Relationships between ranging behaviour and welfare of commercial free-range broiler chickens

Peta S Taylor

BAnVetBioSc. (Hons)

ORCID ID: 0000-0003-3681-5968

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Animal Welfare Science Centre, Faculty of Veterinary and Agriculture

The University of Melbourne, Australia

Free-range chicken meat consumption has increased, partly driven by consumer perception that free-range housing is better for welfare. However, there have been few scientific investigations into the implications of ranging on broiler chicken welfare. Furthermore, how and why chickens access an outdoor range is largely unknown. Previous research has monitored broiler chicken ranging and welfare at the flock level. However, not all chickens access the range when the opportunity is provided. Subsequently, measures at the flock level may not be an accurate assessment of the implications of range use. With the advancement of technology, tracking individual chicken ranging behaviour is now possible. The research presented throughout this thesis was designed to obtain a greater understanding of the relationships between individual ranging behaviour of free-range broiler chickens on commercial farms and the relationships with welfare.

Chapters Three and Four provide descriptive analysis of the environmental factors associated with ranging behaviour. Tracking individual chicken ranging behaviour showed that the proportion of the flock that accessed the range was greater than previously estimated with alternate methodologies. Range use was season dependent with fewer chickens and range visits observed in winter flocks. Heterogeneous flock ranging behaviour was considerable, including chickens that only accessed the range once (8 to 12% of tracked chickens) and high frequency ranging chickens (3 to 9% of tracked chickens) that accounted for more than one third to a half of all range visits within the flock.

Chapters Five and Six investigated relationships between ranging behaviour, individual chicken characteristics and welfare. Few relationships were identified in winter flocks, which may be reflective of minimal range use. In summer flocks, lower weight, better gait scores, increased plumage cover and lower physiological stress responses prior to range access were predictive of subsequent ranging behaviour. These results suggest that individual characteristics and/or early life experience may be partially responsible for heterogeneous flock ranging behaviour. Furthermore, accessing the range was related to welfare in summer flocks after range access; including reduced fear responses and improved gait scores and cardiovascular function.

The study presented in Chapter Seven, investigated relationships between distance ranged from the shed and chicken welfare. Bi-directional relationships between ranging distance and body weight were observed. Frequently ranging further from the shed was associated with improved gait scores, less hock burn, and reduced acute physiological fear responses to confinement after range access was provided. Increased foot pad dermatitis was associated with increased range visits, but not ranging distance. These results suggest that ranging further from the shed had subsequent implications for welfare.

This thesis provides evidence that accessing an outdoor range has bidirectional relationships with chicken welfare. Due to the nature of the research presented in this thesis causation could not be identified. However, the research contributes to the limited knowledge of free-range broiler chicken welfare. As such, the broader understanding of ranging and welfare on commercial farms obtained through this thesis provides industry relevant, hypothesis generating evidence to aid optimal ranging behaviour on commercial farms that promotes good welfare. This is to certify that:

- (i) The thesis comprises only my original work towards the PhD,
- (ii) Due acknowledgement has been made in the text to all other material used,
- (iii) The thesis is less than 100, 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Peta Taylor

P. FS

December, 2017

This thesis has been written with a blended approach, incorporating both submitted and accepted publications and traditional thesis chapters. Chapter's Four to Eight each contain an abstract, introduction, materials and methods, results, discussion and conclusion. Chapters are stand-alone. Peta S Taylor is the first author on all chapters. Assistance given by others is indicated in the Acknowledgement of Contribution statement and in the Acknowledgements of each chapter.

The work presented in this thesis, to the best of my knowledge, is original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at any institution.

Peta S Taylor

P. FS

December, 2017

ACKNOWLEDGEMENT OF CONTRIBUTION TO THE RESEARCH WORK AND AUTHORSHIP

The core theme of this thesis is the behaviour and welfare of commercial freerange broiler chickens, and consists of three original research papers published in peer-reviewed journals, two original research project chapters and one review.

The majority of the work associated with constructing this thesis, including research ideas, development, and writing of all chapters in this thesis were the principal responsibility of the candidate, Ms Peta Taylor, working within the Animal Welfare Science Centre, Faculty of Veterinary and Agricultural Sciences at the University of Melbourne; under the supervision of Professor Jean-Loup Rault (primary supervisor) and Professor Paul Hemsworth (associate supervisor). However, aspects of this thesis required additional advice and support. The inclusion of co-authors reflect active collaboration, including the following contributions:

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The following terms have been used throughout the thesis and are defined at their first use in each chapter.

CR	Close Rangers; chickens that visited range areas close to the
	shed (< 2.7 m) more frequently than range areas further from
	the shed (> 2.7 m)
DR	Distant Rangers; chickens that visited range areas further from
	the shed (> 2.7 m) more frequently than range areas close to
	the shed (< 2.7 m)
FHN	Femoral Head Necrosis
FPD	Foot Pad Dermatitis
FREPA	Free Range Egg and Poultry Australia
GEE	Generalised Estimating Equation
GLIM	Generalised Linear Model
GLM	General Linear Model
GLMM	General Linear Mixed Model
GLIMM	Generalised Linear Mixed Model
HB	Hock Burn
NR	Non Ranging Chickens; chickens that did not access the range
OFT	Open Field Test
R	Ranging chickens; chickens that accessed the range
RFID	Radio Frequency Identification System
SEM	Standard Error of the Mean
TD	Tibial Dyschondroplasia
TI	Tonic immobility

CHAPTER 1 General introduction



Artificial selection of the domestic chicken has resulted in rapid growth rate and high carcass yields of broiler (meat) chickens, such that slaughter weights can be achieved between 35 and 45 days of age. Chicken meat has thus become an affordable source of animal protein; more than 6.2 billion broiler chickens were slaughtered for consumption in 2014 across the globe (FAO, 2017). In addition to genetic effects, various housing and management practices, including increased flock size and specialised diets, have been developed to produce greater quantities of inexpensive chicken meat. However, such intense pressure for rapid growth and increased proportion of muscle mass have resulted in various secondary effects which are detrimental to chicken welfare (Bessei, 2006).

Concern for the welfare of production animals has increased throughout recent history (Schröder & McEachern, 2004; Howell, Rohlf, Coleman, & Rault, 2016; Pettersson, Weeks, Wilson, & Nicol, 2016). Consequently, there has been development in legislation, quality assurance programs and alternate housing systems with the aim of improving the animals' well-being (Matthews & Hemsworth, 2012). With such developments, various marketing initiatives promote higher welfare products aiming to attract animal welfare-conscious consumers.

Free-range products are an appropriate example of such initiatives. Chickens in free-range productions systems are provided with access to an outdoor range during certain periods of their life. The general public typically perceives freerange systems as more natural and better for animal welfare (de Jonge & van Trijp, 2013). Indeed, the consumption of free-range chicken meat has increased in Australia and in various counties around the world (Magdelaine, Spiess, & Valceschini, 2008; Australian Chicken Meat Federation, 2013). However, there is little scientific evidence that access to an outdoor range environment has subsequent implications for chicken welfare. Theoretically, accessing an outdoor range could increase exploration, feed variation, expression of natural behaviours and increase activity levels and is therefore likely to improve chicken welfare. However, individuals are also exposed to diseases, thermoregulation challenges, predation and fear and stress-provoking stimuli otherwise absent in the indoor environment which may have detrimental consequences for welfare.

1.1 Australian free-range chicken meat industry

Perhaps as a result of the relatively small environmental impact, high nutritional value or low cost, chicken meat consumption within Australia has increased tenfold from 4.2 kg per person annually in 1963 to 42 kg for each person in 2011 (Australian Chicken Meat Federation, 2013). Keeping up with consumer demand relies largely on the rapid growth of broiler chickens and the relative intensive nature of modern commercial farms. Under typical Australian commercial conditions day-old mixed sexed chicks are sent to grower farms and housed in flocks of up to 60,000 birds until the majority of chickens are picked up for slaughter at between 45 to 55 days old, dependent on growth rate. To maintain regulated stocking densities, which are typically between 28 kg/m² and 40 kg/m² and to meet specific market requirements, approximately one third of each flock is removed for slaughter at approximately 35 days old referred to hereafter as "partial depopulation" (Australian Chicken Meat Federation, 2013).

Australian broiler chicken production systems currently include conventional (85%), free-range (14%) and organic (< 1%). Unlike laying hens, broilers are not kept in cages in Australia due to the subsequent damage to breast muscle and economic loss; rather chickens are kept on litter in large barns. Conventional housing systems grow broilers on litter, typically rice hulls, wood shavings or straw until slaughter (Australian Chicken Meat Federation, 2013).

Although 85% of chicken consumed in Australia is from conventional systems, the demand for free-range chicken meat in Australia is increasing and has a current market value of \$AUD840 million (Australian Chicken Meat Federation, 2013). The Poultry Code of Practice for the Welfare of Animals (PISC, 2002) dictates broilers in free-range production systems, in addition to indoor shelter, must have access to an outdoor range for a minimum of eight hours a day when chickens are fully feathered. Exceptions to this rule include when weather conditions are adverse, at the farmer's discretion, or in the event of a disease outbreak. Range areas must contain shaded areas and windbreaks. However, there are no restrictions or guidelines as to the specifics of such range resources (PISC, 2002). Free Range Egg and Poultry Australia (FREPA) is the most widely adopted free-range accreditation program in Australia and dictates stocking density must be no higher than 28 to 30 kg/m² depending on the type of shed ventilation and prohibits the use of growth-promoting antibiotics (Free Range Egg & Poultry Australia Ltd, 2015).

Australian free-range commercial farms house faster-growing broiler chickens, such as Cobb or Ross strains, in free-range systems, unlike some European free-range systems which use slower-growing strains due to legislation requiring a minimum slaughter age of 81 days (European Commission, 2008). The behavioural, physiological and morphological differences between broiler strains that differ in growth rate are well known (Bokkers & Koene, 2003a; Dal Bosco, Mugnai, Sirri, Zamparini, & Castellini, 2010; Mikulski, Celej, Jankowski, Majewska, & Mikulska, 2011). Slower-growing broiler strains are more active and more likely to access an outdoor range than faster-growing broiler strains (Bokkers & Koene, 2003a; Nielsen, Thomsen, Sorensen, & Young, 2003). These breed-differences may result in different implications on both range use and welfare of these two types of chickens,

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slower- and faster-growing. Of the limited scientific evidence on broiler chicken ranging behaviour and welfare, very few studies have investigated range use and potential welfare implications for faster-growing broiler strains (Nielsen et al., 2003; Jones et al., 2007; Durali, Groves, & Cowieson, 2012; Durali, Groves, Cowieson, & Singh, 2014).

While it is important to understand the behavioural, physiological and morphological differences between broiler strains, it is equally relevant to acknowledge the differences between broiler chickens and hens, particularly in the field of poultry welfare and free-range housing. Knowledge obtained from laying hen research is often thought of as synonymous with broiler chickens and often reviews of the free-range poultry literature do not distinguish the knowledge obtained from free-range laying hens and that from free-range broiler chickens. However, comparison studies between laying hens and broiler chickens clearly identify the differences in behaviour, physiology and morphology (Hocking, Maxwell, & Mitchell, 1993; Bokkers & Koene, 2003b; Lindqvist, Zimmerman, & Jensen, 2006Buzała, Janicki, & Czarnecki, 2015). These differences are not surprising when the intense selection pressures are taken into consideration, which has resulted in two very distinct production birds (Yamada, 1988).

The extreme morphological differences between broiler chickens and laying hens are shown in Figure 1-1. The purpose of presenting this image is twofold; it highlights the immense morphological differences between laying hens and broiler chickens, although behavioural and physiological differences are not presented. Additionally, the image acts as a visual reminder of the juvenile age of broiler chickens despite their morphology, which is of great importance during investigations of broiler chicken ethology and welfare. As such, this image is provided to display the potential issues when laying hen knowledge is applied to broiler chickens without consideration.



Figure 1-1 A broiler (left) and laying chicken (right) at 38 days old, illustrating morphological differences as a result of intense artificial selection. Although sex of the chicken is unknown, sexual dimorphism does not explain such gross morphological differences. Image source: Australian Chicken Meat Federation (2013).

Indeed, ranging behaviour of laying hens has shown to be much greater than broiler chickens (Gebhardt-Henrich, Toscano, & Fröhlich, 2014; Rodriguez-Aurrekoetxea & Estevez, 2016; Larsen et al., 2017). The contrast in ranging behaviour may reflect genetic differences (morphology, behaviour, physiology) or variations in age and length of ranging opportunities. For this reason, this thesis will focus discussions on broiler chickens, incorporating citations from broiler chicken research. Laying hen research has been cited where relevant broiler chicken literature was not available but only when clearly stated and with caution regarding the application to broiler chickens without further investigation.

Reports of range use in broiler chicken flocks are low and extremely variable. Limitations with estimation methodologies likely accounts for a portion of this variability. However, this variation also illustrates the complexity of factors that modulate ranging behaviour. Of the limited research available on free-range broiler chicken behaviour and welfare, the majority of research comes from Europe; a literature search found only two Australian studies. The climate, housing regulations and commercial broiler strains differ in Australia compared to Europe. Therefore, European research may not be directly applicable to Australian commercial conditions. Moreover, much of the research has been achieved at the flock level which typically involves comparisons between conventional and free-range housing. With recent advancement in technology it is now feasible to monitor individual chicken ranging behaviour, even on commercial farms (Gebhardt-Henrich, Fröhlich, et al., 2014; Gebhardt-Henrich, Toscano, et al., 2014). As such, a clearer understanding of broiler chicken ranging behaviour, including frequency and duration of range visits over time, is now possible and direct implications of range use can be identified.

1.2 Aims of research

The primary focus of this thesis was to obtain a greater understanding of the relationships between broiler chicken ranging behaviour and welfare on Australian commercial farms. The main aims of the research were:

- 1. Quantify patterns of flock and individual ranging behaviour of broiler chickens on Australian commercial farms;
- 2. Identify environmental factors and individual bird characteristics associated with broiler chicken range use;
- 3. Identify relationships between ranging behaviour and broiler chicken welfare.

With so little known about broiler chicken ranging behaviour and welfare, the research presented in this thesis is predominantly descriptive and exploratory. Relationships between ranging behaviour and welfare were investigated and a variety of factors hypothesised to be associated with ranging were considered. This approach permitted a broader understanding of on-farm ranging behaviour and welfare, but could not determine causation. As such, this thesis expands on the limited scientific knowledge of free-range broiler chicken welfare with an observational approach to generate industry relevant hypotheses.

1.3 Outline of thesis

Chapter Two provides a review of the relevant literature, including descriptions of broiler chicken ranging behaviour and modulating factors, scientific approaches to assess animal welfare and the potential implications of ranging behaviour on broiler chicken welfare.

Chapters Three and Four provide descriptions of flock ranging behaviour, intra-individual ranging behaviour and the relationships with environmental factors. These chapters have been published in *Animals*, an open access peer viewed scientific journal.

Chapters Five and Six identify relationships between ranging behaviour, individual chicken characteristics and welfare. Chapter Seven investigates relationships between welfare and ranging behaviour in relation to distance ranged from the shed. Chapter Five has been published in the peer-reviewed *Poultry Science* journal. Chapters Six and Seven are formatted as traditional thesis chapters.

Chapter Eight summarises the main research findings, the potential implications of the research findings and recommendations for future research.

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CHAPTER 2 The behaviour and welfare of free-range broiler chickens: A review



Increased societal concerns regarding animal welfare have persuaded some consumers to support products from free-range housing systems (Toma, McVittie, Hubbard, & Stott, 2011; de Jonge & van Trijp, 2013a) despite little scientific evidence that welfare is improved when access to an outdoor range is provided. Consumers highly value the ability for animals to engage in natural behaviour (Vanhonacker, Verbeke, Van Poucke, & Tuyttens, 2008) which may explain the perception free-range housing systems are better for welfare. However, there is little known about how or why broiler chickens access an outdoor range and even less known about the subsequent consequences for chicken welfare.

It is critical to understand the concept of animal welfare and how welfare can be assessed scientifically before considering the welfare implications of range use. Therefore, the concept and assessment of animal welfare will be reviewed here.

2.1 The concept of animal welfare

There is an increasing trend of public concern for animal welfare, particularly regarding animals which are used for food and fibre (Vanhonacker et al., 2012; de Jonge & van Trijp, 2013b; Howell, Rohlf, Coleman, & Rault, 2016). An agreement on community values and beliefs regarding animal welfare is required for the development of animal-related policies and legislation (Matthews & Hemsworth, 2012). In reality this community agreement is a difficult task as the term "animal welfare" is loaded with underlying beliefs and values influenced by geographies, culture, religion and economics (Phillips et al., 2012). As such, society has looked to science to provide an objective definition and assessment of animal welfare. The science is multidisciplinary, combining physiology, ethology, stress biology, pathology, epidemiology, psychology,

neurobiology and evolutionary theory. Although animal welfare science utilises various assessments to identify welfare risks it is not completely void of valuebased judgments and the scientific definition of welfare has been largely debated (Fraser, 1995; Fraser, Weary, Pajor, & Milligan, 1997; Duncan, 2005; Hemsworth & Coleman, 2011).

2.2 Scientific assessment of animal welfare

An objective definition of animal welfare through science aims to remove value-based decisions but in reality this is a difficult task. There is agreement that welfare is the state of the animal and not something which can be given to it (Broom, 1991). Furthermore, there is agreement that welfare should not be dichotomised (good or bad), rather welfare varies along a continuum from very poor to very good (Broom, 1991). However, the relative importance of an animal's health, feelings or natural living is debated. Broom (1991) defines animal welfare as the state of an animal as it attempts to cope with its environment, an approach including scientific concepts of stress physiology and evolutionary history. But many authors place greater emphasis on an animal's emotions, commonly referred to as "affective state", such as the prevention of suffering and promotion of pleasure. An alternative approach suggests good welfare is beyond the absence of suffering, citing the provision of a relatively natural environment and ability to express all natural behaviour as being best for animal welfare.

Historically, these frameworks have been seen as competing but it is clear such approaches are not mutually exclusive. Modern scientific assessment of animal welfare is achieved with one or a combination of these approaches: a) natural living; b) affective state and; c) biological functioning. These approaches will be discussed in detail here, having also been extensively reviewed elsewhere

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(Fraser, 2008b; Mellor, Patterson-Kane, & Stafford, 2009; Fraser & Nicol, 2011; Hemsworth & Coleman, 2011; Hemsworth, Mellor, Cronin, & Tilbrook, 2015).

2.2.1 Natural living

The natural living approach to welfare suggests animals must be provided with a relatively natural environment and must be able to perform a full repertoire of natural behaviours (Fraser, 2008b; Mellor et al., 2009; Hemsworth et al., 2015; Mellor, 2015c). This proposition suggests the prevention of expressing natural behaviours equates to suffering and compromised welfare. Various philosophers have effectively communicated this approach to the public via popular novels including *Animal Machines* by Ruth Harrison in 1964 and *Animal Liberation* by Peter Singer in 1975. Perhaps as a result, the public often place greater weight on the natural living approach to animal welfare than other stakeholders (Vanhonacker et al., 2008). This is a particularly prominent concept relating to free-range broiler chicken welfare, as consumer support for free-range products is partially driven by the belief that the outdoor range is more natural and thus better for welfare (Fraser, 2006; de Jonge & van Trijp, 2013a; Howell et al., 2016).

Although scientists acknowledge the respect for nature approach to animal welfare is intuitively appealing (Dawkins, 1980), the scientific application of this approach encounters a range of difficulties which are still yet to be addressed (Fraser, 2008b; Hemsworth et al., 2015). One such problem is the definition of "natural", particularly in relation to domesticated species. The broiler chicken is an apt example. Domestication of modern day fowl began approximately 8000 years ago from the Asian junglefowl (*Gallus*) which inhabited dense jungle canopy (Yamada, 1988). More than 8000 years of selection for cockfighting and 50 years of intense selection for egg or meat production characteristics in indoor

systems, has produced a domesticated fowl which has adapted to the captive environment, both at a species and individual level, evident by quantitative changes in behaviour and physiology (Price, 2002). Although domestication has resulted in quantitative behavioural changes in the domestic chicken, Appleby, Mench, and Hughes (2004) suggest no qualitative behavioural changes have occurred, such that all behaviours are apparent to some extent and no new behaviours have arisen. Nonetheless, the "natural environment" of the domestic chicken is an ambiguous term.

Furthermore, one encounters another problem with this approach when reflecting on the challenges associated with "natural" living. Many of the natural behaviours of wild species are responses to deal with predation, scarce food supplies and conserving territories (Price, 2002). It is difficult to fathom that ensuring the expression of such natural behaviours is an effective method to safeguard welfare. Therefore an uncritical approach to providing a "natural" environment and the ability to express all "natural" behaviours can actually result in poor welfare rather than improvements (Mellor, 2015c). There has, however, been rapid progress in the critical assessment of behavioural needs and as such the natural living approach to animal welfare increasingly carries greater weight (Mellor, 2015c).

The concept of behavioural needs acknowledges the suffering which may arise if an animal is restricted from engaging in specific behaviours animals are strongly motivated to perform and equally importantly the positive experiences received when an animal is permitted to express a rewarding behaviour (Mellor, 2015a). The expression of some behaviours are likely related to emotional and motivational states of the animal (section 2.2.3) and thus reflect behaviour critical for survival during the animal's evolutionary past (Mellor, 2015a). For example, predatory behavioural responses are likely related to a negative affective state such as fear and exploratory behaviour is likely associated with positive affective states such as curiosity (Mellor, 2015a). As such, understanding the affective state associated with particular behaviours and the biological consequences of expressing such behaviours may better reflect consequences for welfare than simply the expression of natural behaviours.

The behavioural needs approach to natural living suggests the ability to express important behaviours may require environments which mimic the natural environment, such as stimulus-rich, complex environments which permit the expression of important behaviours such as exploration and play, rather than highly predictable barren environments. However, this may be achieved by artificial means (e.g. environmental enrichment) which permits or encourages the expression of important behaviours rather than explicitly prescribing a "natural" environment per se.

2.2.2 Biological functioning

Assessment of animal welfare with the biological functioning approach has a focus on animal adaption to the environment, suggesting that if adaption is difficult or inadequate then welfare is compromised. Two questions arise from this approach:

i) how much does the animal have to do to try to cope with its environment; and ii) are the attempts to cope successful?

As such, the magnitude of biological responses to a stressor can be assessed and the costs of the attempts to cope quantified. The biological responses in attempts to cope aim to return an animal to a state of homeostasis (a relatively stable internal condition) and include behavioural, physiological and immunological responses.

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Behavioural responses are rapid, energetically economic and are often sufficient to deal with a challenge, for example huddling with conspecifics in response to a cold stressor. Behavioural responses can include the expression of abnormal behaviours such as stereotypies and redirected behaviours (Barnett & Hemsworth, 1990; Mason, 1991). Stereotypies are fixed repetitive behavioural patterns which appear to have no function and are often used as an indicator of an animal's attempt to cope. There is ongoing discussion and research on the welfare significance of stereotypies. However, stereotypies are also viewed as either an adaptive coping response of captive animals in the captive environment or as the inappropriate output in a conflict or thwarting situation (Mason & Latham, 2004).

An animal's major coping mechanism is the physiological response; a twostage process involving activation of the autonomic nervous system and the neuroendocrine system; together they are commonly referred to as the "stress response".

The stress response

The autonomic nervous system is made up of two branches; the parasympathetic branch, which controls "rest and digest" functions and the sympathetic branch which manages "fight or flight" tasks. As an animal perceives a stressor the hypothalamus sends a message via the sympathetic branch of the autonomic nervous system along the sympathetic-adrenalmedullary (SAM) axis to prepare the body for action (Figure 2-1). Catecholamines are secreted from the adrenal medulla; adrenaline and noradrenaline, also known as epinephrine and norepinephrine. The major function of adrenaline and noradrenaline is to liberate energy stores from the liver and skeletal muscle (glycogenolysis) and adipose tissue (lipolysis) and to suppress digestive functions and stimulate cardiac output and respiratory function. As such, the animal diverts energy from biological functions which do not help in an emergency and liberates and delivers oxygen and energy to help behavioural and metabolic processes required for the "fight or flight" response. This response occurs within seconds or minutes.

If the SAM response is unable to deal with the stressor, the second physiological process of the stress responses begins. This can last from minutes to hours and involves the hypothalamic-pituitary-adrenal (HPA) axis (Figure 2-1). The hypothalamus responds to the stressor by releasing the corticotrophinreleasing hormone (CRH), signalling to the anterior pituitary gland to secrete adrenocorticotrophic hormone (ACTH), which travels in the blood to the adrenal cortex where corticosteroids are released. The major corticosteroid released in chickens, along with rodents, reptiles and amphibians, is corticosterone, unlike most mammals (including humans) where it is cortisol. The major function of corticosterone (and cortisol) is to mobilise energy to increase metabolic performance and reduce energy costs of biological functions not required to deal with the stressor, such as digestion and growth and immune and reproduction functions.

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Figure 2-1 A diagrammatic representation of the stress response, including Sympathetic-adrenalmedullary (SAM) and Hypothalamic-pituitary-adrenal (HPA) axes. Adapted from Pearson, 2013.

The physiological stress response, including both SAM and HPA pathways, liberates energy stores and increases cognition, vigilance and arousal. Ideally these functions permit an animal to deal with a stressor and regain a homeostatic state. Thus it is clear that measuring the magnitude of these responses will provide an indication of how the animal is attempting to cope with a stressor. For example, quantifying the amount of corticosterone in the blood relative to basal concentrations after a potential stressor has been introduced will determine the magnitude of the stress response and attempts to cope (Hemsworth & Coleman, 2011).

However, if the stressor is persistent the acute stress response advances to a chronic stress response and this can be very detrimental. Continual secretion of corticosterone is accompanied by liberation of energy and catabolism of muscle and fat stores and corticosteroid-dependent reductions in metabolic efficiency, reproduction and immunity (Broom & Johnson, 1993; Moberg, 2000). The cost will depend on the length of exposure to the stressor, but may result in a prepathological state or ultimately a pathological state and in extreme cases can cause death (Broom & Johnson, 1993).

Evolutionary history highlights the importance of growth, health and reproductive function for animals to pass on their genetic code, i.e. these are critical fitness traits for survival and evolution. Although evolution occurs at the species level, the importance of fitness as an indication of individual adaption has been a critical aspect of the biological function approach to measuring animal welfare (Broom, 1991).

There are constraints within the biological functioning approach to assessing animal welfare which must be considered. The acute stress response should only be used to determine short-term consequences such as comparisons of management practices, as acute stress response in isolation cannot determine the long-term consequences for welfare. Furthermore, evidence suggests exposure to short term stressors during an animal's life can have positive long-term implications, questioning the belief that all stress is bad (Zulkifli & Siegel, 1995). Moreover, it must be noted that the acute stress response occurs in response to positive stimuli in addition to adverse stimuli. For example, plasma corticosteroid concentrations are increased in response to mating in horses (Colborn, Thompson, Roth, Capehart, & White, 1991) and voluntary exercise in humans (Sutton & Casey, 1975) to mobilise energy to support mating and

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exercise. Care must therefore be taken when acute plasma corticosterone responses are interpreted in relation to welfare.

2.2.3 Affective state

Critics of the biological functioning approach to animal welfare have historically argued that welfare is more than just coping within an environment, suggesting how an animal feels contributes or absolutely defines welfare (Desire, Boissy, & Veissier, 2002; Boissy et al., 2007). But the dynamic interactions between emotions (affective states) and biological processes are now widely recognised, particularly the association between emotion and the activation of the SAM and HPA axis (Hemsworth et al., 2015).

Most ethologists agree that animals possess a limited number of basic emotions including fear, pain, frustration, comfort and pleasure and these emotions reflect its needs or wants (Fraser & Duncan, 1998). Fraser and Duncan (1998) hypothesise that affective states are critical evolutionary adaptations which motivate particular behaviours, proposing that negative affective states evolved to protect animals and positive affective states evolved opportunistically when cost is low and provide long-term benefits. A major limitation to this approach is that direct assessments of affective states are not possible. Indirect measures have been used to infer affective state, including pain assessments via self-administered analgesics (Colpaert et al., 2001), cognitive bias (Mendl, Burman, Parker, & Paul, 2009), fear responses (Forkman, Boissy, Meunier-Salaün, Canali, & Jones, 2007) and the expression of reward-motivating behaviours (positive affective experiences) such as play and exploration supported by neuroscience-based evidence (Mellor, 2015a).

If emotional states have evolved to motivate behaviour, behavioural assessments of animal motivation to obtain or avoid specific stimuli should

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provide insight into an animal's affective state. As such, quantifying an animal's choice when presented with alternate resources or environments may indicate its preferences, and therefore may infer affective states. Furthermore, a "cost" can be placed on the preference, for example pushing a door with increasing weight or jumping a barrier with increasing height known as obstruction testing (Olsson & Keeling, 2002), or using operant conditioning training such that an increasing number of pecks are required to access a resource (Lagadic & Faure, 1987).

The "price" an animal is willing to pay is thought to reflect the strength of motivation to gain access or avoid a stimulus. For example, Buijs, Keeling, and Tuyttens (2011) showed that broiler chickens preferred lower stocking density environments and quantified the "cost" broilers were willing to pay to gain access to environments of lower stocking density. Before testing motivation for space allowance, Buijs et al. (2011) utilised a feed test to determine two barrier heights; a "low" barrier which chickens would cross to access feed when they had not been feed-deprived and a "high" one most chickens (>75%) would cross to access feed but only after feed deprivation. These barrier heights were then used to determine motivation to access areas with reduced stocking density, from 40 kg/m² to either 25 or 33 kg/m². Chickens could indicate their spatial preference by moving to a compartment with varying stocking density but were required to cross either the low or high barrier to gain access. More chickens accessed the compartment with lower stocking densities even when gaining access required crossing the high barrier which had previously deterred 25% of feed-restricted chickens to access feed.

From these results, the authors suggested a relatively high motivation for broiler chickens to access areas of increased space allowance. Additionally, the motivation to access areas of lower stocking density increased with age, highlighting the temporal aspects of motivational states. Yet there are methodology issues with this approach which must be taken into account. For example, animals often make choices based on proximal requirements without consideration of long-term consequences; motivations may change over time (as highlighted in the aforementioned Buijs et al. (2011) study, preferences are often biased by familiarity while preference tests may be outside the animal's cognitive capacity and vigilance behaviour may be misinterpreted as a choice (Dawkins, 1977; Hemsworth et al., 2015).

It is clear from this discussion that the concept of animal welfare is complex with no strict dichotomy of good and bad welfare, rather a continuum. The effectors of animal welfare are dynamic and vary in character and intensity and often opposing - as such, trade-offs are often made. By assessing animal welfare through the aforementioned approaches, science is able to address various societal beliefs and welfare concerns. Historically, the scientific study of animal welfare in farm animals has predominantly utilised the biological functioning approach (Hemsworth et al., 2015). A more recent approach has used the biological functioning approach to infer negative affective states such as fear and pain (Green & Mellor, 2011; Mellor, 2015a, 2015b) and as such the previously identified frameworks used to assess animal welfare are no longer seen as competing, rather the dynamic interactions between affective states, biological functioning and behavioural needs is now recognised as fundamental to assessing and improving animal welfare (Hemsworth et al., 2015). The scientific assessment and understanding of animal welfare will continue to evolve as knowledge of animal biology and psychology progress.

Regardless of the approach to assess welfare, it is recommended assessments of animal welfare be achieved with direct measures. Monitoring animal welfare with resource-based measures (RBM) is an indirect assessment, requiring an assumption that the provision of resources will positively affect welfare (Appleby, Hughes, Mench, & Olson, 2011). Animal Based Measures (ABM) are preferred to RBM as the totality of the organism and environment are taken into account including genetics, age, experience and temperament (Fraser, 2008a).

A successful ABM must be valid and reliable. That is, it must accurately reflect animal welfare and be repeatable between assessors and a variety of environments. Although a single measure can indicate poor welfare, a combination of a wide range of indicators is required to provide a more comprehensive and accurate assessment of welfare.

2.3 Broiler chicken ranging behaviour

The term ranging behaviour may be used to describe patterns of the use of space in any designated area. However, throughout this thesis the term 'ranging behaviour' will be utilised to describe the use of an outdoor range, specifically related to range visits, duration of range visits, latency to access the range and in some chapters use of the range area at specified distances from the home shed.

2.3.1 Flock estimates of range use

Estimates of the proportion of broiler flocks that access the outdoor range when provided with the opportunity are inconsistent and highly variable; previous report estimate between 3% and 95% of flocks access the outdoor range (Table 2-1). This variation is reflective of the complexity of factors which modulate broiler chicken ranging behaviour (discussed in section 2.4) but also difficulty in obtaining accurate flock estimates.

Max. (%)	Mean (%)	Strain	Sex	Flock size	Housing	Method of estimation	Age of observation (d)	Location	Season	Reference
N/A N/A	59 45	i657 ⁺ LAB x ⁺	Mixed	102	Transparent tent	Scan sampling	42 to 84	EU	S, A	Christensen, Nielsen, Young, and Noddegaard (2003)
14.3	3.5	Sherwood white ⁺	F	20 000	Commercial sheds	Scan sampling	45 to 53	EU	S,A, W,Sp	Dawkins, Cook, Whittingham, Mansell, and Harper (2003)
51.4	11	Ross 308	F	670	Mobile ark	Scan sampling	38, 45 & 52	EU	S, W	Jones et al. (2007)
75	N/A	Label Rouge ⁺	Mixed	720	Research poultry shed	RFID	35 to 84	EU	W	Chapuis et al. (2011)
70.4 78.7	39.9 68.4	Kosmos 8 Red* White Bresse L40 ⁺	Mixed	25	Research poultry shed	Scan sampling	80 to 108	EU	S, A	Almeida, Hinrichsen, Horsted, Thamsborg, and Hermansen (2012)
95	N/A	Cobb 500	Mixed	270	Commercial shed	RFID	21 to 40	AU	А	Durali, Groves, Cowieson, and Singh (2014)
N/A	36.9	Sasso T44 ⁺	Mixed	1300	Commercial sheds	Focal sampling	42 to 84	EU	Sp, S, A	Rodriguez-Aurrekoetxea, Leone, and Estevez (2014)
100	12.9	Delaware ⁺	Mixed	17	Research poultry shed	Scan sampling	49 & 70	US	Sp	Fanatico et al. (2016)
N/A	35.1 42.8#	Sasso T451 ⁺	Mixed	50	Mobile chicken sheds	Scan sampling	29 to 65	EU	Sp, S, A	Stadig, Rodenburg, Ampe, Reubens, and Tuyttens (2016)

Table 2-1 Summary of estimates of the maximum and mean proportion of broiler chicken flocks that access the outdoor range. Estimates from peerreviewed publications are included in chronological order and highlight similarities and differences of factors that likely affect range use.

N/A flock estimate not reported

+ slower-growing strain than the Ross 308 broiler chicken

* medium-growing strain; slower than Ross 308 but faster than traditional slower-growing strains

F: Female

RFID: Radio Frequency Identification

EU: Europe, US: United States of America, AU: Australia

S: summer; A: autumn; W: winter; Sp: spring

range enrichment provided

Counting the number of chickens on the range at specific intervals over a course of hours, days or weeks, has historically been utilised to determine the level of range use. This method is known as "scan sampling" and is an effective ethological tool (Altmann, 1974), yet is reliant on frequent and short scan intervals (Estevez & Chrisman, 2006). Scan sampling is likely to underestimate flock range use as it is probable not all chickens will access the range at the same time and may rotate between the indoor and range environments multiple times per day. Scan sampling studies have provided critical information regarding broiler chicken ranging behaviour when focusing on relative differences between treatments, for example the effect of range enrichment (Fanatico et al., 2016; Stadig et al., 2016), broiler strain (Nielsen, Thomsen, Sorensen, & Young, 2003) and environmental conditions (Dawkins et al., 2003).

The development of new technologies does provide the opportunity to monitor the ranging behaviour of individual chickens and provide more accurate estimates. Radio Frequency Identification (RFID) technology has been validated and used to track individual ranging behaviour of laying hens on commercial farms (Gebhardt-Henrich, Fröhlich, et al., 2014; Gebhardt-Henrich, Toscano, & Fröhlich, 2014). Durali et al. (2014) were one of the first to track broiler chicken ranging behaviour using RFID technology. By monitoring individual ranging behaviour of faster-growing Cobb 500 broiler chickens, Durali et al. (2014) reported that 95% of the tracked chickens accessed the outdoor range; much higher than previously reported scan sampling estimates of faster-growing broiler chicken ranging studies in Australian commercial conditions; 78% of research that has reported flock estimates of range use was conducted in Europe (Table 2-1).

Although environmental conditions would account for some of the variation in flock ranging behaviour, the Durali et al. (2014) study highlighted potential temporal and spatial methodology constraints with the scan sampling method, such as time availability of observers, visual obstruction and the inability to monitor individual rotation between ranging and shed environments throughout the day and over time. Further support was provided by a research trial in France, which used RFID technology to track the range use of slower-growing Label-Rouge chickens. Although technical difficulties were encountered during the trial, Chapuis et al. (2011) estimated 75% of the flock typically accessed the range each day.

There has also been some work investigating the suitability of global positioning systems (GPS) for tracking individual ranging behaviour (Dal Bosco, Mugnai, Sirri, Zamparini, & Castellini, 2010). However, only a few focal chickens within a flock have been tracked over a short period of time. GPS technology has an advantage in that it can track the location of broiler chickens on the range in addition to the frequency and duration of range visits. But current GPS tracking is limited regarding battery life (3 to 5 days), unit weight (< 5% body weight) and cost. Such limitations currently prevent greater application of these units (Dal Bosco et al., 2010; Siegford et al., 2016). With the rapid development of technology and availability, GPS technology may have greater application in the future.

2.3.2 Individual variation in ranging behaviour within broiler chicken flocks

Various genetic and environmental factors have been shown to be associated with broiler chicken ranging behaviour (section 2.4). It is therefore possible genetic differences and variation in early life experiences between individuals within a flock may result in heterogeneous flock ranging behaviour. Heterogeneous ranging behaviour is evident in free-range laying hen flocks (Campbell, Hinch, Downing, & Lee, 2016; Gebhardt-Henrich, Toscano, et al., 2014; Hartcher et al., 2016; Larsen et al., 2017) as some hens spend more time on the range than others. There has, however, been little investigation of the variation in ranging behaviour within free-range broiler chicken flocks. Durali et al. (2014) are the only authors to date reporting variation in ranging behaviour within commercial broiler chicken flocks, providing evidence that ranging behaviour is variable within flocks and some broiler chickens appear more motivated to access the range and spend more time on the range than others.

2.3.3 *Time spent on the range*

Dal Bosco et al. (2010) monitored the kinetic activity and the amount of range use of 40 focal male broiler chickens from two broiler strains; faster-growing Ross chickens and slower-growing Ancona cross chickens. The amount of time chickens spent on the range was only quantified between 73 to 80 days of age after more than seven weeks of ranging opportunities. Results showed fastergrowing broilers spent an average of 26% of the available time on the range, which was much lower than slower-growing strains which spent 75% of the available time on the range. Faster-growing strains in Australian commercial conditions are not grown to this age and reports of inactivity of faster-growing broiler chickens after five weeks of age are well-known (Bokkers & Koene, 2003). The proportion of time spent on the range may therefore have been greater prior to this age but was not assessed.

Durali et al. (2014) provided the first description of the time broiler chickens spend on the range within a commercial broiler chicken flock. Durali et al. (2014) monitored individual ranging behaviour on 11 days of range access between 21 and 40 days old, although the length of daily range exposure was not reported. Of the 257 tracked chickens, 39% accessed the range for more than 8.7 hours in total, with a maximum duration of 25 hours in total, 36% accessed the range between 1.1 and 8.6 hours in total and 25% accessed the range for less than 1.1 hours in total or not at all. This study provided additional evidence of heterogeneous ranging behaviour within commercial broiler chicken flocks.

2.3.4 Ranging distance

Broiler chickens do not range uniformly and prefer to range close to their home shed (Gordon, 2002; Dawkins et al., 2003; Rivera-Ferre, Lantinga, & Kwakkel, 2007; Dal Bosco et al., 2010; Rodriguez-Aurrekoetxea, Leone, & Estevez, 2014; Fanatico et al., 2016). Chickens will range further from the shed if range resources are provided (Mirabito, Joly, & Lubac, 2001; Gordon, 2002; Dawkins et al., 2003; Fanatico et al., 2016; Stadig et al., 2016). The lack of ranging behaviour in open bare range areas is not surprising in light of the broiler chicken's ancestral habitat; dense vegetative areas with three levels of cover to aid rapid retreat (Collias & Collias, 1967). Rivera-Ferre et al. (2007), suggesting broiler chickens have a critical ranging distance from the shed of 20m which would not be surpassed regardless of resources provided. More recent evidence from Dal Bosco et al. (2010), however, showed slower-growing strains accessed range areas up to 100m from the shed and travelled a distance of 1230m each day (indoor and range activity), much greater than faster-growing strains which ranged a maximum of 25 m and travelled only 125 m each day.

The variation in reports of broiler chicken range use are likely reflective of variations in methodology used to estimate flock use (section 2.3.1). However, this variation also likely reflects the differences in experimental conditions, including broiler strain and housing conditions (Table 2-1), all of which have been shown to influence ranging behaviour.

2.4 Factors which regulate flock range use

What motivates or deters broiler chicken ranging behaviour is relatively unknown and reasons may differ between individuals. There are likely to be overriding circumstances, however, which will affect the majority of broiler chickens' ranging behaviour, such as genetics, strain, age, climate, time of day and the provision of resource on the range.

2.4.1 Broiler strains

European legislation dictates broiler chickens used in free-range production systems must have a maximum growth rate of 45g each day and a minimum slaughter age of 81 days (European Commission No. 543/2008, 2008). Although this can be achieved with fast-growing strains by restricting feed, the majority of European free-range production systems house slower-growing strains of broiler chickens. This legislation does not apply globally and Australian and American free-range production systems typically house the faster-growing broiler strains, such as Ross and Cobb strains. It has been suggested faster-growing strains of broiler chickens are not suitable for use in free-range housing systems (Nielsen et al., 2003; Castellini, Berri, Le Bihan-Duval, & Martino, 2008). Nielsen et al. (2003) observed two strains of broiler chickens in a free-range system; a fastergrowing Ross chicken strain and a slower-growing LAB strain and compared ranging behaviour, health and production characteristics. More LAB chickens were observed on the range and ranged further from the shed than Ross chickens. These observations were associated with higher (i.e. worse) gait scores in Ross chickens and the authors suggested poor leg health contributed to the reduction in ranging behaviour.

But Bokkers and Koene (2003) reported behavioural differences between fastand slow-growing strains within indoor environments; slow-growing strains typically spend more time walking and scratching while fast-growing strains spend more time eating, drinking and sitting. This suggests the latter strains may be less motivated to express active behaviours and thus may be less inclined to explore the range. Castellini, Bosco, Mugnai, and Bernardini (2002) also provided evidence that fewer faster-growing broiler chickens within a flock access the range compared to slower-growing chickens. Faster-growing broiler chickens were also more fearful than slower-growing broiler chickens, which may be related to willingness to access the range. Evidence provided here suggests differences in ranging behaviour between various broiler chicken strains may be multifactorial but the exact mechanism responsible for the reduction in range use is unknown.

The majority of investigations into free-range broiler chicken behaviour and welfare come from Europe and are therefore largely reflective of slower-growing broiler chickens. There is a clear need for research into Australian commercial conditions, including a better understanding of the ranging behaviour, factors which influence ranging behaviour and welfare implications for faster-growing broiler chickens.

2.4.2 Age

Age is likely to have a major role in broiler chicken ranging behaviour, although age is often confounded by the number of ranging opportunities in the literature. The majority of evidence suggests more chickens access an outdoor range as age and ranging opportunities increase; Nielsen et al. (2003) showed an increase range use from 11% to 24% of the flock from seven to ten weeks of age; Christensen et al. (2003) showed an incremental increase in flock range use of 4.6% with each week of range access between 80 to 108 days of age; Stadig et al. (2016) reported an incremental increase in flock range use of 0.3% each day.

Weeks, Nicol, Sherwin, and Kestin (1994) showed variation in range use between each week but no consistent trend with age. Jones et al. (2007) and Fanatico et al. (2016) provided evidence that flock range use decreased with age but was related to time of day; such that the range use is reduced in the afternoon with increasing age, indicating a complex array of factors regulate range use in relation to age.

The increase in flock use of the range area is interesting in the context of the reduction in activity typically shown by aging broiler chickens (Bokkers & Koene, 2003). These results suggest broiler chickens are likely to still access the range although active behaviours on the range reduce with age (Christensen et al., 2003). This may imply there are additional motivations to access the range, in addition to active behaviours which are commonly reported in range areas (Weeks et al., 1994; Jones et al., 2007; Knierim, 2000; Zhao, Li, Li, & Bao, 2014; Fanatico et al., 2016). One might expect the duration of range visits would increase with age and the frequency of range visits reduce due to age-related inactivity. However, ranging behaviour at the level of the individual broiler chicken in relation to age is unknown.

Of note, the aforementioned studies provide descriptions of ranging behaviour by broiler chickens greater than seven weeks of age, but this is older than the typical slaughter age for faster-growing strains. As such, the effect of age/range exposure is relatively unknown in relation to typical age of ranging on Australian commercial farms.

2.4.3 Environmental factors

Season and weather

Weather conditions have been shown to impact broiler ranging behaviour. Gordon (2002) showed broiler chickens are more likely to utilise the range on warm days with no wind or rain. In addition, Dawkins et al. (2003) suggested that direct sun deters range use by broiler chickens. Stadig et al. (2016) showed a linear increase in the number of chickens accessing the range with increasing temperature up to 28°C. The inhibiting ranging effects of rain, increased radiation and wind speed are less pronounced, however, when natural resources are provided on the range (Stadig et al., 2016).

Dal Bosco et al. (2014) indicated the kinetic activity of broiler chickens is greater in summer, relative to winter, which may be linked to reports of greater use of the range in summer relative to winter (Dawkins et al., 2003; Jones et al., 2007). It should be noted that these studies have been conducted in Europe; it is unclear how ranging behaviour is affected by Australian climate conditions, with milder dry winters and greater temperature extremes in summer. These results also highlight the importance of observing broiler chicken range use and behaviour across various seasons.

Time of day

The diurnal pattern of range use is well-documented in the literature, showing peaks of range use in the morning and again in the evening (Christensen et al., 2003; Nielsen et al., 2003; Almeida et al., 2012; Fanatico et al., 2016;. The strength of this pattern varies between broiler strains (Christensen et al., 2003; Almeida et al., 2012;) and age; the evening peak in range use is less pronounced in older broiler chickens (Fanatico et al., 2016). It is likely the diurnal peak in range use is related to diurnal peaks in foraging behaviour observed in broiler chickens (Alvino, Archer, & Mench, 2009), which mimics the diurnal foraging pattern of the domestic chicken's ancestor, the red junglefowl (*Gallus gallus*) (Collias & Collias, 1967). Indeed, the activity levels of free-range broiler chicken

flocks coincide with peaks of ranging activity (Almeida et al., 2012), suggesting that motivation to access the range is partially related to foraging behaviour.

Provision of range resources

The provision of range resources has been shown to affect the proportion of chickens accessing the range within a flock and ranging behaviour (Dawkins et al., 2003; Rivera-Ferre et al., 2007; Dal Bosco et al., 2014; Fanatico et al., 2016; Stadig et al., 2016). Broiler chickens evolved from Asian junglefowl which inhabit dense vegetative areas with three levels of coverage including an understory for brood-rearing cover; it is this cover which is quickly retreated to if junglefowl are disturbed (Collias & Collias, 1967). Although many years of artificial selection in a captive environment challenges the concept of broiler chickens' "natural habitat", the reported effect of range enrichment suggests a preference for this habitat has been conserved. The effectiveness of range enrichment may be related to fear and perceived risk of predation. Indeed, Dal Bosco et al. (2014) showed predation was reduced when the range contained natural forms of enrichment. Fanatico et al. (2016), however, showed temperatures under range structures are much lower than in bare areas of the range and more comparable to the indoor environment. The success of range enrichment in encouraging range use may be related to reduced temperatures in areas on the range, particularly during the heat of the afternoon when range use is typically reduced and when broiler chickens are older and heat stress is more prevalent (Lin, Jiao, Buyse, & Decuypere, 2006).

The provision of range enrichment may increase the length of time broiler chickens spend on the range (Rivera-Ferre et al., 2007; Dal Bosco et al., 2014) and this may reflect the increased resting behaviour on the range when resources are provided (Lubac & Mirabito, 2001). Without monitoring individual range use it

is difficult to determine the effect of range enrichment on the duration of range visits and total time spent on the range.

Young trees have been shown to have no effect on ranging behaviour but older more established trees will increase the proportion of flock on the range (Mirabito et al., 2001; Jones et al., 2007; Dal Bosco et al., 2014). Therefore the characteristic of the resource provided is clearly important. Vertical panels have shown to increase the ranging distance of broiler chickens, highlighting that cover is not the only characteristic which encourages range use (Cornetto & Estevez, 2001). Broiler chickens appear to prefer particular range resources; wigwams are more attractive than windbreaks or straw bales (Gordon & Forbes, 2002), short rotation coppices are more attractive than artificial A-frame structures (Stadig et al., 2016) and olive trees are more attractive than sorghum (Dal Bosco et al., 2014). Yet the fundamental characteristics of range resources which broiler chickens prefer remain largely unknown.

Various authors suggest the provision of range enrichment is associated with improvements to chicken welfare, in particular improved leg health (Dal Bosco et al., 2014; Stadig et al., 2016). This may be related to an increase in ranging distance when resources are provided (Dal Bosco et al., 2014; Stadig et al., 2016), but the mechanisms are difficult to accurately quantify at the flock level.

2.4.4 Individual characteristics

Motivation to access the range or physical restrictions to do so may differ between individuals, such that ranging behaviour may require, or may be enhanced, by optimal health or specific temperament traits. Leg health and temperament traits such as exploration-avoidance, shyness-boldness and activity may be of particular importance. But the relationships between individual characteristics and ranging behaviour remains relatively unknown. This is largely due to the difficulty of tracking individual ranging behaviour of chickens (Siegford et al., 2016) but is also reflective of the lack of assessment prior to range access. Durali et al. (2014) tracked individual ranging behaviour and measured body weight prior to after range access was provided. By assessing weight prior to and after range access, growth rates in relation to range use could be assessed. Yet other health indicators were only assessed after range access and as such the role of good leg health prior to range access on subsequent ranging could not be assessed. Assessing individual characteristics prior to and after providing range access may not only provide insight into specific chicken characteristics which might promote range use but might also clarify the effects of range use by monitoring changes over time.

Understanding the factors modulating range use is important for managing commercial broiler chicken flocks. A greater understanding of these relationships is required to provide optimal ranging opportunities for broiler chickens on commercial farms.

While there have been some comparisons between chickens housed conventionally (indoors without range access) and in free-range systems, there has been very little investigation into the welfare implications directly related to range use.

2.5 Free-range broiler chicken welfare

A comprehensive assessment of free-range broiler chicken welfare should be approached by a combination of the natural behaviours, affective state and biological functioning approaches. Nonetheless, the assessment of free-range broiler chicken welfare may be slightly skewed towards a focus on health characteristics due to the concerns for broiler chicken health and welfare in relation to rapid growth rate (Bessei, 2006).

2.5.1 Behaviour

Free-range housing systems theoretically allow chickens to choose where to spend their time - indoor or on the range - and therefore offer the freedom to exhibit their preference. Monitoring chicken ranging behaviour may provide insight into individual preferences but an animal's preference is greatly affected by previous exposure to an environment so the familiar choice is positively biased (Dawkins, 1976). As range access in Australia is typically not provided until 21 days of age a comparison between indoor and outdoor may not reflect the true preference of the chickens.

Despite the limitations of interpreting the environment preference of freerange broiler chickens, observations of behaviour on the range relative to inside the shed may provide some insight into some of the implications of range use on broiler chicken welfare. Indeed, some behaviours are observed more frequently on the range relative to the shed environment.

The outdoor range appears to promote exploratory behaviour; numerous studies have shown that foraging and pecking behaviours are performed more frequently on outdoor range areas compared to indoor environments (Weeks et al., 1994; Jones et al., 2007; Knierim, 2000; Zhao et al., 2014; Fanatico et al., 2016). Exploration is regulated by both internal and external factors and has been considered a positive affective state (Mellor, 2015b; Mellor, 2016). If it is accepted that exploration is rewarding, then increased foraging in the range environment may be indicative of positive welfare implications of accessing the range. But foraging behaviour on the range may reflect avoidance of social competition for indoor resources such as feed or space, subsequently motivating chickens to access resources elsewhere. The range environment is not synonymous with the expression of behaviours only associated with positive experience. Fanatico et al. (2016) showed increased aggression (peck, threat or displacement) on the range

and some behaviours associated with positive experience including comfort behaviours such as preening were expressed more frequently indoors.

Broiler chickens run and walk more on the range and spend less time sitting and lying than chickens inside the shed (Weeks et al., 1994; Knierim, 2000; Jones et al., 2007). Active behaviours can be further increased if resources are provided on the range (Dal Bosco et al., 2014; Fanatico et al., 2016). But Weeks et al. (1994) and Knierim (2000) show greater levels of activity on the range is typically shortlived for faster-growing broiler chickens; to the extent that lying, resting and active behaviours are similar to conventionally housed chickens after just two weeks of range access.

So reports of increased activity and exploration on the range may reflect behaviours associated with positive experience and thus positive implications of accessing the range for chicken welfare. However, a reduction in resting and comfort behaviour and increased aggression observed on the range may also suggest a more stressful environment where resting and comfort behaviours may be risky to perform.

2.5.2 Health

A major concern with free-range housing systems is that mortality is higher than in conventionally housed flocks; typically in the order of 20% to 50% (Fanatico et al., 2008; Durali, Groves, & Cowieson, 2012; Zhao et al., 2014) but it has been reported as much as three times higher (Weeks et al., 1994). Durali et al. (2012) showed a 45% increase in mortality in free-range systems but mortality was not directly related to range access or use. The major cause of death in freerange systems was from yolk sac infections prior to the provision of range access and was likely due to the restriction of in-feed antibiotics for free-range chickens in Australia (Free Range Egg & Poultry Australia Ltd, 2015). International studies have not reported cause of death (Weeks et al., 1994; Fanatico et al., 2008; Zhao et al., 2014). Contrary to popular opinion, however, increased mortality in freerange housing systems is not likely to be due to the increased risk of predation. Moberly, White, and Harris (2004) surveyed various UK poultry producers and found predation accounted for less than 1% of broiler mortality and can be reduced if range resources are provided (Dal Bosco et al., 2014).

Exposure to the range environment is synonymous with exposure to parasites and pathogens. Such parasites and pathogens are established in the range soil (Rivoal, Ragimbeau, Salvat, Colin, & Ermel, 2005) or introduced by wild birds and other animals (Herman et al., 2003). Rivoal et al. (2005) showed rapid campylobacter infection when chickens were provided range access and the highest load was directly next to the house. Although the disease state of freerange broiler chickens is largely unknown, laying hens with outdoor range access have higher worm burdens than chickens housed in caged or barn systems (Permin et al., 1999). Disease and parasite risks may differ for broiler chickens due to shorter periods of range access and greater periods of rest of the range area between flocks.

Weight

Free-range broiler chickens are lighter after range access than chickens conventionally housed (Weeks et al., 1994; Jones et al., 2007; Wang, Shi, Dou, & Sun, 2009; Durali et al., 2012; Stadig et al., 2016). The mechanism is unknown but appears to be related to increased energy requirements as the reduction in weight gain is often coupled with an increased or similar feed intake (Fanatico et al., 2008; Zhao et al., 2014). The increased energy requirements of free-range chickens may relate to increased activity (Weeks et al., 1994; Knierim, 2000; Jones et al., 2007; Zhao et al., 2014; Fanatico et al., 2016), thermoregulation challenges (May, Lott, & Simmons, 1998) or pasture consumption (Singh & Cowieson, 2013).

Durali et al. (2014) found that chickens accessing the range for a longer period of time had lower body weight and greater gizzard weight than chickens which accessed the range less, suggesting the reduction in growth rate was related to feeding and foraging behaviour. Ingestion of grit and stones have shown to increase gizzard function, permitting further digestion and increasing nutrient absorption (Svihus, 2012). One might therefore expect better growth of chickens frequently foraging on the range. However, forage consumption can dilute nutrient ingestion, impact the gastrointestinal tract and alter physiological processes as a result of high potassium (Singh & Cowieson, 2013). Although the ability to self-regulate nutrition has been observed in chickens (Hughes & Woodgush, 1971; Classen & Scott, 1982), it is not known if nutrient regulation is a motivation for range use.

The effects of forage consumption on broiler chicken health are not well understood. But clover has been shown to increase nutrient uptake (Ponte, Prates, et al., 2008; Ponte, Rosado, et al., 2008) and therefore the type of vegetation on the range must be taken into account. It remains unclear if the ingestion of vegetation is beneficial or detrimental for overall gut health and it is not known if forage consumption is the cause of decreased weight gain associated with range use.

Body weight can be an important indicator of welfare, for reduction in body weight can identify chronically stressed animals (see section 2.2.2) or disease (Preston-Mafham & Sykes, 1970). The importance of body weight for broiler chicken welfare is of particular importance as increased body weight and rapid

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growth rate are often associated with common health and welfare risks, such as metabolic diseases, immobility and poor leg health.

Leg health

Poor leg health has arguably been the greatest historic risk to broiler chicken welfare. Decades of selection for improved leg health has resulted in genetic improvements (Kapell et al., 2012). But a wide range of leg disorders are still present in modern broiler flocks, albeit less prevalent. The two most common leg pathologies of broiler chickens are femoral head necrosis and tibial dyschondroplasia (Sanotra, Lund, Ersboll, Petersen, & Vestergaard, 2001; Dinev, 2009) but broiler chickens also suffer from valgus and varus distortion, spondylolisthesis (kinky back), epiphyseal separation and ruptured gastrocnemius tendons (Mc Geown, Danbury, Waterman-Pearson, & Kestin, 1999; Danbury, Weeks, Chambers, Waterman-Pearson, & Kestin, 2000). Leg deformities and associated pain can result in difficulty reaching feed and water, possibly leading to dehydration and starvation (Butterworth, Weeks, Crea, & Kestin, 2002; Dinev, 2009). Faster-growing broiler strains are particularly susceptible to leg disorders (Kestin, Knowles, Tinch, & Gregory, 1992; Shim et al., 2012).

It is likely these conditions affect the ability to access an outdoor range and may result in chickens never accessing the range or reduce the frequency and duration of range visits. Rodriguez-Aurrekoetxea et al. (2014) provided broiler chickens with perches prior to range access. These were thought to improve leg strength, although there were no detectable morphological changes in leg health. Chickens provided with perches ranged further from the shed in comparison with those not provided with perches. Although there has been some investigation into the relationships between mobility and ranging behaviour, none of the studies have assessed leg health prior to range use (Kestin et al., 1992; Weeks et al., 1994; Fanatico et al., 2008; Durali et al., 2014;). It therefore remains unknown whether poor leg health inhibits ranging behaviour or is positively affected by ranging behaviour.

Improvement to leg health may be achieved with frequent ranging behaviour although previous investigations have produced inconsistent results. Kestin et al. (1992) reared Ross chickens in free-range and conventional conditions. The provision of range access reduced the incidence of poor leg health but did not eliminate the problem; 61% free-range reared broilers had scores of 0 or 1 (better leg health) compared to only 39% reared conventionally. Fanatico et al. (2008) and Zhao et al. (2014) found similar results.

However, Wang et al. (2009) showed that tibial breaking strength was weaker for chickens provided with free-range access when compared to conventionally housed chickens. Other authors show no difference (Weeks et al., 1994; Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005). This inconsistency may reflect differences in the amount of ranging behaviour as improvements to leg health are likely be a result of increased activity, blood flow and improved ossification. This highlights the difficulty of assessing welfare implications of range use at the flock level. Assessment of leg health, for example, may have been measured on individuals which were given the opportunity to range but never did.

Only one study has looked at the implications of ranging behaviour on leg health at the individual level and this found no difference between chickens which accessed the range for more than eight hours in total, compared to those accessing the range for one hour or less (Durali et al., 2014). As leg health is expected to improve with increased activity, frequency of range use rather than

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duration may better detect potential improvements if birds are not continuously active on the range.

Growth related metabolic diseases

Intense selection for greater juvenile weight, efficient feed conversion and increased proportion of breast meat has led to a drastic change in the broiler chicken's anatomy and physiology. With little adaption of oxygen suppling organs, the ratio of heart and lung weight to body weight is lower, reducing oxygen consumption and delivery (Eitan & Soller, 2011). As a consequence of this, growth-related metabolic disorders are a common welfare concern for broiler chickens, in particular ascites and sudden death syndrome. It is possible increased metabolic demand from ranging activity may further exacerbate these metabolic diseases. Chen et al. (2013) showed a reduction in lung weight to body weight ratio in free-range chickens compared to indoor chickens. Ipek and Sozcu (2017), however, found no such difference. Conversely, as ranging behaviour is likely to slow growth rate (see section 2.5.2 'weight') range use may reduce the risk of growth-related metabolic disease.

Ascites affects from 1 to 10% of chickens in commercial flocks (Maxwell & Robertson, 1997; Beheshti, Mousapoor, & Lofti, 2011) and recent findings suggest it is increasing (Part, Edwards, Hajat, & Collins, 2016). Ascites commonly develops as a secondary effect of pulmonary hypertension as incompatible oxygen demands and insufficient pulmonary vascular capacity leads to tissue hypoxia. Pulmonary oxygen exchange must increase in states of hypoxia; unlike mammals, avian lung expansion is prohibited due to attachment to thoracic walls, therefore the avian physiological response is to increase erythrocyte production and constriction of the pulmonary arterioles (Julian, 1998; Eitan & Soller, 2011). The pressure from increased blood viscosity and constricted

pulmonary arterioles makes the workload greater on the right side of the heart leading to hypertrophy of the right ventricle and the atrioventricular valve until valvular insufficiency occurs. Increased pressure in blood vessels forces plasma fluid into peritoneal spaces and interferes with the uptake of fluid by the lymphatic system; it is these secondary effects of fluid accumulation in the abdominal and pericardial cavities which causes ascites.

Death may occur due to accumulation of fluid in the lung (pulmonary oedema), ventricle failure or pressure on air sacs in the lung from fluid accumulation and exacerbated by pressure exhorted from large breast tissue (Julian, 1998; Eitan & Soller, 2011). These conditions are aggravated by high altitude, decreased temperatures and respiratory disease (Julian, 1993). Ascites is likely stressful and possibly painful as health degradation prior to death may occur over an extended period of time from suffocation or starvation (Wideman, Rhoads, Erf, & Anthony, 2013).

There is evidence to suggest conventionally housed chickens have a higher risk of developing ascites than those housed in free-range systems. Herenda and Jakel (1994) completed a survey of broiler abattoirs and quantified the major causes of condemnations from free-range and conventional housing systems. The prevalence of ascites was greater in conventional broiler chickens (0.26%) compared with free-range (0.05%). However, housing differences were confounded with broiler strain and on-farm mortality was not recorded.

Another growth-related metabolic syndrome of concern in the broiler industry is Sudden Death Syndrome (SDS), causing between 2 to 4.5% of flock mortality (Julian, 1986; Olkowski & Classen, 1998). Although a lack of aetiological evidence can make quantifying incidences difficult, death from SDS is likely due to hypoxic conditions (due to previously mentioned reasons) which drive myocardium to become hyperirritable, leading to acute cardiac arrhythmia, ultimately ventricular fibrillation and cessation of effective blood circulation. Although an equal economic concern as ascites for broiler producers, the two conditions likely vary in regards to welfare as death from SDS generally occurs within one minute of symptom onset (Newberry, Gardiner, & Hunt, 1987). Pulmonary hypertension, ascites and SDS can be minimised and potentially prevented by reducing growth rate (Decuypere, Buyse, & Buys, 2000; Bennett, Classen, & Riddell, 2002) and therefore may be an indirect effect of range use related to the reduction in body weight as previously discussed.

Mobility

Reports of minimal range use in broiler flocks are not surprising as fastergrowing broiler chickens spend 50 to 70% of their time inactive and performing most behaviours in a sitting position; comparatively slower-growing chickens spend between 20 to 60% of their time sitting (Bizeray, Leterrier, Constantin, Picard, & Faure, 2000; Bokkers & Koene, 2003). The reason for broiler inactivity is greatly debated. Some authors identify motivation to express active behaviours is a trait altered as a result of intense selection for growth (Bizeray et al., 2000), while others suggest motivation to move is still apparent but chickens are physically constrained due to altered morphology or poor leg health (Rutten, Leterrier, Constantin, Reiter, & Bessei, 2002; Caplen et al., 2013). In reality, reduced activity in broiler chickens is likely to be a combination of factors and age-dependent.

The premise of resource allocation theory in relation to inactivity of broiler chickens suggests energy conservation for growth is achieved by reducing motivation to perform other energy-demanding behaviours such as foraging; indeed, studies with laying hens suggest hen behaviours are modified according to this theory (Schutz & Jensen, 2001). Reduced motivation to perform active behaviours is unlikely a direct risk to welfare, although secondary effects are detrimental, such as dermatitis from sitting on litter for extended periods of time (discussed further below). With inevitable genetic variation within broiler flocks, it may be that lower performing chickens are more active and subsequently more likely to utilise the range area.

Many authors suggest broiler chickens are motivated to perform active behaviours such as foraging, but altered morphology or poor leg health physically restricts them from doing so. Reducing weight load and providing anti-inflammatory drugs has indeed been shown to increase time spent walking and the velocity of walking speed (Rutten et al., 2002; Caplen et al., 2013). This evidence indicates broiler chickens are motivated to express active behaviours but are restricted due to altered morphology, increased weight-bearing or painful leg pathologies. Changes to broiler morphological conformation, including reorientated angle of the breast muscle and decreased pelvic muscle mass, has led to altered gaits in modern broiler chickens to maintain their centre of gravity (Skinner-Noble & Teeter, 2009; Paxton, Anthony, Corr, & Hutchinson, 2010).

Corr, Gentle, McCorquodale, and Bennett (2003) suggest the large breast muscle of broiler chickens interferes with walking ability. Discrediting this theory, Castellini, Mugnai, and Dal Bosco (2002) showed that faster-growing free-range broiler chickens were more active compared to conventionally housed chickens, despite a higher percentage of breast muscle. Skinner-Noble and Teeter (2009) suggest the angle of breast muscle has more of an effect on walking ability than size alone.

Identifying the cause of reduced mobility and the relationship with range use will permit a greater understanding of the implications of range use on welfare. For example, if chickens are motivated to access the range but are physically restricted from doing so they are likely to become frustrated resulting in compromised welfare. If selection has favoured energy-conserving behaviours, however, there may be no direct risks to welfare.

Contact dermatitis

If mobility is affected by range access, or vice versa, the welfare risks associated with inactivity should also be affected, such as contact dermatitis. Contact dermatitis is a skin condition characterised by discolouration, hyperkeratosis and necrosis of the epidermis and severe cases result in swelling, ulcers or lesions (Shepherd & Fairchild, 2010). Broiler chickens can develop dermatitis on the plantar surface of the foot pads (foot pad dermatitis), the back part of the hock joint (hock burn) and on the breast (breast burn). Foot pad dermatitis develops quicker than hock and breast burn (Stephenson, Bezanson, & Hall, 1960). Flock incidence of dermatitis has been reported between 22 to 100% of chickens in faster-growing commercial flocks, although it can be as low as 1% with slower-growing flocks (Hashimoto, Yamazaki, Obi, & Takase, 2011; Bassler et al., 2013). Litter moisture is the major casual factor but dermatitis can be influenced by season, gender, age, lighting, nutrition and climate (Shepherd & Fairchild, 2010). Broiler chickens affected by dermatitis exhibit signs of inappetance indicative of pain (Martland, 1985). Theoretically, dermatitis lesions can heal but this is rarely observed in commercial conditions due to difficulties maintaining litter quality (Martland, 1985).

Free-range broiler chickens have been shown to have less hock burn than conventionally housed chickens (Stadig et al., 2016), possibly related to changes in body weight and leg health as hock burn is positively associated with body weight and lameness (Sørensen, Su, & Kestin, 2000). Reports on the relationships between body weight, lameness and foot pad dermatitis are inconsistent (Shepherd & Fairchild, 2010). However, studies indicate foot pad dermatitis is higher in free-range broiler chickens than the conventionally housed (Haslam et al., 2006; Pagazaurtundua & Warriss, 2006; Dal Bosco et al., 2010). This may be related to housing effects rather than ranging behaviour per se, as litter moisture has shown to be worse in free-range houses (Haslam et al., 2006). Dal Bosco et al. (2014) showed that foot pad dermatitis and breast burn was reduced when range enrichment was provided, perhaps reflective of less time sitting on poor quality litter. Against this, Durali et al. (2014) monitored hock burn and foot pad dermatitis in relation to individual ranging behaviour and found no relationship with the amount of time spent on the range.

2.5.3 Fear

There is a common perception that fearful chickens will not access the outdoor range, although this has received little attention in scientific literature.

Fear and anxiety are emotional and/or motivational states induced by actual or perceived danger (Boissy, 1995). Fear is a powerful state, often outcompeting against other motivational states and it results in both physiological and behavioural fear responses which aid escape or defence (Forkman et al., 2007). Ideally, fear is an adaptive and protective mechanism, yet a chronic state of fear or exaggerated fear responses can be detrimental to welfare (see section 1). Fear may be induced by a variety of extrinsic factors including unfamiliar environments or stimuli (neophobia) (Jones, 2002), evolutionary dangers e.g. open spaces for poultry (Jones, 1996), human contact (Barnett, Hemsworth, & Newman, 1992), social interactions (Jones & Merry, 1988), social isolation for gregarious species (Forkman et al., 2007) and conditioned stimuli (Gray, 1987). It is regulated by a number of intrinsic factors, including sex, breed and strain but can also be altered by experience (Boissy, 1995; Hemsworth & Coleman, 2011). The combination of intrinsic factors leads to variation between naive individuals in the propensity to be frightened. This trait is broadly referred to as general fearfulness (Price, 1984; Boissy, 1995; Jones, 1996; Hemsworth & Coleman, 2011).

As the range environment is novel, general fearfulness may be related to willingness to access the range environment. Gordon and Forbes (2002) increased familiarity of the range environment by providing visual access to an outdoor range and fluctuating temperatures during rearing. Chickens which had visual access to the range during rearing were observed on the range more frequently when access was provided than chickens without visual access during rearing. The effect of the rearing treatment surpassed the effects of providing enrichment on the range, suggesting familiarity and general fearfulness may be a major determinant of ranging behaviour.

Additionally, experiences on the range may alter fearfulness through learning such as habituation, sensitisation or conditioning. Experiences during juvenile stage are more likely to affect general fearfulness (Boissy, 1995) and thus this effect may be particularly prominent in broiler chickens due to the young age they are exposed to the range environment.

There has been some investigation into the relationships between general fearfulness and ranging behaviour, although the results are inconsistent and ranging behaviour has only been assessed at the flock level. Zhao et al. (2014) investigated the fear response of broiler chickens with range access compared to chickens without range access and assessed fearfulness with a tonic immobility (TI) test (description of the test is discussed below). Chickens with access to an
outdoor range had higher fear responses than chickens without range access. However, only five chickens from each flock were tested and whether these focal chickens had accessed the range and if so to what degree was unknown. Stadig et al. (2016) assessed the general fearfulness of free-range and conventionally housed broiler chickens with a TI test before and after range access was provided. The duration of TI before or after range access did not differ between chickens provided with range access and chickens that were not. However, the length of TI prior to range access was related to the proportion of the flock which ranged further from the shed. These results suggest general fearfulness is not related to willingness to access the range but may be related to ranging distance.

Ipek and Sozcu (2017) provided further support that provision of range access does not alter fearfulness in broiler chickens, as heterophil/lymphocyte ratio - a physiological immune measure of chronic stress (Zulkifli, Norma, Chong, & Loh, 2000) - did not differ between free-range and conventionally housed broiler chickens after range access. An interesting finding from Weeks et al. (1994) showed that after range access was provided, fewer attempts to induce TI were required for broiler chickens that were fed on the range, compared with chickens provided with range access but fed indoor and conventionally housed chickens. These results suggest that forcing individuals to access an outdoor range may have detrimental effects on welfare by sensitising their fear response.

It is important to note that all the studies assessed behavioural fear responses with a tonic immobility test. There are a variety of fear tests which have been validated for poultry although the specific fear-provoking stimuli slightly differ (Forkman et al., 2007). Campbell et al. (2016) used a variety of fear tests to assess differences in laying hens in relation to ranging behaviour. Importantly, these authors showed ranging behaviour was not connected to any TI measure but was related to measures from other validated fear tests, such as the open field test (described below). It is therefore important to understand the potential sources of variation between different fear tests and discuss what these measures may reflect. The two most common fear tests used to assess fearfulness in poultry will briefly be outlined for that reason.

Assessing fear responses

Tonic immobility is an innate antipredator response of a catatonic-like state and can be induced by physical restraint (Jones, 1986). This feigning death allows chickens to take advantage of escape opportunities during predation (Thompson et al., 1981). The tonic immobility test typically involves attempting to induce the TI state by physical restraint (commonly for 15 seconds). The number of attempts required to induce the TI state are quantified in addition to the time it takes for a chicken to right itself once the TI state is induced, which can last from seconds to several hours (Jones, 1986). This test has been well-validated, showing the duration of TI is increased after chickens are exposed to procedures associated with fear, such as electric shock and the duration of TI is reduced after habituation and positive handling (Forkman et al., 2007).

It is generally accepted TI measures are positively associated with fearfulness but as the TI state is an innate anti-predator response it is possible TI predominantly reflects fear responses specific to perceived predation. Furthermore, the lack of standardisation between TI tests can account for a large disparity in the scientific literature (Forkman et al., 2007). For example, observers' gaze, presence of conspecifics and the nature of handling prior to the TI test has shown to have effects on the duration of the TI state (Forkman et al., 2007).

The open field test (OFT), also called the novel arena test, involves placing chickens into a novel open arena and monitoring behavioural responses. The OFT test has also been well-validated; increased novelty of the arena or exposure to fear-inducting stimuli prior to the test has shown to increase freezing behaviour and reduce locomotion and vocalisations (Gallup & Suarez, 1980; Jones & Faure, 1982). However, OFT includes elements of novelty and social isolation and therefore interpretation of results can be difficult (Forkman et al., 2007). There are some behaviours more indicative of neophobia than social reinstatement, such as freezing and decreased activity (Jones & Merry, 1988) and neophobia and fear of social isolation can be accurately disentangled by testing chickens in pairs (Forkman et al., 2007).

There are other tests which are utilised to assess fear responses in poultry but they currently have limitations. The emergence test was developed to test fear responses of rodents, but has been adapted for poultry. This test quantifies how long it takes for a chicken to emerge from an enclosed box. The repeatability of the emergence test is relatively unknown and its validity less understood than that of the TI or OFT. In addition, results may be confounded due to an adverse reaction to the brightly lit open field relative to the dark box, and therefore may not accurately reflect fearfulness (Forkman et al., 2007).

The novel object test has been used to assess neophobia and curiosity. A novel object is placed in an arena or home pen and the interaction with the object and the distance between the chicken and the object is assessed over time. A rapid approach (less freezing), shorter distance (approach) and multiple interactions with the novel object infers reduced neophobia and increased curiosity (Forkman et al., 2007). While this test may be particularly useful in relation to free-range broiler chickens, it could be difficult disentangling the effects of physical restraints of older heavier broiler chickens and a lack of motivation to explore. Of interest, the novel object test is the only validated test which has been utilised to investigate curiosity (exploratory behaviour) in chickens. Curiosity has been considered a positive affective state (Mellor, 2016) and may be associated with

range use. There is a need to identify valid assessments of chicken curiosity that can disentangle fear and curiosity.

Of the few investigations into relationships between broiler chicken fear and ranging behaviour only the TI method has been used. Is it unknown if the open field test could clarify relationships between general fearfulness and ranging behaviour. Also, monitoring individual ranging behaviour in relation to fearfulness could determine if fearfulness is directly related to range use or other differences in the environment between conventional and free-range housing.

2.5.4 Individual ranging behaviour and welfare

Only one study has monitored individual range use in broiler chickens and investigated the relationships with welfare. Durali et al. (2014) monitored two flocks of fast-growing broiler chickens in a research pen on a commercial farm in Australia and compared indicators of welfare and productivity between chickens which spent longer than eight hours in total on the range compared to chickens spending one hour or less. Results indicated that chickens which spent longer on the range had reduced growth rate and increased gizzard weight, but there were no differences in foot pad dermatitis, hock burn, leg health, intestinal health scores or the weight of the spleen or bursa of Fabricius. These results could indicate that welfare comparisons between free-range and conventionally housed chickens may be reflective of other differences in housing rather than range use per se. However, the sample size of this study was relatively low (n = 30 each group) and further investigation is required.

2.6 Conclusions

Although there is a perceived benefit in free-range housing systems relating to broiler chicken welfare, the scientific literature infers that range access may have various and opposing effects on welfare which can vary in severity. Much of this information has been gathered in research settings with small flocks in Europe, therefore the application of results to free-range broiler chickens on Australian commercial farms is presumptuous and possibly misleading. The majority of studies have also assessed ranging behaviour and welfare at the flock level, rather than the individual level. As it is likely that ranging behaviour within a broiler chicken flock is heterogeneous, welfare assessments at the flock level may not accurately identify the true implications of range use on chicken welfare. A better understanding of ranging behaviour and the subsequent implications for chicken welfare is required to encourage optimal range use which promotes good welfare. As highlighted through the review of the literature, there are serious welfare concerns in the broiler industry which may be affected by range use and these will be of particular interest alongside the research presented throughout this thesis. This focus includes rapid growth and the associated pathologies, leg disorders and the affective state of fear.

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CHAPTER 3 Ranging behaviour of commercial free-range broiler chickens: Factors related to flock variability



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3.1 Simple summary

Free-range chicken meat consumption has increased. However, little is known about how meat chickens use the outdoor range. Understanding ranging behaviour could help improve management and shed and range design to ensure optimal ranging opportunities. We tracked 1200 individual broiler chickens in four mixed sex flocks on one commercial farm across two seasons. More chickens accessed the range in summer than winter. Chickens that accessed the range in winter did so less frequently and for a shorter period of time daily than chickens ranging in summer. The number of chickens ranging and the frequency and duration of range visits increased over the first two weeks of range access and stabilised thereafter. More chickens ranged in the morning and evening compared to the middle of the day. Ranging behaviour decreased with increased rainfall and shed dew point. This study provides knowledge regarding ranging behaviour in commercial conditions that may guide improvements on farm to provide chickens with optimal ranging opportunities.

3.2 Abstract

Little is known about the ranging behaviour of chickens. Understanding ranging behaviour is required to improve management and shed and range design to ensure optimal ranging opportunities. Using Radio Frequency Identification technology, we tracked 300 individual broiler chickens in each of four mixed sex Ross 308 flocks on one commercial farm across two seasons. Ranging behaviour was tracked from the first day of range access (21 days of age) until 35 days of age in winter and 44 days of age in summer. Range use was higher than previously reported from scan sampling studies. More chickens accessed the range in summer (81%) than winter (32%; p < 0.05). On average, daily frequency and duration of range use was greater in summer flocks (4.4 ± 0.1 visits

for a total of $26.3 \pm 0.8 \text{ min/day}$) than winter flocks ($3.2 \pm 0.2 \text{ visits}$ for a total of $7.9 \pm 1.0 \text{ min/day}$). Seasonal differences were only marginally explained by weather conditions and may reflect the reduction in range exposure between seasons (number of days, hours per day, and time of day). Specific times of the day (p < 0.01) and pop-holes were favoured (p < 0.05). We provide evidence of relationships between ranging and external factors that may explain ranging preferences.

3.3 Introduction

Free-range chicken meat consumption has increased in some countries (Australian Chicken Meat Federation, 2013; Magdelaine, Spiess, & Valceschini, 2008) largely driven by consumer perception that free-range housing is more natural and better for chicken welfare (de Jonge & van Trijp, 2013). However, there is little scientific knowledge regarding how the broiler chickens themselves perceive and utilise the outdoor range, despite access to an outdoor range being a unique feature of free-range housing systems. Theoretically, providing access to an outdoor range provides animals with some control to choose when, where, and how to spend their time. Monitoring these choices can permit an understanding of what free-range broiler chickens want, which is an integral part of defining and safeguarding welfare (Dawkins, 2004). Chickens may access the outdoor range as the range area provides opportunities to explore a more complex environment than the typical indoor shed environment. However, chickens may also access the range to avoid negative stimuli, such as experiences in the shed that may be uncomfortable, frightening, or painful. Hence, monitoring broiler chicken ranging behaviour and modulations in response to environmental factors can provide insights into the factors underlying broiler chickens' motivation to range.

Accessing the range may depend on external stimuli such as weather conditions and range microhabitats. Indeed, previous studies indicate that broiler chicken ranging behaviour is affected by time of day, weather variables (rainfall, direct sunlight, temperature, and wind speed), and resources on the range (e.g., trees and straw huts) (Dawkins, Cook, Whittingham, Mansell, & Harper, 2003; Nielsen, Thomsen, Sorensen, & Young, 2003; Jones, Feber, Hemery, Cook, James, Lamberth, & Dawkins, 2007; Rivera-Ferre, Lantinga, & Kwakkel, 2007). However, how such parameters affect ranging patterns of individual broiler chickens, in terms of frequency and duration of range visits have not been reported.

Historically, research suggests that broiler chicken range use is low, reporting that only 3 to 27% of a flock will access the range (Dawkins et al., 2003; Nielsen, Thomsen, Sorensen, & Young, 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea, Leone, & Estevez, 2014; Fanatico, Mench, Archer, Liang, Gunsaulis, Owens, & Donoghue, 2016). Such investigations into ranging behaviour utilised scan sampling methods, counting the number of chickens on the range area throughout the day at particular points in time (Dawkins et al., 2003; Nielsen et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014; Fanatico et al., 2016). This methodology may underestimate flock range use, as different chickens may access the range at different times in the day. Indeed, recent investigations monitoring individual broiler chicken ranging behaviour suggests that range use is higher than previously reported investigations using scan sampling methods, reporting in the order of 75 to 95% of chickens in a flock accessing the range (Chapuis, Baudron, Germain, Pouget, Blanc, Juin, & Guemene, 2011; Durali, Groves, Cowieson, & Singh, 2014). Therefore, range use is greater than previously thought when assessment is made at the individual level rather than at the flock level.

Understanding ranging behaviour of broiler chickens in response to environmental factors is necessary to develop management practices and shed and range designs that optimise opportunities to range. With this focus, we tracked the individual ranging behaviour of 1200 commercial broiler chickens across four mixed sex flocks on one commercial farm in two seasons to examine the relationships between ranging behaviour and weather and shed conditions, age, and availability of access to the range in terms of hours per day, number of days, and time of day.

3.4 Materials and methods

All animals and experimental protocols used in this study were approved by the University of Melbourne Animal Ethics Committee (Approval Number 1413428.3).

Study site

Four flocks (A–D) of ROSS 308 broiler chickens were studied across two seasonal replicates on one commercial farm in Victoria, Australia: Austral winter (flocks A and B) and summer (flocks C and D). All sheds had chickens from the same hatchery, same feed, same manager, and comparable management practices. Placement of the chicks was made on the same day for winter flocks, and four days apart for summer flocks, with placement day counted as day 0. Each flock contained approximately 6000 (Flocks A and C) or 10,000 (Flocks B and D) broiler chickens kept at a maximum indoor stocking density of 34 kg/m₂, maintained by removing ("thinning") approximately 35% of the flock (chosen based on their location in the shed) for slaughter around 35 days of age, described hereafter as "partial depopulation". The second, and final pick up occurred at 49 days of age and removed all remaining chickens for slaughter, described hereafter as "complete depopulation". Shed one (flocks A and C) measured 40.5

m x 9.3 m and shed two (flocks B and D) 50.5 m x 12.3 m. The sheds were mechanically ventilated with fans. Natural ventilation was provided when automatic curtains were lowered 1 to 2 m on the sidewalls of the shed stopping 1mabove the shed floor. The shed wall was solid from the ground to 1m above, therefore even when the curtains were fully opened chickens could not see the range area except through opened pop-holes. Curtains opened automatically based on shed temperature and humidity and thus varied daily. Brooding occurred in the back half of the shed and was temperature controlled by gas heaters. Feed and water were provided ad libitum inside the shed, but never in range areas. Light (20 to 25 lux) was provided on a 23:1 L:D cycle from 0 to 7 days of age and 16:8 cycle until complete depopulation, excluding the three days prior to partial and complete depopulation when light cycle was 20:4. Two tiered perches were provided in each shed (2.7 m/1000 birds) and plastic red chains were hung (20 cm) from drinker lines spread evenly throughout the shed. In winter flocks, management "turned the litter" during ranging hours on the eighth day of range access. "Turning the litter" was achieved by rotary hoeing the litter throughout the entire shed.



Figure 3-1 Diagram of study sheds and range areas (a) shed one, flocks A and C and (b) shed two, flocks B and D. Pop-holes are numbered (P1–P7) sequentially from the front of the shed (shed access point).

Individual tracking

Individual range usewas tracked by theGantner Pigeon RFIDSystem(2015 Gantner Pigeon Systems GmbH, Benzing, Schruns, Austria), with a bespoke program Chicken Tracker that was developed for the use of tracking chickens and previously validated to track laying hens on commercial farms (Gebhardt-Henrich, Fröhlich, et al., 2014; Gebhardt-Henrich, Toscano, & Frohlich, 2014). Chickens (n = 300/flock) were randomly selected from ten areas evenly spread within the shed; locations varied according to length and width of the shed and distance from pop-holes. Chickens were fitted with a silicone leg band that automatically loosened with leg growth (Shanghai Ever Trend Enterprise, Shanghai, China). Each leg band contained a unique ID microchip (Ø4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered as the chickens walked over antennas. Leg bands were put on three to four days before range access was first provided to allow chickens to habituate to them. Antennas were attached to both sides of each pop-hole (i.e., indoor and outdoor) to determine the direction of movement by each tagged chicken and thus calculate the frequency and duration of range use. Antennas were placed prior to placement of chicks. Chickens were marked with blue or green stock paint (FIL Tell Tail, GEA, New Zealand) on tail and wing feathers to identify tagged chickens in order to retrieve leg bands at the end of the study. In winter flocks, chickens were tracked from the first day that range access was permitted (21 days of age) for 10 days prior to partial depopulation (30 to 33 days of age). Due to logistical reasons, tracking chickens until complete depopulation was prevented. Chickens in summer flocks were tracked daily from the first day that range access was permitted (21 days of age) for 24 days (flock C) and 21 days (flock D) prior to complete depopulation (43 to 45 days of

age). No tagged chickens were removed from the flock during partial depopulation in summer flocks. Chickens were excluded from analysis if tags were not recovered or functional at the end of the trial

Weather conditions

Weather variables were recorded every 10 min on site in summer via a weather station (Ambient Weather, Chandler, AZ, USA). However, due to equipment failure, weather variables were collected twice daily in winter by the Bureau of Meteorology weather station located 20 km from the farm site (Bureau of Meteorology). Climate data loggers were placed inside each shed during the summer replicate and recorded temperature, humidity, and dew point every ten minutes. Climate data loggers were not available for winter flocks.

Statistical analysis

RFID data were cleaned with SASTM (v 9.3, SAS institute Inc., Cary, NC, USA) using a modified macro (Gebhardt-Henrich, Toscano, & Frohlich, 2014). All range visits <10 s were treated as false positives and removed from analysis.

Descriptive ranging data were generated using MATLABTM and Statistics Toolbox Release R2016b (The MathWorks Inc., Natick, MA, USA). Statistical analysis was performed with SPSS statistical software (v 22, IBM Corp, Armonk, NY, USA). Non-ranging chickens were excluded from the analysis that investigated the frequency and duration of range use. In winter flocks, the eighth day of ranging data was analysed separately, due to management turning the litter on that day.

Spearman's rho correlation coefficients were utilised to examine the relationship between latency to access the range and frequency and duration of range use and duration per range visit. As no random variables could be controlled for in non-parametric correlation analysis, analyses were conducted on individual flock data. All other data met the criteria of normality and homogeneity of variance and therefore parametric statistical tests were used. Pearson's partial correlation coefficients were used to examine relationships between cumulative ranging day (relative to first availability) and the frequency and duration of range use and the number of chickens that accessed the range, controlling for flock. Pearson's partial correlation coefficients were also used to identify relationships between the number of hours the range was available daily and the number of chickens on the range, the daily mean duration of a range visit, and the daily frequency and duration of range visits, controlling for flock and age.

Linear regression models were constructed to investigate the chicken and weather variables that predicted the number of chickens that accessed the range. Independent weather variables were included in the analysis if they were correlated with the number of chickens on the range ($p \le 0.10$). A variable was removed from the model if it was strongly correlated with another ($r \ge 0.70$). A maximum of seven variables were included in one model, based on the aforementioned correlation analysis. All possible models were run and the final model included the variables that resulted in the best fit, determined by adjusted r² comparisons and *p*-values indicating significant change in F statistic from a forward stepwise regression analysis. The most parsimonious models are reported with statistically useful variables in the model. Analysis was performed on hourly weather data in summer flocks, but on daily weather data in winter flocks due to technical problems with the onsite weather station in winter.

Analysis of Variance (ANOVA) models were used to investigate the use of each pop-hole in each shed for range entry and exit and the number of ranging chickens between seasons and flocks. Analysis of Covariance (ANCOVA) models were used to determine the effect of time of day on the number of chickens that accessed the range and the frequency and duration of range visits, controlling for age, and included flock and time of day interaction. Post hoc analysis used the Bonferroni method to correct for multiple comparisons. Results are presented as raw means ± Standard Error (SE) unless otherwise noted.

3.5 Results

Range availability

Due to weather extremes, management permitted range access seven out of 10 days prior to partial depopulation in winter flocks for a daily mean of 5.6 ± 0.4 h (total hours: flock A -37.5 h; flock B -37.8 h). Summer flocks had access to the range every day except 2 to 4 days prior to partial depopulation (flock C-10 days; flock D-9 days) and in total 18 and 16 days before complete depopulation for flocks C and D respectively; for a mean of 10.4 ± 0.6 h daily (total hours: flock C-183.8 h; flock D-168.7 h). Range access was not always provided continuously across days, predominantly due to adverse weather conditions. For winter flocks, there was one day of interruption on the sixth day after the range was first available due to adverse conditions (Figure 3-2). For summer flocks, there was two to four intermittent days of interruption between partial depopulation, and two to four intermittent days of interruption between partial depopulation and complete depopulation due to adverse weather conditions (Figure 3-2). Furthermore, range access was provided for only two hours the two days immediately after partial depopulation due to adverse weather conditions





3-2).

Figure 3-2 Bars indicate the proportion of chickens that accessed the range (%successfully tracked chickens; left y-axis) daily in winter (**a**) flock A (**b**) flock B and summer (**c**) flock C (**d**) flock D. Circles indicate time (hours; right y-axis) the range was available each day.

Range use

More than 93% of the 1200 tagged chickens were successfully tracked from day 21 until the end of the study (winter: flock A – 98.3%, flock B – 97.7%; summer: flock C – 93.7%, flock D – 94.7%), indicated by the recovery of functional tags at the end of trial. Figure 3-2 shows the percentage of the flock that accessed the range over time in summer and winter flocks. Fewer chickens accessed the range in the winter than in summer prior to partial depopulation (winter: $32.0 \pm 0.8\%$, summer: $81.4 \pm 6.0\%$; $F_{(1,3)} = 68.0$, p < 0.05). The total number of chickens that accessed the range was relatively similar within season replicates

(winter: flock A-31.2%, flock B-32.8%; summer: flock C-75.4%, flock D-87.3%). Most chickens that accessed the range throughout the study did so prior to partial depopulation, in summer flocks (chickens that accessed the range for the first time before partial depopulation: flock C-92.5%, flock D-94.4% of ranging chickens).

The maximum number of chickens observed on the range at one time was 7.8% and 10.6% in winter, flocks A and B respectively (observed at 27 days of age in both flocks; 12:00 and 13:00 h, flocks A and B respectively) and 36.7% and 32.8% in summer, flocks C and D, respectively (observed at 29 days of age in flock C and 41 days of age in flock D, at 18:00 h in both flocks).

On average, ranging chickens accessed the range $34.4 \pm 6.1\%$ and $50.1 \pm 1.4\%$ of the available days up to partial depopulation (30–33 days of age) in winter and summer flocks, respectively. In summer flocks, ranging chickens accessed the range a mean of $43.5 \pm 1.2\%$ of the available ranging days up to complete depopulation (43–45 days of age). On each available ranging day before partial depopulation, chickens visited the range a mean of 3.2 ± 0.2 and 4.4 ± 0.1 times, for a mean of 7.9 ± 1.0 and 26.3 ± 0.8 min per visit, in winter and summer flocks respectively. After partial depopulation, chickens in summer flocks accessed the range a mean of 4.2 ± 0.1 times daily, for a mean of 23.4 ± 0.9 min per visit.

Latency to access the range

The number of days that it took for a chicken to access the range for the first time, relative to the first day that range access was provided (hereafter referred to as "latency to access the range") varied from the first available day of range access until the last day range access was provided (mean latency winter: flock $A-3.9 \pm 0.2$ days, flock $B-3.9 \pm 0.2$ days; mean latency summer: flock $C-5.9 \pm 0.2$ days, flock $D-3.7 \pm 0.2$ days). The number of chickens that accessed the range

for the first time each day was not correlated with the cumulative number of days the range was previously available (hereafter referred to as "cumulative ranging day") in winter flocks (flock A: $r_{(5)} = -0.11$, p > 0.05; flock B: $r_{(5)} = -0.07$, p > 0.05), but was negatively correlated with cumulative ranging day in summer flocks (flock C: $r_{(13)} = -0.51$, p = 0.05; flock D: $r_{(11)} = -0.90$, p < 0.001).

Ranging behaviour over time

The two days immediately after partial depopulation in summer, flocks were excluded from the analysis as range access was provided ≤ 2 h due to adverse weather conditions. The number of chickens that accessed the range, total daily range visits, mean number of daily range visits/chicken and the mean duration of each range visit before partial depopulation in summer and winter flocks were all positively correlated with cumulative ranging day (p < 0.05), but not between partial and complete depopulation in summer (Table 3-1).

Table 3-1 Pearson's partial correlation coefficients (*r*), controlling for flock, between the cumulative ranging day (relative to first availability) and daily ranging behaviour, before partial depopulation (summer and winter flocks) and between partial and complete depopulation (summer flocks only).

Daily ranging behaviour	Cumulative Ranging Day (from First Access to Partial Depopulation)		Cumulative Ranging Day (from Partial Depopulation to Complete Depopulation)
	Winter (<i>n</i> = 14 days)	Summer (<i>n</i> = 19 days)	Summer (<i>n</i> = 11 days)
Number of chickens that accessed the range	0.86 ***	0.94 ***	0.22
Total daily range visits	0.79 ***	0.76 ***	0.06
Mean daily visits/individual	0.65 *	0.53 *	0.03
Mean duration/visit	0.76 ***	0.79 ***	0.41

Note: * and *** indicates significance at p < 0.05 and 0.001 levels respectively.

Hours available to range

The number of hours the range was available for chickens varied between 3.0 to 7.2 h daily in winter flocks (mean 5.6 ± 0.4 h) and 2.0 to 14.0 h in summer flocks

(mean 10.4 \pm 0.6 h; Figure 3-2). The number of chickens on the range, total visits, total duration, and mean number of visits per chickens in both seasons were positively correlated with the number of hours the range was available (Table 3-2). The number of hours the range was available was positively correlated with the mean duration per visit in summer but not in winter (Table 3-2).

Table 3-2 Pearson's partial correlation coefficients (r), controlling for flock and age, between the number hours the range was available each day and ranging behaviour for winter and summer flocks.

	Range Access (Hours/Day)		
Ranging behaviour	Winter	Summer	
	(n = 14)	(n = 34)	
Number of chickens that accessed the range	0.69 **	0.55 ***	
Total daily range visits	0.74 ***	0.57 ***	
Mean daily visits/individual	0.85 ***	0.62 ***	
Mean duration/visit	-0.06	0.53 ***	

Note: ** and *** indicates significance at the 0.01 and 0.001 level respectively.

Time of day

Time of day had no effect on the number of chickens that accessed the range or the frequency, duration or mean duration per visit in winter flocks. However, range access was only provided between 11:00 h and 16:00 h most days in winter flocks.

In summer flocks, range access was provided inconsistently on some days due to adverse weather conditions (at the farm manager's discretion). Thus, some data were excluded in the time of day analysis, only including data that was reflective of a "typical" ranging day. The criteria for exclusion included length of range access (days with < 2 h range access excluded), hours of range access (hours outside 09:00 and 20:00 were excluded) and day of range access (day one and two of range access (relative to first day range access was provided) were excluded due to unusually low levels of ranging).

There was an interaction between time of day and flock on the number of birds on the range ($F_{(11,290)} = 2.12$, p < 0.05). The number of chickens on the range and number of range visits peaked between 09:00 and 10:00 h and/or 18:00 and 19:00 h in summer flocks (number of chickens: Flock $C - F_{(11,149)} = 2.79$, p < 0.01; Flock $D - F_{(11,141)} = 5.25$, p < 0.001; Figure 3-3; number of visits: Flock $C - F_{(11,149)} = 2.79$, p < 0.01; Flock $D - F_{(11,141)} = 5.10$, p < 0.001). The mean duration of a range visit increased between 16:00 and 20:00 h in flock D ($F_{(11,141)} = 9.76$, p < 0.001) and peaked between 15:00 h and 16:00 h in flock C ($F_{(11,149)} = 2.12$, p < 0.05).



Figure 3-3 Mean number of chickens on the range (± Standard Error (SE)) during ranging hours (9:00 to 20:00 h) for summer flocks; flock C (**a**) and flock D (**b**).

Weather conditions

As expected, weather conditions differed between seasons (Table 3-3).

Variable	Winter	Summer
Minimum outdoor temperature (°C)	3.6 ± 1.2	11.1 ± 0.7
Maximum outdoor temperature (°C)	12.6 ± 0.6	29.9 ± 1.1
Minimum indoor shed temperature (°C)	19.5 ± 0.3	17.8 ± 0.2
Maximum indoor shed temperature (°C)	22.2 ± 0.4	28.5 ± 0.5
Outdoor relative humidity (%)	80.6 ± 3.1	63.9 ± 2.0
Indoor shed relative humidity (%)	68.4 ± 0.3	64.0 ± 0.4
Indoor shed dew point (°C)	-	15.37 ± 0.1
Daily rain fall (mm)	3.4 ± 2.0	1.8 ± 1.3
Wind speed (km/h)	12.2 ± 1.9	4.2 ± 0.6
Ultraviolet radiation (uW/cm ²)	-	853.5 ± 47.4
Sunrise (h)	07:29-07:33	05:51-05:58
Sunset (h)	17:07-17:08	20:29-20:44

Table 3-3 Mean daily (\pm Standard Error (SE)) environmental conditions in winter (n = 8 days) and summer (n = 26 days). Environmental conditions were measured twice daily in winter and at ten minute intervals in summer.

For winter flocks, a multiple regression analysis was used to examine the relationships between the daily number of chickens on the range and outdoor environmental variables (Table 3-4). The model was significant ($F_{(3,13)} = 23.24$, p < 0.001) and accounted for 84.7% of variance in the number of chickens on the range. Rainfall significantly contributed to the model and accounted for 11.2% of the variation, indicating that higher daily rainfall was associated with less chickens on the range. The majority of the variance was explained by age (68.9%).

For summer flocks, a multiple regression analysis was used to examine the relationships between the number of chickens on the range hourly and both outdoor and indoor (shed) environmental variables (Table 3-4). The most parsimonious model accounted for 34.8% of the variance in the number of chickens on the range hourly ($F_{(6,357)}$ = 32.74, *p* < 0.001). Indoor dew point was the greatest predictor of the number of chickens on the range; accounting for 10.9%

of the variation and indicating that increased shed dew point was associated with fewer chickens on the range. Indoor temperature, age and flock contributed to the model; however, each accounted for less than 5% of the variance (Table 3-4).

Table 3-4 Multiple regression analysis on the number of chickens on the range daily in winter, and hourly in summer (adjusted $r^2 = 0.76$ and 0.35 in winter and summer, respectively). Only variables that significantly contributed to the most parsimonious model are presented.

Predictor	Beta Coefficient (Standardised)	t (5, 360)	Partial Correlation Coefficient
Winter			
Rainfall	-0.34 *	-2.46	-0.34
Age	0.83 **	6.06	0.83
Summer			
Indoor dew point	-0.44 **	-8.35	-0.36
Rainfall (daily)	-0.26 **	-4.80	-0.21
Indoor temperature	-0.22 **	-4.19	-0.18
Wind speed	0.12 **	2.70	0.12
Age	0.22 **	4.61	0.20
Flock	0.18 **	4.61	0.20

* and ** indicates significance at the 0.05 and 0.01 level, respectively.

Pop-hole use

Although access to the range was provided via pop-holes evenly spaced along the shed, chickens in flocks A and C (seasonal replicates within shed 1) predominantly used two of the six available pop-holes (P2 and P3 in Figure 3-1a; $F_{(5,269)} = 5.40$, p < 0.05), which accounted for 47.8% of all range visits. These popholes were located at the front of the shed, adjacent to shade cloth on the range. However, these were not the only pop-holes with adjacent shade cloth. Chickens in flocks B and D (seasonal replicates within shed 2) predominantly used two pop-holes located at the front of the shed (P1 and P2 in Figure 3-1b; $F_{(6,313)} = 6.50$, p < 0.01), accounting for 41% of all range visits. The location of one of these popholes was directly adjacent to resources on the range (trees and shade cloth) but not the other. The number of range visits through a specific pop-hole did not differ according to whether a chicken was entering or exiting the range (shed 1: $F_{(1,269)} = 0.10$, p > 0.05; shed two: $F_{(1,313)} = 0.20$, p > 0.05).

Turning the litter

Data from the final day of ranging before partial depopulation in winter were excluded from all analysis as the farmer "turned the litter". Throughout this day, 17.6% (n = 52) and 30.4% (n = 89) of the tracked chickens accessed the range; 38.5% (n = 20) and 43.8% (n = 39) of these chickens had not accessed the range prior to this day in flocks A and B, respectively.

3.6 Discussion

This study tracked individual broiler chickens on a commercial farm without segregating part of the shed, flock or range. Our results show that not all chickens accessed the outdoor range when given the opportunity. Chickens accessed the range on average three to four times for 1.5 to 2 h every two to three days for eight to 26 min per visit. Chickens did not immediately access the range when first given the opportunity, waiting an average of four days before accessing the range. The number of chickens on the range at one point in time was low, particularly in winter flocks, 7.8 to 10.6% in winter and 32.8 to 36.7% in summer, similar to previous studies using scan sampling methods (Dawkins et al., 2003; Dal Bosco, Mugnai, Sirri, Zamparini, & Castellini, 2010; Chapuis et al., 2011; Durali et al., 2014; Fanatico et al., 2016). However, the actual number of chickens that accessed the range over the course of the study was much higher; 31.2 to 32.8% in winter and 75.4 to 87.3% in summer, highlighting limitations in scan sampling method. Clearly, our understanding of commercial free-range broiler chicken ranging behaviour and implications for welfare will improve with advancement of technology. Currently, there is little technology that is affordable, reliable, and feasible for tracking an individual chicken's precise

location (indoor and outdoor) on commercial farms (Siegford, Berezowski, Biswas, Daigle, Gebhardt-Henrich, Hernandez, Thurner, & Toscano, 2016). We found lower flock percentages of range use compared to previously reported RFID studies in Australia (Durali et al., 2014) and internationally (Chapuis et al., 2011), which may reflect differences in management, flock size, range design, strain (growth rate and length of time the range is available), or geographical differences including climate (Dawkins et al., 2003; Nielsen et al., 2003). Segregating part of the flock may also have increased ranging behaviour in the previous RFID studies, given that the provision of vertical panels (e.g., fences) increases ranging behaviour in free-range laying hens (Rault, van de Wouw, & Hemsworth, 2013). Furthermore, the present study was conducted on larger flock sizes than previous studies. We provide evidence of factors that alter ranging behaviour including time and length of range exposure, shed design and shed environment.

The number of range visits and duration of range visits increased over time. Whether this is an effect of range exposure and familiarization and/or a reflection of age and development remains to be determined. The increased frequency of range visits with age we observed, in agreement with other studies (Christensen, Nielsen, Young, & Noddegaard, 2003; Jones et al., 2007), does not reflect broiler chicken age-related inactivity that has been previously reported in indoor and free-range housed broiler chickens (Weeks, Nicol, Sherwin, & Kestin, 1994; Leone, Christman, Douglass, & Estevez, 2010). However, we could not identify activity levels, and ranging visits may include time spent resting and lying down.

More than 90% of chickens that accessed the range in summer flocks did so prior to partial depopulation for slaughter. Furthermore, ranging behaviour (visits and duration) and the number of chickens on the range increased over the first two weeks until partial depopulation, but stabilised between partial
depopulation and complete depopulation in summer flocks. Hence, we found no evidence that additional ranging opportunities provided beyond two weeks further increased ranging behaviour. In Australia, the typical length of time a fast-growing broiler chicken has to access an outdoor range is four weeks, given that range access is typically provided from 21 days of age, but it can be as little as 15 days if the individual is transported for slaughter at partial depopulation. We provide evidence that ranging opportunities prior to partial depopulation for slaughter are sufficient to establish ranging behaviour, relative to chickens that are permitted to range until complete depopulation.

Fewer chickens accessed the range in winter than summer, in agreement with previous studies (Dawkins et al., 2003; Jones et al., 2007). Furthermore, they made fewer visits and spent less time on the range in winter, compared to summer. Ranging behaviour between flocks in the same season was relatively consistent, despite slight differences in flock size and range designs. Weather variables did not explain much of the variance in the number of chickens on the range in either season; rainfall in winter and shed dew point in summer had the greatest effect on ranging behaviour, each explaining less than 12% of the variance. Wind speed, rainfall, and indoor temperature each accounted for less than 5% of the variance in summer flocks. This may reflect the relatively few days of data collection and/or minimal variation within seasons, or that environmental conditions alone do not directly account for most of the variation observed between seasons.

Increased opportunities to range for summer flocks compared to winter flocks may explain differences in ranging behaviour between seasons. The provision of more ranging opportunities (both number of days and hours per day) was linked to a greater number of chickens on the range, visits to the range and time spent on the range. Relationships between increased opportunities and increased ranging behaviour (number of chickens and time spent on the range) have also been reported in laying hens (Campbell, Hinch, Dyall, Warin, Little, & Lee, 2016).

As a consequence of shorter periods of ranging opportunities in winter flocks, the time of day when the range was available also differed between seasons and may partly explain variation in ranging behaviour. Summer flocks showed evidence of time of day effects on ranging behaviour displaying a diurnal ranging pattern in agreement with previous scan sampling studies (Dawkins et al., 2003; Nielsen et al., 2003). Peak ranging times, including the number of chickens on the range and the number of range visits, occurred between 9:00 and 10:00 h and 18:00 and 19:00 h. Diurnal ranging patterns observed in summer flocks likely reflect diurnal rhythms. Broiler chicken foraging behaviour is typically displayed in diurnal peaks in the morning and evening and can be altered with changes in light intensity (Alvino, Archer, & Mench, 2009). The range offers an ideal environment for foraging behaviours; indeed, foraging and ground pecking behaviours have been shown to be greater on the range compared to inside the shed (Taylor, Hemsworth, Dawkins, Groves, & Rault, 2015). Range access was rarely provided during these preferred times throughout winter. The typical pop-hole opening time in winter was between 11:00 and 12:00 h, closing between 16:00 and 17:00 h, compared to summer opening time 9:00 to 10:00 h, closing between 21:00 and 22:00 h. As such, it may be that broilers do not compensate by ranging at alternative times of the day when range access is not provided at favoured ranging times.

Evidence of range use to avoid negative stimuli was anecdotally observed in the current study; as the number of chickens on the range and the number of first time range users increased on the day the litter was turned in winter flocks. Turning the litter is often a critical management practice in commercial broiler sheds to maintain good litter quality and prevent associated effects on chicken welfare (Haslam et al., 2007). However, turning the litter may cause fear and stress, although controlled studies are lacking. The range area could offer an escape from this negative experience, although this was merely an observation on one day (in two sheds). Unfortunately, we did not track broiler chickens after this event and therefore do not know if chickens that accessed the range for the first time during litter turning would continue ranging on subsequent days.

Higher dew point and temperature inside the shed was predictive of fewer chickens on the range in summer flocks; whether this relationship was similar in winter remains unknown as we did not take these measures. This may be additional evidence that range use may be associated with avoiding negative stimuli such as sub-optimal shed conditions. However, causation cannot be inferred in this study, and it is possible that these findings indicate the effect of chickens on the shed environment, through less chickens ranging, hence higher shed stocking density and consequently higher metabolic heat production raising indoor shed temperature and dew point, rather than environmental conditions in the shed encouraging chickens to range. These results do highlight the importance of monitoring the shed environment in relation to ranging behaviour and considering that range access may be related to negative stimuli rather than associated with a positive aspect of the range environment. The influence of the indoor environment is an aspect often overlooked in ranging studies.

We observed a flock preference for specific pop-holes in both sheds (P2 and P3 in shed one, and P1 and P2 in shed two; Figure 3-1). We could not identify the characteristics of preferred pop-hole location and design; this could be related to areas with human disturbance, brooding areas, location of noisy fans at the rear of the shed or protection from weather extremes (wind or UV light). The preferred pop-holes did not appear related to resources on the range such as trees or shade cloths, despite range resources often being the focus of studies of

ranging preferences (Gordon & Forbes, 2002; Dawkins et al., 2003; Rivera-Ferre, Lantinga & Kwakkel, 2007). Two of the favoured pop-holes were directly under shade cloths in shed one, but not in shed two, and not all pop-holes with adjacent shade cloth on the range were favoured in either shed. Characteristics of favoured pop-holes may consequently affect range use and should be investigated further to optimise transition from the shed environment to the range area.

This study provides knowledge regarding ranging behaviour in commercial free-range broiler chickens in relation to age and management and environment variability. Whilst obtaining data on commercial farms have numerous benefits, there are limitations. We make the assumption that the tracked chickens in each flock are representative of ranging behaviour in the whole flock, as careful sampling methods when choosing focal chickens to tag should theoretically provide a representative subsample of the population. Of greater importance, the results were obtained from one farm in one region of Australia, and means of ranging behaviour may not be representative of the most extensive rangers in the flock (see paper two in this series). Furthermore, our study could not assess how far chickens ranged, what range locations are favoured, or activity levels in the shed and range areas. This study was conducted on one strain of broiler chicken that is typically housed in Australian commercial free-range production systems (Ross 308). Results may differ with slower-growing strains used in other countries for free-range production (e.g., Ross 708 or other strains). As there are few scientific investigations regarding ranging behaviour in broiler chickens, this study provides important knowledge to direct further investigations into the factors affecting ranging behaviour.

3.7 Conclusions

This study is the first to monitor individual ranging behaviour of free-range broiler chickens on a commercial farm without altering flock size or shed environment. Tracking chickens through RFID revealed higher estimates of ranging behaviour than previous studies using scan sampling methods. Ranging behaviour increased from first day of range access for two weeks and stabilised thereafter in summer flocks. Fewer chickens accessed the range in winter flocks than summer flocks. Chickens that did range in winter flocks did so less frequently and for a shorter period of time compared to ranging chickens in summer flocks. However, ranging behaviour was relatively consistent within each season. We found little evidence that seasonal differences in ranging behaviour were solely or directly related to variation in weather. Differences in ranging behaviour between seasons may also be due to reduced ranging opportunities in winter (number of days the range was available and length of time) and the time of day the range was available, although these factors are often inherently linked to weather conditions permitting ranging. This study highlights the importance of obtaining a detailed understanding of the influence of range and shed design and environmental and management factors to provide commercial broiler chickens with optimal conditions to range.

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CHAPTER 4 Ranging behaviour of commercial free-range broiler chickens: Individual variation



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4.1 Simple summary

Although the consumption of free-range chicken meat has increased, little is known about the ranging behaviour of meat chickens on commercial farms. Studies suggest range use is low and not all chickens access the range when given the opportunity. Whether ranging behaviour differs between individuals within a flock remains largely unknown and may have consequences for animal welfare and management. We monitored individual chicken ranging behaviour from four mixed sex flocks on a commercial farm across two seasons. Not all chickens accessed the range. We identified groups of chickens that differed in ranging behaviour (classified by frequency of range visits): chickens that accessed the range only once, low frequency ranging chickens and high frequency ranging chickens, the latter accounting for one third to one half of all range visits. Sex was not predictive of whether a chicken would access the range or the number of range visits, but males spent more time on the range per visit. We found evidence that free-range chicken ranging varies between individuals within the same flock on a commercial farm. Whether such variation in ranging behaviour relates to variation in chicken welfare remains to be investigated.

4.2 Abstract

Little is known about broiler chicken ranging behaviour. Previous studies have monitored ranging behaviour at flock level but whether individual ranging behaviour varies within a flock is unknown. Using Radio Frequency Identification technology, we tracked 1200 individual Ross 308 broiler chickens across four mixed sex flocks in two seasons on one commercial farm. Ranging behaviour was tracked from first day of range access (21 days of age) until 35 days of age in winter flocks and 44 days of age in summer flocks. We identified groups of chickens that differed in frequency of range visits: chickens that never accessed the range (13 to 67% of tagged chickens), low ranging chickens (15 to 44% of tagged chickens) that accounted for <15% of all range visits and included chickens that used the range only once (6 to 12% of tagged chickens), and high ranging chickens (3 to 9% of tagged chickens) that accounted for 33 to 50% of all range visits. Males spent longer on the range than females in winter (p < 0.05). Identifying the causes of inter-individual variation in ranging behaviour may help optimise ranging opportunities in free-range systems and is important to elucidate the potential welfare implications of ranging.

Keywords: poultry; pasture; outdoor; range; meat chicken; welfare; Radio Frequency Identification (RFID)

4.3 Introduction

Broiler chicken ranging behaviour remains poorly understood, particularly on free-range commercial farms. A greater understanding of ranging behaviour can assist to ensure optimal opportunities to range through the provision of adequate environment and management practices, and possibly by selecting pertinent chicken characteristics.

The majority of studies on broiler chicken ranging behaviour to date report variability in range use at flock level (Chapuis, Baudron, Germain, Pouget, Blanc, Juin & Guemene, 2011; Durali, Groves, Cowieson, & Singh, 2014; Taylor, Groves, Hemsworth, & Rault, 2016), which has been attributed to environmental conditions such as resources on the range (such as artificial and natural shelters, hay bales, perches and panels (Gordon & Forbes, 2002; Dawkins, Cook, Whittingham, Mansell, & Harper, 2003; Rivera-Ferre, Lantinga, & Kwakkel, 2007; Rodriguez-Aurrekoetxea, Leone, & Estevez, 2014; Fanatico, Mench, Archer, Liang, Gunsaulis, Owens & Donoghue, 2016) and weather variables (including outdoor temperature and Ultra Violet index (Dawkins et al., 2003; Jones, Feber, Hemery, Cook, James, Lamberth & Dawkins, 2007; Rodriguez-Aurrekoetxea et al., 2014). Yet, very little is known about variation between individual broiler chickens within a flock. Genetics and rearing environments have been shown to alter ranging behaviour within a flock (Gordon & Forbes, 2002; Nielsen, Thomsen, Sorensen, & Young, 2003) but relationships with individual ranging behaviour is unknown.

Heterogeneous ranging behaviour may result in variation in individual welfare and reduce uniformity in flocks. There are various beliefs that accessing an outdoor range will impact an animal's welfare state; some consumers believe that accessing an outdoor range will have positive effects on broiler chicken welfare (e.g., increased expression of natural behaviours) and other groups (such as some farmers and veterinarians) are concerned with negative welfare consequences of range access, such as increased health risks due to increased exposure to parasites and extreme weather conditions (de Jonge & van Trijp, 2013; Howell, Rohlf, Coleman, & Rault, 2016). However, there is very little scientific evidence of the impact of range access on broiler chicken welfare. Chicken welfare assessments are often reported as flock averages, however if variation in ranging behaviour exists then chickens within the same flock may have different welfare implications from ranging depending on the degree of variation. If welfare is compromised with increased range use, productivity (growth) may also be affected and result in reduced flock uniformity; an additional challenge for free-range flock management.

In order to assess whether heterogeneous ranging behaviour exists in commercial broiler chicken flocks, we monitored individual broiler chicken ranging behaviour to determine the variation in ranging behaviour between individuals within commercial free-range flocks.

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4.4 Materials and methods

All animals used in this study were approved by the University of Melbourne Animal Ethics Committee (Approval Number 1413428.3). A full description of the methodology is provided in part one of this paper series "Commercial freerange broiler chicken ranging behaviour 1: factors related to flock variability"; however, it is briefly outlined below.

Study site

Four flocks (A–D) of ROSS 308 broiler chickens were studied across two seasonal replicates on one commercial farm during the Austral winter (flocks A and B) and summer (flocks C and D). All sheds had chicks from the same hatchery, same feed, same manager, and comparable management practices. Seasonal replicates occurred within the same sheds (Shed one: 40.5 m x 9.3 m, housing approximately 6000 chickens, flocks A and C; Shed two 50.5m x 12.3 m, housing approximately 10,000 chickens, flocks B and D). Flocks had access to adjacent range areas (54.1 x 13.9 m and 77.9 x 16.4 m adjacent to the shed wall and 13.6 x 9.3 m and 27.5 x 12.3 m at the back of the shed, for shed one and two respectively) accessible through manually operated 1.3 x 0.4 m doors described hereafter as "pop-holes" and spaced 5.65 m apart, with six pop-holes for shed one and seven pop-holes for shed two. Feed and water were provided ad libitum inside the shed, but never in range areas.

Tracking individual range use

Individual range use was tracked by the Gantner Pigeon Radio Frequency Identification (RFID) System (2015 Gantner Pigeon Systems GmbH, Benzing, Schruns, Austria), with a bespoke program, Chicken Tracker. Chickens (n = 300/flock) were randomly selected and fitted with a silicone leg band that automatically loosened with leg growth (Shanghai Ever Trend Enterprise, Shanghai, China). Each leg band contained a unique ID microchip (Ø4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered as the chickens walked over the antenna. Antennas were attached to both sides of each pop-hole (i.e., indoor and outdoor) to determine the direction of movements by each tagged chicken; allowing calculation of the frequency and duration of range visits for each individual.

Chickens were tracked from the first day that range access was permitted (21 days of age) until a few days before partial depopulation (30–33 days of age) in winter flocks due to logistical reasons. However, chickens in summer flocks were tracked until complete depopulation for slaughter (43–45 days of age). Sex and weight of individuals (flock A: n = 83, flock B: n = 97, flock C: n = 280, flock D: n = 290) were recorded at the end of the study when leg bands were removed.

Statistical analysis

RFID data were cleaned with SASTM (v 9.3, SAS institute Inc., Cary, NC, USA) using a modified macro (Gebhardt-Henrich, Toscano, & Frohlich, 2014). All range visits <10 s were treated as false positives and removed from analysis.

Descriptive data are presented for each flock. Statistical analysis was performed with SPSS statistical software (v. 22, IBM Corp, Armonk, NY, USA).

Latency to access the range data did not meet the criteria of normality; therefore, Spearman's rho correlation coefficients were used to examine relationships between latency to first access the range and total frequency and duration of range visits and duration per range visit, relationships between total time spent on the range, total number of range visits and average time spent on the range per range visit. Chi square analysis was used to determine if there was a difference in the number of females and males that accessed the range. Flock could not be included as a random variable in non-parametric Spearman correlations or chi square analysis, but each correlation and chi square analysis was initially performed on each flock and there were no differences in direction or significance values between flocks within season. Hence, flocks were pooled and are presented within season. Ranging data were log transformed and subsequently met the criteria of normality and homogeneity of variance; hence, General Linear Mixed models were used to determine the effect of sex on the total frequency and duration of range visits, average time spent on the range per visit and the number of days an individual accessed the range, with flock and individual nested within flock as random factors, in addition to running the model both with and without final body weight as covariate. General Linear Mixed models were used to compare the average time spent on the range per range visit between chickens that accessed the range only once and chickens that accessed the range more than once, with flock and individual nested within flock included as random factors and weight as a covariate. Results are presented as raw means ± SE unless otherwise noted.

4.5 Results

Range availability

A full description of range availability is provided in chapter four (section 3.5).

Inter-individual variation in ranging behaviour

There was individual variation in ranging frequency and duration within all flocks (Figure 4-1 and Figure 4-2). The mean number of daily visits made by an individual varied between 0–11.8 and 0–12.7 visits in winter and summer flocks, respectively. The mean time an individual spent on the range daily varied

between 0–76.6% and 0–65.7% of the available ranging time, equivalent to 0–4.3 h and 0–6.8 h, in winter flocks and summer flocks respectively.

The total number of range visits made by an individual varied between 0–71 and 0–167 visits over the course of the study in winter flocks and summer flocks respectively. The total duration an individual spent on the range over the course of the study varied between 0–23.0% and 0–40.2% of available overall ranging time, equivalent to 0 to 8.7 h and 0 to 40.7 h, in winter flocks and summer flocks respectively.



Figure 4-1 Frequency of range visits for individual chickens within each flock (winter flocks: A and B; summer flocks: C and D). Patterns within stacked bars represent the number of chickens (% successfully tracked) in each ranging frequency category, daily mean (**a**) and total number of visits throughout the study (**b**) for each flock.



Figure 4-2 Duration of range visits for individual chickens within each flock (winter flocks: A and B; summer flocks: C and D). Patterns within stacked bars represent the number of chickens (% successfully tracked) in each ranging duration category, daily mean (**a**) and total time spent on the range throughout the study (**b**) for each flock.

Latency to access the range

The number of days before an individual first accessed the range after range access was first provided (hereafter referred to as "latency to access the range") was negatively correlated with an individual's total number of range visits (winter: $r_{(188)} = -0.41$, p < 0.001; summer: $r_{(460)} = -0.44$, p < 0.001) and total duration of range visits (winter: $r_{(188)} = -0.34$, p < 0.001; summer: $r_{(460)} = -0.35$, p < 0.001), but not the mean duration per visit. Latency to access the range was also negatively correlated with the number of days an individual accessed the range in summer flocks ($r_{(460)} = -0.33$, p < 0.01), but not in winter flocks.

When individual ranging data were corrected for number of available ranging days remaining after the range was first accessed, to assess ranging patterns after first range access, latency to access the range was still negatively correlated with range use in both seasons (frequency: winter $-r_{(143)} = -0.24$, p < 0.01; summer $-r_{(450)} = -0.34$, p < 0.00; duration: winter $-r_{(143)} = -0.20$, p < 0.05; summer $-r_{(450)} = -0.31$, p < 0.001).

High frequency ranging chickens

A total of 1434 range visits were recorded in winter flocks (flock A: 573 visits; flock B: 861 visits) and 14,008 range visits in summer flocks (flock C: 5644 visits; flock D: 8364 visits). The top 10% of ranging chickens, based on frequency of range visits, accounted for approximately half of the range visits in winter flocks (flock A: 9 chickens accounted for 57% total range visits; flock B: 10 chickens accounted for 47% total range visits) and one-third of range visits in summer flocks (flock C: 21 chickens accounted for 34% of range visits; flock D: 25 chickens accounted for 89–91% of all range visits, irrespective of season (Figure 4-3). Thus, the bottom 50% of ranked ranging chickens accounted for <15% of total visits; summer: flock A – 13.3% of total visits, flock B – 13.5% of total visits; summer: flock C – 4.8% of total visits, flock D – 5.9% of total visits).



Figure 4-3 The proportion of range visits (% total flock range visits) that was attributed to ranked individuals. Ranging chickens were ranked on the total number of range visits and are displayed from lowest to highest percentage of ranging chickens in each flock; solid lines represent winter flocks (flocks A and B), dotted lines represent summer flocks (flocks C and D). Chickens that did not access the range are not included.

The top 10% of ranging chickens, based on the total time spent on the range, accounted for more than half of the total flock time spent on the range in winter flocks (flock A: 70%, flock B: 54%) and more than one-third of the total flock time spent on the range in summer flocks (flock C: 38%, flock D: 37%). The average time spent on the range per visit did not differ between birds ranked in the top 10%, top 11–49% or bottom 50% in either season (winter: $F_{(2,184)} = 0.4$, p > 0.05; summer: $F_{(2,456)} = 0.3$, p > 0.05).

Chickens that accessed the range once

There was a relatively consistent proportion of chickens, across all flocks that accessed the range only once throughout the study (flock A: 12%, flock B: 10%, flock C: 6%, flock D: 8%). The total number of one-time ranging chickens on a particular day was positively correlated with the number of chickens on the range daily in summer flocks ($r_{(30)} = 0.49$, p < 0.05), but not in winter flocks. Conversely, the total number of chickens that ranged only once was positively correlated with age in winter flocks ($r_{(11)} = 0.63$, p < 0.05), but not in summer flocks.

Chickens that accessed the range only once spent longer on the range during that visit than the average time per visit by chickens that accessed the range more than once in summer flocks (one-time ranging chickens: 31.3 ± 12.4 min/visit, more than once ranging chickens: 22.4 ± 4.3 min/visit; $F_{(1,457)} = 11.5$, p < 0.01), but there was no difference in winter flocks (one-time ranging chickens: 9.9 ± 4.7 min/visit, more than one-time ranging chickens: 6.6 ± 0.9 min/visit; $F_{(1,65)} = 3.46$, p > 0.05).

Individual ranging variation and relationships with sex

The proportion of females and males that accessed the range did not differ (winter flocks: female ranging chickens – 52.9%; male ranging chickens – 47.1%; $\chi^{2}_{(1,180)} = 1.43$, p > 0.05; summer flocks: female ranging chickens – 57.3%; male ranging chickens – 42.7%; $\chi^{2}_{(1, 437)} = 0.05$, p > 0.05). The number of days that males and females accessed the range did not differ (winter flocks: females – 3.7 ± 0.4 days; males – 3.3 ± 0.3 days; $\chi^{2}_{(1, 65)} = 0.46$, p > 0.05; summer flocks: females – 7.6 ± 0.3 days, males – 7.0 ± 0.3 days; $\chi^{2}_{(1, 437)} = 2.09$, p > 0.05).

The overall frequency of range visits did not differ between males and females in both seasons (winter flocks: female -18.8 ± 3.2 visits, male -12.8 ± 2.6 visits; summer flocks: female -35.3 ± 2.3 visits; male -24.3 ± 2.0 visits; all p > 0.05). Noteworthy, when weight was not included in the analysis females (lighter in weight than males) accessed the range more frequently than males in summer flocks ($F_{(1, 442)} = 8.18$, p < 0.01) but not winter ($F_{(1, 66)} = 1.26$, p > 0.05).

Males spent longer on the range overall than females in winter flocks (females:2.0 \pm 0.4 h, males:2.3 \pm 0.4 h; F_(1, 66) = 3.92, *p* = 0.052) but not summer

(females:11.8 ± 0.9 h, males:9.5 ± 0.8 h; $F_{(1, 442)} = 1.14$, p = 0.29). Males spent longer on the range per range visit than females in winter (females:12.7 ± 7.1 min, males:17.3 ± 5.7 min; $F_{(1, 65)} = 5.8$, p < 0.05) but not summer (females:20.3 ± 1.2 min, males:27.1 ± 2.5 min; $F_{(1, 442)} = 0.47$, p > 0.05).

4.6 Discussion

Our results showed that ranging behaviour varied greatly between individuals within flocks from the same hatchery, genetic lines, feed composition and availability, management regime, stock people and environmental and range conditions. In all flocks, not all chickens accessed the range and the number of visits and time spent on the range varied greatly between individuals. Although the data clearly identifies a continuum of ranging variation, we have categorized chickens in this paper for simplicity and acknowledge that such categories are arbitrary. We observed chickens that accessed the range only once, high frequency ranging chickens that accounted for one-third to half of all of the range visits (depending on season) and low frequency ranging chickens ranked in the bottom 50% of all tagged chickens that ranged but accounted for less than 15% of all range visits throughout the study.

The variation in ranging behaviour may reflect differences in the motivation to access the range. The high frequency ranging chickens accessed the range more frequently but also for a longer period of time overall and sooner after range access was first provided. High frequency ranging chickens have also been reported in commercial laying hens (Gebhardt-Henrich, Toscano, & Frohlich, 2014; Larsen et al., 2017) and may be of particular interest to industry and consumers. Consumers that support free-range products often feel betrayed with reports of low range use in commercial flocks, leading to controversy and revised labelling regulations of free-range egg products in Australia for instance (Australian Government, 2016). Determining the characteristics that result in high frequency range use may permit early life environmental interventions or breeding programs to encourage range use. A thorough understanding of such interventions is critical and the appropriate application will depend on the characteristics involved and the outcomes on the welfare of the chicken.

Variation in ranging behaviour between individuals may also reflect individual experiences on the range. The most interesting group in this regard is the chickens that accessed the range only once throughout the study (6-12% of tracked chickens). Although the sample size of this group was low within each flock, it was relatively similar between flocks. One-time ranging chickens were not necessarily 'accidental' range users, because the duration of range visits was greater for chickens that accessed the range once compared to those that accessed the range more than once in summer flocks. Perhaps the first range visit was a frightening experience for these chickens, which may have discouraged the chicken from going out again. Indeed, there are reports of links between exposure to a range environment and fearfulness in broiler chickens (Zhao, Li, Li, & Bao, 2014) and the number of days an individual visits the range and fearfulness in laying hens (Campbell, Hinch, Downing, & Lee, 2016; Hartcher, Hickey, Hemsworth, Cronin, Wilkinson, & Singh, 2016; Hernandez, Lee, Ferguson, Dyall, Belson, Lea, & Hinch, 2014). However, the direct relationship between fearfulness and individual broiler chicken ranging behaviour is unknown. It would be interesting to investigate the ranging experience of these particular chickens that accessed the range only once, such as the individual's location, behaviour and environmental stimuli on the range during this single visit.

Our results demonstrated that it is important to disentangle the effects of sex and weight on ranging behaviour of broiler chickens. Females and males did not differ in their ranging frequency when weight was included in the analysis, as lighter chickens accessed the range more frequently. This suggests that weight should always be included when comparing sex effects on ranging behaviour in broiler chickens, given the marked sexual-dimorphic growth of broiler chickens. Our findings differ from Chapuis et al. (2011) who monitored a slower-growing strain of broiler chicken and conversely found that males made up a higher percentage of the top ranging chickens (60%) and females made up the majority of the lowest ranging chickens (70%). Although Chapuis et al. (2011) did not control for weight, sexually dimorphic contrasts are greater in slow growing broiler than fast growing strains (Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005) and it is likely that the difference in findings between the two studies would be exacerbated if growth was controlled for in their study. We hypothesize that our and Chapuis et al. (2011) findings may reflect temperament differences between sexes of chickens. Independent of weight, we found that males spent more time on the range overall and per visit than females, in winter flocks. Hence, sex characteristics other than weight may be associated with ranging behaviour such as those reported in strains of laying chickens, including fearfulness, exploratory behaviour or social behaviour (Jones, 1977; Vallortigara, Cailotto, & Zanforlin, 1990).

We found a high level of variation in range use between seasons and within flocks. These findings highlight the importance of monitoring individual chickens when investigating relationships between range access and welfare. For example, if we were to measure a welfare indicator on our winter flocks, the likelihood of obtaining a measure from an animal that accessed the range at least once would have been only 33%, and a low 10% chance that the chicken would have accessed the range frequently. Clearly, there is a need to determine individual ranging patterns to understand the welfare implications of range use. In addition, the welfare implications of range restriction (during periods of extreme weather conditions or prior to depopulation, a typical commercial practice) on the behaviour and welfare of chickens that differ in their ranging behaviour and motivation remains to be elucidated.

This study provides details of individual variation in the ranging behaviour of broiler chickens on a commercial free-range farm. However, this study was only conducted on one broiler strain and one farm, and the external validity of the findings to other broiler strains, geographical areas, and farms with different flock sizes and range design is unknown. Further investigation is needed to determine the causal factors for this variation, since variation was observed between individuals in the same flock, with the same breeding and hatching history, same shed and range design and similar management practices. This knowledge could lead to science-based improvements in ranging opportunities of commercial free-range broiler chickens.

4.7 Conclusions

Ranging behaviour varied between individuals within the same commercial flocks, revealing chickens that never accessed the range, chickens that accessed the range only once, low frequency ranging chickens, and high frequency ranging chickens, with the latter accounting for a third to a half of all range visits. Males spent more time on the range than females in winter flocks, but frequency of range visits was related to weight rather than sex in summer flocks.

These findings suggest that individual characteristics and/or early life experience partly determine ranging behaviour in commercial conditions, which subsequently results in heterogeneous flock ranging behaviour. The causes for this inter-individual variation in ranging behaviour within flocks should be investigated to ensure that chickens in free-range systems are best suited to such

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housing conditions and thus facilitate optimal ranging behaviour on commercial farms.

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CHAPTER 5 Ranging behaviour relates to welfare indicators pre- and postrange access in commercial free-range broilers



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5.1 Abstract

Little is known about the effect of accessing an outdoor range on chicken welfare. We tracked individual ranging behavior of 538 mixed-sex Ross 308 chickens on a commercial farm across 4 flocks in winter and summer. Before range access, at 17 to 19 days of age, and post-range access, at 30 to 33 and 42 to 46 days of age in winter and summer flocks respectively, welfare indicators were measured on chickens (pre-range: winter N = 292; summer N = 280; post-range: winter N = 131; summer N = 140), including weight, gait score, dermatitis and plumage condition. Post-ranging autopsies were performed (winter: N = 170; summer: N = 60) to assess breast burn, leg health and ascites. Fewer chickens accessed the range in winter flocks (32.5%) than summer flocks (82.1%). Few relationships between welfare and ranging were identified in winter, likely due to minimal ranging and the earlier age of post-ranging data collection compared to summer flocks. In summer flocks prior to range access, chickens that accessed the range weighed 4.9% less (P = 0.03) than chickens that did not access the range. Pre-ranging weight, gait score and overall plumage cover predicted the amount of range use by ranging chickens in summer flocks (P < 0.01), but it explained less than 5% of the variation suggesting other factors are associated with ranging behavior. In summer flocks post-range access, ranging chickens weighed 12.8% less than non-ranging chickens (P < 0.001). More range visits were associated with lower weight (P < 0.01), improved gait scores (P = 0.02), greater breast plumage cover (P = 0.02), lower ascites index (P = 0.01) and less pericardial fluid (P = 0.04). More time spent on the range was associated with lower weight (P < 0.01) and better gait scores (P < 0.01). These results suggest that accessing an outdoor range in summer is partly related to changes in broiler chicken welfare. Further investigations are required to determine causation.

5.2 Introduction

Consumption of free-range chicken meat has increased, partly driven by consumer belief that access to an outdoor range is good for chicken welfare (de Jonge & van Trijp, 2013; Howell, Rohlf, Coleman, & Rault, 2016; Magdelaine, Spiess, & Valceschini, 2008). However, little is known about whether accessing an outdoor range affects the welfare of broiler chickens. Historically, investigating the welfare implications of range use has been difficult; studies that compared chickens housed in free-range and conventional housing have not monitored the individual ranging behavior but rather flock ranging behavior (Jones et al., 2007; Nielsen, Thomsen, Sorensen, & Young, 2003; Stadig, Rodenburg, Ampe, Reubens, & Tuyttens, 2016; Weeks, Nicol, Sherwin, & Kestin, 1994; Zhao, Li, Li, & Bao, 2014). Yet, there can be variation within a flock and not all broiler chickens access the outdoor range when the opportunity is provided (Chapuis et al., 2011; Durali, Groves, Cowieson, & Singh, 2014; Taylor, Hemsworth, Groves, Gebhardt-Henrich, & Rault, 2017a). With the advancement of technology, tracking individual chicken ranging behavior is now possible on commercial farms (Gebhardt-Henrich, Toscano, & Frohlich, 2014; Taylor et al., 2017a; Taylor, Hemsworth, Groves, Gebhardt-Henrich, & Rault, 2017b). Thus, a more thorough investigation of the welfare implications of accessing an outdoor range is now achievable.

The outdoor range provides a more complex environment than the indoor shed and appears to encourage active and exploratory behaviors (Fanatico et al., 2016; Jones et al., 2007; Weeks et al., 1994). Although relationships have been identified between activity and leg health in broiler chickens (Reiter & Bessei, 1996; Thorp & Duff, 1988), it is unknown if broiler chicken ranging behavior on commercial farms is sufficient to result in improved leg health and decreased associated conditions such as dermatitis and breast burn.

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Durali et al. (2014) indicated that Ross 308 broiler chickens that spent more time on the range (> 8.7 hours in total) had lower body weight after range access than chickens that spent less time on the range (< 1.1 hours total). The cause of reduced body weight in relation to ranging behavior is unknown. However, the consequence of lower body weight, reflecting slower growth rate, may improve chicken welfare by reducing the risk of growth related metabolic diseases, such as ascites, sudden death syndrome and deep pectoral myopathy (Julian, 1998, 2005).

Whilst accessing an outdoor range may impact the welfare of broiler chickens, positively or negatively, some welfare conditions may encourage range use. Nielsen et al. (2003) attributes low range use in faster-growing broiler strains, compared to slower-growing strains, to poor leg health. Indeed, poor gait score is often correlated with reduced activity in broiler chickens (Caplen et al., 2014; Weeks, Danbury, Davies, Hunt, & Kestin, 2000). Thermoregulation may be an additional challenge for chickens that are motivated to range. In Australia, chickens are typically permitted access to an outdoor range at 21 days of age based on the appropriate level of plumage cover. However, individual variation in plumage cover could affect ranging behavior in terms of motivation to visit the range or the duration of range visits. Thermal resistance was found to be greater in free-range broiler chickens compared to conventionally housed chickens, and the authors hypothesized that this reflected plumage cleanliness (Ward, Houston, Ruxton, McCafferty, & Cook, 2001), but they did not assess these measures before the chickens were provided with range access or monitor individual ranging behavior.

Thus, ranging may affect the welfare state of an individual broiler chicken or vice-versa. Therefore this paper aimed to identify relationships between the individual ranging behavior of broiler chickens and welfare indicators in commercial situations, with a focus on health. We hypothesized that chicken welfare prior to range access would be related to subsequent ranging behavior when access to the range was provided and that welfare indicators would be altered after range access in relation to ranging behavior.

5.3 Materials and methods

All animals used in this study were approved by the University of Melbourne Animal Ethics Committee (animal ethics approval number 1413428.3).

Mixed sex Ross 308 broiler chicken flocks (n = 4) were studied from 1 farm in Victoria, Australia, across 2 seasons (winter: flocks A and B; summer: flocks C and D). Flocks contained chickens from the same hatchery with comparable management practices. Chickens were housed in mechanically fan ventilated sheds (Figure 5-1) with adjacent range areas. Shed 1 (40.5 m × 9.3 m) housed 6,000 chickens (flocks A and C) and shed 2 (50.5 m × 12.3 m) housed 10,000 chickens (flocks B and D). Additional natural ventilation was provided when automatic curtains were lowered 1 to 2 m on the side walls of the shed stopping 1 m above the shed floor. The shed wall was solid from the ground to 1 m above, therefore chickens had no visual contact with the range area even when the curtains were fully opened, except through opened range doors. Curtains were automatically raised and lowered dependent on the temperature and humidity in the shed. Stocking density in all flocks was kept below 28 kg/m², achieved by removing 1/3 of each flock at 35 days of age, referred to hereafter as 'partial depopulation'. Food and water were provided *ad libitum* inside the shed, but never on the range. Light (20 to 25 lux) was provided on a 23:1 cycle when chicks were aged 1 to 7 days then a 16:8 cycle until slaughter age excluding 3 days before partial depopulation and before all remaining chickens were transported for slaughter

at 45 to 49 days of age, hereafter referred to as 'complete depopulation', when the

light cycle was 20:4.

Figure 5-1 Diagram of the study sheds and range areas (a) shed 1 which housed flocks A and C and (b) shed 2 which housed flocks B and D.



Range areas (Figure 5-1) were accessible through manually operated doors $(1.3 \times 0.4 \text{ m})$ described hereafter as 'pop-holes'. Flocks were raised according to the Free Range Egg and Poultry Australian (**FREPA**) standards which specify that chickens must be fully feathered before range access can be provided (Free Range Egg & Poultry Australia Ltd, 2015). Therefore chickens were first provided with access to the outdoor range at 21 days of age; initial access to the range at 21 days is typical of Australian industry practice. The number of days and hours per day the range was available for ranging was weather dependent and dictated by farm management. Restriction of range access by farm staff was not dictated by 1 variable (e.g. temperature) but often a combination of various variables (e.g.

low temperature, high rain fall and fast wind speed), but the decisions for restricting range access were not recorded. Range areas > 1 m from the shed were covered in grass and was kept at a length of 10 to 20 cm (based on visual observations) by farm management during periods that the chickens had access to the range. The range area for shed 1 was flat. The range area for shed 2 had an approximate 45° slope beginning 7.5 m from the shed wall. Both range areas were fenced; the back fence was 16 m from an adjacent road for shed 1 and another chicken shed in shed 2. Each range contained natural and artificial structures (Figure 5-1). Both range areas contained 2 rectangle shade cloth structures, 7 to 10 m in length that ran adjacent to the shed wall 3 m into the range and 3 m above 3 pop-holes in each shed.

Tracking individual range use

Individual chicken range use was tracked by the *Gantner Pigeon* Radio Frequency Identification *System* (2015 Gantner Pigeon Systems GmbH, Benzing, Schruns, Austria), with a bespoke program, *Chicken Tracker* that was developed for the use of tracking commercial chickens; previously validated and used on a commercial farm to track laying hens (Gebhardt-Henrich, et al., 2014a; Gebhardt-Henrich, et al., 2014b). Between 3 to 5 days before range access was first permitted, chickens (flock A: N = 146; flock B: N = 146; flock C: N = 139; flock D: N = 141) were randomly selected from 10 evenly spread areas within the shed; locations varied according to width and length of the shed and distance from pop-holes. Chickens were fitted with a silicone leg band (Shanghai Ever Trend Enterprise, Shanghai, China) containing a unique ID microchip (Ø4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered as the chickens walked over the antenna. Antennas were attached to both sides of each pop-hole (i.e. indoor and outdoor) to determine the direction of movements by each tagged chicken; hence permitting calculation of ranging frequency and duration. Antennas were placed in the shed before placement of the chicks to minimize disturbance. Chickens were tracked from the first day that range access was permitted (21 days of age) until 30 to 33 days of age in winter flocks (total 9 to 12 days) and 43 to 45 days of age in summer flocks (total 22 to 24 days). Although chickens in winter flocks were provided with access to the range after partial depopulation, logistical concerns from industry participants restricted the tracking of chickens after partial depopulation.

Welfare assessments

Indicators of welfare were assessed prior to range access (17 to 19 days of age; winter: N = 292; summer: N = 280), described hereafter as "pre-ranging" measures, and nine to 12 days after the range was first available (30 to 33 days of age), described hereafter as "post-ranging I" measures in all flocks (winter: N = 131; summer: N = 144). Welfare indicators were also assessed at 42 to 46 days of age in summer flocks (N = 140), described hereafter as "post-ranging II" measures. Post-ranging II measures were not collected for winter flocks for logistical reasons. Timing of data collections were chosen based on typical Australian industry standards; initial range access at 21 days of age (pre-ranging I data collection), partial depopulation at 35 days of age (post-ranging II data collection).

All tracked chickens were caught and placed in a temporary pen the day before data collection. Welfare indicators were measured on randomly chosen chickens before range access in summer and winter flocks. In summer flocks, welfare indicators were measured on randomly chosen chickens at post-ranging I and chickens that were tested before range access but not at post-ranging I were selected for testing at post-ranging II. Due to minimal ranging in winter flocks, post-ranging I welfare indicators were taken from chickens in winter flocks based on the total number of range visits, selecting non-ranger vs. class of relatively high frequency ranging chickens to ensure sufficient sampling for analysis. Chickens were placed into subgroups in temporary pens based on their ranging frequency class, then randomly chosen from this pool. In all flocks, post-ranging analysis only included chickens that were repeatedly measured (e.g. assessed at both pre- and post-ranging data collections).

At all data collection time points (pre-ranging and post-ranging I and II) sex, weight, gait and body condition scores were collected from each chicken. After pre-ranging data collection, the chickens were fitted with a leg band to track range use and sprayed with blue or green stock paint (FIL Tell Tail, GEA, New Zealand) on tail and wing feathers to identify chickens for post-ranging I and II data collection.

Gait scores were assessed by standing directly behind the chicken and when required encouraging the chickens to walk by slow human approach and gentle tactile contact with a clip board. Gait scores were assessed in less than 30 seconds using a 6 point gait score scale (Kestin, Knowles, Tinch, & Gregory, 1992) and later condensed into 3 scores; normal = score 0, affected = score 1 or 2, or lame = score 3 or 4; no scores of 5 were recorded at any time point. Foot pad dermatitis (**FPD**) was scored using a 5 point scale (Welfare Quality ®, 2009), recording the highest score from either foot. The FPD scores were later condensed into 4 scores, only 3 chickens had the maximum score of 4 throughout all flocks. Therefore, scores 3 and 4 were combined. Hock burn (**HB**) was scored on a binary scale; absence or presence on either leg. Breast plumage cover was score 0 a 4 point scale; plumage cover on breast: 75 to 100% = score 1, 50 to 74% = score 2, 25 to 49% = score 3, and 0 to 24% = score 4. Overall plumage cover was scored on a 3 point scale; overall plumage over (excluding breast area): 75 to 100% = score 1, 50
to 74% = score 2, and < 49% = score 3. Vent, breast, and overall cleanliness was assessed using a 3-point scale, modified from Welfare Quality ® (2009); clean = score 1, discolored = score 2, and severe discoloration and mattered, clumped feathers > 10 cm = score 3.

Gait and body condition assessors were trained to score chickens from videos and live assessments prior to data collection. Assessors were blind to chicken's ranging behavior. Inter-observer reliability (N = 3) for body condition scores was measured once. Intra-observer reliability (N = 10) for gait scores were measured at two time points (17 days of age, 31 days of age) in all flocks and additionally at 45 days of age in summer flocks. Kendall's concordance coefficient was used to determine the level of agreement between observers (0.0 to 1.0; complete disagreement to complete agreement). Intra-observer reliability for condition scores ranged from 0.5 to 0.9 (df = 2; agreement P < 0.05). Intra-observer reliability for gait scores ranged from 0.6 to 0.9 (df = 9; agreement P < 0.01).

Autopsy examinations

Post mortem autopsies were conducted at post-ranging I and II data collections from a randomly selected sub-sample of chickens according to ranging behavior (non-ranger vs. class of relatively high frequency ranging chickens) in winter flocks (flock A: N = 73; flock B: N = 97) and summer flocks (flock C: N = 30; flock D: N = 30). Chickens were euthanized using an intravenous injection of pentobarbitone. The same person performed all post-mortem autopsies and was blind to chicken's ranging behavior. Skin was removed from the abdomen and the top of both legs. The prevalence of breast blisters was assessed before legs were removed from hip joint and both hip joints were scored for femoral head necrosis (FHN) using an 8 point scoring system from Wideman et al. (2012). Scores for each leg were summated and categorized as normal = score

1, femoral head separation = score 2, progressive necrosis = score 3 and femoral head necrosis = score 4. Incisions were made into the tibia of both legs and presence and severity of tibial dyschondroplasia (**TD**) was scored using a 4 point scoring system from Garner, Falcone, Wakenell, Martin, and Mench (2002); no signs of TD = score 0, abnormal cartilage under growth plate = score 1, cartilage extended a 1/4 of the way down the tibiotarsus = score 2 and cartilage extended more than 1/4 of the way down the tibiotarsus = score 3. The body cavity was opened and the presence or absence of fluid in the abdomen and pericardial sac were noted. The heart was removed, stored in 70% ethanol and later dissected to obtain right ventricle and total ventricular weights. An ascites index was calculated (right ventricle : total ventricle weight ratio) as an indicator of pulmonary hypertension and a potential preclinical sign of ascites (Wideman & French, 1999).

Statistical analysis

Radio Frequency Identification data were cleaned with SAS[™] (v 9.3, SAS institute Inc., Cary, NC, USA) using a modified macro (Gebhardt-Henrich, Toscano, et al., 2014). All range visits shorter than 10 s were treated as false positives and removed from analysis. Statistical analysis was performed with SPSS statistical software (v22, IBM Corp, Armonk, NY, USA). Ranging behavior varied greatly between seasons and therefore seasonal replicate data were never pooled or compared. Chickens were excluded from analysis if functional tags were not recovered at the end of the trial; sample sizes presented throughout the manuscript are corrected for chickens excluded from the analysis. Normality of data were assessed by Kolmogorov-Smirnov and Shapiro-Wilk normality test statistics and histograms unless otherwise stated.

In the subsequent result section, we first compared welfare indicators from ranging (**R**) and Non-Ranging (**NR**) chickens, and we then investigated relationships between welfare indicators from R chickens and the amount of range use (total number of range visits and total time spent on the range). Chickens that did not access the range throughout the study were classified as NR chickens and chickens that accessed the range at least once were classified as R chickens. Analysis of welfare indicators and the amount of range use were only performed on R chickens. Ranging was minimal in winter flocks, therefore only summer flocks were analyzed to investigate relationships between welfare and the amount of range use (total number of range visits and total time spent on the range). Relationships between ranging and post-ranging I and post-ranging II welfare indicators were similar in summer flocks. Therefore only post-ranging II results are reported, and described as "post-ranging" indicators.

Comparison of pre-ranging welfare indicators between R and NR chickens and relationships with the amount of range use. Relationships between preranging welfare indicators and R and NR chickens or the total number of range visits, total time spent on the range and were investigated using General Linear Mixed Models (GLMM) for continuous normally distributed data or Generalized Linear Mixed Models (GLIMM) with a binary logistic or multinomial logistic link function for binary and ordinal data respectively. Each model included flock, individual nested within flock, sex and weight as random variables where appropriate. Response variables that were significantly associated with the amount of range use at the $P \le 0.1$ level were included in prediction models.

Predicting ranging behavior R and NR chickens with pre-ranging welfare *indicators*. Indicators of welfare that differed at $P \le 0.1$ level were included in a logistic regression to assess the impact of each welfare indicator on the likelihood that chickens would access the range or not. The most parsimonious models are

reported, determined by goodness of fit tests calculated by Omnibus model coefficients and Hosmer and Lemeshow tests and the amount of variation the model accounted for, determined by Nagelkerke R square values.

Predicting the amount of range use by R chickens with pre-ranging welfare indicators. The total number of range visits data did not meet the criteria for normality, even after transformation. Therefore, a GLIMM with a Poisson distribution and log link function was used to predict the total number of range visits when access to the range was provided with pre-ranging welfare indicators. The number of range visits were dependent variables, welfare indicators were the independent variables and flock, individual nested within flock and sex were random variables.

Total time spent on the range was square root transformed and subsequently met the criteria for normality. Thus, a linear regression model was used to predict the total time spent on the range when access was provided with pre-ranging welfare indicators. Total time spent on the range met the criteria for heteroscedasticity and multicollinearity, confirmed by P-P and residual plots. All possible models were run and the final model included variables that resulted in the best fit, determined by changes in F values (P < 0.05) via a forward stepwise regressions analysis and comparisons of adjusted r² values. The most parsimonious models are reported with statistically useful variables in the model.

Comparison of post-ranging welfare indicators between R *and* NR *chickens and relationships with the amount of range use.* Comparisons between R and NR chicken post-ranging welfare indictors and the relationships between the total number of range visits (frequency) or total time spent on the range (duration) and post-ranging welfare indictors were analyzed with GLMM for continuous data that met the criteria for normality or GLIMM for non-parametric variables. The GLIMM with a multinomial logistic or binary logistic distribution and link function were used to assess ordinal and binary welfare indicators respectively. In all models welfare indicators were dependent variables, individuals were the subject variable and time point of data collection (pre- and post-ranging) were the within subject variable. Interactions between time point of data collection (pre- or post-ranging) and range use (R vs NR, frequency or duration) were included to indicate changes associated with range use independent of pre-ranging differences. However, non-significant interactions were removed (P > 0.05) to improve model fit, confirmed by Akaike Information Criterion values. Flock, individual nested within flock, sex and weight were included as random variables where appropriate.

5.4 Results

Ranging behavior

In winter flocks, 32.5% of tracked chickens accessed the range, whereas in summer flocks 82.1% of tracked chickens accessed the range. Ranging behavior varied between chickens within the same flock (Table 5-1). Full descriptions of flock and intra-individual ranging behavior were previously reported (Taylor et al., 2017a, 2017b). Due to the large variation in ranging behavior between seasonal replicates, seasonal replicate data were analyzed and are presented separately.

Table 5-1 Ranging behavior from first day of range access (21 days) until partial depopulation (30 to 33 days of age; winter and summer flocks) and complete depopulation pick-up (42 to 46 days of age; summer flocks only). Data includes chickens that accessed the range a minimum of one time.

	Wi	nter flocks	Sum	mer flocks
	Mean ± SEM	Min - max	Mean ± SEM	Min - max
Ranging before partial depopulation				
Range availability (days)	7		9.5 ± 1.0	(9 – 10)
Daily range availability (h)	5.6 ± 0.6	(3 – 7.2)	10.8 ± 0.6	(2.0 – 13.5)
Total number of range visits	13.8 ± 1.8	(1 – 71)	21.1 ± 1.2	(1 – 116)
Total time spent on the range (hour)	1.8 ± 0.2	(10 s – 8.7 h)	7.3 ± 0.4	(15 s – 42 h)
Mean duration per range visit (min)	11.9 ± 2.8	(10 s – 2.9 h)	23.8 ± 1.0	(115 s – 1.9 h)
Number of days the range was accessed	3.1 ± 0.2	(1 – 7)	4.7 ± 0.1	(1 – 9)
Ranging before complete depopulation				
Range availability (days)			17.0 ± 1.0	(16 – 18)
Daily range availability (h)			10.4 ± 0.6	(2.0 - 14.0)
Total number of range visits			38.6 ± 2.6	(1 – 151)
Total time spent on the range (h)			12.9 ± 0.9	(20 s – 54.7 h)
Mean duration per range visit (min)			21.7 ± 1.1	(20 s – 1.6 h)
Number of days the range was accessed			8.3 ± 0.3	(1 – 17)

5.4.1 Part I: Comparisons of welfare indicators between R vs NR chickens

Comparison of pre-ranging welfare indicators between R *and* NR *chickens.* In summer flocks prior to range access, R chickens weighed less than NR chickens $(F_{(1,274)} = 4.74, P = 0.03; Table 5-2)$ but there was no difference between R and NR chickens before range access in winter flocks (weight P = 0.61; Table 5-2).

In winter flocks prior to range access, R chickens had more breast plumage cover than NR chickens ($F_{(1,127)} = 4.65$, P = 0.03) but there was no difference between R and NR chickens in summer flocks (P = 0.39).

There was no difference in gait scores, FPD, HB, plumage cleanliness or plumage cover between R chickens and NR chickens before range access in either season (gait score: summer P = 0.22, winter P = 0.74; FPD: winter P = 0.41, summer P = 0.51; HB: summer P = 0.35, winter P = 0.87; vent cleanliness: summer P = 0.75, winter P = 0.27; breast cleanliness: summer P = 0.63, winter P = 0.67; overall cleanliness: summer P = 0.65, winter P = 0.91; overall plumage cover: summer P = 0.32, winter P = 0.47; Table 5-2).

Predicting R and NR chickens with pre-ranging welfare indicators. In winter flocks, there were no welfare indicators that could predict R and NR chickens.

In summer flocks, lower pre-ranging weight was predictive of accessing the range (β : - 4.19, CI: 0.00, 0.89, Exp (B): 0.02, *P* = 0.04). The model correctly classified 81.0% of cases ($\chi^{2}_{(3)} = 14.95$, *P* ≤ 0.01). Including sex improved the model (*P* < 0.05) but did not predict range use (*P* = 0.06).

Comparison of post-ranging welfare indicators between R *and* NR *chickens.* In summer flocks, R chickens gained less weight from pre- to post-ranging than NR chickens (interaction between time point (pre- and post-ranging) and range use (R or NR): $F_{(1,270)} = 15.44$, P < 0.001; Table 5-2). There was no interaction

between ranging, time of data collection and sex (P = 0.97) or sex and range use (P = 0.72) on body weight but there was a main effect of sex ($F_{(1,270)} = 76.38$, P < 0.001). In summer flocks, there was no interaction between pre- and post-ranging gait scores and ranging, but there was a main effect of ranging (gait score $F_{(1,262)} = 4.74$, P = 0.03; Table 5-2). There was no difference between in weight or gait scores between R and NR chickens in winter flocks (weight: P = 0.61; gait scores: P = 0.31) but severe gait scores were rare in winter flocks (Table 5-2).

In summer flocks, R chickens had lower vent cleanliness scores (cleaner), ascites indexes and prevalence of pericardial fluid after range access than NR chickens (vent score: interaction between time point and range use $F_{(1,137)} = 6.66$, P = 0.01; ascites index: $F_{(1,51)} = 6.47$, P = 0.01; pericardial fluid: $F_{(1,45)} = 4.78$, P = 0.04; Table 5-2) but there was no difference in vent cleanliness or cardiovascular measures between R and NR chickens in winter flocks (vent score: P = 0.23; ascites index: P = 0.31; pericardial fluid: P = 0.85; Table 5-2).

In winter flocks there was no interaction between pre- and post-ranging breast plumage cover or overall plumage cleanliness and ranging, however there was a main effect, indicating that R chickens had greater breast plumage cover and cleaner overall plumage than NR chickens (breast plumage cover: $F_{(3,252)} = 3.50$, P = 0.02; overall plumage cleanliness: $F_{(1,250)} = 5.11$, P = 0.03; Table 5-2) but there was no difference in plumage between R and NR chickens in summer flocks (breast plumage cover P = 0.46; overall plumage cleanliness: P = 0.80).

Breast cleanliness scores, overall plumage cover, FPD and presence of HB increased after range access in both seasons (all P < 0.05; Table 5-2). However, there was no difference between R and NR chickens (breast cleanliness: winter P = 0.23, summer P = 0.14; overall plumage cover: winter P = 0.311, summer: P = 0.64; FPD: winter P = 0.34, summer P = 0.95; HB: winter P = 0.26, summer P = 0.11).

Post-ranging FHN scores did not differ between R and NR chickens in either season (winter P = 0.79; summer P = 0.95). Breast blisters, tibial dyschondroplasia and abdominal fluid were never observed.

Table 5-2 Prevalence and comparisons of welfare indicators measured pre-range access (17 to 19 days of age) and post-range access (winter flocks: 30 to 33 days of age; summer flocks 42 to 46 days of age) on chickens that accessed the range when access was provided (R) and chickens that did not (NR), in winter and summer flocks.

		Winter				Summer				
		Pre-rang	ge access	Post-range	e access (I)	Pre-rang	e access	Post-range access (II)		
Welfare indicator	Score	NR	R	NR	R	NR	R	NR	R	
Weight (kg)	Female	0.77 ± 0.01	0.75 ± 0.01	1.77 ± 0.03	1.74 ± 0.05	0.81 ± 0.02 a	0.73 ± 0.01 b	2.85 ± 0.07 a	2.45 ± 0.04 b	
	Male	0.86 ± 0.01	0.85 ± 0.02	2.09 ± 0.03	1.99 ± 0.06	0.86 ± 0.03 a	$0.80 \pm 0.01^{\mathrm{b}}$	3.24 ± 0.09 a	2.97 ± 0.06 b	
	Pooled sex	0.80 ± 0.01	0.81 ± 0.01	1.94 ± 0.03	1.88 ± 0.05	0.81 ± 0.01^{a}	0.77 ± 0.01 a	3.05 ± 0.07 b	2.66 ± 0.04 c	
Foot pad	None	65.9 (139)	64.6 (51)	5.8 (5)	4.5 (2)	79.3 (42)	80.4 (181)	56.5 (13)	49.1 (56)	
dermatitis %	Slight	23.7 (50)	24.1 (19)	20.9 (18)	27.3 (12)	7.5 (4)	16.0 (36)	4.3 (1)	14.0 (16)	
(n)	Moderate	8.5 (18)	7.6 (6)	43.0 (37)	47.7 (21)	9.4 (5)	3.1 (7)	8.7 (2)	20.2 (23)	
	Severe	1.9 (4)	3.8 (3)	30.2 (26)	20.5 (9)	3.8 (2)	0.4 (1)	30.4 (7)	16.7 (19)	
Gait score %	Normal	76.1 (124)	67.9 (36)	41.7 (35)	58.1 (25)	38.5 (20) ^a	26.5 (58) ^a	0.0 (0) ^b	16.8 (19) c	
(n)	Affected	23.9 (39)	32.1 (17)	54.8 (46)	39.5 (17)	61.5 (32)	73.1 (160)	73.9 (17)	76.1 (86)	
	Lame	0.0 (0)	0.0 (0)	3.6 (3)	2.3 (1)	0.0 (0) ^a	0.5 (1) ^a	26.1 (6) ^b	7.1 (8) ^c	
Hock Burn %	Absent	86.7 (182)	88.5 (69)	85.9 (67)	97.7 (42)	98.1 (52)	93.7 (208)	62.2 (15)	69.0 (80)	
(n)	Present	13.3 (28)	11.5 (9)	14.1 (11)	2.3 (1)	1.9 (1)	6.3 (14)	34.8 (8)	31.0 (36)	
Vent	Clean	89.2 (189)	91.1 (72)	12.6 (11)	13.6 (6)	88.7 (47)	90.7 (205)	4.3 (1)	22.8 (26)	
cleanliness %	Soiled	9.0 (19)	8.9 (7)	8.0 (7)	13.6 (6)	7.5 (4)	6.2 (14)	8.7 (2)	21.9 (25)	
(n)	Dirty	0.0 (0)	0.0 (0)	79.3 (69)	72.7 (32)	3.8 (2)	3.1 (7)	87.0 (20)	55.3 (63)	
Breast	Clean	65.6 (139)	65.8 (52)	1.1 (1)	4.5 (2)	26.4 (14)	37.8 (85)	0.0 (0)	0.9 (2)	
cleanliness %	Soiled	32.1 (68)	32.9 (26)	5.7 (5)	15.9 (7)	28.3 (15)	34.2 (77)	4.3 (1)	8.7 (10)	
(n)	Dirty	2.4 (5)	1.3 (1)	93.1 (81)	79.5 (35)	45.3 (24)	28.0 (63)	95.7 (22)	90.4 (104)	
Overall	Clean	69.2 (203)	97.4 (76)	8.4 (7)	28.6 (12)	94.2 (49)	91.6 (196)	4.3 (1)	2.6 (3)	
cleanliness %	Soiled	3.3 (7)	1.3 (1)	73.5 (61)	57.1 (24)	5.8 ₍₃₎	7.9 (17)	78.3 (18)	81.7 (94)	
(n)	Dirty	0.5 (1)	1.3 (1)	18.1 (15)	14.3 (6)	0.0 (0)	0.5 (1)	17.4 (4)	15.7 (18)	

Breast	1 (most)	0.9 (2) ^a	3.8 (3) ^b	1.2 (1)	2.3 (1)	26.4 (14)	23.5 (53)	17.4 (4)	11.2 (13)
plumage	2	10.3 (22)	10.1 (8)	3.5 (3)	4.5 (2)	26.4 (14)	26.4 (53)	17.4 (4)	36.2 (42)
cover % (n)	3	36.2 (77)	39.2 (31)	9.3 (8)	22.7 (10)	15.1 (8)	15.1 (32)	43.5 (10)	30.2 (35)
	4 (least)	52.6 (112)	46.8 (37)	86.0 (74)	70.5 (31)	32.1 (17)	32.1 (31)	21.7 (5)	22.4 (26)
Overall	1 (most)	37.1 (79)	40.5 (32)	36.0 (31)	45.5 (20)	73.6 (39)	59.6 (134)	91.3 (21)	95.5 (109)
plumage	2	54.5 (116)	49.4 (39)	60.5 (52)	47.7 (21)	20.8 (11)	27.1 (61)	8.7 (2)	2.7 (3)
cover % (n)	3 (least)	8.5 (18)	10.1 (8)	3.5 (3)	6.8 (3)	5.7 (3)	13.3 (30)	0 (0)	0.9 (1)
Femoral Head	Normal			70.1 (68)	71.0 (49)			25.0 (5)	29.2 (7)
Necrosis	Separation	NI/A		15.5 (15)	15.5 (11)	NI/	٨	20.0 (4)	38.0 (9)
Score % (n)	Progressive	IN/A		5.2 (5)	10.1 (7)	IN/A		30.0 (6)	4.2 (1)
	FHN			9.3 (9)	2.9 (2)			40.0 (8)	29.2 (7)
Pericardial	Absent	NT/A		54.1 (53)	50.7 (35)	NT/	٨	21.1 (4) ^a	52.9 (9) ^b
fluid % (n)	Present	N/A		45.9 (45)	49.3 (34)	IN/A		78.9 (15)	47.1 (8)
Ascites index	Mean ± SE	N/A		0.19 ± 0.01	0.18 ± 0.01	N/.	A	0.17 ± 0.01 a	$0.16\pm0.01^{ m b}$
Sex	Female	46.0 (40)	43.2 (19)			62.3 (33)	55.7 (122)		
	Male	54.0 (47)	56.8 (25)			37.7 (20)	44.3 (97)		

Differing subscript across rows indicates a significant difference between R and NR chickens and time of data collection (pre-ranging or post-ranging).

5.4.2 Part 2: Relationships between welfare indicators of R chickens and the amount of range use

Relationships between the amount of ranging behavior (number of range visits and total time on the range) are only reported in summer flocks due to minimal ranging in winter flocks.

Relationships between pre-ranging welfare indicators of R chickens and the amount of range use. Chickens with lower pre-ranging weight, more normal gait scores and more overall plumage cover subsequently accessed the range more frequently and for a longer time (weight: ranging frequency $F_{(1,207)} = 11.0$, P = 0.001; ranging duration $F_{(1,207)} = 7.63$, P = 0.01; gait scores: ranging frequency $F_{(1,207)} = 8.45$, P = 0.01; ranging duration $F_{(1,164)} = 7.26$, P = 0.01; overall plumage cover: ranging frequency $F_{(1,212)} = 6.10$, P = 0.01; ranging duration $F_{(1,212)} = 5.02$, P = 0.03).

Plumage cleanliness, breast plumage cover, FPD and HB were not associated with the amount of range use (vent cleanliness: ranging frequency P = 0.10, ranging duration P = 0.11; breast cleanliness: ranging frequency P = 0.76, ranging duration P = 0.93; overall cleanliness: ranging frequency P = 0.76, ranging duration P = 0.94; breast plumage cover: ranging frequency P = 0.41, ranging duration P = 0.34; FPD: ranging frequency P = 0.98, ranging duration P = 0.62; HB: ranging frequency P = 0.14, ranging duration P = 0.51).

Predicting the amount of range use with pre-ranging welfare indicators. Lower weight and better gait scores before range access were significant predictors of more subsequent range visits (weight: $F_{(1,214)} = 16.54$, B = -2.74, CI - 4.09, -1.39, P < 0.01; gait score: $F_{(1,214)} = 6.84$, B = 0.39, CI 0.11, 0.67, P = 0.01). Of note, only 1 lame score was observed at this age (Table 5-2).

Normal gait score and more overall plumage cover before range access were predictive of more subsequent time spent on the range ($F_{(2,212)} = 5.68$, $P \le 0.01$) but

only accounted for 4.2% of the total variance (gait score: *t*_(2,212) = -2.29, CI: -8.95, -0.67, Exp(B): -0.154, *P* = 0.02; overall plumage cover: *t*_(2,212) = -2.33, CI: -5.57, -0.47, Exp(B): -0.156, *P* = 0.02).

Relationships between post-ranging welfare indicators of R chickens and the amount of range use. Lower weight gain from pre- to post-ranging was associated with more range visits and more time spent on the range (interaction between time point (pre- and post-ranging) and amount of range use: total number of range visits $F_{(1,226)} = 21.95$, P < 0.01; total time spent on the range $F_{(1,226)} = 9.67$, $P \le$ 0.01). Greater retention of breast plumage cover from pre- to post-ranging was associated with more range visits (interaction between time point and number of range visits: $F_{(1,226)} = 5.71$, P = 0.02) but not with total time spent on the range (P =0.14).

There was no interaction between pre- and post-ranging gait score and ranging, however there was a main effect of range visits and time spent on the range (total number of range visits: $F_{(1,219)} = 5.70$, P = 0.02; total time spent on the range: $F_{(1,215)} = 10.64$, P < 0.01). A lower ascites index was associated with more range visits ($F_{(1,53)} = 8.50$, P = 0.01) but not total time spent on the range ($F_{(1,53)} = 3.80$, P = 0.06). The presence of pericardial fluid was associated with less time spent on the range ($F_{(1,44)} = 4.37$, P = 0.04) but not the total number of range visits (P = 0.10).

Plumage cleanliness (vent, breast and overall) scores, FPD and HB increased after range access (all P < 0.05) and overall plumage cover scores decreased (more plumage cover) (P < 0.01) but none of these measures were related to the number of range visits (cleanliness: vent P = 0.60, breast P = 0.31, overall P = 0.90; FPD P = 0.39; HB P = 0.16; overall plumage cover P = 0.14) or total time spent on the range (cleanliness: vent P = 0.20, overall P = 0.69; FPD P = 0.79; HB P

0.16; overall plumage cover P = 0.09). The amount of range use was not related to FHN scores (ranging frequency: P = 0.72; ranging duration: P = 0.54).

5.5 Discussion

Individual tracking of ranging behavior revealed that ranging chickens in summer flocks had reduced growth, better gait scores and lower ascites index and presence of pericardial fluid than non-ranging chickens after range access. Furthermore, for summer flocks, higher frequency or duration of ranging was linked to reduced growth, retention of breast plumage, improved gait score and better cardiovascular function (reduced ascites index and fewer instances of pericardial fluid) after range access. Weight, plumage cover and gait score before range access were also associated with the broilers' subsequent ranging behavior in summer flocks, although these welfare indicators only explained a small proportion of the variance in ranging behavior, suggesting that other factors were associated with ranging behavior. Ranging chickens in winter flocks had more breast plumage cover before and after range access and cleaner overall plumage after range access compared to non-ranging chickens but no other differences between ranging and non-ranging birds were identified. It is difficult to determine why there were minimal relationships identified between ranging and welfare in winter flocks, but the variation could be related to seasonal effects, the rarity of severe scores (e.g. gait scores) in winter flocks, the age of data collection or minimal ranging behavior. This represents to date the most comprehensive report of the relationship between ranging behavior and the welfare of free-range broilers at the individual level. We discuss below possible explanations for the relationships identified, although we cannot infer causality.

Pre-range access welfare indicators

Typically, consumers believe that accessing an outdoor range positively affects the welfare of an animal (de Jonge & van Trijp, 2013; Howell et al., 2016). Notably, we provide evidence that the welfare state of a broiler chicken may also influence ranging behavior when the opportunity is provided. In summer flocks prior to range access, chickens that later accessed the range weighed less than chickens that would never access the range and lower pre-ranging weight predicted the total number of range visits, greater overall plumage cover predicted the total time spent on the range and pre-ranging gait scores predicted both the number of visits and total time spent on the range.

Gait scores prior to range access ranged between normal and affected, and only 1 lame chicken was observed. Thus the relationship between reduced ranging activity and higher gait scores prior to range access could have different causes. The inability to differentiate painful leg pathologies and impaired walking ability due to unbalanced body conformation limits the interpretation of the gait scoring method (Caplen et al., 2013; Caplen et al., 2012; Sandilands et al., 2011; Skinner-Noble & Teeter, 2009). However, a self-administering analgesic study by Danbury, Weeks, Chambers, Waterman-Pearson, and Kestin (2000) provides evidence that gait scores of 3 and above in broiler chickens are painful. As we saw only 1 gait score above 2 prior to range access, the relationship between ranging and gait score is likely a reflection of differences in weight and body confirmation, rather than painful leg pathologies. Furthermore, we observed no clinical signs of TD and no relationships between ranging and FHN scores. However, despite measuring TD and FHN, the most common leg pathologies in broilers (McNamee et al., 1999; SCAHAW, 2000), chickens may have been suffering from other leg pathologies.

Previous studies have shown a relationship between the provision of range access and broiler chicken weight (Castellini, Mugnai, & Dal Bosco, 2002; Durali, Groves, & Cowieson, 2012) and specifically higher ranging frequency and lower post-ranging weight (Durali et al., 2014). However, this is the first report of weight difference prior to range access and associations with subsequent ranging behavior. Increased body weight has a negative impact on activity levels in broiler chickens (Bokkers, Zimmerman, Rodenburg, & Koene, 2007; Rutten, Leterrier, Constantin, Reiter, & Bessei, 2002). However, the impact of weight on activity is rarely observed at such an early age (between 17 and 19 days of age) (Bokkers & Koene, 2003; Weeks et al., 1994). Hence, the relationship between preranging weight and subsequent ranging frequency may reflect other variables that were not measured, such as motivation to explore and forage. It is possible for instance that ranging individuals are more active in early life and thus have lower body weight, while being simultaneously more likely to use the range.

In summer flocks, pre-ranging plumage cover (overall) was predictive of more time spent on the range. Feathers contribute to heat loss resistance (Deschutter & Leeson, 1986) and therefore it is plausible that the degree of plumage cover may protect chickens from extreme wind speeds and temperatures on the range. However this effect was not seen in winter, when theoretically it would be more pronounced, although it may not have been evident due to minimal ranging and the shorter period of time the chickens were tracked. Alternatively, it could be a seasonal effect on plumage growth, as Yalcin, Settar, Ozkan, and Cahaner (1997) showed that 4 to 7 week old broilers have greater plumage cover in summer compared to winter, despite relatively small differences in temperature (20 - 27°C). This seasonal effect was apparent in the current study as summer flocks had higher plumage scores compared to winter flocks prior to range access. Our results indicate that the likelihood of accessing

the range in summer is *not* related to plumage cover as we found no difference between ranging and non-ranging chickens, but individuals may choose to spend less time on the range if plumage cover is reduced. A greater understanding of these relationships is required before practical recommendations can be made on ranging opportunities relative to plumage cover.

Post-ranging welfare indicators

The differences in post-ranging body weight in relation to range use could be a sustained effect of pre-ranging lower body weight rather than an effect of range use per se, even though pre-ranging weight was controlled for in our analysis. Previous studies report conflicting results regarding the relationships between body weight and ranging behavior which may reflect variation in strains (growth rate) or ranging behaviour (Weeks et al., 1994; Jones, et al., 2007; Durali, et al. 2014; Tong, Cai, Lu, Wang, Shao & Zou, 2015; Stadig et al., 2016). However, our results are in agreement Durali et al. (2014) who individually tracked ranging behavior of faster-growing broiler chickens and reported a reduction in body weight in relation to more time spent on the range on a commercial farm. Weight reduction related to ranging behavior could be due to redirected energy towards thermoregulation, stress responses, activity levels, consuming alternate feed or a combination of factors and further research is required to clarify the mechanism involved.

Lower (better) gait scores after range access were more prevalent in ranging chickens and were associated with more range visits and more time spent on the range. Such relationships suggests that accessing the outdoor range may improve leg health, in agreement with previous studies using scan sampling methods (Fanatico et al., 2008; Jones et al., 2007). As foraging and active behaviors are observed more frequently on the range compared to the indoor shed (Fanatico et al., 2016; Jones et al., 2007; Weeks et al., 1994), accessing an outdoor range has the potential to improve muscle and bone strength through increased activity (Reiter & Bessei, 1996; Thorp & Duff, 1988). We did not monitor activity or muscle and bone characteristics and thus cannot identify the potential mechanism of the relationships identified. As we did not find any association with FHN or TD we find no evidence that ranging behavior is related to predominant broiler chicken leg pathologies. Furthermore, we cannot infer causation; although we statistically controlled for pre-ranging gait scores we cannot reliably rule out that good leg health encourages ranging or that relationships with gait scores are a reflection of morphological differences (see previous discussion of gait scoring methodology). Nonetheless, we further highlight an important relationship between gait scores and range use that warrants further investigation.

In summer flocks, the ascites index and presence of pericardial fluid was lower in chickens that accessed the range compared to chickens that never accessed the range and was related to the amount of range use. Such results may indicate that better cardiovascular function enables chickens to be more active and subsequently increase range use, alternatively these relationships may indicate improvements to cardiovascular function in response to ranging, although scores remained below the suggested index indicative of subclinical ascites of 0.29 (Wideman, 2001). Herenda and Jakel (1994) indicated that conventionally housed chickens had higher instances of ascites at slaughter age than free-range chickens, but they did not monitor individual ranging behavior or other causes for it. An alternative measure of cardiovascular function that does not require euthanasia should be investigated to infer a causal relationship.

This study highlights the importance of monitoring individual ranging behavior, rather than flock ranging behavior. Tracking the frequency of range use permitted a clearer understanding of relationships between individual ranging behavior and welfare states. However, our findings were only relationships. Evidence of causality would require controlled experiments. Nevertheless, this study provides guidance for future controlled research with outcomes applicable to commercial situations. We observed the welfare implications on 4 flocks across 2 seasons, but all trials were completed on only one farm and 1 broiler chicken strain and thus the extrapolation of these results relate to other farms should be carefully considered, especially as a number of factors influence ranging behavior. However, as post-ranging welfare parameters reported in the current study were measured between 30 and 33 days of age in winter flocks and 42 and 46 days of age days in summer flocks, it is not surprising to find disparity in the presence and severity of some measures as many of the welfare measures are affected by age and growth; ascites (Julian, 1993), leg health (Vestergaard & Sanotra, 1999), dermatitis (Kjaer, Su, Nielsen, & Sørensen, 2006), plumage scores and body weight (Gous, Moran Jr, Stilborn, Bradford, & Emmans, 1999).

5.6 Conclusions

We identified a number of relationships between ranging behavior and welfare, such as improvements in breast plumage cover, gait scores and cardiovascular function, and a reduction in weight which require further research to understand causation and the mechanisms involved. A greater understanding of these relationships will allow for science-based improvements in the welfare of commercial free-range broiler chickens.

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CHAPTER 6 Accessing an outdoor range reduces short term fear responses in commercial free-range broiler chickens



6.1 Abstract

The complex environment of the outdoor range, in contrast to the relatively consistent indoor shed environment, may elicit varying fear responses in individual chickens, which subsequently may account for some of the ranging variation previously reported in free-range broiler chicken flocks. I investigated relationships between fear responses and range use of free-range mixed sex broiler chickens on a commercial farm. I hypothesised that general fearfulness would regulate range use or subsequently would be modulated by range use. Individual range use was tracked (n = 413 chickens) with Radio Frequency Identification (RFID) technology across four flocks and two seasons. Behavioural and physiological indicators of general fearfulness were measured in an Open Field Test (OFT) before range access (winter flocks: n = 145 chickens; summer flocks: n = 269 chickens) and after range access (winter flocks: n = 131 chickens; summer flocks: n = 146 chickens). Before access to the range was provided, there were no differences in fear responses between chickens that subsequently accessed the range and chickens that never accessed the range. However after range access, chickens that accessed the range showed reduced behavioural fear responses compared to chickens that never accessed the range, evident by more vocalisations and lines crossed during the post-ranging OFT (p < 0.01). Furthermore, I identified relationships between the frequency and duration of range visits and incremental reductions of physiological and behavioural fear responses. Hence, accessing an outdoor range reduces general fearfulness in broiler chickens, highlighting the potential positive welfare implications of range use.

6.2 Introduction

Broiler chickens show motivation to explore new environments (Newberry, 1999) and exploratory behaviour is observed more frequently in an outdoor

range environment compared to the indoor shed (Weeks, Nicol, Sherwin, & Kestin, 1994; Knierim, 2000). Nevertheless, not all broiler chickens will access an outdoor range when access is provided (Durali, Groves, Cowieson, & Singh, 2014; Taylor, Hemsworth, Groves, Gebhardt-Henrich, & Rault, 2017a).

Broiler chickens in modern free-range production systems are reared in highly controlled and stable environments often lacking complexity and variation, until access to the outdoor range is permitted. Previous research suggests that animals housed in environments that lack complexity are more likely to be fearful of novel stimuli (Meehan & Mench, 2002). Therefore, the increased novelty, complexity or predation risk when broiler chickens are exposed to the range environment could elicit a potent fear response. Fear is a powerful emotional and motivational state that often outcompetes other motivational systems such as feeding, sex and seeking behaviours (Jones, 1996). Various external stimuli can evoke a state of fear including novel environments or stimuli, evolutionary dangers, conditioned stimuli or social interactions (Gray, 1987; Jones, 1996, 2002). Social isolation is also fear-provoking for social species (Forkman, Boissy, Meunier-Salaün, Canali, & Jones, 2007). Therefore, fear, particularly of novelty and social separation, may limit range use in poultry.

Some individuals are more likely to be frightened of non-specific novel stimuli (Jones, 1996; Hemsworth & Coleman, 2011) which may account for some of the variation in heterogeneous flock ranging behaviour (Durali et al., 2014; Taylor et al., 2017a; Taylor, Hemsworth, Groves, Gebhardt-Henrich, & Rault, 2017b). The propensity for naive individuals to be frightened of novel stimuli is referred to as "general fearfulness" (Price, 1984; Jones, 1996; Hemsworth & Coleman, 2011). General fearfulness is influenced by intrinsic factors including sex, breed or strain (Hemsworth & Coleman, 2011) but may also be modulated by the environment, particularly in the juvenile period (Boissy, 1995). Therefore,

general fearfulness may regulate range use, and additionally may be affected by experiences during range use, perhaps by repeated exposure to novelty (Jones & Waddington, 1992) or the occurrence of frightening experiences (Gallup & Suarez, 1980). The outcome will depend on the intensity and nature of range experiences.

Over-stimulation of fear eliciting stimuli can have detrimental effects to animal health, biological functioning and affective state (Jones & Boissy, 2011) therefore reducing fear is an integral part of safeguarding welfare (Brambell, 1965; Mellor & Beausoleil, 2015). However, exposure to fear inducing stimuli that are of moderate intensity and duration can lead to physiological adaptations that prime animals to cope with stressors encountered later in life (Gross & Siegel, 1980; Khajavi, Rahimi, Hassan, Kamali, & Mousavi, 2003). Chickens in commercial conditions are inevitably exposed to fear-inducing stimuli, such as novel stock person behaviour or clothing, litter management regimes, feed restriction, catching and transport. Accessing an outdoor range may be an effective method to stimulate an adaptive fear response, subsequently allowing chickens to better cope when unavoidable stressors arise from routine management procedures.

Whilst there has been some investigation into the welfare implications of accessing an outdoor range in broiler chickens, relationships between fear and range use are inconclusive. Zhao, Li, Li, and Bao (2014) provided evidence that broiler chickens with range access were more fearful than chickens without access to an outdoor range. Conversely, Stadig, Rodenburg, Ampe, Reubens, and Tuyttens (2016) suggest that broiler chickens with access to an outdoor range were *less* fearful compared to chickens without access to a range. However, neither study monitored individual ranging behaviour. As such, results may reflect differences in various housing aspects between the chickens provided

with or without range access in these studies, rather than direct relationships between fear and range use.

With increased development and accessibility of tracking technology (Siegford, Berezowski, Biswas, Daigle, Gebhardt-Henrich, Hernandez, Thurner, & Toscano, 2016), identifying individual ranging behaviour is now possible. This technology in conjunction with assessment of fear responses prior to and after exposure to the outdoor range may provide a clearer understanding of the relationship between ranging and fear. Such relationships have been shown in individual laying hens (Campbell, Hinch, Downing, & Lee, 2016; Hartcher, Hickey, Hemsworth, Cronin, Wilkinson, & Singh, 2016) but not broiler chickens.

Fear, like other emotional and motivational states, cannot be directly measured. However fear responses can be quantified. Fear responses include both behavioural and physiological modifications in response to fear-inducing stimuli that aid escape or defence, including freezing or avoidance behaviour and activation of the autonomic nervous and neuroendocrine systems (Forkman et al., 2007).

I aimed to identify relationships between ranging behaviour and fear in commercial free-range broiler chickens, by measuring behavioural and physiological fear responses to an Open Field Test (OFT) before and after range access. In addition chronic stress was assessed with faecal glucocorticoid metabolite concentrations. I hypothesised that more fearful chickens prior to range access would not access the range, and that accessing the range more frequently and for a longer period of time would reduce fearfulness after range access.

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6.3 Materials and methods

All animals used in this study were approved by the University of Melbourne Animal Ethics Committee (animal approval number 1413428.3).

Study site

Shed one (flocks A and C) measured 40.5 m × 9.3 m and shed two (flocks B and D) 50.5 m × 12.3 m. Each shed had access to an outdoor range (54.1 × 13.9 m and 77.9 \times 16.4 m adjacent to the shed wall and 13.6 \times 9.3 m and 27.5 \times 12.3 m at the back of the shed, for shed one and two respectively) opening to the west in shed one and north in shed two (Figure 6-1). The maximum outdoor range stocking density was 6.8 birds/m² and 6.2 birds/m² in shed one and two respectively. The range was accessible through manually operated doors $(1.3 \times$ 0.4 m), described hereafter as "pop-holes", spaced 5.65 m apart, with six popholes for shed one (1.3 cm/bird) and seven pop-holes for shed two (0.9 cm/bird). Both range areas were fenced; the back of the range was approximately 16 m from an adjacent road for shed one and another chicken shed in shed two. The range area for shed one was flat. The range area for shed two had a maximum 45° slope beginning 7.5 m from the shed wall. Both ranges were covered with grass, starting one to two meters from the shed wall, kept 10 to 20 cm long throughout periods when the range was available. Anecdotally, no visible degradation of grass greater than one to two meters from the shed was observed during ranging periods. Each range contained natural and artificial structures. Two trees in the shed one range area and three trees in the shed two range area (two to three meters high) were present three to four meters from the shed in each range, located behind the artificial shade cloth (Figure 6-1). Both range areas contained two rectangle shade cloth structures, seven to 10 m in length that ran adjacent to the shed wall three meters into the range and three meters above three pop-holes in each shed (Figure 6-1).



Figure 6-1 Diagram of study sheds and range areas (a) shed one, flocks A and C and (b) shed two, flocks B and D. Pop-holes are numbered (P1–P7) sequentially from the front of the shed (shed access point).

Animals and husbandry

Four flocks (A–D) of mixed sex Ross 308 broiler chickens were studied across two seasonal replicates on one commercial farm in Victoria, Australia: Austral winter (flocks A and B) and summer (flocks C and D). All sheds had chickens from the same hatchery, same feed, same manager, and comparable management practices. Placement of the chicks was made on the same day for winter flocks, and four days apart for summer flocks, with placement day counted as day 0. Each flock contained approximately 6,000 (Flocks A and C) or 10,000 (Flocks B and D) broiler chickens. Indoor stocking density in all flocks was kept below 28 kg/m², achieved by removing approximately one third of the flock (chosen based on their location in the shed) for slaughter around 35 days of age, referred to hereafter as "partial depopulation". The second and final depopulation occurred at 49 days of age and removed all remaining chickens for slaughter, described hereafter as "complete depopulation". The sheds were mechanically ventilated with fans. Natural ventilation was provided when automatic curtains were lowered one to two meters on the side walls of the shed stopping one meter above the shed floor. The shed wall was solid from the ground to one meter above, therefore even when the curtains were fully opened chickens could not see the range area except through opened pop-holes. Curtains opened automatically based on shed temperature and humidity and thus varied daily. Brooding occurred in the back half of the shed and was temperature controlled by gas heaters. Feed and water were provided ad libitum inside the shed, but never on the range. Light (20 to 25 lux) was provided on a 23:1 (Light : Dark) cycle when chicks were aged one to seven days, then a 16:8 cycle until complete depopulation excluding the three days prior to partial and complete depopulation when light cycle was 20:4. A two tiered metallic perch (2.7m long) was provided for every 1000 birds in each shed and plastic red chains were hung (20 cm) from drinker lines spread evenly throughout the shed. In winter flocks, management "turned the litter" during ranging hours on the eighth day of range access. Turning the litter was achieved by rotary hoeing the litter throughout the entire shed.

Chickens were first provided with access to the outdoor range at 21 days of age; according to the Free Range Egg and Poultry Australian (FREPA) accreditation standards chickens must be fully feathered before range access can be provided (Free Range Egg & Poultry Australia Ltd, 2015). Providing first access to the range at 21 days is typical of Australian industry practice. Pop-holes were manually operated by farm staff. The number of days and hours per day the range was available for ranging was weather dependent and dictated by farm management. Restriction of range access by farm staff was not dictated by one variable (e.g. temperature) but often a combination of various variables (e.g. low temperature, high rain fall and fast wind speed). Data regarding reasons for range restriction was not collected, but such management practices are typical of Australian commercial free-range production.

In winter flocks, chickens were tracked from the first day that range access was permitted (21 days of age) for 10 days prior to partial depopulation (30 to 33 days of age). Due to logistical reasons, tracking chickens in winter flocks until complete depopulation was prevented. Chickens in summer flocks were tracked daily from the first day that range access was permitted (21 days of age) for 24 days (flock C) and 21 days (flock D) prior to complete depopulation (43 to 45 days of age). Leg bands were put on three to four days before range access was first provided to allow chickens to habituate to them. No tagged chickens were removed from the flock during partial depopulation in summer flocks.

Tracking individual range use

Individual range use was tracked by the Gantner Pigeon RFID System (2015 Gantner Pigeon Systems GmbH, Benzing, Schruns, Austria), with a bespoke program Chicken Tracker that was developed for the use of tracking chickens; previously validated and used on a commercial farm to track laying hens (Gebhardt-Henrich, Fröhlich, Burose, Fleurent, Gantner, & Zähner, 2014; Gebhardt-Henrich, Toscano, & Frohlich, 2014). Chickens were randomly selected from 10 areas evenly spread within the shed; locations varied according to length and width of the shed and distance from pop-holes. Chickens were fitted with a silicone leg band that automatically loosened with growth (Shanghai Ever Trend Enterprise, Shanghai, China; Figure 6-2). Each leg band contained a unique ID microchip (Ø4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered as the chickens walked over the antenna. Antennas were attached to both sides of each pop-hole (i.e. indoor and outdoor) to determine the direction of movement by each tagged chicken; hence permitting calculation of ranging frequency and duration. Antennas were placed in the shed before placement of the chickens to minimise disturbance. Chickens were marked with blue or green stock paint (FIL Tell Tail, GEA, New Zealand) on tail and wing feathers to identify tagged chickens in order to retrieve leg bands at the end of the study. Chickens were excluded from analysis if tags were not recovered or functional at the end of the trial.



Figure 6-2. Photograph of leg band, with individual identification noted on the exterior. Leg bands contained an individual radio frequency identification microchip that is not visible in this photograph.

Ranging behaviour was tracked on 1,200 chickens but indicators of fearfulness and physiological stress were measured on a sub-sample of these chickens (n = 315 chickens). A Chi-square comparison of the number of chickens that accessed the range and Mann U Whitney non-parametric tests comparing ranging behaviour indicated no significant differences in ranging behaviour between the 1200 chickens reported in chapters four and five and the subpopulation of the experimental flock used in this study (number of chickens that accessed the range: winter p = 0.79; summer p = 1.00; frequency of range visits: p = 0.08, summer p = 0.33; duration of range visits: winter p = 0.39; number of days the range was accessed: p = 0.07, summer p = 0.29).

Quantifying fear responses

Behavioural and physiological fear responses were assessed prior to range access (between 17 and 19 days of age), described hereafter as "pre-ranging" measures, and 9 to 12 days after range access was provided (between 30 and 33 days of age), described hereafter as "post-ranging" measures.

Chickens were randomly chosen prior to range access (winter: n = 145; summer: n = 269) and after range access (winter: n = 131; summer: n = 145). Behavioural and physiological fear responses to an Open Field Test (OFT) were quantified. Faecal glucocorticoid metabolite (FCGM) concentration was measured as an indicator of chronic stress. Chickens were then weighed, fitted with a leg band to track range use and sprayed with blue or green stock paint (FIL Tell Tail, GEA, New Zealand) on tail and wing feathers to identify chickens for post-ranging data collection.

Open field test (OFT)

Chickens were randomly selected from the temporary holding pen and transported out of the shed by hand five to 10 m to an Open Field Test (OFT) arena within a fully enclosed marquee. Square test arenas were 2400 mm² with solid panels 450 mm high, placed on bare ground covered with grass three to seven cm long. Chickens were placed in the centre of the front third of the arena and left for three minutes. A recording of white noise played continuously through a portable speaker located above the test arena to minimise external noise distractions. Behaviour was recorded via GoPro digital camera (model Hero3, GoPro Inc., San Mateo, CA, USA) mounted directly above the arena. At a later time, videos were analysed by two trained observers. A transparent film was overlayed on the video footage and the arena was divided into nine equal size squares and the number of lines crossed was assessed; a line was considered

crossed when both feet passed over a line. Other OFT measures included latency to move, number of vocalisations and binary records (yes/no) of ground pecking and escape attempt. An escape attempt was defined as two legs leaving the ground and jumping into, onto or over the arena wall. Intra-observer reliability ranged from 0.93 to 0.96 (Intraclass Correlation Coefficient: $F_{(1,9)} = 232.1$; p < 0.01; $F_{(1,9)} = 28.6$; p < 0.01).

Blood collection

Chickens were placed on their side on a table with legs extended and the handler lightly covered the chickens head. Approximately, 2 mL of blood was collected from the brachial wing vein with an S-monovette (Sarstedt AG & Co, Nümbrecht, Germany). Blood was collected in less than two minutes as not to reflect handling effects. After collection, blood samples were spun on site at 10 000 rpm for five minutes. The supernatant was collected, stored on ice and frozen at - 20° C for later analysis.

Faecal glucocorticoid metabolite analysis (FCGM)

The secretion and accumulation of stress hormones can be measured in excreta. In poultry, such faecal metabolites reflect secretion of an approximate four hour delay (Dehnhard et al., 2003). Thus FCGM concentration were assessed to reflect basal stress hormone concentrations prior to the OFT challenge. When available, faecal samples were collected from the OFT arena. Faecal samples were only analysed for FCGM concentration in summer flocks. Faecal samples were prepared for analysis by drying samples at 103°C overnight. Cooled samples were ground into a fine powder. A portion (0.2 g) of the ground faecal sample was weighed and placed into 5 mL Eppendorf tube. Two mL of 80% ethanol was added to the sample and placed on an automatic shaker for 30 minutes, supernatant was removed and stored in an Eppendorf tube; this step was

repeated once. Supernatant was centrifuged at 5000 rpm for 15 minutes. The final supernatant was removed and dried with a nitrogen-dryer for four to five hours. Samples were reconstituted in 100µl of 80% ethanol and placed on a shaker for 30 minutes. Glucocorticoid metabolite concentrations were analysed with a commercially available double antibody radioimmunoassay kit using methods outlined in section **Error! Reference source not found.** of the general ethodology chapter.

Corticosterone concentration analysis

Plasma corticosterone concentrations and faecal glucocorticoid metabolite concentrations were measured using a commercially available double antibody radioimmunoassay kit (MP Diagnosistics, Orangeburg, NY, USA) as per manufacturer's instructions with the exception of the dilution factors; 1:4 for plasma and 1:100 for faecal samples dilution factors were required to fall within the standard curve. Duplicates with a coefficient of variation greater than 5% were reanalysed.

Statistical analysis

Radio Frequency Identification data were cleaned with SAS[™] (v 9.3, SAS institute Inc., Cary, NC, USA) using a modified macro (Gebhardt-Henrich, Toscano, et al., 2014). All range visits shorter than 10 s were treated as false positives and removed from analysis.

All statistical analysis was performed with SPSS statistical software (v22, IBM Corp, Armonk, NY, USA). Normality of data were assessed by Kolmogorov-Smirnov and Shapiro-Wilk normality test statistics and histograms unless otherwise stated. Ranging behaviour varied greatly between seasons and therefore seasonal replicate data were never pooled or compared. Chickens were excluded from analysis if functional tags were not recovered at the end of the
trial. Data are presented as seasonal replicates, with flock included in each statistical model.

Comparisons of pre-ranging fear responses between Ranging (R) and Non-Ranging (NR) chickens were achieved with Chi-square analysis for binary data and ANOVAs or non-parametric Mann U Whitney tests for continuous data dependent on the distribution. Normality of data were assessed by Kolmogorov-Smirnov and Shapiro-Wilk normality test statistics and histograms. Latency to move during the OFT was analysed with Cox survival analysis to account for censored data and included weight and flock as covariates.

Due to minimal ranging in winter flocks, relationships between the amount of range use and fear responses were only analysed with data from summer flocks. Indicators of the amount of range use included the total number of range visits (frequency), the total time spent on the range (duration) and latency to access the range, defined as the number of days that it took for a chicken to access the range the first time, relative to the first day that range access was provided. Plasma corticosterone and FCGM data met the assumptions of normality and were analysed with a general linear mixed model (GLMM). The number of lines crossed and vocalisations during the OFT were analysed with a generalised linear mixed model (GLIMM) with a Poisson distribution and a log link function. Escape attempt, defecation and ground pecking during the OFT were analysed with a GLIMM with a binary distribution and logit link function. All models included the amount of range use as fixed factor and flock, sex, weight and individual nested within flock as random variables where appropriate. Latency to move during the pre-range OFT was analysed with Cox survival analysis and included amount of ranging as a fixed factor, weight, sex and flock as covariates. Response variables that were significant at the $p \leq 0.1$ level were included in a linear regression model to predict the amount of range use. Frequency data were log transformed and duration data were square root transformed and subsequently met the criteria for normality, heteroscedasticity and multicollinearity, confirmed by residual plots. A maximum of five variables were included in the regression model. The most parsimonious models are presented with statistically useful variables in the model, determined by goodness of fit tests calculated by Omnibus model coefficients and Hosmer and Lemeshow tests and the amount of variation the model accounted for, determined by Nagelkerke R square values.

Post-ranging data were analysed with the aforementioned GLMM or GLIMM models, however included repeated measures to identify changes associated with range use independent of pre-ranging differences. All models included time point of data collection (pre- or post- ranging) and range use (R vs NR or frequency, duration or latency of range use) as fixed factors and interactions, but non-significant interactions were removed to improve the model fit, confirmed by Akaike Information Criterion values.

6.4 Results

Ranging behaviour

Fewer chickens accessed the range in winter flocks (30.7% tagged chickens) than summer flocks (81.5% tagged chickens). Chickens that did access the range in winter, did so less frequently and for a shorter period of time than ranging chickens in summer flocks (Mean frequency: winter 10.8 ± 2.0 range visits, summer 32.5 ± 2.2 range visits; Mean duration: winter 1.3 ± 0.3 h, summer 11.3 ± 0.1 h). Individual ranging behaviour varied within each flock. A full description of ranging behaviour is reported in chapter four and five.

6.4.1 Comparisons of fear responses between Ranging (R) and Non-Ranging (NR) chickens

Pre-ranging fear responses

Behavioural and physiological fear responses to the OFT did not differ between R and NR chickens before range access in either season (latency to move: winter p = 0.21 summer p = 0.59; number of vocalisations: winter p = 0.66 summer p = 0.46; number of lines crossed: winter p = 0.98 summer p = 0.20; escape attempt: winter p = 0.54 summer p = 0.66; ground pecking: winter p = 1.00 summer p = 0.75; defecation: summer p = 0.54; plasma corticosterone concentration: winter p = 0.94summer p = 0.62; Table 6-1). Faecal Glucocorticoid Metabolites (FGCM) did not did not differ between R and NR chickens before range access in summer (p =0.68; Table 6-1).

Post-ranging fear responses

In both seasons, NR chickens crossed fewer lines and vocalised less during the post-ranging OFT compared to R chickens (interaction between time point (pre- and post-ranging) and range use: winter: lines crossed $F_{(1,202)} = 6.5$, p = 0.01; vocalisations $F_{(1,109)} = 7.7$, p = 0.01; summer: lines crossed $F_{(1,262)} = 13.8$, $p \le 0.001$; vocalisations $F_{(1,132)} = 4.1$, p = 0.04; Table 6-1). In both the pre- and post-ranging OFT, R chickens were more likely to ground peck compared to NR chickens in winter flocks ($F_{(1,116)} = 6.9$, p = 0.01; Table 6-1) but not summer flocks ($F_{(1,263)} = 2.8$, p = 0.09).

Escape attempt, defecation, latency to move and corticosterone concentrations did not differ between R and NR chickens after range access in either season (escape attempt: winter p = 0.91; summer p = 0.12; defecation: summer p = 0.06; latency to move: winter p = 0.09; summer p = 0.62; acute plasma

corticosterone response: winter p = 0.54; summer p = 0.96; FGCM: summer p = 0.86; Table 6-1).

In summer flocks, chickens that defecated during the post-ranging OFT were more likely to attempt to escape the arena (Escape attempt: defecating chickens 62.1% (n = 18); chickens that did not defecate 48% (n = 26); $\chi^{2}_{(2,109)} = 7.73$, p = 0.005). This may be reflect a sampling bias. However, no other measure of fearfulness differed between chickens that defecated during the OFT and those that did not (plasma corticosterone: $F_{(1,206)} = 0.11$, p = 0.75; latency to move: $F_{(1,212)} = 0.20$, p =0.65; lines crossed: $F_{(1,212)} = 3.43$, p = 0.07; vocalisations: $F_{(1,212)} = 0.39$, p = 0.53; Ground pecking: $\chi^{2}_{(2,108)} = 6.79$, $p \le 0.001$). Table 6-1 Raw means and standard error (SE) of fear responses during the OFT before and after range access in winter and summer flocks and Faecal Glucocorticoid Metabolites (FGCM) in summer flocks. Chickens are categorised based on range use (R = accessed the range at least once; NR = did not access the range).

		Winter			Summer				
		Pre-range	e access	Post-ran	ge access	Pre-rang	ge access	Post-ran	ge access
Fear Response		R	NR	R	NR	R	NR	R	NR
Latency to move (s)		139.3 ± 12.1	123 ± 7.1	49.1 ± 6.6	73.7 ± 6.6	33.4 ± 3.3	31.5 ± 4.3	14.4 ± 2.7	19.2 ± 5.5
Lines crossed		2.2 ± 0.5^{a}	1.8 ± 0.3^{a}	3.3 ± 0.5^{b}	1.3 ± 0.2^{a}	8.2 ± 0.6^{a}	6.0 ± 0.8^{a}	$9.2 \pm 0.8^{\mathrm{b}}$	$5.1 \pm 0.8^{\circ}$
Vocalisations		55.1 ± 8.3^{a}	59.4 ± 4.8^{a}	55.5 ± 4.5^{a}	$43.4 \pm 3.9^{\mathrm{b}}$	138.1 ± 3.4^{a}	131.3 ± 6.3^{a}	$91.7 \pm 4.3^{\mathrm{b}}$	$77.6 \pm 6.7^{\circ}$
Escape attempt % (n)	No	100.0 (38)	98.1 (105)	90.9 (40)	94.3 (82)	84.4 (179)	82.5 (47)	73.4 (80)	83.3 (30)
	Yes	0 (0)	1.9 (2)	9.1 (4)	5.7 (5)	15.6 (33)	17.5 (10)	26.6 (29)	16.7 (6)
Ground pecking % $_{(n)}$	No	89.5 (34)	98.1 (105)	58.1 (25)*	81.6 (71)*	92.9 (197)	96.5 (55)	74.0 (77)	88.6 (31)
	Yes	10.5 (4)	1.9 (2)	52.9 (18)*	$18.4_{(16)}^{*}$	7.1 (15)	3.5 (2)	26.0 (27)	11.4 (4)
Plasma corticosterone (ng/mL)		19.3 ± 0.9	20.4 ± 0.5	7.0 ± 0.6	7.7 ± 0.4	17.2 ± 0.4	16.1 ± 0.7	9.4 ± 0.5	9.1 ± 0.8
Defecation (%) (n)	No	N/2	A	N/A		39.1 (43)	33.3 (12)	40.0 (44)	13.9 (5)
	Yes					60.9 (67)	66.7 (24)	60.0 (66)	86.1 (31)
FGCM (ng/mL)		N/2	A	N	/A	40.7 ± 1.3	42.6 ± 2.9	47.4 ± 2.4	47.5 ± 3.9

* indicates significant difference between R and NR chickens within season and time point of data collection (e.g. pre- or post-ranging). Different letters across rows within season indicate interactions between R and NR chickens and time point of data collection.

6.4.2 Relationships between the amount of range use and fear responses

Relationships between the amount of range use and fear responses were only analysed for the R chickens in summer flocks, due to minimal ranging in winter flocks.

Relationships between pre-ranging fear responses and the amount of range use

Pre-ranging acute plasma corticosterone response to the pre-ranging OFT and FCGM concentration were negatively correlated with the subsequent time spent on the range (acute plasma corticosterone response: $F_{(1,201)} = 5.2$, p = 0.02, estimate - 0.002, SE 0.0001, t = - 2.3, CI - 0.003, - 0.001; FCGM: $F_{(1,110)} = 4.0$, p = 0.05, estimate - 0.005, SE 0.002, t = - 2.0, CI - 0.0105, - 1.483⁵) but not the subsequent total number of range visits (acute plasma corticosterone response: p = 0.22; FCGM: p = 0.08) or latency to access the range (acute plasma corticosterone response: p = 0.30; FCGM: p = 0.30; FCGM: p = 0.58).

Chickens that subsequently accessed the range more frequently and for longer were less likely to defecate during the pre-range OFT (frequency: $F_{(1,207)} = 8.5$, $p \le 0.001$, estimate - 0.019, SE 0.007, t = - 2.8, CI - 0.032, - 0.006; duration: $F_{(1,205)} = 5.1$, p = 0.03, estimate - 0.001, SE 0.001, t = - 2.3, CI - 0.001, - 9.045⁵). Latency to access the range was not associated with the likelihood of defecating during the OFT (p = 0.07).

There were no relationships identified between the latency to move, number of lines crossed or vocalisations, escape attempt, defecation or ground pecking during the pre-ranging OFT and the number of range visits, time spent on the range or latency to access the range (latency to move: frequency p = 0.40; duration p = 0.50; latency p = 0.11; number of lines crossed: frequency p = 0.41; duration p= 0.51; latency p = 0.85; number of vocalisations: frequency p = 0.41; duration p =0.61; latency p = 0.49 escape attempt: frequency p = 0.76 duration p = 0.67; defecation: frequency p = 0.21; duration p = 0.36; latency p = 0.55; ground pecking: frequency p = 0.93; duration p = 0.79).

Predicting the amount of range use with pre-ranging fear responses

The most parsimonious model that predicted the number of range visits included pre-ranging acute plasma corticosterone response, pre-ranging FCGM concentration, the number of vocalisations during the pre-ranging OFT and pre-ranging weight ($\chi^{2}_{(2,108)} = 6.79$, $p \le 0.001$) and accounted for 17.7% of the variance. Lower acute plasma corticosterone response, more vocalisations during the pre-ranging OFT and lower body weight before range access were predictive of more subsequent range visits (acute plasma corticosterone response: $\beta = -0.21$, p < 0.05; vocalisations: $\beta = 0.25$, p = 0.01; weight: $\beta = -0.26$, p = 0.01). FCGM concentration improved the model fit but was not predictive of the total number of range visits ($\beta = -0.12$, p = 0.17).

The most parsimonious model that predicted the total time spent on the range included pre-ranging acute plasma corticosterone response and pre-ranging FCGM concentration ($\chi^{2}_{(2,112)} = 5.42$, p = 0.01) and accounted for 7.3% of the variance. Lower acute plasma corticosterone response and FCGM concentration before range access were associated with subsequently longer time spent on the range (acute plasma corticosterone response: $\beta = -0.22$, p < 0.05; FCGM concentration: $\beta = -0.20$, p = 0.05).

Relationships between post-ranging fear responses and the amount of range use

More vocalisations during the post-ranging OFT was associated with more range visits and time spent on the range (interaction between time point and range use: frequency $F_{(1,202)} = 22.5$, p < 0.001, estimate - 0.004, SE 0.001, t = - 4.74, CI - 0.005, -0.002; duration $F_{(1,193)} = 4.8$, p = 0.03; estimate - 0.024, SE 0.011, t = - 2.13, CI - 0.047, - 0.001). More lines crossed during the post-ranging OFT was

associated with more time spent on the range (interaction between time point and range use: $F_{(1,193)} = 4.8$, p = 0.03; estimate - 0.017, SE 0.0080, t = - 2.18, CI - 0.033, - 0.002) but not the number of range visits ($F_{(1,204)} = 0.1$, p = 0.87).

Lower acute plasma corticosterone response to the pre- and post-ranging OFT was associated with more range visits ($F_{(1,102)} = 4.2$, p = 0.04, Estimate - 0.042, SE 0.02, t = - 2.1, CI - 0.083, - 0.0014) but not time spent on the range (p = 0.056).

The amount of range use was not related to FCGM concentration (frequency: p = 0.48; duration: p = 0.53), latency to move (frequency: p = 0.53; duration: p = 0.90), ground pecking (frequency: p = 0.35; duration: p = 0.34) or escape attempt (frequency: p = 0.49; duration: p = 0.85) during the post-ranging OFT.

A summary of study results is provided in Table 6-2.

Table 6-2 Summary of results: p-values identify differences in behavioural and physiological fear responses between Ranging (R) Non-Ranging (NR) chickens in winter and summer flocks. Significance p < 0.05 indicated by grey shading. Relationships between number of range visits and total time spent on the range and behavioural and physiological fear responses for R chickens in summer flocks (r values indicate the strength and direction of relationship, significant relationships at p < 0.05 significance level are shaded grey).

Fear Response	Winter R v NR	Summer R v NR	Summer Total number of range visits	Summer Total time spent on the range
Pre-ranging				
Open field				
Latency to move (s)	NS	NS	NS	NS
Lines crossed	NS	NS	NS	NS
Vocalisations	NS	NS	NS	NS
Escape attempt	NS	NS	NS	NS
Ground pecking	NS	NS	NS	NS
Plasma corticosterone	NS	NS	NS	$0.02~(\mathrm{LD} < \mathrm{SD})$
Defecation		NS	$\leq 0.001~({\rm HF}$ < LF)	0.03 (LD < SD)
FCGM		NS	NS	$0.05~(\mathrm{LD} < \mathrm{SD})$
<i>Post-ranging</i> Open field Latency to move (s)	NS	NS	NS	NS
Lines crossed	0.01 (R > NR)	< 0.01 (R > NR)	NS	0.05 (LD > SD)
Vocalisations	0.01 (R > NR)	0.05 (R > NR)	< 0.001 (HF > LF)	< 0.01 (LD > SD)
Escape attempt	NS	NS	NS	NS
Ground pecking	0.01 (R > NR)	NS	NS	NS
Plasma corticosterone	NS	NS	$0.04~(\mathrm{HF} < \mathrm{LF})$	NS
Defecation		NS	NS	NS
FCGM		NS	NS	NS

NS denotes no difference (p > 0.05). FGCM denotes Faecal Glucocorticoid Metabolites. HF and LF denotes high and low frequency of range visits respectively. LD and SD denotes long and short duration of range visits respectively.

6.5 Discussion

After only 9 to 12 days of ranging opportunities, chickens that accessed the range were less fearful in the post-ranging OFT than those that did not access the range. In addition, lower post-ranging fear responses were related to higher frequency and duration of range visits. There was no difference between ranging and non-ranging chickens prior to range access, suggesting that fearfulness diverged during the time of range exposure.

OFT behaviour

The magnitude and characteristic of fear responses in the OFT are considered to reflect the underlying propensity to be frightened of novel non-specific stimuli (Forkman et al., 2007). Studies that exposed chickens to intuitively fear-eliciting stimuli prior to an OFT, such as an electric shock or anxiogenic drug administration, resulted in decreased vocalisations and locomotion (Gallup & Suarez, 1980; Moriarty, 1995). Conversely, the provision of enrichment has shown to subsequently increase OFT locomotion, vocalisations and ground pecking (Jones & Waddington, 1992) and anxiolytic drug administration decreased latency to move (Salvatierra & Arce, 2001). Whilst interpretation of behavioural fear responses is complex, based on the aforementioned OFT findings I interpret fewer vocalisations, longer latency to move and fewer lines crossed during the OFT as indicative of increased general fearfulness. In addition to the above literature, the correspondence between the OFT measures in our study provides some validity for the interpretation of differences in fear.

Comparisons of behavioural fear responses between ranging and non-ranging chickens

The OFT fear responses of chickens that subsequently accessed the range later in life did not differ before range access from those that did not access the range. Fear responses of free-range laying hens and broiler chickens are often measured after range access is provided (Campbell et al., 2016; Hartcher et al., 2016; Zhao et al., 2014) and thus it is difficult to determine if such relationships between fear responses and range use are related to individual characteristics and/or are altered by accessing the range. I provide support for the latter, including significant interactions between range use and time. Chickens that accessed the range were more active and vocalised more during the post-range OFT than chickens that did not access the range. Such behavioural responses indicate that accessing the range reduces general fearfulness relative to chickens that never access the range (Forkman et al., 2007). However, I fully acknowledge that such results indicate relationships and not causation. It is possible that the reduction in fear responses were related to alternative variables during the period of time that range access was provided. Further controlled investigations can determine causation and identify the mechanisms involved.

Our results contradict Zhao et al. (2014) who provided evidence that chickens with access to an outdoor range were more fearful than chickens without access, and Stadig et al. (2016) who suggested that fearfulness after range access was not related to the provision of range access. I provide evidence specifically related to individuals accessing the range, whilst Zhao et al. (2014) and Stadig et al. (2016) could not assess which chickens accessed the range. Housing differences between groups with or without range access may have accounted for the differences in fearfulness in the aforementioned studies, rather than range use per se. Furthermore, Zhao et al. (2014) and Stadig et al. (2016) quantified fear responses using Tonic Immobility (TI) enforced by manual restraint. As TI is an innate antipredator response, such behavioural responses to the TI test may reflect specific fear responses related to predators (Jones, 1986) which may differ to OFT stimuli that elicit fear responses to novelty. However, novelty, handling and social isolation are commonalities between the two tests and fear responses between TI and OFT are often correlated or accumulative (Suarez & Gallup, 1981; Jones & Mills, 1983). Reduced locomotion during the OFT observed in this study by nonranging chickens is in agreement with findings from free-range laying hen OFT studies (Hernandez et al., 2014; Campbell et al., 2016). Importantly, Campbell et al. (2016) monitored individual hen ranging behaviour and quantified fear responses with a variety of tests, identifying relationships between OFT

behaviour and range use but not TI. Results from the current study suggest that range use reduces general fearfulness as assessed by the OFT.

Exposure to novelty and fear-provoking stimuli are unavoidable in commercial broiler chicken production systems due to the necessity of routine management procedures, such as turning the litter, feed restriction and transportation. The reduction in fear responses associated with range use observed in this study may enable ranging chickens to cope better when such stimuli are encountered. A reduction in fear responses to such inevitable challenges may highlight positive implications of accessing an outdoor range on broiler chicken welfare, and should be further investigated.

Relationships between behavioural fear responses and the amount of range use

The frequency and duration of range use was related to OFT behaviour. Acute and chronic physiological fear responses to the pre-ranging OFT and the likelihood that a chicken would defecate during the pre-ranging OFT were related to the amount of subsequent range use and significantly contributed to ranging prediction models. However, prediction models explained less than 8% and 18% of the variance in total duration and total frequency of range visits respectively, suggesting that other factors modulate ranging frequency and duration. It should be recognised that in addition to fear-provoking stimuli, physical stressors such as reduced feed intake and increased activity can elicit an acute stress response (Cockrem, 2007).

Fear responses during the post-ranging OFT were associated with the amount of range use, with incremental reductions in fear responses as range use increased. Repeated exposure to novel stimuli in the range environment likely resulted in habituation to novelty and subsequently reduced general fearfulness. Numerous studies provide evidence that moderate exposure to a stressor is beneficial; decreasing morbidity and mortality relative to unstressed counterparts (Zulkifli and Siegel, 1995). However, exposure to intense and chronic stressors inhibit adaptation and compromise welfare. Therefore exposure to moderate fear inducing stimuli should be present in an environment to benefit chicken welfare. I provide evidence that the range environment may be a suitable stressor.

Limitations

Fear responses were measured by a well validated OFT (Forkman et al., 2007). The repeated exposure to the OFT arena may have altered behavioural responses. For instance, great tits have been shown to increase exploration during an OFT arena after repeated exposure due to learning and habituation (Dingemanse, Both, Drent, van Oers, & van Noordwijk, 2002) and repeated exposure to OFT arenas reduces broiler chicken ambulation and vocalisations (Balážová & Baranyiová, 2010). Although the two tests in the current study were separated 11 to 16 days apart, it is difficult to disentangle effects from repeated exposure and age (Weeks et al., 1994). Although habituation to the arena may have occurred, differences related to ranging behaviour were still apparent between chickens that accessed the range compared to chickens that did not.

Although there is a general consensus that the OFT assesses general fearfulness (Forkman et al., 2007), there is an additional element of social isolation that may have influenced fear responses. Testing chickens in pairs in addition to social isolation would help disentangle fear responses specific to novelty (general fearfulness) and social isolation.

6.6 Conclusions

I found no evidence that general fearfulness is related to willingness to access the outdoor range in broiler chickens. However, after range access was provided, the fear response of chickens that accessed the range was reduced relative to chickens that never accessed the range, and relative to the amount of ranging, suggesting that general fearfulness is modulated by exposure to the complex range environment. Whilst controlled experiments are required to elucidate the mechanisms involved, these results suggest that accessing an outdoor range may have positive implications for broiler chicken welfare by reducing fearfulness.

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CHAPTER 7 Frequent range visits further from the shed are positively related to broiler chicken welfare



7.1 Abstract

Ranging chickens often aggregate in range areas close to the shed causing detrimental effects to the range environment. However, the relationship between ranging distance and broiler chicken welfare are largely unknown. The ranging behaviour of 305 mixed sex Ross 308 chickens was tracked on a commercial farm from the second day of range access to slaughter age (16 to 42 days of age). Chickens were categorised into close-ranging (CR) or distant-ranging (DR) categories based on the frequency of visits less than or greater than 2.7 m from the home shed, respectively. Half of the tracked chickens (n = 153) were weighed at seven days of age, and from 14 days of age their body weight, foot pad dermatitis, hock burn and gait scores were assessed weekly. The remaining tracked chickens (n = 152) were assessed for fear and stress responses, only prior to and after range access was provided, at 12 and 45 days of age respectively. Fear and stress responses included, physiological stress responses to 12 minute confinement in a transport crate followed by behavioural fear responses to a tonic immobility test. Weight at seven and 14 days of age predicted DR chickens (p =0.05). After range access was provided, DR chickens weighed less every week (p = 0.001), had improved gait scores (p = 0.01), lower prevalence and severity of hock burn (p = 0.02) and reduced acute plasma corticosterone response to confinement (p < 0.05) compared to CR chickens. The relative growth rate of male DR chickens was slower in the first week of range access and faster in the final week of range access, than male CR chickens (p < 0.05). Distant ranging was confounded with the amount of range access; longer and more frequent range visits were correlated with the number of visits further from the shed (p < 0.01). Thus the relationships identified may reflect ranging frequency and duration rather than ranging distance per se. Further studies are required to disentangle such effects and to identify causation.

7.2 Introduction

Chickens prefer to range close to their home shed (Rivera-Ferre, Lantinga, & Kwakkel, 2007; Dal Bosco, Mugnai, Sirri, Zamparini, & Castellini, 2010; Fanatico, Mench, Archer, Liang, Gunsaulis, Owens, & Donoghue, 2016). The aggregation of chickens in range areas close to the shed can have detrimental effects on the range environment and chicken welfare due to accumulation of excreta and nutrient load, reduced vegetation and increased pathogens (van de Weerd, Keatinge, & Roderick, 2009). Therefore, encouraging uniform ranging distribution in free-range commercial broiler chickens flocks is important to safeguard chicken welfare and environment sustainability. Ranging distance can be encouraged by providing range enrichment, both natural structures such as trees and bushes (Mirabito, Joly, & Lubac, 2001; Dawkins, Cook, Whittingham, Mansell, & Harper, 2003; Stadig, Rodenburg, Ampe, Reubens, & Tuyttens, 2016) or artificial structures such as roosts, screened shelters and wigwams (Gordon, 2002; Fanatico et al., 2016). Despite the success of range enrichment to encourage chickens to range further from the shed in the aforementioned studies, preferences to range close to the shed were still apparent.

Individual characteristics may significantly impact on the willingness of a chicken to range further from the shed, and therefore may play an important role in promoting uniform ranging distribution. Stadig, Rodenburg, Ampe, Reubens, & Tuyttens (2016) found evidence that general fearfulness, measured via a tonic immobility test, was related to the number of chickens that ranged further than 5 m from the shed, suggesting that the propensity to be frightened prior to range access impeded ranging distance. Furthermore, particular health characteristics may also impede ranging further from the shed. Ranging greater distances may require good leg health or lower body weight. Indeed, chickens housed indoor show reduced activity with increasing weight and poor leg health (Reiter &

Bessei, 1996; Weeks, Danbury, Davies, Hunt, & Kestin, 2000; Rutten, Leterrier, Constantin, Reiter, & Bessei, 2002). Furthermore, Rodriguez-Aurrekoetxea, Leone, and Estevez (2014) showed that that the provision of perches altered tibial morphological characteristics and increased ranging distance. Few studies have assessed chicken welfare before range access as well as after range access is provided. Therefore, it is difficult to tell if particular aspects of welfare, such as leg health, are affected by, or encourage, ranging distance.

Individual broiler chicken ranging behaviour was tracked including distance ranged from the shed on a commercial farm and welfare was assessed prior to and after range access, with a focus on health and general fearfulness. It was hypothesised that chickens would exhibit inter-individual preferences for ranging distance, and that chickens that prefer to range further from the shed would be less fearful before range access and have better leg health and lower body weight after range access than chickens that ranged closer to the shed.

7.3 Materials and methods

This experiment was approved by the South Australian Research and Development Institute Animals Ethics Committee in accordance with the Australian Code of Practice for the Care and User of Animals for Scientific purposes (ethics approval number 3-16).

Study site and subjects

This study was conducted on one flock on a commercial farm in South Australia during summer. A tunnel ventilated shed (160 m × 16 m) with cooling pads and range areas (156 m × 17.3 m) located on both sides of the shed contained approximately 39,740 mixed sex Ross 308 broiler chickens placed at one day old with a stocking density kept below 28 kg/m². Radio frequency identification (RFID) equipment was used to track individual chicken ranging behaviour. Due

to a finite amount of RFID equipment, a study area within the commercial shed was partitioned off. The study area (96 m²) was located in the middle of the shed, partitioned with mesh fencing 0.5 m high, extending eight meters from the south wall of the shed and 12 m wide. Range access in the experimental area was provided via two south facing range doors (3.8 m wide), hereafter referred to as "pop-holes". Pop-holes were spaced 3.8 m apart. The experimental range area $(17.3 \text{ m} \times 12 \text{ m})$ contained a 0.8 m high, 12 m \times 3.5 m rectangle horizontal shade cloth that covered the range area 6.1 m to 9.6 m from the shed width wise (Figure 7-1). Two 0.3 m high immature trees were present 12 m from the shed, close to the experimental fence. The experimental area housed approximately 1580 chickens that were randomly caught at four days of age from various areas within the shed, based on location relative to the front of the shed and distance from pop-holes. Chickens were caught by corralling approximately 50 to 100 individuals at pre-determined sampling location using portable fencing. Chickens were then chosen randomly, placed in a crate, transported and released in the experimental area. Stocking density was consistent with the commercial flock (28 kg/m²) and was maintained by depopulating (removing approximately one third of the flock for slaughter) at 35 days of age. Chickens in the experimental flock were manually depopulated, removing unmarked chickens from the experimental flock into the commercial flock. The litter was turned manually in the experimental area at 14 and 36 days of age at the same time as the rest of the shed litter was turned with machinery for litter management purposes.



Figure 7-1 Shed and experimental pen dimensions and layout. Experimental range area indicates range areas separated by Radio Frequency Identification (RFID) antennas differing in distance from the shed wall, defined as close-range (< 2.7 m from shed wall), mid-range (2.7 to 11.2 m from the shed wall) and far-range (11.2 to 15.3 m from the shed wall).

Chickens were permitted range access from 15 days of age. Management provided access to the range at their discretion, often dictated by the shed environment (e.g. increased relatively humidity) which was difficult to control during extreme external weather conditions when the pop-holes were opened. Due to the climate in South Australia in summer (maximum temperature range: 25.7 °C to 29.1 °C; minimum temperature range: 12.3 °C to 14.3 °C) pop-holes were typically open at sunrise (06:00 am) when temperatures were lower and closed as daily temperature and humidity increased (12:00 pm). Range use was provided an average of 4.8 ± 0.9 days weekly for 5.5 ± 0.6 hours daily. However, range availability varied each week, dictated by weather conditions (Table 7-1). In the final week of the study, increased temperatures and thunderstorms subsequently increased humidity and opening pop-holes became a welfare risk

to chickens inside the shed, consequently range access was not permitted between 39 to 42 days of age.

Table 7-1 Mean weekly ranging conditions during each week of range access, including availability of range and range weather conditions. Morning weather conditions were measured at 9am by an Australian Government Bureau of Meteorology weather station located 0.6 km from the farm.

Mean weekly ranging	Week 3 Week 4		Week 5	Week 6	
conditions					
Number of days the range was	5	7	4	3	
available					
Total number of hours of range availability	28.6	45.1	19.8	11.5	
Hours of daily access	5.7 ± 1.6	6.4 ± 1.0	5.0 ± 0.7	3.8 ± 0.7	
Time of day range was available	7:48 – 12:12	6:51 – 13:17	5:40 - 10:20	6:00 – 13:20	
Morning temperature (°C)	19.9 ± 2.3	18.5 ± 1.4	23.3 ± 1.8	19.9 ± 0.8	
Morning relative humidity (%)	55.4 ± 7.9	61.0 ± 7.2	50.3 ± 3.4	87.0 ± 6.1	
Morning wind speed (km/h)	12.0 ± 4.9	8.2 ± 0.8	5.5 ± 2.9	4.0 ± 0.0	
Min. daily temperature (°C)	15.8 ± 2.1	13.6 ± 1.0	17.1 ±1.7	17.9 ± 0.6	
Max. daily temperature (°C)	30.9 ± 2.5	31.4 ± 1.1	36.7 ± 0.8	29.9 ± 0.6	

Tracking individual range use

Range use was tracked on a subpopulation (n = 305) within the experimental pen. Chickens were randomly selected from the experimental area, based on the location across the width of the experimental area and distance from pop-holes.

Individual range use was tracked by the Gantner Pigeon RFID System (2015 Gantner Pigeon Systems GmbH, Benzing, Schruns, Austria), with a bespoke program Chicken Tracker that was developed for the use of tracking chickens; previously validated and used on a commercial farm to track laying hens (Gebhardt-Henrich, Fröhlich, Burose, Fleurent, Gantner, & Zähner, 2014; Gebhardt-Henrich, Toscano, & Frohlich, 2014). Chickens were randomly selected from 10 areas evenly spread within the shed; locations varied according to length and width of the shed and distance from pop-holes. Chickens were fitted with a silicone leg band that automatically loosened with growth (Shanghai Ever Trend Enterprise, Shanghai, China; Figure 7-2). Each leg band contained a unique ID microchip (Ø4.0/34.0 mm Hitag S 2048 bits, 125 kHz) that registered as the chickens walked over the antenna. Antennas were attached to both sides of each pop-hole (i.e. indoor and outdoor) to determine the direction of movement by each tagged chicken; hence permitting calculation of ranging frequency and duration. In addition, two rows of RFID antennas were placed in the range at 2.7 m and 11.2 m from the shed wall (Figure 7-1). The placement of antennas in single rows at various distances from the shed permitted identification of the maximum distance for each range visit but not the duration of time spent in areas further from the shed. Antennas were placed in the shed before placement of the chickens to minimise disturbance. Chickens were marked with blue or green stock paint (FIL Tell Tail, GEA, New Zealand) on tail and wing feathers to identify tagged chickens in order to retrieve leg bands at the end of the study. Chickens were excluded from analysis if tags were not recovered or functional at the end of the trial.



Figure 7-2. Photograph of leg band, with individual identification noted on the exterior. Leg bands contained an individual radio frequency identification microchip that is not visible in this photograph.

Chickens were tracked from the second day of range access until depopulation for slaughter (16 to 44 days of age). Although the range area was

first available to access at 15 days of age, technical problems prevented tracking range use on this day.

Throughout the study 97% of tagged chickens were successfully tracked, indicated by functioning tags recovered after the experimental period. Four chickens were found dead during the study, two tags were dysfunctional and three tags were never recovered. Ranging and welfare data from these nine chickens were excluded from analysis.

Welfare indicators

Investigating relationships between ranging distance and welfare included assessments of health and fear responses. To minimise the effects of handling prior to measuring indicators of fear responses, tracked chickens (n = 305) were randomly allocated to either the health (n = 153) or fear (n = 152) aspect of this project. A full set of welfare data was not collected on a small population of tracked chickens (n = 11) for various reasons, subsequently these chickens were included in the analysis of ranging behaviour but not welfare.

Health measures

At seven days of age, 153 focal chickens were randomly selected, based on locations across the width of the experimental area and distance from pop-holes. Foot pad dermatitis and hock burn were scored using the methodology outlined in section 3.5.1. Chickens were weighed, tagged with wing band with a unique identification number (Jiffy Wing Bands 893, National Band and Tag, Newport, KY, USA), sprayed with blue stock paint (FIL Tell Tail, GEA, New Zealand) and released into the flock. At 14 days of age, focal chickens were tagged with a leg band containing a unique ID microchip to monitor range use (a detailed description of RFID microchips and leg bands is provided in section **Error! eference source not found.** of the general methodology chapter). Gait, foot pad dermatitis and hock burn were scored and chickens were weighed each week from 14 to 42 days of age (a detailed description of scoring methodology for gait and body condition is provided in section **Error! Reference source not found.** of he general methodology chapter). Assessors were blind to an individual's ranging behaviour.

Fear responses

To minimise the effects of previous handling, fear responses were assessed on different focal chickens (n = 152) once before range access was provided (12 days of age) and once after range access was provided (45 days of age). Chickens were caught the night before testing and segregated in a smaller pen within the shed with *ab libitum* access to food and water. On the day of testing, chickens were randomly chosen from six evenly distributed areas of the holding pen and placed in a transport crate in groups of three in a quiet room adjacent to the shed. Exactly 12 minutes after chickens were placed in crates, chickens were removed and a 2 mL blood sample was collected from the brachial wing vein. Chickens were placed on their side on a table with legs extended and the handler lightly covered the chickens head. Approximately, 2 mL of blood was collected from the brachial wing vein with an S-monovette (Sarstedt AG & Co, Nümbrecht, Germany). Blood was collected in less than three minutes as not to reflect handling effects. After collection, blood samples were spun on site at 10 000 rpm for five minutes. The supernatant was collected, stored on ice and frozen at - 20° C for later analysis. All chickens were bled within two minutes of removing the chicken from the crate to avoid an acute stress response to handling influencing basal plasma corticosterone concentrations (Broom & Johnson, 1993). Plasma corticosterone concentrations were measured using a commercially available double antibody radioimmunoassay kit (MP Diagnosistics, Orangeburg, NY, USA) as per manufacturer's instructions with the exception of the dilution factors; 1:4 plasma dilution factor was required to fall within the standard curve. Duplicates with a coefficient of variation greater than 5% were reanalysed.

Physiological stress responses to the novel environment during confinement should reflect the underlying propensity to be frightened or 'fearful' in general (Boissy, 1995), that is, general fearfulness or fear of unfamiliar stimuli. However, handling prior to confinement may have contributed to corticosterone responses (Forkman, Boissy, Meunier-Salaün, Canali, & Jones, 2007).

Immediately after blood samples were obtained, each chicken was transported by hand 5 to 10 m to an independent room to conduct a tonic immobility (TI) test. A state of tonic immobility is an antipredator response and therefore likely reflects fearfulness related to predation but has also shown to be reflective of general fearfulness (Forkman et al., 2007). Additionally, tonic immobility test also includes additional stressors such as handling and social isolation (Forkman et al., 2007). All three TI testing rooms were temperature controlled, isolated from weather extremities and identically designed. The chicken was inverted and restrained gently on its back in a U shaped cradle with light pressure applied to the sternum and the head was lightly covered by the handler for 15 s. A maximum of three attempts were made to induce the TI state. A successful induction was considered when the chicken remained in TI for more than 15 s after the handler released pressure. The length of time chickens remained in TI was recorded. Chickens were permitted to remain in a TI state for a maximum of 360 s after which they were gently righted. If TI was not induced after three attempts, that chicken was given a score of zero. If breathing appeared laboured or restricted the chicken was brought out of TI immediately and excluded from analysis (n = 2 post range access). Experimenters remained out of chicken's field of view after TI was induced. A white noise recording played continuously in each of the TI rooms and crating room to minimise any outside sound disturbance.

Following the TI test, each chicken was weighed, marked with green livestock spray marker (FIL Tell Tail, GEA, New Zealand) and returned to the experimental flock. Handlers were blind to an individual's ranging behaviour.

Statistical analysis

Ranging behaviour

Ranging behaviour of focal chickens from the health and fear parts of this study were compared with non-parametric Kruskal Wallis tests. The total number of range visits, the number of range visits to the close-, mid- and far-range areas did not differ between focal chickens from the two sub-populations (total number of range visits: p = 0.78; total time spent on the range: p = 0.57; first day the range was accessed: p = 0.13; number of visits to the close-range: p = 0.30; number of visits to the mid-range: p = 0.57; number of visits to the far-range: p = 0.11). Therefore, ranging behaviour data from all tracked chickens were pooled for analysis and are presented together.

The relationships between percentage of visits to close-, mid- and far-range areas and overall range use (frequency and duration) were analysed with Spearman's correlation analysis. The peak number of weekly range visits was analysed with a generalised linear model (GLIM). The number of birds that displayed increased, decreased or stabilised maximum ranging distance each week from the preceding week was analysed with Chi-square analysis with the Bonferroni correction method for multiple comparisons.

The effect of sex on the total number of range visits and overall percentage of visits to the close- and mid-range areas, were analysed using a general linear

model (GLM). Sex was included as a fixed factor and final body weight as a covariate. Due to minimal far-range visits, binary GLIM comparisons (accessed far-range vs did not access far-range) were used to compare sex.

Categorising ranging chickens

To identify relationships between ranging distance and welfare indicators, chickens were categorised into groups based on the maximum distance ranged from the shed per visit. Chickens that accessed the close-range (maximum 2.7 m from the shed) more frequently (> 50% of total visits) than the mid- or far-range areas (greater than 2.7 m from the shed) throughout the study were categorised as "Close Rangers" (CR). Conversely, chickens that accessed the mid-and far-range areas (greater than 2.7 m from the shed) more frequently (> 50% of total visits) were classified as "Distant Rangers" (DR). Few chickens never accessed the range throughout the experiment (n = 16) and thus were excluded from all analysis.

Comparisons between close- and distant-ranging chickens

Relative growth rate was calculated by dividing the difference in body weight from the previous week, by the previous week's body weight, for example

$$\frac{\Delta W eight_{Week 3} W eight_{Week 4}}{W eight_{week 3}}$$

Analysis of pre-range access data

Relative growth rate, weight at 7 and 14 days of age and acute plasma corticosterone concentrations were analysed with GLM to determine differences between CR and DR chickens, including sex and weight as random factors where appropriate. Ordinal and binary data, such as gait, foot pad dermatitis and hock burn scores, the number of TI attempts, failure to induce a TI state (maximum attempts) and the number of chickens remaining in TI for the maximum duration were analysed with generalised linear mixed models (GLIMM) to determine differences between CR and DR chickens. Due to the censored nature of TI duration (maximum 360 s) data were analysed with Cox regressions, with ranging category as a fixed factor, handler, sex and number of attempts to induce TI as random factors, and time of day and weight as covariates. Binary logistic regressions were used to predict CR and DR chickens. Pre-ranging data were included in a binary logistic regression if p-values were ≤ 0.1 . A variable was removed from the regression model if it was strongly correlated with another variable (≥ 0.7) with a maximum of three variables included. The most parsimonious models are reported with statistically useful variables in the model, confirmed by goodness of fit tests calculated by Omnibus model coefficients and Hosmer and Lemeshow tests and the amount of variation the model accounted for, determined by Nagelkerke R square values.

Analysis of post-range access data

Post-ranging gait, foot pad dermatitis and hock burn scores and number of TI attempts were analysed with an ordinal logistic generalised estimating equation (GEE) accounting for repeated measures with a robust estimator autoregressive working correlation matrix; Wald statistics are reported. Post-ranging weight and acute plasma corticosterone responses were analysed with a general linear mixed model (GLMM), accounting for weekly repeated measures with an autoregressive covariance structure and individual as the subject. All GLMM and GEE models included ranging distance category (CR or DR), week and the interaction between ranging distance category and week as fixed factors and sex, weight and handler as random variables where appropriate. Furthermore, pre-ranging weight (14 days of age) and number of range visits were included as covariates in all GLMM and GEE models to control for any differences prior to

range access and frequency of range use respectively. Non-significant interactions were removed from models (p > 0.05).

Failure to induce a TI state and the number of chickens remaining in the TI state for maximum duration were analysed with a binary logistic generalised linear mixed model (GLIMM). Post-ranging TI duration censored data were analysed with Cox regressions, with handler, sex and number of attempts to induce TI included as random factors and time of day, weight and pre-ranging TI duration included as covariates.

Raw means ± SEM are reported unless otherwise noted.

7.4 Results

Ranging behaviour

Not all chickens accessed the range (non-rangers: 5.4% (n = 16) tracked chickens; Figure 7-3). On average, individuals accessed the range 12.9 ± 0.2 days, 52.5 ± 1.3 times, for a total duration of 8.9 ± 0.2 hours during the study (Table 7-2).

Table 7-2 Ranging behaviour (mean \pm standard error of the mean (SEM)) of focal chickens throughout the study period.

	Mean ± SEM	Minimum	Maximum
Total number of range visits	52.5 ± 1.3	1	185
Total time spent on the range (h)	8.9 ± 0.2	0.02	44.5
Days the range was accessed (% available)	67.9 ± 1.1	5.3	94.7
Visits to close-range (% range visits)	61.9 ± 0.8	8.1	100
Visits to mid-range (% range visits)	41.3 ± 0.7	1.1	83.2
Visits to far-range (% range visits)	5.7 ± 0.2	0.7	17.1

Few ranging chickens (13.2%, n = 37) were never detected at the RFID antenna 2.7m from the shed (Figure 7-3). The majority of tracked chickens (62.5%, n = 175)

visited close-range areas more frequently (more than 50% of range visits were a *maximum* distance of 2.7 m). Less than half of the tracked chickens (37.5% of tracked chickens, n = 105) visited the mid-range area more frequently (more than 50% of range visits were greater than 2.7 m but less than 11.2 m). No chicken visited the far-range area more frequently (more than 50% of all range visits were distance greater than 11.2 m); the maximum percentage of an individual range visits to the far-range area was 17.1%.

More visits to the mid- and far-range areas were observed when more chickens were on the range (mid-range: $r_{(109)} = 0.84$, $p \le 0.001$; far-range $r_{(109)} = 0.48$, $p \le 0.01$). Chickens accessed the mid- and far-range areas more if they accessed the range more frequently (mid-range: $r_{(280)} = 0.62$, p < 0.001; far-range: $r_{(280)} = 0.60$, p < 0.001) and for a longer period of time (mid-range: $r_{(280)} = 0.78$, p < 0.001; far-range: $r_{(280)} = 0.73$, p < 0.001).



Figure 7-3 Percentage of tracked chickens that accessed the close-, mid-and far-range areas or did not access the range each week when range access was provided.

The number of weekly range visits and visits to the close- and mid-range areas peaked at four weeks of age (total range visits: $\chi^{2}_{(3, 280)} = 255.9$, p < 0.001; close-range visits: $\chi^{2}_{(3, 280)} = 189.8$, p < 0.001; mid-range visits: $\chi^{2}_{(3, 280)} = 303.3$, p < 0.001; Figure 7-4). The number of visits to the far-range area peaked at five and six weeks of age ($\chi^{2}_{(3, 280)} = 168.4$, p < 0.001; Figure 7-4).



Figure 7-4 Mean (± standard error of the mean (SEM)) number of weekly range visits per chicken overall (black line) and separated (grey lines) into visits to the close- (square), mid- (cross) and far- (triangle) range areas per chicken from week three to week six.

Almost all of the tracked chickens (98%) continued to access the range every week after their first range visit. Only 2% of chickens (n = 6) accessed the range during one week but not again for the remainder of the study (n = 2 each week, excluding week six). An individual's maximum ranging distance rarely decreased between weeks; 9.3% of chickens (n = 27) reduced their maximum ranging distance from one week to the next throughout the study. The number of chickens that reduced the maximum ranging distance did not differ each week (p > 0.05; Figure 7-5). The number of chickens that increased the maximum ranging distance was greater at four weeks of age than five and six ($\chi^{2}_{(4,888)} = 104.7$, p < 0.001; Figure 7-5). Conversely, the number of chickens that neither increased nor decreased the maximum ranging distance was greater at five and six weeks of age.



Figure 7-5 Percentage of tracked chickens that decreased (black bars), increased (white bars) or did not increase or decrease (grey bars) the maximum ranging distance each week. Significant differences (p < 0.001) between ranging behaviour and week are indicated by different letters.

The number of females and males tracked throughout the study were similar (females n = 142, males n = 136, unknown n = 18). There were no differences between the sexes in the likelihood of accessing the far-range (p = 0.93), the proportion of CR or DR rangers (p = 0.89), the total number of range visits (p = 0.40) or percentage of visits of visits to the close-, mid- or far-range areas (close-range visits: p = 0.20; mid-range visits: p = 0.14; far-range visits: p = 0.27). There was no interaction between sex and week on total weekly range visits (p = 0.11), mid-range visits (p = 0.19) or far-range visits (p = 0.30). However, there was an
interaction between sex and week on the number of close-range visits ($F_{(93, 198)} = 3.08$, p = 0.03) indicating that at three weeks of age males visited the close-range area more than females but between four and six weeks of age females visited the close-range more than males.

7.4.1 Comparisons of pre-range welfare indicators

Body weight and health

The body weight of DR chickens (n = 47 chickens) was lower than CR chickens (n = 86 chickens) before range access (weight at 7 days: $F_{(1,128)} = 5.70$, p = 0.02; weight at 14 days: $F_{(1,127)} = 5.74$, p = 0.02; Table 7-3). Prior to range access at 14 days of age, gait, foot pad dermatitis and hock burn scores did not differ between CR and DR chickens (p > 0.05). High gait, foot pad dermatitis and hock burn scores were rare at 14 days of age (Table 7-3).

Table 7-3 Mean (\pm standard error of the mean (SEM)) body weight at 7 and 14 days of age and pre-ranging growth rate (mean \pm SEM) and prevalence of gait, food pad dermatitis and hock burn scores at 14 days of age, prior to range access. Data are presented in groups of chickens that accessed the range area close to the shed more frequently (Close Rangers) and chickens that accessed areas further from the shed more frequently (Distant Rangers). * indicates significant difference between close rangers and distant rangers at *p* < 0.05.

Measure	Score	Close Rangers	Distant Rangers
Day 7 weight (g)	Female	190.5 ± 3.5	$178.3 \pm 5.0^{*}$
	Male	200.2 ± 2.7	193.9 ± 4.3
	Mixed sex	195.6 ± 2.2	$186.0 \pm 3.5^{*}$
Day 14 weight (g)	Female	508.5 ± 7.9	490.0 ± 10.3
	Male	561.9 ± 6.1	$541.7 \pm 7.0^{*}$
	Mixed sex	534.0 ± 6.0	$515.3 \pm 7.3^{*}$
Pre-ranging	Female	1.6 ± 0.1	1.8 ± 0.0
growth rate	Male	1.8 ± 0.1	1.8 ± 0.1
	Mixed sex	1.7 ± 0.1	1.8 ± 0.1
Gait score % (n)	1 - normal	61.9 (52)	72.3 (34)
	2 – affected	38.1 (32)	27.7 (13)
	3 – lame	0 (0)	0 (0)
Foot pad	1 – none	86.9 (73)	89.4 (42)
dermatitis % (n)	2 – slightly affected	11.9 (10)	8.5 (4)
	3 – moderate	0 (0)	2.1 (1)
	4 – severe	1.2 (1)	0 (0)
Hock burn % (n)	1 – none	86.9 (73)	89.4 (42)
	2 – slight	13.1 (11)	10.6 (5)
	3 – severe	0 (0)	0 (0)

Fear responses

There was no difference in any TI measure between CR (n = 79 chickens) and DR (n = 52 chickens) before range access (failure to induce TI: p = 0.33; number of inductions: p = 0.25; TI duration: p = 0.56; maximum TI duration: p = 0.74; Table 7-4). Pre-ranging acute plasma corticosterone response to confinement did not differ between CR and DR chickens (p = 0.16; Figure 7-9). There was no effect of body weight or sex on any fear response measure (p > 0.05).

Tonic Immobility measure	CR	DR	
Failure to induce TI (%)	25.6 (n=20)	34.5 (n = 19)	
Inductions required to induce TI	2.0 ± 0.1	2.3 ± 1.1	
Duration of TI (s)	119.1 ± 13.8	135.9 ± 20.5	
Maximum TI duration (%)	10.0 (n=6)	13.9 (n=5)	

Table 7-4 Pre-range access Tonic Immobility (TI) measures for close-ranging (CR) and distant-ranging (DR) chickens.

Predicting the likelihood of distant-ranging with pre-ranging welfare indicators

Weight prior to range access predicted CR and DR chickens at both seven days of age ($\chi^{2}_{(1, 133)} = 5.6$, p = 0.02) and 14 days of age ($\chi^{2}_{(1, 133)} = 5.2$, p = 0.02) correctly classifying 67.2% and 65.4% of cases respectively. Pre-ranging growth rate or sex did not predict CR and DR chickens (p > 0.05).

Acute plasma corticosterone response to confinement and TI measures prior to range access did not predict CR and DR chickens (p > 0.05).

7.4.2 Relationships with ranging and post ranging welfare indicators

Health

Four chickens were found dead inside the shed during the study between 17 and 35 days of age. Two of the four chickens had accessed the range prior to death for two to 10 visits on two days, spending a total of 6.5 mins to 1.8 hours on the range. Two of the chickens found dead never accessed the range. All four birds were excluded from analysis.

Weight

Every week of the study, body weight was lower for DR chickens than CR $(F_{(3,130)} = 5.6, p = 0.001;$ Figure 7-6). In addition, more range visits, lower pre-ranging weight and females were associated with reduced weekly body weight

(number of weekly range visits $F_{(1,130)} = 10.9$, p = 0.001; sex $F_{(1,130)} = 27.5$, p < 0.001; pre-ranging weight $F_{(1,130)} = 4.8$, p < 0.001). There was no three-way interaction between DR and CR, sex and week on body weight (p = 0.22).



Figure 7-6 Raw mean body weight (kg) \pm standard error of the mean (SEM) for close-ranging (black lines) or distant-ranging (grey lines) chickens from the first week of range access (week three) to the final week of range access (week six). Data are separated into sex; female (solid lines) and males (dotted lines). * indicates a significant difference between close- and distant-ranging chickens at p < 0.01.

There was a three way interaction between ranging distance, week and sex on relative growth rate ($F_{(3,362)} = 4.97$, p < 0.01): male DR chickens grew slower the first week of range access (2 to 3 weeks of age) compared to male CR chickens ($F_{(1,63)} = 13.7$, p < 0.001; Figure 7-7) but slower during the final week of range access ($F_{(1,59)} = 5.56$, p = 0.02; Figure 7-7). Ranging distance was not related to the relative growth rate of females ($F_{(1,124)} = 1.96$, p = 0.16). More range visits were associated with lower relative growth rate, regardless of sex ($F_{(3,320)} = 17.0$, p < 0.001).



Figure 7-7 Female (a) and male (b) mean relative growth rate \pm standard error of the mean (SEM) for close-ranging (black lines) or distant-ranging (grey lines) chickens from the first week of range access (week three) to the final week of range access (week six). * indicate significant difference between close- and distant-ranging chickens at p < 0.05.

Gait score

Overall, CR chickens had higher (worse) gait scores than DR chickens (χ^2 (1, 132) = 6.9, $p \le 0.01$; Figure 7-8). Increased body weight was associated with higher (worse) gait scores (χ^2 (1, 132) = 43.7, p < 0.001). There was no effect of the total number of range visits or sex on gait scores (p > 0.05).



Figure 7-8 Percentage of close- (grey bars) and distant- (white bars) ranging chickens with normal, affected or lame gait scores pooled from weeks 3 to 6. Dotted lines within bars indicate the percentage of raw gait scores. * indicate significant difference between close- and distant-ranging chickens at $p \le 0.01$.

Contact dermatitis

Hock burn increased over time but CR chickens had higher (worse) scores than DR chickens after range access (interaction between ranging distance and week: $\chi^{2}_{(3,130)} = 9.4$, p = 0.02; Table 7-5). Increased body weight was associated with higher hock burn scores ($\chi^{2}_{(1,130)} = 24.3$, p < 0.001). There was no effect of sex or the total number of range visits on hock burn (p > 0.05).

HB Score	Ranging category	Week 3	Week 4	Week 5	Week 6
1 – none % (n)	CR	84.7 (72)	91.7 (77)	72.3 (60)	36.1 (30)*
	DR	95.7 (44)	84.4 (38)	84.8 (39)	56.5 (26)
2 – slight % (n)	CR	15.3 (13)	15.6 (7)	16.9 (14)	65.4 (17)
	DR	4.3 (2)	4.3 (2)	15.2 (7)	19.6 (9)
3 – severe % (n)	CR	0.0 (0)	1.2 (1)	10.8 (9)*	43.4 (36)*
	DR	0.0 (0)	0.0 (0)	0.0 (0)	23.9 (11)

Table 7-5 Weekly Hock Burn (HB) scores for close-ranging (CR) and distant-ranging (DR) chickens. * indicates significant difference between CR and DR chickens each week at p < 0.05.

There was no effect of ranging distance on foot pad dermatitis (p = 0.71). However, foot pad dermatitis scores increased over time ($\chi^2_{(3, 132)} = 46.2, p \le 0.01$) and higher scores were associated with more range visits and lower weight (total number of range visits: $\chi^2_{(1, 132)} = 8.2, p < 0.01$; weight $\chi^2_{(1, 132)} = 10.9, p \le 0.01$).

Fear responses

There was no difference in any TI measure between CR and DR after range access (failure to induce TI: $\chi^2_{(1, 126)} = 0.02$, p = 0.54; number of inductions: $\chi^2_{(1, 100)} = 2.52$, p = 0.23; TI duration: $\chi^2_{(1, 100)} = 0.27$, p = 0.60; maximum TI duration: $\chi^2_{(1, 100)} = 0.01$, p = 1.00; Table 7-6). There was no effect of body weight or sex on any TI measure (p > 0.05).

Table 7-6 Post-range access Tonic Immobility (TI) measures for close-ranging (CR) and distant-ranging (DR) chickens.

Tonic Immobility measure	CR	DR	
Failure to induce TI (%)	21.1 (n=16)	20.0 (n = 10)	
Inductions required to induce TI	2.0 ± 0.1	2.0 ± 0.1	
Duration of TI (s)	203.9 ± 14.5	198.0 ± 19.1	
Maximum TI duration (%)	19.4 (n = 12)	19.5 (n = 8)	

The acute plasma corticosterone response to confinement was lower in all chickens after range access but a greater reduction was observed in DR chickens than CR chickens (interaction between time of data collection and ranging distance: $F_{(1,130)} = 4.3$, p = 0.04; Figure 7-9).



Figure 7-9 Acute plasma corticosterone response to confinement in a transport crate for 12 minutes, prior to range access (pre-ranging) and after range access (post-ranging). Differing letters indicate significant differences at p < 0.05 level.

7.5 Discussion

Monitoring the ranging distance of individual broiler chickens on a commercial farm over time revealed that chickens that accessed the range area more frequently and furthest from the shed (distant-ranging chickens) were lower in body weight than chickens that accessed the close-range more frequently, both before and after range access. Furthermore, distant-ranging chickens had better gait scores, less hock burn and lower corticosterone response to confinement after range access. Such results suggest that ranging further from the shed may have positive implications for broiler chicken welfare. However,

only relationships were identified and causation cannot be inferred. Furthermore, ranging distance was confounded with the frequency of range visits. With these limitations in mind, I discuss potential mechanisms.

Relationships between ranging distance and welfare

Body weight prior to range access was predictive of ranging further from the shed and distant-ranging chickens weighed less at all time points than closeranging chickens, even after range access was provided. Lighter birds may be restricted from accessing resources inside the shed due to social competition and subsequently access the range in search of feed or water. However, Estévez, Newberry, and De Reyna (1997) provide evidence that monopolisation of feeders by a few chickens does not occur in broiler flocks. Rather weight differences may reflect temperament differences such as activity or motivation to explore. Growth rate between close- and distant-ranging chickens differed only after range access, suggesting a bi-directional relationship between body weight and ranging behaviour. It is unclear why the relationships between relative growth rate and distant-ranging differed between the sexes, but it could be reflective of sex related differences in ranging behaviour. Male distant-ranging chickens accessed the close range more frequently in the first week of range access compared to female distant-ranging chickens, coinciding with the divergent growth rate in males between distant- and close-ranging chickens. Of note, range access was restricted during the last four days of the study (from 39 to 42 days of age) due to weather conditions. This coincided with the only week that distant-ranging male chickens grew faster than close-ranging male chickens. This may be an indication that the relative growth rate of males is indeed a ranging effect, but further investigations are required to identify causation.

Gait score, hock burn and food pad dermatitis were not related to or predictive of ranging distance. Of note, poor gait scores and hock burns are usually rare at this young age (Vestergaard & Sanotra, 1999; Knowles et al., 2008; Bassler et al., 2013). However, after range access, chickens that ranged further from the shed more frequently had better gaits scores and less hock burn that chickens that ranged closer to the shed. Gait scores and hock burn are often positively correlated (Kestin, Su, & Sorensen, 1999; Sørensen, Su, & Kestin, 2000; Kristensen et al., 2006) and thought to reflect poor leg health, as increased gait scores (worse mobility) are associated with more time sitting on soiled litter, increasing the risk of hock burn. However, it is difficult to identify the reason for poor mobility/locomotion with the gait scoring methodology, i.e. leg health vs. growth morphology (Skinner-Noble & Teeter, 2009; Sandilands et al., 2011; Caplen, Hothersall, Murrell, Nicol, Waterman-Pearson, Weeks, & Colborne, 2012; Caplen, Colborne, Hothersall, Nicol, Waterman-Pearson, Weeks, & Murrell, 2013). As such, the effects of weight and leg health cannot be disentangled. Nevertheless, ranging distance was related to improved mobility.

Foot pad dermatitis was not related to ranging distance but was more prevalent and severe in chickens that accessed the range more frequently. This is in agreement with Pagazaurtundua and Warriss (2006) who showed that broiler chickens with access to an outdoor range had more foot pad dermatitis compared to chickens without outdoor range access. The mechanism of this relationship is unclear. High frequency ranging chickens may be more likely to damage their feet when in the range which has been shown to increase the risk of foot pad dermatitis (Shepherd & Fairchild, 2010) or they may be more active, spending more time standing or walking on litter. There is some evidence that foot pad dermatitis is painful (Sinclair, Weber Wyneken, Veldkamp, Vinco, & Hocking, 2015; Weber Wyneken, Sinclair, Veldkamp, Vinco, & Hocking, 2015), and therefore further research into causation is required to improve the welfare of ranging broiler chickens.

General fearfulness, assessed with TI before and after range access, was not related to ranging distance. However, distant-ranging chickens had reduced physiological stress response to confinement after range access compared to chickens that ranged close to the shed. Although, the disagreement between the fear response tests may reflect other differences between close- and distantranging chickens that trigger the non-specific physiological stress response, such as increased activity (Cockrem, 2007). Moreover, the TI and confinement test are thought to reflect general fearfulness, however there is variation in the nature of the fear eliciting stimuli between the two tests, such as social isolation and increased handling and restraint during the TI test. As such, these results may rather suggest that fear responses specific to novelty are reduced after range access but fear responses to social isolation, predation and handling are not. Our results contradict Stadig et al. (2016) that report a reduction in general fearfulness, measured by TI, as more chickens ranged further from the shed (> 5 m) although they did not assess individual ranging behaviour. However, pre-test handling, including confining chickens for 12 minutes, various forms of human contact and collecting blood samples, likely impacted our tonic immobility (TI) results (Jones, 1992). Indeed, the average duration of TI in the current study was greater than previously reported in free-range broiler chickens studies (Zhao, Li, Li, & Bao, 2014; Stadig et al., 2016). However, the reduced physiological stress response in relation to ranging distance observed in the current study support the behavioural fear response found by Stadig et al. (2016) and suggest that ranging further from the shed reduce the magnitude of fear responses in broiler chickens after range access.

Ranging behaviour

This study provides evidence that broiler chickens ranged relatively far from the shed, although this took time, in agreement with Rodriguez-Aurrekoetxea et al. (2014). Yet, Fanatico et al. (2016) showed that ranging distance of chickens decreased with age and Weeks, Nicol, Sherwin, and Kestin (1994) showed no consistent trend in ranging distance with age. Such inconsistencies between studies are likely related to the strain of broiler chicken, ranging opportunities (age of exposure and length), maximum ranging distance permitted (14 m to greater than 50 m), flock size, housing conditions, provision and type of range resources present, weather variation and geographical location. I provide evidence of the effects of age on ranging distance, specific to Ross 308 broiler chickens under south-eastern Australian commercial free-range conditions. Furthermore, I provide the only description from data that continuously tracked individual ranging behaviour.

Visits to the far-range area peaked during five and six weeks of age and few chickens (8%) decreased their maximum ranging distance from week to week. This suggests that the first visit further from the shed may be the biggest hurdle for broiler chickens. It may be that once the range area further from the shed is reached, the far-range is rewarding for chickens and thus reinforcing use. In addition, the number of chickens that ranged further from the shed was related to the number of chickens on the range, which may suggest an increase in perceived safety with a larger group size, motivation for intra-individual space, or simply physical pressure to move further from the shed as crowding increased.

Despite an increase in ranging distance with age, there was an overall preference to range closer to the shed; 13.2% of ranging chickens (n = 37) never ventured further than 2.7 m from the shed, more than half of all the range visits

(61.9%) were a maximum of 2.7 m from the shed and most of the tracked chickens (62.4%) visited the close-range more frequently than the mid-or far-range areas. This evidence supports previous results from various scan sampling studies that chickens stay in the vicinity of the shed/close to the shed (Weeks et al., 1994; Mirabito & Lubac, 2001; Christensen, Nielsen, Young, & Noddegaard, 2003; Fanatico et al., 2016). The implications of crowding in range areas closer to the shed has known effects on land degradation and disease risk (van de Weerd, Keatinge, & Roderick, 2009) but may also restrict range use if close-ranging chickens act as physical barriers to conspecifics. However, only nine birds never accessed the range suggesting that crowding closer to the shed unlikely prevented chickens from accessing the range.

The number of tagged chickens that never accessed the range (5.2%; n = 16) throughout the study was lower than previously reported internationally (25% - Chapuis et al. (2011)) but similar to local reports on segregated flocks in Australia (5% - Durali, Groves, and Cowieson (2012)) but not unaltered flocks (18 to 68% - Taylor, Hemsworth, Groves, Gebhardt-Henrich, and Rault (2017)). The early age of first range access (15 days) compared to previous studies (21 days - Durali et al., (2012); Taylor et al., (2017)) may have positively affected ranging behaviour, as the number of chickens that accessed the range increased overtime. Alternatively, the subsequent effects of segregating the experimental flock from the commercial flock, such as decreased flock size and fencing in the range may also have increased range use, as previously reported in laying hens (Rault, van de Wouw, & Hemsworth, 2013; Gebhardt-Henrich, Toscano, & Frohlich, 2014). Nevertheless, the aim of this experiment was not to provide descriptive ranging behaviours on commercial farms, but to identify the relationships between ranging distance and indicators of welfare.

Limitations

Chickens were categorised based on their overall ranging behaviour. Categorising chickens was required for statistical analysis due to the relatively low number of range visits. The method of categorisation did not mean that a close-ranging chicken never accessed the far-range and clearly the distantrangers had to cross the close-range area to reach the mid- and far- range areas. As technology advances the exact location and ranging behaviour may be tracked at an individual level permitting a better insight into the relationships observed. Furthermore, the frequency of range visits was positively related with the number of visits to the mid- and far-range, but could not disentangle the effects of ranging frequency and ranging distance on welfare.

7.6 Conclusions

Monitoring individual ranging behaviour over time in relation to distance ranged from the shed provided evidence that ranging further from the shed may have had positive implications for chicken welfare, including improved gait scores, a reduction in hock burn and physiological stress response to confinement and bi-directional relationships with weight. Further research is required to identify causation because of the implications of these results on improved welfare and increased ranging distribution on commercial free-range farms.

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CHAPTER 8 General discussion



8.1 Reviewing the aims of the thesis

There is little known about the relationships between ranging behaviour and broiler chicken welfare, particularly in Australian commercial conditions. This thesis contains observational hypotheses generating research which is applicable to commercial broiler chicken farms.

The research aims addressed in this thesis include:

- 1. Quantify patterns of flock and individual ranging behaviour of broiler chickens on Australian commercial farms;
- 2. Identify environmental factors and individual bird characteristics associated with broiler chicken range use;
- 3. Identify relationships between ranging behaviour and broiler chicken welfare.

The main objectives from each of the five experimental chapters contributed to the overall aims, including: 1) provide accurate descriptions of flock ranging behaviour and relationships with environmental factors; 2) examine intra-individual ranging behaviour within commercial flocks; 3) identify relationships between ranging behaviour, individual chicken characteristics and welfare in relation to health; 4) investigate bi-directional relationships between ranging behaviour and general fearfulness and; 5) investigate relationships between welfare and ranging behaviour in relation to distance ranged from the shed.

This chapter will summarise the main research findings and consider the contribution this research makes to the scientific understanding of free-range broiler chicken welfare. Practical implications, limitations and recommendations for future work will also be discussed.

8.2 Summary of research findings and implications

This thesis includes descriptive analysis of ranging behaviour on commercial farms and identifies external and internal factors associated with ranging behaviour and welfare. As there is little known about ranging behaviour and broiler chicken welfare, this thesis can provide direction for future scientific investigations or practical assistance on farm management strategies to help optimise ranging opportunities on commercial farms and improve the welfare of free-range broiler chickens.

8.2.1 Ranging behaviour of commercial broiler chickens

Results presented in Chapter Three suggest scan sampling methodologies under-represent the actual percentage of broiler flocks that access the range. Estimating range use at the flock level using scan sampling methods in Chapter Three would have inferred range use in broiler chicken flocks was relatively low, as the maximum number of chickens accessing the range at one time was between 8 to 10% in winter flocks and 33 to 37% in summer flocks; comparable to previous scan sampling estimates (11 to 59% flock estimates - (Christensen, Nielsen, Young, & Noddegaard, 2003; Dawkins, Cook, Whittingham, Mansell, & Harper, 2003; Fanatico et al., 2016; Jones et al., 2007; Nielsen, Thomsen, Sorensen, & Young, 2003; Rodriguez-Aurrekoetxea, Leone, & Estevez, 2014; Stadig, Rodenburg, Ampe, Reubens, & Tuyttens, 2016).

However, by tracking individual chicken ranging behaviour a more accurate estimate of flock range use was achieved; 32% of winter flocks and 81% of summer flocks accessed the range; in agreement with other studies which tracked individual range use (Chapuis et al., 2011; Durali, Groves, Cowieson, & Singh, 2014). It is not clear why more chickens do not access the range at the same time but it does highlight the error encountered when inferring flock range use by

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counting the number of birds on the range at one time without taking into account individual rotation between indoor and range environments throughout the day and with age. Based on the data presented in Chapter Three, including the maximum number of chickens observed on the range at one time (i.e. scan sampling estimate) and the more accurate estimate when individual range use was monitored, previously utilised scan sampling methodologies likely predict, at best, 25% to 46% of the actual flock that uses the range. However, more frequent scan samples may improve reliability (Estevez & Chrisman, 2006).

Further limitations of the scan sampling methodology are that the frequency and duration of range visits cannot be determined. Average flock estimates by tracking individual chicken ranging behaviour (Chapter Three) indicate broiler chickens waited four days after the range was first available before accessing it, visited the range 3.3 to 4.4 times for 7.9 to 26.3 minutes each day in winter and summer flocks, respectively, and preferred to range closer to the shed (Chapter Seven). Yet there are serious limitations with this approach. Reporting pooled flock averages does not account for the temporal dynamics of ranging behaviour, as the frequency, duration and distance from the shed increased over time (Chapters Three and Seven).

More importantly, reporting average flock ranging behaviour is inaccurate as there does not appear to be an "average ranger" in commercial broiler flocks (Chapters Four and Seven). Although flock estimates suggest broiler chickens visit the range between 3.3 and 4.4 times a day, some accessed the range 13 times for up to 6.8 hours (Chapter Four) and visited range areas further from the shed more frequently than close to the shed (Chapter Seven). These chickens did not appear to be outliers, but rather part of sub-populations within the flock, albeit arbitrarily defined in this thesis. Sub-populations included a relatively consistent population between flocks and across seasons which accessed the range only once and conversely relatively high frequency rangers that accessed the range soon after the opportunity was provided and accounted for one third to a half of all range visits (Chapter Four). This variation is particularly important when assessing the effects of range use on welfare. It is unlikely the implications of range use on chicken welfare would be the same for chickens accessing the range only once and chickens which visited multiple times per day from an early age. As such, comparisons between housing systems which do not quantify individual range use may misrepresent the implications of accessing an outdoor range on chicken welfare.

Descriptive analysis of broiler chicken ranging behaviour presented throughout this thesis challenges the previous reports of low range use in broiler chicken flocks and the notion that faster-growing strains of broiler chickens exhibit low motivation to access an outdoor range and subsequently are not suitable for use in commercial free-range industries (Jones et al., 2007; European Commission, 2008).

8.2.2 Factors that influence broiler chicken ranging behaviour

Environmental and management factors

The need for free-range broiler research specific to Australian conditions is evident in reports that show broiler chicken ranging behaviour is regulated by weather conditions (Gordon & Forbes, 2002; Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014; Stadig et al., 2016). Indeed, results presented in Chapter Three highlight seasonal effects on ranging; broiler chickens were less likely to access the range in winter flocks and chickens which did visit did so less frequently and for a shorter period of time than chickens in summer flocks. This is the first description of the magnitude of seasonal differences in ranging behaviours by monitoring individual ranging chickens; a reduction of flock range use in the order of 40%. Only a small amount of flock ranging behaviour in each season was explained by weather conditions. Rather the amount of ranging opportunities and age (confounding factors) explained much more of the variance and time of day the range was available significantly affected ranging behaviour. The seasonally related reduction in ranging behaviour may reflect a threshold for the number of ranging opportunities required to achieve higher levels of flock range use on commercial farms, or that broiler chickens do not compensate by ranging at alternate times of the day when access is not provided at preferred times.

Accessing the range to avoid negative stimuli was observed (Chapter Three). Litter treatment and sub-optimal shed conditions, including high dew point and temperature, were associated with more chickens on the range (Chapter Three). With an increasing amount of research aiming to increase the proportion of range use in a flock, this data acts as a reminder that range use is not always synonymous with positive motivation and highlights the importance of monitoring all aspects of the free-range environment, both indoors and outdoors in free-range research. Furthermore, the data suggest that allowing chickens the choice of where to spend their time is an important and dynamic aspect of free-range housing. The mere fact chickens are provided with some choice and control may in itself have positive implications for welfare (Nicol, Caplen, Edgar, & Browne, 2009; Perdue, Evans, Washburn, Rumbaugh, & Beran, 2014).

Individual characteristics

Early life differences associated with ranging suggest an inherent characteristic associated with range use which may be explained by intra-

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individual variation in temperament. The term temperament is often used interchangeably with behavioural syndrome, personality and coping style. Temperament traits have been grouped into five major categories; shynessboldness, exploration-avoidance, activity, sociability and aggressiveness (Réale, Reader, Sol, McDougall, & Dingemanse, 2007) and are related to individual variation in morphology (Biro & Stamps, 2008) and physiology (Cockrem, 2007). Temperament traits such as boldness or sociability are unlikely to be related to range use as there was no evidence pre-ranging fear responses before range access to a novel and socially isolating arena were related to ranging behaviour. However, differences in activity or exploration traits may explain why body weight, leg health and physiological fear responses prior to range access predicted range use (Chapters Five and Six).

This thesis presents a comprehensive analysis of environmental and individual factors associated with modulations of broiler chicken range use. Yet ranging prediction models which included weather variables and shed conditions or plumage condition, leg health and body weight explained very little of the variance in ranging behaviour. This suggests alternative factors modulate the motivation to range in broiler chickens on commercial farms.

8.2.3 Broiler chicken ranging behaviour and subsequent effects on welfare

In all research presented in this thesis, chickens were only included in postranging analysis if they were assessed both pre- and post-range access, with the exception of post-mortem measures. Statistically controlling any differences in welfare indicators prior to range access and quantifying the interactions with ranging behaviour enabled an assessment of changes to welfare in relation to range use. This method attempted to disentangle the effects between individual characteristics which encourage range use and implications of ranging behaviour, providing a greater understanding of these relationships.

In summer flocks, when more ranging was observed, ranging chickens gained less weight between pre- and post-ranging data collections, compared to chickens which never accessed the range. Furthermore, growth was negatively related to the number of range visits, time spent on the range and distance ranged from the shed - although such results may be reflective of individual characteristics such as an active temperament and therefore would be present prior to range access. But alterations in the weekly growth rate of male ranging chickens during range access (Chapter Seven) provides evidence that ranging behaviour may exacerbate relationships with body weight. It is unclear why this pattern in weekly growth rate was not observed in females, but may reflect sex differences in ranging behaviour. The mechanism for the relationship between body weight and ranging is unclear, but may be related to increased active behaviours on the range (Weeks, Nicol, Sherwin, & Kestin, 1994), time away from the feeder (Knierim, 2000), pasture consumption (Singh & Cowieson, 2013) or increased thermoregulation requirements (Yahav & McMurtry, 2001). Clearly, the implications for welfare depend on the cause and direction of the relationship.

Accessing the outdoor range, particularly frequently for a relatively long time and further from the shed, appeared to improve chicken welfare. Improvements in gait scores, hock burn, cardiovascular function and breast plumage cover were observed after range access in ranging chickens, and improvements were related to the amount of range use and distance ranged from the shed. Previous research had provided evidence of such improvements to welfare when access to an outdoor range is provided, compared to chickens housed without range access (Herenda & Jakel, 1994; Ward, Houston, Ruxton, McCafferty, & Cook, 2001; Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005; Stadig et al., 2016).

However, research in this thesis provides evidence of improvements specifically related to the amount of range use without confounding factors of differences in housing conditions. Of importance, poor leg health, reduction in cardiovascular function, and increased hock burn are associated with rapid growth, feed intake and body weight (Julian, 1998; Kestin, Gordon, Su, & Sørensen, 2001; Kjaer, Su, Nielsen, & Sørensen, 2006). The relationships identified between range use and welfare may be indirect effects of ranging and directly related to weight. But increased activity is often observed in the range environment (Weeks et al., 1994; Jones et al., 2007; Zhao, Li, Li, & Bao, 2014; Fanatico et al., 2016) which may directly influence each of the welfare indicators associated with range use. This would suggest the welfare of broiler chickens housed in conventional sheds may also be improved by increasing activity.

Further evidence to suggest range access has positive implications for broiler chicken welfare was the observed reduction in behavioural and physiological fear responses to novelty and social isolation after range access. A reduction in fear responses after range access was observed in chickens visiting the range and moreover were negatively associated with the amount of use (Chapter Six). General fearfulness was reduced in ranging chickens even in winter flocks when range use was low, suggesting a relatively low threshold of range use required for such benefits to welfare.

The increased risk of foot pad dermatitis (FPD) associated with ranging frequency (Chapter Seven) highlights the welfare risks associated with accessing an outdoor range and is in agreement with previous studies (Haslam et al., 2006; Pagazaurtundua & Warriss, 2006; Dal Bosco, Mugnai, Sirri, Zamparini, &

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Castellini, 2010). It had been suggested that increased foot pad dermatitis is specifically related to differences in housing conditions, such as poor litter quality in free-range sheds compared to conventional sheds (Nielsen et al., 2003). This thesis provides evidence of incremental increases in FPD risk specifically related to frequency of range use which may be associated with activity.

8.3 Practical relevance of the work

Reports of low range use in free-range poultry flocks often lead to controversy and allegations that broiler free-range chickens are "free-range" in name only (Nicol et al., 2017). The research presented in this thesis shows that previous estimations of flock range use with scan sampling methodologies misrepresents actual range use and monitoring individual ranging behaviour showed a higher proportion of commercial broiler flocks access the range, albeit season dependent. Perhaps of greater importance, this thesis provided evidence that accessing an outdoor range may be good for chicken welfare, even in winter flocks when range use was relatively low i.e. reduced fear responses after only nine days of available range access (Chapter Six).

With evidence that ranging may be good for chicken welfare, factors associated with increased range use should be further investigated to ensure optimal ranging opportunities for broiler chickens on commercial farms. Such factors include pop-hole design and increased opportunities to range which may be achieved by providing access at an earlier age or providing range access during preferred times of the day.

8.4 Challenges and limitations

The major limitation of work presented throughout this thesis is that causation cannot be determined. This has been highlighted in each chapter and therefore will only be briefly discussed here. Various methods were used to clarify the relationships between range use and chicken welfare, such as repeated measures before and after range use. However, the relationships I report may be an indirect effect of range use or reflective of range relationships with one single variable - for example, improved gait scores associated with reduced body weight or related to a variable which was not assessed. Furthermore, cardiovascular function was only assessed with post-mortem examination after range access and detecting leg pathologies is difficult when broiler chickens are young (i.e. prior to range access). So it is unclear whether the relationships observed between ranging and welfare, particularly mobility and cardiovascular function, are reflective of positive effects of accessing the range or existing traits that encourage range use. Yet, with such little scientific knowledge regarding free-range broiler chicken ranging behaviour and welfare, the research approach of this thesis permitted a broad investigation into commercially relevant relationships. As such, this thesis provides a good foundation for future industry relevant research.

It is unclear why few relationships between ranging and welfare were observed in winter flocks, but may be due to lower levels of ranging in winter flocks or a seasonal effect of the relationships between welfare and range use. Indeed, Dal Bosco et al. (2014) provided evidence that kinetic activity is much greater in summer flocks than winter. If the effects of accessing the range were related to activity, then perhaps broiler chickens are not active enough during winter to reach the level of ranging required for such improvements to health and welfare.

Tracking individual broiler chicken ranging behaviour on commercial farms with the use of RFID technology provided a better understanding of relationships between broiler chicken welfare and ranging behaviour. However, the economic and labour costs associated with RFID use on commercial farms prevented the inclusion of more flocks or farms. The results presented throughout this thesis are only from five flocks across two farms in Victoria and South Australia, so care must be taken in applying knowledge derived from these studies to alternate broiler chicken strains, housing conditions or geographical locations. Having said that, these relationships identified in this research are most likely to reflect those at least in typical south-east Australian free-range broiler farms.

8.5 Recommendations for future work

The research presented throughout this thesis provides the most comprehensive assessment of the relationships between individual broiler chicken ranging behaviour and welfare, but there are still many unanswered questions. This body of work generates hypotheses requiring further investigation to ensure the expansion of knowledge for free-range broiler chicken production and to continuously improve broiler chicken welfare. Potential research priorities in this field are outlined in this section.

Whether or not the relationships identified between broiler chicken ranging behaviour and welfare in this thesis have a causal basis which needs to be examined, including ranging behaviour and mobility, cardiovascular function, fear responses, hock burn and foot pad dermatitis. Understanding the causation of the relationships between ranging behaviour and body weight are a clear priority for the industry in relation to productivity and flock uniformity. Yet causation is equally important to identify in regards to welfare; reduced growth rate may reflect poor welfare if energy resources were diverted from growth to physiological stress responses (section 2.2.2), but if weight differences are reflective of individual differences such as an active temperament this would not be a concern for chicken welfare in relation to range use. Relationships between temperament traits and ranging behaviour has been investigated in laying hens (Campbell, Hinch, Downing, & Lee, 2016) but not broiler chickens. Evidence presented throughout this thesis suggests individual temperament may indeed be an important factor in ranging behaviour of broiler chickens, particularly activity and exploration and should be further investigated.

Identifying the specific characteristics of high frequency range users or chickens which prefer to range further from the shed may lead to breeding or management programs which optimise the number of chickens accessing the range on commercial farms. These sub-populations of chickens may become frustrated, however, when range access is restricted, which is often dictated by weather conditions at the discretion of the farmer. This may compromise the welfare of broiler chickens which are highly motivated to range and therefore the effects of range restriction on these sub-populations should be further investigated.

A reduction in fear responses after accessing an outdoor range has the potential to directly and indirectly safeguard welfare, as high levels of fear can lead to stress, increased injury, morbidity and mortality (Jones, 1996). Reducing fear responses has the potential to increase productivity and reduce downgrades at slaughter in addition to improving welfare. It is important to understand if the observed reduction in fear responses help broiler chickens cope when commercially relevant stressors are encountered, such as litter turning, feed restriction, catching and transport. Furthermore, the mechanism should be identified to determine if similar effects can be achieved in conventionally housed broiler chickens by providing an indoor environment which mimics the specific range characteristic responsible for changes in fear responses, such as environmental complexity or novelty.

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Monitoring individual range access with RFID technology permitted a more comprehensive understanding of individual ranging behaviour on commercial farms. But the behaviours expressed and experiences on the range could not be quantified with this method. The development of remote monitoring technology is rapid and future investigations should work towards incorporating such technologies, including accelerometers and portable heart rate monitors, to gain a better understanding of individual experiences on the range and how this may relate to welfare. Identifying range experiences of the one-time range users in particular (Chapter Four), may help to understand what prevents further range use, which may include frightening experiences on the range or a lack of rewarding experiences.

If further research provides conclusive evidence that accessing an outdoor range improves chicken welfare, further studies should address how to increase use of the range. This thesis suggests pop-hole design and age of first access may be good tools to encourage range use but these require further investigation. Free Range Egg & Poultry Australia Ltd (2015) regulation advises that access to the outdoor range must be provided only when chicks are fully feathered, typically 21 days old, although the level of plumage cover varies within a flock. The degree of plumage cover was not related to whether a chicken would access the range or not, nor the frequency of range visits (Chapter Five). Rather chickens with less plumage cover prior to range access spent less time on the range. Restricting range access before 21 days of age based on plumage cover may not be required if individuals self-regulate the length of visits according to thermoregulation requirements. The effects of exposing chickens to the range environment at an earlier age are unknown and are likely season-dependent.

Chickens were provided with range access at 15 days in the study presented in Chapter Seven, rather than the typical industry practice of 21 days. The

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number of chickens accessing the range and their amount of range use were much greater than reported in Chapter Three, which may be related to the age of first exposure to the range environment. However, multiple confounding factors could explain the relative differences, such as climate, vertical fencing panels and flock size, all of which have been shown to increase ranging behaviour in broiler chickens or laying hens (Dawkins et al., 2003; Gebhardt-Henrich, Toscano, & Frohlich, 2014; Rault, van de Wouw, & Hemsworth, 2013). The provision of wintergardens, as is often provided to laying hens, may protect chicks from extreme weather conditions and predator risk (perceived or actual) and encourage range use. As such, wintergardens may be a suitable option to provide range access to chicks at a younger age without compromising welfare and should be further investigated.

Evidence that pop-hole design affects ranging behaviour has been investigated in laying hens (Harlander-Matauschek, Felsenstein, Niebuhr, & Troxler, 2006; Gilani, Knowles, & Nicol, 2014) but not broiler chickens. The preference to enter and exit the range area through specific pop-holes (Chapter Three) suggests pop-hole design may be an important factor in accessing the range. The preference of specific pop-holes were not related to resources on the range, despite range enrichment being the focus of most range-related broiler research. Rault and Taylor (2017) showed that increasing pop-hole availability increased the number of chickens on the range at one time, although individual chickens were not monitored and pop-hole availability was confounded by a proportionate increase in ranging area.

8.6 Conclusions

Range use of commercial free-range broiler chickens was much greater than previously reported, although intra-individual variation within a flock was considerable. This challenges previous suggestions that faster-growing broiler chickens are not suitable for free-range production systems. Individual chicken characteristics, including lower body weight, better mobility, greater plumage cover and lower physiological stress responses predicted ranging behaviour and causation of these predictors should be investigated to ensure broiler chickens in free-range production systems are best suited to their environment. Season, suboptimal shed environment and shed design were related to range use and a greater understanding of these relationships may lead to optimal ranging opportunities on commercial farms. Accessing an outdoor range, including frequency, duration and distance ranged from the shed were associated with chicken welfare, including improved cardiovascular function, mobility, hock burn and reduced fear responses. However, ranging behaviour was also associated with increased foot pad dermatitis. Ranging behaviour had a bidirectional relationship with body weight but the mechanisms were not determined.

This thesis generates industry-relevant hypotheses which must be further investigated by controlled studies to help understand causation and lead to science-based industry-relevant improvements to free-range broiler chicken welfare.

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APPENDIX I

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Indoor side fidelity and outdoor ranging in commercial free-range chickens in single- or double-sided sheds

Jean-Loup Rault*, Peta S. Taylor

Animal Welfare Science Centre, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, VIC 3010, Australia

ARTICLE INFO	A B S T R A C T
Keywords: Behaviour Broiler Design Outdoor Poultry Welfare	The ranging behaviour of broiler chickens kept in free-range housing systems remains poorly understood, despite access to the outdoor range being their main feature. We investigated the impact of allowing chickens to have range access on both sides vs. one side of the shed, using 24 flocks of approximately 40,000 Ross 308 chickens of mixed sex on one commercial farm across winter and summer. Sheds were identical and pseudo-randomly allocated to either double-sided (no modification) or single-sided (by keeping one side closed at all time) range access treatment. Flocks were first provided with range access from 15 to 17 and 21 to 27 days of age for summer and winter flocks, respectively. Live outdoor observations were conducted daily for the first week after first range access and every other day from the second week onwards until the day prior to depopulation (44 days of age), twice daily in each morning and evening during anticipated peaks of range use. Indoor side fidelity was also assessed by spray marking 320 chickens in total on the right- and left-hand side of the shed, one colour each side, and conducting two to four repeated counts of the colour-marked chickens had 50% chance of being found on either side of the shed (P < 0.001 from a side preference), and therefore did not support the hypothesis that chickens haw indoor side fidelity in commercial conditions. Consequently, we could not elucidate whether an individual chicken would cross to the opposite side of the shed to access the range. Winter flocks had infrequent range access and low number of birds on the range (49 \pm 175 chickens wut chickens were observed on the range being affected by the interaction of treatment and age (P < 0.001) incore chickens were observed on the range being affected by the interaction of treatment and age (P < 0.001) incore chickens were observed on the range being affected by the interaction of treatment and age (P < 0.001) incore chickens were observed on the range being affected by the interaction of treatment and

1. Introduction

The ranging behaviour of free-range chickens remains poorly understood, despite access to the outdoor range being the main feature of this housing system. Free-range production has increased rapidly, driven by consumer perceptions of free-range housing as more animalwelfare friendly (de Jonge and van Trijp, 2013; Howell et al., 2016). However, free-range farms vary markedly in housing design, range design and management. There is still much to learn about the animal, housing and management factors that influence ranging behaviour in broiler chickens.

For instance, it is not known whether offering range access on a single side of the shed affects ranging behaviour compared to range

access on both sides of the shed. Various mechanisms may result in increased ranging behaviour when range access is offered on both sides of the shed, such as an increased number of entry and exit points of the shed onto the range, often referred to as 'pop-holes', which is linked with increased range use in laying hens (Gilani et al., 2014), or a shorter distance to travel to access the range.

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Broiler chickens may also be reluctant to move away from their 'home' environment in order to access the outdoor range. For instance, laying hens took longer to access a resource when they were required to pass or interact with an unfamiliar conspecific (Grigor et al., 1995). It has been hypothesised that chickens in large flock sizes, as seen in commercial conditions, remain within a limited 'home' area where they can recognise their neighbours and avoid or minimise agonistic

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^{*} Corresponding author. Present address: Animal Welfare Science Centre, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, VIC 3010, Australia. E-mail addresses: raultj@unimelb.edu.au, jl.rault@gmail.com (J.-L. Rault)

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interactions (McBride and Foenander, 1962). However, there has been evidence to disprove the theory of 'home' areas within commercial flocks of broiler chickens (Estévez et al., 1997), showing that they utilise large areas within commercial sheds (Preston and Murphy, 1989). Interestingly, Newberry and Hall (1990) showed that broiler chickens stayed in small 'home' areas but these areas move over time.

An additional feature of providing range access on both sides of the shed is usually a greater surface area for ranging. Broiler chickens increase space use when additional space is provided in larger pens (Newberry and Hall, 1990) or via an outdoor patio (Estévez et al., 1997); although such observations have not been investigated in flock sizes above 3000 individuals, which are most common on commercial farms. Broiler chickens are also motivated to access areas of low stocking density in controlled experiments (23 or 32 kg/m²)(Buijs et al., 2011), and decreased stocking density was found to be linked to higher ranging behaviour in laying hens (Campbell et al., 2017).

We hypothesised that providing access to an outdoor range on two sides of the shed would result in more chickens using the range as compared to providing access on a single side of the shed. We also investigated the effect of a potential shed 'home' environment on ranging behaviour, hypothesising that chickens located in areas closer to the pop-holes would range more than chickens further away in the shed.

2. Materials and methods

This experiment was approved by the South Australian Research and Development Institute Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.1. Site and subjects

The study was conducted on one commercial farm in South Australia with twelve sheds, grouped in blocks of six parallel sheds across two sites 1 km apart. Twenty-four flocks were studied across two replicates; 12 winter flocks and 12 summer flocks. All sheds had chickens from the same hatchery, same feed, and comparable management practices but with a different manager on each site. Placement of the chickens was made progressively over eight days, with placement day counted as day 0. Each flock contained approximately 39,740 Ross 308 broiler chickens placed at day-old, at a stocking density less than 34 kg/m² maintained through partial depopulation (also called thinning or first pick-up) of approximately 35% of the flock around 35 days of age. All sheds were identically built, 160 m \times 16 m, with tunnel ventilation and cooling pads. Brooding occurred in the front half of the shed.

Each shed had separate 156 m \times 17.3 m outdoor ranges on each side (*i.e.* double-sided), accessible through 14 pop-holes (3.8 m \times 0.4 m) spaced every 3.8 m on each side, apart from one pop-hole which was located at the middle length between the cooling pads occupying the front 57.2 m of the shed. The range was fenced, with a fence shared across two adjacent shed range areas. Six 0.8 m high 12 m \times 3.5 m rectangle horizontal shade cloth artificial covers were located on the range 6.1 m from the shed walls, and trees (1–2 m high at Site 1 and 1 m at Site 2) were present 12 m from the shed walls and spaced out approximately 5–10 m apart along the shed.

2.2. Chicken indoor side fidelity

To study whether broiler chickens maintain side fidelity or conversely move randomly between sides within the shed, 320 chickens in total on the right-hand side (n = 160 chickens) and left-hand side of the shed (n = 160 chickens) in ten summer flocks and nine winter flocks were marked either blue or green using livestock spray-paint (FIL Tell Tail, GEA, New Zealand) within four days prior to first range access

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(around 12 and 18 days of age in summer and winter flocks, respectively). The colour markings were randomly allocated between sides across flocks. Chickens were sampled from various locations in the shed to obtain a representative sample of the population, at regular intervals approximately 20 m apart, alternating between an alley within 1.3 m to the wall side, and another alley between 2.6 and 4.0 m from the wall side on each side (the 'alleys' being visible based on feeder and drinker lines spacing). Hence, the 50% of the shed floor surface in between each side was ignored as it was a less clear-cut area. Chickens were caught by corralling approximately 25–40 individuals at the sampling location using portable fences, then randomly picking up and marking 20 chickens with livestock spray-paint on the tips of their wings and top of the rump where most adult feathers were present at this age.

2.3. Pop-hole treatments

The sheds were allocated to either the double-sided pop-hole treatment without modification or to the single-sided pop-hole treatment by keeping one side closed at all times. Sheds were allocated to treatment groups to ensure an equal representation of treatments across sites. The sheds were oriented West-East, with pop-hole openings on the North and South sides. Single-sided pop-hole sheds were split equally between sides opening on the North or South sides across sites. Pophole opening times were automatically programmed but were at times manually overridden at the manager's discretion according to outdoor temperature forecast and feathering of the chickens. Chickens always ventured outside on their own will and were never forced outside.

2.4. Data collection

2.4.1. Indoor counting

In order to assess indoor side fidelity, live indoor observations were conducted twice within one week for winter flocks and every second day for one week for summer flocks, starting from the day after marking. One of three observers, blind to treatment, walked slowly to minimise disturbance in the middle of the shed and counted the number of marked chickens of each colour on one side of the shed. The 25% of floor space in the middle of the shed was ignored as this could not strictly be said to be on the right or left sides. The observer then walked back counting the number of marked chickens from each colour on the opposite side of the shed. Indoor counting was conducted after the outdoor count to avoid interfering with the number of chickens seen outside.

Chickens in 19 out of the 24 flocks were colour-marked due to some flocks being too young for marking at the time, with 54 counts conducted. However, in three of the summer flocks, chickens started feather pecking colour-marked conspecifics, with a strong bias toward pecking green-marked conspecifics. Hence, green-marked chickens were segregated in these flocks for animal welfare reasons, and data for these flocks collected after a feather pecking event (8 counts) were discarded as they were significantly lower than other data points for the number of non-segregated green-marked chickens recorded $(t_{(7)} = 2.33, P < 0.05)$. This left 46 counts from 17 flocks: six summer flocks with full dataset of four counts, a summer flock with three counts, another summer flock with one count the day after marking, and nine winter flocks with full dataset of two counts. Other chickens were noticeably scared of the blue-marked conspecifics upon release, with a visible flight zone surrounding blue-marked conspecifics when they moved, but they appeared to settle the following day with no notable pecking marks on blue-marked chickens.

2.4.2. Outdoor counting

Live outdoor observations were conducted daily for the first week after range access was first permitted and every other day from the second week onwards until the day prior to depopulation (44 days of age). Observations were conducted twice daily at anticipated peaks of

range access, in each of the morning and evening. For morning sessions, counting started 15 min after pop-holes opened or within the first hour of range access if daylight was not sufficient to accurately count chickens. The period of observation for morning count was determined based on a parallel experiment in which we observed range access through radio frequency identification (RFID) tracking of 300 individuals in one flock on the same farm, which showed that the number of chickens on the range was highest during the first three hours after range access was first available ($F_{(23,391)} = 90.5$, P < 0.001) (Taylor et al., unpublished). For the evening sessions, chickens were counted from 1800 h. One observer conducted the winter counting observations alternating between days.

The observer always remained outside the range area and used binoculars to count chickens, approaching slowly within 10 m from the fence to avoid scaring the chickens. A chicken was only counted as ranging if it had both feet on the ground outside of the pop-hole, which meant that 'pop-hole sitters' (sitting at the pop-hole between the shed and the range) were not counted as ranging. 'Counts' were conducted by counting individual chickens up to 50, estimating the number of chickens in increments of 10 from 50 to 200 chickens, and above that estimating a density of chickens in one area (usually between 50 to 100 chickens) and extrapolating that according to the range surface, but ensuring the density was uniform across the range. The observer counted the number of chickens on each range successively without adhering to a determined inter-observation interval as the aim was to undertake the counting within the narrowest time range possible. Marked chickens of each indoor side colour observed ranging were also counted.

For the first week of range access, counts were repeated three times in each session to enhance accuracy. The repeated counts were recorded from the front of the range (with the observer staying outside the range, *i.e.* observer never entered nor crossed the range), then from the back of the range, returning to the front of the range for the final count. The change of location aimed to minimise the effect of blind spot and distance estimate bias.

For the second week onwards, the repeated counts were limited to two, using a similar method but with the observer first counting ranging chickens from the six flocks at one site from the back of the range, then traveling to the other site to undertake a count the same way, before repeating the counts from the front of the range at both sites in succession. This was necessary from the second week onwards to minimise the interval between counting at each site, because all flocks were given access to the range at similar times, whereas the first week of range access was staggered due to the age difference between flocks. The flocks on each site were always counted in the same sequential order.

The winter data set was incomplete due to inclement weather conditions resulting in infrequent range access (nine days of range access out of 21 days studied; 65 counts; average outdoor temperature: 17.2 ± 0.2 °C on days chickens had range access), for an unrecorded amount of time but often less than a few hours and small numbers of chickens observed ranging in winter flocks (see Section 3.2. below). Therefore, analyses comparing double vs. single sided-shed treatments were conducted on the summer data only, as previously done for free-range broiler chickens (Dawkins et al., 2003)

Across all summer flocks, 592 counts were recorded across 19 days, from first range access from 15 to 17 days of age until 44 days of age. Average outdoor temperature on days chickens had range access was 22.4 \pm 0.8 °C. Chickens were not necessarily provided range access all day, as it was often too hot in the afternoon and evening (outside ambient temperature > 30 °C), which resulted in evening counts on only seven days out of 19 days, but morning counts on all days. Furthermore, range access times depended on weather conditions and time of sunrise (between 06:45–07:08 h) and sunset (19:34 h–20:06 h) throughout the course of the study. As a result, time varied between $06{:}32\ h$ and $14{:}24\ h$ for 'morning' counts and between $17{:}33\ h$ and $19{:}01\ h$ for evening counts.

For the first week, not all flocks reached the feathering stage to permit range access, and opening times were sometimes staggered between flocks according to feathering stage and outdoor temperature. Therefore, each round of repeated observations took approximately 22 min for each site, including travel time between sites, ensuring counts from both sites started as close as possible to 15 min after first range access. For the second week onwards, each round of observations took about 22 min for each site despite reducing from three to two counts per session as more chickens were observed on the range from the second week onwards, taking longer to count.

2.5. Statistical analyses

Indoor count data were compiled as the number of marked chickens recorded on the side of the shed they were initially marked ('same side') divided by the total number (*i.e.* both sides of the sheds) of marked chickens recorded during a count for each colour. Data were analysed using one-sample *t*-tests, comparing the observed proportion to the hypothesised mean of 1 (*i.e.* all recorded marked chickens on the same side of the shed).

Outdoor counts were cumulative, i.e. representing the sum of the chickens observed on the two range sides for double-sided pop-hole sheds but the count for the only range side accessible for single-sided pop-hole sheds. Outdoor count data were averaged per counting session and period (morning or evening) and square-root transformed, after which they met the criteria for normality and homogeneity of variance. Outdoor counts were analysed using a mixed model (Proc Mixed, SAS v9.3). For comparison between seasons, the model included the fixed effects of season and accounted for repeated measures on flocks as subjects. Flocks were considered the experimental unit and were independent across seasons because sheds were depopulated at 45 days of age. For comparison between treatments and to identify other factors that significantly affected ranging behaviour, the model included day of observation, double-sided vs. single-sided pop-hole treatment, age and the interaction of treatment \times age. The effect of period (morning or evening), time of observation, observer and site were not significant and were therefore removed from the model.

To further investigate the effect of age on ranging behaviour, outdoor count data were broken down into epochs of eight days, from the start of range access until the day prior to depopulation (44 days of age; the fourth and last epoch containing 6 rather than 8 days), and data analysed in a mixed model with epoch as main effect, accounting for repeated measures.

Results are reported as Least Square (LS)-means \pm SE unless stated otherwise.

3. Results

3.1. Indoor side fidelity

On average, $50.0 \pm 1.7\%$ of blue-marked chickens and $42.5 \pm 1.3\%$ of green-marked chickens were successfully identified on a side of the shed during counts, as opposed to being in the middle 25% of the area or being missed during live observations. However, marked chickens of either colour did not show side fidelity by staying on the side where they were initially marked ($t_{(46)} = -52.47$, P < 0.001 and $t_{(46)} = -51.14$, P < 0.001 for blue- and greenmarked chickens, respectively; Fig. 1), rejecting the null hypothesis that chickens maintain indoor side fidelity.

3.2. Outdoor counts in summer and winter

According to weather conditions and chicken feathering stage, flocks were first provided range access from 15 to 17 days of age for



Fig. 1. Indoor side fidelity for colour-marked chickens. Colour markings were randomly allocated between sides across flocks, on 320 chickens per flock in 19 flocks. Live observations were conducted two to four times over the week following marking to record the side each colour-marked chickens were on.

summer flocks, and from 21 to 27 days of age for winter flocks.

Season affected the number of chickens observed on the range ($F_{(1,22)} = 12.04$, P = 0.002), with 656 \pm 81 chickens observed ranging at one time on average in the summer and 49 \pm 175 chickens observed ranging at one time on average in the winter.

3.3. Summer outdoor counts according to double- vs. single-sided pop hole treatment and other factors

Single- vs. double-sided treatment had the strongest effect on the number of chickens observed on the range in summer flocks ($F_{(1,10)} = 110.62$, P < 0.001), but age ($F_{(26,125)} = 9.59$, P < 0.001) and the interaction of treatment × age ($F_{(26,125)} = 2.55$, P < 0.001) were also significant, with the mixed model accounting for 72.5% of the variance in the number of chickens observed on the range. More chickens were observed on the range for double-sided pop-hole sheds compared to single-sided pop-hole sheds from the seventh day of range access onwards, with significant differences on some but not all of these days (Fig. 2). Day of observation also had a significant effect ($F_{(18,159)} = 5.98$, P < 0.001), which may have been linked to weather conditions or related management practices such as changes in range

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access time. There was a strong correlation between the number of chickens observed on the range and age/number of ranging days (r = 0.73, P < 0.0001), as age and the number of ranging days were linked to each other.

A separate statistical analysis for chickens in the double-sided sheds revealed that the number of chickens observed on the range characteristically differed between 8-days epochs ($F_{(3,12)} = 144.73$, P < 0.001; Fig. 2). Between 24 and 264 chickens were observed on the range on average during each counting session in the first eight day of range access epoch (P < 0.001 compared to all later periods), which was the lowest out of all four epochs. Approximately 1000 chickens were observed on the range on average in the second eight day epoch (i.e. from 23 days of age; P < 0.001 compared to all other epochs). The number of chickens observed on the range continued to increase with time (age/day of range access) with between 2000 and 4000 chickens observed on the range on average in the third eight day epoch of range access (i.e. from 31 days of age; P < 0.001 compared to all previous epochs) and more than 4000 chickens on the range on average over the last four counting days, with a final estimate of up to 6000 chickens per flock observed on the range at one time on average at 44 days of age just prior to depopulation (equivalent to an average of 28% of the flock on the range at one time by that stage after a 35% partial depopulation of the flock around 35 days of age, and a minimum of 21% and a maximum of 37% of the flock across flocks). There was no statistically significant difference in the number of birds on the range after 31 days of age, between the third eight day epoch and the last six day epoch (P = 0.24).

The orientation of the range (North vs. South) did not affect the number of chickens observed on the range ($F_{(1,53)} = 0.82$, P = 0.37), considering both single- and double-sided pop-hole sheds.

3.4. Outdoor ranging side fidelity

Colour-marked chickens were observed during 10.3% of the observations on the range, with a maximum of four chickens for both colours at any one time, but predominately observations of only one marked chicken. Given that chickens were not found to maintain indoor side fidelity in the first place, and the scarcity of data, this information was not subjected to statistical analysis. Furthermore, the marked birds



Fig. 2. Average number of chickens observed on the outdoor range according to treatment (double vs. single-sided pop-hole sheds) and age (days) in the summer flocks. Counts are cumulative, *i.e.* representing the sum of the chickens observed on the two range sides for double-sided pop-hole sheds but the count for the only range side accessible for single-sided pophole sheds. Data were analysed using the square root transformation but are presented as non-transformed. * indicates significant differences between treatments at that time point based on the statistical analysis on the transformed data, not the non-transformed in the graph.

were difficult to see for longer than one week after marking, as the paint wore off and new feathers grew on top of the markings.

4. Discussion

We did not find evidence of side fidelity inside the shed. Consequently, we could not elucidate whether an individual chicken would cross the shed to access the range on the opposite side. The number of chickens observed on the range increased continuously from the second week of range access until depopulation. Sheds with range access on only one side had fewer chickens on the range than doublesided sheds during the period of high ranging activity, which suggests that shed design can limit ranging in free-range broiler chickens.

4.1. Indoor side fidelity

Chickens are known to prefer particular areas of sheds in which they are housed, such as near the cooling fans, walls, or entrance door when a shed is considered lengthwise, which is likely linked to environmental conditions (e.g. temperature) or disturbance levels (Newberry and Hall, 1990; Rodriguez-Aurrekoetxea et al., 2014). In our case, we assessed side fidelity widthwise through the marking of chickens on quarters of the shed on opposite sides of the shed, which are often comparable in the features they offer, and because access to the outdoor range is often provided length-wise along the sides of the shed. The results showed that chickens from each side spread evenly across the shed, with 50% chance of being found on either side. However, limitations exist to our assessment method as we could not ensure that the method of catching the chickens for marking did not disturb them, which would have resulted in not sampling them from their 'home' area. As a related example, flock sampling procedure has been shown to have a large impact on the observed prevalence of impaired walking ability in broiler chickens (Marchewka et al., 2013). Anecdotally, the chickens usually ran away from the catching location following marking, which could have disturbed their preferred location. However, similar situations occur frequently when farmers checked the flock daily through walking rounds or when they till/turn the litter, which may in itself disrupt any existing site fidelity (Newberry and Hall, 1990).

The movement of broiler chickens in commercial indoor sheds remains relatively unknown, partly due to the difficulty to identify and track individuals in large flocks. Farmers often report anecdotal cases of the infrequent black chicken in white strains (e.g. Ross or Cobb), who is always located in the same area of the shed, but our observations support the hypothesis that chickens move between sides. The scientific literature itself has provided conflicting results, with some studies suggesting that chickens stay close to their brooding site (Crawford, 1966; Tribe, 1981), that ranges overlap extensively (Appleby et al. (1985); broiler breeder flocks), that chickens staved in small 'home' areas which move over time (Newberry and Hall, 1990), or that chickens disperse (Preston and Murphy, 1989). Whether this discrepancy in findings between studies is related to differences in brooding location (e.g. one large area vs. multiple smaller brooding sites), brooding methods (e.g. length of confinement in brooding site), flock size or stocking density is unclear. One study suggested that site attachment strengthens with longer confinement in the brooding area (Tribe, 1981).

The feather pecking tendency towards green-marked chickens was not expected, and the two colours have been used in the past and recommended by industry contacts. It may be that the colour green stimulated a pecking response in chickens as they would show toward other green-colour items like grass. This behavioural response would be innate since this occurred prior to range exposure, while confined in the shed with no other green items in their environment. Preston and Murphy (1989) reported that chicks dyed green spread more quickly from their brooding area than chicks dyed blue, with no identifiable causes, whereas our findings suggest this may have resulted from pecking from conspecifics. Based on our results, we recommend avoiding green-colour marking.

Chickens did not seem to maintain side fidelity inside the shed. Therefore, it was not possible to elucidate whether chickens crossed further than 12 m to the opposite side of the shed to access the range.

4.2. Double- vs. single-sided pop-hole sheds

When range access was available only on one side of the shed compared to both sides of the shed, fewer chickens simultaneously ranged, from the second week of range access onwards. This effect was more pronounced as ranging increased with age and/or the number of ranging days (not dissociable from each other in this study, as chickens aged as they were provided more days of range access). The maximum outdoor range stocking density capacity was probably reached sooner in single-sided sheds compared to double-sided sheds, with the later offering both twice the range area but also twice the amount of popholes to move between the shed and the range. In addition, broiler chickens are often reluctant to move away from the shed, even when shade or cover such as trees are provided (Dawkins et al., 2003; Jones et al., 2007), and therefore higher outdoor stocking density closer to the shed may impair movement for other chickens in that area and those wanting to enter and exit the shed.

Although the comparison in this study was well controlled under commercial conditions with identical sheds, extrapolation of the results to other farms needs to be undertaken with caution because in Australia for instance, sheds providing range access on a single side are commonly older and narrower sheds (hence with a reduced distance to the range), whereas sheds providing range access on both sides of the shed are often newer and wider.

4.3. Factors affecting ranging behaviour

We found a strong positive correlation between the number of chickens observed on the range and the number of ranging days, consistent with previous studies (Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). Few chickens were observed ranging during the first week after range access was first provided, but increasingly more chickens were observed ranging in the subsequent days, continuing to increase until prior to depopulation at 44 days of age. By that stage, an average of 28% of the flock was observed ranging at one time when both sides of the range were accessible, and there were no clear signs that ranging abated at any point although it dropped around 33 days of age when flocks had interrupted range access due to partial depopulation of the flock. Studies using Radio Frequency Identification (RFID) system in broiler chickens (Durali et al., 2014; Taylor et al. 2016) showed that individuals alternate between the shed and the range throughout the day. Therefore, we cannot infer the exact proportion of the flock that accessed the range over the course of the day or the study, because we did not identify individuals to determine whether the same or different chickens were observed on the range across different counts.

At present, range access is often provided when the chickens are deemed sufficiently feathered to cope with outdoor weather conditions (usually by 21 days of age in Australia). During this study, flocks were provided range accessed between 15 and 27 days of age depending on weather conditions. Whether there is an optimal or critical age to introduce chickens to the range matters remains to be investigated, as we had little variability in age at first range access between flocks within season in the current study.

We counted the number of chickens on the range, shortly after range access was first provided in the morning (when sufficient light allowed counting) and within two hours of sunset in the evenings, which are known to be preferred times for ranging (Dawkins et al., 2003; Jones et al., 2007). Daily range access was often first available to the chickens approximately 30 min prior to sunrise. The influence of the time of day

at which range access is first provided on the motivation to range, possibly in conjunction with indoor and outdoor conditions (e.g. light, temperature), merits further research.

More chickens ranged in the summer flocks compared to the winter flocks, consistent with previous studies (Dawkins et al., 2003; Jones et al., 2007). Whether greater ranging is dependent on weather conditions, a habituation effect and/or greater opportunities remains to be investigated, given that chickens have usually frequent range access in the summer but irregular range access in the winter, with varying daily durations according to weather. This is an important question given that free-range systems are inherently affected by weather conditions. We did not find a significant effect of the range compass orientation on the numbers of chickens ranging, similarly to Dawkins et al. (2003), or between morning and afternoon when the sun position varied.

5. Conclusions

More chickens were observed on the range simultaneously when range access was available on both sides of the shed compared to a single side during the period of high ranging activity. We found no evidence to support the hypothesis that chickens show indoor side fidelity in commercial conditions. Ranging behaviour was governed by complex interactions between housing, management and environmental variables. To this end, a number of factors need to be taken in consideration before extrapolating the results of this study to other farms (e.g. shed size, pop-hole access, range features, weather variation).

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