores of the subpopulations, we used non-parametric multiple comparison plot (Steel-Dwass test).

4.2.5 Results

4.2.5.1 Range use

During the three-week monitoring period when hens were 18-21 weeks of age, there were significant differences between the number of days the stayers, roamers and rangers spent on the range (Figure 4-12). Range use significantly increased over time and resulted in no significant difference between rangers and roamers, whereas stayers still preferred most of their available days in the shed when comparing the range use during 22–72 weeks of age (Figures 4-13 and 4-14).

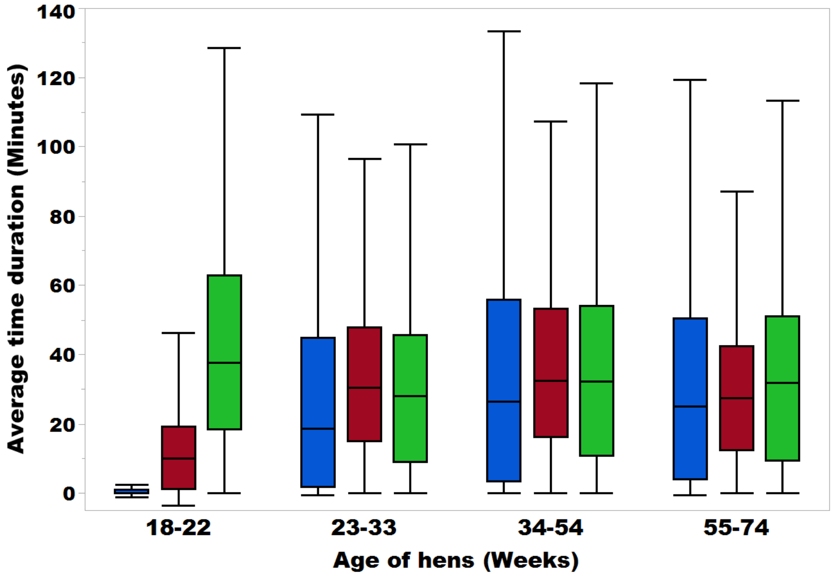

Figure 4-12 The time duration that hens spent on the range during the four production periods

The blue, red and green boxplots represent the stayers, roamers and rangers, respectively.

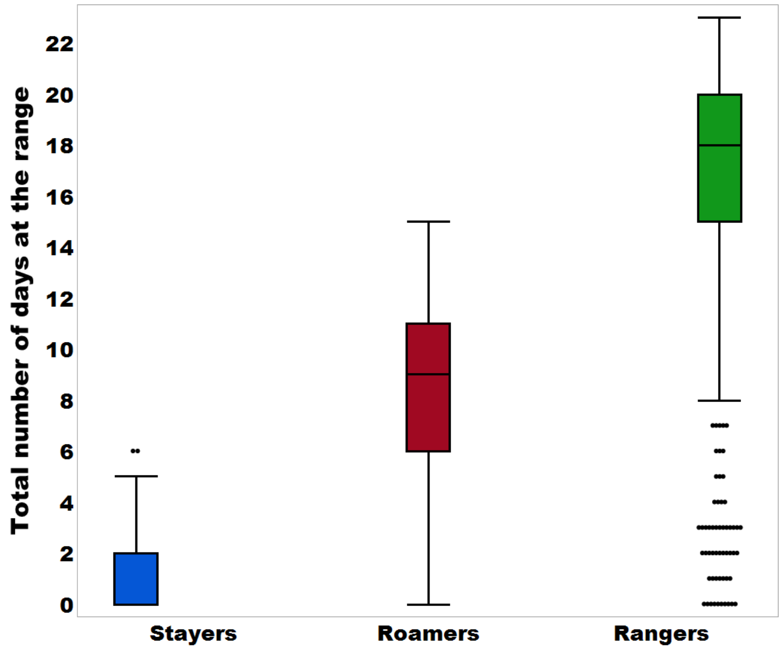

Figure 4-13 Range use distribution of the flock subpopulations of free range laying hens – stayers (n = 977), roamers (n = 1169) and rangers (n = 1283) from 18 to 21 weeks of age

The blue, red and green boxplots represent the stayers, roamers and rangers, respectively.

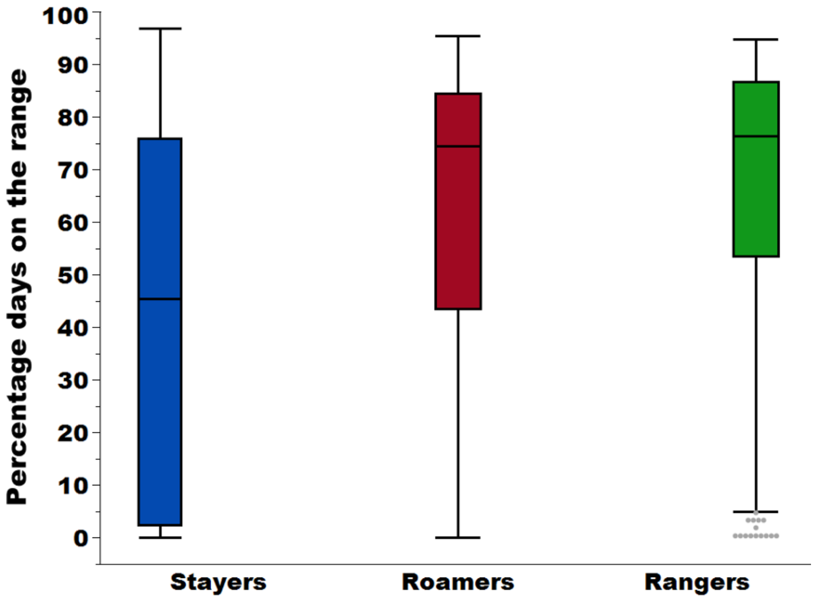

Figure 4-14 Range use distribution of the subpopulations of stayers (n = 977), roamers (n = 1169), and rangers (n = 1283) in Lohmann Brown free range laying hens from 22 to 72 weeks of age evaluated in 3 flocks

4.2.5.2 Body weight and egg laying performance

The rangers had a higher body weight at 16 and 22 weeks of age compared to the stayers and roamers, but not at 72 weeks of age (Figure 4-15). Hens that spent more time on the range ('rangers'), came into lay earlier compared to hens that preferred to stay in the shed ('stayers'). For example, at 22 weeks of age, rangers enjoyed a laying rate of 89.5 ± 2.8%, while stayers performed at 72.9 ± 8.5%. Stayers did not achieve the performance of rangers until they were older than 52 weeks of age.

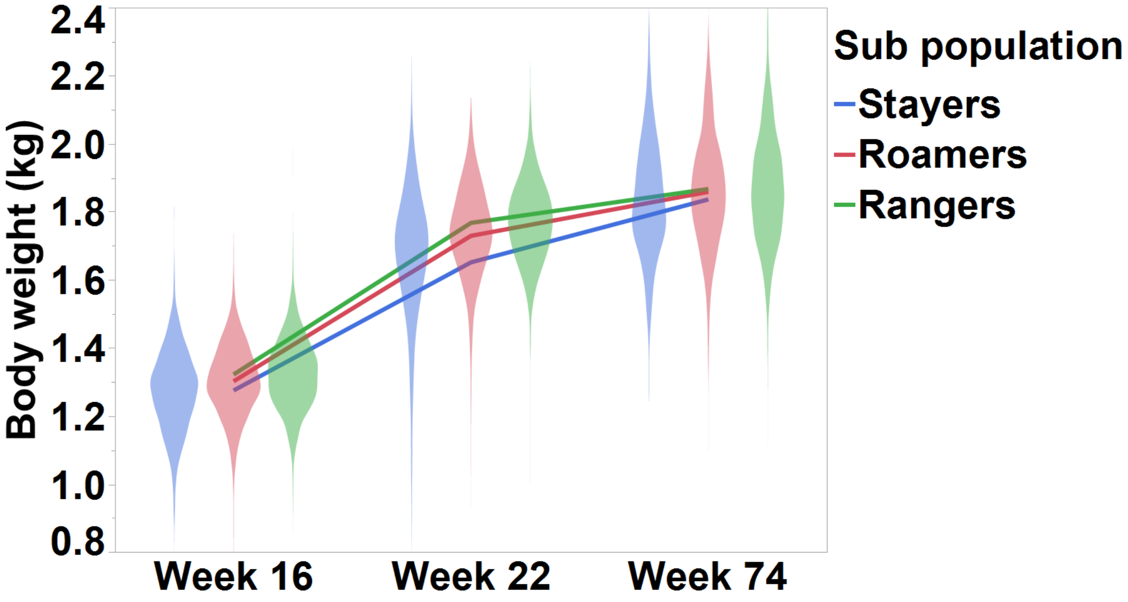

Figure 4-15 Violin plot with an overlay of line graph representing body weight of stayers, roamers and rangers at 16, 22 and 74 weeks of age. The blue, red and green colour shades represent the stayers, roamers and rangers, respectively.

4.2.5.3 Egg laying performance and egg quality

Egg weight did not differ between stayers and rangers, while egg weight changed over time (P = 0.0001; Figure 4-16).

Similarly, albumen height did not differ between stayers and rangers (P = 0.62), while albumen height changed over time (P = 0.005; Figure 4-16).

Yolk colour was not affected by range use (P = 0.37) but decreased significantly over time, indicating paler yolk as hens aged (P < 0.001; Figure 4-17). There was an observed subpopulation x time interaction (P = 0.025).

The Haugh unit was significantly higher in stayers compared to rangers (P = 0.0076) and decreased in both groups significantly over time (P < 0.001; Figure 4-17).

Analysis of eggshell breaking strength revealed no significant effect between rangers and stayers (P = 0.00790) but there was no significant effect of treatment and treatment-age of hen interaction (P = 0.614; Figure 4-18).

Table 4-3 The difference between the laying performance of stayers, roamers and rangers at 22, 32, 42, 52, 62 and 72 weeks of age

Week/Sub-group	Stayers	Roamers	Rangers
Week 22	78.2 ± 1.9^{c}	83.9 ± 1.6^{b}	88.0 ± 1.1^{a}
Week 32	93.5 ± 1.1^{a}	86.1 ± 1.5^{b}	91.6 ± 1.0^{a}
Week 42	90.8 ± 2.3^{a}	91.7 ± 0.8^{a}	93.9 ± 0.7^{a}
Week 52	87.1 ± 1.8^{a}	83.8 ± 2.3^{b}	87.3 ± 1.9^{a}
Week 62	89.7 ± 3.0^{b}	94.6 ± 1.3^{a}	85.2 ± 1.5^{c}
Week 72	95.5 ± 0.9^{a}	89.9 ± 1.4^{b}	85.1 ± 0.9^{c}

The letters a, b, c are to denote statistically significant difference – observations that do not share these letters exhibit statistically significant difference.

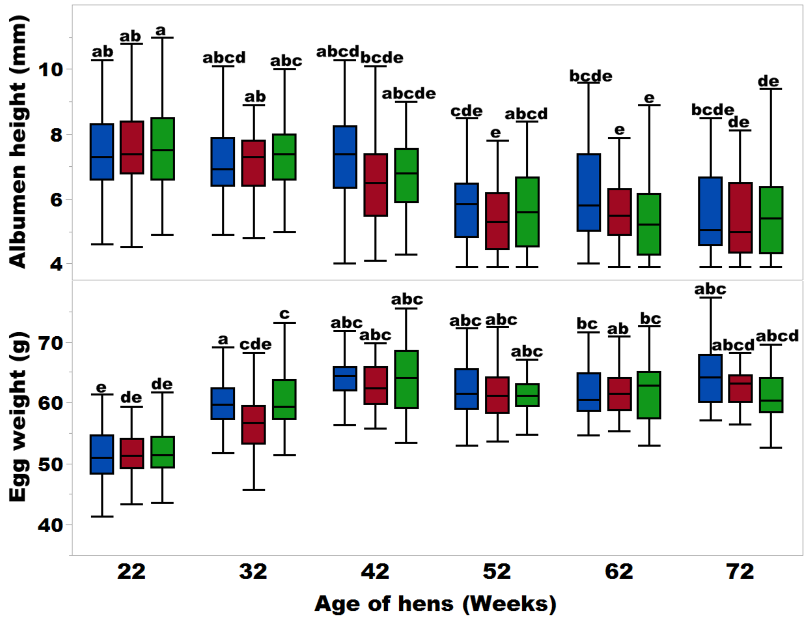

Figure 4-16 Box plots representing the difference between the egg weight and albumen height of the stayers, roamers and rangers at 22, 32, 42, 52, 62 and 72 weeks of age

The blue, red and green colour shade represent the stayers, roamers and rangers, respectively.

The different superscripts indicate difference between the age of hens.

The letters a, b, c, d, e in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

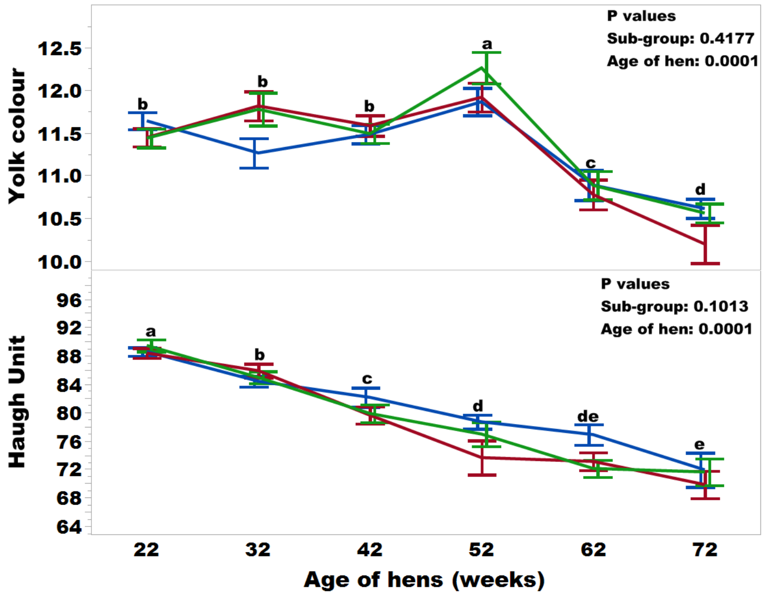

Figure 4-17 The difference between the egg yolk colour and Haugh unit of the stayers, roamers and rangers at 22, 32, 42, 52, 62 and 72 weeks of age

Lower yolk colour scores indicate paler colour.

The blue, red and green colour shade represent the stayers, roamers and rangers, respectively.

The different superscripts indicate difference between the two subpopulations.

The letters a, b, c, d, e in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

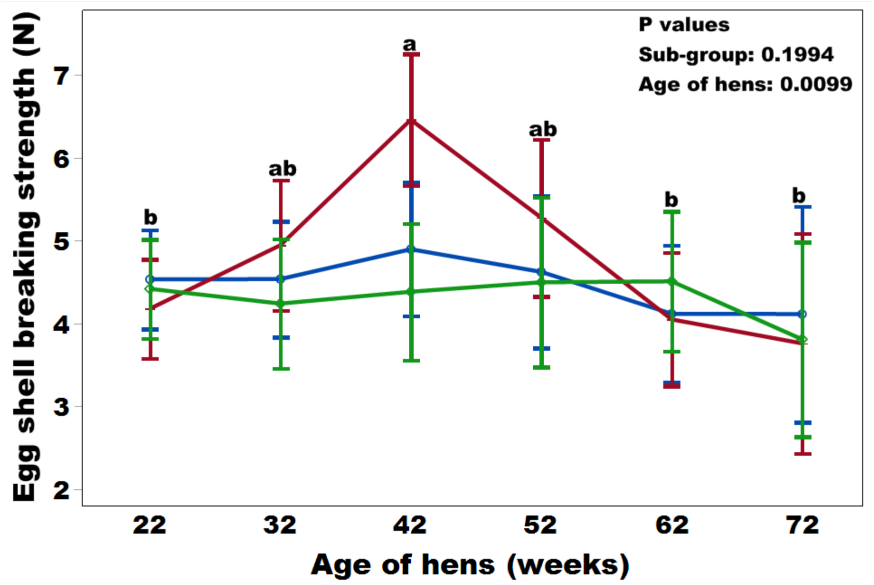

Figure 4-18 The difference between the eggshell breaking strength of the stayers, roamers and rangers at 22, 32, 42, 52, 62 and 72 weeks of age

The blue, red and green colour shade represent the stayers, roamers and rangers, respectively.
The different superscripts indicate difference between the weeks.
There was no difference between the subpopulations in all weeks.
The letters a, b are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

4.2.5.4 System, floor and waste eggs

The proportion of system, floor and waste eggs laid by the subpopulations is presented in Figure 4-19. There was a significant difference between the subpopulations, but also in between age of hens and subpopulation x age of hen interaction (P = 0.0001, 0.0001, 0.0177), respectively. There was a decrease in the proportion of eggs laid on the system as the hen age increased. In all subpopulations, the highest proportion of eggs laid on the system was observed at 22 weeks of age, while the lowest percentage of eggs laid on the system was observed at 72 weeks of age. There was a significant difference in system eggs between subpopulations at 32 and 42 weeks of age (P = 0.0001).

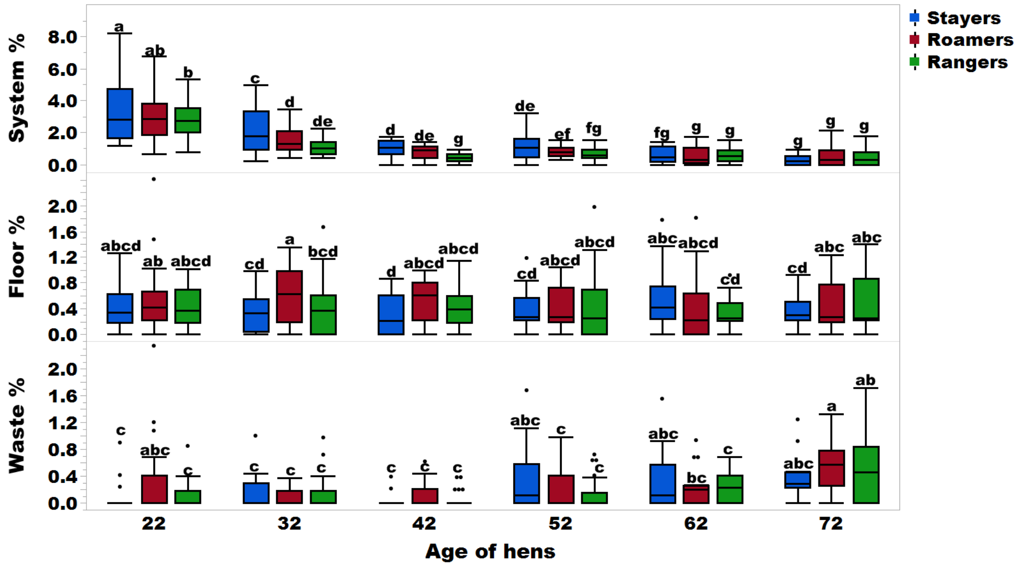

Figure 4-19 The proportion of system, floor and waste eggs laid by the subpopulations at all time points

The blue, red and green colour shades represent the stayers, roamers and rangers.
The letters a, b, c, d, e in the graphs are to denote statistically significant difference – the plots that do not share these letters exhibit statistically significant difference.

4.2.5.5 Health and welfare

Rangers had significantly more gastrointestinal parasites (A. *galli* and cestodes), more often spots on the liver but less often fatty liver syndrome. The proportion of hens with different keel bone scores and the prevalence of gastrointestinal parasites are shown in Table 4-5. There was a significant subpopulation effect and flock effect on keel bone damage. Overall, feather cover was significantly better in ranging hens.

Table 4-4 The mean score of the health and welfare of stayers, roamers and rangers in all flocks pooled

	Stayers	Roamers	Rangers	P-value
Keel bone score	0.937 ± 0.025	0.931 ± 0.022	0.909 ± 0.021	0.6668
Cestodes infestation score	0.352 ± 0.012^{c}	0.389 ± 0.012^{b}	0.436 ± 0.011^{a}	0.0001
A. *galli* score	0.102 ± 0.008^{b}	0.138 ± 0.008^{a}	0.130 ± 0.008^{a}	0.0048
Spots on the liver score	0.050 ± 0.006^{c}	0.061 ± 0.006^{bc}	0.086 ± 0.006^{a}	0.0001
Fatty liver score	0.264 ± 0.015^{a}	0.203 ± 0.012^{b}	0.219 ± 0.012^{b}	0.0026
Egg follicle score	3.886 ± 0.012^{c}	3.899 ± 0.011^{ab}	3.903 ± 0.010^{a}	0.3091
Neck feather score	3.599 ± 0.019^{c}	3.710 ± 0.015^{ab}	3.719 ± 0.014^{a}	0.0001
Chest feather score	2.799 ± 0.022^{c}	2.923 ± 0.019^{b}	3.017 ± 0.017^{a}	0.0001
Wing feather score	3.174 ± 0.020^{c}	3.271 ± 0.016^{a}	3.216 ± 0.016^{bc}	0.0019
Back feather score	3.520 ± 0.020^{c}	3.640 ± 0.016^{ab}	3.659 ± 0.016^{a}	0.0001
Vent feather score	3.656 ± 0.017^{c}	3.776 ± 0.012^{a}	3.733 ± 0.013^{ab}	0.0001

The letters a, b, c are to denote statistically significant difference – observations that do not share these letters exhibit statistically significant difference.

Table 4-5 The number and proportion (%) of hens with keel bone damage or with gastrointestinal parasites (Cestodes and *A.galli*) – for each of the range use sub-groups per flock

Sub-populations	Keel bone damage; n (%) No damage	Minor damage	Severe damage	Cestodes; n (%) Present	Absent	A. *galli*; n (%) Present	Absent
Stayers	731 (**48.6**)	135 (**8.98**)	637 (**42.4**)	489 (**32.5**)	1014 (**67.5**)	153 (**10.2**)	1350 (**89.8**)
Roamers	861 (**48.3**)	182 (**10.2**)	738 (**41.4**)	692 (**38.9**)	1089 (**61.2**)	246 (**13.8**)	1535 (**86.2**)
Rangers	983 (**49.0**)	222 (**11.1**)	801 (**39.9**)	875 (**43.6**)	1131 (**56.4**)	261 (**13.0**)	1745 (**87.0**)
Total	2575 (**48.7**)	539 (**10.2**)	2179 (**41.2**)	3234 (**61.1**)	2056 (**38.9**)	660 (**12.5**)	4630 (**87.5**)
P-value Flock		0.0001		0.0001		0.0001	
Range use		0.0544		0.0001		0.0134	
Flock x Range use		0.1230		0.0155		0.1022	

Table 4-6 The number and proportion (%) of hens with fatty liver and spots on the liver – obtained from four different commercial laying hens 74 week of age

Subpopulations	Fatty liver; n (%) Normal	Mild	Severe	Spots on the liver; n (%) Absent	Present
Stayers	1212 (**80.6**)	185 (**12.3**)	106 (**7.05**)	1428 (**95.0**)	75 (4.99)
Roamers	1513 (**85.0**)	175 (**9.83**)	93 (**5.22**)	1672 (**98.9**)	109 (**6.12**)
Rangers	1685 (**84.0**)	202 (**5.8**)	119 (**5.93**)	1833 (**91.4**)	173 (**8.62**)
Total	4410 (**83.4**)	562 (**10.6**)	318 (**6.01**)	4933 (**93.3**)	357 (**6.75**)
Subpopulation		0.1316		0.0188	
Flock		0.0001		0.0001	
Flock x sub-population		0.0142		0.0009	

The numbers in the bold represent the proportion (%) of hens with different liver condition scores and the presence and absence of the spots on the liver.

4.2.5.6 Egg follicle score

There was a significant effect of flock on the egg follicle scores but there was no effect of the sub-group and flock x sub-group interaction. Overall, 93.2% to 94.4% of all hens/flock investigated were still in full production (Table 4-7). On average, 93.2% of the stayer hens were in full production compared to the 94.2% and 94.4% of the roamer and ranger hens, respectively (P = 0.001). Stayer hens had the highest percentage of hens on early regression (4.06%) compared to the 3.14% and 2.69% (Table 4-7). This significant difference could be observed in every single flock.

Table 4-7 The number and proportion (%) of hens with different egg follicle scores – in light, medium and heavy free range laying hens at 74 weeks of age in the four flocks

Subpopulation	Egg follicle observation; n (%) No follicles	Late regression	Early regression	Full egg production
Stayers	26 (**1.73**)	16 (**1.06)**	61 (**4.06**)	1400 (**93.2**)
Roamers	27 (**1.52**)	21 (**1.18**)	56 (**3.14**)	1677 (**94.2**)
Rangers	23 **(1.15**)	36 (**1.80**)	54 (**2.69**)	1892 (**94.4**)
Total	76 **(1.44)**	73 **(1.38)**	171 **(3.23)**	4969 **(93.9)**
Subpopulation			0.1503	
Flock			0.0001	
Flock x subpopulation			0.1503	

4.2.6 Discussion and conclusion

This current study demonstrates that hen subpopulations differ in their egg laying performance, health and welfare, and understanding the characteristics and behaviours of these subpopulations is highly relevant to the achievement of outstanding results for the whole flock. With rangers clearly outperforming stayers at 22 weeks of age, the question needs to be raised about how the subpopulation of stayers in a shed can be minimised. Furthermore, what is the impact of these observations if hens are housed for an extended laying period, for example until 100 weeks of age? The lack of differences in egg quality between stayers and rangers indicates that there would be limited disadvantage of housing rangers only.

Free range flocks have been commonly described as performing poorly compared to barn and caged hens (Englmaierová et al. 2014; Tumova & Ebeid 2003). However, the current results lead to the suspicion that the overall reduced laying performance of free range flocks may be attributed to the subpopulation of stayers, rather than the housing system as such. A first attempt to investigate the metabolic needs of hens that prefer to stay in the shed and hens that range frequently was performed by Kolakshyapati et al. (2019a). Selecting commercial laying hens based on their range usage during 18–74 weeks of age and measuring their metabolic energy in a closed-circuit calorimetry chamber, it became evident that hens that prefer to stay in the shed had significantly higher metabolisable energy (ME) intake (P = 0.025), heat production (P = 0.005), and heat increment/body weight$^{0.75}$ (P = 0.005) compared to hens that accessed the range frequently. This led to the conclusion that hens that prefer to stay in the shed had significantly higher maintenance energy requirements and were less energy efficient compared to hens that used the range.

The age of the hen influenced the proportion of system, floor and waste eggs laid by the sub-groups. The highest proportion of system eggs was laid at week 22 and the proportion reduced as the age of the hens increased, most probably because the hens learnt to use the nest box with time. There was a significant difference between the subpopulations, age of hens and sub-group x age of hen interaction (P = 0.0001, 0.0001, 0.0177), respectively. In all subpopulations the highest proportion of eggs laid on the system was at 22 weeks, while the lowest percentage of eggs laid on the system was observed at 72 weeks of age. There was significant difference in system eggs between subpopulations at 32 and 42 weeks of age (P = 0.0001).

There was significant effect of subpopulations in the proportion of the eggs laid on the floor in 32 weeks of age (P = 0.0148). There was no significant effect of the age of the hen on the floor eggs (P = 0.5355). However, the age of the hen had an effect on the waste eggs that the hens produced, with the highest proportion of waste eggs produced at 72 weeks of age (P = 0.0001).

5 Objective B: Flock dynamics of commercial free range laying subpopulations

5.1 Defining subpopulations of differential resource usage in free range laying hens

Clustering is a common data mining methodology used for improved subject understanding. The aim of this study was to identify subpopulations of laying hens housed in an aviary system in order to understand the use of feed chains, which can affect hen performance and welfare. A total of 9375 Lohmann Brown free range laying were housed amongst 3 commercial flocks in a shed equipped with a 3-tier aviary system and individually monitored from 18–21 weeks of age using RFID technology. The individual body weights of all hens were obtained at 16, 22, and 74 weeks of age. K-Means cluster analysis optimised with the Calinski-Harabasz criterion was performed.

Hens of cluster 1 (n = 2442 hens) spent significantly more time on the lower tier feed chain (14.5 ± 2.36 hours/hen/day) compared to hens of cluster 2 (n = 2 083; 6.9 ± 2.4 hours/hen/day) and hens of cluster 3 (n = 1116; 2.0 ± 1.9 hours/hen/day), respectively ($P < 0.05$). Hens of cluster 3 spent 10.9 ± 3.6 hours/hen/day at the top tier feed chain compared to hens of cluster 1 and 2 (0.9 ± 1.1 and 3.6 ± 2.1 hours/hen/day respectively; $P < 0.05$).

Hens of all clusters were of comparable body weight distributions at 16, 22 and 72 weeks of age. Hens of cluster 3 spent the least time on the range and the most time on the upper tier feed chain of the upper tier ($P < 0.05$), however, there was no significant impact on weight gain between 16 and 72 weeks. We conclude that several subpopulations of hens can be identified in the aviary system and that these subpopulations result in an uneven load on the resources (e.g. feed chains). Further analysis of the data using classification models based on support vector machines, artificial neural networks and decision trees is warranted to investigate the contribution of other parameters of hen performance.

5.1.1 Introduction

In free range systems, hens are allowed to express their natural individual behaviour and this implies freedom of choice. Freedom of choice allows hens to show individual variation. There is growing evidence that there is individual variation in resource usage by the hen (Rufener et al. 2018). Individual hen differences in their physiology, phenotypic appearance, epigenetics, interaction with the environment and resource usage are reflected in the formation of subpopulations, while the differences in subpopulations reflect the differences in flocks. The subpopulations are more evident in free range hens because hens are allowed to express their individual behaviour. We previously demonstrated that range use subpopulation differs in terms of parameters such as body weight and production performance, however, current flock management practices are based on the flock average, although there is growing evidence of the existence of subpopulations within flocks.

Understanding subpopulation differences in the use of key resources such as the feeders is of paramount importance, as it can impact welfare and production performance of individual hens and hen subpopulations. Accessing different resources by hen subpopulations means that some hens are more vulnerable to exposures than others.

One of the predicaments faced by the scientific community is the complexity of individual hen behaviour and the measurement of key resource usage use. Use of the radio frequency identification

(RFID) system has offered a better solution to the problem through its capability to collect individual hen movement data at every second of the day in the aviary system and on the range. With the increasing availability of cheaper data storage devices, these data can be stored on the farm and can be used for further analysis, allowing us to collect a relatively big dataset with more dimensions. The feasibility of various technological solutions to be adapted for poultry has been shown in research facilities, and also under semi-commercial conditions (Singh & Cowieson 2013; Gebhardt-Henrich et al. 2014a; Larsen et al. 2017; Sibanda et al. 2019). By matching individual hen movement with nest box access, RFID systems have also demonstrated their value in recording individual hen performance (Marx et al. 2002; Thurner et al. 2006; Icken et al. 2008, Icken et al. 2013). Allowing the accumulation of big data for data mining, clustering, and machine learning has great potential not only for real-time data and flock management, but can also be extended to large-scale poultry disease warnings and poultry risk classifications (Feiyang et al. 2016).

High dimensional cluster analysis divides random data into groups (clusters) for the purposes of identifying structures within the data, improved understanding and data compression. More specifically, it tries to identify homogenous groups of cases if the grouping is not previously known. Because it is exploratory, it does not make any distinction between dependent and independent variables. In behavioural science, clustering is very important in order to understand the competition for resources, which has a direct implication for the welfare of hens. It has been proven that there are subclasses of hens within the range usage. The hens are usually clustering as low, medium and high range users according to the time they spend on the range. This time spent on the range is, however, correlated with the time spent on the upper tier of the three-tier aviary system. Thus, clustering hens using the time spent on the range only is not enough to explain the variation in hen movement behaviour in free range systems.

Poultry datasets contain many traits measured for the same bird, and this allows the use of multivariate statistical analysis for various purposes. These methods can be used to analyse phenotypic records or the breeding values of traits. Non-hierarchical cluster analysis is a multivariate technique that uses a k-means algorithm to partition individuals into groups, where k denotes the number of clusters. To conduct a k-means analysis, the number of clusters needs to be specified at the start (Rencher 2002). This could be used to separate the patterns of resource usage within a population of hens. In this study, we are forming clusters or subpopulations according to their time spent on the top tier, middle tier, nest box and range. The aim of this study was to use unsupervised machine learning to detect clusters or flock subpopulations in commercial free range flocks, and to evaluate the use of high dimensional clustering for animal behavioural studies.

5.1.2 Materials and methods

5.1.2.1 Data description

The primary data for this analysis were taken from the study (Sibanda et al. 2019). Range use and aviary system usage are critical measures in free range laying hens. The data used in the experiment were taken from five separate flocks. The data contained 13,716 individual hens that were tracked over period of three weeks after placement using a UHF-RFID System. From the dataset we obtained a description for each hen consisting of 11 variables including body weight (three variables), time (four variables), and number of events (four variables).

5.1.2.2 Identifying subpopulations using k-means and agglomerative clustering

The k-means and agglomerative analysis were done in MATLAB. Cluster validation was done using the Calinski-Harabasz criterion. The difference between the population mean and subpopulation means was determined using ANOM analysis.

The k-means clustering algorithm is described by the following equation:

$$\text{objective function} \leftarrow J = \overset{\text{number of clusters}}{\overset{\uparrow}{\sum_{j=1}^{k}}} \overset{\text{number of cases}}{\overset{\uparrow}{\sum_{i=1}^{n}}} \underbrace{\left\| \overset{\text{case } i}{\overset{\uparrow}{x_i^{(j)}}} - \overset{\text{centroid for cluster } j}{\overset{\uparrow}{c_j}} \right\|^2}_{\text{Distance function}}$$

The clustering algorithm selected the random three hens and set them as the initial centroids of the k clusters that are to be found. Then it computed all the Euclidean distances of the remaining hens to the k centroids. The hens were then assigned to each cluster with the smallest distance. Then the algorithm computed cluster centroids as the mean value from all the hens that belong to the cluster, with the objective to minimise the time index. This process was repeated until the variations on the cluster centroids were almost similar. The Calinski-Harabasz criterion was used to select the optimum number of clusters. The k-means analysis was done using Matlab, while normal mixture cluster analysis was done using JMP version 14. The biplot graphs and histograms were produced using JMP version 14.

5.1.2.3 Visualisation of the clusters

In order to understand the data, visualisation was done in RStudio with the ggplot2 package and JMP 14. t-Distributed Stochastic Neighbour Embedding (t-SNE) is a technique for visualising high-dimensional data in two dimensions through a non-linear dimensionality reduction algorithm (van der Maaten & Hinton 2008).

5.1.3 Results

5.1.3.1 K-means and agglomerative cluster characteristics

The Calinski-Harabasz criterion identified the three clusters as the optimum number of clusters from the data. The k-means algorithm identified 1470, 3473 and 2301 hens as Clusters 1, 2, and 3 respectively, while the agglomerative algorithm identified 979, 3501 and 2764 as Clusters 1, 2, and 3 (Tables 5-1 and 5-2).

Table 5-1 Descriptive statistics of the k-means clusters of the free range flocks

	Summary statistics	Average lower feeder time (mins)	Average upper feeder time (mins)	Average nest box time (mins)	Average range use time (mins)
Cluster 1 (n = 1470)	**Mean ± SEM**	108.7 ± 2.28	498.0 ± 4.16	71.91 ± 1.37	6.18 ± 0.32
	SD	87.35	159.68	52.50	12.11
	Skewness	0.85	0.89	1.41	3.07
	Kurtosis	0.07	0.31	5.31	12.23
	CV	80.34	32.06	73.01	196.18
	Median	89.74	458.34	63.06	0.01
Cluster 2 (n = 3473)	**Mean ± SEM**	302.4 ± 1.80	143.8 ± 1.57	78.11 ± 1.16	30.00 ± 0.45
	SD	106.22	92.67	68.35	26.59
	Skewness	-0.31	0.42	3.54	1.02
	Kurtosis	-0.66	-0.55	18.70	1.19
	CV	35.12	64.44	87.50	88.59
	Median	312.14	132.19	61.19	26.07
Cluster 3 (n = 2301)	**Mean ± SEM**	648.5 ± 2.50	46.28 ± 1.17	59.03 ± 1.05	26.82 ± 1.20
	SD	119.77	56.15	50.19	29.67
	Skewness	0.70	1.80	2.61	1.20
	Kurtosis	0.32	4.89	11.93	0.94
	CV	18.47	121.32	85.03	110.62
	Median	635.07	26.06	47.99	16.94

Table 5-2 Descriptive statistics of the agglomerative clusters of the free range flocks

	Summary statistics	Average lower feeder time (mins)	Average upper feeder time (mins)	Average nest box time (mins)	Average range use time (mins)
Cluster 1 (n = 979)	**Mean ± SEM**	88.91 ± 2.5	571.8 ± 4.6	68.17 ± 1.6	4.799 ± 0.4
	SD	76.65	144.07	49.27	11.37
	Skewness	0.99	0.87	0.79	3.88
	Kurtosis	0.48	0.14	0.44	19.42
	CV	86.21	25.20	72.27	236.94
	Median	65.33	539.58	60.45	0.00
Cluster 2 (n = 3501)	**Mean ± SEM**	264.1 ± 1.9	178.7 ± 1.94	72.88 ± 0.9	26.59 ± 0.4
	SD	109.9	114.55	52.57	25.36
	Skewness	-0.169	0.320	2.637	1.098
	Kurtosis	-0.436	-0.865	12.22	1.392
	CV	41.61	64.12	72.13	95.40
	Median	273.5	164.9	61.08	22.39
Cluster 3 (n = 2764)	**Mean ± SEM**	611.7 ± 2.6	55.30 ± 1.1	69.07 ± 1.4	27.94 ± 0.6
	SD	137.16	58.45	72.42	30.08
	Skewness	0.474	1.030	3.573	1.169
	Kurtosis	0.001	0.178	18.49	0.866
	CV	22.42	105.7	104.8	107.7
	Median	602.1	36.99	51.40	18.81

In K-means clustering method, hens of cluster 1 (n = 1470 hens) spent significantly more time on the upper feeding chain (498.0 ± 4.16 min/hen/day) compared to hens of cluster 2 (n =3473; 143.8 ± 1.57 min/hen/day) and hens of cluster 3 (n = 2301; 46.28 ± 1.17 min/hen/day), respectively ($P < 0.05$). Hens of cluster 3 spent 648.5 ± 2.50 min/hen/day at the lower feeder chain compared to hens of cluster 1 and 2 (108.7 ± 2.28 and 302.4 ± 1.80 min/hen/day respectively; $P < 0.05$). The hens from all the clusters spent least time at the range and at the nest box. The clusters 1 and 2 spent comparable time on the range ($P > 0.05$) while cluster 3 hens spent the least time on range (6.18 ± 0.32 min/hen/day; $P < 0.05$).

5.1.3.2 Agreement between the k-means and agglomerative clusters

Table 5-3 Contingency table of hen classification by the agglomerative and k-means clustering algorithms with Kappa agreement statistics

		Agglomerative clustering			
		Cluster 1	**Cluster 2**	**Cluster 3**	**Total**
K-means	**Cluster 1**	979 (**66.6**)	491 (**33.4**)	0 (**0**)	**1470**
	Cluster 2	0 (**0**)	2992 (**86.15**)	481 (**13.85**)	**3473**
	Cluster 3	0 (**0**)	18 (**0.78**)	2283 (**99.2**)	**2301**
	Total	**979**	**3501**	**2764**	**7244**
Kappa coefficient	**Kappa**	**SEM**	**Lower 95%**	**Upper 95%**	
	0.7794	**0.0065**	**0.7667**	**0.7922**	

Of the hens classified by the k-means as Cluster 1, 2 and 3 hens, the agglomerative algorithm classified 66.6%, 86.15% and 99.2% of the hens as Clusters 1, 2, and 3. There was a strong agreement in the classification of hens into Clusters 1, 2, and 3 between k-means and agglomerative, as indicated by a kappa coefficient of 0.7794.

5.1.3.3 Visualisation of the clusters

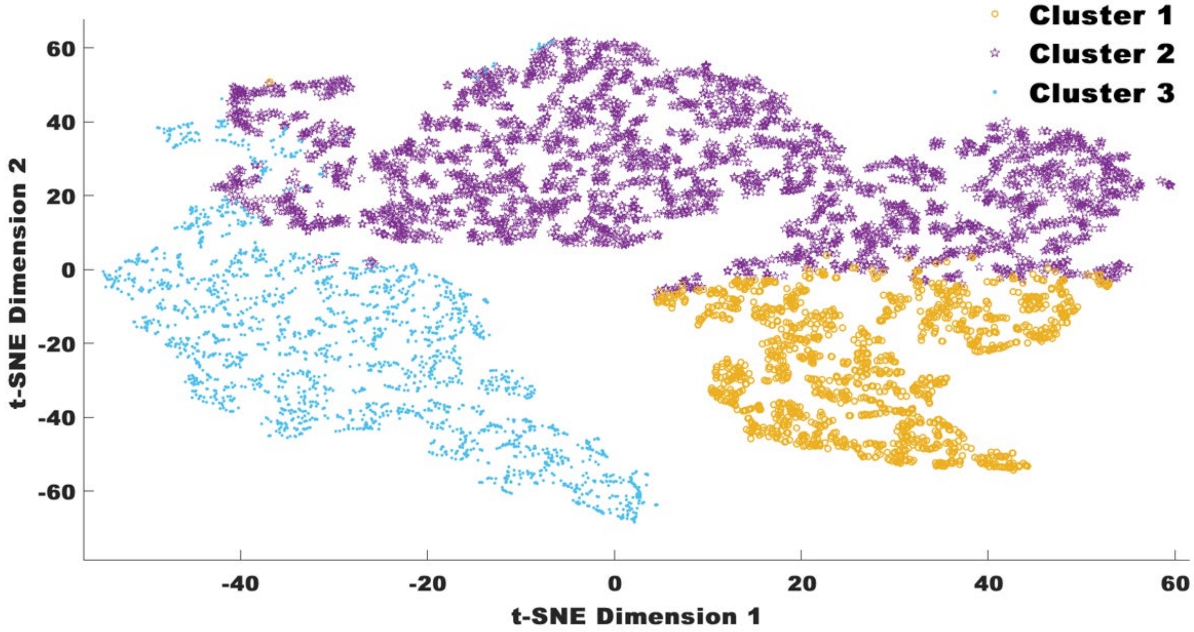

Figure 5-1 2D scatter plot of t-SNE visualising cluster assignments of individual hens by a k-means algorithm

Cluster 1 hens (yellow circles), Cluster 2 hens (purple star) and Cluster 3 hens (blue dot) are grouped according to their similarity in the time duration in the feeders, nest boxes and on the range.

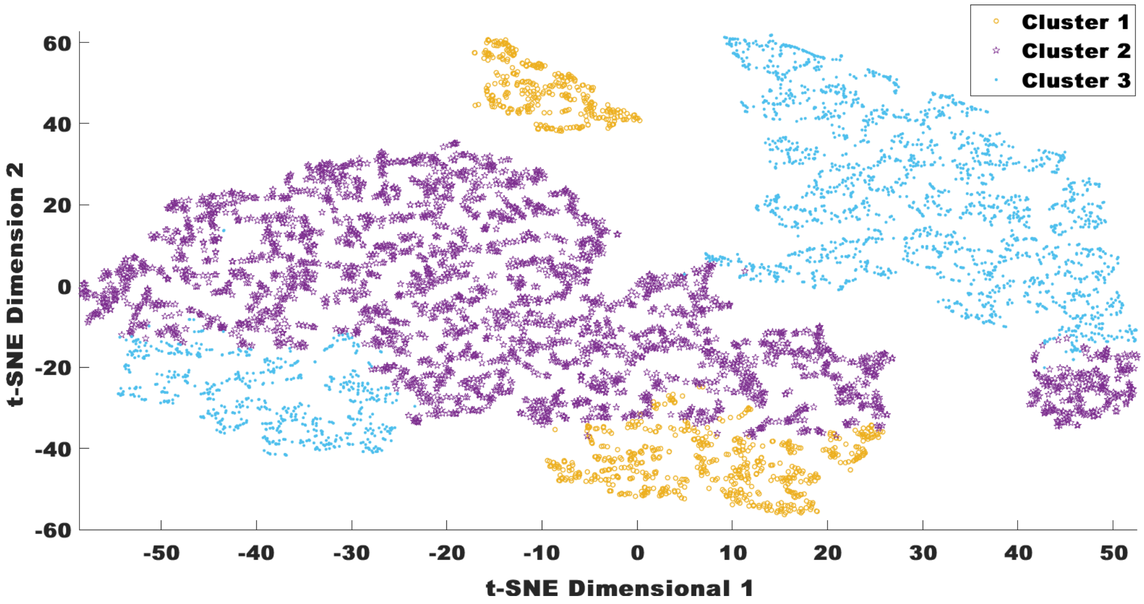

Figure 5-2 2D scatter plot of t-SNE visualising cluster assignments of individual hens by an agglomerative algorithm

Cluster 1 hens (yellow circles), Cluster 2 hens (purple star) and Cluster 3 hens (blue dot) are grouped according to their similarity in the time duration in the feeders, nest boxes and on the range.

5.1.3.4 Feeder occupancy over time

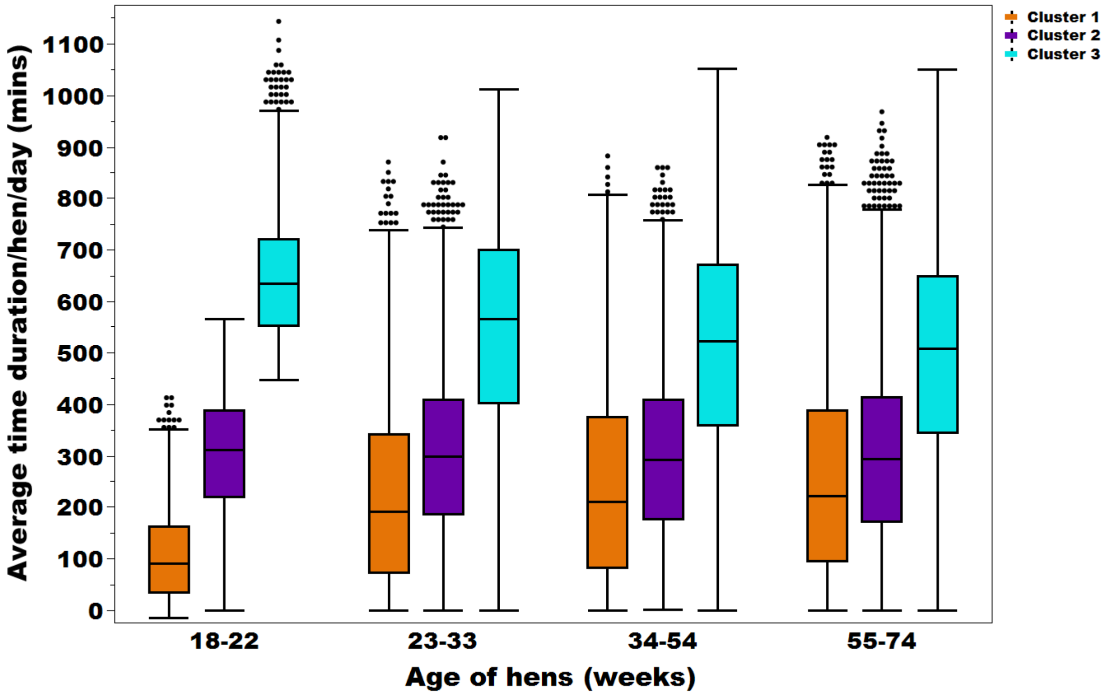

Figure 5-3 Box plots representing the time duration that the hens of each cluster spent on the lower feeder tiers – from during pre-laying period (18–22 weeks), to peak laying period (23–33 weeks), late laying period (34–54 weeks) and end of laying period (55–74 weeks)

Cluster 1 hens spent the least time on the lower feeder compared to the hens of Cluster 2. While the hens of Cluster 1 were increasing the time they spent on the lower feeder the hens of Cluster 2 reduced their time duration on the lower feeder.

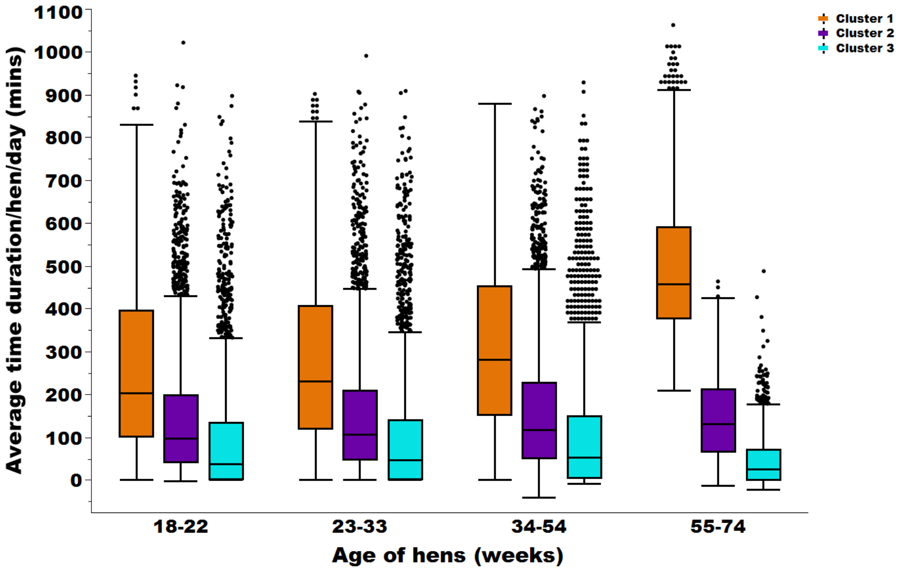

Figure 5-4 Box plots representing the time duration that the hens of each cluster spent on the upper feeder tiers – from during pre-laying period (18–22 weeks), to peak laying period (23–33 weeks), late laying period (34–54 weeks) and end of laying period (55–74 weeks)

5.1.3.5 Nest box occupancy over time

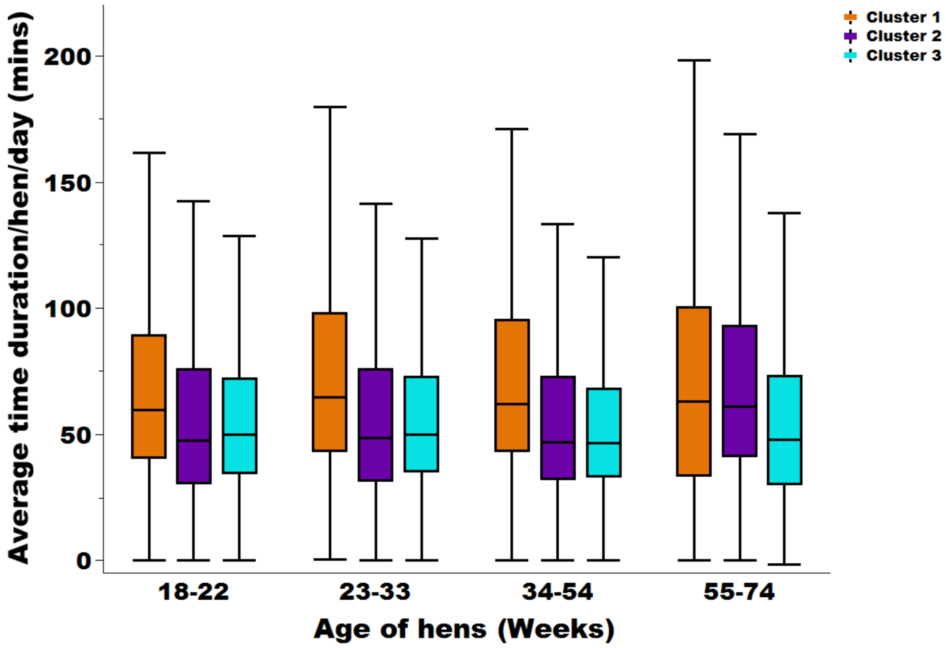

Figure 5-5 Box plots representing the time duration that each hen cluster spent in the nest box

5.1.3.6 Range use over time

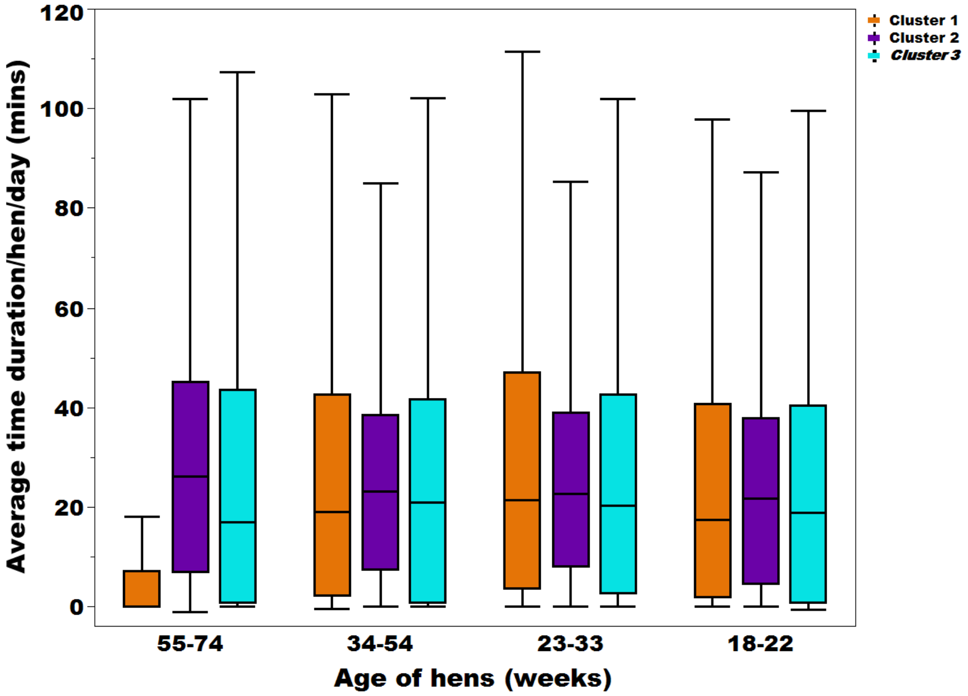

Figure 5-6 Box plots representing the time duration that each hen cluster spent on the range

5.1.3.7 Body weight distributions of each cluster

There was significant difference in the average body weight of the 3 clusters at 16 and 22 weeks of age ($P < 0.05$; Figure 5-7) but not at week 74. The average body weight of hens was significantly different at 16 weeks of age for clusters 1, 2, and 3, being 1.27 ± 0.10 kg , 1.32 ± 0.11 kg and 1.30 ± 0.11 kg, respectively.

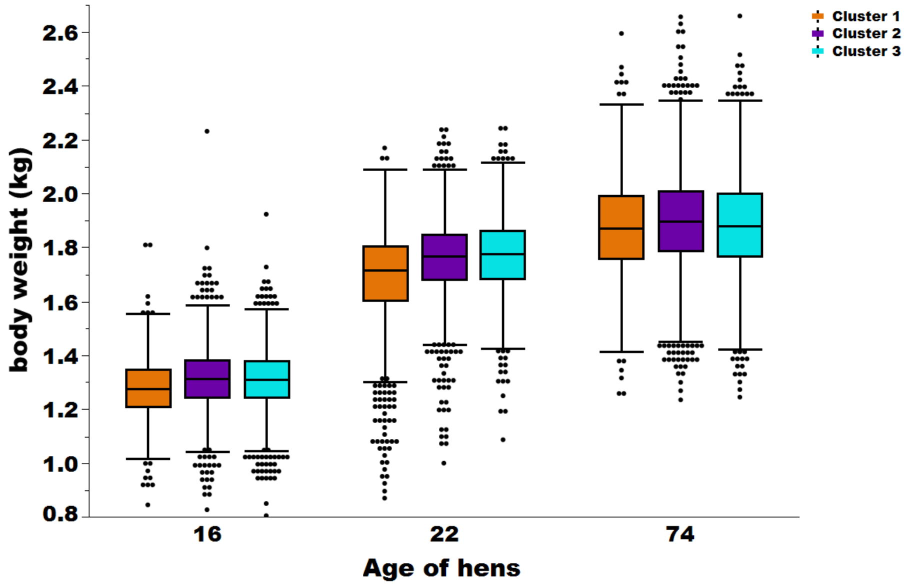

Figure 5-7 Box plots representing the body weight for each hen cluster at 16, 22 and 74 weeks of age

5.1.4 Discussion and conclusion

Automatic classification of hens is an innovative idea to support farmers in offering different management decisions for different laying hen subpopulations within flocks, thereby improving animal welfare. In this study, we demonstrated that there is individual variation and subpopulation difference in access to the key resources such as nest boxes, feeders and the range. These results clearly indicate the existence of flock subpopulations within the hen house. These findings were consistent in all three flocks investigated. The formation of clearly distinct subpopulations was associated with the location of hens in different areas of the aviary system, which would allow for different management strategies based on hens' geographic location. This study demonstrates that the type of cluster characteristics depends on the method used for its determination. Clusters of hens might be caused by social cohesion around using resources, in this case feeders, nest boxes and the range. It is imperative to understand the social dynamics of free range hens as it has a direct implication for the welfare of hens.

With ever-increasing farm sizes and consumer awareness, producers are often challenged with having to project how flock management decisions will affect production performance and welfare outcomes in a complex system. Hen movement data have the potential to be used for the prediction of health and welfare outcomes. In these predictive models, we should also recognise the complexity inherent in behaviour, physiological and biological systems. Without be blindsided by unexpected results, a systematic, comprehensive way of analysing, modelling and simulating complex behavioural data is warranted to predict unanticipated outcomes. However, comprehensive models of complex systems or RFID data for poultry are currently unattainable.

The impact of hen management and stockmanship skills on hen performance, health and farm economics cannot be underestimated (Blokhuis et al. 2007). If the skillset and knowledge about flock management are extended to a substantial understanding of the differences and needs of various flock subpopulations, the use of loose husbandry systems will become more sustainable and profitable. Modern technology, such as integrated RFID systems that allow for constant and automatic flock monitoring, will help to determine the load on resources, detect unusual movement patterns, and provide indicators about the localisation of different performance groups. For example, the employment of RFID systems in poultry breeder feeding systems has been used to increase flock uniformity to 100% (Zuidhof et al. 2017).

Automated feeding stations that recognise individual birds and use body weight information may be part of future flock management in a way similar to the dairy industry, where cows are fed with supplements based on their daily milk yield. However, being able to determine the proportion of hens that are not using the nest boxes, not accessing certain feeder lines, and the proportion of those that are using the range in real time, can allow for adequate changes in diet manipulation. Furthermore, offering different diets or feed additives through different feeder lines may directly target the different requirements of hens that favour these specific feeder line locations. It is not only of the highest interest to investigate how feed technology can be used to support underperforming subpopulations, but also how to ensure that overperforming hens are truly nurtured to their best long-term care.

5.2 Characterisation of subpopulations of differential body weight in free range laying hens

5.2.1 Summary

Previously, we demonstrated that hens with higher range use at placement were of higher body weight compared to the minimum range users. This was an indication that body weight affected the behaviour of the hens, thereby influencing the health and welfare of the hens. The aim of this study was to assess the welfare and health status of commercial free range laying hens of various body weights.

A total of 7708 Lohmann Brown hens at 74 weeks of age were obtained from four commercial free range flocks housed on the same location/farm under the same housing conditions. All hens were humanely sacrificed and evaluated for body weight, feather cover, keel bone damage, gastrointestinal parasites, liver appearance, and egg follicle status. Hens were classified, according to their body weight distribution percentile, as light (1.65 ± 0.002 kg), medium (1.86 ± 0.010 kg), and heavy (2.08 ± 0.002 kg) hens. All parameters were analysed using nominal regression models, with flock, body weight, and their interactions as the predictors.

In summary, 55.8% of heavy hens had single or multiple keel bone fractures compared to 48.9% and 50.7% of medium and light hens, respectively ($P = 0.0001$). Light hens had a significantly ($P < 0.05$) better feather cover on their chest (3.02 ± 0.018) compared to medium and heavy hens with scores of 2.96 ± 0.013 and 2.87 ± 0.018 respectively, but significantly less neck feather cover. Furthermore, when flocks were pooled, light hens had the highest prevalence of gastrointestinal helminths (both $P < 0.05$), compared to the medium and heavy hens. Heavy hens had the highest incidence of fatty liver syndrome compared to medium and light hens. Heavy and medium hens were significantly ($P < 0.05$) more often in full egg follicle production (95.3% and 94.8%, respectively) compared to the lighter hens (90.0%). There was a significant flock effect for all parameters investigated.

In conclusion, heavy (2.08 ± 0.002 kg) and medium (1.86 ± 0.010 kg) hens appeared healthier, more resilient towards infectious diseases and persistent in their egg follicle production. However, heavier hens suffered significantly more often from fatty liver syndrome. Overall, inter-flock variation was more significant than body weight differences within the flock. Hens weighing on average 1.65 kg were the least economic subpopulation. Further research about the ideal management of these hens is required.

5.2.2 Introduction

There is a growing global trend in the use of non-caged egg production systems, as well as in Australia. In 2018, free range egg production accounted for 45.4% of the total Australian egg production, while barn eggs accounted for 9.1% (Australian Eggs 2019). This trend is influenced by increasing awareness of animal friendly production systems.

The increased space availability and opportunities to exercise that are offered by cage-free housing systems have resulted in better leg bone health and a reduced prevalence of osteoporosis and gait problems, as well as increased tibia quality (Knierim 2006; Aguado et al. 2015). It seems to be of minor relevance for leg health if the exercise is performed in the shed or on the range (Sibanda et al. 2019; Kolakshyapati et al. 2019c). However, uncontrolled hen activity in non-caged housing systems also increases the likelihood of collision with the furnishing, and failed landings commonly result in keel bone damage with an observed prevalence of 20–83% (Moinard et al. 2004; Wilkins et al. 2004; Leyendecker et al. 2005; Rodenburg et al. 2008; Käppeli et al. 2011; Toscano et al. 2018). This landing impact force is not only a serious welfare concern but it also has a direct impact on farm economics, as keel bone damage can be associated with reduced egg production and egg quality (Nasr et al. 2012a; Nasr et al. 2012b). In addition, free range egg production is known for its biosecurity risk, and has been associated with increased prevalence of bacterial and viral diseases such as avian influenza, *Salmonella*, Spotty Liver Disease (SLD), and gastrointestinal parasites.

Furthermore, non-caged systems are known to produce fewer and poorer quality eggs compared to caged systems (Aerni et al. 2005; Golden & Arbona 2012; Singh et al. 2009). Egg follicle production and eggshell quality decreases with age and, while it is known that heavier hens produce eggs of poorer shell quality, the impact of body weight on the egg production rate is unknown (Johnson et al. 1986; Eluera 1997). This is especially important when considering the industry's current target of extending flock life until 100 weeks of age.

For many reasons, body weight and flock uniformity are two of the most important productive traits in poultry production, and trigger decision-making and management actions. For example, body weight is important in determining the onset of egg production as well as egg size (du Plessis & Erasmus 1972; Summers & Leeson 1983). Body weight is also one of the physical attributes considered to determine the hen`s aggressive behaviour, interaction frequency and subsequent social status in a flock (Cloutier & Newberry 2000; Bradshaw 1992). The social status of a hen can in turn significantly impact hen health and welfare. Other phenotypic appearance factors influencing the social status of hens includes feather colour, comb size and comb colour. Body weight is also known to affect the health status of a hen, where undernourished hens can have compromised immune function or inadequate nutrient resources to initiate ovulation or maintain egg production.

A breed-specific standard body weight is recommended by hen breeders, and a flock uniformity of at least 80–85% is desired. However, the relationship between hen body weight and hen health and welfare parameters under commercial free range conditions has rarely been investigated, and it may be of further interest to determine upper and lower thresholds, which can support key management decisions supporting hen health (e.g. modified vaccination schemes, nutritional diet adaptation).

Therefore, the objective of this study was to assess the health and welfare status of hens of different body weights, in laying hens housed under commercial free range conditions.

5.2.3 Materials and methods

5.2.3.1 Animals and sample collection

All procedures were approved by the University of New England's Animal Ethics Committee (AEC 16-087). The study results include 7708 Lohmann Brown hens obtained from four commercial free range flocks (Flocks A-D), subject to similar housing and management conditions. All experimental hens were housed amongst flocks of 40,000 hens during December 2017 and February 2019. All hens were individually identified using numbered leg bands (Impinj, Inc. – Seattle, WA, USA) and weighed at 16, 22 and 74 weeks of age using poultry weighing scales (BAT 1 – VEIT Electronics, Moravany, Czech Republic) with a precision of 0.001 kg. Of the initially placed 3125 hens/flock, those individuals that were still alive, and still equipped with its identification leg band at 74 weeks of age, were then investigated resulting in 2015, 1804, 1918 and 1971 hens of Flocks A, B, C, and D, respectively.

5.2.3.2 Body weight subpopulations

After data collection, the pooled body weight distribution of all of the flocks was assessed, and the hens were classified according to the lowest 25% body weight quantile (light; 1.65 ± 0.002 kg), hens above the 25% body weight quantile until the 75% body weight quantile (medium; 1.85 ± 0.001 kg), and hens above the 75% body weight quantile (heavy; 2.08 ± 0.002 kg). Figure 5-8 shows the weight distribution of all hens in each flock and provides detailed information about body weight classification. The hens of lighter and medium body weight were below the recommended Lohmann Brown breeder standard (2.03 kg), while the hens of heavier body weight had a higher body weight compared to the mean of the breeder standard (Lohmann 2019).

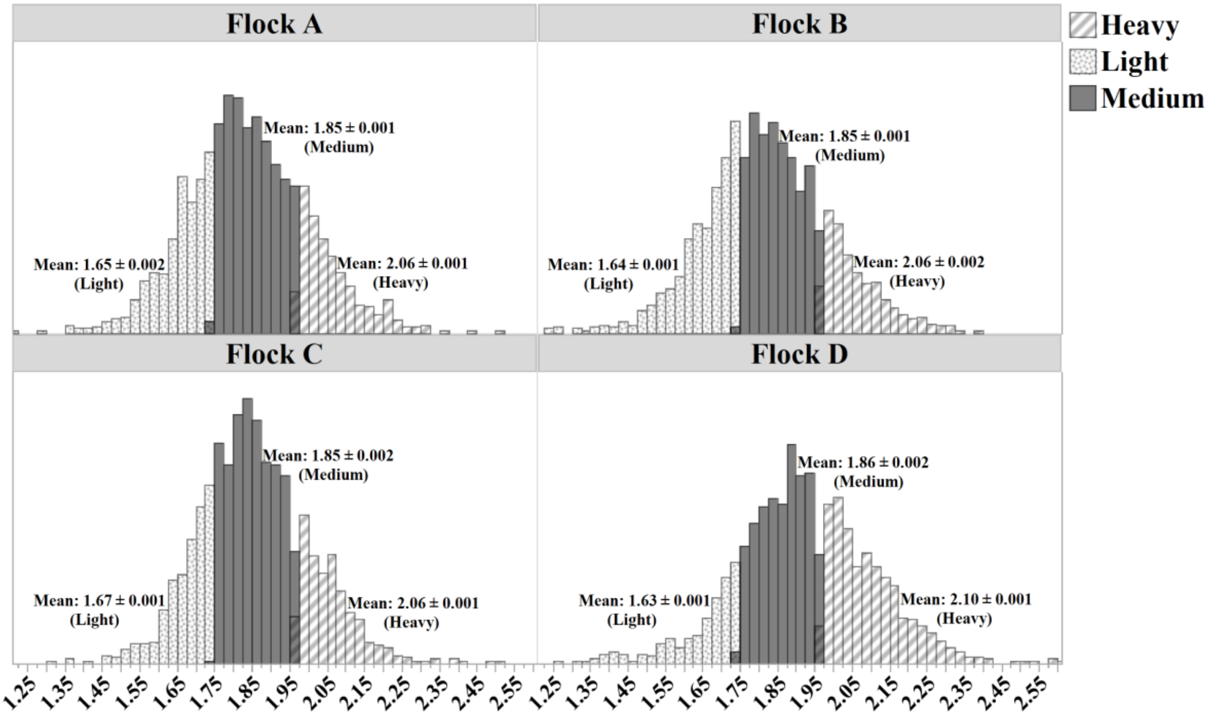

Figure 5-8 Histograms of the body weight distribution for the four free range flocks A–D

Four different free range flocks (Flocks A, B, C and D) were evaluated at the age of 74 weeks.
The mean body weight of the heavy hens (2.06–2.10 kg) meets the target standard body weight for hens of that age, as outlined by the breeder recommendations.

5.2.3.3 Health and welfare parameters

The feather cover, keel bone, health status of the liver, prevalence of gastrointestinal parasites, egg follicles was measured according to materials and methods in Section 4.2.3.

5.2.4 Statistical analyses

All the data were analysed using JMP Statistics software (version 14 – SAS Institute Inc., Cary, NC, 1989-2019). Boxplots were created to show the difference between hens of light, medium and heavy weight at 16, 22 and 74 weeks of age. To determine the effect of body weight, flocks and their interactions, on keel bone damage, fatty liver scores, egg follicle scores and feather scores, a nominal logistic regression model was used. For fatty liver and spots on the liver scores, an additional analysis was carried out to compare the overall population mean using Analysis of Means – Transformed Ranks (ANOM). ANOM graphically tests the equality of means on count data and allows multiple comparisons of the subgroups to the overall population mean.

5.2.5 Results

The descriptive statistics and distribution of hen body weight at 16, 22 and 74 weeks of age are shown in Table 5-4 and Figure 5-9. The hens of light, medium and heavy weight were significantly different at 16, 22 and 74 weeks of age ($P < 0.05$). The difference between hens of light and heavy body weight was 0.126 kg, 0.157 kg and 0.422 kg at 16, 22 and 74 weeks of age, respectively.

Table 5-4 Descriptive statistics of light, medium and heavy commercial free range laying hens at 74 weeks of age

Group	Descriptive statistics	Week 16	Week 22	Week 74
Light	Mean ± SEM (kg)	1.252 ± 0.003	1.659 ± 0.004	1.653 ± 0.002
	SD	0.111	0.18	0.107
	CV	8.849	10.88	6.488
	Skewness	-0.806	-4.552	-6.54
	Kurtosis	9.09	38.25	88.388
Medium	Mean ± SEM (kg)	1.308 ± 0.002	1.738 ± 0.003	1.855 ± 0.001
	SD	0.105	0.18	0.061
	CV	8.008	10.39	3.304
	Skewness	-0.886	-5.351	-1.128
	Kurtosis	14.77	46.97	3.304
Heavy	Mean ± SEM (kg)	1.378 ± 0.003	1.816 ± 0.006	2.075 ± 0.002
	SD	0.112	0.255	0.097
	CV	8.128	14.02	4.693
	Skewness	-1.899	-4.9	8.094
	Kurtosis	24.16	32.78	8.095
Pooled	Mean ± SEM (kg)	1.312 ± 0.001	1.738 ± 0.002	1.860 ± 0.002
	SD	0.117	0.209	0.171
	CV	8.917	12.04	9.206
	Skewness	-0.879	-4.455	-0.3
	Kurtosis	11.1	34.46	5.95

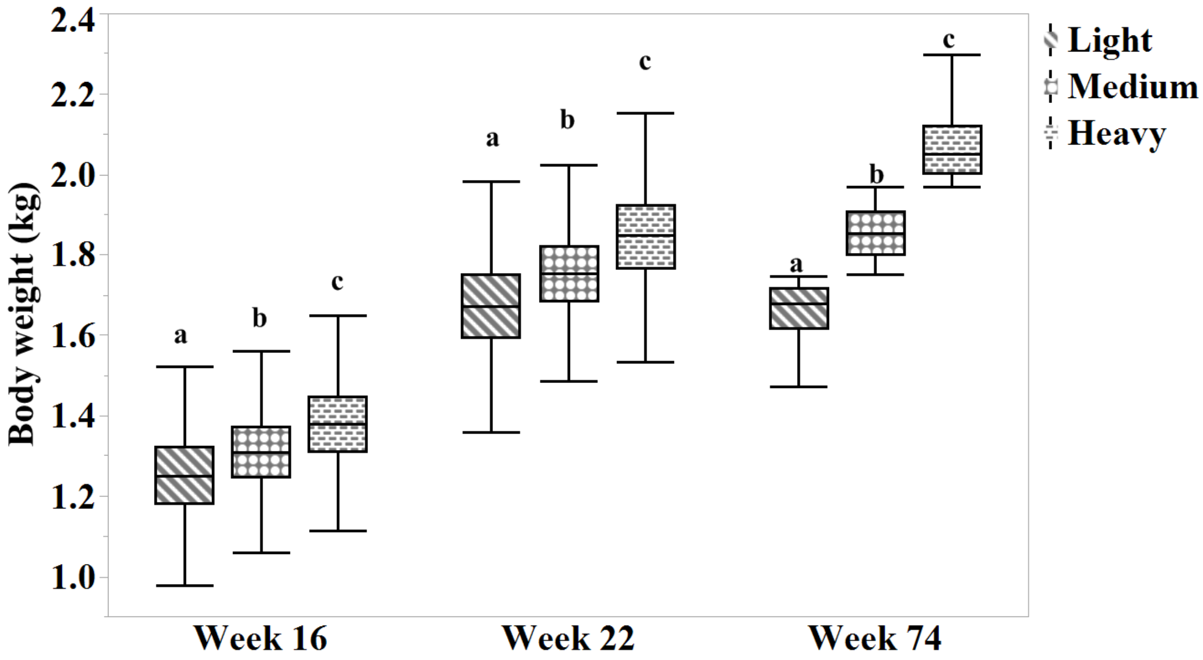

Figure 5-9 Retrospective classification of light (n = 1916), medium (n = 3879), and heavy (n = 1913) hens after being assessed and grouped at 74 weeks of age

Since each individual hen was also weighed at 16 and 22 weeks of age, and identified using individual numbered leg bands, the average body weight and body weight distribution of each categorised group are known when hens were placed in the layer house (16 weeks of age) and at point of lay (22 weeks of age).
Superscripts of different value represent statistically significant differences for the respective age.

5.2.5.1 Feather score

There was a flock and body weight effect on the feather score (P = 0.0001), while there was no flock x body weight interaction (P > 0.005). Heavier hens had the highest feather score on the neck compared to the hens of all other groups (P = 0.0071), while on the other hand lighter hens had the highest chest feather cover compared to all other groups (P = 0.0001, Figure 5-10). There was no significant difference in feather cover between light, medium and heavy hens on the wing, back and vent.

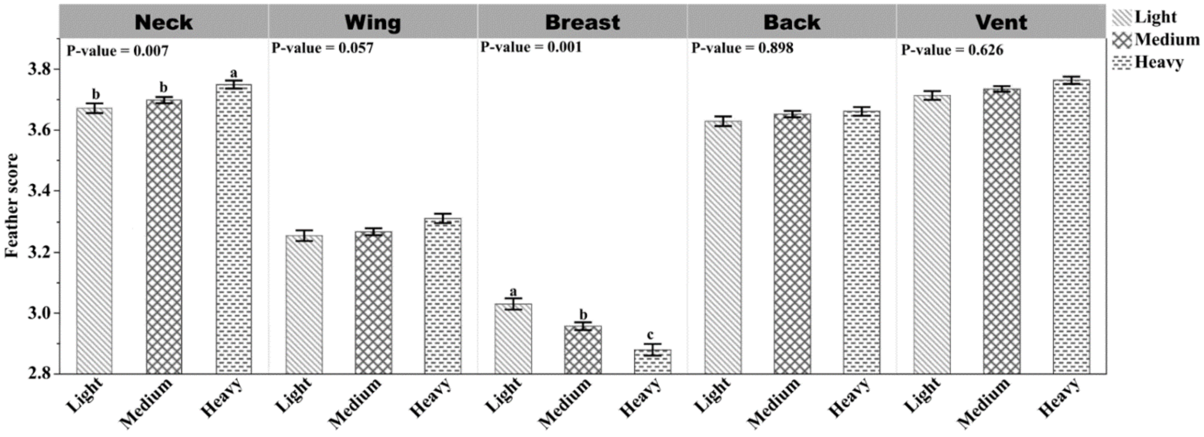

Figure 5-10 Feather scores of the different body parts obtained from light (n = 1916), medium (n = 3879), and heavy (n = 1913) commercial laying hens (pooled) at 74 weeks of age

Means within the same row with different letter superscripts are significantly different ($P < 0.05$).
The feather score ranges from 0.0 (no feather cover) to 4.0 (full feather cover).
While heavy hens had significantly better feather cover on their neck, they experienced significantly less feather cover on their chest.
Superscripts of different value represent statistically significant differences.

5.2.5.2 Keel bone score

The prevalence of keel bone damage ranged from 33.2% to 76.7% across Flocks A–D (Table 5-5). A significant flock difference could be observed, with Flock A having the highest prevalence of keel bone damage compared to all the flocks (P = 0.0001; Table 5-5). When the flocks were pooled together, heavy hens had the highest proportion of keel bone damage compared to all other groups of hens (P = 0.0001, Table 5-5).

5.2.5.3 Prevalence of gastrointestinal parasites

The prevalence of gastrointestinal helminths across all four flocks is shown in Table 5-5. A total of 14.7% of the hens in all of the four flocks had *A. galli* infection, while 39.9% had cestode infections. However, there was high inter-flock variation in the prevalence of gastrointestinal helminths. Flock A had a significantly higher prevalence of *A. galli* compared to all other flocks ($P < 0.0001$, Table 5-5).

Although there was variation in the prevalence of *A. galli* in the flocks, there was an overall significant difference between light, medium and heavy hens (P = 0.001). When the flocks were pooled together, lighter hens had 18.4% *A. galli* prevalence compared to 14.4 and 11.4% of medium and heavy hens, respectively (P = 0.0001). In Flocks A and D light hens had the lowest cestodes prevalence, while in Flock B heavy hens had the lowest cestodes prevalence. The medium body weight had the highest cestodes infestation in Flocks A, B and D. However, in Flock C, 59.2% of the light body weight hens were infested by cestodes compared to 53.1% and 55.7% hens of the medium and heavy body weight respectively.

Table 5-5 The number and proportion (%) of hens with keel bone damage or with gastrointestinal parasites (cestodes *and A. galli* infections) – for each of the light, medium and heavy body weight sub-groups per flock

Flock	**Body weight**	**Keel bone damage n** (%)			**Cestodes n** (%)		***A. galli* n** (%)	
		No damage	**Single fracture**	**Multi-fractures**	**Present**	**Absent**	**Present**	**Absent**
Flock A	Light	175 (**30.7**)	2 (**0.35**)	393 (**69.0**)	88 (**15.4**)	482 (**84.6**)	302 (**53.0**)	268 (**47.0**)
	Medium	326 (**31.7**)	1 (**0.10**)	701 (**68.2**)	219 (**21.3**)	809 (**78.7**)	459 (**44.6**)	569 (**55.4**)
	Heavy	97 (**23.3**)	2 (**0.48**)	318 (**76.3**)	84 (**20.1**)	333 (**79.9**)	168 (**40.3**)	249 (**59.7**)
	Total	598 (**28.6**)	5 (**0.31**)	1412 (**71.2**)	391 (**19.4**)	1624 (**80.6**)	929 (**46.1**)	1086 (**53.9**)
Flock B	Light	254 (**56.6**)	2 (**0.45**)	193 (**43.0**)	202 (**32.4**)	422 (**67.6**)	43 (**6.89**)	581 (**93.1**)
	Medium	677 (**62.2**)	3 (**0.28**)	409 (**37.6**)	326 (**35.4**)	594 (**64.6**)	80 (**8.70**)	840 (**91.3**)
	Heavy	227 (**52.4**)	2 (**0.46**)	204 (**47.1**)	106 (**28.3**)	268 (**71.7**)	32 (**8.56**)	342 (**91.4**)
	Total	1158 (**57.1**)	7 (**0.40**)	806 (**42.6**)	634 (**32.0**)	1284 (**68.3**)	155 (**8.05**)	1763 (**91.9**)
Flock C	Light	115 (**43.6**)	95 (**35.2**)	60 (**22.2**)	266 (**59.2**)	183 (**40.8**)	2 (**0.45**)	447 (**99.6**)
	Medium	367 (**43.6**)	336 (**40.0**)	139 (**16.5**)	578 (**53.1**)	511 (**46.9**)	7 (**0.64**)	1082 (**99.4**)
	Heavy	292 (**42.2**)	280 (**40.5**)	120 (**17.3**)	241 (**55.7**)	192 (**44.3**)	5 (**1.15**)	428 (**98.9**)
	Total	774 (**43.1**)	711 (**38.6**)	319 (**18.7**)	1085 (**56.0**)	886 (**44.0**)	14 (**0.75**)	1957 (**99.3**)
Flock D	Light	401 (**64.3**)	1 (**0.2**)	222 (**35.6**)	127 (**47.0**)	143 (**53.0**)	4 (**1.48**)	266 (**98.5**)
	Medium	615 (**66.8**)	2 (**0.22**)	303 (**32.9**)	474 (**56.3**)	368 (**43.7**)	11 (**1.32**)	831 (**98.7**)
	Heavy	233 (**62.3**)	0 (**0**)	141 (**37.7**)	387 (**55.9**)	305 (**44.1**)	12 (**1.73**)	680 (**98.3**)
	Total	1249 (**64.5**)	3 (**0.14**)	666 (**35.4**)	988 (**53.1**)	816 (**46.9**)	27 (**1.51**)	1777 (**98.5**)
Pooled	Light	945 (**49.4**)	100 (**5.23**)	868 (**45.4**)	683 (**35.7**)	1230 (**64.3**)	351 (**18.3**)	1562 (**81.7**)
	Medium	1985 (**51.2**)	342 (**8.82**)	1552 (**40.0**)	1597 (**41.2**)	2282 (**58.8**)	557 (**14.4**)	3322 (**85.6**)
	Heavy	849 (**44.3**)	284 (**14.8**)	783 (**40.87**)	818 (**42.8**)	1098 (**57.2**)	217 (**11.3**)	1699 (**88.7**)
	Total	3779 (**48.3**)	726 (**9.62**)	3203 (**42.1**)	3098 (**40.2**)	4610 (**59.8**)	1125 (**14.6**)	6583 (**85.4**)
P-value flock			0.0001		0.00001		0.00001	
P-value body weight			0.0001		0.00026		0.02564	
P-value flock x body weight			0.3560		0.00327		0.85790	

5.2.5.4 Prevalence of fatty liver and spots on the liver

The difference in the proportion of hens with fatty liver and livers with spots is shown in Table 5-6. When all the four flocks were pooled together, 9.14% of the hens had spots on the liver. A total of 89.6% of the hens from the four flocks were affected by fatty liver. Flock B had the highest proportion of hens with mild and severe fatty liver score. Lighter hens had the highest proportion of livers with spots compared to all other groups (P = 0.0001). The mean score (spots on the liver) for light and heavy hens was significantly above the population average (Figure 5-11).

The difference in the proportion of hens with fatty liver and spots on the liver is shown in Figure 5-11. When all flocks were pooled together, heavier hens had a significantly higher proportion severe fatty liver compared to medium and light hens (P = 0.0001; Table 5-6) but there was no significant difference between the lighter and medium hens (P = 0.6734). Heavy hens had significantly more often fatty livers than the population mean, whereas it was opposed for medium and light hens (Figure 5-11b).

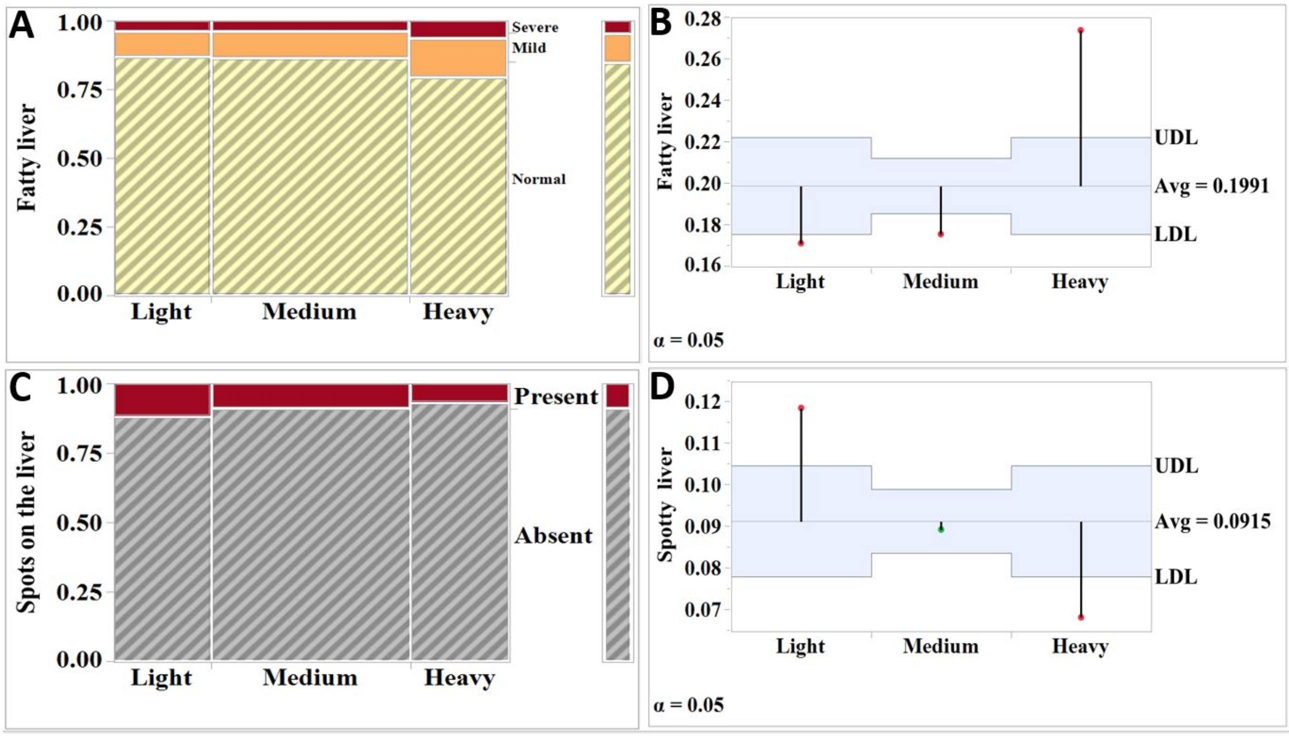

Figure 5-11 Mosaic plots representing the relative percentage of fatty liver (A) and spots on the liver (C) obtained from 74 weeks old laying hens (pooled)

The graphical analysis of means (ANOM) (B and D) for the relative percentage of fatty liver and spots on the liver indicates the upper decision line (UDL) and the lower decision line (LDL) at the 95% deviation level from the population mean (mean prevalence = 0.19 equal to 19% and 0.09 equal to 9%, respectively).

The red dots at the end of the decision line indicate significant difference (P < 0.05) from the population mean, the green dot at the end of the decision line indicates no statistical significance.

Heavy hens had significantly more often fatty livers than the population mean, whereas it was opposed for medium and light hens.

Heavy hens had also significantly less frequently spots on the liver, while light hens had significantly more often spots on the liver.

Table 5-6 The total number of hens with fatty liver and spots on the liver obtained from four different commercial laying hens 74 week of age

Flock	**Body weight**	**Fatty liver n** (%)			**Spots on the liver n** (%)		
		Normal	**Mild**	**Severe**	**Absent**	**Present**	**n**
Flock A	Light	537 (**94.2**)	33 (**5.8**)	0 (**0**)	432 (**75.8**)	138 (**24.2**)	570
	Medium	979 (**95.2**)	47 (**4.57**)	2 (**0.19**)	823 (**80.0**)	205 (**20.0**)	1028
	Heavy	376 (**90.2**)	37 (**8.87**)	4 (**0.96**)	357 (**85.6**)	60 (**14.4**)	417
	Total	1892 (**93.9**)	117 (**5.8**)	6 (**0.3**)	1612 (**80.0**)	403 (**20.0**)	2015
Flock B	Light	475 (**76.1**)	95 (**15.2**)	54 (**8.65**)	556 (**89.1**)	68 (**10.9**)	624
	Medium	674 (**73.3**)	169 (**18.4**)	77 (**8.37**)	820 (**89.1**)	100 (**10.9**)	920
	Heavy	240 (**64.2**)	90 (**24.1**)	44 (**11.8**)	324 (**86.6**)	50 (**13.4**)	374
	Total	1389 (**72.4**)	354 (**18.5**)	175 (**9.1**)	1700 (**88.6**)	218 (**11.4**)	1918
Flock C	Light	392 (**87.3**)	33 (**7.35**)	24 (**5.35**)	430 (**95.8**)	19 (**4.2**)	449
	Medium	912 (**83.8**)	115 (**10.6**)	62 (**5.62**)	1053 (**96.7**)	36 (**3.3**)	1089
	Heavy	322 (**74.4**)	78 (**18.0**)	33 (**7.62**)	419 (**96.8**)	14 (**3.2**)	433
	Total	1626 (**82.5**)	226 (**11.5**)	119 (**6.0**)	1902 (**96.5**)	69 (**3.50**)	1971
Flock D	Light	260 (**96.3**)	9 (**3.33**)	1 (**0.37**)	268 (**99.3**)	2 (**0.74**)	270
	Medium	793 (**94.2**)	29 (**3.44**)	20 (**2.38**)	836 (**99.3**)	6 (**0.71**)	842
	Heavy	580 (**83.8**)	66 (**9.54**)	46 (**6.65**)	685 (**99.0**)	7 (**1.01**)	692
	Total	1633 (**90.5**)	104 (**5.8**)	67 (**3.7**)	1789 (**99.17**)	15 (**0.83**)	1804
Pooled	Light	1664 (**87.0**)	170 (**8.89**)	79 (**4.13**)	1686 (**88.1**)	227 (**11.9**)	1913
	Medium	3358 (**86.6**)	360 (**9.28**)	161 (**4.15**)	3532 (**91.1**)	347 (**8.95**)	3879
	Heavy	1518 (**79.2**)	271 (**14.1**)	127 (**6.63**)	1785 (**93.2**)	131 (**6.84**)	1916
	Total	6540 (**84.8**)	801 (**10.4**)	367 (**4.8**)	7003 (**90.9**)	705 (**9.14**)	7708
P-value body weight		0.0001			0.83744		
P-value flock		0.0001			< 0.0001		
P-value flock x body weight		0.04			0.05398		

The numbers in the bold represent the proportion (%) of hens with the different liver condition scores, and the absence and presence of the spots on the liver.

5.2.5.5 Egg follicle score

Overall, 93.1% to 96.0% of all hens/flocks investigated were still in full production (Table 5-7). On average, 90.0% of the lighter hens were in full production compared to the 94.7% and 95.3% of the medium and heavier hens, respectively (P = 0.001). Lighter hens had the highest percentage of hens with no follicles (2.77%) compared to 0.39% and 0.73% for medium and heavy hens, respectively (P = 0.001). This significant difference could be observed in every single flock.

Table 5-7 Proportion of hens with different egg follicle scores in light, medium and heavy free range laying hens at 74 weeks of age in the four flocks

Flock	**Body weight**	**Egg follicle observation** n (%)			
		No follicles	**Late regression**	**Early regression**	**Full egg production**
Flock A	Light	7 (**1.23**)	17 (**2.98**)	31 (**5.44**)	515 (**90.4**)
	Medium	8 (**0.78**)	19 (**1.85**)	33 (**3.21**)	968 (**94.2**)
	Heavy	2 (**0.48**)	11 (**2.64**)	11 (**2.64**)	393 (**94.2**)
	Total	17 **(0.84)**	47 **(2.33)**	75 **(3.72)**	1876 **(93.1)**
Flock B	Light	16 (**2.56**)	12 (**1.92**)	38 (**6.09**)	558 (**89.4**)
	Medium	1 (**0.11**)	4 (**0.43**)	43 (**4.67**)	872 (**94.8**)
	Heavy	6 (**1.60**)	2 (**0.53**)	15 (**4.01**)	351 (**93.9**)
	Total	23 **(1.20)**	18 **(0.94)**	96 **(5.01)**	1781 **(92.9)**
Flock C	Light	10 (**2.23**)	13 (**2.90**)	15 (**3.34**)	411 (**91.5**)
	Medium	6 (**0.55**)	31 (**2.85**)	36 (**3.31**)	1015 (**93.3**)
	Heavy	2 (**0.47**)	5 (**1.17**)	21 (**4.91**)	400 (**93.5**)
	Total	18 **(0.92)**	49 **(2.49)**	72 **(3.66)**	1826 **(92.9)**
Flock D	Light	20 (**7.41**)	6 (**2.22**)	6 (**2.22**)	238 (**88.2**)
	Medium	0 (**0**)	2 (**0.24**)	24 (**2.85**)	816 (**96.9**)
	Heavy	4 (**0.58**)	2 (**0.29**)	9 (**1.30**)	677 (**97.8**)
	Total	24 **(1.33)**	10 **(0.55)**	39 **(2.16)**	1731 **(96.0)**
Pooled	Light	53 (**2.77**)	48 (**2.51**)	90 (**4.70**)	1722 (**90.0**)
	Medium	15 (**0.39**)	56 (**1.44**)	136 (**3.51**)	3671 (**94.7**)
	Heavy	14 (**0.73**)	20 (**1.05**)	56 (**2.93**)	1821 (**95.3**)
	Total	82 (**1.06**)	124 (**1.61**)	282 (**3.66**)	7214 (**93.6**)
P-value body weight		0.0001			
P-value flock		0.0001			
P-value flock x body weight		0.0007			

5.2.6 Discussion

The aim of this study was to assess the health and welfare status of hens of different body weight housed in a large-scale commercial free range farm. In the current study, we managed to detect body weight subpopulation differences for keel bone damage, feather cover, cestode and *A. galli* infections, fatty liver damage, and egg follicle production. While the relative weight status between the body weight subpopulations did not alter with age, the absolute difference between light and heavy hens increased. This emphasises the crucial importance of high-quality pullet rearing in achieving the highest flock uniformity possible. Parkinson et al. 2015 demonstrated previously that egg producers frequently increase the average body weight of a flock in an effort to reduce the number of underweight (and potentially underproductive) hens, even though this does not improve flock uniformity. All this implies that the pullet body weight rearing targets should be more rigorously set, and that their achievement be much more closely monitored than is currently the case.

The prevalence of keel bone damage in the flocks investigated in the current study ranged between 33.2% and 76.7% (Table 5-5), and is therefore comparable to other commercial flocks of similar size housed in aviary systems. Body weight had a significant impact on the overall prevalence but also the severity of the damage (Heerkens et al. 2016). Compared to cage systems, a single-tier aviary system has more keel damage due to the provision of key resources such as nest boxes, water lines and feeders in elevated positions, increasing the risk of keel bone damage through hen collision and fall (Wilkins et al. 2004; Campbell et al. 2016a).

Heavy hens had higher proportions with either a single or multiple keel bone fractures, and this might be explained by the fact that the pressure load of the heavy body weight on the keel bone is higher during a collision of the hen with a perch or other shed furniture (Pickel et al. 2011). However, the impact of body weight on keel bone damage could not be observed when comparing hens of light and medium weight. Similarly, Fleming et al. 2004 also found that there was no clear relationship between the severity of keel bone damage and body weight, comparing LSL White Leghorn hens of 1.703 ± 0.005 kg and 1.641 ± 0.008 kg body weight.

Similarly, while all hens were of remarkably good feather cover compared to other published studies, the feather cover of heavy hens on their breast was significantly less compared to medium and light hens. This may be due to increased wear and tear during perching, feeding or resting, where the breast might have been placed more frequently on the shed equipment or the bedding material (Tauson 1984; Kjaer 2000; Yamak & Sarica 2012). By contrast, the feather cover of the neck was poorer in lighter hens, which might possibly be a result of their being dominated by heavier or more aggressive hens (Cloutier & Newberry, 2000).

The likelihood and severity of liver lesions associated with Spotty Liver Disease in this study may be attributed to the fact that lighter hens are more likely to experience a compromised immune system. The farm veterinarian had diagnosed Spotty Liver Disease via PCR in every sampled flock at some stage during its housing period, and characteristic lesions could be observed during our investigation in up to 24.2% of hens/flocks. Spotty Liver Disease lesions affected lighter hens significantly more often in every single flock. By contrast, well-nourished heavier hens being close to the recommended breeder standard exhibited lesions significantly less often. These heavy hens had, however, a higher prevalence for Fatty Liver Syndrome. This is not surprising when referring to the egg production status – in every single flock, heavy hens had significantly more individuals with full follicle production, and likewise in every single flock, light hens had significantly more ovaries with no egg follicle production compared to medium and heavy hens. The maximum percentage of hens without active egg follicles occurred for light hens in Flock D, with a difference of 6.83% observed compared with heavy hens. This indicates that light hens present a significant economic loss for the farmer, and the target threshold of minimum body weight, to ensure persistent egg production, should be set around at least 1.86 ± 0.010 kg (medium body weight).

The presence of Fatty Liver Syndrome is directly associated with the fat mobilisation required for egg yolk production and genetically predisposed (Couch 1956; Butler 1976; Ivy & Nesheim 1973). It is also known that hepatic lipogenesis is increased in heavy birds compared to their lighter counterparts (Saadoun & Leclerq 1987). As such, obtainment of body condition scoring rather than simple absolute body weight in this study might have allowed for more detailed conclusions (Gregory & Robins 1998). The persistent egg production of medium and heavy hens observed in every flock is highly relevant for the egg producer, highlighting at the same time that heavy hens may benefit from nutritional support for reducing the severity and impact of the fatty liver on systemic body function (Grobas et al. 1999).

Because we investigated the ovulation status at 74 weeks of age, one can only speculate about the impact of body weight on egg quality. However, we have previously demonstrated that heavier hens use the range significantly more often at 17 weeks of age, which may influence the ovulation rate and onset of lay (Sibanda et al. 2018). The underlying mechanism may be due to the increased UV light exposure that stimulates sexual maturation by inducing photo sexual response, increasing serum follicle-stimulating hormones and 17β-estradiol, enhancing growth, and the number of ovarian follicles (Lewis et al. 2007; Hassan et al. 2014). Heavier hens have also been found to have better bone quality, which may impact calcium metabolism and eggshell quality; this needs further investigation (Kolakshyapati et al. 2019b).

In this study, heavier hens had not only less Spotty Liver Disease lesions, but also a significantly lower proportion of hens with *A. galli* infection compared to the light hens. This is similar to the study from Das and Gauly (2014) who also found heavier hens to be more resistant to infection compared to lighter hens, where lighter hens had more worm burden and a higher infection intensity, which might be due to the difference in nutrient allocation and nutrient availability for immune system function and production.

Significant inter-flock variation in different parameters is frequently observed and has, for example, been reported in research conducted in the Netherlands and Denmark in organic free range housing systems (Bestman & Wagenaar 2014; Hegelund et al. 2006). The reasons for this may include external factors (e.g. varying age of the parent flock, exposure to different weather conditions, exposure to various pathogens, the experience of the responsible stock person) or internal factors (e.g. genetic variation, rearing experience, flock dynamics) (Van de Weerd & Elson 2006). In order to exclude as many of these variables as possible, all hens subject to this study were of the same breed, reared in the same facility setting, placed in sheds of the same design, at the same geographic location, in sheds with the same orientation, and managed by the same personnel. However, the age of the breeder hens and associated epigenetic factors would have varied, as well as exposure to different weather conditions due to the flocks' sequential placement within 12 months' difference.

5.2.7 Conclusion

In conclusion, we demonstrated that the subpopulation of hens weighing close to the recommended mean breed standard, and hens of 1.85 ± 0.010 kg, were healthier, more resistant to infectious diseases and more persistent in their egg follicle production. However, heavier hens (2.08 ± 0.002 kg) exhibited significantly more often Fatty Liver Syndrome. Overall, inter-flock variation was more significant than body weight differences within the flock. Despite the fact that all hens were reared, managed and housed under the same conditions, the health and welfare status differed significantly between flocks and body weight subpopulations. The causation of inter-flock and subpopulation variation requires further investigation, and investigation of how to manage and ideally prevent the occurrence of subpopulations are highly warranted.

6 Objective C: Testing various feeding strategies for subpopulations of free range layers

6.1 Testing various feeding strategies to improve hen production, health and welfare

6.1.1 Summary

The energy requirements of birds that range frequently are higher compared to hens that prefer to stay in the shed, due to the increased metabolic activity required for locomotion and thermoregulation. The objective of this research was to develop and validate the impact of various feed strategies on hen performance, health and welfare. A total of 5625 hens, placed amongst three flocks of a total of 40,000 birds were selected, based on their frequent ranging activity (rangers). These rangers were split into three groups to be fed a standard commercial diet, to have access to an outdoor feeder, or to be fed with a diet containing +10% Metabolisable Energy (ME). Parameters evaluated included individual hen body weight at 16, 22 and 74 weeks of age, as well as egg production at 22, 32, 42, 52, 62 and 72 weeks of age. Feeding a diet of higher metabolisable energy (+10%) and elevated amino acid concentration (up to 10%) resulted in significantly higher laying performance at 52 and 62 weeks of age compared to hens that were fed conventionally. In conclusion, subpopulations of free range laying hens require individual nutrient support to achieve outstanding performance. Performance-based feeding allows for an efficient and responsible use of resources, and applied solutions that can be integrated on farm are highly warranted.

6.1.2 Introduction

Following the banning of conventional cages in the EU and the global trend of increased barn and free range production, the dynamics and impact associated with hen movement are of relevance to the majority of egg producers. The benefits of space availability include increased opportunities to exercise, which results frequently in better leg bone health, and a reduced prevalence of osteoporosis and gait problems (Knierim 2006; Leterrier et al. 2008; Aguado et al. 2015). It seems to be of minor relevance for leg health if the exercise is performed in the shed or on the range (Sibanda et al. 2019b). However, uncontrolled hen activity in non-caged housing systems increases the likelihood of collision with the furnishing and failed landings commonly resulting in keel bone damage, with an observed prevalence of 20-83% (Moinard et al. 2004; Wilkins et al. 2004; Leyendecker et al. 2005; Rodenburg et al. 2008; Käppeli et al. 2011). This is not only a serious welfare concern but it also has a direct impact on farm economics, as keel bone damage is associated with reduced egg production and egg quality (Nasr et al. 2012a; 2012b).

The nutritional requirements of hens that range frequently may differ compared to hens that prefer to stay in the barn. Furthermore, the diet composition that free range hens consume is complex. This makes it difficult to develop a feeding strategy to meet their nutrient requirements. Ideally, the amount of supplement required should be based on the amount of nutrient foraged and the total nutrient requirement. However, there is limited information on the forage intake of free range birds during the season. A better understanding of foraging behaviour and the forage intake of free range hens will enable producers to develop an economic feeding system. Given that free range birds consume a significant amount of forage, the nutritive value of forages for free range hens will be crucial for the development of a supplementary feeding system. In addition, there is a trend of increasing the dietary fibre content in the poultry diet in intensive systems, to reduce pecking problems and improve animal welfare. The evaluation of fibre resources for poultry production is required by the industry. Moreover, hens that range are exposed to inherently variable climatic conditions, and thermoregulation is energy consuming.

Free range poultry nutrition is key to improving egg production. Management of nutrition in free range laying hens is very multifarious due to irregular diet content (varied quality and quantity of pasture intake), differing ranging behaviours, and exposure of hens to different climatic conditions. To understand the contribution of feeding strategies to the nutrition of free range laying hens, there is a need for more information about the range use by free range laying hens.

The likelihood of increased distances between the hen and the feed resources available in the shed increases with the size of the available range area and may compromise frequent feeder access. The energy requirements of birds that range frequently are higher compared to hens that prefer to stay in the barn. Not only is additional energy for increased metabolic activity required, but exposure to the changing climate has to be taken into account.

The energy requirements of birds that range frequently are higher compared to hens that prefer to stay in the shed due to the increased metabolic activity required for locomotion and thermoregulation. The additional energy requirement for maintenance has been estimated to be 10% (floor-housed) or 15% (free range) higher compared to hens housed in cages (Aerni et al. 2005; Tiller 2001). On-range feeding is an alternative feeding strategy commonly used in Australia. In Australia, up to 47.5% of free range egg producers provide feed on the range (Singh et al. 2017). While this strategy may be beneficial for the hens on the range, the biosecurity risk associated with this practice cannot be overestimated.

Furthermore, alternative housing systems face welfare problems in terms of feather pecking, biosecurity and cannibalism. Growing evidence shows that feather pecking is caused by genetic factors (Kjaer et al. 2001), dietary deficiencies (Ambrosen & Petersen 1997; Kalmendal & Bessei 2012) and environmental conditions (Savory 1995; Kjaer & Sørensen 2002). Range use has been reported to reduce the incidences of feather pecking because the hens have more space, and it gives more opportunity for foraging. On the downside, free range egg production is notoriously known for poor biosecurity and has been associated with the increase in prevalence of Spotty Liver Disease (SLD) in Australia. Egg production and egg follicle production are also reduced by age (Johnson et al. 1986), and therefore the question remains whether range use and diet formulation can increase egg production in the late egg production period. It is important to achieve a 100-week layer hen. However, in order to investigate the potential nutritional benefit of additional feed sources other than in the shed, the objective of this research was to develop and validate the impact of various feed strategies on hen performance, health and welfare.

The objective of this study is: (1) to compare the production growth, performance and health status of subpopulations of free range laying hens at 74 weeks of age; and (2) to determine the effects of using three different strategies on the health status of the hen.

6.1.3 Materials and methods

6.1.3.1 Animal housing and treatment diets

Five commercial free range laying flocks were subject to this research. In each shed, 3,125 Lohmann Brown hens were housed amongst their 36,875 flock companions. These hens were individually monitored using the radio frequency identification (RFID) system described in Chapter 3 and classified according their daily range usage from 18 to 22 weeks of age. At 22 weeks of age, the top 60% of range users were selected into three groups consisting of 625 hens, each allowing for comparable stocking density. Hens of Group 1 (control) were housed under conventional conditions and fed a standard commercial diet (Table 6-1). Hens of Group 2 (outdoor feeder) were housed under standard conditions but had a biosecure outdoor feeder available (Figure 6-1). The outdoor feeder was placed at a 25 m distance from the shed on the range, gravity filled with the standard diet and *ad libitum* available during range access times (9 am–8 pm). Hens of Group 3 (+10% ME) received a diet that was formulated using the same ingredients than hens of Groups 1 and 2, but the diet included +10% metabolisable energy and approximately +10% essential amino acids. In order to keep the diet balance in respect to the amino acid profile, not all individual components could be elevated by 10%. For further details please see Table 6-1. The diet was administered through feed chains serving the pens of Group 3 hens only. The individual range usage of all hens was continuously monitored until hens were 72 weeks of age.

Egg production per pen (stayers, roamers, rangers) was determined at 22, 32, 42, 52, 62 and 72 weeks of age. During these collection weeks, all eggs laid were collected for the duration of seven consecutive days and subject to on-farm grading.

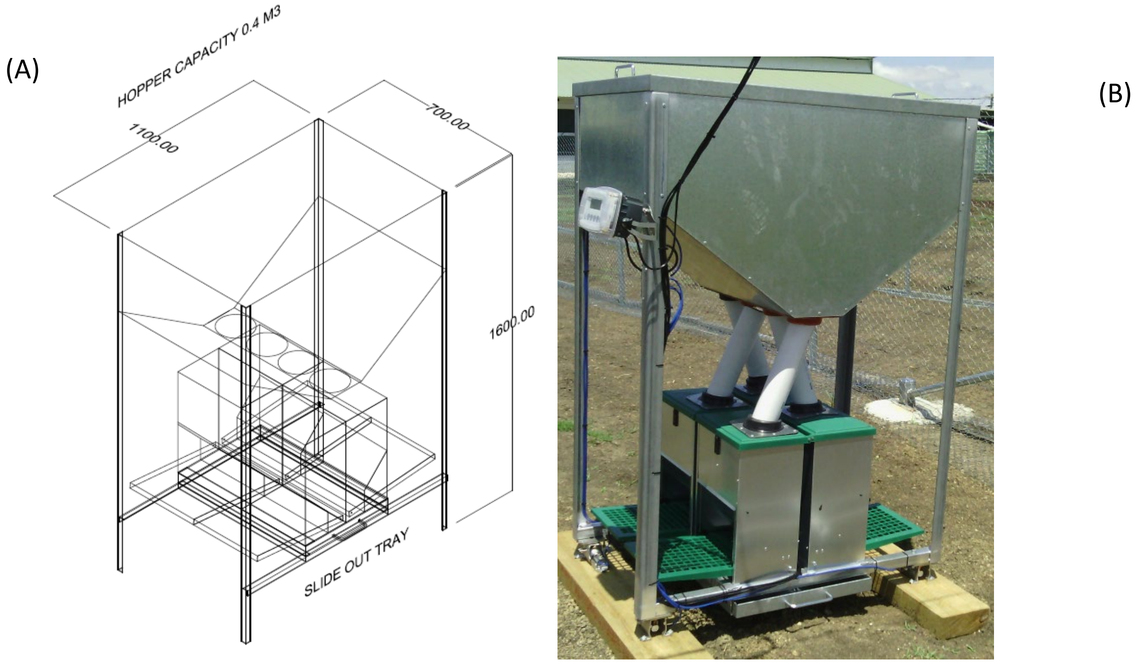

Figure 6-1 Biosecure outdoor feeder

Picture (A) provides technical details of the biosecure outdoor feeder, which comprised: (1) a slide out tray to collect and prevent feed spill on the range; (2) feeder platforms that would allow the operation of a leaver for feed access, only if a hen of at least 1.5 kg in weight were placed on the platform; and (3) netting cover to prevent roosting activity from flying wild birds.

Picture (B) shows the feeder set up on the range.

Table 6-1 Nutrient content of experimental diets

Item	Units	Conventional diet (control)	Treatment diet (+10%)
Nutrient content of the diet			
Metabolisable Energy (ME)	kcal/kg	2728.9	3,013.8
ME including enzyme activity	kcal/g	2775	3,099.5
Crude protein	%	18.9	18.9
Lysine	%	0.92	0.994
Methionine	%	0.49	0.586
Methionine + Cysteine	%	0.79	0.898
Threonine	%	0.69	0.702
Isoleucine	%	0.70	0.808
Leucine	%	1.58	1.599
Tryptophan	%	0.21	0.240
Arginine	%	1.11	1.089
Histidine	%	0.45	0.470
Valine	%	0.88	0.940
T.Dig. Lysine	%	0.83	0.931
T.Dig.Methionine	%	0.45	0.529
T.Dig. Methionine + Cysteine	%	0.70	0.793
T.Dig.Threonine	%	0.60	0.652
T.Dig.Isoleucine	%	0.64	0.746
T.Dig.Leucine	%	1.40	1.407
T.Dig.Tryptophan	%	0.18	0.208
T.Dig.Arginine	%	1.00	0.979
T.Dig.Valine	%	0.79	0.849
Crude fat	%	4.10	8.271
Linoleic acid	%	1.21	1.918
Crude fibre	%	3.41	2.848
Starch	%	38.5	39.13
Total Xanthophyll	%	0.001	0.001
Red Xanthophyll	%	0.001	0.001
Phytate phosphate	%	0.23	0.206
Ash	%	12.4	12.11
Calcium	%	3.90	3.908
Av. Phosphate	%	0.63	0.617
Total phosphate	%	0.76	0.727
Sodium	%	0.21	0.255
Chloride	%	0.21	0.225
Potassium	%	0.65	0.548

T.Dig. = Total digestible; Av. = Available.

6.1.3.2 Data collection

Egg production per pen (control hens, outdoor feeder hens, and +10% ME hens) was determined at week 22, 32, 42, 52, 62 and 72 weeks of age. All eggs laid were collected for the duration of seven consecutive days during each of the collection week and were recorded each week, and the laying performances were extrapolated to the entire week.

6.1.3.3 Statistical analysis

The pen was considered a statistical unit, allowing for the investigation of three replicates, using the age of the hens as covariate. The data on range use, egg laying performance, and egg quality were analysed by ANOVA with a completely randomised design by JMP version 14 (SAS Institute Inc., Cary, NC, 1989-2019).

6.1.4 Results

6.1.4.1 Egg laying performance

When investigating strategies to increase the laying performance of rangers in more detail, feeding a diet of higher metabolisable energy (+10%) and elevated amino acid concentration (up to 10%) resulted in a significantly higher laying performance compared to hens that were fed conventionally (Table 6-2).

There was no significant difference between the treatment groups at 22, 32 and 42 weeks of age. The rangers with a conventional diet were significantly outperformed by the rangers with extra 10% metabolisable energy (ME) and 10% amino acids (AA) by 14.1%, 15% and 11.6% at 52, 62 and 72 weeks of age, respectively (Table 6-2).

Table 6-2 Rate of lay (%) of hens that frequently visit the range (‘rangers’) – difference in egg laying performance between hens with a conventional diet, hens using an outdoor feeder, and hens with a diet with extra 10% metabolisable energy and 10% amino acids

Age of hens	Week 22	Week 32	Week 42	Week 52	Week 62	Week 72
Control group	89.5 ± 2.8^{a}	90.3 ± 0.2^{a}	90.0 ± 1.5^{a}	77.9 ± 3.7^{b}	75.3 ± 4.7^{b}	71.6 ± 1.1^{c}
Access to outdoor feeder	89.9 ± 3.4^{a}	97.2 ± 0.9^{a}	90.7 ± 1.4^{a}	79.0 ± 4.2^{b}	81.1 ± 3.7^{b}	77.7 ± 1.5^{b}
+10% ME; +10 % AA	86.7 ± 3.7^{a}	98.7 ± 2.7^{a}	94.9 ± 2.6^{a}	92.0 ± 3.8^{a}	90.3 ± 1.5^{a}	83.2 ±3.8^{a}
P-value	0.839	0.149	0.357	0.006	0.012	0.003

Numbers with different superscripts in the column represent significant difference ($P < 0.05$).
Three replicates with 625 hens/replicate were evaluated.

6.1.4.2 Eggs and quality

The age of the hens had an effect on the albumen height (P < 0.001), but adding an extra 10% ME and inclusion of the outdoor feeder had no effect on the albumen height (P = 0.5560). At 22 weeks, albumen height that was greater by 1 mm compared to all the other weeks.

Analysis of yolk colour revealed that age of the hen, and treatment had an effect on the yolk colour (P = 0.204, P < 0.0001), and their interaction also had an effect (P = 0.0206). The rangers fed with an extra 10% ME had a higher yolk colour index value compared to all other treatment groups.

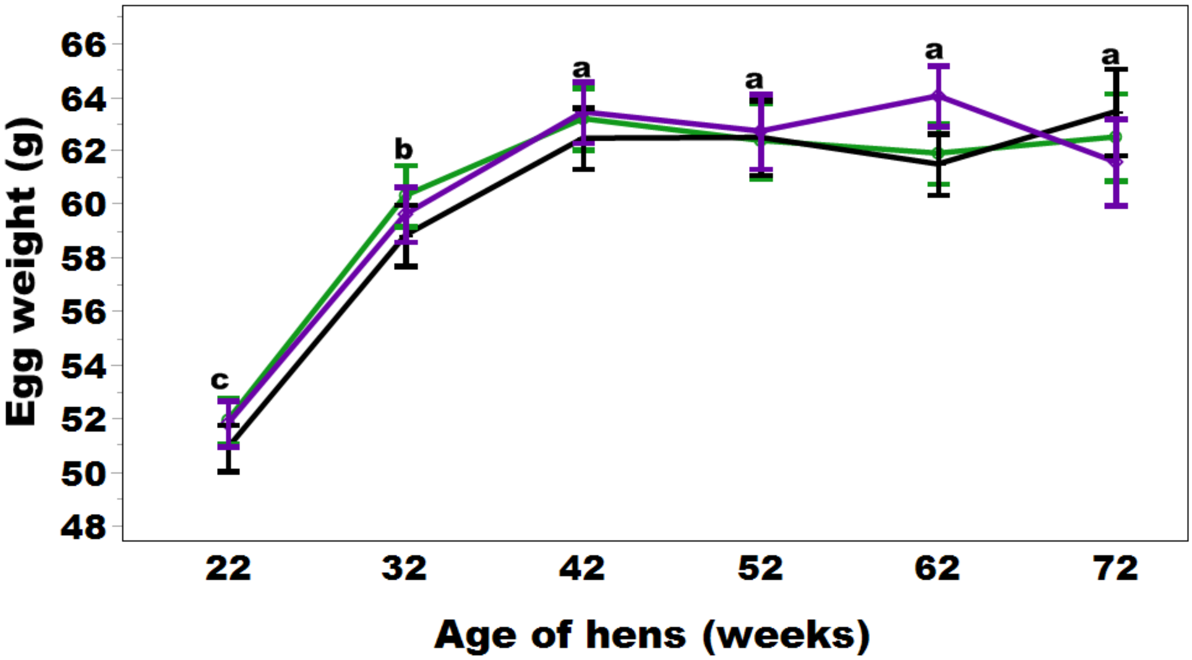

Figure 6-2 The difference between the egg weight of the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% metabolisable energy – at 22, 32, 42, 52, 62 and 72 weeks of age

The green, black and purple lines represent rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% ME, respectively.
The different superscripts indicate difference between the weeks (P = 0.0001).

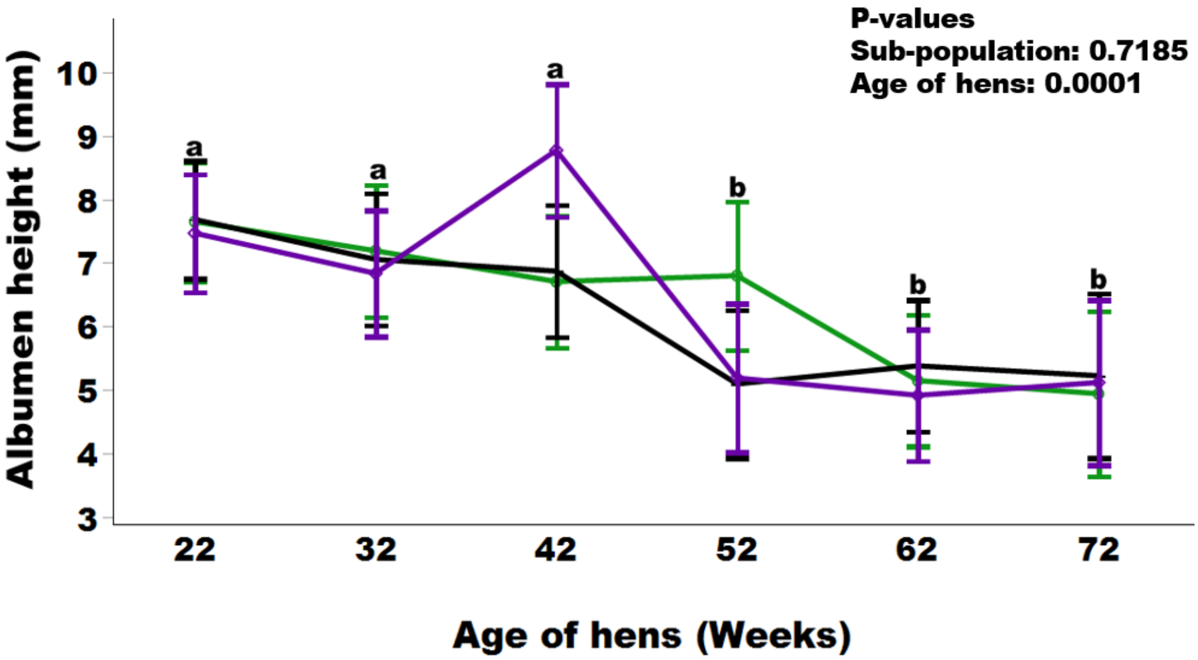

Figure 6-3 The difference between the albumen height of the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% metabolisable energy – at 22, 32, 42, 52, 62 and 72 weeks of age

The green, black and purple lines represent rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% ME, respectively.

The different superscripts indicate difference between the weeks (P = 0.0001).

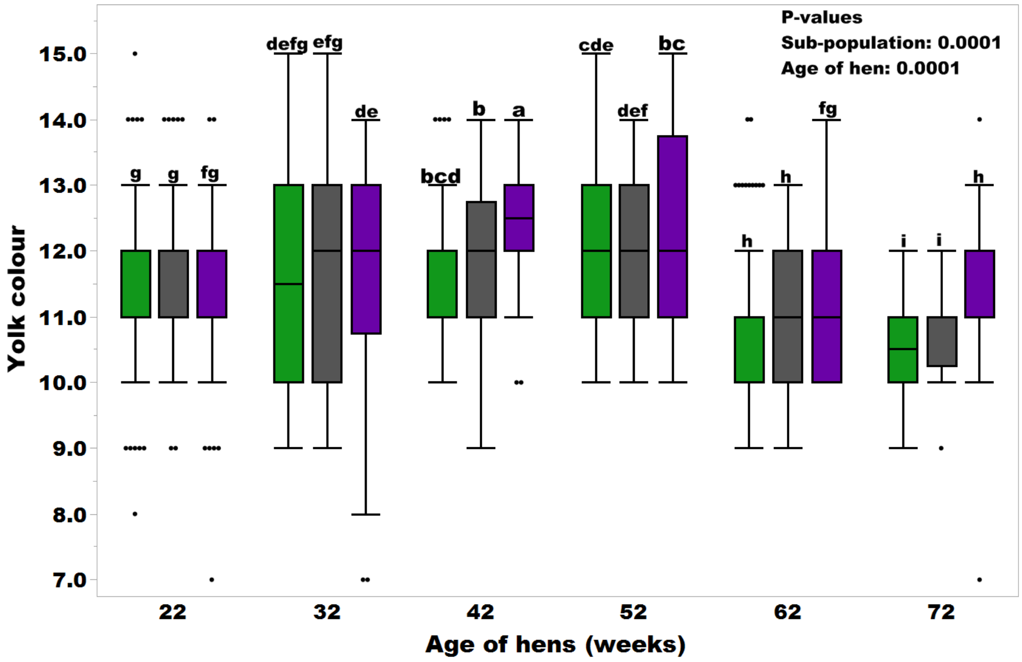

Figure 6-4 The difference between the yolk colour of the rangers control group, rangers with access to the outdoor feeder and rangers with extra 10% metabolisable energy – at 22, 32, 42, 52, 62 and 72 weeks of age

Lower yolk colour scores indicate paler colour.

The green, grey and purple box plots represent the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% ME, respectively.

The different superscripts indicate difference between the subpopulations and the age of the hens.

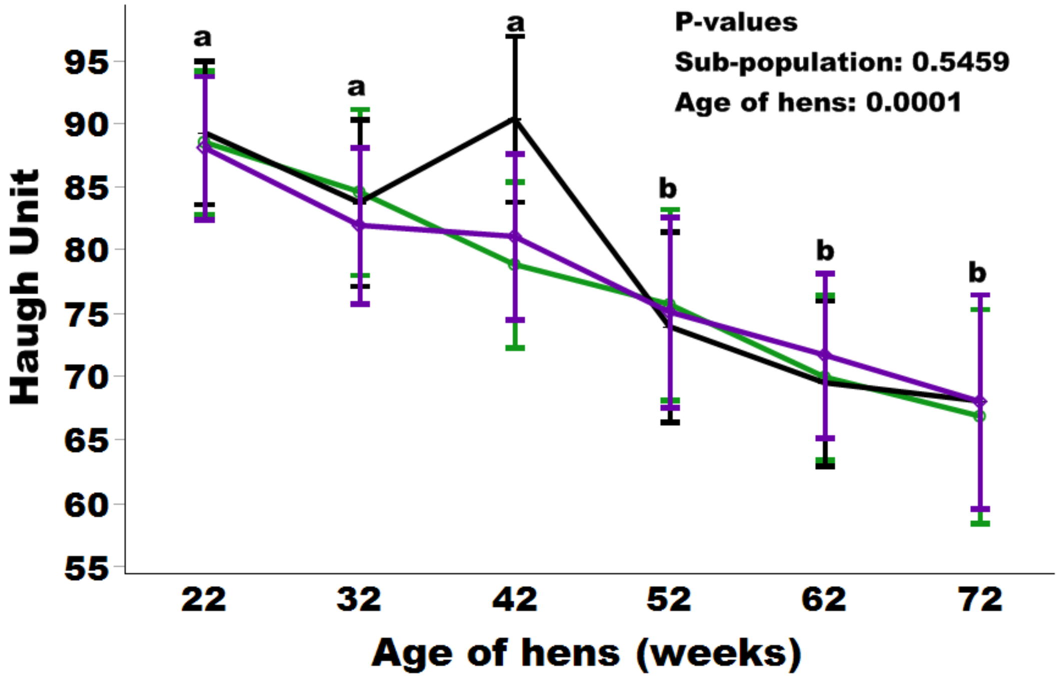

Figure 6-5 The difference between the Haugh unit of the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% metabolisable energy – at 22, 32, 42, 52, 62 and 72 weeks of age

The green, black and purple lines represent rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% ME, respectively.

The different superscripts indicate difference between the weeks.

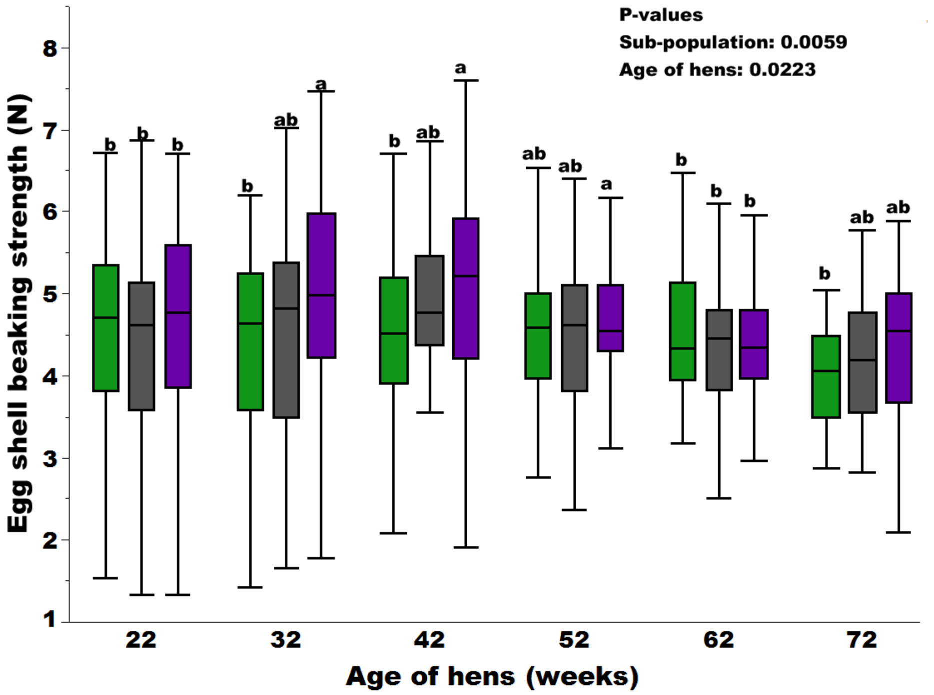

Figure 6-6 The difference between the eggshell breaking strength of the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% metabolisable energy – at 22, 32, 42, 52, 62 and 72 weeks of age

The green, grey and purple box plots represent the rangers control group, rangers with access to the outdoor feeder, and rangers with extra 10% ME, respectively.

The different superscripts indicate difference between subpopulation and age of hens.

6.1.4.3 Health and welfare

Table 6-3 shows the analyses of variance for the different subpopulations and treatments investigated in this study. The rangers control group had the highest score on the chest (P < 0.0010; Table 6-3).

The proportion of hens with keel bone damage was not significantly different between the subpopulations (P = 0.326; Figure 6-7B). The proportion of hens with severe keel bone damage in all treatment groups ranged from 46.6% to 50.9%, which was similar to the proportion of hens without keel bone damage ranging from 48.9% to 53.1% (Figure 6-7A). The proportion of hens with a moderate score of 1 was significantly lower, as it ranged from 0.09 to 0.47%.

The incidence of tapeworm infection of hens from the rangers control group and rangers with an outdoor feeder was 5.8% and 3.6% more than that of the rangers with the 10% extra ME group (P = 0.001). By contrast, the rangers with the 10% extra ME had a significantly lower incidence of tape worm infection. The rangers control group did not significantly differ from the population mean (Figure 6-8B). Lesions associated with Spotty Liver Disease were not significantly different in all the treatment groups. (Figure 6-9).

Table 6-3 Feather score of rangers control group, rangers with outdoor feeder, and rangers with 10% ME

Parameter	Rangers – control	Rangers + outdoor feeder	Rangers + 10% ME	P-value
Neck	3.72 ± 0.014	3.71 ± 0.014	3.74 ± 0.014	0.3626
Chest	3.11 ± 0.021[a]	3.01 ± 0.021[ab]	2.96 ± 0.021[b]	0.0010
Wing	3.27 ± 0.019	3.29 ± 0.020	3.35 ± 0.020	0.1651
Back	3.71 ± 0.016	3.67 ± 0.018	3.73 ± 0.017	0.3976
Vent	3.76 ± 0.016	3.74 ± 0.017	3.76 ± 0.016	0.5369

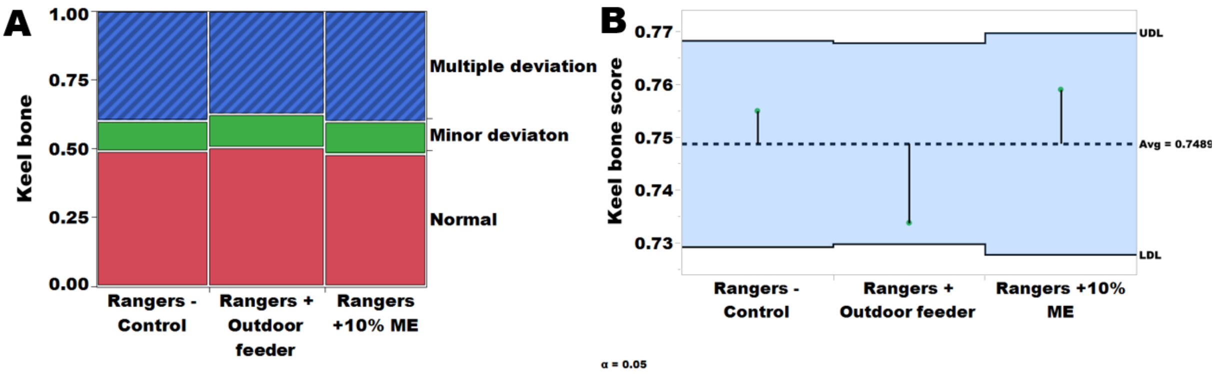

Figure 6-7 Mosaic plot (A) showing the proportion of hens with keel bone damage in 5 different treatments of Lohmann Brown hens

The graphical analysis of means (ANOM) (B) for the keel bone damage showing the upper decision line (UDL) and the lower decision line (LDL) at 95 % deviation level from the population mean (avg = 0.9801).

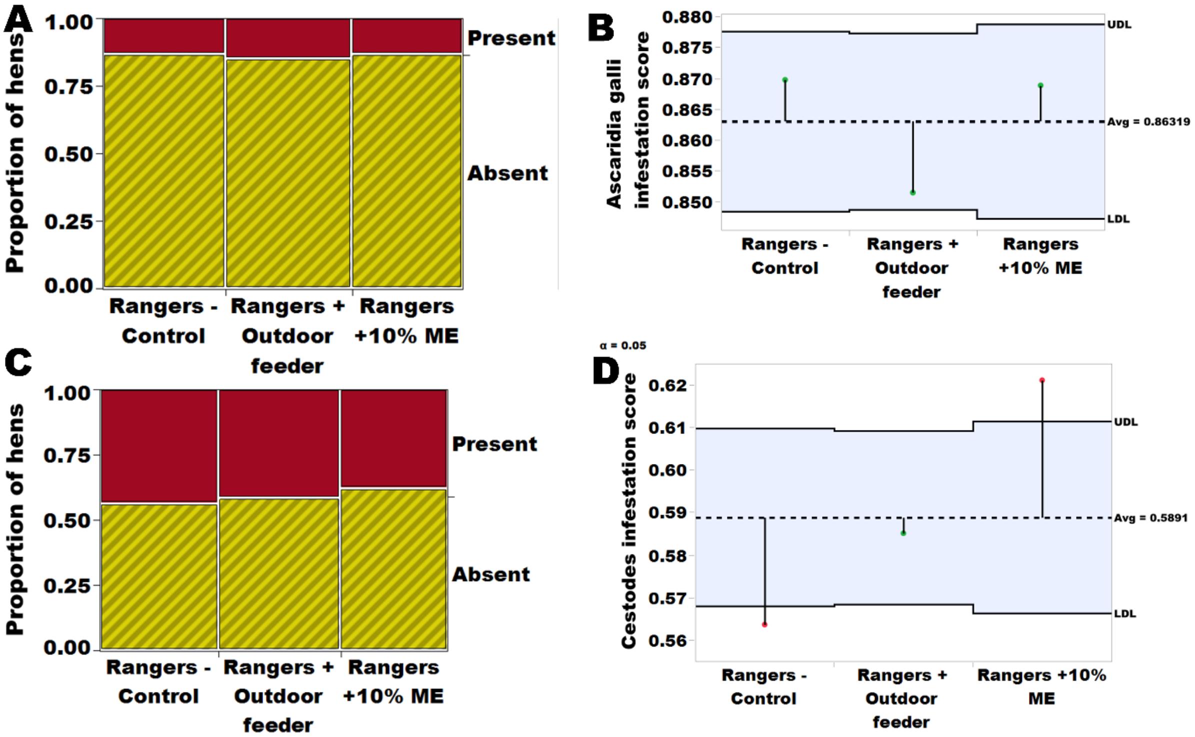

Figure 6-8 Mosaic plots (A and C) showing the proportion of hens with *A. galli* and cestodes infestation in 3 different treatments of Lohmann Brown hens

The graphical analysis of means (ANOM) (B and D) for the *A. galli* and cestodes infestation showing the upper decision line (UDL) and the lower decision line (LDL) at 95% deviation level from the population mean (avg = 0.3570 and 0.1878, respectively).

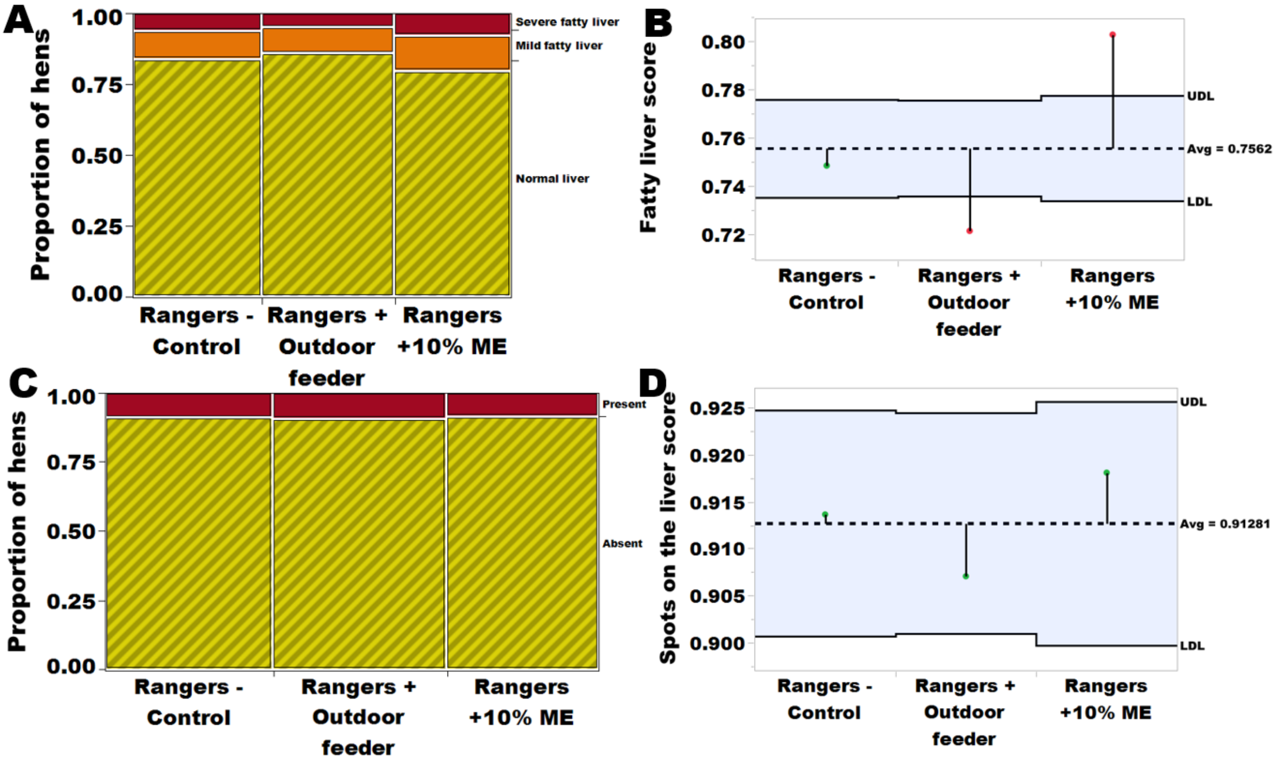

Figure 6-9 Mosaic plots (A and B) showing the proportion of hens in 3 different treatment of Lohmann brown hens with fatty liver and spotty liver respectively

The graphical analysis of means (ANOM) C and D for the fatty liver and spotty liver showing the upper decision line (UDL) and the lower decision line (LDL) at 95% deviation level from the population mean (avg = 0.2203 and 0.1177, respectively).

6.1.5 Discussion and conclusion

The results indicate that even though rangers are already performing at the expectations of the breed standard, their genetic potential can further be utilised when providing them with additional energy and essential amino acids. Economic calculations based on feed cost/tonne and difference in egg output will lead to the decision if a diet with higher energy and amino acids would be beneficial. Providing feed in an outdoor feeder was of no additional benefit in respect of the laying performance. It was previously demonstrated that an outdoor feeder also had no effect on increasing range usage, therefore we can conclude that using outdoor feeders is not only a considerable biosecurity risk but seems to be of no benefit to hens or farm economics at all.

Reduced body condition and nutrition may result in many of the challenges that face free range hens. The SLD results from this study, which seem to suggest that range access does not increase the Spotty Liver prevalence, are different from what is commonly believed. Body weight has a significant association with the susceptibility to infectious challenges and subsequently the health status of a hen (Sibanda et al. 2019b). Under-nourished hens or hens with an imbalanced nutrient supply are more likely to develop infectious diseases (Gross 1992). Furthermore, the impact of feed quality, the nutritional status of the hen, and hen body weight affects internal and external egg quality (Leeson & Summers 2009; Roberts 2004; Sahin et al. 2002). In summary, in order to maintain animal health and productivity it is crucial to measure, control and modify the nutrient intake and utilisation of commercial free range laying hens. Nutritional intervention is highly warranted when specific challenges, such as heat stress, have an impact on the health status of laying hens (Lin et al. 2006; Bollengier-Lee et al. 1998). In fact, diet and efficient feed utilisation can influence the stress level of the hen, as well as hen behaviour, reflected in frustration and aggression (Braastad & Katle 1989).

In conclusion, subpopulations of free range laying hens require individual nutrient support to achieve outstanding performance. Performance-based feeding allows for an efficient and responsible use of resources, and applied solutions that can be integrated on farm should be considered. This study shows that the amount of time the hens spend on the range does not have an effect on keel bone damage as there was no difference between the range use subpopulations.

6.2 The impact of range use on bone health

6.2.1 Summary

Bone tissue adapts continuously to metabolic calcium demands, as well as to external forces due to physical weight loading and hen movement. Limited calcium metabolism and subsequently limited availability from the medullary bone is one major factor contributing to reduced eggshell quality in hens at the end of lay. Increasing physical activity and biomechanical loading during hen rearing has been demonstrated to increase bone strength, enhancing bone mass as well as endocortical and periosteal bone metabolism. Presently, the consequences of daily range access during lay on bone quality characteristics in laying hens is unknown.

The aims of this study were to: (1) characterise tibiotarsal bone indices in commercial free range laying hens; and (2) evaluate the impact of range usage during lay on tibia bone quality in commercial free range laying hens. In this exploratory study we described and analysed the volumetric measurements, morphological mechanical and trabeculae indices of the tibiotarsal bone of 48 Lohmann Brown laying hens at 74 weeks of age. All bone parameters were obtained using micro computed tomography, plotted using JMP version 14, and correlated with individual range use.

The study found that range use was not associated with tibial trabecular architecture (bone volume, bone volume fraction, trabecular thickness, trabecular connectivity density, and structural model index), or any other morphological characteristics (breaking strength, diaphyseal diameter, bone weight and bone mineral density) of the tibia ($P > 0.05$) at 74 weeks of age. The results demonstrate a large variation in individual bone characteristics and suggest that range use had a minor effect on the bone quality of the commercial laying hens used in this study. In conclusion, the bone health of free range commercial laying hens may be predominantly positively impacted by other features such as hen genetics, feed, the quality of pullet rearing, perch availability or other shed equipment, and these variables exceed the effect of daily range access.

6.2.2 Introduction

Osteoporosis is the most common metabolic bone disorder in modern commercial laying hens, resulting in a fracture prevalence of up to 30% over the duration of the laying period (Whitehead & Fleming 2000). Osteoporosis is characterised by bone mass loss due to microstructure degradation that causes fractures during hen movement and hen transportation. Bone mineral density (BMD) is responsible for 60–70% of the variability in bone strength. The remaining 30–40% of variation in bone strength is associated with genetic and epigenetic factors such as the geometry and microarchitecture of the cortical and trabecular bones (Ammann & Rizzoli 2003).

Calcium mobilisation for eggshell formation places the largest demand on BMD (Whitehead 2004; Li et al. 2015; Guo et al. 2017). Commercial laying hens need to mobilise approximately 2 g of calcium daily for eggshell formation, equivalent to 10% of the total body calcium (Miller 1992). Calcium mobilisation from the medullary bone is responsible for one third of the calcium required for eggshell formation in the uterus, while the remaining two thirds of calcium is generated directly from the digestive tract (Nys & Guyot 2011). Limited calcium metabolism and availability from the bone is the major factor contributing to reduced eggshell quality in hens during the egg production cycle (Elaroussi et al. 1994). Bone quality is negatively correlated with overall egg production and eggshell quality (Riczu et al. 2004; Kim et al. 2005). The reduced ability of hens to deposit calcium for the eggshell, and the subsequent impact on eggshell quality, is responsible for the economically driven decision to depopulate a layer flock and replace the old birds with younger ones that produce more high-quality eggs. Maintaining calcium homeostasis and understanding bone calcification is therefore of paramount importance to improve hen welfare, the economics of egg production, as well as ethical and sustainable food production.

While the egg producer has limited capacity to control the quality of pullets received, several management options are or may be available to maintain and improve the health status of layers, and to ensure adequate egg production. For example, feeding a pre-lay diet has been proven to contribute significantly to egg quality at the end of lay (Summers & Leeson 1994). The freedom of movement in cage free systems can stimulate structural bone formation to avoid mechanical failure, but it also provides a high-risk environment for uncontrolled hen movement and subsequently (bone) injury (Leyendecker et al. 2005; Rodriguez-Navarro et al. 2018).

While the availability of perches can enhance bone mineral density, they have also been found to be associated with significantly higher incidences of keel bone damage, and dislocated and broken toe nails (Hester et al. 2013; Olsson & Keeling 2002). Therefore, univocal conclusions about the benefit of perches in layer houses remain in question (Appleby et al. 1993; Donaldson & O'Connell 2012).

Range access provides hens with horizontal space that allows for various exercise activities including running and flying, while reducing the likelihood for collision, smothering, and falling from vertical structures. Additionally, range usage allows hens to be exposed to daylight, stimulating hormone and vitamin D production, which is known to improve bone mineralisation and prevent calcium depletion in eggshells in free range laying hens (Kuhn et al. 2014). We therefore hypothesise that range usage may have beneficial effects on bone health. The aim of this study was to characterise tibiotarsal bone indices in commercial free range laying hens and to evaluate the impact of range usage during lay on these tibia bone parameters.

6.2.3 Materials and methods

6.2.3.1 Animals and sample collection

All procedures were approved by the University of New England Animal Ethics Committee (AEC 16-087). Fifty Lohmann Brown hens at 74 weeks of age were selected from a commercial free range egg producer. The experimental hens were housed amongst a 40,000-layer flock and monitored for range usage using a custom-made radio frequency identification (RFID) system (Science and Engineering Workshop at the University of New England, Armidale, NSW, Australia) as described in Chapter 3. Briefly, a randomly selected subpopulation of hens was equipped with individually numbered RFID leg bands (Monza R6 UHF-RFID leg band – Impinj, Inc., Seattle, WA, USA) at the age of 16 weeks, and monitored for range access daily until 72 weeks of age. The range use was monitored using RFID antennae placed along the pop holes and also across the width of the range at a 25 m distance, parallel to the shed. All hens were subject to the same management and environmental conditions.

6.2.3.2 Morphometric and mechanical parameters of the tibiotarsal bones

At 74 weeks of age, hens were humanely killed, and the left tibia was removed. Muscles and tendons attached to the tibiae were manually removed and the bones stored at -20°C until measurements were to be taken. The length of the tibia was measured from its intercondylar eminence to the lateral malleolus, and the diaphyseal diameter was measured in the mid-diaphyseal region using a digital Vernier Calliper (0.01 mm precision – Kincrome Australia Pty Ltd, Scoresby, Victoria). For biomechanical testing, the left tibiae were subjected to 3-point bending to failure. The bones were mounted on a mechanical testing frame (600LX – Instron, Norwood, MA, USA) across supporting beams with gaps of 50 mm, and a perpendicular load applied to the midpoint. The breaking strength was recorded as the peak force (N) required to reach structural failure.

6.2.3.3 Volumetric bone measurements and bone density

Prior to breaking, quantification of the cortical bone mineral density, total bone volume, relative bone mineral density, proportion of blood vessels and relative marrow volume was performed on all left tibiae using a GE-Phoenix V|tome|xs 240 micro CT scanner (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). Tibiae and two calibration calcium hydroxyapatite phantom equivalent (0.25 and 0.75 g.cm-3 Bruker-MicroCT, Melbourne, Australia) were mounted on a rotating stage, and imaged using the predetermined optimal X-ray tube settings (160 kV, 120 mA, 200 ms integration time per projection, focal spot 4 mm diameter) respectively. The projections were captured using a 1000 x 2000-pixel detector array (DXR-250) (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) set to 3600 projections (in full 360-degree rotation), which resulted in an isotropic voxel side length of 124.91 µm when reconstructed. All scans were captured using the GE constant rotation CT function to reduce acquisition time. Tomographs were projected across the 16-bit depth dynamic range and VGStudio Max 2.0 (VolumeGraphics 2009) was used to concert the images to a TIFF stack for import into imageJ (Schneider et al. 2012). Volumes were imported into FIJI, ImageJ version 2.0.0.0-rc-15/1.49k, Java 1.6.0_65, and the ‘threshold’ tool was used to isolate pixels representing different phases (bone, marrow and air) for a sample from each scan. These pixels were then used to create a mask of classified pixels and any misclassification was manually removed. These masks were then used to train a classifier in the ‘trainable Weka segmentation’ machine learning toolkit (v3.2.29) available in ImageJ (Frank et al. 2016). This classification algorithm determined for the training sample was assessed for accuracy and, when acceptable, was applied to the remainder of the dataset. Voxel counting methods were used for volumetric analysis and the BoneJ plugin was used for trabeculae indices analysis (Doube et al. 2010).

6.2.3.4 Trabeculae bone architecture

A second set of randomly collected left tibiotarsal bones (n = 10) was used to analyse the trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), connectivity density (Conn.Dens), and structure model index (SMI). To assess the trabecular and cortical bone differences in response to range use, further subregional scanning of the tibiae was performed on the diaphyseal region. The bone in the trabecular space was assumed to reflect medullary changes (Saunders-Blades et al. 2009). The tibiotarsal bones were imaged using the X-ray tube settings and (170 kV, 120 mA, 200 ms integration time per projection, focal spot 4 mm diameter) respectively, resulting in a higher resolution voxel size of 9.8 µm. Following a similar process as before, images were imported into ImageJ, and BoneJ was used to segment trabecular bones from cortical bones using a manual hand-contouring approach before calculating the above parameters.

6.2.3.5 Statistical analysis

All statistical analyses were performed using JMP Statistics software (version 14 – SAS Institute Inc., Cary, NC, 1989-2019) and in R version 3.5.0 R (R Core Team 2018). The mean, standard error of the mean, median, standard deviation, minimum, maximum, range, and the coefficient of variation were used to describe volumetric bone measurements, bone morphological indices and the trabeculae architecture. Descriptive statistics regarding the range use of hens were visualised using histograms and box plots created using the ‘ggplot2’ package (Wickham 2016) within Studio (RStudio Team 2016). The Fisher-Pearson coefficient of skewness and the kurtosis were used to evaluate normality of the variables using the ‘e1071’ package (Meyer et al. 2017) in R. Bivariate linear regression models in JMP were used to investigate the relationships between the different properties of the tibia and range use.

6.2.4 Results

While 50 hens were sampled for this research, the RFID tags of two hens were found to be malfunctioning and therefore excluded from the range use descriptives, as well as the correlation statistics.

6.2.4.1 Range usage

Details about the range use of the sampled hens, while the hens were 18–74 weeks of age, are summarised in Figure 6-10. The hens visited the range for a minimum of 0 days to a maximum of 242 days. The number of days spent on the range show bimodality, with two local maxima peaks during days 1–25 and days 200–225, while the time and the number of visits to the range peaked at 40–60 minutes duration and 2–3 visits per hen per day, respectively. Although the number of visits has a single peak, the distribution is skewed to the left as indicated by the skewness value of 1.75 and a high kurtosis of 5.35. Co-efficiencies of variation of 53.4%, 43.4% and 48.2% were observed for the days that hens accessed the range, the time they spent on the range, and the number of visits to the range, respectively.

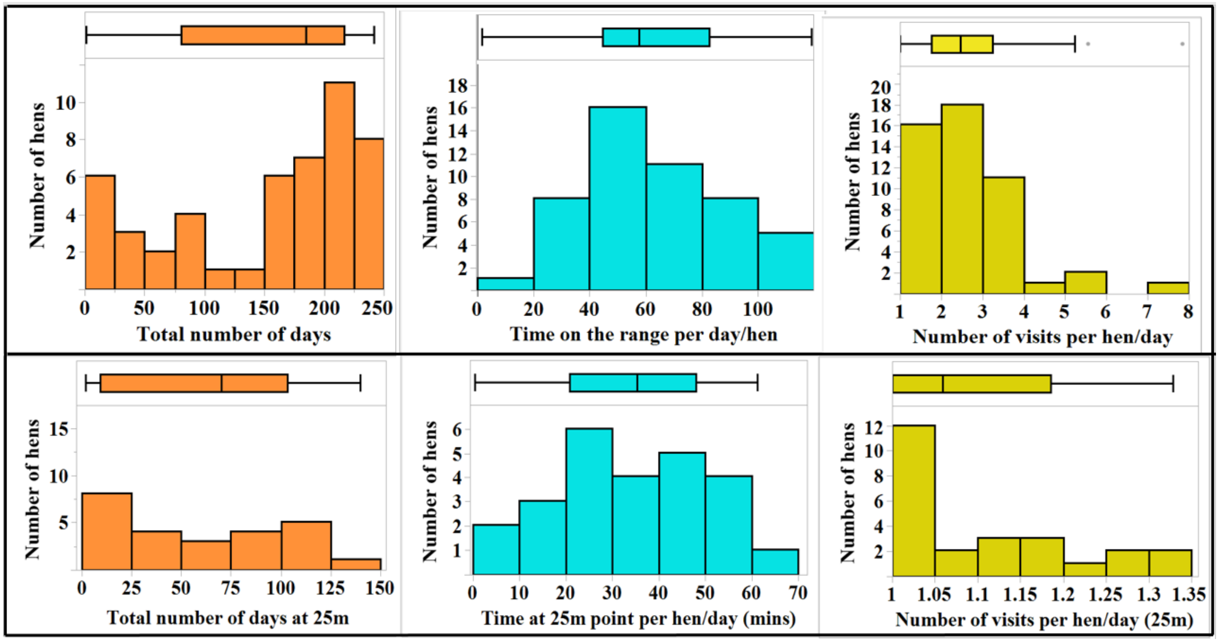

Figure 6-10 Histograms representing information about the range use of commercial free range laying hens (n = 48)

The upper row of figures (A, B and C) refers to the total number of days (A), the time on the range (B), and the number of visits (C) that the hens accessed the range through the pop hole.
The lower row of figures (D, E and F) refers to the total number of days (D), the time on the range (E), and the number of visits (F) that hens accessed the range at a 25 m distance from the shed.

6.2.4.2 Bone measurements descriptives

Descriptive statistics of volumetric bone measurements of the whole tibial bone obtained from all 48 hens are presented in Table 6-4. Figure 6-11 provides two examples demonstrating the large variety of blood vessels branching, which could be observed in our study population.

The total bone volume ranged from 7059 to 11,008 mm^3 with a mean of 8484 ± 128.3 mm^3. The highest coefficient of variations (27.3% and 19.3%) were found in and total bone volume and total marrow volume, respectively, while the lowest coefficients of variation were observed in the total bone volume (10.6%) and the cortical bone volume (14.0%; Table 6-4).

The mean bone breaking strength of the hens was 157.3 ± 7.9 N with a coefficient of variation of 35.9%. By contrast, bone length and diaphyseal diameter had a coefficient of variation of 3.28% and 4.95%. The mean bone mineral density of the cortex was 484.2 ± 7.36 [343.1-613.2] mg/cm^3 with a coefficient of variation of 10.5% (Table 6-4). The descriptive analysis of the trabeculae bone structure at the mid diaphysis of the tibiae are presented in Table 6-4. Figure 6-12 gives an example of the cross section of the tibia used for the trabeculae analysis. The bone volume fraction, connectivity density, and the structure model index had a very high variation of 89.3%, 91.2%, and 79.8%, respectively.

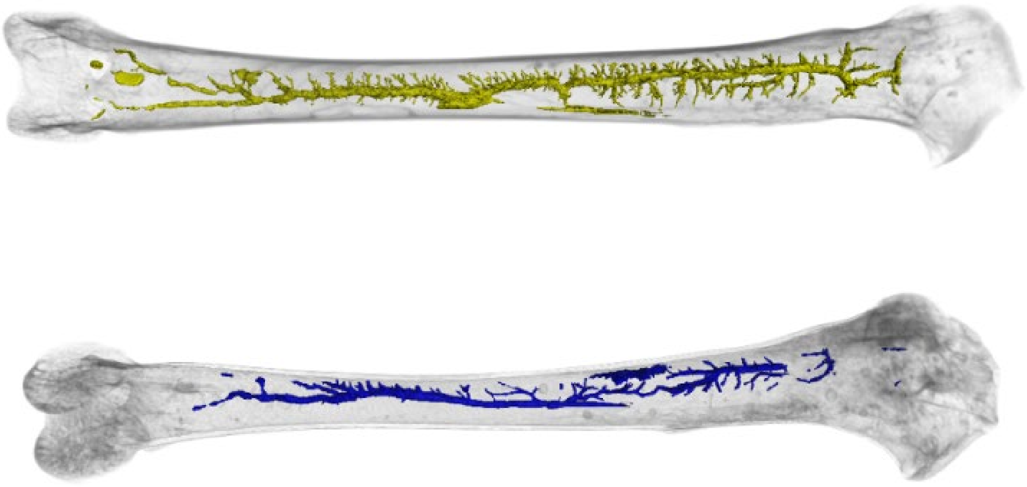

Figure 6-11 Three dimensional projection of the drained blood vessels in the tibia

The upper tibia was obtained from a hen that ranged 84% of its available days (210 days in total; yellow), and the lower tibia was obtained from a hen that ranged only 50% of its available days (125 days in total; blue).
Segmented from micro CT scans.

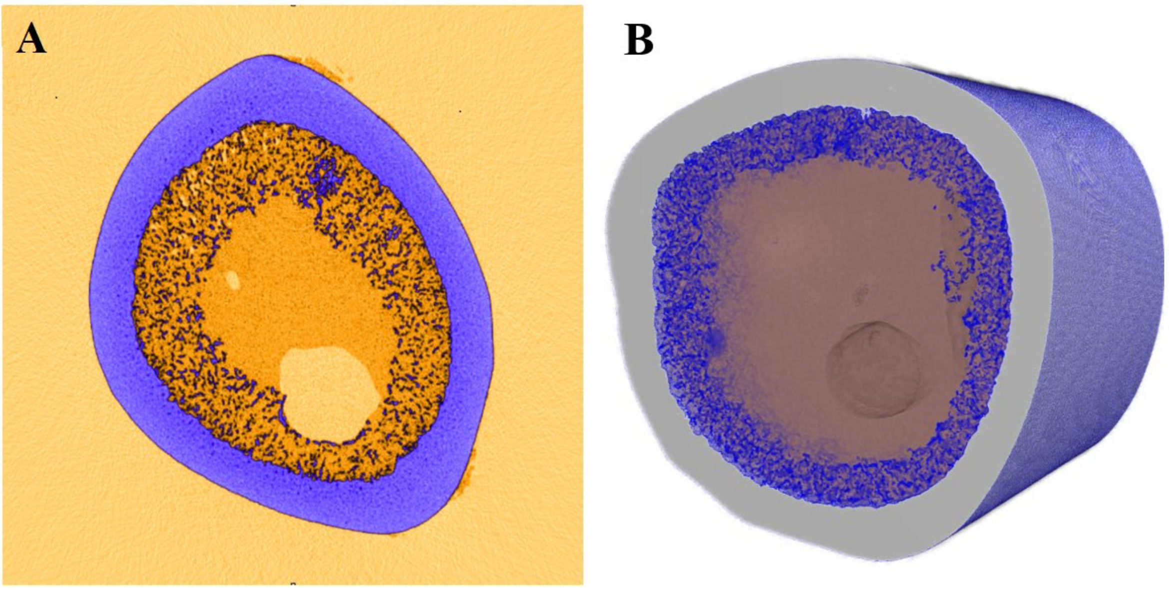

Figure 6-12 Cross-section of the tibia in 2-(A) and 3-(B) dimensions at the mid-diaphyseal shaft of a 74 week old commercial Lohmann Brown hen

Table 6-4 Descriptive statistics of volumetric, morphometric, trabeculae measurements of the bone marrow, cortical bone and blood vessels of 48 commercial free range laying hens at 74 weeks of age

Volumetric	*Mean ± SEM*	*Median*	*SD*	*Min*	*Max*	*Range*	*CV*	*Skewness*	*Kurtosis*
Total bone volume (mm^3)	8484 ± 128.3	8351	898.4	7059	11008	3949	10.6	0.792	0.572
Cortical bone volume (mm^3)	4582 ± 91.7	4442	641.9	3574	6256	2683	14.0	0.800	0.154
Total bone marrow volume (mm^3)	3442 ± 94.8	3440	663.6	2199	5184	2985	19.3	0.324	0.085
Total volume of blood vessels (mm^3)	214 ± 8.32	218	58.2	68.0	330	262	27.3	-0.439	-0.220
Morphometric and mechanic									
Bone breaking strength (N)	157 ± 7.90	157.1	56.4	54.6	342	342	35.9	0.217	1.85
Bone length (mm)	122 ± 0.56	124	4.01	110	131	20.6	3.28	-0.869	0.99
Diaphyseal diameter (mm)	8.29 ± 0.06	8.31	0.411	7.23	8.97	1.74	4.95	-0.392	-0.21
Bone mineral density (g/cm^3)	484 ± 7.36	478	127	343	613	270	10.5	0.374	0.24
Bone weight (g)	12.8 ± 0.18	12.7	1.28	10.4	17.0	1.49	10.0	0.884	1.61
Trabeculae bone architecture									
Total volume (mm^3)	99.3 ± 6.04	119.1	97.1	69.7	142.3	72.6	19.3	0.50	-0.56
Bone volume (mm^3)	5.15 ± 1.05	3.33	4.28	2.62	13.7	11.1	64.5	1.36	2.14
Bone volume fraction BV/TV (%)	5.80 ± 1.60	5.20	4.70	1.81	19.7	17.9	89.3	2.25	8.09
Trabecular thickness (mm)	0.050 ± 0.01	0.046	0.045	0.04	0.090	0.05	31.9	2.73	9.85
Connectivity density (mm^3)	154 ± 44.4	140.3	90.1	45.7	449	403	91.2	1.42	1.69
Structure index model (1/mm^3)	3.65 ± 0.92	2.90	4.07	-4.17	6.27	10.4	79.8	-1.42	6.26

6.2.4.3 Regression analysis of range use and volumetric, morphologic and mechanical tibia characteristics

There was no significant relationship between the total time that individual hens spent on the range and any of the bone volumetric measurements (Figure 6-13). The R^2 values were 0.088, 0.066, 0.067, and 0.052 for total bone volume, % blood vessel, % bone marrow, and % cortical bone, respectively. The relationship between range use, the morphometric and mechanical measures are presented in Figure 6-14. There was no significant linear or curvilinear relationship between range use, bone breaking strength, bone mineral density, and diaphyseal diameter. There was a significant cubic relationship between bone length and the total number of days spent on the range. There was no significant linear relationship between range use, trabeculae thickness, connectivity density and bone volume fraction (Figure 6-15). There was also no significant linear or curvilinear relationship between range use, bone breaking strength, bone mineral density, bone length and diaphyseal diameter (Figure 6-16).

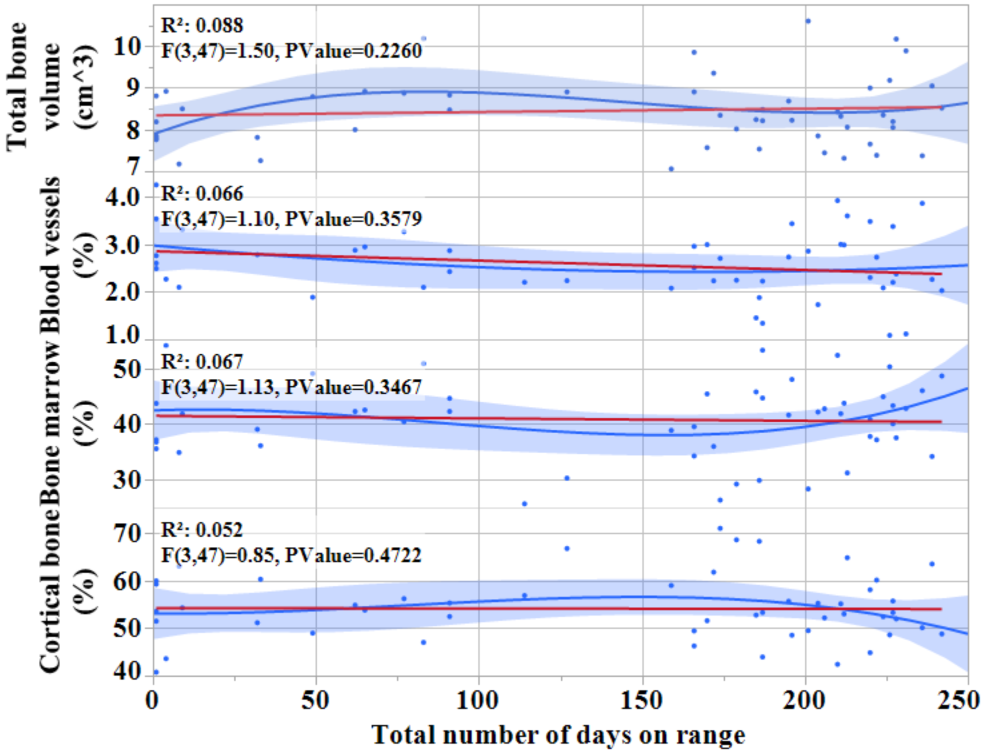

Figure 6-13 Relationship between range use and the proportion of cortical bone, bone marrow, blood vessels and total bone volume of Lohmann Brown free range laying hens at 74 weeks of age (n = 48)

Every dot represents one hen.
The red and blue lines represent the linear and the third order quadratic fit, respectively.
The shaded area represents the 95% prediction interval.

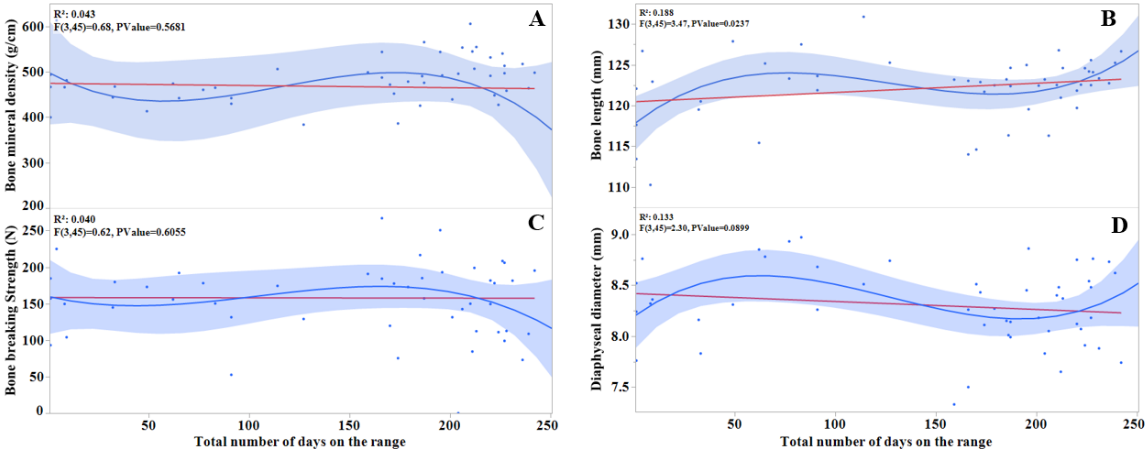

Figure 6-14 Relationship between range use and bone mineral density (A), bone length (B), bone breaking strength (C), and diaphyseal diameter (D)

All bones were obtained from Lohmann Brown free range laying hens at 74 weeks of age (n = 48).
Every dot represents one hen.
The red and blue lines represent the linear and the third order quadratic fit, respectively.
The shaded area represents the 95% prediction interval.

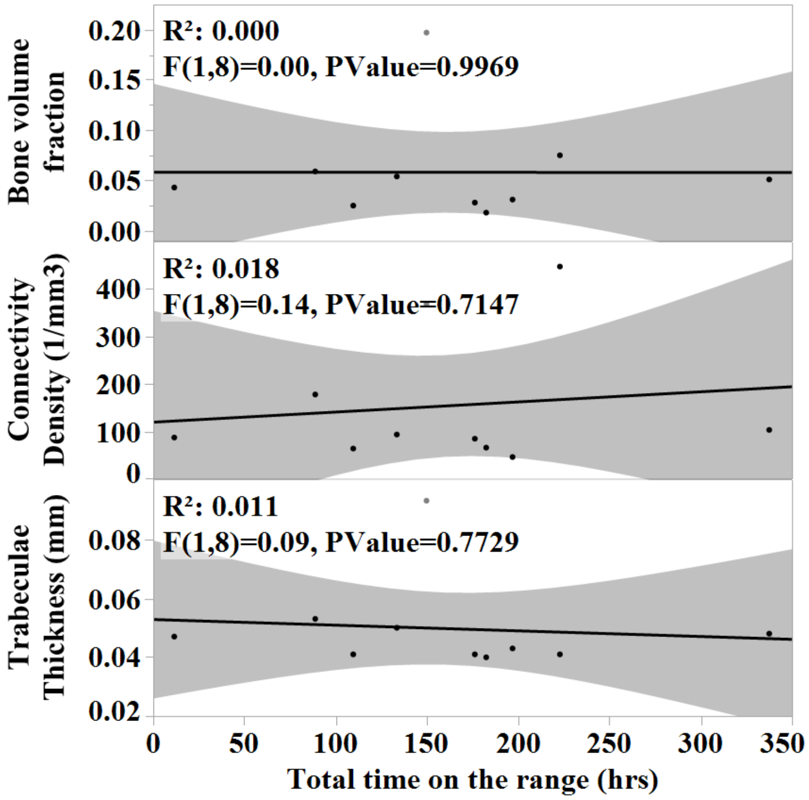

Figure 6-15 Relationship between the total number of hours that individual hens spent on the range and the trabeculae characteristics of tibiae obtained from commercial Lohmann Brown free range laying hens at 74 weeks of age (n = 45)

Every dot represents one hen.
The shaded area represents the 95% prediction interval.

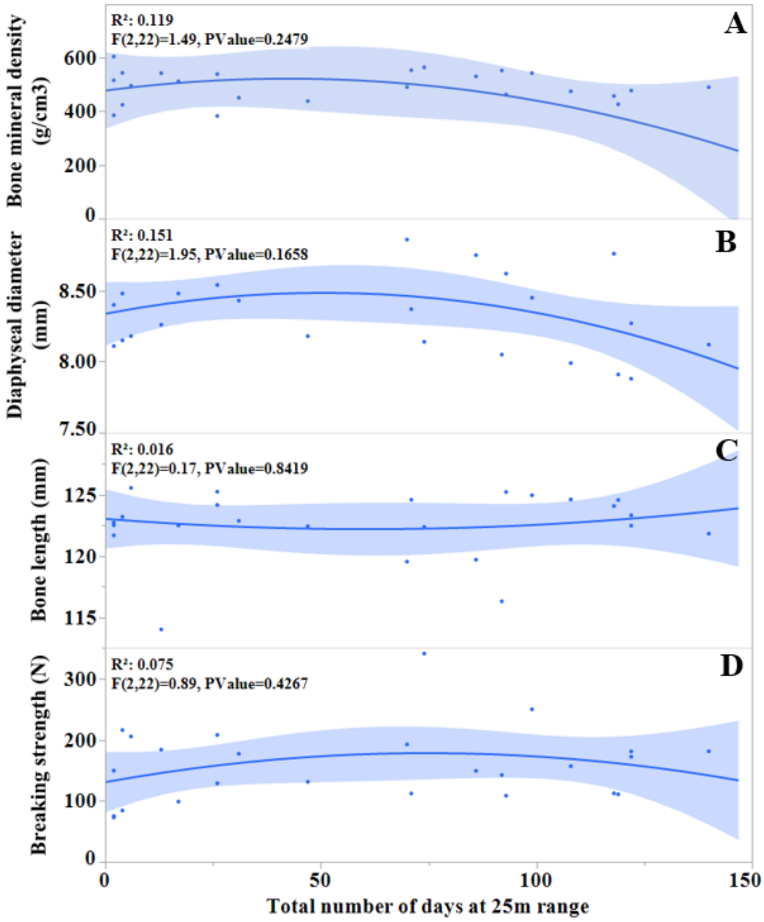

Figure 6-16 Regression analysis between the total number of days on the range at a distance of at least 25 m from the shed and bone mineral density (A), diaphyseal tibia diameter (B), tibia length (C), and tibia breaking strength (D) of Lohmann Brown free range laying hens at 74 weeks of age (n = 24)

Every dot represents one hen.
The solid line indicates the best fit, while the shaded area represents the 95 % prediction interval.

6.2.5 Discussion

The aim of the study was to describe the tibiotarsal bone structure including parameters of volumetric, morphometric, mechanical and trabeculae architecture of free range laying hens under commercial conditions, allowing for comparison with other research studies in the future. Bone breaking strength and the risk of bone fracture is determined by the structural properties and the morphological characteristics of the bone. The results of this study demonstrated that the bone length and diaphyseal diameter of these commercial Lohmann Brown laying hens were similar to the bone quality observed of hens of the Lohmann White breed (Regmi et al. 2015).

Range use was not significantly correlated to bone quality. This is somewhat surprising giving the fact that whippets, thoroughbred race horses and human athletes are frequently subject to improved bone quality and mineral density when performing horizontal locomotion activities (Hart et al. 2017). Hens that had been exercised on a tread mill for the duration of four weeks also experienced an increase of bone weight. As such, it might be possible that once tibiae experienced a certain level of mineralisation, the quality of the bone could not be further improved by voluntarily range use. Another potential explanation why range usage was not beneficial to bone health parameters may be that hens, rather than being active running on the range, may have spent their time dustbathing, sleeping, sitting, and resting. These activities may be considered as less strenuous to the bone cells, which are responsive to mechanical strain. Even hens that range as far as 25 m on to the range did not exhibit beneficial effects on any of their bone parameters. One possible explanation would be that hens that preferred to spend their available time in the shed would have experienced vertical structures such as perches, nest boxes and an aviary system, allowing for physical exercise, while hens on the range had only a horizontal range space available. Similarly, most hens are motivated to use perches (Olsson & Keeling 2002), although perch use differs between individuals and strains (Faure & Jones 1982). Regmi et al. (2016) found that laying hens kept in an aviary system had better bone quality compared to caged hens. This suggests that range access, no matter how much time was spent on the range, does not compensate for the use of vertical structures such as perches or aviaries. Future research should be directed at measuring not only hen access to certain shed/range areas, but should also include detailed horizontal and vertical movement, which would allow for an estimation of physical load on the skeletal system.

The tibiae collected from free range laying hens in this study had a relatively high coefficient of variation in trabeculae architecture indices, indicating a higher individual variability. Despite the fact that the hens used in this study were of the same genetic breed, and had been reared and housed in the same environment, individual differences in their range usage were evident. This high level of variation that occurs innately in the flock is seemingly greater than the effect of the limited range of clonal fish with identical genotype (Bierbach et al. 2017), but is not fully understood. How and why individual animals of the same background and similar experience prefer different environments requires further research. The individual differences observed in range use might also apply to individual differences of the rearing or hen house environment, and ultimately could be responsible for the large variation of bone characteristics such as the trabecular architecture, bone volume fraction and connectivity density. Complex dynamics in personality traits such as aggressiveness, avoidance of novelty, boldness, exploration and sociality are likely to have an impact on the intensity of exercise that an individual undertakes. It can be assumed that this may significantly contribute to hen physiology including bone quality. Kolakshyapati et al. (2019b) characterised individual hens that prefer to use the range or prefer to stay in the shed based on their fearfulness, and reported that fewer range visits during the first three weeks of range access was associated with increased fearfulness at the end of lay. Those individual behavioural characteristics may be able to be influenced by early life experience and therefore contribute significantly to the use of the provided resources/range (Campbell et al. 2019).

The low bone mineral density and low bone volume fraction of 5.8 % observed in hens of this research is similar the parameters described by Fleming et al. (1998) on the proximal tarsometatarsus with a bone volume of 6.6–7.3% in commercial hens at 70 weeks of age. While range usage showed no effect on tibiae obtained from hens at 74 weeks of age, a positive impact of range use on bone quality and/or calcium metabolism might have been possible during onset of lay, peak of lay or any other age. During the onset of lay, when hens are between 16 and 18 weeks of age, hens rapidly deposit their available calcium in the medullary bone, which has less structural integrity than the cortical bone (Whitehead 2004). In rats, physical activity and biomechanical loading is thought to enhance endocortical and periosteal formation, and reduces endocortical resorption thereby increasing overall bone quality, especially during this developmental stage (Birkhold et al. 2016). Therefore, it would be interesting to investigate the impact of range use on bone and egg quality when hens are younger, e.g. 18–-26 weeks of age.

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8 Plain English Summary

Project Title:	**Nutritional management of free range laying hens**
Australian Eggs Limited Project No	1UN151
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Objectives	The objectives of the project were to: a) characterise subpopulations of free range laying hens b) determine the dynamics of free range subpopulations c) develop and validate feeding strategies for subpopulations of free range layers.
Background	Free range poultry production is a rapidly growing sector and therefore of increasing impact to the egg industry. Range usage depends on flock size, the number of pop holes, shelter on the range, weather conditions, age and experience of the flock. The individual freedom to range results in the development of several subpopulations within one flock. Previous studies revealed that a certain percentage of birds rarely leave the hen house, while others spend the majority of their time ranging. As a result, free range flocks experience reduced flock uniformity and sub-optimal nutrition and, as a consequence, sub-optimal egg production. Understanding the complexity of hen movement and the interaction of hens within their environment provides an opportunity to limit the disadvantages associated with housing in non-cage husbandry systems, and aids in decision-making.
Research	The research was conducted at a commercial farm over a three-year period. In order to quantify individual hen usage of the range and the aviary system, a Radio Frequency Identification (RFID) system was custom built to monitor a total of 18,450 Lohmann Brown hens housed in six identical commercial free range sheds. Health and welfare parameters of all hens were obtained at different ages, and from 22 weeks of age hens were grouped according their range usage into 'stayers', 'roamers' sand 'rangers'. Rangers were subject to different feed treatments. Range use, egg laying performance and various health parameters were obtained until the animals were 72 weeks of age. The performance of hens that accessed the range frequently exceeded the expected performance of the breed standard. Egg quality, however, differed only on rare occasions between the subpopulations and is therefore of less concern.

	The use of the range was significantly correlated with the use of the aviary system, whereas stayers preferred to use the upper tiers of an aviary system and rangers accessed predominantly the lower tiers. The development of stayer and ranger subpopulations resulted in an uneven use of resources, and the dynamics of these distinct subpopulations can predict range use. Feeding a diet of higher metabolisable energy (+10%) and elevated amino acid concentration (up to 10%) resulted in significantly higher laying performance compared to hens that were fed a conventional diet. Range use did not increase bone health. Subpopulations of free range laying hens require individual nutrient support to achieve outstanding performance. Performance-based feeding would allow for an efficient and responsible use of resources. Applied solutions that can be integrated on farm are highly warranted.
Outcomes	Hens preferred to spend most their time near feed chains rather than on the range or in the nest boxes. Range use was positively associated with the use of the lower feeder tier of the aviary system, which allows egg producers to manage the subpopulations of a flock using various strategies. Furthermore, there was evidence that the amount of time that hens spent ranging or using the aviary system is related to differences in hen welfare and egg production. Rangers came into lay significantly earlier, while stayers outperformed the rangers at the end of lay. While flock subpopulations varied within their health and welfare status, these parameters were measured only at 74 weeks of age. It would be interesting to study the impact of ranging or aviary system use difference during peak egg production, and also investigate flock mortalities. Modifying the diet to suit frequent range users showed significant improvement not only in egg laying performance but also in hen health and welfare.
Implications	Using big data and computer learning will be a powerful tool to allow for an in-depth understanding about hen usage of the aviary system and assist in decision-making regarding the height, width, design and the number of tiers to ensure the achievement of desired performance and welfare outcomes. Being able to determine the percentage of hens that are not using the nest boxes, not accessing certain feeder lines, and the proportion of those that are using the range, can allow for adequate changes in dietary manipulation. Furthermore, offering different diets or feed additives through different feeder lines may directly target the different requirements of hens that favour these specific locations. This is especially true when aiming for flock longevity and laying persistence beyond 100 weeks of age. Investigation of the reasons and the key events that are associated with the development of flock subpopulations needs to be extended to the rearing facilities, as well as to the consequences of modified rearing strategies on the use of the hen house, including the aviary system and other resources.

Key Words	Aviary, egg quality, feed, layer, production, poultry, RFID
Publications	**Journal Articles** Sibanda TZ, Walkden-Brown SW, Kolakshyapati M, Dawson B, Schneider D, Welch M, Iqbal Z, Cohen-Barnhouse A, Morgan NK, Boshoff J, Ruhnke I, (2019). **Flock use of the range is associated with the use of different components of a multi-tier aviary system in commercial free-range laying hens.** *British Poultry Science, 61:2, 97-106, DOI: 10.1080/00071668.2019.1686123* Ruhnke I, Boshoff J, Cristiani IV, Schneider D, Welch M, Sibanda TZ, and Kolakshyapati M. 2019. **Free-range laying hens: using technology to show the dynamics and impact of hen movement**. *Animal Production Science. https://doi.org/10.1071/AN19256* Sibanda TZ, Kolakshyapati M, Walkden-Brown SW, de Souza Vilela J, Courtice J, Ruhnke I. 2019. **Body weight subpopulations are associated with significant different welfare, health and egg production status in Australian commercial free-range laying hens in an aviary system.** *European Poultry Science **84** https://doi.org/10.1399/eps.2020.295* Sibanda TZ, Flavel R, Kolakshyapati M, Schneider D, Welch M, Ruhnke I. 2019. **The association between range access and tibial quality in commercial free-range laying hens.** *British Poultry Science, DOI: 10.1080/00071668.2020.1759786.* Sibanda T.Z**.**, M. Kolakshyapati, M. Welch, D. Schneider, J. Boshoff and I. Ruhnke**.** 2020. **Characterising Free-Range Layer Flocks Using Unsupervised Cluster Analysis.** Animals 2020, 10(5), 855; https://doi.org/10.3390/ani10050855 Sibanda T.Z., M. Kolakshyapati, M. Welch, D. Schneider, J. Boshoff and I. Ruhnke**. 2020. Managing free-range laying hens - Part A: Frequent and non-frequent range users differ in laying performance but not egg quality.** *Animals 2020, 10(6), 991;* https://doi.org/10.3390/ani10060991 Ruhnke I., Sibanda T. 2019. **Feeding hens in a loose husbandry system- challenges and consequences on performance and welfare.** *European Poultry Science Symposium, Gdansk, Poland, 2019. 22: 21-55* **Conference Abstracts** Sibanda TZ, Dawson B, Welch M, Schneider D, Kolakshyapati M, Boshoff J, Ruhnke I. **Validation of a radio frequency identification (RFID) system for Aviary systems.** APSS, Sydney, Australia. Accepted, February 2020. Sibanda TZ, Kolakshyapati M, de Souza Vilela J, Courtice J and Ruhnke I. **Body weight is associated with welfare, health and egg production status in commercial free-range laying hens.** RAAN*,* Armidale, Australia. Submitted, October 2019.

Sibanda TZ, Welch M, Kolakshyapati, Schneider D, Ruhnke I. **Unsupervised exploratory cluster analysis of free-range laying hens to determine the use of aviary feed chains and range access.** *European Poultry Science Symposium, Gdansk, Poland, 2019, pp 162.*

Sibanda TZ, Flavel R, Kolashyapati M, Ramirez-Cuevas S, Welch M, Scheider D, Ruhnke I. **Outdoor feeder increased range usage but not bone quality in commercial free-range laying hens.** *Congress of the International Society for Applied Ethology, Norway, 2019.*

Boshoff J, Christiani IV, Sibanda T, Kolakshyapati M, Schneider D, Welch M, Dawson B, Ruhnke I. **Development of interactive visualisation software for research communication.** *30th Australian Poultry Science Symposium, Sydney, Australia, 2019.*

Sibanda TZ, Flavel R, Kolakshyapati, Schneider D, Welch M, Boshoff J, Ruhnke I. **Is range usage at the onset of egg production associated with tibial bone mineral density at the end of lay?** *30th Australian Poultry Science Symposium, Sydney, Australia, 2019.*

Sibanda TZ, Ruhnke I. **Nutritional management of laying hens.** *Poultry Ideas Exchange and Australasian Milling Conference, Gold Coast, Australia, 2018.*

Sibanda TZ, Kolakshyapati M, Boshoff J, Ruhnke I. **The effects of body weight on the diurnal range use and latency to use the range in free-range laying hens.** *Congress of the International Society for Applied Ethology, Canada, 2018.*

Sibanda T, Schneider D, Welch M, Iqbal Z, Cohen-Barnhouse A, Kolakshyapati M, Morgan N, Ruhnke I. **The impact of range use on flock uniformity in commercial free-range laying hens.** *29th Australian Poultry Science Symposium, Sydney, Australia, 2018.*