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Experimental Improvements in Pullet Rearing

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Experimental Improvements

in Pullet Rearing

Kate India Norman

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of PhD in the Faculty of Health Sciences, Bristol Veterinary School, January 2021.

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Abstract

An increasing number of laying hens are being housed in more complex loose-housed systems but there is a lack of research focusing on the rearing period to prepare them for the laying shed. The first aim of this thesis was to investigate the provision of access ramps during the rearing period to encourage movement between elevated levels. Reduced behaviours indicative of hesitancy was found in young pullets that were reared with previous ramp access. The second aim of the thesis was to look at the effects of rearing in complex three-dimensional environments on the spatial cognitive ability of chicks. If reared with access to complex structures chicks showed improved spatial navigational ability in the detour test and a suggested improved ability to utilise intra and extra maze cues in the rotated floor test. The third aim was to apply these results to a commercial laying farm to look at the application of access ramps to elevated structures during the rearing period and if this had effects on movement between levels in the laying shed. Results demonstrate rearing with access ramps improves structure use at rear and reduces behaviours indicative of hesitant transitions down ramps in the laying shed. Chicks reared with access ramps were found to have better plumage scores at 40 weeks of age compared to the non-ramp reared chicks, suggesting there may be benefits to welfare. The final study aimed to summarise the rearing practices currently used in the UK, highlighting common practices that may require further investigations. In summary, this thesis demonstrates that rearing layer chicks in more complex environments and providing access via ramps to elevated areas may have beneficial impacts on spatial development, movement and welfare in commercial laying systems.

Acknowledgements and Dedication

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Authors Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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List of Abbreviations

AP – Aggressive pecking

BM – Brood and move

CI – Confidence Interval

CR – Control reared

ES – Elevated structure

FAWC - Farm Animal Welfare Committee

GFP – Gentle feather pecking

GR - Grid Ramp

LED – Light emitting diode

LR – Ladder Ramp

MDF – Medium density fibreboard

NRA – No ramp area

RA – Ramp area

RFT – Rotated floor test

RR – Ramp reared

RSPCA – Royal Society for the Prevention of Cruelty to Animals

SD – Standard Deviation

SFP – Severe feather pecking

WAIR – Wing assisted inclined running

Chapter 1 - General Introduction

General Introduction

1.1 Aim and objectives

The overarching aim of this thesis was to improve the rearing environment in commercial laying hen systems and specifically, to investigate methods that could be applied to rearing farms to improve bird movement in the commercial laying system. These systems can be complex for birds to navigate with resources such as food, litter and nest boxes located throughout different levels in the environment. There were four main objectives of this work; the first was to investigate the effect of the provision of ramps as resources that could potentially be applied to commercial rearing farms to improve movement in the rearing and laying system. The second was to study spatial navigation in the first four weeks of life to understand the developmental effects of rearing in complex environments. The third objective was to apply these ramp resources within commercial rearing and laying systems to assess effects on movement and welfare outcomes. The final objective of this thesis was to identify areas lacking in research by conducting a small survey of rearing producers to elicit information on current housing design and management.

1.2 UK laying industry

In 2018 around 41 million pullets and laying hens were farmed in the United Kingdom (BEI, 2018). An increase in free range production from 27% to 54% in the past 14 years reflects the increase in consumers' demand for free range eggs (BEI, 2018). Hens kept in these increasingly popular free range systems have access to the outside therefore the opportunity to perform a range of natural behaviours. However these free range systems have greater levels of mortality (Weeks et al., 2012, Weeks et al., 2016), pose a greater

risk of disease and have higher reported problems of injurious pecking and cannibalism (Tauson, 2005) when compared to colony cage systems. Research has been conducted on free range laying hen systems to reduce production losses and increase the welfare of laying hens (Lambton et al., 2010, Lambton et al., 2013). However, recent evidence drawn together in a review by Janczak and Riber (2015) suggests a knowledge gap about the effect that early life experiences of laying hens may have on their behaviour and welfare later in life.

UK Regulations and Council Directive 1999/74/EC set out the minimum standards for housing of laying hens that must be followed, with the aim of ensuring good welfare. There is no mention of the rearing systems in these standards that are legally binding for all producers. The UK government issues codes of practice which include information on the best practice for rearing laying hens but these codes are not legally binding. However, in the UK there are assurance schemes that outline higher standards that producers can adopt to add value to the eggs produced. These include RSPCA Assured (RSPCA, 2018) and Soil Association (SoilAssociaton, 2019). These assurance schemes include specifications for the rearing period of laying hen production, based on scientific research and best practice (see Table 1-1).

Table 1-1. A comparison of details from Soil association (SoilAssociaton, 2019) and RSPCA (RSPCA, 2018) assured standards for pullets.

	RSPCA Assured	Soil Association
Feeders and	- Grit should be provided from 3	- Forage must be added to diet
drinkers	weeks of age	
	- 2.5cm feeder track per chick	
	- 1 nipple drinker per 12.5 pullets	
	- Supplementary drinkers at 1-4 days	
	of age	
Stocking	- 15 weeks = 15 pullets per m ²	- In fixed housing: 10birds/m², with a
density	- $16 \text{ weeks} = 14 \text{ pullets per m}^2$	maximum of 21 kg liveweight/m ²
	- $17 \text{ weeks} = 13 \text{ pullets per m}^2$	- In mobile housing of less than 150m
	- $18 \text{ weeks} = 12 \text{ pullets per m}^2$	floor space: 16/m ² , with a maximum
	- Or 20kg/m ² at 16 weeks of age	of 30 kg liveweight/m ²
		- Max of 2000 birds per house
		- Range 4m ² /bird
Lighting	- Minimum of 10 Lux at pullet head	- Continuous dark period of 8 hours
	height	(does not apply to chicks)
		- Must provide a dim period of at least
		10 minutes
Perch Space	- 7 days at the latest	- 15 cm/bird aerial perch space
	- Not less than 5cm per pullet	
Litter	- Access to litter at all times.	- 50% of flooring must be solid (not
	Information on suitable litter types	slatted)
		- Suitable litter material provided
Mutilations	- Must be conducted before 24 hours	- Not allowed
(beak	old	
tipping)		
Free range	- If to be free range access given by	- Access by 12 weeks of age
	12 weeks of age	- Mainly covered with vegetation
	- Shade and shelter must be provided	- Provide protective shelter at all times
		- Permits poultry easy access to
		adequate numbers of drinking and
		feeding troughs.

1.2.1 Housing systems at lay

Laying hens are housed in a variety of systems in the UK, broadly split into cage and loose-housed. The current cage systems are called furnished, or colony cages, generally housing between 80 to 100 hens. The Council directive (1999/74/EC) requires 750cm² of cage area per bird, a nest area, litter (often in the form of a scratch mat) and 15cm per bird of perch space. These colony cages replaced conventional cages in 2012 in all EU member states. Conventional cages required 550cm² per bird but no other enrichment type or furnishing. The colony cages were designed to improve welfare in the commercial laying hen industry by providing hens with the opportunity to express more behavioural needs (Weeks and Nicol, 2006). Although caged egg production contributes up to 44% of laying egg production (BEI, 2018), from 2025 onwards many large retailers have pledged not sell caged eggs owing to pressure from consumers who want to purchase cage-free eggs.

The alternative loose-housed systems in the commercial laying industry allow hens more freedom of movement throughout the system, and indeed require hens to move to different areas to access resources such as food and water. These systems tend to be either (i) single-tier with resources such as feed, water and nest boxes on a raised slatted area and ground level litter area or (ii) multi-tier with multiple blocks of slatted areas up to 4 tiers high with resources on each tier and a ground level litter area. These types of housing design are used for free range, organic and barn laying systems. In free range systems birds have freedom of movement throughout the house and access to an outside area through pop holes that open in the sides of the sheds. Organic systems are very similar, but birds must be housed in smaller flocks and at a lower stocking density with free range access. Barn systems allow hens freedom of movement within the system but

do not provide outside ranging access. Free range systems are most common in the UK and contribute approximately 52% of the UK egg production with organic at 2% and barn systems contributing 2% (BEI, 2018). With the likely reduction in the number of cage egg demands following 2025 there may be an increase in the loose-housed barn systems as an alternative to cage egg production.

1.2.2 Housing systems at rear

For the purpose of this thesis the term chick will refer to young layer strain birds to 8 weeks of age and the term pullet to describe young layer strain birds from age 9 weeks to point of lay (around 19 to 20 weeks of age). Laying chicks are incubated and hatched at commercial hatcheries then transported to the rearing facility approximately 48 hours after hatching. There is evidence that the environment and experiences during incubation and hatching can have developmental impacts. Riedstra and Groothuis (2004) found prenatal exposure to light increased the levels of feather pecks observed in chicks. Although there is evidence of prenatal effects on development and behaviour, this is not the focus of this thesis as all chicks were obtained from the same commercial hatchery under the same conditions. In the commercial laying industry hens will commonly be reared in a separate building to the laying system up to 16 weeks of age. These rearing systems are either cage or loose-housed. The loose-housed systems are often fully littered barns with raised perches and sometimes raised slatted platforms. If destined for multi-tiered laying systems, it is now recognised that chicks will fare better if reared in a similar set up. Compared to floor-reared birds, multi-tier reared birds show better use of raised areas, nest boxes and perform more movements between raised areas (Colson et al., 2005), all of these movements are required in multi-tier laying systems. Some producers will rear chicks in multiple rearing sheds over the 16-week rearing period, a process called brood and move (BM). The BM process means that chicks will be housed in an indoor barn with heat sources then transferred around 8 weeks of age to a larger environment that is often configured like a laying shed. This rearing set up is commonly used by large organic rearing producers so they can provide pullets with outside range access from 12 weeks of age whilst brooding the next flock of chicks.

The rearing period constitutes a fifth of a laying hen's commercial lifespan and includes the period from hatch to 16 weeks of age. During this time much of the birds' neural development and learning takes place. Neural development for spatial navigation has been found to take place as early as 14 days of age (Rogers, 2014), demonstrating the importance of rearing in a similar environment to the laying system. It is well researched that early experience has long term effects on learning and behavioural development, for example, preferences for ground pecking and dustbathing occur between 3 to 10 days of age (Vestergaard and Lisborg, 1993, Vestergaard and Baranyiova, 1996, Vestergaard et al., 1999, Nicol et al., 2001). There is evidence of early experiences having long lasting impacts on welfare (Janczak and Riber, 2015, Campbell et al., 2019). For example, rearing chicks on wire floor compared to access to substrate has been found to increase the risk of feather pecking developing at lay (Blokhuis and Vanderhaar, 1989). The development of welfare problems at rear can increase the risk of welfare problems at lay (Gilani et al., 2013) therefore efforts must be made to provide a suitable environment to ensure good welfare at rear.

1.3 Welfare of laying hens

The definition of animal welfare has been much debated over the years (Dawkins, 1990, Fraser, 1999, Fraser, 2003, Dawkins, 2003, Dawkins, 2008, Broom, 2011). Broadly people agree that it must encompass functioning (physical health), mental health

(emotions or feelings) and behavioural needs of the animal. For this thesis behavioural and physical measures will be used to assess animal welfare. Setting standards for farm animal welfare has been developed over many years. The Brambell Report in 1965 first introduced the concept that animals should have the freedom to stand up, lie down, turn around, groom themselves and stretch their limbs, becoming known as the Five Freedoms. These 5 freedoms were later broadened by the Farm Animal Welfare Committee (FAWC): Freedom from hunger and thirst; from discomfort, pain, injury and disease; to express normal behaviour; from fear and distress (FAWC, 2009). The five freedoms still form the basis of many assurance schemes such as RSPCA Assured in an attempt to specify what is needed to meet the Five Freedoms.

1.3.1 General welfare and behavioural needs

Recording animal behaviour is useful to inform animal welfare, some background to the welfare measures used in this thesis will be discussed and the importance of access to specific resources required in the laying hens environment. To achieve good welfare for laying hens we need to meet their basic needs such as physical or biological requirements. But we must also consider psychological needs, behaviours that the animal may 'want' to perform. Weeks and Nicol (2006) defined behavioural needs as behaviours that will be performed even in the absence of an optimum environment or resource but this may also include behaviours that animals are highly motivated to perform (Jensen and Pedersen, 2008). Dustbathing is an example of a behavioural need, as in the absence of suitable litter i.e. wire flooring, birds will perform sham dustbathing (Vestergaard et al., 1999, Olsson and Keeling, 2005). As discussed, we can record animals' behavioural responses to identify behavioural needs by asking the animal what it wants but we can also record 'abnormal' behaviours in association with restriction

from a resource. In laying hens we see abnormal behaviours in suboptimal environments such as displacement preening or redirected foraging (Cooper and Albentosa, 2003). Recording these negative types of behaviour together with positive 'wanting' behaviours can help to assess welfare.

Dustbathing is a highly motivated behaviour and laying hens perform the behaviour in the absence of a suitable resource, making it a behavioural need for laying hens. The function of dustbathing is to remove lipids from feathers and parasites from skin (Martin and Mullens, 2012). If restricted from access to suitable dustbathing material we still observe the 'sham dustbathing' behaviour on wire flooring (Olsson and Keeling, 2005, Lindberg and Nicol, 1997). Although this sham behaviour may not satisfy the function, if laying hens were housed with a less suitable substrate i.e. wood shavings then transferred to a more suitable substrate i.e. sand, dust bathing was recorded more often and for longer (Vanliere et al., 1990), suggesting that the wood shaving material was lacking an aspect that the hens required to fully satisfy the dustbathing behaviour. Substrates with smaller particles are preferred as the small particles can penetrate the plumage (Scholz et al., 2010, de Jong et al., 2007). In commercial systems it is therefore important that laying hens can access suitable litter material to enable the performance of dustbathing behaviour. If chicks, pullets or laying hens are restricted from these resources, due to difficulty in navigating complex environments, this will impact welfare.

Foraging behaviour takes up a large proportion of the domestic hen's time budget suspected to be similar to semi-wild Red Jungle fowl recorded foraging at 60% of active daylight hours (Dawkins, 1989). In commercial systems hens have a continuous feed supply yet foraging behaviour is still observed. Bubier (1996b) found that pecking,

scratching and food searching behaviour was performed equally on litter containing food and litter without, suggesting a valued behaviour regardless of food accessibility. Bubier (1996a) also found the time budget that hens spent in woodchip foraging areas was not altered if hens had to pay a 'cost', squeezing through a narrow gap to gain access, suggesting foraging as a high priority behaviour. In loosed-housed systems laying hens are provided with foraging material in the litter and out on the range, if free range. It is therefore important to ensure hens can access these resources with ease by providing access routes throughout the laying system. If access is thwarted to resources that enable the performance of these high priority behaviours, we may see abnormal or displacement behaviours as a result of frustration which can indicate poor welfare (Weeks and Nicol, 2006). In commercial systems abnormal behaviours such as injurious feather pecking may be observed in an unsuitable environment (Lambton et al., 2010), for example if there is a lack of suitable foraging opportunities. This is measurable by behaviour and assessing plumage cover.

Nesting instinct has been identified as a behavioural priority in laying hens. Hens will select a nest site by nesting material (Huber et al., 1985), enclosure (Appleby and McRae, 1986), light intensity (Appleby et al., 1984b) and social conditions (Tahamtani et al., 2018, Appleby et al., 1984a). Hens are highly motivated to access a nest site; this increases during oviposition (egg laying time). Studies have found hens will squeeze through narrow gaps to access a nest site (Cooper and Appleby, 1996) and push through weighted doors (Cooper and Albentosa, 2003) showing greater motivation for nest sites over food during oviposition. In commercial systems nest boxes can be situated on upper levels and in different locations to other resources such as the ground level litter areas. If access to a suitable nest site is inhibited, frustration behaviours may be expressed such as vocalisations (Meijsser and Hughes, 1989), pacing (Yue and Duncan, 2003) and

displacement preening (Duncan and Woodgush, 1972). Thus, making restriction from suitable nesting sites a clear welfare concern in commercial situations and a clear importance of ensuring movement between the resources provided in the laying system. Frustration behaviours have been observed if access to perches for night-time roosting is prevented (Olsson and Keeling, 2000). Hens will push through a weighted door to access a perch at night (Olsson and Keeling, 2002). Behaviours such as stepping on the spot, pacing, and head movements have been identified in studies as behaviours that may indicate frustration as a result of restricted access to a resource (e.g. a perch) or reward (Davies et al., 2014, Pettersson et al., 2017a, Pettersson et al., 2017d, Lambe et al., 1997). Measuring hens balancing behaviour on various perching materials it appears a larger diameter and rubber material are best suited for hens (Pickel et al., 2010). However, hens show no preference for specific aspects of a perch (Appleby et al., 1998) and if no perches are provided hens will choose to perch on the highest fixtures and fittings accessible (Appleby et al., 1988), height is an important aspect of a perch (Schrader and Mueller, 2009). In commercial settings the function of daytime perching may be to escape aggressive interactions (Donaldson and O'Connell, 2012) by providing a refuge point for lower ranking birds to avoid higher rank birds (Cordiner and Savory, 2001). Access to perches from a young age may be beneficial to laying hens by improving development of spatial ability (Gunnarsson et al., 2000, Wichman et al., 2007) and increasing perch use for night-time roosting, an important aspect for commercial systems (Heikkila et al., 2006).

1.3.2 Feather pecking

Feather pecking is the plucking of feathers from conspecifics and is thought to be an abnormal behaviour in poultry (Huber-Eicher and Audige, 1999). There are two forms

of feather pecking. Gentle feather pecking (GFP), the gentle pecking at the tips of feathers and severe feather pecking (SFP), the pecking and pulling of feathers, often with removal of the feather. SFP can result in injuries or even death due to cannibalism. This is a welfare and economic concern which can reduce productivity due to defeathering leading to increased feed intake (Huber-Eicher and Sebö, 2001, Lambton et al., 2010, Lambton et al., 2013).

Lambton et al. (2010) carried out a study to identify the risk factors associated with gentle and severe feather pecking in loose-housed laying hens. The authors identified that feather pecking was more likely to occur in flocks with poor range use, poor rearing, frequent diet changes and restricted litter access. In a later study Lambton et al. (2013) looked at the effect of a bespoke management package on the levels of injurious pecking in loose-housed flocks. Individually tailored management strategies were created for farms in the treatment groups. Strategies included litter quality, diet, range quality, flock health, perching and other environmental enrichments. It was found that the more management strategies that were applied on each farm, the lower was the level of injurious pecking. Many of the management strategies such as perch provision, litter access, range access, younger age of arrival and lower stocking density (Bestman et al., 2009, Bestman and Wagenaar, 2003, Knierim, 2006, Nicol et al., 2013, Pettersson et al., 2016) have been found to be protective factors for feather pecking in other studies. This shows the importance of management on farms and the influence of environment on hens' welfare.

Research has demonstrated how the rearing environment can impact feather pecking, both directly and indirectly. Studies have identified many environmental factors that, if experienced at rear, can be predictive of feather pecking in the laying environment. Some examples of risk factors outlined in studies are high ammonia, carbon dioxide,

light and noise levels (Drake et al., 2010, Gilani et al., 2013). Frequent changes in diet have been associated with high levels of feather pecking in the laying environment (Lambton et al., 2010) and this has also been found in rear, with the risk of SFP increasing 64 fold with every diet change (Gilani et al., 2013). Bestman et al. (2009) identified some factors in the rearing environment that may impact the levels of feather pecking at lay, finding that in 90% of cases flocks that showed feather damage at rear continued to show feather damage at lay. High levels of feather pecking in rear have been associated with high levels of SFP at lay (de Haas et al., 2014a, Drake et al., 2010). Feather damage has been recorded at rear; in one study 12% of 34 flocks had feather damage at the end of rear (Gilani et al., 2013). Not all flocks appeared to have feather damage, indicating an influence of management, genetics and environment at rear. If management strategies can be applied to rearing flocks this may have beneficial effects on the levels of feather pecking at lay. Management of the rearing house can have an effect on feather pecking for example, disruption of litter access in the first four weeks of life can increase SFP at lay (de Haas et al., 2014a). Behavioural traits of birds can also influence feather pecking: fearful chicks in an open field test showed more GFP and SFP as adults (Rodenburg et al., 2013) showing links with behaviour at rear and resultant behaviours as adults. As discussed here the rearing environment can have an impact on the development of feather pecking. When making environmental changes to the early rearing period recording measures such a plumage cover and injurious feather pecking behaviour can be a useful indicator for welfare and therefore will be used as a recording in this thesis.

1.3.3 Keel bone fractures

Keel bone fractures are another welfare concern in loose-housed laying systems. Recent studies have suggested the prevalence of keel bone fractures at end-of-lay can be up to 95% (Wilkins et al., 2011). The prevalence of birds with old breaks varies between systems (see Table 1-2). Wilkins et al. (2011) assessed the prevalence of keel bone fractures in different housing systems using palpation, finding that the proportions of birds with keel bone fractures ranged between 28-89% in non-cage systems, compared with 22-44% in colony cage systems.

A large proportion of birds sustaining injuries is a welfare concern. There is evidence that healed keel fractures are a source are chronic pain, as hens with healed fractures have been shown to form a place preference by association if an opioid analgesic drug was previously administered in that place, whereas hens with no fractures did not show such preferences (Nasr et al., 2013). This outlines the importance of reducing the prevalence of keel bone fractures. Legislation states that laying hens must be provided with adequate perches allowing 15cm per bird (1999/74/EC). With the addition of perches into loose-housed systems there is an increased risk of colliding with structures in the house which may result in keel bone fractures.

Table 1-2. Adapted from Wilkins et al. (2011), showing the difference in percentage of keel bone fractures from palpation and dissection in different systems.

System type	n	% old break palpation	% old break dissection	Mean severity of breaks
Free range	12	62	67	1.91
A frame perches with additional horizontal bars	7	68	78	2.15
A frame perches with aerial suspended perches	6	87	86	2.59
Organic mobile	8	39	45	1.61
Organic mobile with fixed aerial perches	4	67	84	2.26
Organic house with single tier of slats	11	51	59	1.83
Barn system	10	53	63	1.80
Colony cage	9	32	36	1.45

Wilkins et al. (2011) saw high impact velocity landings from elevated structures in loose-housed systems, which may be the cause of at least some fractures to the keel bone. This correlates with the high percentage of old breaks (87%) found by palpation in the free range housing systems equipped with suspended aerial perches (Wilkins et al., 2011). Unfortunately, there appear to be trade-offs required between opportunity to perform natural behaviours (perching and ranging outdoors) and the risk of sustaining injuries or disease. These problems must be addressed in order to provide hens with the highest welfare possible. One hypothesis is that laying hens are not exposed to suitable environments during rear (Janczak and Riber, 2015), if the features of these environments are identified we may be able to reduce keel fractures.

Research into early life has included aspects of skeletal growth and spatial navigational development. Chicks reared in more spatially complex environments such as aviaries show improved bone mineral density (Hester et al., 2013, Casey-Trott et al., 2017a,

Casey-Trott et al., 2017b) and cognitive skills (Tahamtani et al., 2015, Wichman et al., 2007). Research into feather pecking, skeletal health and cognitive development has produced useful outcomes that could be applied to commercial systems and improve the welfare of hens at both rear and lay. In this thesis where applicable keel bone palpations will be conducted to assess if there are any effects of rearing experience on keel bone fractures.

1.4 Movement in loose housed systems

As previously discussed, access to resources throughout commercial systems is important for laying hens to express behavioural needs. In loose-housed systems the resources are spread out throughout the house requiring birds to move up and down levels to reach them. If access is restricted from these resources, we may see frustration behaviours, injurious feather pecking or injuries due to collisions. It is therefore important to ensure that birds have ease of access to resources in commercial laying systems.

1.4.1 Physical ability and physical consequences

In commercial systems an increased number of collisions with the environment are observed when a level change is included (Harlander-Matauschek et al., 2015). Stratmann et al. (2015) modified aviary systems to include more perches, ramps and platforms. The authors found that the inclusion of ramps in the systems resulted in a reduction in the number of collisions by 59% and falls by 45% and birds showed 44% more controlled movements. The benefits of providing access ramps in commercial systems has been shown by Pettersson et al. (2017a) who observed fewer behaviours indicative of hesitancy in laying systems with ramps spanning the full length of a level

change. Laying hens also show a preference for using grid ramps over ladder ramps shown by reduced latency to transition and fewer behaviours indicative of hesitancy when transitioning on a grid ramp (Pettersson et al., 2017d). By ensuring a good access route to move up and down between levels we may improve accessibility of important resources such as the range and litter areas.

Limited research has been conducted on the rearing environment in relation to structural enrichment. Colson et al. (2008) found if birds were reared with vertical structures, similar to those in the laying shed, they performed more long distance flights and had fewer collisions and falls. There is also an association with rearing environment and the number of keel bone fractures recorded with up to 18.8% fewer fractures recorded in aviary reared birds compared to cage reared birds (Casey-Trott et al., 2017a). In a review Harlander-Matauschek et al. (2015) suggested that when moving up and down ramps chicks perform wing assisted incline running (WAIR) which may be important for development of the wing muscles that attach to the keel bone. Campbell et al. (2019) reviewed environmental enrichments for rearing laying hens, summarising that further research is needed in commercial settings to identify practical cost-effective enrichment for rearing.

1.4.2 Cognitive ability and cognitive consequences

Laying hens spatial ability to navigate the environment may also be a contributing factor to resource accessibility. Studies have found that rearing laying hens in aviaries compared to floor systems improved the number of successful long-distance flights (Colson et al., 2008) and improved spatial cognition when tested using a hole board task (Tahamtani et al., 2015). Improving spatial cognition may result in improved use of

the laying system, especially in Aviary systems where resources can be distributed throughout many levels.

The age that layer chicks require access to more complex structures is yet to be investigated in detail. Kozak et al. (2016) looked at locomotion activity of layer chicks over a period of 1 to 9 weeks of age. The authors observed chicks using the low-level perch (10cm) and low-level platform (70cm) by 2 weeks of age. In semi-natural conditions chicks have been observed to show a peak in perching behaviour at 10 days of age (Workman and Andrew, 1989). There appears to be evidence of a sensitive period for experiencing environmental complexity. Freire and Rogers (2007) found if visual barriers were provided between 8 to 9 days there was no effect on performance in detour tests or food reward tests, however, if visual barriers were provided between 10 to 11 days of age chicks performed better in the tests. This sensitive period for the development of cognitive skills may be applicable for perching behaviour. Wichman et al. (2007) investigated whether spatial navigational ability is innate. They were unable to disentangle this question but did find an association with spatial skill in a twodimensional test and performance in a three-dimensional test situation. Further work is needed to assess whether early access to complex three-dimensional environments improves spatial ability of layer pullets.

In summary, if spatial navigation of chicks is improved through complex rearing this may have beneficial effects on bird movement and navigation in commercial laying systems. This may result in reduced collisions and falls, shown to reduce the number of keel bone fractures which will be beneficial to laying hens welfare. Improved navigation through the environment should result in improved access to important resources such

as nest boxes and litter material for foraging behaviours, in turn reducing the performance of frustration behaviours or feather pecking.

1.5 Application to commercial systems

Much of the research discussed here has been from small experimental studies. Fundamental research to address initial investigations is important, as this can be conducted in controlled experimental conditions. However, it is not easily applied to commercial situations where there are many variables that may influence the results. Applied research on farms has been conducted either observationally to compare aspects of different systems (Pettersson et al., 2017a, Campbell et al., 2016c, Campbell et al., 2016a, Campbell et al., 2016b) or making changes to the system and comparing outcomes (Gilani et al., 2012, Zimmerman et al., 2006). Studies can also be carried out on commercial style experimental facilities where smaller groups of hens can be housed but in situations that aim to replicate a commercial setting (Stratmann et al., 2015, Harlander-Matauschek et al., 2006). Conducting research on commercial farms with good numbers of replication is an important step to ensure fundamental research is applicable to commercial settings and will have an impact. Chapters 2 and 3 report the results of studies conducted in such settings.

Summarising current practices in the commercial laying sector is also important for developing further research or identifying gaps in knowledge where research is needed. The use of questionnaires or surveys have been used to collect information from producers and prove useful in consolidating information in previous studies (Hane et al., 2000, Huber-Eicher, 1999, Green et al., 2000). By including commercial producers in these surveys, we can identify if research outcomes are being applied to commercial systems.

This literature review draws together research conducted to improve the welfare of laying hens. With an increase in the number of free range and loose-housed egg production systems we see different causes to the welfare problems that arise such a keel bone fractures and feather pecking. There appears to be a lack of research conducted on the rearing environment, especially for improving navigation and movement throughout the rearing and laying systems. This thesis will therefore aim to investigate methods to improve the rearing systems for loose-housed laying hens.

1.6 Objectives and progression of studies

Investigations begin in Chapter 2 by looking at the effects of rearing with ramp access on the ability to move between raised levels in an experimental setting (Norman et al., 2018). In Chapter 3 further investigations are conducted into spatial navigation of layer chicks and if there are impacts of rearing with access to complex elevated structures on navigational ability at a young age (Norman et al., 2019). Chapter 4 applies this research on a commercial organic rearing and laying farm to assess whether rearing with ramps and early access to elevated structures improves movement and reduces keel bone fractures and plumage damage in the laying shed. A questionnaire summarising rearing methods of pullet producers is described in Chapter 5. Finally, Chapter 6 discusses the results of studies reported in Chapters 2 to 5 and outlines future research ideas.

Chapter 2 - The effect of experience of ramps at rear on the subsequent ability of layer pullets to negotiate a ramp transition

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The effect of experience of ramps at rear on the subsequent ability of layer pullets to negotiate a ramp transition

Abstract

In commercial situations, laying hens must negotiate levels to reach resources such as food, water and litter. Providing ramps in aviary systems reduces collisions and resultant keel bone fractures in adults. We investigated whether providing ramps during rear improved the ability of birds to transition between levels.

Chicks were reared commercially in two flocks both of which provided access to raised structures from three weeks of age. One flock had no ramps, but the other flock was provided with additional access to two types of ramp (wooden ladders, and grids formed from commercial poultry slats placed at an angle). At 8 weeks of age, 64 birds (32 from each rearing condition) were transferred to an experimental facility. At 10 weeks of age, 32 pullets from each group were trained to run to a food reward. During testing at 12-14 weeks of age the pullets accessed the food reward by moving up or down a ramp. The pullets' behaviours and time taken to complete the task were recorded. Ramp use over three days was also observed in a room replicating a small-scale single-tier system. Four groups of 16 birds aged 12-14 weeks were housed for three days and the number of transitions between the raised tier and litter were recorded.

For upward transitions, more ramp-reared birds than control birds succeeded in reaching the food reward for both ladder (52% vs 13%) and grid ramps (74% vs 42%). Birds from the ramp-reared group took significantly less time to complete an upwards transition (68.8s \pm 49.3) than the control group (100s \pm 37.6) (p = 0.001). In addition, the control group showed more behaviours indicative of hesitancy (moving away, head

orientations, ground pecking and crouching) before transitioning, and signs of difficulty when making upward transitions (crouched walks, pauses, turning, returning and escape attempts). In the group housing observations, the ramp reared groups had almost double the number of transitions between the slats and litter on day one compared to the control group. This difference was reduced by day three.

In summary, this suggests there are positive effects of providing ramp experience during rear shown by any combination of bird mobility, strength or cognitive ability leading to an increase in apparent confidence in older pullets. It is not known whether these benefits persist through to the laying period, but no detrimental effects were noted so we suggest that ramps should be included from the early rearing period onwards.

2.1 Introduction

In loose housed laying systems in many European countries, research has been conducted on the inclusion of ramps to help birds with level changes in their environment (Heerkens et al., 2016, Pettersson et al., 2017a, Stratmann et al., 2015). There are two principal loose housing systems for laying hens: single tier (flat deck) and multi-tier (aviary) systems. Single tier systems comprise a raised slatted area containing food, water and nest boxes with a drop down to reach the litter and range. Multi-tier (aviary) systems contain multiple tiers stacked on top of each other with food, water and nest boxes. There can be vertical drops of up to 90cm between tiers, including to the ground level litter.

In loose housed systems, increased collisions with the environment have been observed when a level change is included (Harlander-Matauschek et al., 2015). Collisions and falls from heights can lead to injuries such as keel bone fractures (Stratmann et al., 2015). Birds with keel fractures show restricted movements and reduced willingness to jump down from perches (Nasr et al., 2012a, 2012b). Experimental work has shown that mobility is partially restored if analgesic drugs are administered (Nasr et al., 2015) suggesting that untreated keel bone fractures are painful. In commercial systems fracture rates can be as high as 80% of the flock at end of lay in more complex housing with aerial perches (Wilkins et al., 2005, 2011). The addition of ramps in the laying house has been shown to reduce falls and collisions by 45% and 59% respectively, along with 44% of birds showing more controlled movements if provided with ramps (Stratmann et al., 2015). When negotiating a level change fewer hesitancy behaviours have been recorded in laying flocks provided with ramps spanning the full width of the lower tier (Pettersson et al., 2017a), suggesting that ramps can aid transitions between levels.

Birds' ability to negotiate ramps of different design has also been trialled, showing easier transitions on a grid ramp and a preference for a grid ramp over a ladder ramp (Pettersson et al., 2017d).

Before transfer to the laying system at 16 weeks of age, pullets destined for loose-housing systems in Europe are commonly reared in large areas with litter covered floors and some perches and raised structures to give them experience of navigating in three dimensions. Rearing in complex environments, such as aviaries, which provide opportunities for exercise, can reduce the proportion of keel bone fractures measured during lay. For example, Casey-Trott et al. (2017a) found a fracture rate of 41.5% in aviary reared birds compared with 60.3% for cage reared birds.

The cognitive effects accruing from perch or tier provision also seem to be enhanced when birds are reared with these structures, rather than encountering them for the first time when moved to the laying system. For example, Gunnarsson et al. (2000) found that rearing birds with perches to 8 weeks of age, improved their ability to negotiate a series of raised platforms to reach a food reward. Because the difference in performance between the two rearing groups increased with task difficulty, the authors argued that the rearing conditions may have influenced spatial navigational ability, and that the results could not easily be explained only by differences in physical strength. This was tested more directly by Tahamtani et al. (2015) who compared the influence of cage versus aviary rearing on spatial cognition using a two-dimensional hole board task thereby eliminating the confounding factor of physical ability. These authors reported that birds reared in the more barren cage environment had poorer working memory (i.e. short-term memory used to recall the location of food rewards in a hole board task). Further, Colson et al. (2008) showed that birds reared with vertical structures, similar to those later encountered in the laying shed, performed more long distance flights (100cm

to 300cm), accounting for 40% of all flights compared to 35% for floor reared birds. Generally, indirect effects due to improved spatial navigation are likely to be complemented by direct effects of additional exercise or improved physical ability (Gunnarsson et al., 2000). Overall it seems that, despite gaps in knowledge about possible longer- term cognitive effects, rearing birds with vertical structures has both physical and cognitive benefits, and the provision of ramps for adult laying hens aids smooth transitions between levels.

There is less evidence for the effects of ramp provision during the first few weeks after chick's hatch. In a review, Harlander-Matauschek et al. (2015) suggested that the provision of ramps at a young age may promote wing-assisted inclined running, which could affect the development of the keel bone and muscles and improve balancing abilities. Kozak et al. (2016) reared chicks in complex aviaries with ramps, low level platforms and perches. Ramp use peaked at 2 weeks of age when chicks started to use the upper levels. In this study the effect of ramps and low-level perches were confounded, and it was not clear if chicks utilised the ramps to gain access to the upper levels. LeBlanc et al. (2017) looked at the effect of ramp angle and found that from 2 weeks of age all birds were successful on inclines up to 40° which continued to 36 weeks of age. From some preliminary findings from Chapter 4, we have shown that providing ramps during the first week of age can increase the use of other raised structures in commercial systems (Norman et al., 2017).

Improving the mobility and confidence of young birds could have beneficial effects during the stressful transfer to the laying system. With resources spread throughout the house, birds must navigate the system effectively as soon as possible, to avoid welfare problems (Pettersson et al., 2016). Given that ramps appear to encourage better access and use of perches, tiers and vertical structures during the laying period, and that there

are some indications of beneficial effects of ramp provision during rear, it is important to consider at what stage ramps should be provided during the rearing period. The aim of this study was to determine whether experience of inclined ramps during the early rearing period would improve birds' subsequent ability to negotiate similar ramps towards the end of rear.

The specific objectives were to compare the effects of rearing birds from 3 to 8 weeks of age with or without ramp access to elevated platforms on:

- i. Individual latency to move up or down a ramp at 12-14 weeks of age.
- ii. Individual behaviour at 12-14 weeks of age when traversing a ramp for a food reward.
- iii. The number of ramp transitions made by groups of birds aged 12-14 weeks over a period of three days.

2.2 Materials and Methods

2.2.1 Animals and housing

For this study, British Black Tail pullets (*Gallus gallus domesticus*) from the same parent flock were reared to 8 weeks of age in two flocks of 2,000 pullets in adjacent sheds (12.5m by 8m) on a commercial rearing farm. Housing comprised a fully littered floor, gas brooders, track feeders and bell drinkers. At three weeks of age, as normal rearing practice, both flocks were provided with four A frame perches (L:2m, H:0.5m) and two elevated platforms (L:360cm, W:60cm, H:50cm) to encourage vertical movement in preparation for the laying house. Platforms consisted of metal frames with white plastic slats on top. One flock (ramp-reared) was additionally provided with two grid ramps (GR) and two ladder ramps (LR) that were attached to the platforms at an angle of 61 degrees (to fit between the drinker lines in the rearing sheds), with the other

flock used as a control. Each GR consisted of a white plastic poultry slat (Jansen) attached to a sheet of medium density fibreboard (MDF) for support. Each LR was constructed from hardwood timber with three rungs (4.4cm square) 30cm apart.

At 8 weeks of age, 32 birds from each flock (ramp reared or control) were randomly selected from different areas of the rearing sheds and transported to a research facility at the University of Bristol. Upon arrival birds were weighed and keel palpated using the method of Wilkins et al. (2004). No keel bone fractures were detected. The birds were kept in their rearing groups and were housed separately in two similar rooms (3.66m by 3.05m) each with floors covered in wood shaving litter, two feed hoppers (30cm diameter) and two bell drinkers (30cm diameter). Birds were fed *ad libitum* on chick crumb and gradually moved onto a layer mash. Lighting was on a 12h dark:12h light cycle, with room temperature maintained around 19-22°C and fan ventilation. Each room contained one identical raised platform (L:120cm, W:60cm, H:50cm). The ramp reared group was provided with a GR and LR (identical to those provided in the commercial rearing system) leading up to the platform at an angle of 61° (Figure 2-1).



Figure 2-1. Photograph of the platform (L:120cm, W:60cm, H:50cm) with the ladder ramp (right) and grid ramp (left) attached in the ramp reared group home pen.

2.2.2 Negotiation of a ramp by individuals

The aim of the individual bird tests was to measure whether there was a difference between ramp reared and control birds in the individual latency to transition a ramp and to compare behaviour before and during a transition down or up a ramp.

A separate room was used for individual testing, which used a narrow pen (3.02m by 0.65m) set up at the side of the room with one long side fenced off with a wooden frame covered in chicken wire. During the first stage of habituation and training a white plastic slat was positioned on the floor at one end of the pen (Figure 2-2, section A) with shavings covering the concrete flooring (Figure 2-2, section B). During testing the ground level slat was replaced with a raised structure (90cm high) with the plastic slat fixed on top. Either a GR or a LR (L:120cm by W:57cm) were attached (angle 45°) (See Figure 2-3). The LR had three central rungs 30cm apart. For downward transitions hens

were placed via a cardboard door in the wire framework onto the raised slatted platform. For upward transitions birds were lifted over a wire barrier onto the shavings (Figure 2-2 section B). A CCTV camera was installed at a raised position on the wall facing the ramp to record behaviour during up and down transitions. The birds' preference for ramp type (GR or LR) has been reported (Pettersson et al. (2017d).

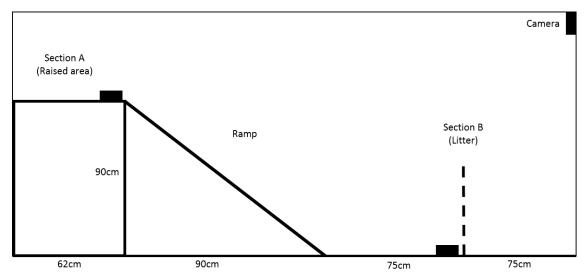


Figure 2-2. A side view diagram of Individual testing pen. The black boxes represent the bowls at starting position A for up transitions and B for down transitions. The dashed line represents the barrier used to shorten the starting box for up transitions.

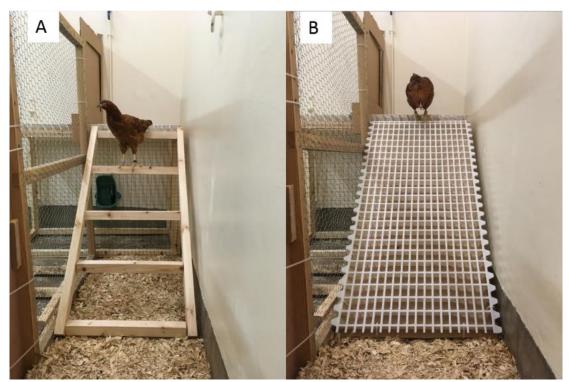


Figure 2-3. Photograph of individual testing pen with both ramp types. (A) Ladder ramp (LR) and (B) grid ramp (GR).

Habituation took place over the first 14 days (8 to 10 weeks of age) where birds were introduced to handling and a food reward (tinned sweetcorn). Over the first 7 days birds were fed *ad libitum* sweetcorn from two ceramic bowls (black outside with white inside) in the home rooms. Once all birds were eating sweetcorn from the bowls, the birds were introduced to the individual testing room (days 8-14). Two birds were carried to the individual testing room and were fed *ad libitum* sweetcorn from the bowls placed on the ground. This progressed to feeding inside the test pen in pairs, then feeding individual birds in the test pen. Habituation to the testing room was complete when all birds were eating calmly from the bowl when alone in the testing pen.

Training took place over 14 days (10-12 weeks of age). Following habituation to the testing room, each bird was carried to the testing room and placed at one end of the

testing pen. The starting position (litter or a ground level slat) was balanced across individuals. A bowl containing 5 pieces of sweet corn was already in position at the other end of the testing pen where a researcher tapped on the bowl twice with a pencil to attract the bird's attention. Once the bird reached the bowl and had eaten the sweetcorn, the procedure was repeated in the other direction. Each bird received this training once a day from days 15-25. The starting direction was alternated for each bird. During the last three days 26-28 whether the bird succeeded in reaching the bowl within two minutes was recorded. To meet the training criteria birds had to be successful in 5 out of 6 of these tests (3 in each direction).

2.2.3 Individual Testing protocol

All birds experienced a recap day and two days of testing. The recap day involved all hens experiencing the training protocol (no raised levels) in both directions, as there was a break between training and testing for most birds.

Testing took place during days 30-44 when birds were 12-14 weeks old. 16 birds from the same rearing group were tested over three days, this was repeated four times, alternating between the rearing groups, so all birds could be tested. The testing pen was set up with the raised slatted area and a ramp attached with cable ties. Each bird experienced four tests GR-DOWN, GR-UP, LR-DOWN and LR-UP. Testing order of ramp type and direction was systematically balanced to account for first experiences. See Figure 2-4 for example testing procedure for an up transition.

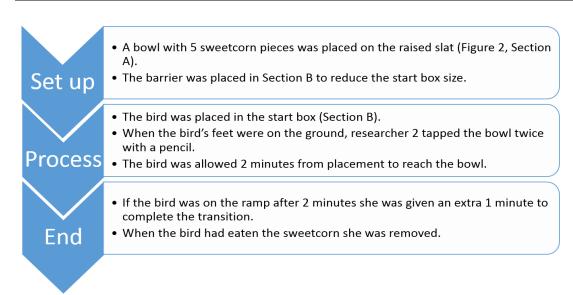


Figure 2-4. Flow chart of the testing procedure for an upwards transition in the individual tests.

Videos of the testing days for each bird were watched using VLC media Player (VideoLAN, France). The pre-transition period was recorded from the start of the trial until a transition was started. Behaviours indicative of hesitancy were recorded (as used in Pettersson et al. (2017a), Lambe et al. (1997)) such as head orientations, crouches, steps and pacing. The transition period (defined as any movement from the starting position moving to the reward) was recorded from when the bird had started a transition to reaching the bowl. A transition was defined as ended with both feet were on the litter or slats (dependent on direction). Each bird was followed to record if it completed a transition or attempted one without successful completion. Behaviours whilst transitioning were also recorded such as moving straight down, jumping, half jumping, crouch walk, running etc. Table 2-1 lists the behaviours recorded.

Table 2-1. Ethogram of the behaviours recorded in the individual tests.

	Pre-transition behaviours
Behaviour	Description
Start (latency)	Release of the bird, both feet placed on slat/floor
Orientation (incidence)	Rotates head in the direction of movement
Foot raise (incidence)	Raising foot to initiate movement
Step on the spot	Raising foot and replacing on the same spot
(incidence)	
Pace (incidence)	Walking along the top of the slat, looking down the structure
Crouch (incidence)	Lowering body to the ground, preparing for a jump
Move away (incidence)	Bird turns away from ramp for more than 3s
Ground peck (incidence)	Bird pecks ground (bouts separated by 3s)
	Transition behaviours
Transition start (latency)	One foot placed on ramp
Straight (incidence)	Smooth transition on the ramp
Rung step (incidence)	Stepping between rungs on the ladder ramp
Rung Jump (incidence)	Jumping between rungs on the ladder ramp
Zig zag (incidence)	Moving across the ramp
Sidestep walk	Moving in a straight line with the wing side facing down
(incidence)	
Crouch walk (incidence)	Moving with body low to the ramp
Wing flap (incidence)	Wing extended and flapping
Balance (incidence)	Wings slightly away from the body
Half jump (yes/no)	Moving halfway down the ramp then jumping
Jump (yes/no)	Jumping from the top of the structure
Run (incidence)	Fast paced movement on the structure
Pause (incidence)	Stops mid transition
Turn (incidence)	Turns on ramp
Return (incidence)	Goes back to the start position without completing transition
Structure collision	Collides with structure impacting movement
(yes/no)	
Landing Collision	Collides at the end of the transition movement
(yes/no)	
Hit bowl (yes/no)	Collide into bowl
No attempts (yes/no)	Does not attempt a transition on the ramp
Escape (incidence)	Bird orientates head looking to escape (hand needed to stop
	escape)
End (latency)	Bird reaches bowl and starts eating

2.2.4 Use of ramps in a group setting over three days

The aim of the group test was to compare the effect of early rearing experience on the number of successful transitions birds made going up and down ramps and whether this changed over time.

Group testing took place in a room (3.66m by 3.05m) identical to the home pens. Six wooden frames each designed to support 6 plastic slats (L:120cm by W:57cm) were joined together to create a raised slatted area (W:366cm x D:120cm and H:90cm). Chicken wire was used to block access to underneath the frames. The floor area (366cm x 185cm) was covered with wood shavings. A LR and GR (L:120cm x W:171cm) were attached to the wooden frames of the raised area by cable ties at a height of 85cm, resulting in an angle of 45°. The LR had three central rungs 30cm apart. There was a small gap between the ladder ramp and the wall of 24cm, present in both positions. A feed hopper (right) and bell drinker (left) were installed above the slatted area. Two CCTV cameras were attached to the walls, to provide a full view of each ramp (Figure 2-5).



Figure 2-5. The raised area in the group testing room. Grid ramp (GR) on the left and ladder ramp (LR) on the right. Raised slatted area 90cm high with ramp angles of 45° .

2.2.5 Group testing protocol

Group testing commenced with the first group of 16 birds after they had completed the individual tests. Between 8.30am and 9.00am the following day 16 birds were placed on the raised slatted area of the group testing room. The birds were left in the group testing room for three days, during which the next group of 16 was run through individual testing. Videos were recorded from 9.00am to 5.00pm for the three consecutive days that each group was housed in the testing room, as this was a time period when the birds would be undisturbed. To minimise any side bias, each day the birds were removed from the group testing room and put into crates whilst the ramp positions were swapped. The side that the ladder ramp and grid ramp started on were balanced for the two groups. Birds were always replaced on the raised slatted area. Once the birds were replaced by 9.00am the rooms were not disturbed until 8.30am the next

morning. Throughout the day birds could be inspected through a small peep hole so as not to disturb them. On the fourth day birds were removed, the ramps reset, and the next group was placed in the group testing room. This was repeated until all four groups had completed three consecutive days in the testing room.

From video recordings, the total number of successful up and down transitions completed within a minute was recorded for each ramp type. Recording periods were from 9:00 h to 17:00 h, every successful transition over the 8-hour time period was recorded. A transition began when a bird placed both feet on the ramp or on one of the ladder rungs and ended when both feet were either on the litter or on the raised area. Every bird that started a transition was tracked and it was recorded whether it completed a transition or returned to the starting area within the minute. A 1-minute time period was chosen to exclude birds that stopped/perched on a ramp. If the bird was still on the ramp after one minute the observation was terminated. Further records such as number of jumps or collisions were taken. Additional scan sample recordings were taken at tenminute intervals between 9:00 h and 17:00 h to count the number of birds' stationary on each ramp type to determine the extent to which blocking might influence ramp use.

2.2.6 Statistical analysis

All data were analysed using SPSS 23 (IBM). Data were analysed separately for up and down transitions (see table 2-1 for response variables). Two birds were removed from analysis, one for not completing the training criterion and the other as it was tested incorrectly. For pre-transition behaviours, all 62 birds were included in the analysis. For transition behaviours, only birds that attempted a transition were included (N = 58) for analysis. Some birds had to be removed from analysis for certain variables, for example, if they completed the transition by jumping, they could not perform certain behaviours

on the ramps such as pausing or returning. All variables were tested for normality and square root and log transformations were made to try and meet the assumptions, but these were not achieved. Therefore, a non-parametric alternative to an independent ttest was used, two sided Mann-Whitney U test, to perform exploratory statistics to test whether the rearing groups were equal. Due to the nature of the data multiple tests were conducted which increased the risk of a type 1 error. The significant results were considered in light of this to ensure they pointed to the same interpretation of how the rearing groups performed when transitioning the ramps. Mean and standard deviation will be reported in this analysis. Some of the data were nominal so they could not be analysed on an individual level such as successful transitions and jumps. Percentages and graphs are reported to indicate any trends in the results. Statistical analysis could not be performed for the group tests as N=2, so simple summary statistics are presented.

2.2.7 Ethical approval

The University of Bristol's Animal Welfare and Ethical review body approved this study under UIN: UB/17/046.

2.3 Results

2.3.1 Individual testing results

For the results, upwards and downwards transitions will be reported separately. Both ramp types were analysed together unless there was a statistically significant difference between the two. See Table 2-2 for detailed results. When considering the time taken to start a transition (start latency) the control group took longer when faced with the Ladder ramp but there was no significant difference for the grid ramp. The time taken

to transition when negotiating a ramp (transition latency) was significantly longer in the control group compared to the ramp reared group with both ramp types combined. When looking at the total latency to start and complete a transition of a ramp, the control group took significantly longer than the ramp reared group for upward transitions. For the downwards ramp transitions there was no significant difference in latency between the rearing groups.

When comparing pre-transitions behaviours (i.e. orientation, foot raise, step, pace, crouch, move away, ground peck), the number of head orientations per bird was greater in the control group compared to the ramp group when transitioning up a ramp. Significantly more control birds moved away from the bottom of the ramp before attempting an upwards transition. There was no significant difference between groups for foot raises, steps, pacing, crouching and ground pecking for upwards transitions. There was no significant difference between rearing groups for pre-transitions behaviours during downwards transitions. See Table 2-2 for statistical results.

The time taken before transitioning and the number of behaviours birds showed, allowed the rate to be calculated for pre-transitions. For the LR-UP there was a significant difference in the rate of ground pecks being greater in the control group compared to the ramp group. When considering the rate of pre-transition behaviours occurring when moving down ramps, differences between the rearing groups appeared when tested with the ladder ramp. For LR-DOWN there was a greater rate of head orientations in the control compared to the ramp reared group. This was opposite for the rate of crouching which was lower in the control group compared to the ramp group. See Table 2-2 for results.

For the behaviours recorded (see Table 2-1) during the upward transitions, the control group displayed significantly more crouch walks than the ramp reared group. More pauses, returning to the starting position and turning around on the ramps were recorded in the control group compared to the ramp reared group. For the ladder ramp the number of rung steps and rung jumps were recorded. There was a significant difference between the groups, with the ramp reared group showing more rung jumps compared to the control group. A difference in the number of birds showing escape behaviours in the control group compared to the ramp reared group was found. Ramp reared birds had a greater mean number of attempts compared to the control group for LR-UP transitions. No significant difference in the behaviours when transitioning down the ramps was found between rearing groups. See Table 2-2 for the significant results.

Table 2-2. A table listing the significant results for latency, pre-transition behaviours and transition behaviours separated for direction. Results that were analysed separately for ramp type are noted with LR or GR. The Mann Whitney U test n1=control group and n2= Ramp reared group.

Upwards transitions			
	Mean ± SD	Mean ± SD	Test result and
	Control	Ramp reared	Significance level
Total Latency	$100s \pm 37.6$	$68.8s \pm 49.3$	U = 1182, n1 = 62
			n2 = 62, p = 0.001
Transition	30.24 ± 26.66	14.10 ± 14.78	U = 207.5, n1 = 17
latency			n2 = 39, p = 0.027
Start latency	$66.80s \pm 35.40$	$30.10s \pm 23.54$	U = 32.00, n1 = 10
(LR)			n2 = 17, p = 0.008
Head	2.98 ± 1.42	2.29 ± 1.63	U = 1265, n1 = 62
orientations			n2 = 62, p = 0.001
Move away	0.94 ± 0.903	0.42 ± 0.560	U = 1302, $n1 = 62$
			n2 = 62, p = 0.001
Rate of Ground	0.0107 ± 0.0122	0.0035 ± 0.011	U = 47, n1 = 10
pecks (LR)			n2 = 17, p = 0.018
Crouched walks	0.56 ± 0.698	0.26 ± 0.447	U = 232.5, n1 = 27
			n2 = 27, p = 0.009
Pauses	0.97 ± 0.928	0.27 ± 0.447	U = 460.5, $n1 = 37$
			n2 = 45, p = 0.001
Returns	0.57 ± 0.647	0.11 ± 0.318	U = 512.5, n1 = 37
			n2 = 45, p = 0.001
Turns	0.57 ± 0.647	0.13 ± 0.344	U = 529.5, n1 = 37
			n2 = 45, p = 0.001
Rung jumps	0.2000 ± 0.42164	1.4706 ± 1.1245	U = 25.00, n1 = 10
(LR)			n2 = 17, p = 0.001
Escapes	0.19 ± 0.568	0.03 ± 0.254	U = 1707, n1 = 62
			n2 = 62, p = 0.017
Attempts (LR)	0.35 ± 0.551	0.61 ± 0.495	U = 350.5, n1 = 31
			n2 = 31 p = 0.035
Downwards trans			
	Mean ± SD	Mean ± SD	Test result and
	Control	Ramp reared	Significance level
Rate of head	1.01 ± 0.799	0.588 ± 0.418	U = 136.5, n1 = 22
orientations			n2 = 19, p = 0.039
(LR)	0.004 0.000		
Rate of	0.284 ± 0.681	0.345 ± 0.404	U = 137, n1 = 22
crouching (LR)			n2 = 19, p = 0.047

When comparing the rearing groups, of the 62 birds tested, 23 birds (74%) in the ramp reared group and 13 birds (42%) in the control group had successful transitions for the GR-UP. For the LR-UP, in the ramp reared group 16 birds (52%) were successful compared to 4 birds (13%) in the control group. Irrespective of prior ramp experience 70-80% of birds successfully negotiated down the ramps, however 38% of birds without ramp experience collided upon landing compared to 10% in the ramp reared group. See Figure 2-6 for a graph of results.

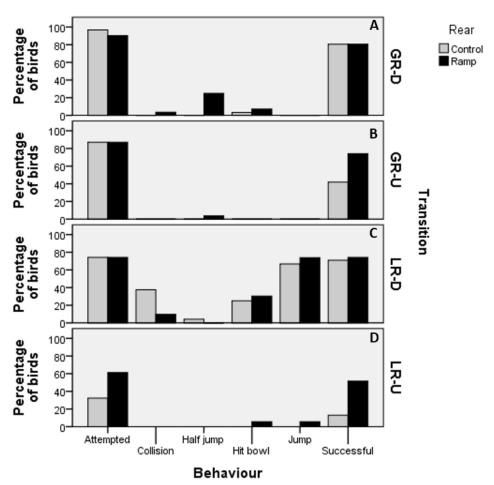


Figure 2-6. Graphs comparing the percentage of birds performing behaviours during different transitions. (A) Grid ramp transitions down (GR-D), (B) Grid ramp transition up (GR-U), (C) Ladder ramp transitions down (LR-D) and (D) Ladder ramp transitions up (LR-U).

2.3.2 Group Testing Results

Owing to the nature of the data, some could only be obtained at a group level so with only 2 groups per treatment the data are presented descriptively. On their first day in the group testing room there were more up and down transitions on the ramps in the ramp reared groups (14.72 ± 9.95) compared to the control groups (7.22 ± 9.304) . The mean number of up transitions was greater in the ramp reared groups (15.69 ± 11.79) compared to the control groups (6.34 ± 5.597) on day 1. This difference reduced by day 3 with the control groups having a mean of 12.31 ± 9.282 and the ramp reared group having a mean of 11.81 ± 6.855 for down transitions. For up transitions the control group had a mean of 10.50 ± 6.520 and the ramp group had a mean of 13.31 ± 8.495 (see Figure 2-7). In total, 17 jumps in the control groups and 39 in the ramp groups were recorded. Two collisions in the control group and 9 in the ramp group were also observed.

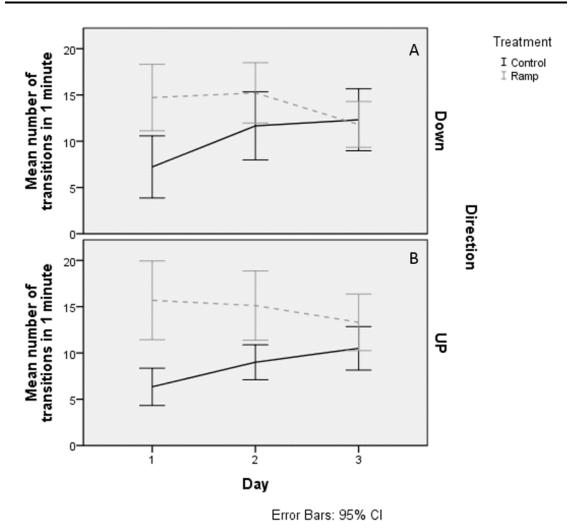


Figure 2-7. Graphs showing the difference in the mean number of transitions per day for ramp-reared and control birds in the group tests over three days. (A) Downwards transitions, (B) upwards transitions.

2.4 Discussion

2.4.1 Individual Differences in latency

The first aim of the study was to determine whether individuals with different experience of ramps during 3 to 8 weeks of age had an altered latency to move up or down two types of ramp.

For upward transitions, there was a significant difference between individuals from the two rearing conditions. Latency to start moving up a ladder ramp was significantly longer in the individuals with no ramp experience compared to the ramp reared

individuals. This difference in latency suggests that birds without previous experience may be more cautious in starting a transition. Scott et al. (1999) looked at birds jumping to perches for a food reward and found birds that were successful in jumping tended to jump within the first 20 seconds. This suggests hesitant birds may take longer in starting a transition.

Birds without rearing experience of ramps took longer to transition a ramp than birds with previous ramp experience. A greater number of ramp reared birds completing a transition than non-ramp reared birds, suggests that birds without previous experience of ramps had difficulty transitioning up both types of ramps. An increased latency to transition may cause blocking on ramps in commercial systems which could also prevent other birds from transitioning. There were no clear differences between individuals in the latency for down transitions, suggesting no effect of rearing conditions.

In a commercial laying house, it is important for birds to easily access the litter and to be able to move up to food and water on the raised areas. If birds are hesitant in moving between levels, this could cause crowding and blocking on ramp areas which will limit access for other birds. Crowding on raised areas of the shed can increase the risk of collisions or pushing from conspecifics which could lead to keel bone fractures (Stratmann et al., 2015). If blocking or difficulty in negotiating a level change restricts access to resources, this could reduce bird welfare and lead to unwanted behaviours such as feather pecking (Nicol et al., 2013, Alm et al., 2015).

One factor to consider is that food was used as a motivator in individual bird testing. This may have influenced their level of caution in negotiating down the ramps, as many birds were observed to have a collision on a downwards descent of a ramp. Individual differences in levels of food motivation were not tested in this study. There is evidence

of individual differences in motivation for a food reward (Scott et al., 1999) which may have a confounding effect on the results, with some birds showing more 'riskey' behaviours to reach a food reward if highly motivated to reach the food.

2.4.2 Individual pre-transition behaviours

For upward transitions there were higher numbers of head orientations and moving away in the control individuals. Multiple head orientations have been shown to indicate hesitancy when transitioning ramps (Pettersson et al., 2017a). Head orientations were found to be precursors for jumping down from perches by Scott et al. (1999). A greater number of head orientations may be indicative that the control group took time looking at the food reward before attempting the transition. The greater number of birds that moved away from the bottom of the ramp in the control group could possibly indicate that they were less willing to transition and looked for a different route up or lost interest in the reward (Lambe et al., 1997, Scott et al., 1999). As with the latency, there was no difference between individuals from the two rearing conditions in behaviours prior to moving down either ramp type.

2.4.3 Individual rates of pre-transition behaviours

The rate of head orientations was greater in the control groups when transitioning down the ladder ramp suggesting more heistancy.. Corroborated by Lambe et al. (1997), where the rate of head movements was positively correlated with the time taken to jump down from a perch. The rate of crouching was greater in the ramp reared group. Crouching tends to indicate a transition will be made, therefore a higher rate of crouching suggests birds are more likely to make a transition (Lambe et al., 1997, Scott et al., 1999), and indeed more ramp reared birds made the transitions down onto the litter.

For LR-UP there was a greater rate of ground pecks in the control group. Ground pecking has been identified as a redirected behaviour when a goal is not achieved (Kuhne et al., 2011). Therefore, it could have been a redirected behaviour from not obtaining a food reward. It was more likely to occur in the upwards transition owing to the litter covered floor. Birds that abandoned transitioning were more likely to ground peck and only 13% successfully transitioned the LR-UP in the control group compared to 52% in the ramp reared group. As the control group seemed to have more difficulty with the upwards transition than the downwards one, it could be reflective of this increased difficulty.

2.4.4 Behaviours recorded whilst transitioning a ramp

Birds seemed to express similar behaviours whether reared with or without ramps when transitioning down. When moving up the ramps birds without prior ramp experience showed a higher number of crouched walks. A crouched walk was recorded when birds' bodies were held low to the structure; it appeared to the observers that these birds lacked confidence when transitioning. The control birds also paused, turned and returned to the litter more frequently than the ramp reared group. This suggests they were unsure of making an upward transition. Confirmed by the fact that more birds in the ramp reared group successfully completed an upwards transition. The individuals in the control group showed a higher number of escape attempts. This suggests that they struggled with transitioning the ramp and looked for alternative routes to leave the testing pen. These behavioural recordings suggest that control birds are less confident in using the ramps when making the first transition upwards. Few studies have looked at upward transitions on ramps in laying hens. LeBlanc et al. (2017) observed chicks use ramps from 1 week of age, and training and testing may help with bone and muscle

development. When looking at rearing experience with inclined structures Kozak et al. (2016) reared chicks in a complex environment, and found chicks used the inclined surfaces from 2 weeks of age. However, no previous studies have considered the consequences of previous experience of ramps during rearing.

2.4.5 Other behaviours

When looking at the nominal data, the ramp reared group showed a greater percentage of successful and attempted transitions compared to the control group. This suggests that previous experience with ramps may encourage use of ramps when first exposed to them. With the downward transitions, there was a greater percentage of collisions in the control group compared to the ramp reared group. This suggests that birds with no previous ramp experience may be at more risk of colliding with the floor when transitioning down the ladder ramp. Studies have found with more ramps in a system there is a reduction in the number of falls, collisions and keel bone fractures (Stratmann et al., 2015). By introducing ramps at an early age birds may be more likely to use these structures as a route for level changes.

2.4.6 Group recordings

When tested in groups, the ramp reared groups transitioned more than the control groups. However, the number of successful transitions in control groups increased over the three days to almost the same as the ramp reared group (Figure 2-7). This suggests that, despite having exposure to ramps during individual testing, the control group were less confident in using the ramps to begin with, but as they gained experience, they were more able to move between the slats and litter. This is important in a laying shed as movement between the litter and raised areas is essential for birds to forage and reach

food and water. If thwarted this can have negative consequences such as feather pecking (Nicol et al., 2003, 2013).

2.4.7 Conclusion

Overall increased movement on ramps was seen in older pullets with previous ramp experience, shown by differences in pre-transition, transition behaviours and latency. The previous ramp experience may have increased any combination of bird mobility, strength or cognitive ability leading to an increase in apparent confidence. We have found that early experience of ramps influences pullets up to 14 weeks of age. From the group observations the difference in transitions appears to reduce over three days, so it is possible that non-ramp reared birds can learn to use ramps relatively quickly. But the time this may take in a more complex commercial system with older birds is unknown. The lack of replication of the rearing treatments is a limitation of this study, thus further replication is required to ensure there was no group effects from the rearing treatments. In all, only positive effects were noted with early ramp experience, so we suggest that ramps should be included from the early rearing period onwards and that mechanisms for the observed effects should be elucidated with further studies.

In summary providing chicks with experience of ramps from 3 weeks of age has shown to reduce the number of behaviours that appear to indicate hesitancy before and during a transition of a ramp. As concluded if installed in commercial systems, in particular multi-tier rearing, this may encourage the use of elevated levels in the system resulting in improved access to important resources. However, the importance of providing experience of complex three-dimensional environments and the age that differences in spatial cognition may be observed has not be studied. The impacts of rearing in more

complex environments versus simple environments on chick's spatial navigational ability will be investigated in Chapter 3.

Chapter 3 - The impact of early structural enrichment on spatial cognition in layer chicks

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Jessie Adriaense provided technical assistance.

The impact of early structural enrichment on spatial cognition in layer chicks

Abstract

The aim of the study was to determine whether early access to elevated structures affects spatial navigational abilities. Ninety-six-day-old chicks were reared in 16 pens. Eight pens were provided with A-frame perches with an attached platform and a ramp. Eight pens had no elevated structures. At 14-15 days of age 48 chicks (24 from each rearing group) were tested in a battery of navigational tasks: a detour test, jump test and rotated floor test (RFT). The remaining 48 chicks (24 from each rearing group) received the same tests at 28-29 days of age. Chicks reared with elevated structures were faster at completing the detour test (*P*=0.045). Older chicks were more likely to turn left in the detour test (p=0.013) and were more successful in the jump test (69% vs 31% completion, p=0.001). There was no treatment effect on use of intra or extra-maze cues in the RFT, but the proportion of chicks using intra-maze cues declined between the first (0.76) and second (0.43) repeat of the RFT (p=0.038), particularly for chicks reared with elevated structures. We conclude that bird age or developmental stage may have a predominant influence on spatial navigation and physical ability, but early experience of elevated structures had some mediating effects which require further investigation.

3.1 Introduction

A growing proportion of laying hens are being housed in non-cage systems. Whether single, or multi-tier (aviary), these systems can be complex for birds to navigate. Resources such as food, water, nest boxes and litter are distributed throughout the system and on multiple levels in aviary houses. Successful movement within these systems and into outdoor areas, if available, will be influenced by the birds' general cognitive abilities and their specific spatial navigation skills.

Most hens are reared in separate facilities to 15-18 weeks of age, then transferred to the laying system. It is recognised that rearing environment should be tailored to the laying environment (Janczak and Riber, 2015). For example, birds reared in aviaries show reduced keel bone fractures when also housed in aviaries at lay compared to cage reared birds (Casey-Trott et al., 2017a). If chicks destined for aviary systems cannot be reared in aviaries, then they may instead be provided with elevated surfaces within a singletier house. These structures tend to be table platforms and perches. There is evidence that early access to perches prior to 4 weeks of age reduces floor eggs and cloacal cannibalism and may have other welfare benefits (Appleby et al., 1986, Gunnarsson et al., 1999, EFSA, 2015). However, more research is needed to explore the importance of timing and the exact type of experience needed with elevated structures to minimise later welfare problems. The RSPCA standards state that birds must have perches by 10 days of age, and recommend perches are raised 25cm off the floor (RSPCA, 2018). However, 10-day old chicks might not use perches at this recommended height to their full potential, and earlier access to lower perches might increase usage. Heikkila et al. (2006) observed the first chick using perches at a height of 20cm from the floor by 8 days of age, and all chicks were using perches by 22 days of age, suggesting a desire to perch from a young age. Facilitated access (using ramps or staged perches) may be even more beneficial. Our own work has shown that access to ramps during the rearing period improved ramp transitions in older pullets, shown by reduced hesitancy behaviour and shorter transitioning latencies (Norman et al., 2018). A preference for grid ramps over ladder ramps was found suggesting the type of ramp should also be considered (Pettersson et al., 2017d).

The provision of elevated structures to young birds in commercial systems has primarily been driven by the desire to facilitate more movement, so that when pullets are moved to the laying system, they are more mobile. This may in turn reduce welfare problems such as later cloacal cannibalism (Gunnarsson et al., 1999), keel bone damage (Wilkins et al., 2005, Wilkins et al., 2011) and feather pecking (Lambton et al., 2010, Nicol et al., 2013, Lambton et al., 2013, Campbell et al., 2019). However, elevated structures can also be regarded as a form of environmental enrichment which is likely to affect brain development and cognition (Campbell et al., 2019). Exposure to an enriched environment compared to a barren one from a young age can improve spatial memory in mammals (Sneddon et al., 2000, Kobayashi et al., 2002). For example, increased neurogenesis in the dentate gyrus of the hippocampus, a brain region important for memory, is observed when rodents are housed in enriched conditions (Kempermann et al., 1997). Similarly, chicks reared with ground-level visual barriers (opaque screens) demonstrate improved spatial memory (Freire et al., 2004), and have longer dendrites and more spines per dendrite in the hippocampus, which may lead to improved encoding of relative positional information (Freire and Cheng, 2004).

Further work is needed to understand if the elevated structures now commonly provided for chicks have any influence on their spatial cognitive abilities. Gunnarsson et al. (2000) reared chicks with or without perches to 8 weeks of age and tested them at 16 weeks of age in a navigational task requiring birds to jump between raised platforms to reach a food reward. Birds raised with or without perches were equally likely to jump to a 40cm platform, but birds raised with perches were both more likely to jump to a single 80cm platform (suggesting improved physical competence) and more likely to jump from a 40cm to an 80cm platform. This latter result implies improved navigational ability; however, it cannot be excluded that this result was also due to perch-reared birds possessing greater confidence in jumping at height due to increased physical strength. For this reason, further work using tasks that more clearly separate potential physical and cognitive components of navigation would be useful.

The age at which structures are provided may also be highly relevant. A rise in perching behaviour at 9 to 10 days of age followed by a fall at 11 days of age coincides with a shift in brain activity to the right hemisphere in the hippocampus (Workman and Andrew, 1989). At this same age, chicks voluntarily seek to move out of sight of the hen and then to regain contact, providing them with experience of visual occlusion. The provision of artificial visual barriers at 10-12 days of age improves chicks' ability to use external cues in detour tests and hidden food reward tests, but these improvements in spatial cognition do not occur if visual barriers are provided at 8 days of age and removed by 9 days (Freire and Rogers, 2007). This provides evidence of a sensitive period during which experience of visual occlusion is particularly beneficial in brain development for spatial navigation. The importance of gaining perching experience during this shift in brain activity has not been explored.

Cognitive tasks where differences in physical strength are unlikely to play a major role include the hole board task, detour tasks and rotated floor tasks. The hole board task

requires a subject to use both reference and working memory to locate food rewards hidden in various cups on the floor. Tahamtani et al. (2015) compared the performance of birds reared in either cages or aviaries in a hole board task and detected better working memory and reversal learning in aviary reared birds tested at 16 weeks of age. In a study looking at associations between cognition and feather pecking Nordquist et al. (2011) also used the hole board task with 25 day old chicks. However, one problem with this test is that it can take up to 12 days of habituation and 28 days for the different training and test phases. Experience during long term training may influence navigational development in the control chicks and impact their ultimate test performance. The detour test requires a subject to navigate out of a U-shaped pen to gain access to its companions. Other studies have detected early experience effects on spatial cognition using detour tests (Freire et al., 2004, Wichman et al., 2007).. The rotated floor test (RFT) requires a subject to associate intra- or extra-environmental cues with the location of its companions, see (Wichman et al., 2009). Both of these have been used previously in studies with chickens, with very little training required.

In this study we therefore used both the detour and RFT to look at the effect of elevated structures on different aspects of navigation during the first four weeks in the chick's life. Both tests require minimal training and assess birds in a two-dimensional environment where differences in physical ability should play a minor role. We also examined the effect of elevated structures on performance in a three-dimensional jump test adapted from Gunnarsson et al. (2000). This test confounds physical and cognitive ability but provides a good comparison point with the previous work by Gunnarsson et al. (2000) on older birds.

3.2 Methods

3.2.1 Animals and housing

Ninety-six British Black Tail (*Gallus gallus*) day-old female chicks were used for testing. Four extra chicks were purchased to account for any mortalities, and two of these were used to replace two chicks that died in the first few days. The day after hatching chicks arrived at the facility and were housed in four rooms with four pens per room (1.5m x 1m) and 6 chicks per pen (two treatment pens had 7 chicks per pen, these extra chicks were not tested). All pens had wood shavings for litter and a heat lamp. Chicks were fed *ad libitum* chick crumb (in small troughs and upgraded to feed hoppers by 2 weeks of age) and provided with bell drinkers. The light schedule was 12 hours on 12 hours off (07.30 to 19:30). Room temperature was slowly reduced as the chicks aged and the heat lamps were removed at 2 weeks of age.

Eight treatment pens (two per room) had elevated structures (see Figure 3-1). These comprised 8 wooden perches (each length 60cm, 2cm diameter) arranged in an A-frame (three perches at height 10cm, two at 25cm, two at 40cm and one at 60cm) and a plastic grid platform (width:30cm x length:30cm) situated on one side of the A-frame, partially covering the perches at height 25cm. A plastic grid ramp (length:40cm x width:30cm at 40° from ground level) led from the floor to the platform on the A-frame. The other 8 pens were controls with no elevated structures. From day 1 chicks were encouraged to feed and drink by tapping on feed bowls and dipping their beaks in water. Between 5 and 9 days of age chicks were handled once per day and bowls of feed were placed in the pen to encourage habituation to human presence. From 10 to 27 days of age (excluding the 4 testing days) all chicks were habituated to handling by feeding oats in a bowl and transferring them to cardboard boxes regularly. At 12 days of age coloured

leg rings were fitted to each chick to allow individual identification. Chicks were weighed each week to ensure even growth.



Figure 3-1. Enrichment structure comprising 8 wooden perches, a plastic platform and a ramp.

3.2.2 Use of elevated structures

Recordings of use of the elevated structures in the treatment pens were taken each day from 1 to 26 days of age. Eight GoPro cameras (Hero5) were set up in each treatment pen to take pictures of the structures during the light period every 10 minutes over three hours each day, giving 19 recording time points over the day. Recording times were either in the morning or afternoon to avoid disturbance by the feeding or habituation schedule of the day. The earliest recording period was 09:00 to 12:00 and the latest was 16:30 to 19:30. All eight cameras were set up to record at the same time across all

treatment pens. The numbers of chicks on the perches, platforms and ramps were counted.

3.2.3 Navigational tests

Chicks were tested in three navigational tasks: the detour test, jump test and rotated floor test (RFT). Forty-eight individually identifiable chicks (3 per pen) were tested on all three tests at 14-15 days of age and 48 (the remaining 3 chicks per pen) were tested on all three tests at 28-29 days of age. The navigational tests were carried out by two researchers over two days. The tests were systematically balanced for order to account for differences in experience. The researchers worked at the same time testing 12 chicks each from two of the rooms in one day, swapping between all three navigational tests. Each navigational test was video recorded using a GoPro camera. The ID and pen number of each chick was read out loud before the start of the test to ensure identification.

3.2.4 Detour test

The detour test (Wichman et al., 2007, Freire et al., 2004, Wichman et al., 2009) (see Figure 3-2) was conducted within a cardboard arena (L 60cm x W 60cm x H 43.5cm). Three familiar chicks (reared in the same pen) were placed in the holding compartment at point A (25cm x 25cm) with a view into the arena. Some oats were sprinkled on the floor and once all chicks were pecking at the oats the test chick was moved to the detour compartment at point B (20cm x 22cm) and placed on a central cross facing the companions through the mesh. The detour compartment was rectangular, with two solid sides and a front wire mesh side facing the holding compartment. The aim of the test was to assess the chick's ability to navigate out of the detour compartment to reach its companion chicks. Orientation errors were recorded whenever a chick's head or whole

body crossed the orientation error lines then re-crossed without crossing the goal lines. The time taken for the chick's head to cross the goal lines was recorded. Each chick was given a maximum time of 5 mins, at the end of testing or upon completion the test chick was returned to the holding compartment and the next chick was placed in the detour compartment.

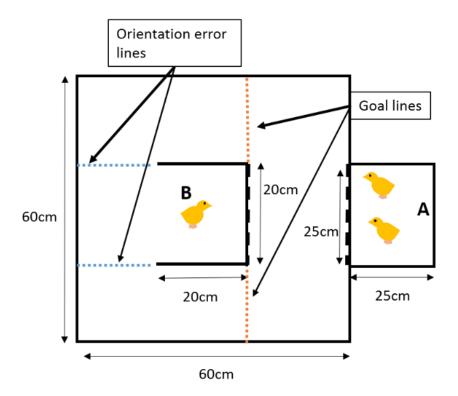


Figure 3-2. The detour test: The apparatus was made from cardboard. Point A shows the holding compartment and point B shows the detour compartment. The dotted lines on the holding compartment and detour compartment indicate the mesh viewing windows. Orientation error lines and goal lines are labelled

3.2.5 Jump test

The jump test adapted for chicks (Gunnarsson et al., 2000)(see Figure 3-3) was conducted within a wooden box with a mesh front (W 40cm x L 40cm x H 60cm). There were two trials, in the first trial, chicks had to negotiate one level to regain contact with companions placed within a small wire mesh holding compartment (22cm x 22cm) on

the lower platform (point A1). In the second trial they had to negotiate two levels to regain contact with companions placed within the holding compartment on the upper platform (point A2). Once all chicks were ground pecking at oats on the floor of the holding compartment the test chick was removed and placed at point B on the floor. The latency for each test chick to jump up to the platform was recorded. A maximum of 5 minutes was given, if the chick did not jump it was returned to the holding compartment.

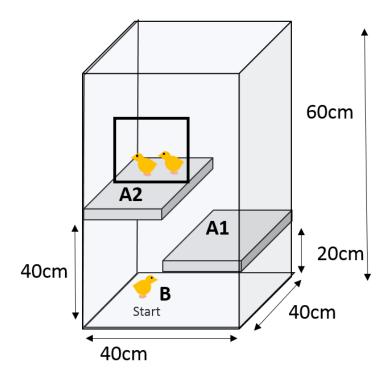


Figure 3-3. The Jump test: The three solid sides and the platforms were made from MDF. The front side was made from removable mesh. Point A1 shows where the companion birds were placed within a small compartment on the lower platform, and point A2 where they were placed on the upper platform. Point B shows the start position of the test chick.

3.2.6 Rotated Floor Test

The RFT (Wichman et al., 2009, Freire and Rogers, 2005, Freire and Rogers, 2007) (see Figure 3-4) provided a training phase so that chicks could learn that their companions were behind one of two screens. They were then tested to see if they were relying on intra or extra-maze cues. A chick was recorded as using intra-maze cues if it used the

screens as the cue to find its companions and extra-maze cues if the chicks used overall position as the cue. The apparatus was a rectangular area (L 175cm x W 61cm), with two screens placed at 42cm from each short side of the arena. The floor was covered with cardboard that could be wiped down or replaced to avoid any marks being left by previously tested chicks. A holding compartment could be placed behind either of the screens. One screen had a coloured pattern (red, blue, yellow and green), the other screen was a plain grey colour and the position of the screens was swapped for each chick. A circular starting compartment (20cm diameter made of wire mesh) was placed in the centre of the arena at point B. The initial training took on average 6 minutes and the first test took on average two minutes. The chicks had a two-minute resting period between the training and testing, where all three chicks were transferred to a holding box outside the RFT arena, so the arena could be cleaned and set up for the next subject.

For the first training session all 3 chicks were initially placed in a holding pen behind one of the screens at point A1 or A2 (counterbalanced), with colour and position cues balanced across treatments. The test chick was then removed and placed in the central starting compartment, facing forwards with the screens to the left and right. After 5 seconds the starting compartment was removed. The test chick was given 1 minute to explore the arena. A chick was considered to have learnt the task when it went behind the screen where its companions were, within 1 minute, in two successive training trials (the training was stopped at this point). The chick was given 30 seconds to remain with its companions then replaced in the companion holding pen. If a chick did not go behind the screen with the companion chicks the researcher gently nudged the chick in the right direction using their hand. Each chick could experience a maximum of 6 training trials within the first training session. Any chick that had not reached the learning criterion after 6 trials, was classified as having not learnt the task and was not tested further.

The test phase for chicks that met the learning criterion followed the first training session. All chicks were removed from the arena, the screens were swapped to the opposite sides from training and the central starting compartment was replaced. The test chick was placed into the central starting compartment but this time there were no companion chicks. After 5 seconds the starting compartment was removed, and the chick could explore the arena. A maximum time of two minutes was allowed for the test. Recordings were taken if the chick crossed the marker lines, and the screen colour was noted down. If the chick chose the screen that the companions had previously been behind (opposite location) this was noted down as using intra-maze cues. If the chick chose the location where companions had previously been found (opposite screen colour) it was noted to be using extra-maze cues. The test was stopped when the chick went behind a screen. The chick was then removed from the arena and the training and testing sessions were repeated with the screens in the same positions as the first session, so each chick received two training sessions and two tests on one day.

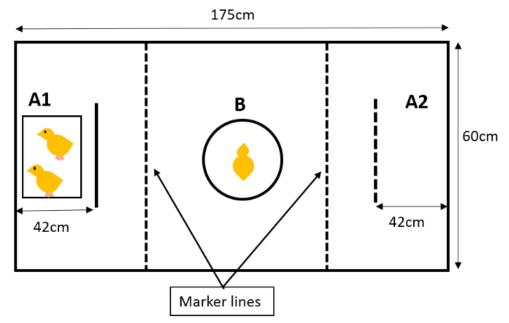


Figure 3-4. The rotated floor test: The arena was made from cardboard and the holding compartments were made from mesh. Points A1 and A2 indicate possible positions of the companion holding compartment behind the screens. Point B shows the position of the test chick within the observation compartment. The marker lines are labelled showing the point a chick had to cross to indicate its first choice.

3.2.7 Statistical analysis

Before analysis each video was re-coded by an independent researcher to ensure blinding of the videos. All data were analysed using both MLwiN (3.0) and SPSS 24 (IMB). All data met the assumptions of the statistical tests used; no transformations were required. Data pertaining to the use of the elevated structures were tested to look for a correlation between structure use and age. The number of chicks that did not complete the detour test or jump test was similar between the treatments, so chicks were removed from the analysis of latency to complete the tests to obtain a normal distribution. General Linear Models (GLMs) were used, with treatment and age as fixed factors, to look for differences in the latency to complete the detour, jump test and RFT. A binomial model (generalised linear model) was used to look at direction chosen for the detour test with treatment and age as variables. Binomial tests were used to compare the proportion of

chicks choosing intra or extra-maze cues in the RFT. Count data (orientation errors) were analysed using a Poisson model. No interaction effects were found for any of the data. Only main effects are presented, and all results are presented in the format mean \pm SD.

3.2.8 Ethical approval

The University of Bristol's Animal Welfare and Ethical review body approved this study under UIN: UB/18/012

3.3 Results

3.3.1 Structure use

Within the treatment pens, the use of the elevated structures increased over time. A strong, positive correlation between age and total structure use was found (r = 0.870, n = 24, p = 0.001), (Figure 3-5). The total mean (over 19 observations/day) number of chicks observed on the structures over each day was calculated. There was a gradual increase in structure use with a peak at 10 days of age (0.21 average chicks/pen) followed by a fall at 11 days of age (0.11 average chicks/pen). This was followed by the highest peak at 12 days of age (0.27 chicks/pen) and continual fluctuations to 26 days of age. Figure 3-5 shows that over the first three days the ramp was predominantly used by chicks. Use of the platform increased up to 10 days of age and from 8 days of age there was a gradual increase in the use of the highest perches (Perch 3 and Perch 4).

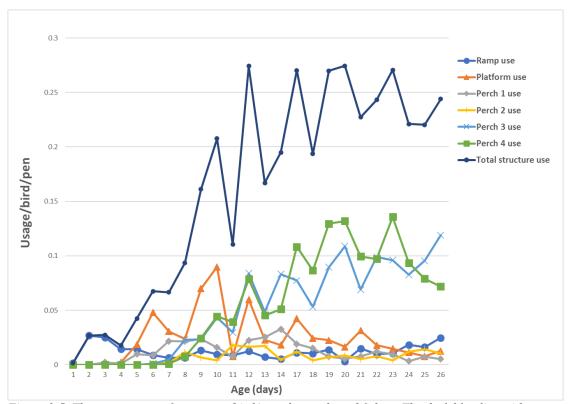


Figure 3-5. The mean usage of structures/bird/pen observed ovr 26 days. The dark blue line with a small circle ID represents the total structure usage by chicks.

3.3.2 Detour test

Out of the total 96 chicks that were tested at 14-15 and 28-29 days of age, 67 completed the detour test in under 5 minutes. Of the successful chicks 33 were from an enriched environment and 34 were from a control environment. The latency to complete the detour test was significantly lower in the enriched chicks (58.42 ± 52.52) compared to the control chicks $(89.91 \pm 73.73; z = -2.005, n = 67, p = 0.045)$ (Figure 3-6). There was no effect of age on the latency to complete the test (z = -1.091, n = 67, p = 0.275). Using a binomial model, there was a significant effect of age on the direction in which chicks tended to leave the start box (z = -1.113, n = 67, p = 0.013). At 14-15 days of age 37.5% went left out of the detour box when viewd from above, whereas at 28-29 days of age 62.9% went left out of the detour box (Figure 3-7). There was no effect of treatment on direction to leave the start box.

There was a difference in the number of orientation errors between the groups tested at different ages, with fewer orientation errors at 14-15 days (0.17 ± 0.43) compared to 28-29 days $(0.77 \pm 1.23; z = 3.928, n = 96, p = 0.001)$. There was no significant difference between treatments (z = 1.33, n = 96, p = 0.183). There was a significant difference in the number of orientation errors and the completion of the detour test within 5 minutes (z = -4.384, n = 96, p = 0.001), with the successful chicks showing fewer orientation errors (0.27 ± 0.60) compared to the unsuccessful chicks (0.93 ± 1.41) .

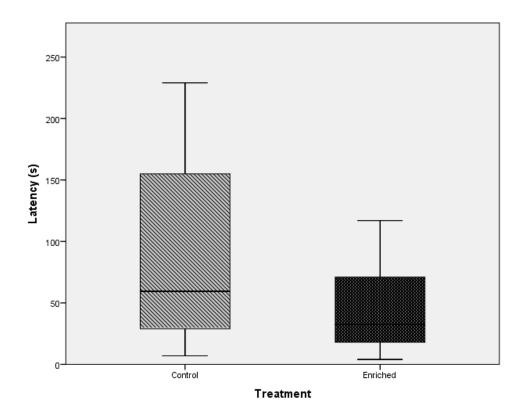


Figure 3-6. Box plot showing the difference in latency between the control and enriched group for the detour test.

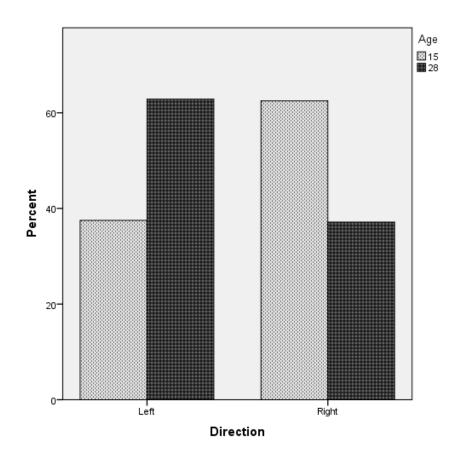


Figure 3-7. Direction of choice for successful chicks in the detour test,, separated by age groups at 15 and 28 days of age.

3.3.3 Jump test

There was a significant difference between ages for both trials of the jump test, but no effect of rearing treatment. Trial 1 (jumping up to 1 platform) was completed by 31% of birds at 14-15 days and 69% at 28-29 days of age (z = 3.909, n = 96, p = 0.001). Trial 2 (jumping up both platforms) was completed by 23.4% of birds at 14-15 days and 76.6% at 28-29 days of age (z = 5.412, z = 96, z = 0.001). There was no overall difference in the latency to complete the jump test between treatment or age.

3.3.4 Rotated floor test

When considering the number of chicks passing the training criteria for the first training session of the rotated floor test, 72.9% were successful at 14-15 days of age compared

to 91% at 28-29 days of age. The successful chicks showed good consistency with only 3.8% of the previously successful chicks being unsuccessful in the second training session. However, the number of training sessions was similar for the successful chicks, with an average of 2.65 ± 0.98 for training session one and 2.21 ± 0.62 for training session two. There was no effect of rearing experience on the success of passing either training session.

Of the chicks that passed the training criteria and were tested at 15 days of age, a chisquared test indicates there was a significant difference in the proportion of chicks that
made no choice in the control rearing group (35%) compared to the enriched rearing
group (6.6%) in the first RFT when required to cross the marker lines ($X^2(1, n = 35)$)
= 3.902, p = 0.048). There was no difference between the ages or treatments in the
second test to cross the marker lines or when required to go behind the screen to make
a choice. Chicks that did not make a choice were removed from further analysis as this
had no effect on results.

For test 1 65.2% of chicks used intra-maze (the screens) cues when crossing the marker lines. Similarly, 76.3% of chicks used intra-maze (the screens) cues when required to go and look behind a screen. These differences were not attributed to age or treatment.

For test 2 it was found that 57.4% of the chicks were relying on extra-maze cues when crossing the marker line, and 57.1% when going behind a screen. There was no significant difference between the use of extra or intra-maze cues for age or treatment.

The use of intra-maze (the screens) cues between test 1 and test 2 showed a significant difference in the use between the first (76.3%) and second test (42.9%), suggesting a shift to extra-maze cues. Looking at treatment effects, 75% of the control chicks made the same choice in test 1 and test 2, whilst only 50% of the treatment chicks made the

same choice (Figure 3-8). This result shows a trend towards signficace(see Table 3-1 for summary of results).

Table 3-1. A summary of the RFT results testing if the proportion of chicks that chose the intra-maze cues (screens) differed significantly by chance.

Test	Proportion of chicks	Test Statistic			
Did intra-maze cue use differ from chance (50	0% of chicks making the ch	oice) in test 1 and test 2			
Test 1 Cross marker lines	0.65 intra-cues	0.50, p = 0.019*			
Test 1 Behind screen	0.76 intra-cues	0.50, p = 0.001*			
Test 2 Cross marker lines	0.43 intra-cues	0.50, p = 0.275			
Test 2 Behind screen	0.43 intra-cues	0.50, p = 0.568			
Did intra-maze cue use differ significantly bet	tween test 1 and test 2				
Comparing use of intra-maze cues for test 1 and test 2 to cross marker lines.	0.43 intra-cues	0.65, p = 0.005*			
Comparing intra-maze cues for test 1 and test 2 behind screen	0.43 intra-cues	0.76, p = 0.038*			
Did the control chicks make the same choice more than by chance across test 1 and test 2					
Comparing choice by control chicks for test 1 and test 2	0.75 same choice	0.50, p = 0.077			

For the first test phase, of the chicks that made a choice, chicks at 14-15 days of age took longer to cross the marker lines and choose a screen (54.43 ± 50.23) compared to 28-29 days (29.50 ± 38.44 ; z = -2.024, n = 66, p = 0.043). There was no effect of treatment. The latency to go behind a screen was not affected by age or treatment. For the second test phase there was no difference in latency to cross the marker line or go behind a screen between age or treatment.

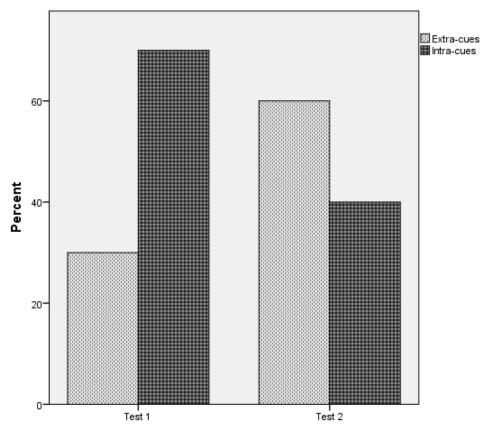


Figure 3-8. Graph for the rotated floor test, showing the difference between test one and test two in the use of intra-maze cues (the screens) or extra-maze cues.

3.4 Discussion

Using a battery of tests allowed more of an overview of the effects of providing accessible elevated structures than has previously been obtained. We found high use of simple structures from a young age, with a greatly increasing ability and preference to use higher perches as birds aged. We obtained different results from the three navigational tests. Chicks reared in a complex three-dimensional environment were faster at completing the detour test and more enriched chicks made a choice at 15 days of age in the RFT but there was no difference between the rearing groups in performance in the jump test.

The strong correlation between age and structure use corroborates previous studies showing an increase in structure use over time. Kozak et al. (2016) reared chicks in complex aviaries with low level platforms, perches and ramps finding a peak in elevated structure use around two weeks of age. Heikkila et al. (2006) observed all chicks in their study using perches by 22 days of age but the first chicks were observed on perches by 8 days of age. Our results show that access ramps and lower level structures up to 10cm were utilised in the first few days of age. Providing lower and more varied elevated structures may encompass the ability of all chicks and from a younger age, as seen in our study. Simply by adding an elevated ramp leading up to 25cm high perches we may provide access to all chicks by 10 days of age.

The chicks reared with elevated structures had shorter latencies to complete the detour test. This might suggest improved spatial cognition. However, there was no difference between treatments in the number of orientation errors or the successful completion of the test within 5 minutes. Given the difference in the latencies between the rearing groups, it was unexpected that an equal number of chicks from each group did not complete the detour test. One explanation for this is that some of the chicks might have been satisfied with being able to see the companion chicks through the mesh as the chicks would have had auditory and visual contact, this may not have differed with the rearing treatments. Faster completion of the detour test could be in indicator of improved spatial cognition, but it could also be a measure of motivation if chicks reared with elevated structures were more motivated to regain contact with pen mates. Speed of decision making has shown to correlate highly with other measures of motivation. Browne et al. (2010) tested chickens in a T-maze to ascertain their preferences for environments. Chickens with strongest preferences for an environment also had the shortest latencies to choose. Although only studied in adult laying hens, synchronous

perching may be one possible reason why chicks reared with elevated structures may have had a stronger motivation to regain access to their companions. Synchronous perching at night may have strengthened affiliative bonds or reduced aggression (Eklund and Jensen, 2011, Donaldson and O'Connell, 2012). However, if the treatment chicks were highly motivated to regain access to their companions, we would expect to see significant differences in the latencies reflected in all three tests. There was no difference in the latency to reach companions in the RFT or the jump test, suggesting no difference in motivation between the rearing groups.

More orientation errors were made by older chicks, although latency and completion of the detour test within 5 minutes were not different between the age groups. Impulsivity is found to reduce over 7 to 19 days of age when chicks were trained to peck green or blue beads for a food reward (Amita and Matsushima, 2011). In our study it may be the case that younger chicks were more impulsive and made quick simple decisions based on strong imprinting. Errors may increase as older birds take account of more information, acquired with experience.

There was an interesting effect of age on direction taken when leaving the starting box. Evidence has shown there is increased activity in the right hemisphere of the brain at 10 and 11 days of age but by 15 days of age there is no specific hemispheric learning, suggesting both hemispheres are used equally (Rogers, 2014). In this study at 14-15 days of age 62.5% of chicks went right out of the detour box when facing their companions and viewed from above but when tested at 28-29 days of age 62.9% of the chicks went left out of the detour box. Studies have shown that domestic chicks tend to have a left bias when interacting with their environment. When tested on ordinality 5 day old chicks were more successful at locating the correct order when tested from left

to right (Rugani et al., 2007). Similarly Regolin (2006) found 9 day old chicks will peck more to the left, suggesting an asymmetry that may be found in the bird brain. Vallortigara et al. (1999) tested chicks in a detour test at 4 days old with either the left or right eye covered or with binocular vision. They found that binocular chicks tended to detour to the left side of the barrier suggesting they use the right eye (chicks will turn their head to look forwards and walk in the direction their beak is facing), therefore the left hemisphere of the brain. In our study at 14-15 days more chicks appeared to be using the right hemisphere of the brain, whereas at 28-29 days of age more chicks were using the left hemisphere of the brain. This may indicate that changes in hemispheric dominance may be sustained for longer than 15 days of age. There is no research looking at the interactions between behaviour and brain lateralisation beyond 15 days of age so further work is needed to explore this.

In the Jump test, older chicks (28-29 days) were more successful than the younger birds (14-15 days) at accessing both levels of the test. Unlike Gunnarsson et al. (2000) we found no rearing treatment effect, although our control group had never had experience with raised structures. However, our procedures differed. Gunnarsson et al. (2000) compared birds reared with or without perches to 8 weeks of age and conducted the jump test at 16 weeks. We tested birds at a much younger age, so differences may have been minimised, with the control chicks only experiencing four weeks without elevated structures. Gunnarsson et al. (2000) also provided higher perches up to 120cm, our maximum height was 60cm, again minimising differences between our groups. The results of the jump test did demonstrate the challenge for 14-15-day old chicks to access a platform 20cm from the ground level as only 31% were successful at jumping to this first level regardless of the rearing treatment. This is important to consider when rearing

chicks commercially and we have demonstrated in this study that chicks will access raised structures if given the opportunity via lower level access ramps or perches.

The rotated floor test did not reveal any difference between rearing treatments in the success of meeting the training criterion. Despite testing only chicks that had passed the training criterion, a significantly smaller percentage of enriched chicks (6.6%) at 14-15 days of age failed to make a choice by crossing the marker lines compared to the control chicks when cues were confounded when the internal screens were rotated so the internal and external cues no longer matched.

For chicks that successfully completed the tests, there was no treatment effect in use of intra or extra-maze cues during the two test sessions. Previous studies have found that chicks with experience of visual occlusion between days 10 to 12 tend to use distal or extra-maze cues in the test phase of the RFT, whereas chicks without such experience tend to use intra-maze cues (Freire and Rogers, 2005, Freire and Rogers, 2007, Wichman et al., 2009). We expected that access to elevated structures might provide incidental experience of visual occlusion and therefore that our treatment chicks would show a greater tendency to use extra-maze cues than our controls, but this was not the case. In the first RFT, the majority of our successful chicks from both rearing groups (76.3%) used intra-maze cues. It is possible that the elevated structures did not in fact provide chicks with sufficient experience of visual occlusion to have influenced their initial cue preference. One point to mention is that the previous studies that use the RFT rotate the entire arena. In contrast in our study only the screens were swapped. Therefore, in our case the markings on the wall of the arena would provide extra-maze cues, whereas in previous studies these would provide intra-maze cues. There are therefore stronger extra-maze cues in our study compared to the cited studies (Freire and Rogers, 2005, Freire and Rogers, 2007, Wichman et al., 2009). Our finding that the chicks used mainly intra-maze cues with this approach suggests even more strongly that they were not using extra-maze cues.

In the second RFT we found that the proportion of chicks choosing the same location was no longer significantly different from random, with 57.1% using extra-maze cues, and a larger shift in preference observed in the treatment chicks (p=0.07). All chicks may have shifted their cue use preference because tests (companions absent) were nonrewarded. Chicks will therefore have experienced their initial test response as incorrect, possibly leading to a devaluation of the reliance initially placed on intra-maze cues and a shift in cue-use even after an intervening re-training schedule. Under short intervals of retention, specifically concerning objects and location, information is retained in both hemispheres of the brain (Regolin et al., 2005), possibly accounting for the shift in the cue use preference we observed in the second test. Wichman et al. (2009) and Freire and Rogers (2007) also interspersed training sessions with repeated non-rewarded tests, but they did not explore whether chicks shifted cue use across the repeated tests, so we cannot compare our results. The increased tendency of treatment chicks to shift cue use may be because their experience of elevated structures provided them with a greater capacity to attend to cues of both types. Further exploration of the cue preferences and reversal learning capacities of chicks reared with and without elevated structures would be useful.

3.4.1 Conclusion

In other studies, we (Norman et al., 2018) and others (Gunnarsson et al., 2000) have found that early experience of structures such as ramps improves the use of the structures compared to birds that have had no experience, illustrating the importance of early life

experiences. However, in these previous studies chicks were reared for a longer time (up to 8 weeks or more) under different rearing conditions. In contrast, the work presented here found that rearing with elevated structures up to 28-29 days of age had only subtle effects. There was a shorter latency in the detour test which might indicate improved spatial cognition or altered social motivation, although differences in the rearing groups' motivation was not reflected through all three tests. We found a tendency for treatment chicks to show a greater shift towards the use of extra-maze cues in repeated RFT. However, the battery of tests used did not reveal any substantial general effects on physical ability, social motivation or spatial cognition. It appears that age and development over time may have a stronger influence on spatial navigation and physical ability than short-term early experience of elevated structures.

The results from Chapter 2 and 3 provide interesting results that demonstrate the importance of early experience of structures to encourage movement during the rearing period on both physical ability and spatial cognitive ability. These findings are useful to the commercial laying industry, but it is important to ensure the results are applied in a commercial setting. Chapter 4 continues research on rearing with elevated structures but applies this to a commercial rearing and laying farm in an attempt to conduct a controlled farm study.

Chapter 4 - Influence of ramp provision at rear on hen movement, behaviour and welfare on a commercial farm

Influence of ramp provision at rear on hen movement, behaviour and welfare on a commercial farm

Abstract

In commercial systems laying hens must move between levels to reach resources. Recent studies have shown the importance of early life experiences on the development of behaviours. However, more research is needed to look at whether providing ramps during rear can improve the ability of birds to transition between levels at lay.

This study examined 12 organic commercial flocks (2000 birds/flock) on a multi-age site, studied from 1 to 40 weeks of age. There were 6 visits at 1, 3, 15-16, 16-17, 24 and 40 weeks of age, where transitioning behaviour and use of the elevated structures were recorded along with welfare data such as keel bone and feather scores. Six cycles of flocks were reared in two sheds (12 flocks), then moved to two laying sheds of identical facility design. At 4 days of age, both rearing sheds were provided with 6 identical elevated structures (ES) comprised of 9 perches and two plastic platforms. In one rearing shed three ES were additionally fitted with plastic rampsleading up to the low perch and the remaining three ES had ramps leading up to the middle perch. Laying sheds had a drop down to the litter where intermittent ramps were installed, this allowed comparison of transitions and ramp use of the rearing groups.

Data were analysed in using normal or binomial models in MLwIN. The number of chicks observed on the ES was greater in the groups with ramp access (z = 3.621, n = 192, p = 0.001) and structure use increased over 1 to 16 weeks of age regardless of treatment. At lay more pacing (15% vs 2.5%) and crouching (69.2% vs 36%) were observed in the control reared birds before transitioning down a ramp. For upwards

transitions on a ramp more wing flapping during transitions was observed in the control reared chicks (8.4% vs 2.1%). For welfare measures, a greater percentage of birds had keel bone fractures in the control reared groups compared to the ramp reared at 40 weeks of age, suggesting an effect of rearing with ramps.

Rearing with access to ramps appears to increase the use of elevated structures at 1 and 3 weeks of age. When transferred to the laying shed, flocks reared without ramps show more behaviours indicative of hesitancy when transitioning such as crouching, pacing and wing flapping. Generally, a higher number of birds transitioned using ramps, suggesting a preference to use ramps to move between levels in a commercial system. Keel bone fractures were lower in the ramp reared groups suggesting an effect of rearing experience on bone development or mobility between levels, suggested by the reduced hesitancy type behaviours observed in the ramp reared groups when transitioning.

4.1 Introduction

Cage-free commercial laying hens are housed in complex systems, where resources such as food, water, nest boxes, perches and litter are commonly distributed on different levels. Additionally, in free range systems, access to the outdoor range can sometimes mean moving through elevated pop-holes. Resources such as litter and the outside range are required for the performance of foraging (Nicol et al., 2013) and dust bathing; which, together with night-time perching have been identified as behavioural needs (Weeks and Nicol, 2006). Restriction from these resources can reduce welfare and lead to the development of problems such as feather pecking, fearfulness and aggression (Cordiner and Savory, 2001, Newberry et al., 2001, Donaldson and O'Connell, 2012). It is thus important that hens can move freely throughout such cage-free systems so that each resource is accessible and that welfare benefits are maximised.

Currently, evidence suggests that movement is compromised, with reduced ranging observed in birds that roost furthest from pop-holes (Pettersson et al., 2017c) and also that collisions and falls are common in commercial systems (Harlander-Matauschek et al., 2015). The resultant keel bone injuries restrict movement (Nasr et al., 2012a) and are painful, as shown when Nasr et al. (2015) provided analgesics to birds with keel bone fractures and found that movement between perches was partially restored. In commercial systems as many as 80% of laying hen will have evidence of past keel bone fractures (Wilkins et al., 2011). It is thus important to investigate whether bird movement can be facilitated by improved bird rearing practices and structural design within rearing and laying houses.

Some studies have looked at access to ramps in the laying shed in facilitating movement and reducing injuries. Pettersson et al. (2017a) observed more behaviours indicative of

hesitancy such as crouching, pacing and stepping on the spot when birds attempted to move between levels in commercial houses with no ramp provision, than in houses containing ramps. Pettersson et al. (2017d) also looked at ramp type preference in experimental conditions. They found that layer pullets showed fewer hesitancy behaviours and smoother transitions when using a grid ramp over a ladder ramp (both common types of ramps found in commercial poultry systems), information which could be usefully applied to commercial systems. Alongside these benefits, the installation of ramps and platforms in commercial aviary systems has been found to reduce collisions by 59%, falls by 45% and keel bone fractures by 23% at 60 weeks of age (Stratmann et al., 2015). Similarly, the installation of ramps between perches has shown positive effects on the reduction of keel bone fractures in experimental aviary systems (Heerkens et al., 2016). Because providing birds with access to ramps facilitates movements and reduces the number of fractures at lay, it is important to consider how to maximise these benefits and to explore factors that could further increase ramp use.

The importance of rearing in an environment similar to the laying system has been acknowledged (Janczak and Riber, 2015). There are a variety of rearing methods used in the commercial industry for rearing laying hens. These include large single level barn systems, jump start systems where increased complexity is included as the chicks get older and aviary rearing systems that tend to match systems used in lay. In commercial systems, as awareness of the importance of matching the rearing and laying system has grown, it is now common practice to rear with elevated structures to encourage the development of the behaviours that will be required for chicks to navigate a three-dimensional system at lay. There has been limited research about the best types of

elevated structure to provide for younger birds, and in commercial rearing systems it is most usual for relatively simple structures such as raised perches to be included.

Given the growing evidence that ramps provide benefits for laying hens, we have been studying the effects of ramp provision for younger birds too. In a small-scale study, we found that chicks reared with ramp access from 3-weeks-old in a commercial farm utilised ramps to a greater extent and with more confidence when tested at 14 weeks of age within a small experimental facility than birds reared without ramps (Norman et al., 2018). One reason why ramps may not routinely be provided in commercial systems to young chicks is because producers want chicks to stay near the heat source, food and water, or because they believe chicks will not use elevated structures during the first few days of life. However, we have shown that chicks will use ramps in the first few days of life, then low-level perches (10 cm) at approximately 5 days of age, and they will slowly progress to higher levels (60cm) by 8 days of age (Norman et al., 2019). In addition, young chicks provided with easy access to elevated structures were subsequently faster to solve a detour task and appeared to more effectively utilize both hemispheres of their brain to attend to intra and extra maze cues in a rotated floor task than control chicks (Norman et al., 2019).

The effects of rearing in more complex environments is an area of growing interest (Janczak and Riber, 2015, Campbell et al., 2019), especially in regards to bird development and improving movement in commercial houses (Pettersson et al., 2017a, Stratmann et al., 2015). Our studies have highlighted benefits of rearing with ramps under experimental conditions (Norman et al., 2019, Norman et al., 2018) but these results have not yet been replicated on a commercial scale. Indeed, replicated longitudinal studies on commercial rearing and laying farms are still rare and in a

commercial setting it can often prove difficult to use controlled experimental designs. However, by working with a commercial partner we were able to design a long-term, replicated study using matched flocks of laying hens. The aim of our study was to assess the extent to which knowledge and results obtained from experimental studies would be applicable within commercial systems. Our specific aims were to examine (i) the effects of ramp provision at rear on the use of elevated structures by young pullets, (ii) the effects of ramp provision at rear on the ability and confidence to use ramps provided to all birds during the laying period and (iii) the effects of ramp provision at rear on welfare parameters including plumage condition, keel bone fractures and floor eggs.

4.2 Methods

4.2.1 Experimental Design

Over three years 6 pairs of organic British Blacktail flocks were visited from 1 to 40 weeks of age. Six of which were ramp reared (RR) and six control reared (CR) without ramps. All flocks were kept on one farm which possessed two rearing houses and six laying sheds of approximately 2000 birds per flock. The site was multi-age meaning of the six laying sheds there were three different ages on the site at one time.

This set up enabled us to design an experiment whereby we allocated two rearing treatments (i) ramps provided to access elevated structures and (ii) control with elevated structures but not ramps and to alternate these treatments between the two rearing houses available to avoid treatment x house confounding. Each rearing flock was moved independently to a laying house with no mixing, so we were able to continue data collection and examine any long-term effects of the rearing treatment during the laying

period. Rearing flocks were systematically allocated to laying houses so that each laying house received one RR flock and one CR flock during the experiment.

Observations were made at three time points during the rearing period at 1, 3 and 15-16 weeks, and three in the laying period at 16-17, 24 and 40 weeks of age. See Table 4-1 for a summary of experimental design, flock and housing information.

Table 4-1. Rearing and laying shed set ups for each ramp reared and control reared flock for the 6 replicates.

Replicate	Rearing	Laying	Treatment at	Ramp angle at	Arrival
	house	house	rear	lay (degrees)	
1	R1	B2	RR	30	15/04/2016
	R2	B1	CR	30	15/04/2016
2	R1	C2	CR	30	20/09/2016
	R2	C1	RR	30	20/09/2016
3	R1	A1	RR	45	24/01/2017
	R2	A2	CR	45	24/01/2017
4	R1	B2	CR	30	08/06/2017
	R2	B1	RR	30	08/06/2017
5	R1	C2	RR	30	07/11/2017
	R2	C1	CR	30	07/11/2017
6	R1	A1	CR	45	27/03/2018
	R2	A2	RR	45	27/03/2018

The rearing sheds were static with a curved roof with 142.7m² of floor space covered with wood shavings. Rearing sheds were both set up with feed tracks giving mini pellet feed up to 11 weeks of age then pellet grower feed and 7 nipple drinker lines. The lighting schedule was 23 hours light in the first day reducing gradually over the rearing period to 10 hrs light at 7 weeks of age. Temperature was maintained at 30 °C during the first few days then slowly reduced to match the temperature in the laying sheds. Heat was provided by gas spot lamps, whole shed heating through hot pipes running along the length of the shed and hot air fans run by a biomass boiler. All flocks had access to

the outside range by 10 weeks of age through two pop holes (each L: 2m by H: 0.4m). Flocks were moved from rear to lay between 15 to 16 weeks of age in one night using transport modules.

All rearing flocks had access to six elevated structures (ES) (see Figure 4-1) from four days of age, when the chicks were released from the brooding circles. Each ES comprised 9 metal perches (length 302cm, width 3.5cm), with three perches (25cm apart) at three different heights (43cm, 73cm and 103cm). Two plastic slats (width 60cm, length 115cm) were fixed within the ES to provide platforms at different heights (Figure 4-1). In each replicate, the RR flock had one ramp attached to each ES. Three of the ES were fitted with plastic ramps (width 60cm, length 74cm, angle 35.5°) leading up to the low perch and three ES had ramps (width 60cm, length 115cm, angle 40°) leading up to the middle perch. The CR flock had six ES without ramps.

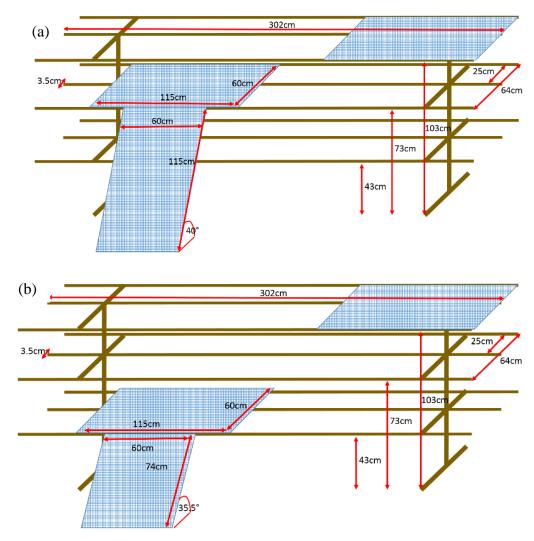


Figure 4-1. Elevated structure dimensions used in the ramp reared sheds(RR), (a) shows high ramp (b) shows the low ramp. The control reared (CR) sheds elevated structures were identical to these but without ramps.

The six laying houses on site were mobile organic units with approx. 345m² of floor space. See Figure 4-2 for schematic diagram. Four of the sheds (Figure 4-2a) were set up with a plastic slatted area spanning the whole width of the shed down to 50% of the length. In two of the sheds (Figure 4-2b) the wood chip covered litter area stretched down the sides of the slats. Nest boxes ran down the centre of the raised slatted area, dividing this into two sections. All had a raised slatted area (approx. 70cm from the litter) and a ground level litter area. Intermittent ramps were installed at the level change, resulting in 4 metres of ramp access and 4 metres without ramps in the shed with litter and the end and 8 meters of ramp access and 13m without ramps in the shed with litter at the sides. There were four pop holes with two on each side of the house (L: 2.35m by H: 0.4m) leading to the range from the litter area on both sides of the sheds. All sheds had aerial perches at 1 meter high with 18cm of perching space per bird resulting in approx. 360m of perch length running down the length of the slatted area. Feed tracks and drinker lines matched those in the rearing sheds. See Figure 4-2 for shed layouts. The lighting schedule was 16 hours of light and varied between summer and winter with the lights set to turn off at the same time as dusk. The birds were fed on a high peak mini pellet throughout lay. Enrichment was provided to the flocks in the form of pecking objects such as buckets and boots. Replicates 5 and 6 were provided with pecking blocks and alfalfa hay nets on the litter area.

Chapter 4

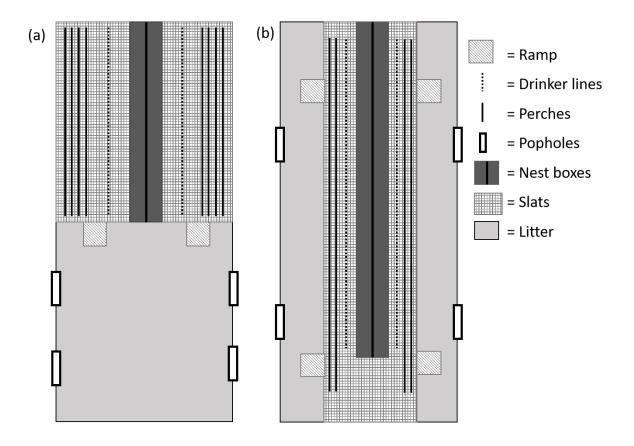


Figure 4-2. Laying shed layout (a) showing all shed set up for replicates 1, 2, 4 and 5 and (b) showing shed set up for replicates 3 and 6. Images not to scale.

4.2.2 Behavioural Recordings

Observations were made at three time points during the rearing period at 1, 3 and 15-16 weeks. On the first visit at 1 week of age the total number of chicks on the ES was counted. At 3 and 15-16 weeks, scan samples were taken to look at the movements up and down the ES. Three of the six structures were chosen at random in each shed. The number of chicks present on the different parts of the ES were counted at the beginning and end of the recording period. The recording period involved 5-minute scan sampling where all movements down the ES were recorded and the area the chicks moved down from was noted. This was then repeated for movements up the ES. Focal recordings were taken at 3 and 16 weeks of age. For each structure focal birds making a movement on the ES were studied. When 10 focal birds were observed (approximately 30 birds per

flock), or 10 minutes had passed recordings stopped. A focal bird was chosen if it was performing orientation behaviour indicating a downwards or upwards transition. This was described as the bird rotating its head to look in the direction of movement. Behaviours performed after the orientation behaviour were tallied, thus recorded as counts per behaviour (see Table 4-2). Recordings were stopped if birds completed a transition or moved away from transitioning.

Table 4-2. An ethogram of behaviours of focal birds during up and down movements.

Behaviour	Description			
Orientation	Bird rotates head to look in the direction of movement			
Pacing	Bird raises one or both feet and replaces on ground or perch, or bird moves along structure followed by head orientation, crouch or movement down/up			
Crouching	Lowers body to ground, or lowers body on ramp			
Straight down	Bird walks or runs straight down the ramp			
Zig Zag down	Bird crossed ramp in a zig zag motion to get to the bottom or top			
Wing flap	Bird flaps wings during movement			
Move away	Bird moves away from initial position without showing further intention of traversing within 10 seconds			
To ramp	Bird moves away but to ramp			

At 16, weeks of age three types of interactions were recorded for feather pecking. These included severe feather pecking (SFP), gentle feather pecking (GFP) and aggressive pecks (AP) (Lambton et al., 2010). An area 2 metres by 2 metres was randomly selected, the number of birds in the area were counted at the beginning and end of the recording period. The number of SFP, GFP and AP were recorded over three minutes of continuous recordings in three different areas of the house, selected randomly at either end and the middle of the shed. Feather pecks and aggressive pecks were recorded as bouts: a series of pecks not separated by more than five seconds (Lambton et al., 2010). Rates of pecking were calculated as the number of pecks per bird per second.

In the laying shed at 17 and 24 weeks of age 3-minute continuous scan samples and focal recordings were taken for transitions between the slats and litter. Four recordings were taken at 2-metre lengths along the slats: two areas with ramps (RA) and two areas without ramps (NRA) were selected. Separate recordings were taken for upwards and downwards movements and the number of birds in the recording area were counted at the start and end of the scans.

At 17 and 24 weeks of age, feather pecking observations were taken using the same procedure as for the 15-16-week observations during rear.

At 16-17 and 24 weeks of age the attitude of the flocks were assessed using approach distance and reactions to novel objects developed by Whay et al. (2007). Distance to approach birds before they moved away was recorded by walking through the house selecting a bird at random and counting two birds to the left. The bird had to be standing up and facing the researcher, they approached the bird at a steady pace and estimated the distance before the bird moved away. This was repeated on 20 birds in each flock. Reactions to a novel object (blue folder at 17 weeks of age and a white and blue tub at 24 weeks of age) were recorded by placing a novel object on the ground and recording the time taken for a bird to first interact with it and then how many birds were within 30cm radius after 60 seconds. The novel object test was repeated four times on each flock.

Range use was recorded by counting the number of birds near to the house (5 meters) in the middle range (5-20 meters) and far (the rest of the range).

4.2.3 Welfare assessments rearing phase

Feather scores of 20 birds per flock were recorded at 16, weeks of age by walking in a straight line down the centre of the shed, selecting a bird at random then counting two birds to the left and visually feather scoring that bird. Birds were not handled to minimise disturbance and plumage was scored using the method from Bright et al. (2006). The neck, back, rump, tail and wings were scored using a four-point scale 0 (best) to 4 (worst). The criterion for body feathers and flight feathers differed slightly.

4.2.4 Welfare assessments laying phase

Feather scores of 20 birds per flock were recorded at 17 and 24 weeks of age using the same procedure as for the 16-week assessment for birds at rear.

At 40 weeks of age feather cover and keel bone fractures were scored. Up to 100 birds per shed were caught from four different locations (25 litter, 25 slats, 25 perches, 25 nest boxes). In four sheds only 50 birds were caught as the birds were fearful and showed signs of distress. Feather cover was scored by picking the bird up and scoring the body and flight feathers separately using a three-point scale 0 (best) to 2 (worst). The keel damage was then scored using a 0 (no damage) to 2 scale based on the technique used by Wilkins et al. (2004). Validation for keel bone palpations were conducted. A score of 94% matched scores compared to a gold standard assessor and 85% match at dissection for scoring a break. Suggesting a good level of keel score assessment. At 24 weeks of age the number of floor eggs were counted over 1 day.

4.2.5 Statistical analysis

Data were analysed using SPSS 24 (IMB) or MLwiN 3.0. The statistical package MLwiN was chosen as it is designed for multilevel modelling and can therefore

accommodate data nested within levels with repeated measures. Four levels were used for behavioural data models (Bird within shed within occasion within replicate). All residuals were checked for normal distributions using Shapiro-Wilks test or plotted graphically and transformed to meet the assumptions of the tests. If normality could not be achieved the non-parametric alternative was used. For analysis of the numbers on structures and scan data at rear and lay a normal model was used in MLwiN. Due to the low occurrence of behaviours during the focal recordings for transitions up and down the ramps, data were coded as yes or no, and a Binomial model was used for analysis. Ordinal data such a keel bone fracture scores and feather cover were converted to binomial data due to a lack of data for some scores, these were therefore analysed using a binomial model in MLwIN with two levels (Bird within shed)..

To report the data for the scan samples and numbers on the structures at rear, means were calculated for each treatment. For the focal bird behaviours of birds transitioning at rear and lay, the percentage of each behaviour was calculated for the birds in the recording session for the two rearing treatments. The direction (up or down) was analysed separately. For the focal birds at lay all were included in analysis for the pretransitioning behaviours, only birds that attempted a transition were included for analysis of the transitioning behaviours. Pre-transition behaviours for birds that moved away and did not transition were analysed separately. Means were calculated for the welfare recordings for the rearing treatments.

During the 16 weeks recordings in the final rearing flocks the lighting inside the shed was considerably reduced compared to previous flocks. This resulted in poor visibility for feather cover and feather pecking observations, so these were not taken during this

visit. Data were not obtained on keel fractures and feather cover scores at 40 weeks for the first laying flocks visited as their sheds were destroyed by strong winds.

4.2.6 Ethical Approval

Ethical approval for this project was approved by the University of Bristol's Animal Welfare and Ethical review body under UIN: UB/16/040.

4.3 Results

4.3.1 Numbers on structures at rear

The total number of chicks observed using the elevated structures was significantly different between the treatment groups (z = 3.621, n = 192, p = 0.001) and the three recording ages (z = 2.617, n = 192, p = 0.009) with no interaction effects. More chicks were observed on the structures in the RR groups (21.50 ± 17.44) compared to the CR groups (13.99 ± 14.25). Post hoc test revealed an increase in total structure use between 1 and 3 weeks of age and 1 and 16 weeks of age. But there was no difference in structure use between 3 and 16 weeks of age (See Table 4-3). Although there appears to be some differences between the rearing treatments at the three ages, an interaction effect was not found, which may be due to the sample size of 12.

Table 4-3. The raw mean number of chicks observed on the elevated structures (totalled for each area of the ES excluding the ramps)

Age	Ramp	Control	Total
(weeks)	$(mean \pm SD)$	$(mean \pm SD)$	$(mean \pm SD)$
1	2.38 ± 5.85	0.00 ± 0.00	1.19 ± 4.27
3	31.84 ± 13.55	17.76 ± 13.91	24.81 ± 15.36
16	28.20 ± 13.55	23.43 ± 10.95	25.82 ± 12.45
Total	21.50 ± 17.44	13.99 ± 14.26	17.74 ± 16.33

Considering the components comprising the elevated structures separately (perches and slatted platforms), significantly more RR chicks (9.58 ± 8.62) were observed on the slatted platforms compared to the CR chicks $(3.62 \pm 4.50; z = 4.249, n = 192, p = 0.001)$. There were no effects of age on the number of chicks using the slats. There was no significant difference with the number of chicks observed on the perches between the rearing groups. However, there was a difference between the three age points (z = 3.436, n = 192, p = 0.001). Post hoc tests revealed a significant increase in the number of chicks using the perches with age: 0.008 ± 0.065 at 1 week, 13.52 ± 9.07 at 3 weeks and 18.19 ± 8.84 at 16 weeks.

4.3.2 Scan samples of movement up or down structures at rear

For the route taken to move up and down the structures there were some main effects of age and treatment. For access up the structures there was a difference between treatments with more CR chicks using the low perch $(5.306 \pm 3.82 \text{ vs } 2.33 \pm 2.44; Z = 3.534, n = 72, p = 0.001)$, and regardless of treatment a greater overall use of the low perch at 3 weeks compared to 16 weeks of age $(4.77 \pm 4.01 \text{ vs } 2.86 \text{ vs } 2.66; Z = -2.279, n = 72, p = 0.023)$. More CR chicks were also observed using the low slat to move up the structures $(1.00 \pm 1.43 \text{ vs } 0.44 \pm 0.97; Z = 1.961, n = 72, p = 0.050)$. There was no difference between age or treatment for use of the middle perch, high perch, middle slat or high slat to access the structures. Although no comparison between treatments could be made for ramp use, as a reference the mean number of chicks moving up the low-level ramp at 3 weeks of age was 6.22 ± 7.27 and 2.39 ± 3.55 at 16 weeks of age. The mean number using the middle ramp at 3 weeks of age was 6.63 ± 7.49 and 4.56 ± 4.68 at 16 weeks of age.

For access down the structures more CR chicks were observed using the low perch (3.47 \pm 2.37 vs 2.39 \pm 2.63; Z = 1.951, n = 72, p = 0.051) and regardless of treatment more chicks transitioned down using the low perch at 3 weeks of age (4.08 \pm 2.85 vs 1.78 \pm 1.51; Z = -4.152, n = 72, p = 0.001) compared to 16 weeks of age. There were no interaction effects for age and treatment. No age or treatment effects were seen for movements down from the middle perch, high perch, low slat, middle slat and high slat. For reference the number of RR chicks using the low ramp for access down was 2.94 \pm 3.59 at 3 weeks of age and 0.94 \pm 1.63 at 16 weeks of age. The mean number using the middle ramp at 3 weeks of age was 4.94 \pm 5.61 and at 16 weeks of age 3.22 \pm 3.34 for the middle ramp.

4.3.3 Focal behaviours at rear

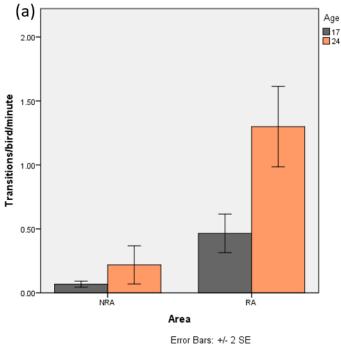
For focal movements up the structures there was a greater percentage of pacing recorded at 3 weeks of age (3.49%) compared to 16 weeks of age (0.27%; Age Z = -2.464, n = 704, p = 0.014) but no treatment differences were observed. Crouching before a transition up showed a trend towards a higher percentage in the CR group compared to the RR group (34.3% vs 13.3%; Z = 1.860, n = 704, p = 0.063). We also found more crouching behaviour at 3 weeks of age compared to 16 weeks of age (39% vs 8.8%; Z = -3.181, n = 704, p = 0.001). More wing flaps were observed in the CR groups (39.8%) compared to the RR groups (17.7%; Z = 2.583, n = 704, p = 0.010), and at 3 weeks of age (43.9%) versus 16 weeks of age (13.8%; Z = -2.567, n = 704, p = 0.010). The CR group performed more jumps up to perches (83.1% vs 32.2%; Z = 6.820, n = 704, p = 0.001), similarly more jumps up to the slats were recorded at 16 weeks of age (18.3%) versus 3 weeks of age (5.52%; Z = 2.753, n = 704, p = 0.006).

When recording focal movement down the elevated structures a greater percentage of crouching was observed at 3 weeks of age (54.8%) compared to 16 weeks of age (21.2%; Z = -3.444, n = 695, p = 0.001). More chicks were observed wing flapping at 3 weeks of age compared to 16 weeks of age (82.2% vs 24%; Z = -2.838, n = 695, p = 0.005) and a greater percentage of jumps down from perches in the CR groups (82.7%) compared to the RR groups (42.1%; Z = 9.348, n = 695, p = 0.001) were recorded. For jumps down from the slats more CR chicks were observed compared to the RR chicks (17.3% vs 7.5%; Z = 3.206, n = 695, p = 0.001) and more jumps at 16 weeks of age (15.5%) compared to 3 weeks of age (8.8%; Z = 2.222, n = 695, p = 0.026).

4.3.4 Level Transitions at Lay

There was no treatment effect on the number of birds transitioning up or down between the slatted area and the litter. Generally, hens transitioned more at 24 weeks of age (0.013 ± 0.013) compared to 17 weeks of age $(0.004 \pm 0.005; Z = 3.193, n = 96, p = 0.001)$. More birds were also observed transitioning up at 24 weeks of age (0.008 ± 0.008) than at 17 weeks of age $(0.004 \pm 0.004; Z = 2.329, n = 96, p = 0.020)$ but there were no age differences for transitioning down.

Interestingly we found significant differences between areas with ramps and those without in the number of birds that transitioned per second (adjusted for the number of birds in the recording area) between the slats and litter. More birds transitioned down using a RA (0.015 \pm 0.012) compared to a NRA (0.002 \pm 0.004; Z = 8.741, n = 96, p = 0.001). There was also a greater number of birds transitioning up in the RA (0.008 \pm 0.007) versus NRA (0.003 \pm 0.005; Z = 5.399, n = 96, p = 0.001). See Figure 4-3 for results.



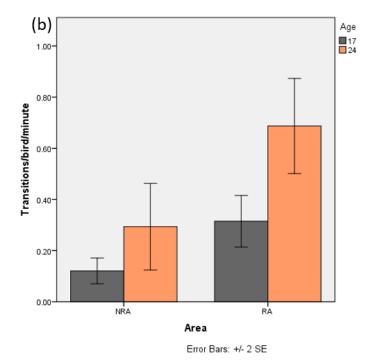


Figure 4-3. Transition per bird per minute in ramp area (RA) and no ramp areas (NRA) for (a) downwards and (b) upwards transitions comparing transitions at 17 and 24 weeks of age

For focal behaviour recordings at lay we first looked for differences in transitions between a RA and NRA. If a difference was found, we continued analysis separately for these areas. Table 4-4 illustrates the statistically significant results for upwards and downwards transitions. Figure 4-4 illustrates these differences graphically comparing the ramp areas, age and treatment groups.

For downwards pre-transitions we observed more pacing at the NRA compared to the RA. In the RA there was more pacing in the CR compared to the RR birds. The percentage of pacing observed also reduced from 17 weeks of age to 24 weeks of age. A greater percentage of crouching before transitions was observed in the NRA compared to the RA. An interaction between age and treatment was found, further analysis looked at the simple effects for age and treatment. More crouching was observed in the RA at 17 weeks of age in the CR compared to the RR birds, however, no treatment differences were observed at 24 weeks of age or in the NRA. Overall, more birds were recorded performing multiple pacing behaviour in the NRA compared to RA with large differences at 17 weeks of age in NRA but not at 24 weeks. No differences were found for any other recorded behaviours.

For transition behaviour moving down in a RA more CR birds wing flapped compared to the RR birds. The number of birds that transitioned in a straight line down the ramp appeared to be approaching significance, more RR birds compared to the CR birds went straight down a ramp. For comparing the difference over age more birds were observed to zig zag down a ramp at 17 weeks of age compared to 24 weeks of age and more birds moved straight down the ramp at 24 weeks of age compared to 17 weeks of age.

Overall, more birds moved away from transitioning down NRA compared to a RA. For birds that moved away from transitioning more pacing was recorded at 17 weeks

compared to 24 weeks. Similarly, more crouching was recorded at 17 weeks of age compared to 24 weeks.

For upwards pre-transition behaviours more pacing was observed in the NRA compared to the RA and more crouching in the NRA, however there were no treatment or age effects.

For behaviours observed when transitioning up a RA the RR birds showed fewer wing flaps compared to the CR birds and more wing flaps were observed at 17 weeks of age compared to 24 weeks of age. There were no differences in transitions at the NRA or for any other behaviours recorded.

For transitions up more birds moved away in a NRA compared to a RA. More 24-weekold birds moved away to a ramp to transition up. More multiple head orientations were observed at 17 weeks of age compared to 24 weeks of age.

Table 4-4 Results for focal behaviour transitions. Separated for downwards and upwards transitions. Only significant results are presented.

DOWNWARDS					
Pre transition					
Pacing	Area : 33.5% (NRA) vs 8.8% (RA), Z=9.153, n=957, p=0.001 Treatment in RA : 15% (CR) vs 2.5% (RR), Z=3.058, n=480, p=0.002 Age in RA : 31.9% (17 weeks) vs 10.3% (24 weeks), Z=-6.088, n=408, p=0.001				
Crouching	Area: 54.1% (NRA) vs 26.9% (RA), Z=8.402, n=957, p=0.001 Interaction treatment*age in RA: At 17 weeks 69.2% (CR) vs 14% (RR), Z=7.131, n=480, p=0.001				
Multiple pacing	Area: 5% (NRA) vs 1% (RA), Z=3.242, n=957, p=0.001 Age 17 weeks: 8.8% (NRA) vs 1.3% (RA); Z=-2.486, n=479, p=0.013 No other significant differences				
	Transition behaviours				
Wing Flaps	Treatment in RA: 1.7% (RR) vs 19.2% (CR), Z=4.621, n=467, p=0.001				
Straight down	Treatment in RA : 88.7% (RR) vs 83.0% (CR), Z=-1.893, n=467, p=0.058 Age in RA : 78.9% (17 weeks) vs 92.5% (24 weeks), Z=2.668, n=467, p=0.008				
Zig zag	Age in RA: 22% (17 weeks) vs 7.9% (24 weeks), Z=-2.563, n=467, p=0.010				
	Move away behaviours				
Move away	Area: 42.8% (NRA) vs 1.3% (RA), Z=9.650, n=210, p=0.001				
Pacing	Age: 43.4% (17 weeks) vs 12.5% (24 weeks), Z=-3.739, n=210, p=0.001				
Crouching	Age: 50.0% (17 weeks) vs 5.8% (24 weeks), Z=-3.178, n=210, p=0.001				
UPWARDS					
Davina	Pre transition				
Pacing	Area: 14.5% (NRA) vs 0.83% (RA), Z=5.748, n=927, p=0.001 No significant treatment or age effects				
Crouching	Area: 58.6% (NRA) vs 2.7% (RA), Z=12.913, n=927, p=0.001				
	No significant treatment or age effects				
Transitions					
Wing flaps	Treatment in RA: 2.1% (RR) vs 8.4% (CR), Z=3.025, n=477, p = 0.002				
	Age in RA: 9.2% (17 weeks) vs 1.3% (24 weeks), Z=-2.808, n=477, p=0.005				
Move organ	Move away behaviours				
Move away	Area: 15.7% (NRA) vs 0.2% (RA), Z=4.515, n=71, p=0.001				
To ramp Multi head	Age: 88.5% (24 weeks) vs 35.6% (17 weeks), Z=2.632, n=71, p=0.008 Age: 28.9% (17 weeks) vs 3.8% (24 weeks), Z=-2.294, n=71, p=0.022				
orientations	Age. 20.7/0 (17 weeks) vs 3.0/0 (24 weeks), 2.—-2.274, 11—71, p=0.022				

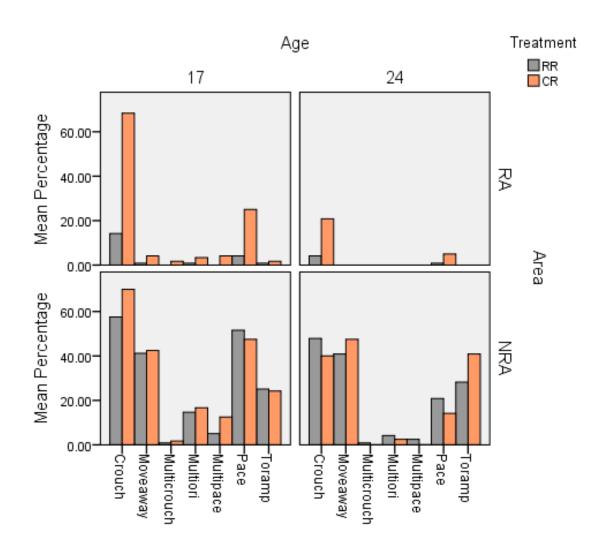


Figure 4-4. Graphs showing the percentage focal behaviours for downwards transitions allowing comparisions between age (17 and 24 weeks of age), area (ramp area = RA or no ramp area = NRA) and treatment (ramp reared = RR amd control reared = CR)

4.3.5 General welfare measures

We observed no treatment effects for feather cover or feather pecking when measured at 16, 17 and 24 weeks of age. For the fearfulness tests we did not observe any treatment differences in the novel object test for the time to approach or number of birds interacting with the novel object after 60s and no differences in approach distance. There were no differences in the number of floor eggs between the rearing treatments. There were no differences in feather cover scores between the rearing treatments at 40 weeks

of age, however a difference between treatments in birds recorded with keel bone fractures at 40 weeks of age was observed, (Z = -2.193, n = 800, p = 0.028) with 64.8% of birds recorded with fractures in the CR compared to 52% in the RR groups (figure 4-5).

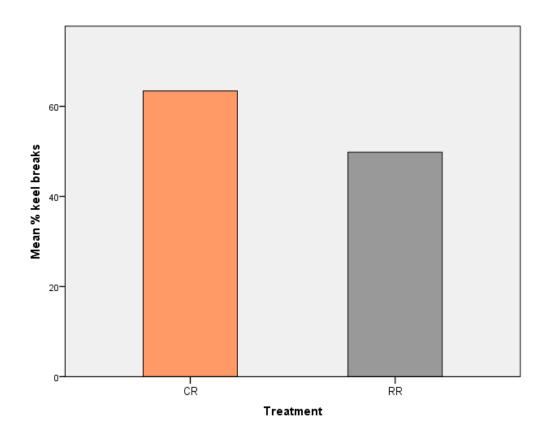


Figure 4-5. Mean percentage of birds with keel bone damage in the control reared (CR) and ramp reared (RR) treatments.

We observed some differences in the welfare measures over the three recordings ages. For feather cover recordings there was a significant difference between the age points $(Z=5.183,\,n=640,\,p=0.001)$, post hoc test reveal poorer feather cover at 24 weeks compared to 16 weeks of age and 17 weeks of age. For feather pecking there was a significant difference between the ages and the total pecks observed per bird per second $(Z=-1.966,\,n=126,\,p=0.049)$. Post hoc tests reveal an increase in the total number of pecks between 16 and 17 weeks of age and a reduction in pecks between 17 and 24

weeks. There was also a difference between ages in the number of GFP per bird per second (Z = -3.007, n = 126, p = 0.003). Post hoc tests show a reduction in GFP between 16 and 24 weeks of age and a reduction between 17 and 24 weeks of age. For the approach test we observed a shorter response distance of 10.21 ± 12.87 at 24 weeks of age compared to 16.27 ± 15.93 at 17 weeks of age (Z = -2.326, n = 240, p = 0.020).

4.4 Discussion

The results from this study show that providing ramps during rear enables chicks to access elevated structures during their first week of life. Chicks have a preference to use the ramps to access elevated structures and this is observable throughout the rearing period. When transferred to the laying shed a preference for ramps to transition between levels is shown regardless of the rearing treatment. When accounting for rearing experience we can see that there are benefits to rearing with ramps seen during the first few weeks in the laying shed. This is shown by a greater percentage of hesitancy behaviours (pacing and crouching before transitioning and wing flaps during a transition) in birds without prior experience of using ramps, suggesting they are less confident in transitioning down ramps in the laying shed. There was a difference in keel bone damage recorded at 40 weeks of age with the ramp reared group recorded to have a lower percentage of keel bone fractures compared to the control group.

4.4.1 Rear

Our findings clearly demonstrate that providing low level access ramps to perches or other elevated structures enables access to raised areas of 25cm during the first week. Very few chicks were seen on the structures at 1 week of age in the flocks not provided with ramp access. These findings correspond with some of our previous work where we

observed chicks from 1 day old using structures with low level perches (10cm) and access ramps. We saw an increase in ramp use over 1 to 3 days of age followed by an increase in use of higher structural elements up to 26 days of age (Norman et al., 2019). These observations in experimental and commercial situations suggest that if provided with low level access points, elevated structures will be accessed during the first week of life and greater use persists to 3 weeks of age.

These differences in structure use and experience during the early stage of development may have longer term cognitive effects. Experimental studies have found that chicks with early access to perches show improved spatial skills when tested. Norman et al., (2019) used a battery of tests to look at the effect of early access to three-dimensional structures. We tested chicks using a detour test and found chicks with structure access were faster at completing the test. Structure reared chicks appeared to utilise both hemispheres of the brain, displaying the ability to attend to both intra and extra maze cues in the rotated floor test compared to the chicks reared in a barren environment. We also tested chicks in a three-dimensional test looking at both navigational and physical ability. Regardless of rearing experience, fewer chicks succeeded in reaching a 20cm high level at 2 weeks of age than at 4 weeks of age (Norman et al., 2019). The results reported here confirm the findings under commercial farming conditions where fewer chicks were observed on the elevated structures at 1 and 3 weeks of age than at 16 weeks. From 3 weeks of age onwards the chicks without ramps accessed the higher parts of the elevated structures by using the low-level perches and platforms. Chicks with ramps also used this method but more than twice the number used the access ramps. Chicks reared without ramps showed a trend towards more crouching before transitioning up and more wing flapping behaviour than those reared with ramps. We also observed more low perch jumps up the structures and more jumps down from the low-level perches and slats in the control groups. These differences in hesitancy behaviours between the rearing groups suggests that providing access ramps improves the confidence to transition up and down structures. We found that regardless of treatment the recordings of hesitancy behaviours (crouching, wing flaps, pacing) reduced from 3 to 16 weeks of age when moving up the structures and the occurrence of crouching and wing flaps reduced for movements down the structures.

Encouraging the use of ramps during the rearing period may also have benefits in bone and muscle development. Dial (2003) discussed wing assisted incline running (WAIR) observed in wild Galliformes during movements up inclines. Dial and Jackson (2011) also suggest that adult Galliformes may prefer WAIR rather than jumping or flying up to elevated areas. WAIR has been recorded in domestic laying hens when transitioning inclined ramps of 40 degrees (LeBlanc et al., 2018). This wing flapping and running up inclined structures may improve muscle and bone development (Rath et al., 2000).

4.4.2 Lay

When transferred to the laying environment differences between the rearing groups were identified in the percentage of hesitancy behaviours observed when transitioning down the ramps. These behaviours have been identified and used in both commercial and experimental studies to look at ramp transitions (Pettersson et al., 2017a, Norman et al., 2018, Pettersson et al., 2017d). The control group showed more crouching, pacing and wing flapping behaviours before transitioning down a ramp. This suggests that they were more hesitant before transitioning down a ramp compared to groups reared with ramps. In our previous experimental study we found birds with ramp experience showed fewer hesitancy behaviours before transitioning (fewer head orientations), during a

transition (fewer pauses, turns and returns) and were faster to complete a transition (Norman et al., 2018). These results are reflected on farm, although there was more focus on the pre-transition behaviours. We know from the results at rear that the ramp reared birds were using the ramps to access the elevated structures, so it could be assumed that they learnt to transition on an inclined surface. It is important to note that the recordings on farm were taken after 1 week of acclimatisation to the laying shed, this shows a strong effect of rearing with ramps. By 24 weeks of age we saw significantly more straight down transitions regardless of rearing treatment suggesting improved movement on ramps with more experience. The importance of matching the rearing and laying environment have been outlined in various reviews (Janczak and Riber, 2015, Rodenburg et al., 2008). Hesitancy or difficulty in transitioning the ramps (as seen in the control group) could result in restricted access to resources, which are provided at different levels and therefore unsatisfied behavioural needs which could result in frustration and welfare consequences (Weeks and Nicol, 2006). Although not quite significant, a greater percentage of ramp-reared birds moved straight down the ramp compared to the controls, which tended to zig zag at 17 weeks and also wingflapped significantly more. This suggests smoother transitions and more controlled movements down the ramps.

We observed 42.8% of birds moved away from transitioning down levels without ramps compared to 1.3% birds in areas with ramps. Similarly, for upward transitions, 15.7% of birds moved away from areas without ramps compared to 0.2% in areas with ramps. This shows an overall preference for using ramps to transition both up and down between the litter and raised slatted area. It appears birds prefer to walk up and down an inclined surface rather than jump or fly. With an increase in the number of multi-tier

laying systems this is an important observation. If birds have trouble in transitioning between levels, they may not access some resources. By simply adding ramps into the system we may improve movement throughout. There is evidence that birds with keel fractures are less willing to jump down from a perch for food (Nasr et al., 2012a). Installing ramps in multilevel systems may be important to aid movement of birds with fractures. By creating an easy route to navigate the system as shown by Stratmann et al. (2015) we may also reduce the overall keel bone fracture rate and incidence of falls and collisions.

4.4.3 Welfare measures at lay

An important finding of this study was a difference in the percentage of birds with keel bone fractures between with rearing groups. In the CR groups 64.8% of the birds were recorded with keel bone fractures and 52% in the RR groups. There is research that suggests rearing in complex environments helps to develop bone mineral density (Hester et al., 2013, Casey-Trott et al., 2017a), spatial navigation (Norman et al., 2019, Wichman et al., 2007). Our study provided early access to structures requiring spatial navigation and therefore more movement during the first weeks of life. Further work is required to disentangle these theories. Previous research has found that access to ramps in the laying period reduces the number of keel bone fractures (Stratmann et al., 2015, Heerkens et al., 2016), thus by providing ramps during rear and lay we may dramatically reduce the overall percentage of birds sustaining keel bone fractures. It is important to replicate this work in systems such as multi-tier with high reported percentages of fractures (Wilkins et al., 2011, Wilkins et al., 2005).

Restriction from resources such as litter and the outside range have been shown to increase the risk of feather pecking developing within a commercial flock (Lambton et

al., 2010) and it will be observed earlier with more dramatic changes in the environment (Drake et al., 2010). We found no difference in feather pecking and feather cover when measured at 17, 24 and 40 weeks of age. We had hypothesised that a differences in feather cover may be observed due to improved access to the litter and range upon transfer to the laying shed, with improved ease and experience of transitioning using ramps. Early access to perches has been suggested to reduce fearfulness in laying flocks (Donaldson and O'Connell, 2012). Further, birds reared in more complex environments, such as aviaries, show reduced fearfulness (Brantsaeter et al., 2017). With the difference in feather cover we might expect to see a difference in the outcome of the fearfulness tests in the respective rearing treatments: as this can be a predictor to feather pecking (de Haas et al., 2014a, Krause et al., 2006), however we did not see any differences in the approach distance, the response to a novel object or feather cover. This may be due to the simplicity of the system with only one transition level for birds to access the litter area and range. If this study was replicated in a multi-tier system with up to 4 levels, we may see different results.

There were no differences in floor eggs between the rearing groups. Gunnarsson et al. (1999) found that access to perches prior to 4 weeks of age reduced floor eggs at lay. All chicks had the opportunity to use the structures by 1 week of age, but structure use was lower in the CR chicks. We also recorded floor eggs at 24 weeks of age so may have missed any differences at the start of the laying period when more floor eggs were present.

4.5 Conclusion

In conclusion, we found if ramps are provided at rear chicks will utilise raised structures from an earlier age and show fewer hesitancy behaviours when transitioning at lay. An overall preference to use a ramp for upwards and downwards transitions suggests that by increasing the number of ramps in the rearing and laying system we may encourage more movement between the litter and raised slatted areas, especially important for multi-tier systems where birds must travel further and make more transitions to access the litter (and range). A lower percentage of keel bone damage in the RR groups illustrates the importance of rearing with early access to elevated structures and provides an applicable change that commercial producers can implement in their rearing systems with an aim to reduce keel bone damage. Overall, our previous experimental results appear to have translated well into commercial systems giving confidence in the benefits of rearing with ramps in commercial systems.

Chapter 4 demonstrates the importance of applying research in a commercial situation where it will have the largest impact. Throughout this thesis very little information on the rearing practices of commercial producers has been found. It is not known how much scientific research is applied in the commercial industry or the current practices used in the rearing of laying hens. This will be investigated in Chapter 5 by using an online questionnaire.

Chapter 5 - A Descriptive Chapter:

Exploring the Common Practices for

Rearing Laying Hens Commercially

A Descriptive Chapter: Exploring the Common Practices for

Rearing Laying Hens Commercially

Abstract

The purpose of this study was to explore the rearing practices for commercial laying hens currently used in the UK. An online questionnaire was designed to be distributed among rearing producers in the UK through large egg producing companies and at poultry producer meetings. There were 16 responses representative of 5.8% of the sampled population. Due to the low response rate only summary descriptive statistics were used.

The questionnaire was structured to obtain general background information such as farm size and system type. Questions were asked about the rearing house design, lighting, enrichment and feeders and drinkers. Questions were either short responses or tick boxes.

In general, there appeared to be three sizes of rearing farms large (>10000), medium (>10000) and small (>1000). There was a positive correlation between farm size and the multiple types of rearing systems and for a higher number of external laying hens producers. The smaller rearing farms tended to rear for their own laying farms and appeared to provide ramps and ladders at a younger age than the larger rearing producers. Descriptions of the management strategies show some variation between farms and some current practices that may need further research into.

Due to the small sample size the results are not representative of the population of rearing farms and the questionnaire would benefit from some modification to ensure it is used correctly by producers. However, the importance of sampling the population has been highlighted here and allows identification of areas requiring further research or where application of research findings is lacking in the commercial industry of laying hens.

5.1 Introduction

The welfare of laying hens housed in intensive production systems has received much global research. Investigations have been carried out into behaviour, performance, system design and management of the laying system. Recently there has been increasing recognition that welfare problems can be influenced by the environment and/or experiences laying hens undergo during the rearing period from hatch up to 16 weeks of age (Janczak and Riber, 2015, Campbell et al., 2019), and that these can have long term effects. UK legislation covers very few aspects of the rearing of laying hens, and The Welfare of Farmed Animals (England) Regulations does not cover the rearing period for laying hens. Rearing is not mentioned in the Council Directive 1999/74/EC, however, recently more information and suggestions on rearing pullets for lay has been published in the DEFRA Code of Practice for the welfare of laying hens and pullets 2018, but these are not enforceable and are used only as guidelines for good welfare. The Codes of Practice do provide some information and suggestions about the brooding and rearing environment recommending, for example, the provision of perches from 7 days of age and mentioning the benefits of rearing with dark brooders (accessible dark resting boxes). Assurance schemes have set out more detailed guidelines for producer compliance, such as the Soil Association organic standards and the RSPCA Assured Welfare standards which give more details, such as, brooding methods and suitable enrichments. But one reason for little mention of rearing practices in assurance schemes, Codes of Practice or legislation is because there has been limited applied research on rearing practices in commercial systems on which to base recommendations.

Janczak and Riber (2015) reviewed management and environmental factors applied during the rearing period, they highlighted various changes that could be made to

improve both behaviour and welfare of layers such as, access to litter as early as possible, rearing in similar environments to lay and early access to perches from 7 days of age. Campbell et al. (2019) also conducted a review focusing on the environmental enrichment provided during rear and the impacts on behavioural and physiological development. The authors concluded that further research is needed to understand chick's early life development and what environmental enrichment needs to be included during the rearing period. Housing systems can vary greatly from enrichment, design of perching structures, feeding and drinking systems, and general management. With an increase in consumer demand for eggs from loose-housed hens many companies are developing new rearing systems to prepare laying hens for housing in these more complex loose-housed systems. These can be loose-housed aviary style rearing systems (Figure 5-1a), fully littered barn type systems (Figure 5-1b) or brood and move (BM) systems where chicks are housed in one system up to 8 weeks of age (Figure 5-1c) then housed in a second system that is matched to laying system (Figure 5-1d). Research has identified that rearing in systems that match the laying set up is beneficial in reducing the risk of fearfulness (Brantsater et al., 2016), improving bone development (Casey-Trott et al., 2017a, Casey-Trott et al., 2017c, Casey-Trott et al., 2017b) and improving spatial navigation (Tahamtani et al., 2015). This research has translated well into commercial set ups and it is now recognised that if birds are to be housed in more complex loosed-housing aviaries they should be reared in similar set ups.



Figure 5-1. Images of rearing setups (a) multi-tier aviary rearing, (b) floor barn rearing, (c) brood and move shed 1 and (d) brood and move shed 2.

The literature discussed in Chapter 1 highlights the range of research that has been conducted on poultry welfare and environmental set up. However, the extent to which many of these practices are applied to commercial rearing farms has not been investigated. The value of applying simple surveys to obtain background information for farming practices has been demonstrated in various studies. Green et al. (2000) used a survey to assess associations of feather pecking with management and disease. Hane et al. (2000) used a survey to look at laying hens husbandry practices in Switzerland and this was also conducted for pullets (Huber-Eicher, 1999). With an increase in loose-housed laying system throughout the UK to 54% of production it is not known how these changes are reflected in the UK rearing systems. This study aims to summarise the current rearing practices adopted in the UK and draw conclusions of areas that may need investigating to contribute to the best rearing practices.

5.2 Methods

5.2.1 Design

An online questionnaire was designed to explore the current rearing practices in the UK. The questionnaire was designed to take about 10 minutes to complete to encourage as many responses as possible. The questionnaire was created using Bristol Online surveys. It was distributed using an online link and password. Paper copies were also taken to events and distributed. Companies were emailed and asked to spread the link throughout their rearing producers. The questionnaire was live from June 2017 to September 2019. Figure 5-2 shows the navigation through the questionnaire.

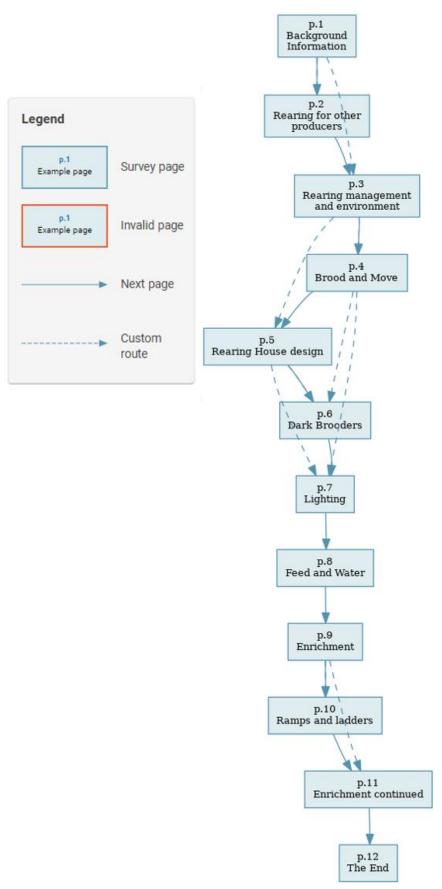


Figure 5-2. Flow diagram of questionnaire map and set up. Dependant on the responses to questions producers may have followed a custom route through the questionnaire.

The first section was used to collect general background information for each farm.

Respondents were requested to give:

- The systems that the farm reared for were listed and respondents were asked to tick all that applied.
- ii) The total number of birds at rear on the site
 - The number of birds per rearing shed
 - The number of rearing sheds
- iii) The total number of birds in lay on site (if any)
 - The number of birds per laying shed
 - The number of laying sheds on site
- iv) If they reared for other producers

If respondents reported rearing for other producers, they were taken to the next section of the questionnaire. The next questions were:

- i) The number of other producers
- ii) If the rearing systems were matched to the laying set up
- iii) How long the driving time was to the furthest producer.

They were then taken to a section on rearing management and environment and asked:

- i) Do you rear in an environment similar to the laying shed
- ii) Do you get smothers at rear, the options of 'No, Rarely, Frequently'
- iii) The age in weeks that smothers were most common
- iv) Following set of questions asked if the birds stayed in one shed throughout rearing

If the respondent answered that they reared their pullets in multiple sheds throughout rear they were taken to a section about BM rearing methods, if not they skipped to the next section. The questions asked were:

- i) Do you brood and move, 'Yes, No, Sometimes'
- ii) The first environmental set up, 'Fully littered, Raised areas, Other'
- iii) The second environmental set up 'Fully littered, Raised areas, Other'
- iv) The age chicks were moved to the second environment
- v) The age of access to raised areas
- vi) Age of transfer to the laying shed
- vii) How the rearing sheds are heated
- viii) If dark brooders were used

Producer that reared in one shed were asked:

- i) The setup of the rearing environment, 'Fully littered, Raised areas, Other'
- ii) Age of access to raised areas
- iii) Age of transfer to laying shed
- iv) Heating of the rearing shed
- v) If dark brooders were used

If producers answered 'no' to dark brooding they skipped to the next section, if they answered 'yes' they were asked:

- i) If they used whole house heating or just heated under the dark brooders
- ii) How they heated under the brooders
- iii) The materials the dark brooders there made of
- iv) If the dark brooders had curtains

- v) How many per shed
- vi) The dimensions of the dark brooders

The next set of questions were about the lighting in the sheds. Producers were asked:

- i) The lighting cycle they started the chicks on
- ii) How the dark period was increased
- iii) Do they dim the lights before the dark period
- iv) Do they use coloured light
- v) What type of lights are used in the sheds

Questions on the feed and water were asked. Respondents were requested to give:

- The type of feeders used: Chain feeders, pan feeders, pan and chain feeders, other.
- ii) The type of drinkers used: Nipple drinkers, bell drinkers, bell and nipple drinkers and other.
- iii) If the feeders and drinkers were matched to the laying environment
- iv) How many diet changes there are during rear
- v) The form of feed given at rear: crumb, mash, pellet and other
- vi) The form of feed given at lay: crumb, mash, pellet and other

The next question asked if respondents rearing with ramps or ladders if 'yes' they were taken to the section on ramps and ladders if 'no' they were taken to the enrichment section. For the ramps and ladders respondents were asked:

- i) The ramp or ladder design: plastic slatted ramp, wooden slatted ramp, metal slatted ramp, plastic ramp, wooden ladder, metal ladder
- ii) The age of ramp or ladder access

The enrichment questions were continued. The respondents were asked:

- i) What enrichment do they provide at rear
- ii) What age do they give access to elevated structures
- iii) What elevated structures are the chicks exposed to: plastic slats, metal slats, wooden slats, plastic perches, metal perches, wooden perches, plastic platforms, metal platforms, wooden platforms.
- iv) The height of the highest perch
- v) The material provided for litter: straw, wood shavings, soil, woodchips and grass

5.2.2 Statistical Analysis

In 2006 there were 275 rearing farms registered with the BEIC (Consultant, 2006), there were no other accessible more current records found for the number of rearing farms in the UK. Responses were received from 16 producers, giving a response rate of 5.8% of the population, a low response rate. A sample size of 161 would give us a confidence level of 95% with an error margin of 5%.

The following data are presented in a descriptive way due to the small sample size. Summary statistics were calculated in SPSS 24. Pearson's Correlations were performed for continuous data. Producers were asked to fill out a single questionnaire for each different type of rearing system they had on their farm. However, consulting the answers it appears the questionnaire was answered for multiple types of rearing systems on one site. It therefore is assumed that if any of the systems had aspects corresponding to the question asked it would be answered for that system.

5.3 Results

For the background information 10 of the 16 producers reared in multiple types of rearing systems, resulting in 29 different rearing systems. See Figure 5-3 for percentage of systems reared in. The number of chicks housed per farm varied from 2,000 to 7,500,000 with a mean of 550,344. A variation in size of rearing farm was observed, therefore these farms were categorised as large (>100,000), medium (>10,000) or small (>1,000). See Table 5-1 for summary of rearing information. There was a significant correlation between the number of chicks housed at rear and the number of different systems the rearing farms had (r(16) = 0.710, p = 0.002), with larger rearing farms having more varied rearing systems, see Figure 5-4 for scatter graph. A significant correlation was found between the number of chicks housed at rear and the number of different producers reared for (r(16) = 0.958, p = 0.001), showing producers rearing more chicks tended to rear for a greater number of external laying hen producers, see Figure 5-5.

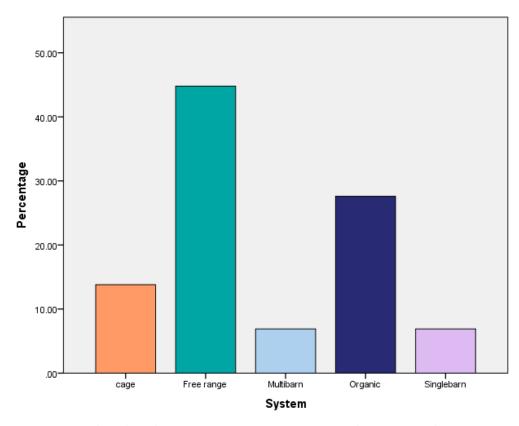


Figure 5-3. Graph to show the percentage of rearing systems producers reported.

Table 5-1. Summary statistics for questions separated for large, medium and small producers. Where averages were taken results are displayed as mean \pm SD.

Producer size	Large > 100,000	Medium > 10,000	Small > 1,000	Total
Number of producers	6	6	4	16
Average number of rearing systems	2.16 ± 0.983	1.83 ± 0.753	1.25 ± 0.500	1.81 ± 0.834
Average total number	1425167±	3667 ±	4125±	550344±
of birds in rear	3263586	27218	2394	1856015
% producers with layers on site	33.3	33.3	100	50.0
Average number of birds in lay on site	7920 ± 17710	23333 ± 42521	9300 ± 6529	14453 ± 26149
% rearing for other producers	83.3	66.6	0	56.3
Average journey time to furthest producer (hours)	3.40 ± 1.39	2.90 ± 2.84	n/a	3.16 ± 2.02
% Rear in multiple sheds	16.6	16.6	0	12.5
% Rear with raised platforms or slatted areas	100	50.0	50.0	68.8
Age access to raised platforms or slatted areas (days)	9.70 ± 8.04	10.2 ± 8.87	14.5 ± 15.6	11.1 ± 10.2
Average age Pullets transferred to laying sheds (weeks)	16.0 ± 0	15.7 ± 0.820	15.8 ± 0.500	15.8 ± 0.540
% Dark brooders	0	33.3	50.0	25.0
Average starting light cycle (hours)	22.2 ± 1.17	22.7 ± 1.37	23.3 ± 0.500	22.6 ± 1.15
Average number of diet changes at rear	3.50 ± 0.550	2.75 ± 0.710	3.00 ± 0	3.23 ± 0.600
% Access to ramps at rear	100	100	25.0	81.3
Average age of first access to ramps or ladders (days)	13.0 ± 7.87	11.8 ± 6.91	1.00 ± 0	11.5 ± 7.49
Average age of first access to perches (days)	13.0 ± 7.87	11.8 ± 6.91	14.5 ± 15.6	12.9 ± 9.29
Average highest perch chicks can access (cm)	158 ± 54.6	158 ± 65.5	113 ± 28.7	147 ± 54.9

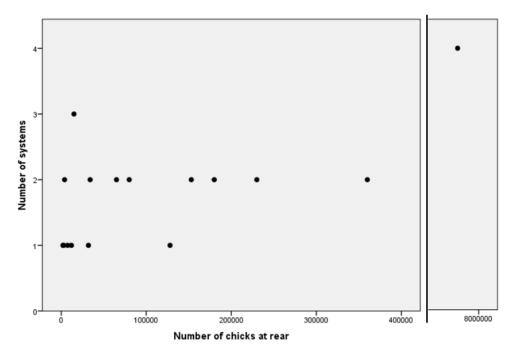


Figure 5-4. Scatter graph of number of chicks housed per rearing farm and the number of different rearing systems. The y axis has been compressed to include the largest producer.

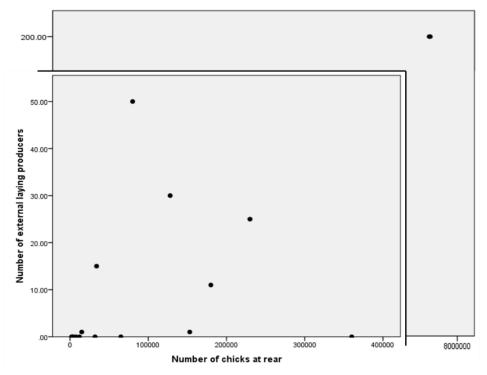


Figure 5-5. Scatter plot correlating the size of producer with the number of external producers they rear for. The x and y axis have been compressed to include the largest producer.

5.3.1 House design

Only two of the producers transferred pullets to a new house during the rearing period using the BM method. These chicks were moved to the second environment between 6 and 8 weeks of age. The age that chicks had access to raised platforms or slatted areas varied between farms from day 1 to 28 days. The maximum journey time to laying producers was estimated to be 7 hours and the minimum was 30 minutes, no correlations were found between rearing farm size and journey time to producers. Smothers were not reported as a frequent occurrence by any of the producers. However, if they were observed producers tended to see them between 5 to 12 weeks of age.

5.3.2 Brooding and lighting

In the questionnaire many producers reported to use a combination of heating methods, 11 used gas brooders, 3 oil space heaters, 4 biomass and 1 underfloor heating. Four producers reported dark brooding, only three followed through with further answers. For heating the house when dark brooding two producers reported heating the whole shed and under the dark brooders, one producer only heated the shed.

For whole house lighting the average number of light hours on day one was 22.6 hours. The maximum light period was 24 hours with the minimum of 20 hours. All producers gradually increased the dark period over rear and 10 of the producers dimmed the lights before the dark period. One of the producers reported using green coloured light in the rearing sheds. Many producers reported using light-emitting diodes (LED), of the 12 producers that responded to the types of lights used, eight reported using LED lights, and the remaining five used various other forms of bulbs.

5.3.3 Feeders and drinkers

Producers tended to use chain feeders (8) or a combination of pan and chain feeders in the rearing house (6). One producer just used pan feeders and one used tube feeders. Eight producers used bell and nipple drinkers and 6 used only nipple drinkers. One producer reared with only bell drinkers and one selected other, but the type was not specified. Ten of the producers matched the feeders and drinkers to the laying environment. Regarding the number of diet changes at rear only 13 of the producers answered this question. There was a maximum number of 4 changes and a minimum of 2, with an average of 3.2 changes. Considering the type of feed, there were a variety of feeds from crumb, mash and pellet. With 10 out of 16 of the producers feeding a variety of these throughout the rearing period. However, when asked about the feed at lay, of the 15 producers that answered, 13 said the birds were fed on a mash diet.

5.3.4 Enrichment

For elevated enrichment the minimum age that structures were provided was 1 day of age and the maximum was 28 days of age (see Table 5-1 for average values). Variations on material and type of elevated structures provided were recorded, for example plastic slats, metal slats, metal perches and wooden perches. The minimum perch height provided was 50cm and the maximum was 250cm (see Table 5-1 for average values).

The material used for ramps in the rearing period varies from plastic slatted ramps, metal slatted ramps, metal ladders, metal mesh ramps, and wooden slatted ramps. There was a large variation in the age that producers provided ramp access from 1 day of age to 21 days of age (see Table 5-1 for average values).

For enrichment that the producers reported providing there were some common themes such as: Lucerne blocks, hanging buckets, egg trays, hanging bottles, pecking blocks and straw bales. Ten of the producers provided wood shavings as litter, 3 provided straw and 3 provided both wood shavings and straw.

5.4 Discussion

Overall, the results of this questionnaire show various rearing factors that appear to be well utilised in the industry such as pecking and structural enrichments, but we have also found some rearing strategies that may need further research such as lighting and dark brooding to ensure the right practices are being continued within the rearing industry to improve welfare at rear.

5.4.1 House design

A large variation in the size of rearing farms was identified and further analysis was conducted to investigate the differences in rearing set up between the size of rearing producers. It was found that larger rearing farms tended to rear chicks in multiple systems and for multiple laying producers. This suggests there is a difference in the management style of these large rearing producers and the smaller producers, who appear to rear for their own farms. Smaller producers between 1,000 and 10,000 birds in rear on site, tended to have more laying birds on site and did not rear for other producers. This may suggest that they could tailor the rearing environment to match the laying environment. Although no significant correlation was found, producers that were categorised as large with over 100,000 birds on site had longer journey times to laying hen producers farms. Drake et al. (2010) identified a reduction in the risk of feather pecking if pullets stayed on the same farm for rearing and lay, one suggestion for this

was that journey time was reduced. Transportation is a stressful process for laying hens with increased levels of corticosterone measurable after just one hour of transportation (Knowles and Broom, 1990). Feather pecking in laying hens has been associated with stress (El-Lethey et al., 2000) thus long transport times may increase the risk of the onset of feather pecking.

Considering the environmental set up it appeared that more of the larger rearing producers provided the chicks with raised platforms and ramps compared to the smaller producers. The larger producers appeared to provide access from a younger age. There may be some effects of age of access to elevated structures, Rogers (2014) identified at 10 days of age chicks start to pay attention to distal and spatial cues and our own research suggests improved spatial navigation in a detour task if reared with elevated structure from day 1 (Norman et al., 2019). It appears to be important to provide access to elevated structures as early as possible.

Very little work has been conducted on piling and smothering during rear. Piling can be caused by panic and hysteria resulting in hens crowding or piling in particular areas which can result in smothering or injuries but could also be more of a 'creeping' type of behaviour (Bright and Johnson, 2011, Richards et al., 2012, Rayner et al., 2016) suggested to be cause by a source of attraction for birds (Campbell et al., 2016b). There have been reports in some studies of smothering resulting in higher levels of mortality in some rearing flocks (Gilani et al., 2012), but this does not appear to be of high concern within the producers questioned. Producers did not report smothering as a problem in their rearing systems.

5.4.2 Brooding and lighting

There is very little research on brooding chicks in the first few days of life. There are a few main methods each one will be discussed and explained in detail: Gas lamps, biomass, underfloor, hot air, dark brooders. Gas lamps are a common brooding method, they are mobile and provide a spot of heat source that can easily be adjusted in temperature by adjusting the height of the lamp. They are used to bring the whole house up to temperature and then provide direct areas of heat. Whole house heating tends to be through pipes running through the shed providing an even heat throughout. This prevents cold spots but there is no direct heat area for chicks to seek out a varying temperature gradient. Whole house heating methods via pipes reduce the amount of bioproducts released into the shed as a result of burning for example. We found that producers used a varied number of methods with gas spot lamp brooders being the most common. There is no research looking at brooding methods when rearing chicks in commercial environments this may need to be explored to assess the possible effects of whole house heating to create an even heat distribution or spot heating resulting in temperature variations.

Dark brooders are another addition to the rearing environment. These are boxes of varying sizes raised from the ground to allow chicks access underneath, a fringed curtain is often used to create dark area under the brooder. The brooders often have a heat source underneath, but this is not always the case. There has been research conducted both experimentally and on commercial rearing farms to look at the benefits of rearing with dark brooders. There appear to be positive effects of on feather pecking (Gilani et al., 2012, Riber and Guzman, 2017) and stress responses (Riber and Guzman, 2016a). Despite only positive effects of rearing with dark brooders they are not commonly used

in commercial rearing systems as demonstrated here with only 4 producers using them in the rearing system.

The average lighting schedule to start the chicks on was 22.6 hours of light. The risk of SFP developing during rear was found to decrease with longer photoperiods (Gilani et al., 2013), other studies have also reported less fear in a tonic immobility test during the first few days of life (Newberry and Blair, 1993) and have a more active lymphocyte response than chicks with a shorter photoperiod (Mashaly et al., 1988). However, chicks brooded with heat lamps with longer photoperiods have been shown to have longer periods of activity associated with this (Riber et al., 2007). Synchronous resting has been stimulated in a study that alternated 40 minutes light period and 40 minutes dark period. This has no effect on feed intake or weight gain but chicks showed more synchronicity in active behaviour (Malleau et al., 2007). Rearing with short alternated photoperiods or dark brooders may help to promote longer active and inactive phases and encourage the development of synchronicity within groups of chicks. Separating the active and inactive chicks may go some way to prevent the development of SFP by reducing opportunities to peck resting individuals (Riber and Forkman, 2007) or prevent the 'mis-imprinting' on feathers for foraging material (Jensen et al., 2006).

There has been very little scientific research on the most suitable aspects of lighting for rearing laying hens. The wavelength of light has been shown to play an important role in the development of behavioural asymmetry (Rogers and Krebs, 1996). One producer reported using green coloured lights. Khosravinia (2007) tested chicks' preference for yellow, orange, red and green light reporting a preference for green light measured by a time spent in the area, number of droppings and food consumed. Green light may be a

good alternative in commercial systems but the possible effects of changing the colour of light when moved to laying sheds or given outside range access must be considered. Many producers used LED's in their rearing sheds. One of the drivers behind using LED's is that they produce monochromatic light and have high energy efficiency, good reliability and long life (Parvin et al., 2014). Some studies have found benefits to using LED's compared to fluorescent lamps in productivity measures of feed consumption, feed conversion, bird weight and mortality (Mendes et al., 2013). However, a lot of this research has been conducted on broilers further work is needed to apply this to laying hens.

5.4.3 Feeders and drinkers

There are many variants of feeders and drinkers. Most producers have automated feeders, these tend to be chain feeders that will drag feed through the troughs around the whole rearing shed. Some provide pan feeders in the early stages of rear to encourage feeding. The producers questioned here tended to use chain feeders or a combination of chain and pan feeders. Drinkers can be either large bell drinkers or nipple drinkers. Producers reported to use nipple drinker lines or a combination of bell and nipple drinkers. There has been research looking at the risk factors for welfare problems that start at rear. Certain feeder and drinker types can cause problems, as well as the number of feed changes and feed type at rear (Gilani et al., 2013, Drake et al., 2010). For 62.5% of the producers the feeders and drinkers were matched to the laying environment. This has been ranked as an important environmental factor to ensure smooth transitions between the rearing and the laying environment and a risk factor in the development in feather pecking at lay (Drake et al., 2010).

Regarding the number of diet changes at rear only 13 of the producers answered this question. There was a maximum number of changes of 4 and a minimum of 2, with an average of 3.2 changes. This has been associated with an increase in the risk of feather pecking in epidemiological studies for both rearing and laying farms (Lambton et al., 2010, Gilani et al., 2013). Changing the feed type is essential for commercial producers to help with the growth and development of the pullets to get them up to weight to start laying. Each feed change will have different nutritional components. However, there are risk factors associated with diet types (Gilani et al., 2013, Lambton et al., 2010). The type of feed can also be a risk factor, the most used feed types are crumb, pellet and mini pellet feed. Considering the type of feed form is important as hens can be selective in their feeding so may not receive a complete balance of nutrients. Considering the type of feed there were a variety of feeds from crumb, mash and pellet. With 10 out of 16 of the producers feeding a variety of these throughout the rearing period. However, when asked about the feed at lay, of the 15 producers that answered 13 said the birds were fed on a mash diet. This corresponds with the literature suggesting that birds fed on mash show improved feather cover at lay (Savory et al., 1999).

5.4.4 Enrichment

(Campbell et al., 2019) conducted a review of environmental enrichment provided at rear to laying hens. They classed enrichments as "environmental modifications that have demonstrable impacts on the birds including perches, toys, functioning, and more complex alternative rearing systems". Structural enrichment is shown to be important during rear for the development of spatial navigation and learning to use the environment (Norman et al., 2019, Wichman et al., 2007). All producers provided elevated enrichment by 28 days of age. However, our research has suggested improved

spatial navigation at 4 weeks of age if elevated structures are provided from day 1 (Norman et al., 2019), it may be important for some of the producers to introduce raised structures from a younger age. Thirteen of the producers reported providing ramps at rear with an average age of 11.6 days of age. We have found the provision of ramps at rear encourages earlier access to elevated structures that may otherwise not be accessible by the chicks until an older age and if chicks experience ramps at rear they are better at using them (Norman et al., 2018).

The average height of perches was 146.88cm however some of the producers only provided perches up to 50cm. Research has demonstrated the importance of perching in laying sheds to reduce fearfulness in flocks and provide a place for refuge by conspecifics (Cordiner and Savory, 2001, Donaldson and O'Connell, 2012). Most producers provide perches during rear, as it is now a requirement at lay. Rearing in environments that match the laying system, such as rearing aviaries, improves movement at lay and reduced collisions and falls (Colson et al., 2005). The provision of more simple elevated enrichments may be important as it encourages chicks to jump up and down between elevated areas, performing actions that will be required in the laying shed.

There has been very little focus of pecking enrichments during the rearing stage. The results of feather pecking are commonly observed at the later stages of lay, therefore there is not a focus on providing alternative pecking devices during rear. There is evidence that feather pecking can develop in the rearing sheds (Gilani et al., 2013). If access to foraging opportunities is limited the likely-hood of feather pecking developing during rearing is increased, theorised to be a form of re-directed foraging (Blokhuis,

1986). Providing litter substrate to stimulate foraging during rear is important to prevent feather pecking (de Haas et al., 2014a, de Haas et al., 2014b).

Enrichment to stimulate pecking behaviour can also be provided in the forms of objects. For enrichment that the producers reported providing there were some common themes such as: Lucerne, hanging buckets, egg trays, hanging bottles, pecking blocks and straw bales. Ten of the producers provided wood shavings as litter, 3 provided straw and 3 provided both wood shavings and straw. McAdie et al. (2005) provided string to chicks from 1 day of age and found a reduction in gentle and severe feather pecking at 8 weeks of age. UK farm assurance schemes such as the Welfare standards for pullets (RSPCA, 2018) state environmental enrichment in the form of pecking and/or foraging objects must be available by 15 days of age. Some examples provided are hanging bottles, boots and pecking blocks. A concern about the use of pecking objects is that they may lose novelty, therefore may need rotating with new objects. Pettersson et al. (2017b) provided a resource packed to laying hens to study the effects on feather pecking in commercial flocks. Wind chimes and pecking pans were included as pecking enrichments. Use of the wind chimes was variable between flocks with 6 flocks showing no interactions with the chimes at all. However, the pecking pans were used consistently across flocks suggesting as an enrichment, the pans are generally more attractive to laying hens. Some objects such as string or carboard may be better suited as these will change when pecked creating novelty. Edible enrichments are becoming increasingly common, Lucerne for example is being use more readily by the industry. It is a form of alfalfa hay and can be purchased in compact bales that take time to break down and consume. It appears to have some nutritional benefits alongside acting as a physical pecking enrichment. Alfalfa is good source of fibre rich in vitamins, minerals and amino acids (Gerard, 2008), so may act as a source of additional nutrition that is lacking in the

organic poultry diets. Providing alfalfa bales as a source of enrichment and forage is suggested in the Featherwel pecking project. There is evidence to suggest that rearing with a high source of fibre is beneficial for gizzard development and nutrient absorption (Svihus, 2011).

There were some limitations to this study, the first was the low response rate. As only 16 responses were received there was not a representative sample of the population, therefore, only descriptive statistics could be calculated. The second limitation was that the producers only filled out one questionnaire, even if they reared for multiple types of systems. It was also not possible to obtain specific information on membership of assurance schemes, this may have affected the standards to which producers were held and should be considered in future investigations. The initial aim was for producers to fill out a questionnaire per rearing system which would have provided us with more information. In future studies answers could be restricted so only one rearing system could be ticked, thus answers to the subsequent questions would have to be for that one specific system. Finally, the design of the questionnaire could be modified, having received the responses some questions were missed out, a response should have been required before respondents could move onto the next section. Some questions could have been developed to obtain more information from the producers.

5.5 Conclusions

This chapter identifies several areas where research has been applied to commercial systems but also where research is lacking. This highlights the value of obtaining an overview of the current practices used in commercial systems. We have identified differences in the set up and designs of different sized rearing farms. Considering the

responses from the producers and the research discussed some suggestions for further research can be made.

- Transfer time to laying farms may be putting birds at increased risk of severe feather pecking. Reducing transport time and research to improve catching methods and transport modules is required.
- The translation of research that highlights the importance of rearing with access to environmental complexity appears to be used in the commercial industry as all of the producers provided elevated areas for their chicks to use. However, the age that chicks had access to these elevated areas may need to be addressed as some producers were not providing access until 28 days of age.
- Heat provision during the brooding period needs to be explored, a variety of methods are used and there is little research to advise on this.
- Dark brooders appear to show benefits in reduced fearfulness and feather
 pecking in experimental conditions and commercial farms. These are not used
 widely on commercial farms, further research into the fundamental of dark
 brooders is needed.
- The lighting period for commercial systems needs more research into encouraging synchronicity in chicks and the effects of extended light periods during the first few days after hatch.

These areas identified above are not well understood in commercial systems and novel alternative research may be beneficial when focused on the early rearing period of commercial laying hens. Alongside further research, improved translation of research to commercial farms is needed to ensure knowledge is applied.

Chapter 6 - General Discussion

General Discussion

The aim of this thesis was to investigate if there were effects of changes in the rearing environment of laying hens, specifically by aiming to encourage the use of elevated structures at rear to improve general movement through the laying system. Following on from the literature review in Chapter 1 it became clear that there was a lack of research on the use of elevated structures and chick movement at rear. Results from studies looking at laying hens movement in commercial sheds showed encouraging results from the addition of ramps. Chapter 2 explored the effects of rearing experience with ramps versus no ramp experience on the ability to transition a ramp. The results suggested benefits in transitioning ability if reared with ramps, however, the age that ramps should be provided was not known. This led onto the question of the importance for chicks to experience environmental complexity during early life for the development of spatial cognition, addressed in Chapter 3. Following on from this, some aspects from the experimental studies were applied to a commercial rearing and laying farm. The effects of early experiences of ramps and elevated structures at rear on the movement between the tiers at lay were considered in Chapter 4. In Chapter 5 the current commercial rearing management practices were explored using a questionnaire distributed to laying hen rearing producers, with the aim to summarise management strategies and identify areas where further research may improve bird welfare at rear.

6.1 Methodologies

Throughout this research four methodologies were used either as a combination or individually in a study: Applied experimental research, applied longitudinal research,

behavioural recordings and an online questionnaire. Each methodology will be discussed.

6.1.1 Experimental Research

There are benefits to conducting research in experimental conditions, for example, this allows for a controlled study design with many variables accounted for. Experimental studies were carried out in Chapter 2 and Chapter 3 of this thesis. In Chapter 2 this allowed individual pullets to be tested in their transitioning ability after a short training period. By conducting this research in an experimental facility video recording equipment could be used to allow detailed behavioural observations which would not be possible in farming situations.

The study discussed in Chapter 3 was also conducted in an experimental facility. For this study many replicates of pens were required to remove any pen effect on the data. 16 pens with 8 pens per treatment were used and then each chick within these pens was tested. In a commercial situation there are many chicks housed in one shed so effects of the housing cannot be accounted for, which is possible in experimental conditions as it allows for multiple pens to be set up.

The main concern with small-scale experimental studies is that they have been conducted under conditions that may not be representative of commercial practice and therefore may not translate into 'real' world situations. The effects may be reduced and unnoticeable in situations with many differing external variables. However, as demonstrated in this thesis, experimental studies are useful for initial detailed investigations with a specific focus in mind.

6.1.2 Longitudinal Research

In Chapter 4, an on farm longitudinal study was conducted to assess the effects of rearing with ramps on farm. This study was conducted on a multi-age site with matched treatment and control flocks reared in two separate sheds. Each flock was studied up to 40 weeks of age and in total the study took 3.5 years to complete. Due to the set up on this farm the study could be run under controlled conditions with the treatment flocks undergoing an intervention at rear with no other differences between the two rearing sheds. The benefits of conducting a longitudinal study are that allowances can be made for confounding factors by matching controls and treatments (Coggon et al., 2009). The commercial farm study setup of two matched rearing sheds allowed for effects of weather, seasons, management, parent flocks and other confounding factors to be accounted for. Each rearing flock was from the same breeder flock and hatchery thus accounting for any early life experiences that may influence results. The study was conducted on one farm which controlled for the management style and any changes that were made during the rearing and laying periods as changes were always made to both rearing and laying sheds.

There is very little research conducted on commercial farms where interventions are made and the effects studied. Commonly multiple farms are used for commercial farm studies, but this results in much more variation in housing set up, management styles, age and breeds. Therefore, a large sample size is required to account for the variation in environments and laying hens' experiences. The sample size for the farm study was 12, appearing relatively small compared to other commercial studies (Pettersson et al., 2017a, Pettersson et al., 2017b, Brendler and Schrader, 2016, Stratmann et al., 2019). However, due to the controlled environment and experiences of each flock that made up

the sample size this was sufficient for the aims that we had set out for the study. Conducting research over a long time period can also have drawbacks. Pettersson et al. (2017b) looked at two consecutive flocks in commercial farms (a baseline followed by a treatment flock). The longitudinal design of this study resulted in some changes in genotypes between flocks which must be accounted for.

Conducting research in commercial settings has the benefits of not needing to obtain any extra subjects. In line with the 3R's, which aim to reduce, refine and replace, by using subjects from commercial settings there is no need to use any extra subjects as they are already in production. This must be considered when researching animal welfare as the use of live animals for research purposes requires ethical consideration to ensure animals lives are not used unnecessarily.

6.1.3 Behavioural Recordings

In Chapters 2 and 4 detailed behavioural recordings were taken to assess the movement of chicks and laying hens up and down ramps and level transitions without ramps. These recordings were developed from various other studies (Pettersson et al., 2017a, Pettersson et al., 2017d, Lambe et al., 1997, Scott et al., 1999, Scott et al., 1997). In the experimental conditions for Chapter 2 video cameras were set up to capture bird transitions. The videos were re-coded to ensure blinding of the treatments (ramp reared, or not ramp reared) to prevent observer bias when recording the transition behaviours from the videos. Using video cameras also allowed more detailed behaviour to be recorded, such as the latency to transition. Conducting similar recordings in a commercial setting (Chapter 4) proved to be more challenging, therefore the number of focal behaviours recorded had to be reduced and there were further limitations to taking live recordings. It was not possible to be blinded for the on-farm recordings as all

behavioural recordings were taken by one observer. An ethogram was created to ensure recordings were taken using the same protocol for each flock. Taking live behaviour recordings may have created some observer effects in the number of birds transitioning up or down between the raised areas. Care was taken to minimise these effects by keeping a 2-metre distance from the recording location and waiting for the chicks or laying hens to adapt to the presence of the observer. To reduce this effect completely, installing a camera on site would have been a good alternative to capture the behaviour, however this creates more time required to watch videos and logistics of setting up a camera and protecting it in a commercial setting.

6.1.4 Questionnaire

The questionnaire discussed in Chapter 5 provided an interesting summary of rearing practices in the UK. The questionnaire was straightforward to design and followed a simple structure aiming to gain information about rearing practices in the UK. It was accessible through an online link and password that was sent out to contacts in the laying hen industry. Unfortunately, the response rate was only 5.8% of the population of rearing farms. Previous surveys of producers have received responses of 51.5% in laying hen producers (Green et al., 2000) and 42.6% for pullet rearing survey in Switzerland (Huber-Eicher, 1999). However, these farms were visited and farmers interviewed which may have encouraged a greater response rate. There were some limitations to this method of data collection for the specific sample of respondents. In the rearing industry there are many different farming setups, with some large companies rearing in multiple houses of different designs and then smaller producers rearing for themselves. The questionnaire was not designed for producers with multiple types of rearing set ups. The aim was for producers to complete one questionnaire per rearing system, but this was

not explained clearly in the questionnaire. If this questionnaire was to be repeated more work would be needed to recruit producers. Targeting the larger rearing companies would be an option and UK wide advertising in poultry magazines could help to increase the response rate.

6.2 Results and implications for rearing pullets

Investigating in detail the rearing requirements of chicks for access to elevated structures has provided some useful insight that is applicable to commercial rearing farms. The overall aim of this thesis was to introduce improvements during the rearing period with the specific focus on movement and navigation. Chapters 2, 3 and 4 have achieved this aim and demonstrate the importance of rearing in complex environments with the opportunity to access elevated areas and the practical application of this in commercial systems.

The results from these studies have wide implications to many different rearing systems. In multi-tier rearing chicks will commonly be confined within the system and let down onto the litter at a later age. However, our results clearly show that chicks will utilise ramps during the first week of life and this results in an increase in the use of elevated structures at 3 weeks of age. If ramps are provided between the levels of multi-tier rearing system, producers may feel encouraged to let chicks utilise different levels and access the litter areas from a younger age. By promoting the use of the three-dimensional environment, we are aiding the chick's development of spatial navigation ability (Norman et al., 2019). This developmental period is important to ensure that chicks and laying hens are prepared for the environments in which they are housed and required to navigate (Colson et al., 2005, Tahamtani et al., 2015). The evidence for improved spatial development and structure use can be disseminated to rearing producers and used to

demonstrate the importance of chicks being provided access to complex threedimensional structures from a young age.

The results from the questionnaire suggest that the age chicks have access to elevated structures is very variable between producers. The advice to producers would be to install ramps during the first week of life at rearing in all systems with perches or raised areas, as our results from the jump test (Chapter 3) suggest 15 day old chicks show difficulty in reaching levels up to 20cm high. The results from the on farm study show that chicks will use ramps as an access route to elevated structures if given the opportunity. This suggests a possible preference for ramps, reflected in the laying shed by a greater number of transitions in the ramp areas. Previous studies conducted on the use of ramps by laying hens have yielded similar results (Pettersson et al., 2017a, Pettersson et al., 2017d). Fewer hesitancy behaviours have been found in pullets when transitioning grid ramps (plastic commercial slats) verses ladder ramps showing that simple plastic slats, that many producers have access to, will suffice for a ramp (Pettersson et al., 2017d). Thus, the introduction of ramps into commercial systems need not be costly to producers. A concern for some producers could be that the addition of ramps may result in a cluttered system and possibly cause clustering of chicks away from heat sources. Conducting this research on a commercial rearing farm has given no evidence of negative consequences of providing access to ramps during the first week of rearing.

This research has identified possible frustration behaviours related to restriction from a resource. Behaviours such as pacing, stepping on the spot and head movements were recorded as pre-transition behaviours to moving up or down a ramp or making a level change, developed from previous studies (Lambe et al., 1997, Scott et al., 1999). These

behaviours have been used in other studies, for example Davies et al. (2014) recorded increased head movements when laying hens were able to view a food reward but not access it, these could be frustration related or anticipatory behaviours. Increased head movements were recorded in the control group in Chapter 2 when tested in a downwards ramp transition to reach a food reward. Although initially recorded as head orientations to look down or up a ramp, with the introduction of a food reward they may have indicated frustration or anticipation due to restricted access to a reward, as Davies et al. (2014) observed in their study. In commercial settings if laying hens are expressing frustration-related behaviours in response to restriction from a resource these may trigger abnormal behaviours such as injurious pecking, for example, restriction or interrupted access to litter (Gilani et al., 2013, de Haas et al., 2014b). In the on farm study (Chapter 4) improved feather cover was recorded at 40 weeks in the laying hens reared with ramp access. Feather pecking is a multi-factorial problem that has been identified to be clearly related to feeding and foraging behaviour (Rodenburg et al., 2013). If initial stressors were experienced during early life (de Haas et al., 2014a), such as frustration due to difficulty in accessing foraging resources i.e. the litter, this may have resulted in enhanced difficulty in coping with environmental stressors throughout the laying period. Sharing these findings with commercial producers is important due to the multi-factorial nature of injurious pecking, to reduce some of the factors may be beneficial in expression of this behaviour.

6.3 Limitations

Specific limitations associated with the methodologies used in each chapter have been discussed throughout. Some overarching limitations for this thesis were sample size, breed selection and confounding factors.

6.3.1 Sample size

Regarding the two experimental studies (Chapter 2 and 3), most of the behavioural recordings were on an individual bird level, resulting in a large sample size per treatment. However, in the first study looking at rearing with ramps (Chapter 2), there were only two group housing rooms for the chicks, one for each treatment. This does not account for the effect of room on the treatments. This was a problem when reporting on the group housing results and only summary statistics could be performed due to there being two groups per treatment. Consideration also needs to be made for the individual tests results as treatment is confounded with room (only two rooms were used for housing due to space limitations. When studying spatial cognition (Chapter 3) an effort was made to ensure there were multiple replicates for each rearing treatment to account for pen effect, resulting in 8 pens per treatment. As with much research conducted on commercial farms the sample size was a limitation of this research. Twelve flocks were followed from rear until lay with 6 flocks per treatment. Owing to this being a longitudinal study conducted over a 3.5-year period, time was a limiting factor. It was decided that as the study was only conducted on one farm the number of confounding variables would be reduced. The response rate for the questionnaire was low with only 5.8% response rate for the population. The factors influencing this were the distribution of the questionnaire which could be improved by utilising advertising and with more time visiting poultry events and collecting questionnaire responses in person or over the phone may yield a greater number of responses.

6.3.2 Bird Genotype

This research was funded by a company that use a British Black tail genotype, a cross between Rhode Island Red and a light Sussex genotypes, specifically produced for the company. Therefore, this research is not widely applicable to the whole of the commercial industry. Studies have shown differences in behaviour and welfare outcomes between different breeds of birds (Ali et al., 2019a, Ali et al., 2019b), thus caution not to generalise results to all breeds is needed. Replication of these studies on different genotypes and in different environments such as multitier systems in required.

6.3.3 Confounding Factors

Throughout the studies there were multiple confounding factors. Experimental studies (Chapters 2 and 3) shared a common problem during testing that individual birds varied in motivation for either a food reward (Chapter 2) or companionship (Chapter 3). When training pullets to run to a food reward down the testing corridor differences in speed to reach the reward were noticed between individuals (Chapter 2). These differences were increased when the level change was introduced, thus using latency as a measure of hesitancy to transition may have been misleading in some cases as some highly food motivated pullets may have been more hesitant when transitioning without a food reward. When studying spatial cognition (Chapter 3), motivation to reach companions was used due to chicks being young. However, during the RFT a large variation in time to reach companions was observed during the training and testing. This may have indicated differences in motivation.

In Chapter 4 the aim was to conduct a controlled farm study to apply the results investigated in Chapters 2 and 3. In commercial settings there are some factors that are out of the researcher's control. Due to the time scale of the project changes to the farm practices and set up may have occurred. To account for confounding factors such as management practices, the weather and age of outside range access, a matched-pairs design for treatment and control flocks was used to reduce the effects. Variation within

the replicated flock cycles may have occurred but the matched-pairs for treatments will have reduced the effects of this. Conducting research studies in experimental conditions allows for the control of many more variables as in Chapter 2 and 3. However, in the on farm study (Chapter 4) it was demonstrated that replicating research in a commercial setting can provide confidence in the outcome of the results.

6.4 Future research

As discussed in the Introduction, the laying industry has been gradually changing from predominantly cage to loose-housing systems (BEI, 2018). The application of the research to commercial laying systems is an important aspect of this thesis. In the onfarm study aspects of the experimental work conducted in Chapters 2 and 3 were applied to a commercial rearing and laying farm. The next steps would be to apply this research to more complex rearing and laying systems. In systems with multiple levels for laying hens to transition it is important that there are no obstacles that may prevent access to important resources. Current research has found a reduction in keel bone fractures by installing ramps on commercial farms (Stratmann et al., 2015, Heerkens et al., 2016). By applying the current knowledge gained here we know there are effects of rearing experience with elevated structures on movement between levels and on access ramps. We recorded lower percentages of keel bone damage in the RR groups in our on farm study (Chapter 4), which follows the findings of previous studies that have reported rearing in environments such as aviaries result in reduced levels of keel bone fractures within a flock (Casey-Trott et al., 2017a, Casey-Trott et al., 2017b, Casey-Trott et al., 2017c). Work should be conducted on developing more complex rearing systems that challenge chicks' spatial navigation during the first four weeks of life to improve

cognitive development and their physical ability to navigate commercial housing systems throughout the birds' life.

6.4.1 Behavioural research on commercial farms

There is a lack of applied research *in situ* on commercial laying farms. Recording the behaviour of laying hens whilst transitioning between levels has been a success of this thesis. The application of this work to more complex laying systems would be useful. Recording behaviour during transitions may allow the identification of areas that create difficulty for laying hens to transition thus restricting access to resources and possibly resulting in frustration or injury. This work is relevant for both the rearing and laying systems and should inform changes to house design that may ease transitions, such as the application of ramps in the rearing and laying systems. A challenge of research on commercial farms is the identification of individual birds and tracking movement through the laying system. Recent work has explored the use of accelerometers, light meters and Infra-red radio tags to measure individual birds locations and movements (Rufener et al., 2019, Siegford et al., 2016, Buijs et al., 2018, Buijs et al., 2019). However, there are still challenges to be resolved when tracking birds in commercial systems, not least the loss of fitted equipment.

6.4.2 Behavioural research in semi-natural conditions

There has been very little research on the behavioural development of domesticated laying hens ancestor, the Red Jungle Fowl. Collias and Collias (1967) observed Jungle fowl in their natural habitat in North-Central India. They observed chicks flying up into trees from a very young age in response to alarm calls from the mother hen. The responses of commercial chicks to vocalisations has not been studied. The use of (recorded) vocalisations to encourage perching behaviour may be an interesting step in

development and promoting the use of more complex environments from a young age. Australian bush turkey chicks show a preference for performing WAIR rather than flight (Dial, 2003) suggested to be used to gain access to elevated tree branches for predator avoidance. The results of performing these behaviours on steep slopes on the skeletal and muscular development of the domestic chick has not been looked at in detail and the effects this may have on keel bone fractures. More research is needed to investigate navigation, skeletal and muscular development in laying hens.

6.4.3 Spatial Cognition

Further work is needed to investigate the effects of rearing in complex systems on the spatial cognitive development of layer chicks. The specific age that access to complex three-dimensional environments is required for development of spatial navigation is not known. Workman and Andrew (1989) observed changes in behaviour associated with ages where changes in lateralization of the brain occur in chicks reared in semi-natural conditions. More recent research has identified changes in hemisphere dominance associated with the development of behaviours (Freire and Rogers, 2005, Freire and Rogers, 2007, Rogers, 2008, Rogers, 2014). In commercial systems chicks may be confined to single level rearing systems and may lack experience during a critical developmental window. With an increase in the number of hens kept in loose-housed systems such as complex multi-tier or aviary systems, it is important that understanding of the development of spatial navigation is researched further. Testing chicks in tasks that are more representative of the commercial setting may be more applicable. Buijs et al. (2018) tested laying hens' movements over obstacles. This could be applied to chicks by creating a complex obstacle course for them to navigate to reach an important resource, with the aim to test specific cognitive and physical skills required for the laying environment. This should be carried out in conjunction with a two dimensional spatial task such as the radial arm maze used by Whiteside et al. (2016). Further work is needed to identify whether there are continued differences in brain lateralisation during development and the effects that rearing in enriched environments has on the brain structure. By improving birds navigation, we may consequently improve welfare such as reducing the number of keel bone fractures and birds with plumage damage due to injurious pecking.

6.4.4 Commercial practices

The rearing producer questionnaire identified current practices conducted by commercial pullet rearers that may need further researching to identify the best practices for rearing laying hens. Aspects found from the questionnaire consist of the age that chicks require access to elevated structures, in line with the suggested future research from the other chapters included in this thesis. Further work is needed on the brooding of chicks. There is no recent research addressing the optimum brooding temperature, heating set up and lighting schedule required during the first few days of life. With evidence of the importance of early life experiences (Janczak and Riber, 2015) more research is needed to update current practices. Recent research has identified benefits of rearing with dark brooders both experimentally and on commercial rearing farms (Gilani et al., 2012, Riber and Guzman, 2016b, Riber and Guzman, 2017), however, the results of the questionnaire suggest that dark brooders are not used widely on commercial rearing farms. Further work is needed to explore the use of these on commercial farms and the aspects of dark brooders that may be beneficial to chicks.

6.5 Summary

This thesis has explored the effects of rearing experience and environment on movement and navigational ability of laying hens in commercial systems. The studies discussed have concluded that the provision of simple resources such as ramps during rear can increase the early use of elevated structures thus improving spatial cognition of layer chicks. The application of this research to commercial laying systems is important as this could have impacts on the behaviour and welfare of laying hens now housed in increasingly complex systems. Recommendations include providing low level perches and access ramps during the first week of rear to encourage use of the three-dimensional environment, this is a feasible low-cost addition to loose-housed environments. It is suggested that further research should go back to basics to answer some fundamental questions of domestic laying hens' navigational, skeletal and muscular developments by comparisons with semi-natural rearing conditions. Further knowledge could be applied to develop a more suitable rearing environment to challenge the development of commercial laying chicks.

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Appendix 1 – Questionnaire



Rearing Practices for Laying hens

Background Information
What housing system do you rear for? (Tick all that apply)
□ Organic
☐ Free range
Single Tier Barn
Multi Tier Barn
Cage
Other
If you selected Other, please specify:

	Appendix 1
Total number of birds in rear?	
Number of birds per rearing shed?	
Total number of rearing sheds on farm?	

Total number of birds in lay on farm?

Number of birds per laying shed?

Total number of laying sheds on farm?

Do you rear for other producers?

C Yes

C No

Rearing for other producers
How many different producers?
Do you tailor rearing to match different producers environmental set up?
How long is the driving time to the furthest producer?

Rearing management and environment

	Do you rear in an environment similar to the lay	ving shed set up?
	Do you get smothers at rear?	
O No	C Rarley	Frequently
weeks	At what age are smothers most common?	
	Do the birds stay in one shed throughout rearin	g?
O Yes		

Brood and Move

Do you brood and move?
□ More info
C Yes
C No
Sometimes
Is the first envronment a fully litter/scratch floor or does it comprise of raised areas?
☐ Fully littered Raised
areas Other
If you selected Other, please specify:

Is the second environment a fully litter/scratch floor or does it comprise of raised areas?
☐ Fully littered Raised ☐ areas Other ☐
If you selected Other, please specify:

At what age do you move the chicks to the second environment/shed?
Weeks
At what age do chicks have access to raised platforms/slatted areas?
weeks
At what age do you transfer pullets to the laying shed?
weeks
How do you heat the rearing shed?
Do you use dark brooders?
C Yes
C No

Rearing House design

Does the rearing shed have a full litter/scratch floor or does it also have raised platforms/slatted areas?

□ Fully littered floor□ Raised areas
At what age do chicks have access to raised platforms/slatted areas?
weeks
At what age do you transfer pullets to the laying shed?
weeks
How do you heat the rearing shed?
Do you use dark brooders?
C Yes C No

Dark Brooders

Do you heat the whole house or just the area under the dark brooders?
How do you heat under the dark brooders?
What are your dark brooders made of?
□ Wood□ Plastic
✓ Metal✓ Other
If you selected Other, please specify:

Do your dark brooders have curtains?

_	Appendix 1
O Yes	
C No	
	How many dark brooders are in a shed?
	Please give the dimensions of dark brooders? Height, width and length
200	
cm	

Lighting	
What light cycle do the chicks start on?	
□ More info	
How is the dark period increased?	
Do you dim your lights before the dark period?	
Do you use coloured lights?	
What type of lights do you use in the sheds?	

Feed and Water

What type of feeders do you use a rear?

Chain feeders Pan feeders Pan and chain feeders Other
If you selected Other, please specify:
What type of drinkers do you use at rear? ☐ Nipple drinkers
☐ Bell drinkers
Bell and nipple drinkers Other
If you selected Other, please specify:
Are the feeders and drinkers the same as in lay?
C Yes
C No
© Sometimes

Appendix 1

How many diet changes are there during rear?	
What form of feed do you give at rear?	
□ Crumb□ Mash□ Pellet□ Other	
If you selected Other, please specify:	
What feed form are the birds given atlay?	
☐ Crumb ☐ Mash ☐ Pellet ☐ Other	
If you selected Other, please specify:	

Appendix 1

Enrichment

Do your chicks get experience with ramps or ladders leading up to elevated structures?

C Yes

O No

Ramps and ladders

Please specif	y ramp	or	ladder	design?

☐ Plastic slatted ramp
☐ Wooden slatted ramp
☐ Metal slatted ramp
☐ Plastic ladder Wooden
□ ladder Metal ladder
Other
If you selected Other, please specify:
At what age do the chicks first have access to ramps or ladders?
weeks

Enrichment continued
What enrichment do you provide for your birds during rear?
At what age do you provide access to evevated strucutres?
weeks
What elevated structures are the birds exposed to before being moved to the laying house? (Tick all that apply)
☐ Plastic slats Metal ☐ slats Wooden slats ☐ Plastic perches ☐ Metal perches ☐ Wooden perches ☐ Plastic platforms/tables ☐ Metal platforms/tables ☐ Wooden platforms/tables ☐ Wooden platforms/tables ☐ Other ☐

	Appendix 1
If you selected Other, please specify:	
What is the highest perch your birds can access?	
cm	
What materials do you provide for litter at rear?	
 □ Straw □ Wood shavings □ Soil Woodchips □ Grass □ Other 	

If you selected Other, please specify:

The End

Thank for completing this questionnaire